Delivering precision health: the role of molecular diagnostics

In recent years there has been an unmistakable trend towards precision health, a broad, all-encompassing approach that uses innovations in technology, diagnostics, and bioinformatics to enhance disease prevention, diagnosis, treatment, and monitoring. Molecular diagnostics approaches are now instrumental in delivering precision health to patients in many clinical areas, and their role is likely to become even more prominent in years to come.

Revolutionizing healthcare

The 'All of Us' program was established in 2015. This $215 million program is an ambitious effort launched by the US government and the National Institutes of Health (NIH) to gather data from one million people "with the ultimate goal of accelerating research and improving health". In this program, "researchers will use data from the program to learn more about how individual differences in lifestyle, environment, and biological makeup can influence health and disease (1)."

The All of Us program fits well within a broader trend of initiatives and collaborations being launched by governments, hospitals, and industry to revolutionize healthcare at a fundamental level. These projects take a multidisciplinary approach to gather, collate, and analyze data for improving population health and wellbeing. Many of them aim to contribute to delivering precision health.

What is precision health?

Precision health can be defined as an approach to healthcare that is patient-centric and patient-specific at every step. It applies this personalization to disease prevention, diagnosis, treatment, and monitoring.

Understanding the nuances of an individual's genetics and epigenetics is key to achieving many aspects of precision health. This improved understanding, combined with increasingly easy access to genetic information, has led to a vastly expanded role of genetics in the clinic. The term 'precision medicine' is frequently used to refer to the use of genetics to help tailor patient therapies.

The potential benefits of implementing a precision health approach are wide ranging. Not only can it result in improved survival rates for diseases such as cancer, it can also improve prevention through screening, reduce adverse effects of treatments, and avoid money being wasted on treatments that are ineffective for a given patient.

Fig 1. Understanding the nuances of an individual's genetics and epigenetics is key to achieving many aspects of precision health.

Precision through molecular diagnostics

The genetic information required to deliver precision health can come from a range of clinical diagnostic tests. Advances in molecular in vitro diagnostic assays (IVD assays) have been a key driver for better genetic information, including increased precision, faster turnarounds, and reduced costs.

Assays based on polymerase chain reaction (PCR) currently have the highest market share among the most common molecular diagnostic assays for detecting genetic abnormalities. Other established methods include DNA microarrays and fluorescent in situ hybridization (FISH).

DNA sequencing methods such as Sanger sequencing also have a long track record in finding mutations, but Sanger sequencing has limitations in throughput. Now, the massively parallel sequencing capabilities of next generation sequencing (NGS) approaches have largely replaced Sanger sequencing in many research applications and are making fast inroads into the clinic.

These technologies contribute to precision health by identifying the genetic abnormalities underlying diseases, resulting in diagnoses and treatments based on more than just symptoms. In oncology for example, the genetic makeup of tumors can be highly variable, even when the physical appearance is similar. Characterizing tumors genetically can therefore contribute substantially to personalizing treatments and maximizing their effectiveness.

The ways in which clinicians use molecular diagnostics to deliver precision health vary greatly between different clinical areas. For example, decisions on treatment for infectious diseases might require analyzing bacterial or viral genomes, whereas cancer diagnostics focus on genome markers in germline or tumor DNA.

The following sections provide an overview of these two clinical areas, describing how molecular IVD tests contribute to precision health, giving examples of current approaches, and outlining future perspectives.

Precision health in cancer

High-precision approaches are well suited to personalized cancer therapy due to high variability, not just in the causes of cancer, but also in treatments and their side effects. Molecular diagnostics already play a substantial role in oncology, one which in the future is likely to increase at a rapid pace.

Worldwide, an estimated 18.1 million people are diagnosed with cancer every year, leading to 9.6 million deaths. The most common types are lung, breast, and colorectal cancer. For each of these cancers, molecular assays are widely used to enable reliable screening or to guide clinicians in making critical decisions for personalized cancer treatment (2).

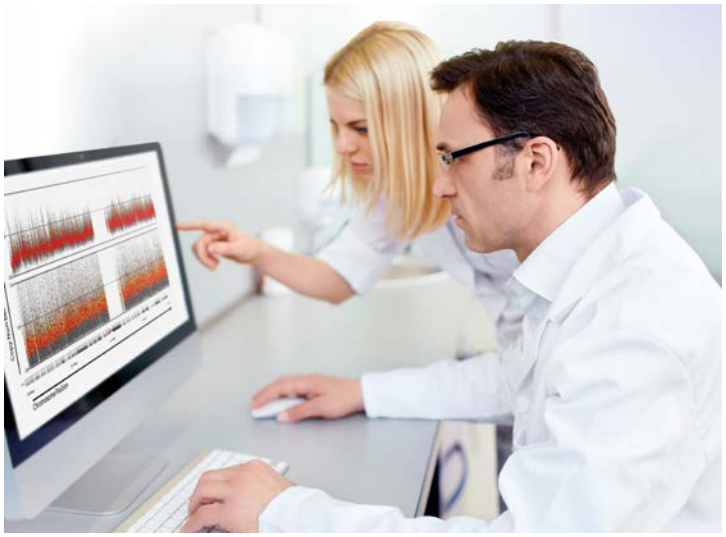

Fig 2. Scientists in a lab analyzing sequencing data.

Lung cancer

Lung cancer is the most common cause of cancer-related death in the US (3). Clinicians tend to classify lung cancers by cell type: small cell lung cancer and non-small cell lung cancer (NSCLC). NSCLC is responsible for around 85% of cases and, as a result, is the primary focus in the development of personalized therapies (4).

Molecular diagnostic techniques can play an instrumental role in determining the underlying genetic cause of NSCLC, and have a direct impact on treatment. The genetic makeup of lung cancers is extremely variable, with mutations in hundreds of genes linked to the disease. However, to warrant routine molecular testing for a biological marker, the key question is whether the test outcome can enable more targeted treatment (i.e. whether the mutation is 'actionable').

For example, patients with NSCLC can have multiple single nucleotide polymorphisms (SNPs) in EGFR, which are responsible for around 10% of cases. Patients found to have one or more of these mutations in EGFR are likely to respond well to EGFR tyrosine kinase inhibitors, which block EGFR signaling. Examples include gefitinib, erlotinib, and afatinib. In patients without these mutations, EGFR is likely to function normally, so these drugs are unlikely to have any effect (4,5).

A similar approach can be taken for translocations in the *ALK* gene, which affect around 5% of patients with NSCLC. Patients with *ALK* translocations respond well to treatment with crizotinib, a tyrosine kinase inhibitor which blocks the transmission of growth signals to the cell nucleus (4).

Role of molecular diagnostics in lung cancer

Molecular diagnostic tests for gene abnormalities in EGFR and *ALK* are widely used to guide treatment. These tests include fluorescent *in situ* hybridization (FISH) analysis and PCR-based tests, such as quantitative PCR (qPCR, also known as real-time PCR) and droplet digital PCR (ddPCR) (5,6).

NGS panels are a suitable option for targeting many genes simultaneously. Their massively parallel sequencing capabilities make it possible to use one assay to study not only EGFR and *ALK*, but also less commonly affected genes for which specific treatments exist, such as *ROS1, ERBB2, BRAF, MET,* and *RET*. It also enables targeting *KRAS*, which is a common cause for lung cancer, but difficult to target—thereby providing more clarity on treatment (5).

Colorectal cancer

Colorectal cancer affects one million people each year and has a high ability to metastasize.

The EGFR gene also forms a key target for a precision approach in the treatment of colorectal cancer, but clinicians must also take mutations in other genes into account. Patients found to be positive for EGFR mutations normally respond well to monoclonal antibodies that block EGFR, such as cetuximab and panitumumab. However, the efficacy of anti-EGFR treatment depends strongly on mutations in MRAS and KRAS (also known as the RAS genes) (7,8).

The RAS genes are involved in downstream intracellular signaling. If a tumor genome contains mutations in both EGFR and the RAS genes, standard anti-EGFR therapy will not be effective and can, in some cases, make matters worse. Studies are ongoing on whether a similar effect applies to tumors with *BRAF* and *PIK3CA* mutations. Therefore, analyzing the status of both EGFR and the RAS genes is essential in implementing a genomics-based approach (7,9).

Role of molecular diagnostics in colorectal cancer

qPCR is the test of choice for single-gene analysis due to its speed and simplicity. NGS has the ability evaluate a range of genes that might affect the efficacy of anti-EGFR therapy, including *NRAS, KRAS, BRAF,* and *PIK3CA*. These assays enable informed decision-making on whether to opt for anti-EGFR treatment or less specific alternatives, such as anti-VEGF drugs (bevacizumab, aflibercept, ramucirumab).

The tests mentioned here all focus on identifying the best avenues for treatment of advanced cancers. Molecular diagnostics also play a role when it comes to screening for early detection of colorectal cancers.

For example, one stool sample-based screening test (Cologuard™, Exact Sciences) uses PCR alongside other techniques to assess the risk of developing colorectal cancer. It estimates cancer risk by studying hyper-methylation in the promoter regions of *NDRG4* and *BMP3*, as well as point mutations in *KRAS* (10). These and other tests underline the role of molecular assays at different stages of cancer development and their contributions to precision health.

Breast cancer

The fight against breast cancer is more complex and multi-faceted than many other cancers, and molecular diagnostics have long played a central role in this fight. Since the mid-1990s, Sanger sequencing has been used to identify inheritable mutations in *BRCA1* and *BRCA2*, two key genes in predicting familial breast cancer (11).

Treatment for breast cancer often follows a personalized approach that considers the state of the tumor as well as the patient's individual characteristics and choices. From a genetic point of view, key characteristics of breast cancer are chromosome abnormalities in three genes: estrogen receptor, progesterone receptor, and *HER2* (11).

To test whether a tumor expresses estrogen or progesterone receptors, clinicians generally use nonmolecular methods, such as immunohistochemistry (IHC). If a tumor is hormone receptor-positive, recommended treatments usually include tamoxifen and aromatase inhibitors (12).

Testing for HER2

The third receptor, *HER2*, is responsible for 20 to 30% of breast cancers. In the majority of these cases, the cause is amplification (a form of copy number variation, CNV) of the *HER2* gene; the remaining cases are characterized by overexpression of the protein without amplification. *HER2* abnormalities can be tested with immunohistochemistry, but when the abnormality is caused by amplification, FISH provides a more suitable alternative (12,13).

A range of targeted treatment options exist for *HER2*-positive tumors that substantially improve outcomes for patients. These options include the antibodies trastuzumab, pertuzumab, and TDM-1, and the small molecule drug lapatinib (11).

When a tumor is negative for all three receptors (so-called triple-negative breast cancer), none of these treatments is likely to be effective, highlighting the need to understand the exact genetic makeup of the tumor. For triple-negative tumors, NGS provides a suitable means to interrogate large numbers of genes to search for causative mutations (12).

Future developments in oncology

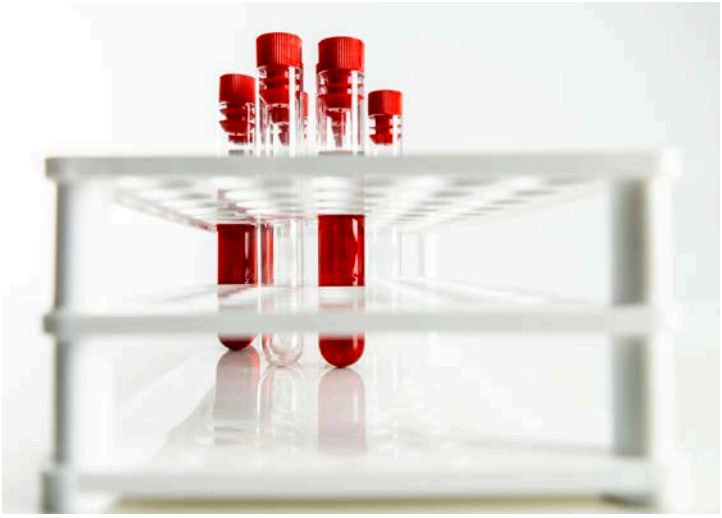

Fig 3. Clinical trials are underway aiming for early stage disease detection of a range of common cancers through liquid biopsies.

In the coming years it is expected that advances in cancer genomics and drug discovery will spur the discovery of new actionable mutations. These discoveries could expand the molecular IVD market in delivering better precision health approaches for more types of cancer.

In addition, increased capabilities and the precision of molecular diagnostic assays have the potential to improve patient outcomes. As assay sensitivities improve, one development that holds much promise is the use of liquid biopsies to test for genetic abnormalities. In cancer patients, liquid biopsies, for example a blood sample, often contain a small amount of fragmented, cell-free DNA (cfDNA) originating from the tumor. With sufficiently sensitive assays, this so-called circulating tumor DNA (ctDNA) can serve as input for molecular assays.

Improved sensitivity alone, however, will not be sufficient to make sure there is broad applicability of liquid biopsies. Because of the low availability of ctDNA in a sample and its highly fragmented nature, large amounts of data and sophisticated bioinformatics techniques are needed to apply the method clinically.

Molecular diagnostics companies that are currently developing blood-based tests to detect a range of cancers from a single sample include Guardant Health and Grail. Since 2014, Guardant Health has had a diagnostic liquid biopsy-based test for late stage cancers, and in 2019 it launched a research-use-only test for early-stage cancer and cancer recurrence (14).

Grail is currently running a series of clinical trials aiming for early stage disease detection of a range of common cancers through liquid biopsies. These and other companies are all working towards the key goal of liquid biopsies: to create a single blood test to detect as many cancers as possible, as early as possible.

Precision health in infectious disease

The precision health approach is also being applied to the treatment and control of infectious diseases. As of 2019, the FDA has approved 320 molecular assays for identifying microorganisms. This is more than double the number of approved human genetic tests (15).

Genetic tests reliably identify pathogens—or specific mutations in pathogens—to help clinicians develop or select tailored treatments. In certain cases, studying the patient's own DNA can also provide information to predict the efficacy of treatments against infectious diseases.

Antibiotics can treat many bacterial infections, but are ineffective against viruses, which can present similar symptoms. It is therefore important to exclude viral causes before initiating any antibiotic treatment. Selecting the most suitable type of antibiotic based on genetic tests can both improve the efficacy of the treatment and help minimize the risk of antibiotic resistance.

For these reasons, correct pathogen identification enables high-precision treatment. Two examples of infectious diseases where genetic information holds the key to high-precision treatment are sepsis and HIV.

Sepsis

Sepsis is a life-threatening condition caused by the host's disproportionate immune response to a pathogen. When left untreated, it can lead to septic shock, organ failure, and death. Bacteria are the cause of most sepsis cases, however viruses or fungi can also be responsible. The most suitable treatment for any specific case of sepsis depends highly on the type of pathogen, meaning species identification is critical to treatment efficacy (16).

First-line management of suspected sepsis cases includes blood culture for pathogen identification and broad-spectrum antibiotics. The antibiotic treatment might include multiple drugs to be effective against a variety of common causes for sepsis. These causes include gram-positive and gram-negative bacteria (*E. coli, S. aureus, K. pneumoniae, S. pneumoniae*) and potentially fungi (*Candida*). Once the pathogen has been identified, more targeted treatment should replace broad antibiotics (17).

Fig 4. Analyzing whole genomes or single cells can help to predict the efficacy of treatments against infectious diseases.

In the fight against many infectious diseases, fast test results are essential for the usefulness of the test, both for the patient and from the perspective of outbreak control. In the case of sepsis this need is well-demonstrated; survival rates improve dramatically with faster disease diagnosis. However, blood culture results (pathogen identification and resistance pattern) can take several days, potentially delaying the start of individualized treatment (18).

Role of molecular diagnostics in sepsis

Multiplex qPCR assays can detect the presence of a range of bacteria, fungi, and viruses in a blood sample without the need for culture. As a result, clinicians can get information about the pathogen involved within 4 to 6 hours. In addition, sequencing following PCR can provide an even more detailed analysis. Sanger sequencing is currently part of diagnostic testing, but NGS is also trialed as an alternative because of its high sensitivity (18,19).

Information from molecular tests makes a substantial contribution to precision treatment. Fast pathogen identification helps start targeted treatment earlier, which improves patient outcomes, especially when the broad-spectrum antibiotics are not suitable for the identified pathogen. This approach also helps avoid antibiotic overuse, and can therefore contribute to combating antibiotic resistance (17).

HIV

In the 40 years since the discovery of HIV in humans, there has been substantial progress in managing the condition. However, as HIV cannot currently be cured, existing treatments focus on slowing disease progress and preventing transmission, for example, by preventing the virus from entering T cells.

HIV-1 (the most common form of HIV) uses the CD4 receptor to enter a T cell, but also needs a co-receptor. It can use CCR5, CXCR4, or both.

In 2007, the FDA approved the first receptor antagonist (maraviroc) for *HIV-1* treatment, but it is only effective against infections that exclusively use the co-receptor CCR5. Due to this limitation, personalized HIV tropism testing is needed to make sure miraviroc treatment is likely to be effective (20,21).

Role of molecular diagnostics in HIV

There are both phenotypic and genetic assays available for HIV tropism testing. Among the genetic assays, Sanger sequencing (e.g. Trofile™, Monogram Bioscience) is the most widely used technique. However, Sanger sequencing has a limit of detection of around 20% population frequency, whereas evidence indicates that minority populations as low as 1% can influence drug sensitivity (21).

As a result, NGS-based assays have entered the market offering better sensitivity without compromising on accuracy. This improved sensitivity leads to better detection of low-level minority variants, and therefore better targeted treatments that avoid ineffective use of medications (22).

Future developments in infectious diseases

Expansion of the role of molecular diagnostics in diagnosing and treating infectious disease will highly depend on technological developments in speed, simplicity, and robustness (23).

For example, the sensitivity of molecular assays could bring a more personalized, targeted approach to point-of-care testing. However, this type of testing usually requires easy-to-use devices that give results in less than 1 hour, which is out of reach of most existing molecular tests.

A potential solution might be to use isothermal amplification as an alternative to thermal cycling. This could reduce waiting times compared to existing molecular tests, but could also offer a more accurate alternative to nonmolecular point-of-care tests that are fast but less precise, for example lateral flow assays (23).

NGS is also likely to continue its growth in infectious disease applications. Its ability to generate unbiased, high-throughput genomic data with single-nucleotide sensitivity makes it well-suited for sequencing large regions of a pathogen genome.

This technique is already applied in tackling disease outbreaks due to food-borne pathogens. As sequencing costs decrease and awareness of NGS in the clinic increases, the scope for NGS-based IVD assay development could grow rapidly (21).

Precision health moving forward

The precision health approach will undoubtedly become a mainstay of healthcare. With new genetic factors for diseases being discovered in rapid succession, molecular diagnostics will play an ever-increasing role.

Molecular assays are already enabling a personalized, high-precision approach to health in the clinical areas discussed here, as well as in Mendelian diseases, complex diseases, and reproductive health. In areas where the role of genetics is currently less clear, molecular assays could see a steady increase in years to come.

One such example is Alzheimer's disease. Many cases of Alzheimer's do not have a known cause, but specific genes and mutations are increasingly linked to the disease. The causes of Alzheimer's are also highly variable, which means that the likelihood of finding a 'magic bullet' treatment, effective for all forms of the disease, is unlikely (24).

Instead, the principles of precision health appear to be highly suited to the prevention and management of Alzheimer's. And although this individualized approach extends far beyond genetics, molecular diagnostics could play a role in prevention as well as in management, or even treatment, of Alzheimer's.

Summary and conclusions

The move towards precision health is at the forefront of modern medicine. Personalization in all aspects of health has a clear potential to lead to better prevention, diagnostics, treatments, and generally improved well-being.

Molecular assays have helped researchers and clinicians in improving personalized healthcare for more than 40 years, and the speed of technological developments in molecular biology means that molecular diagnostics have far from reached their full capability.

Oncology and infectious diseases are two areas where molecular techniques have not only proved their value, but are also likely to play an increasing role in the future. However, the reach of molecular diagnostic tests does not end there, with many other areas following suit, aiming to deliver on the promises of precision health.

Fig 5. NGS generates unbiased, high-throughput, highly-sensitive genomic data.

References

1. All of Us Research Program, World Health Organization [Online] https://allofus.nih.gov. Accessed 7 May 2019.
2. Latest global cancer data: Cancer burden rises to 18.1 million new cases and 9.6 million cancer deaths in 2018, The International Agency for Research on Cancer (IARC) [Online] www.who.int/cancer/PRGlobocanFinal.pdf. Accessed 7 May 2019.
3. Common Cancer Types, National Cancer Institute [Online] www.cancer.gov/types/common-cancers. Accessed 7 May 2019.
4. Personalised Medicine at a Glance: Lung Cancer, European Society for Medical Oncology [Online] www.esmo.org/Patients/Personalised-Medicine-Explained/Lung-Cancer. Accessed 7 May 2019.
5. Sholl, L., Molecular diagnostics of lung cancer in the clinic. *Transl Lung Cancer Res* **6**, 560–569 (2017).
6. Ilie, M. *et al.* Optimization of EGFR mutation detection by the fully-automated qPCR-based Idylla system on tumor tissue from patients with non-small cell lung cancer. *Oncotarget* **8**, 103055–103062 (2017).
7. Personalised Medicine at a Glance: Colorectal Cancer, European Society for Medical Oncology [Online] www.esmo.org/Patients/Personalised-Medicine-Explained/Colorectal-Cancer. Accessed 7 May 2019.
8. Kafatos, G. *et al.* RAS mutation prevalence among patients with metastatic colorectal cancer: a meta-analysis of real-world data. *Biomark Med* **11**, 751–760 (2017).
9. Chen, G. *et al.* Molecular Diagnostics for Precision Medicine in Colorectal Cancer: Current Status and Future Perspective. *Biomed Res Int* (2016). [Online].
10. Decision Memo for Screening for Colorectal Cancer - Stool DNA Testing (CAG-00440N), Centers for Medicare & Medicaid Services [Online] www.cms.gov/medicare-coverage-database/details/nca-decision-memo.aspx?NCAId=277. Accessed 7 May 2019.
11. Personalised Medicine at a Glance: Breast Cancer, European Society for Medical Oncology [Online] www.esmo.org/Patients/Personalised-Medicine-Explained/Breast-Cancer. Accessed 7 May 2019.
12. Hageman, I. S., Molecular Testing in Breast Cancer: A Guide to Current Practices. *Arch Pathol Lab Med* **140**, 815–824 (2016).
13. Mitri, Z. *et al.* The HER2 Receptor in Breast Cancer: Pathophysiology, Clinical Use, and New Advances in Therapy. *Chemother Res Pract* (2012). [Online].
14. Guardant Health Launches Blood-Based Early Cancer Assay for Research Use, Genetic Engineering News [Online] www.genengnews.com/news/guardant-health-launches-blood-based-early-cancer-assay-for-research-use. Accessed 7 May 2019.
15. Nucleic Acid Based Tests, U.S. Food and Drug Administration [Online] www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/InVitroDiagnostics/ucm330711.htm. Accessed 7 May 2019.
16. Diagnostics Assessment Programme, Sepsis: The LightCycler SeptiFast Test MGRADE, SepsiTest and IRIDICA BAC BSI assay for rapidly identifying bloodstream bacteria and fungi, National Institute for Health and Care Excellence [Online] www.nice.org.uk/guidance/dg20/documents/sepsis-the-lightcycler-septifast-test-mgrade-sepsitest-and-iridica-bac-bsi-assay-final-scope2. Accessed 7 May 2019.
17. Schmidt, A. S. and Mandel, J. Evaluation and management of suspected sepsis and septic shock in adults, UpToDate [Online] www.uptodate.com/contents/evaluation-and-management-of-suspected-sepsis-and-septic-shock-in-adults. Accessed 7 May 2019.
18. Suberviola, B. *et al.* Microbiological Diagnosis of Sepsis: Polymerase Chain Reaction System Versus Blood Cultures. *Am J Crit Care* **25**, 68–75 (2016).
19. Brenner, T. *et al.* Next-generation sequencing diagnostics of bacteremia in sepsis (Next GeneSiS-Trial): Study protocol of a prospective, observational, noninterventional, multicenter, clinical trial. *Medicine (Baltimore)* **97** (2018).
20. Archer, J. *et al.* Use of four next-generation sequencing platforms to determine HIV-1 coreceptor tropism. *PLoS One* **7** (2012).
21. BCC Research. Next-generation Sequencing: Emerging Clinical Applications and Global Markets (2017).
22. Raymond, S. *et al.* Performance comparison of next-generation sequencing platforms for determining HIV-1 coreceptor use. *Sci Rep* **7** (2017). [Online].
23. Kozel, T. R. and Burnham-Marusich, A.R. Point-of-Care Testing for Infectious Diseases: Past, Present, and Future. *J Clin Microbiol* **55**, 2313–2320 (2017).
24. Hampel, H. *et al.* A Precision Medicine Initiative for Alzheimer's disease: the road ahead to biomarker-guided integrative disease modeling. *Climacteric* **20**, 107–118 (2017).

gelifesciences.com

GE Healthcare Bio-Sciences AB, Björkgatan 30, 751 84 Uppsala, Sweden
GE Healthcare UK Limited, Amersham Place, Little Chalfont, Buckinghamshire, HP7 9NA UK
GE Healthcare Europe, GmbH, Munzinger Strasse 5, D-79111 Freiburg, Germany
GE Healthcare Bio-Sciences Corp., 100 Results Way, Marlborough, MA 01752 USA
GE Healthcare Japan Corporation, Sanken Bldg., 3-25-1, Hyakunincho Shinjuku-ku, Tokyo 169-0073 Japan
For local office contact information, visit gelifesciences.com/contact

KA8251180619WP