The AHSN Network

Genomic innovation: technologies for personalised medicine

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Foreword

The increasing personalisation of medicine will have a considerable impact on the way we deliver healthcare. In the future, health and social care will utilise a whole raft of new technology to deliver improvements in personalised medicine, which administers care based on the needs of the individual rather than a 'one-size-fits-all' approach.

The AHSN Network has made a significant contribution in the development and deployment of innovative technologies, medicines and therapies. As part of the AHSN national prioritisation process, AHSNs have outlined a specific interest in the development of personalised medicine, and in particular in genomics technologies. Genomics is a key component in the delivery of the aspirations around personalised medicine, as the effectiveness of a drug or therapy on a patient can differ considerably according to their individual genetic make-up.

By using genomics to understand the most effective mode of care for the individual, we can improve the impact of treatment. Genomics is a step change in personalised medicine, but it should be understood that it is not personalised medicine per se. It should be viewed as part of an innovative personalised medicine toolkit alongside other diagnostics that has now expanded to include a wider family of genomics-related and other 'omics technologies, such as proteomics, transcriptomics and pharmacogenomics.

Within this new world of personalised medicine and genomics as outlined in this report, the AHSN Network has a key role to play in developing commercial partnerships, new technologies and helping to speed up implementation and adoption into service. AHSNs have the ability to broker and network between industry, academia and the public sector, to help to understand the evidence requirements for implementation and adoption, encourage and promote the relevant engagement of commissioners, support the redesign of existing patient pathways, stimulate the development of new patient pathways and work to develop innovative roles and responsibilities within the health and social care workforce.

I hope this timely and informative report will also be of value to the wider health and social care system in guiding future developments and action.

Tony Davis

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Executive summary

Introduction

This review for the AHSN Network builds on the 2018 report *The Personalised Medicine Technology Landscape* and presents an evidence review of genomics and genomics-related technologies that will have an impact on the delivery of personalised medicine within two to three years.

The aim is to support the Academic Health Sciences Network's (AHSN) thematic programme on personalised medicine, which has been established to support the implementation of novel diagnostic and treatment approaches that make use of genomics and other 'omics technologies.

This review has been informed by desk-based research and analysis of public sources of information including grey literature, peer-reviewed literature, and interviews with expert stakeholders.

technologies that make use of genomics approaches are beginning to have greater impact on the delivery of healthcare

Genomic technologies in the NHS

With the 2019 launch of the Genomic Medicine Service and National Genomic Test Directory in England, genomics and technologies that make use of genomics approaches are having an increased impact on the delivery of healthcare in the short to mid-term.

Given these developments, and the genomics focus of the AHSN Network's personalised medicine work programme, technologies with an 'omics component were selected for analysis in this report.

Technology opportunities

For each of the technologies described, there is an opportunity for the AHSN Network to support innovation adoption and spread.

Circulating tumour DNA testing for cancer is a fast moving technology area and one type of companion diagnostic test is already available for lung cancer treatment via the National Genomic Test Directory. In the next three years there is potential for implementation of further companion diagnostic testing in other cancers and the use of ctDNA testing as a monitoring tool is showing great promise.

Pharmacogenomics testing will be included in the National Genomic Test Directory in the near future and as such there are a number of pilot projects to explore which gene-drug pairs are most ready for clinical implementation. The opportunities in this area will come once the pilot projects are complete and there is a need for the implementation of pharmacogenomics to be realised in the NHS.

Transcriptomics is another 'omics technology where three tests are already available to support clinical decision making in women with breast cancer. There are a number of other clinical areas where further support and evidence gathering is needed, for example in the area of rare disease diagnosis.

Near patient testing to support antimicrobial stewardship, including rapid diagnostic testing for infectious disease, is an area of varied and intense activity in terms of technology development and application. In particular, technologies that support antimicrobial stewardship have the potential to contribute to global efforts to mitigate antimicrobial resistance. Disease areas where there is already potential to support innovation and implementation efforts include influenza, urinary tract infections and sepsis.

Genetically modified regenerative medicines are an extremely complex subset of innovative regenerative. A number of GMRMs have already been approved for use within the NHS, including innovative CAR-T therapies for blood cancers, and a gene therapy for a rare immuno-deficiency disorder, ADA-SCID. The opportunities to support innovation include further developments in gene therapies, and over a longer term, in genome editing approaches. Due to the rare nature of many of the diseases treated with regenerative medicine, consideration needs to be given to evidence requirements and collection, which can take time with small patient numbers. This will also have an impact on specialised commissioning approaches for these rare disease therapies.

Conclusions

Effective coordination between the AHSN Network, the NHS and other stakeholders is required to address the challenges ahead. In meeting these challenges there is great opportunity to transform pathways, patient care and workforce engagement with new technologies.

Effective coordination between the AHSN Network, the NHS and other stakeholders is required to address the challenges ahead

industry needs to work closely with the NHS to develop the interventions and applications which best meet NHS needs

These challenges include:

- **Understanding evidence requirements.** Industry should work more closely with NHS services in order to develop interventions and applications which best meet specific NHS needs. In addition, NHS and NICE evidence requirements will need to be addressed in order to facilitate effective and systemic health system adoption of innovation.
- **Engagement of commissioners** is essential to support understanding of the nature of new technologies, how they can benefit patients and clinical services, their requirements and specific implementation approaches to achieve the desired outcomes.
- **Pathway transformation** can occur as new technologies are implemented, in contrast to less disruptive interventions, which can be added to established care pathways whilst still delivering the expected benefit. In both scenarios special attention and coordinated effort is required to implement new interventions in healthcare. Without taking these steps there is unlikely to be equitable access to these new interventions.
- **Engagement and training** of the workforce is vital. In in terms of genomics, this should continue through the ongoing efforts of Health Education England to embed genomic literacy in the workforce. Engagement for other technology areas should be considered.

Delivering on the promise

Should all the key elements be in place, there are a number of benefits.

For patients these include:

- More precise diagnosis and prognosis
- More targeted treatment
- Fewer side effects and improved clinical outcomes

For the health system these include:

- More efficient use of resources
- More streamlined care delivery
- Improved health outcomes

The implementation of healthcare services based on the new technologies in development will be occurring in a health system that is also undergoing technological transformation and infrastructural change. New healthcare technologies will require further developments in major digital services and infrastructure, which are vital to ensure their successful implementation.

As the single biggest integrated healthcare system in the world, the NHS is uniquely placed to transform healthcare at a population level. There is therefore a valuable opportunity for the AHSN Network to play a key role in supporting the implementation and spread of personalised medicine technologies in the NHS and to help realise the benefits to patients and the NHS.

as the single biggest integrated healthcare system in the world, the NHS is uniquely placed to transform healthcare at a population level

Recommendations

Circulating tumour DNA testing

Given that the available evidence for use of ctDNA testing varies broadly across different cancer types, as well as across different applications, approaches to assess the clinical validity and utility of ctDNA tests will need to be considered on a case by case basis.

Further work is needed to determine the most clinically and cost effective way of using EGFR ctDNA testing for non-small cell lung cancer in the UK health system. When new tests become available with improved sensitivity and specificity, they should be evaluated against current tests to ensure that the most suitable technology is used and implemented across the health system.

For clinical indications where there is an unmet need and a ctDNA companion diagnostic test has been identified, consideration should be given at an early stage of test development to gathering the evidence required to support best practice and integration of the test into clinical pathways.

Further work is needed to support gathering of evidence around the clinical utility of ctDNA monitoring approaches, through clinical research studies and trials.

Pharmacogenomics

With pharmacogenomic information interpretation of results is not always straightforward and requires careful consideration. The most up to date evidence from databases and recent guidelines should be used to support clinical decision making.

Further work is needed to determine for which situations reactive or pre-emptive pharmacogenomics testing will best meet the needs of patients and the health system and how such approaches could be delivered.

Consideration needs to be given to the evidence requirements to support implementation of pharmacogenomics testing, and to supporting test developers to understand these requirements.

Support for collaborations that facilitate sharing of resources and data are needed to underpin the information networks required to enable correct prescribing.

Clinical decision support systems for pharmacogenomics testing are a key area that requires further development and support.

Transcriptomics

Further support is needed for evidence-gathering of clinical utility of gene expression profiling tests, particularly in terms of patient outcomes.

Ongoing research is required to identify the most promising applications for transcriptomics in rare disease, such that these can be supported in terms of gathering evidence of clinical effectiveness.

Consideration should be given to how current DNA sequencing pipelines and infrastructure can be utilised and altered to support RNA sequencing efforts, should further evidence for its use arise.

Support for research into standardisation of RNA analysis methods is key to ensuring that evidence gathered is reproducible, accurate and reliable.

Near patient testing to support antimicrobial stewardship

With the use of their existing networks and by close collaboration with patient safety collaboratives AHSNs are well placed to support the identification, implementation and dissemination of new diagnostic tests as part of their work programme on AMR.

The AHSNs could help support further implementation and broader use of influenza point of care tests in several ways, through supporting dissemination and implementation of tests with sufficient evidence, to helping generate new evidence where needed.

In order to support the timely and effective implementation of point of care tests, test developers should work with the health system to understand evidence requirements early in the development process. This will require not only understanding the analytical evidence required, but also consideration of the health economic impact and changes to service models.

Genetically modified regenerative medicine

Consideration needs to be given to the levels of evidence required on the clinical effectiveness of therapies that treat diseases with low patient numbers and how that evidence can support specialised commissioning of these therapies.

Introduction

Since the publication of NHS England's vision for personalised medicine in September 2016[1], the concepts and principles of personalised medicine and care, and in particular the application of genomics technologies to achieve this, have been introduced into a number of recent policy documents or announcements including:

- *Generation Genome* – the 2016 annual report of the Chief Medical Officer for England[2]
- House of Commons Science and Technology Committee report *Genomics and Genome Editing in the NHS* (April 2018[3], response July 2018[4])
- Prevention green paper[5]
- National genomic healthcare strategy (due early 2020)

Against this backdrop of moving towards personalised medicine as 'business as usual', in 2017 NHS England commissioned the PHG Foundation to undertake a review of the personalised medicine technology landscape, in order to understand the near term opportunities and challenges of realising the benefits to patients of the delivery of personalised medicine. The aim of the report, *The Personalised Medicine Technology Landscape*, (PMTL) was to inform how the health system could deliver improvements in care for patients in the next 2–3 years and what policies needed to be in place to realise those benefits[6].

This document:

- Reviewed developments in biomedical and digital technologies that have been proposed to contribute to the personalisation of medicine
- Identified and described specific examples of technologies that have a sufficiently well-developed evidence base for validity and utility, such that they would be able to underpin the delivery of personalised medicine within the 2–3 year time frame
- Analysed how some of these approaches could be integrated most effectively within the NHS and highlighted key considerations for action that NHS England could take to develop and deliver personalised medicine

Given the opportunities presented in the report of the 'omics technologies underpinning many of these developments in personalised medicine, the Academic Health Science Networks (the AHSN Network) is seeking to explore how the spread of these technologies can be supported in the NHS.

The AHSN Network: supporting innovation and technology spread

AHSNs were established by NHS England to 'identify and spread health innovation at pace and scale', supporting the adoption of innovative technologies into the health system for the benefit of patients.

In 2018 the AHSN Network established a series of thematic programmes to provide a mechanism of focusing on specific topics which could be supported by collaborative work. One of these themes is Personalised Medicine – while recognising the broad definition of personalised medicine, this programme has a scope that is more focused on novel diagnostic and treatment approaches that make use of genomics and other 'omics technologies.

the scope of the AHSN personalised medicine programme is focused on novel diagnostics and treatment approaches that make use of genomics and other 'omics

Against the background of the completion of the 100,000 genomes project and the establishment of the Genomic Medicine Service in England, the AHSN personalised medicine programme has developed a plan of work to meet AHSN objectives in terms of national personalised medicine policy.

This plan involves:

- Working with academic and industry partners
- Identifying early wins that may form part of future AHSN adoption and spread programmes
- Describing and agreeing the role that AHSNs may play in future strategy implementation in this area of work.

To support this plan of work the AHSN Network aims to build on the findings of the PMTL. The objective of this follow up report is to support further understanding of how the personalised medicine technology landscape will continue evolving in the next 2–3 years and also to consider how the support of the AHSNs and other stakeholders can accelerate the implementation and uptake of proven personalised medicine technologies, in particular those arising from innovations in genomics ore related 'omics approaches

Personalised medicine in the NHS: an update

For the 2018 report, a long list of twenty-five technologies was drawn up based on internal research and intelligence gathering.

These technologies were placed into one of four categories:

- Technologies for greater molecular level characterisation
- Technologies for personalised therapeutic interventions
- Technologies for personalised disease and health monitoring
- Underpinning and enabling technologies

Following further research and consultation with experts a short list of technologies ready for implementation was drawn up, these were:

- Circulating tumour DNA testing
- Pharmacogenomics
- Transcriptomics
- Pathogen genomics
- Regenerative medicine (genome editing/therapy and stem cell therapy)
- 3D imaging and printing
- Machine learning for image analysis and digital pathology

The report also outlined underpinning digital and supportive technologies that are needed for the implementation of personalised medicine.

Since 2018, significant developments have taken place in the provision of genetic and genomic testing in England. With the 2019 launch of the National Genomic Medicine Service and the National Genomic Test Directory, genomics testing will be delivered by one of seven Genomic Laboratory Hubs that will offer the tests listed on the Directory, ensuring that all patients in England will receive equitable access to tests.

since 2018, significant developments have taken place in the provision of genetic and genomic testing in England

Given the focused definition of personalised medicine defined by the AHSN programme, technologies with a strong 'omics component were selected from the original review for further analysis in this report.

these technologies are:

- Circulating tumour DNA testing in cancer management
- Pharmacogenomics
- Transcriptomics
- Near patient testing to support antimicrobial stewardship
- Regenerative medicine – with a focus on genetically modified regenerative medicines.

Objectives

The specific objectives of this report are:

- To inform of recent developments in key biomedical (and digital) technologies identified in the PMTL report and a look forward to short-term readiness (2–3 years) of these technologies for clinical implementation.
- To identify organisations and initiatives supporting the development of personalised medicine technologies
- To provide recommendations for actions that the AHSNs, NHS and other stakeholders could take to support technology development.
- While this report primary focuses is on genomic technologies, a small number of the highlighted technologies are not genomic. This is a reflection of the technologically interlinked nature of this area.

this report aims to understand how the personalised medicine technology landscape will continue evolving in the next two to three years

Scope and definitions

For this work, the NHS England definition of personalised medicine will be used:

‘a move away from one size fits all approach to the treatment and care of patients with a particular condition, to one which uses new approaches to better manage patients’ health and target therapies to achieve the best outcomes in the management of a patient’s disease or predisposition to disease’, with a particular focus on: ‘aspects of novel diagnostic and treatment approaches relating to the genome’.

The specific time-frame is of technologies that have approached or are near implementation ready within the next 2–3 years. This review is focused on developments in England. Where appropriate, a summary of up and coming applications that have future potential is provided.

This report does not cover in detail the social, ethical, legal, regulatory or economic issues relevant to the delivery of personalised medicine, however where relevant these topics are raised in the context of specific technologies.

Methodology

Desk based research and analysis using a combination of official publications from governmental and non-governmental organisations, grey literature and peer-reviewed literature have been used to update knowledge on technology developments since March 2018.

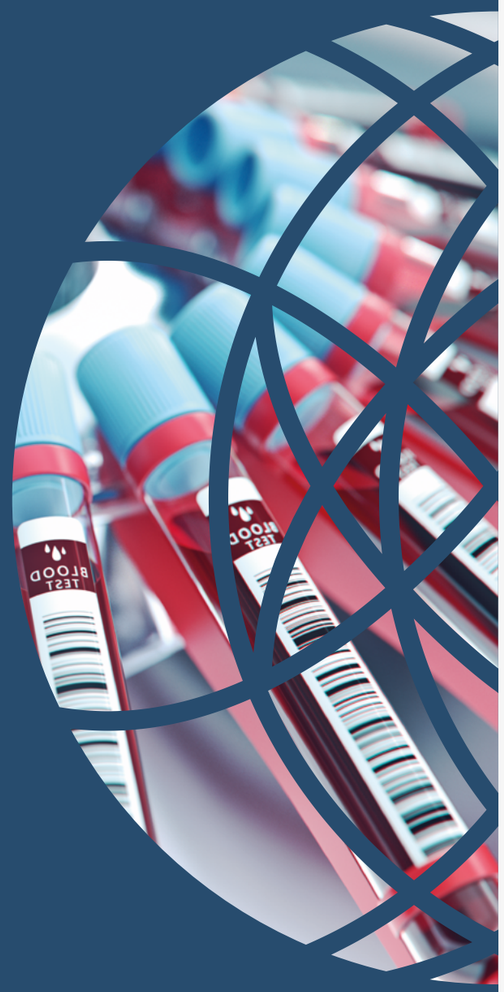
Updates to clinical utility evidence have been considered including a look forward to wider implementation potential within a three-year timeframe.

Where appropriate, in-depth interviews (telephone or in person) have been conducted with experts and relevant stakeholders to understand the enablers and barriers to implementation and adoption of the applications identified within the English NHS, including identification of areas where further research support is needed. These consultees are acknowledged in Appendix 1.

Information gathered from desk and interview-based research has been synthesised and analysed by the PHG team to develop the report’s conclusions.

1. ctDNA testing

Circulating tumour DNA testing for cancer is a fast moving technology area with one type of companion diagnostic testing already available for lung cancer treatment in the NHS in England via the National Genomic Test Directory. In the next three years there is potential for implementation of further companion diagnostic testing in other cancers



Cells can release fragments of their DNA into bodily fluids such as blood and urine, where it becomes known as cell-free DNA (cfDNA). This typically occurs during cell death but can also occur as a result of active cellular secretion[7]. Circulating tumour DNA (ctDNA) refers to the cell free DNA specifically produced by tumour cells. In a process commonly referred to as a liquid biopsy, a sample of a bodily fluid such as blood can be taken from a patient and tested for ctDNA. Both the amount of ctDNA present in the blood, and the genetic alterations it contains, can provide diagnostic and prognostic information about a patient's cancer.

Why is ctDNA testing useful?

For solid tumours the current approach to obtaining genetic information about a patient's cancer is to take a biopsy of tumour tissue and analyse its DNA for genetic alterations. This is often necessary for prescribing therapies that target specific mutations in tumours. Additional mutations that make tumours resistant to therapy can also arise during treatment; in these situations a patient may have to undergo biopsy more than once to determine if this has occurred. Any findings will support decision-making about ongoing clinical management.

ctDNA liquid biopsy provides an alternative to use of solid tumour biopsy samples. Use of ctDNA has several advantages over solid biopsies, as well as providing new opportunities for managing cancer patients:

Less invasive: Body fluid samples such as blood and urine can be obtained using less invasive techniques than those required for a solid tissue sample. Therefore ctDNA testing typically does not require a specialist appointment and is safer for the patient than taking a solid biopsy.

Rapid process: The more rapid process of obtaining and analysing a ctDNA sample could deliver faster results if used instead of a solid biopsy, and makes frequent testing feasible.

Repeatable: The less invasive and simpler nature of collecting a sample for ctDNA testing means that tests can be repeated at multiple time points, or in instances when the original result is inconclusive.

Captures tumour heterogeneity: ctDNA is thought to be derived from all areas of a tumour and from all tumours in the body, more accurately reflecting the genetic heterogeneity of a cancer than solid biopsies obtained from a limited number of sites.

ctDNA liquid biopsy has several advantages over solid tumour biopsies

These features allow ctDNA testing to contribute to more personalised cancer management approaches in the following ways:

Increasing accessibility to companion diagnostic testing and to targeted therapy. A sample of blood or other fluid can be collected when a solid biopsy is not possible due to tumour location, or when a patient is too unwell or unwilling to undergo the procedure.

Providing a prognostic indicator, to help identify patients with residual disease who are most at risk of relapse, and guide treatment decisions accordingly.

Allowing clinicians to regularly perform ctDNA testing in order to **monitor and respond to changes in disease course.**

Facilitating the stratification of patients into molecularly targeted clinical trials, and helping to investigate drug mechanisms during trials.

In the longer term, ctDNA testing could be used as **a screening and/or early detection tool** for cancer.

Methods for ctDNA analysis

high quality pre-analytical sample collection and preparation methods are essential to success in ctDNA analysis

The development and implementation of ctDNA tests is dependent on the capability of technologies to accurately and reliably analyse extremely small quantities of tumour DNA in a plasma sample. In addition, high quality pre-analytical sample collection and preparation methods are also essential in determining the test's success. The vast majority of tests require a blood sample, which needs to be collected in a specialised blood collection tube containing a preservative. This prevents white blood cells in the sample breaking down and releasing their DNA into the sample, which would swamp the ctDNA signal and make analysis very difficult. The blood sample then needs to be transported to a clinical laboratory within 24 hours, where the ctDNA is extracted from the plasma fraction of the blood and analysed[8]. Standardisation of the methods involved in each stage of sample preparation and analysis is essential to ensure a consistent and quality service.

There are several different methods underpinning the ctDNA tests in use or in development, which measure different properties of ctDNA. These are summarised in Table 1.

The parameters of different testing methods determine their suitability for a certain application. For example some types of tumours and earlier stage tumours tend to release smaller quantities

Table 1: Types of test technology

Technology	Description	Detection limit (MAF*)	Advantages	Disadvantages
Quantitative PCR (qPCR)	Quantitative PCR method based on amplification of DNA fragments in sample in real time. Several variations available to improve sensitivity, e.g controlling temperature to amplify preferred sequences (COLD-PCR)	>0.1 with standard methods to ~ 0.001% with COLD-PCR	▪ Fast ▪ Relatively inexpensive	▪ Relies on known variants ▪ Basic method not sensitive enough for many applications.
Digital droplet PCR (ddPCR) [9-11]	Based on qPCR but partitions sample into many parallel reactions to reduce background noise. Variants exist, including BEAMing, to increase sensitivity	<0.001 for standard ddPCR to 0.0002 with BEAMing	▪ Most sensitive PCR method ▪ Standard methods affordable ▪ Fast.	▪ Relies on known variants ▪ BEAMing complex and costly
UltraSEEK	PCR based method that multiplexes several samples at the same time, then uses MALDI/TOF mass spectrometry to identify mutations.	0.001	▪ Sensitive ▪ Allows multiplexing	▪ Relies on known variants ▪ More complex protocol, requires mass spec equipment
Targeted next generation sequencing (NGS) methods	Sequencing based methods that target to specific regions of a gene. Many variations in development to increase sensitivity e.g. CAPP-Seq	<0.01 standard methods to ~0.002 % with sensitive methods such as CAPP-Seq	▪ High throughput, so can be used in large panels ▪ Doesn't rely on known variants ▪ Can identify more types of genetic variation	▪ More expensive ▪ Currently lower sensitivity than ddPCR methods

*MAF = mutant allele fraction, the ratio between the mutant and wild type gene variants (alleles) in a sample e.g. MAF 0.1 means that the technology is sensitive enough to detect the allele if it is present in 10% of the sample.

of ctDNA, requiring a more sensitive PCR based test. Next generation sequencing (NGS) based methods are more suitable for certain types of genetic alterations such as structural rearrangements, or for identifying de novo mutations. New methods with increased sensitivity or that measure alternative features of ctDNA, such as DNA fragment length or methylation, are in development.

There is also the possibility in the future to combine multiple ctDNA testing methods with other biomarkers such as proteins.

Current and future implementation of ctDNA testing

ctDNA tests are at different stages of clinical implementation for the applications outlined above, and their use varies widely across different cancer types. For some types of cancer, companion diagnostic ctDNA tests for treatment selection are already in use or close to implementation, whilst tests for monitoring residual disease and tumour progression are showing promise in clinical trials for certain applications. On the other hand, early detection applications are still in an early clinical research phase with much more development required.

early detection applications are still in an early clinical research phase with much more development required

There are several reasons why the implementation for ctDNA testing varies so broadly for different applications, including:

- **Tumour biology**: Some cancers release more ctDNA than others, and larger tumours tend to produce more ctDNA – research is ongoing to better understand tumour biology and the reasons behind these differences. Therefore the tiny levels of ctDNA released by early stage tumours or those beginning to recur after surgery are technologically more challenging to detect than the larger amounts of ctDNA that tend to be released from later stage, metastatic tumours.
- **Impact on clinical pathways**: For some applications of ctDNA testing, such as companion diagnostic testing, ctDNA tests are used as a substitute when testing of a solid tumour sample is not possible – overall, changes required to the clinical pathway to facilitate this are minor. In contrast, potential uses such as monitoring and early detection are more novel and could result in disruption of, or redesign of, current clinical pathways. More disruptive technologies are harder to implement.
- **Evidence of clinical utility**: The volume of evidence supporting clinical utility varies between applications. ctDNA companion diagnostic tests often have demonstrated utility in terms of increasing patient access to targeted drugs. There is a need for more evidence to support the utility of monitoring and early detection applications.

Recommendation

Given that the available evidence for use of ctDNA testing varies broadly across different cancer types, as well as across different applications, approaches to assess the clinical validity and utility of ctDNA tests will need to be considered on a case by case basis.

Use of ctDNA tests as companion diagnostics for treatment selection

Improving outcomes for people with cancer is one of the key aims of the NHS *long term plan*, with faster diagnosis and access to more effective tests and treatments two of the ways proposed to achieve this[12]. Targeted therapies offer patients more tailored treatment based on the specific biological features of their tumours, which can provide better outcomes with fewer side effects. Often these therapies are designed to target specific mutations or genetic alterations, requiring genetic analysis of a tumour sample through use of a companion diagnostic test. ctDNA testing can provide benefits over solid tumour sample testing for the reasons outlined earlier, increasing accessibility to targeted therapies for patients and potentially increasing the speed of diagnosis.

Current state of implementation

The only application for which ctDNA testing is currently available across the NHS in England is as a companion diagnostic test in advanced non-small cell lung cancer (NSCLC), to support the prescription of tyrosine kinase inhibitor (TKI) drugs that target tumours with *EGFR* mutations[13]. ctDNA testing is currently used both at diagnosis in case of solid biopsy failure, and as a first-line test when tumours in patients on 1st/2nd generation TKIs progress, to determine if resistance to therapy is caused by an additional mutation in *EGFR* called p.T790M, for which a 3rd generation TKI, osimertinib, is available.

currently, ctDNA testing is only available across the NHS in England as a companion diagnostic test in advanced non-small cell lung cancer

Since the publication of PHG Foundation's 2018 report *The personalised medicine technology landscape* (where the use of ctDNA testing in NSCLC was described in depth) ctDNA testing for *EGFR* hotspot mutations in NSCLC has been listed on the NHS England National Genomic Test Directory for cancer, meaning that all patients should have access to the test.

Understanding why *EGFR* ctDNA testing in NSCLC was the first ctDNA test to be used in the UK is useful in helping to understand the required evidence, resources and environment needed for implementation of ctDNA tests in other areas. The following were several contributing factors:

- The expectation of NICE's positive recommendation of the targeted therapy osimertinib (3rd generation TKI), providing an opportunity for ctDNA companion diagnostic testing
- Availability of a CE-IVD (*in vitro* diagnostic) marked kit, Roche cobas®, for testing and other technological advances in the sensitivity and usability of ctDNA technologies
- ctDNA testing complemented solid tumour testing for *EGFR* already performed in laboratories
- The availability of blood stabilising tubes improving the logistics of sending blood samples from the clinic to laboratories
- Clinical unmet need – patients not accessing genetic testing, and targeted therapy, due to solid biopsy failure
- Laboratories willing to develop testing, supported by clinicians and pharmaceutical companies

Future work required for ctDNA EGFR testing in NSCLC

Whilst *EGFR* ctDNA testing for NSCLC has now been rolled out, there is still room for further improvements. Although NICE released a Medtech innovation briefing in January 2018[14], ctDNA testing is still not included in official NICE guidance for management of NSCLC. Currently ctDNA testing is only carried out as a test for treatment naïve cancers if a solid biopsy test fails to provide a result. Evidence suggests it may be more clinically and potentially cost effective to use ctDNA testing as a first line test for all treatment naïve cancers, with solid biopsies only used if the test fails or is negative[15,16]. In addition ctDNA testing technologies are constantly evolving, therefore ongoing review of the best test to use is required. The new Genomic Laboratory Hubs could aid this process.

Recommendation

Further work is needed to determine the most clinically and cost effective way of using EGFR ctDNA testing for non-small cell lung cancer in the UK health system. When new tests become available with improved sensitivity and specificity, they should be evaluated against current tests to ensure that the most suitable technology is used and implemented across the health system.

evidence suggests it may be more cost and clinically effective to use ctDNA as a first line test for all treatment of naïve cancers

Treatment selection-future areas approaching implementation

Many of the factors which drove implementation of *EGFR* ctDNA testing in NSCLC now apply to other uses of ctDNA as a companion diagnostic test, both within NSCLC and for other cancers. Examples of applications near to clinical implementation are highlighted below. For all these applications laboratories currently can choose between in house development and validation, or to purchase commercial tests.

Advanced Colorectal cancer

The need: Patients with metastatic colorectal cancer require genetic testing of their tumour to determine whether they are eligible for treatment with cetuximab, as the presence of *KRAS* or *NRAS* mutations means the drug is likely to be ineffective. ctDNA testing could widen access to patients unable to provide a solid biopsy sample. In future, testing for the *BRAF* V600E resistance mutation in patients who become resistant to initial therapies may also be important to allow prescription of new targeted therapies without the need for repeat solid biopsies. The triple therapy encorafenib in combination with binimetinib and cetuximab is currently in phase 3 trials for *BRAF* V600E mutant metastatic colorectal cancer[17].

The evidence: The All Wales Medical Genetics Service already offers in house droplet digital PCR based ctDNA testing for the most common mutations in *KRAS* and *NRAS* to patients with metastatic colorectal cancer who have no solid biopsy sample available, or where the sample has failed molecular analysis[18]. However, an international pilot external quality assessment scheme found higher error rates for *RAS* testing than *EGFR* testing, potentially because best practice for *RAS* testing is less established[19]. Clinical trials have demonstrated that *BRAF* testing using droplet digital PCR is suitable for ctDNA

analysis of *BRAF* V600E mutations in advanced colorectal cancer, but evidence on clinical utility is lacking.

Commercial tests Several CE-marked kits are available for *RAS* and/or *BRAF* testing, which use different technologies, measure different parameters and have different sensitivities:

- Inostics Oncobeam RAS CRC kit (Sysmex Inostics)
- IdyllaTM ctNRAS-BRAF mutation test
- IdyllaTM ctKRAS mutation test (Biocartis)
- Guardant 360 panel (Guardant Health)

Implementation readiness: The current use of *KRAS* and *NRAS* testing in Wales suggests this technology is ready for implementation in the UK, however this requires careful evidence based consideration of the best type of test to use with appropriate quality control. Though the technology for *BRAF* V600E mutation detection is available, more evidence is required on clinical utility before the test is implemented.

Advanced Ovarian Cancer

The need: Currently ovarian cancer patients without a known germline *BRCA* mutation receive a *BRCA* test on tissue, which makes them eligible for maintenance treatment with the PARP inhibitors Nirapib or Olaparib[20]. As in NSCLC, ctDNA testing can increase patient access to therapies when tissue is not available for a genetic test. Patients can also become resistant to PARP inhibitors through acquiring secondary mutations in *BRCA1/2*. Testing for these resistant mutations could help clinicians decide when to switch therapies or change a treatment plan.

implementation requires careful evidence based consideration of the best type of test to use with appropriate quality control

The evidence: NGS based ctDNA testing to detect both germline and somatic *BRCA1/2* mutations has been shown to be feasible, though studies so far have been relatively small[21,22]. It has shown been shown that secondary resistance mutations can also be detected using ctDNA, however more studies are required on both the validity and utility of these tests[22,23].

Commercial tests: Guardant Health's Guardant 360 panel test is the only CE-marked test available, however there is limited evidence on its use in ovarian cancer.

Implementation readiness: There is a lack of validated commercial tests available for detection of *BRCA1/2* mutations in ovarian cancer, although tests could be developed and validated in laboratories. More evidence is needed on clinical utility of these tests. ctDNA testing for resistance mutations requires more evidence on both clinical validity and utility before it is ready for implementation.

Advanced NSCLC - other mutations

The need: Targeted therapies are available for first line treatment of NSCLC patients with structural rearrangements of *ALK* and *ROS1* genes. ctDNA testing could be used to determine *ALK* and *ROS1* mutation status at diagnosis, widening access to targeted therapies to patients with no solid tumour biopsy sample available[24]. In addition resistance mutations in *ALK* and other genes arise in patients treated with ALK inhibitors. In future, detection of these resistance mutations could be used to guide treatment decisions.

The evidence: Structural rearrangements in ctDNA are harder to analyse using PCR based methods than simple mutations, but there is rapidly accumulating evidence from clinical trials that this can be achieved using NGS based tests and has clinical utility[25-27]. *ALK* resistance mutations can be more easily detected using current methods, but as yet the clinical benefit of this approach is uncertain as current second line therapies do not require genetic analysis of tumour samples[24,28,29]. No cost analysis has been undertaken for either test.

Commercial tests: Guardant Health's Guardant 360 panel test is the only CE-marked test that can measure both *ALK* and *ROS1* alterations. Other commercial tests are available in the US (see Table 2).

Advanced Breast Cancer

The need: Patients with *PIK3CA* mutations are more likely to respond to Novartis' drug Piqray (alpelisib), which was recently approved by the US Food and Drug Administration (FDA). Apelisib is not currently available on the NHS but NICE guidance on its use for treating advanced hormone-receptor positive, HER2-negative, *PIK3CA*-positive breast cancer is currently in development, with an expected publication date of 9th December 2020[30]. In order for Alpelisib to be prescribed, companion diagnostic testing will be needed for *PIK3CA* mutations. Use of ctDNA testing could ensure all patients are able to access alpelisib without relying on a solid tumour biopsy sample.

The evidence: In May 2019 Qiagen's Therascreen PIK3CA RGQ PCR Kit was approved by the FDA for use as a companion diagnostic to identify *PIK3CA* mutations in both tissue and blood samples of patients with advanced breast cancer. As of February 2020 the test has also gained CE marking and is commercially available in Europe. PIK3CA testing for breast cancer is not currently carried out in the

use of ctDNA testing could ensure all patients are able to access alpelisib without relying on a solid tumour biopsy sample

NHS, and evidence is needed on how it could fit into the current clinical care pathway.

Commercial tests: The Guardant 360 panel (Guardant Health) is the only CE marked approved test. Qiagen's Therascreen PIK3CA RGQ PCR Kit is FDA approved but does not yet have a CE mark.

Implementation readiness: The technology is available for implementation, however the need for implementation will depend upon recommendation of alpelisib by NICE. Potentially the test will be ready for implementation in the near future, subject to NICE approval of Alpelisib and evidence on its utility in the NHS.

Recommendation

For clinical indications where there is an unmet need and a ctDNA companion diagnostic test has been identified, consideration should be given at an early stage of test development to gathering the evidence required to support best practice and integration of the test into clinical pathways.

ctDNA for detection of minimal residual disease and monitoring

Cancers can recur after the original tumour is removed, despite there being no visible sign of its presence. This is due to small amounts of cancer cells remaining, known as residual disease. In addition, during treatment tumours often become resistant to cancer therapies and the patient may relapse. Current imaging methods are unable to detect residual disease, and are too costly and time consuming to be used for frequent monitoring of patient tumour burden during treatment. In addition monitoring for specific resistance mutations would require repeat solid biopsies, which is not realistic for most cancers.

The less invasive and simpler nature of ctDNA testing means that regular testing to monitor tumour burden and resistance could become possible, whilst increasingly sensitive technologies means that ctDNA can be used to detect residual disease before other clinical symptoms are apparent. This could mean earlier, more effective interventions for some patients, whilst potentially helping to reduce the anxiety of those with no sign of recurrence.

As described in more detail below, the use of ctDNA for monitoring is still in clinical research stages, with evidence of utility in particular needed. A commercial test currently used in the clinical research context is Natera's Signatera assay, which is being trialled in a number of cancers for a range of monitoring purposes (see Table 2). More commercial tests are likely to be developed both for research and clinical use as ctDNA monitoring becomes more established.

ctDNA could be used to detect residual disease before other clinical symptoms are apparent and regular testing could help with tumour burden and resistance monitoring

Monitoring for residual disease and relapse

The presence of ctDNA has been shown to accurately predict tumour relapse up to several months before it is clinically detectable by other methods, depending on the cancer type. There is robust evidence that ctDNA can be used to detect cancer recurrence early and reliably in breast, colorectal and lung cancers[31]. Further prospective studies are now required to generate evidence in large patient populations for these cancer types. There is also an urgent need for evidence on whether it is clinically actionable and useful to predict and/or detect cancer recurrence earlier, especially if tumours are still too small to be confirmed by traditional imaging methods. Answers to these questions are necessary before ctDNA can be routinely implemented for monitoring. The UK based c-TRACK TN randomised trial for moderate or high risk early stage triple negative breast cancer may help start to provide answers of the utility of ctDNA monitoring[32]. This is a phase 2 trial of 150 patients, to understand if detection of minimal residual disease though ctDNA monitoring is useful in triggering intervention though treatment with pembroluzimab.

Example: Detecting residual disease in colorectal cancer

In a recent prospective study of 150 patients with localised colorectal cancer, ctDNA monitoring for recurrence was suitable for use in 80% of patients and able to detect recurrence on average 10 months before radiological methods[33]. ctDNA has also shown to be a useful prognostic marker in patients with advanced CRC, with those with ctDNA present prior to chemotherapy being more likely to relapse afterwards[34]. The Royal Marsden NHS Foundation Trust recently started recruiting colorectal cancer patients into the TRACC multi-centre, prospective translational research study[35]. This is a large study of 1000 patients which aims to understand if ctDNA can be used to identify minimal residual disease and relapse earlier than existing methods. This will be helpful in understanding how monitoring can impact clinical pathways for these cancer patients.

the presence of ctDNA has been shown to accurately predict tumour relapse several months before it is clinically detectable by other methods

Table 2: Examples of ctDNA tests for solid tumours currently available for clinical use

Test	What it measures	Technology	CE mark	FDA status	Available in UK*
		Diagnostic			
Cobas EGFR Mutations Test v2, Roche	42 mutations in exons 18, 19, 20 and 21 of the epidermal growth factor receptor (*EGFR)* gene, including the T790M resistant mutation.	RT-PCR	✓	Approved (for NSCLC)	✓
Guardant 360, Guardant Health	Specific mutations, amplifications and fusions in over 70 genes in different solid tumour types	NGS panel	✓	Breakthrough device & LDT	✓
OncoBEAM RAS CRC Kit, Sysmex Inostics	*KRAS* and *NRAS* mutations in colorectal cancer	Digital PCR	✓	LDT	✓
Therascreen EGFR RGQ Plasma PCR kit, Qiagen	Exon 19 deletions and exons 20 and 21 substitutions (T790M and L858R respectively) in the *EGFR* gene	RT-PCR	✓	Approved (for NSCLC)	✓
Super-ARMS EGFR mutation test, AmoyDx	42 *EGFR* mutations in NSCLC, in Exons 18, 29, 20 & 21	RT-PCR	✓	n/a	✓
Invision First- Lung (Inivata)	36 genes relevant to the care of patients with advanced NSCLC.	Tagged amplicon seq	x	LDT	x
Target selector lung & breast panels (Biocept)	Actionable mutations in lung and breast cancers	NGS	x	LDT	x
Foundation One Liquid (Foundation medicine)	Genomic alterations and MSI status in over 70 genes in different solid tumour types	NGS panel	x	Breakthrough device & LDT	x
Qiagen Therascreen PIK3CA RGQ PCR Kit	Detection of 11 mutations in the phosphatidylinositol 3-kinase catalytic subunit alpha (*PIK3CA*) gene	PCR	✓	Approved (advanced breast cancer)	x
		Monitoring			
Signatera (Natera)	Personalised monitoring test based on 16 tumour variants from solid biopsy sample	NGS & PCR	x	Breakthrough device & LDT	x
Colvera (Clinical Genomics)	Methylation of *BCAT1* and *IKZF1* in colorectal cancer	qPCR	x	LDT	x
		Early detection			
Epi proColon (Epigenomics)	Methylated cytosine residues in the *SEPTIN9* gene for detection of colon cancer	RT-PCR	✓	Approved (Colon cancer)	✓

*correct as of February 2020

Clinical Genomics have already launched their Colvera test in the US for monitoring for recurrence of colorectal cancer (see Table 2).

Monitoring of treatment response

There is evidence that using ctDNA for regular monitoring of overall tumour burden or for detection of resistance mutations may allow responses to treatment to be evaluated sooner and emergence of resistance to be detected earlier than would otherwise be possible. ctDNA monitoring has also shown to be able to predict treatment response and relapse in a number of cancers. Research is most advanced and closest to clinical application in colorectal cancer and NSCLC, though most studies are exploratory and results from larger trials are needed[34,36-38]. A small trial is also being run by The Christie NHS Foundation Trust in Manchester to help determine if ctDNA tracking is useful for indicating when to switch therapies in melanoma[39]. Further work required for all applications in this area includes:

- Establishing guidelines on how often testing should be carried out and the most suitable tests to use
- How useful it is to clinicians in making treatment decisions and in improving patient outcomes
- The impact on the overall treatment cost

> given the complexity and scale of studies required, it is unlikely that all of these factors affecting ctDNA monitoring studies will be addressed within the next three years

Given the complexity and scale of studies required, it is unlikely that all of these factors will be addressed within the next three years.

Example: Monitoring in NSCLC

In a study of 122 NSCLC patients, increased ctDNA levels were shown to precede or coincide with disease progression, with an average lead time of 2.7 months[40]. In addition two specific resistance mutations could also be detected an average 1.4 months in advance of disease progression. ctDNA monitoring has also shown to be possible in NSCLC patients with non-targetable mutations, as the concentration of ctDNA as measured by tracking other mutations specific to a patient's cancer can still predict treatment response[37].

The US based LIBERTI observational trial is currently enrolling 500 patients with early stage NSCLC resected cancer, to gather more information about how levels of ctDNA change in response to tumour treatment, as well as to further understand the relationship between ctDNA levels after surgery and disease free survival[41]. More evidence is now needed on the utility of monitoring ctDNA to help predict and determine treatment response in NSCLC, for example whether it is appropriate to change treatment course before clinical symptoms emerge.

Recommendation

Further work is needed to support gathering of evidence around the clinical utility of ctDNA monitoring approaches, through clinical research studies and trials.

Screening/early detection

The use of ctDNA testing as a cancer screening tool or as a tool to detect early disease is the focus of research and not likely to be considered for clinical implementation for some time.

There are several large clinical trials that have been launched or are planned by commercial companies to understand the potential and feasibility of this application. These include Grail's study of a multi-cancer early detection blood test[42], Guardant Health's plan to launch a study for colorectal cancer screening[43] and the newly launched DETECT study of Thrive Earlier Detection's CancerSEEK test to detect multiple cancer types in healthy people[44].

Using ctDNA for early diagnosis is still in an early stage of clinical research for most cancers, with as yet unproven validity and utility. The exception to this is the FDA approved Epi proColon assay (Epigenomics AG) for detection of the methylated promoter of *SEPT9*, which has evidence showing its utility in colorectal cancer screening. This test is CE marked and available in the UK but has not been shown to have any clear advantages over the FIT test (a non-'omics based test) currently used in NHS screening[45].

when considering implementation of ctDNA analysis laboratories should plan ahead to consider which techniques are likely to be required for future applications of ctDNA analysis

General considerations

Choice of test method

The most common analysis method currently in clinical practice is PCR which is the most sensitive technique to identify known mutations of interest. However Next Generation Sequencing (NGS) based methods are becoming increasingly sensitive and in the future may replace PCR methods. NGS methods do not restrict a test to a few predefined mutations and are more suitable for detecting other types of genetic alterations that occur in cancer.

Tests can be developed in house by clinical laboratories or be purchased from the increasing number of companies developing commercial tests. The choice of testing method depends on the capabilities, equipment and budget of the clinical laboratory performing the test. When considering implementing ctDNA analysis, laboratories should be aware of the different options available and select the technique most appropriate for the applications required, as well as plan ahead to consider which techniques will likely be required for future applications of ctDNA analysis.

Guidelines

In order to ensure that ctDNA testing is offered to all eligible patients equitably it is important that evidence based guidelines specific to the UK health system are produced and kept updated as technologies mature.

it is important that evidence based guidelines specific to the UK are produced and updated as technologies mature

Current NICE clinical guidelines for management of NSCLC does not cover *EGFR* ctDNA testing, however a 2018 Medtech innovation briefing outlines uses as well as the uncertainties surrounding choosing the best test[14]. Other regions in the UK have developed clinical guidelines. The All Wales Medical Genetics Service (AWMGS) has produced documents on when and how to submit samples for *EGFR* ctDNA testing in NSCLC testing, as well as for *KRAS* and *NRAS* testing in colorectal cancer[8, 18]. The Scottish Medicines Consortium guidance on Osimertinib for treatment of NSCLC recommends ctDNA testing to detect the p.T790M *EGFR* mutation, with a tissue test as a second line test if needed[46].

International guidelines for NSCLC are being produced and kept updated by The International Association for the Study of Lung Cancer (IASLC) in collaboration with the College of American Pathologists (CAP) and the Association for Molecular Pathology (AMP)[47], as well as the European Society for Molecular Oncology (ESMO)[28]. These could be used to inform the development of guidelines on ctDNA testing in NSCLC in England.

As further ctDNA testing becomes available, clinical laboratory guidelines and clinical management guidelines will be required to:

- Advise on the most appropriate test to use for different applications
- Outline standardised sample preparation and testing protocols
- Guide clinicians on when to test
- Inform appropriate clinical actions as a result of testing

Quality Assurance

It will be important that External Quality Assurance (EQA) programmes are established to ensure that testing carried out in laboratories is consistent and meets minimum required standards across the NHS. An international EQA pilot scheme for analysis and reporting of ctDNA is already being tested in several European laboratories including the UK[19].

Changes in regulation

It is important to be aware that in May 2022 the future Health Institution Exemption (HIE) Regulation for *In Vitro* Diagnostic Medical Devices is set to replace the current Medical Devices Directive[48]. This may place more restrictions on laboratories wishing to develop their own ctDNA tests.

ctDNA could help more patients access targeted drug therapies, without having to rely on an invasive and potentially unsuccessful tissue biopsy

Conclusions

There are a number of promising applications of ctDNA testing nearing implementation in the next few years. Use of ctDNA as a companion diagnostic test for targeted therapies is ready for or approaching clinical use in several cancers, in addition to its current use in NSCLC. This will help more patients access targeted drug therapies, without having to rely on an invasive and potentially unsuccessful tissue biopsy procedure. There are a number of tests and technologies already available to deliver companion diagnostic ctDNA testing, and the lessons learned from implementation of a ctDNA testing service for NSCLC can be applied to similar tests for other cancers.

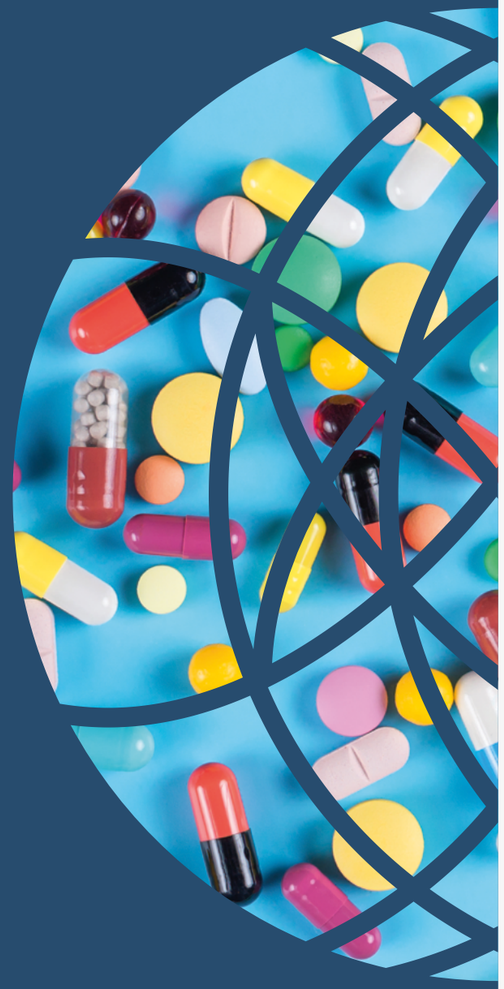
The use of ctDNA testing for monitoring cancers is also showing great promise, with evidence of the feasibility of monitoring for recurrence and treatment response rapidly accumulating for several cancers. More work is now essential to determine how monitoring can be implemented in clinical practice, including how and when to test as well as how to act upon the results. In the more distant future ctDNA testing for the early detection of cancers may become a reality, with several large clinical trials currently underway in this area.

ctDNA testing is already becoming an established technology in the health service; *EGFR* testing in NSCLC is now on the National Genomic Test Directory, Genomics England is investigating the use of ctDNA in the healthcare system using samples from the 100,000 genomes project, and use of ctDNA testing is planned in the new Accelerating Detection of Disease (ADD) cohort of five million healthy people.

Therefore now is an ideal time to focus on broader implementation of ctDNA services, whilst generating the necessary evidence and providing the infrastructure for future ctDNA testing requirements.

2. Pharmacogenomics

Pharmacogenomics testing will be included in the NHS England National Genomic Test Directory and there are a number of pilot projects already exploring which gene-drug pairs are most ready for clinical implementation



Pharmacogenomics (PGx) is one application of 'omics technologies that is likely to be key to the success of personalised medicine. PGx is defined as 'the study of variation of DNA and RNA characteristics as related to drug response'[49] and PGx studies look at how genetic variation impacts on the pharmacokinetics and pharmacodynamics of drugs. These two concepts describe overall response to drug exposure:

Pharmacokinetics defines variability in the process of how drugs are absorbed, distributed, metabolised, and excreted by the body, and in their toxicity.

Pharmacodynamics describes variability in drug action by modifying their effects on cell receptors and downstream biochemical pathways.

PGx testing can be considered at a disease diagnosis, or as part of the diagnostic pathway. Another term often used in conjunction with PGx is companion diagnostics (also known as pharmacodiagnostics or theranostics). This term has been adapted by different regulatory authorities to describe a predictive biomarker assay – for PGx this is a genomic or molecular assay – developed in parallel to a specific drug, often a targeted therapy. Companion diagnostics testing is discussed in the context of cancer and liquid biopsy in chapter 1.

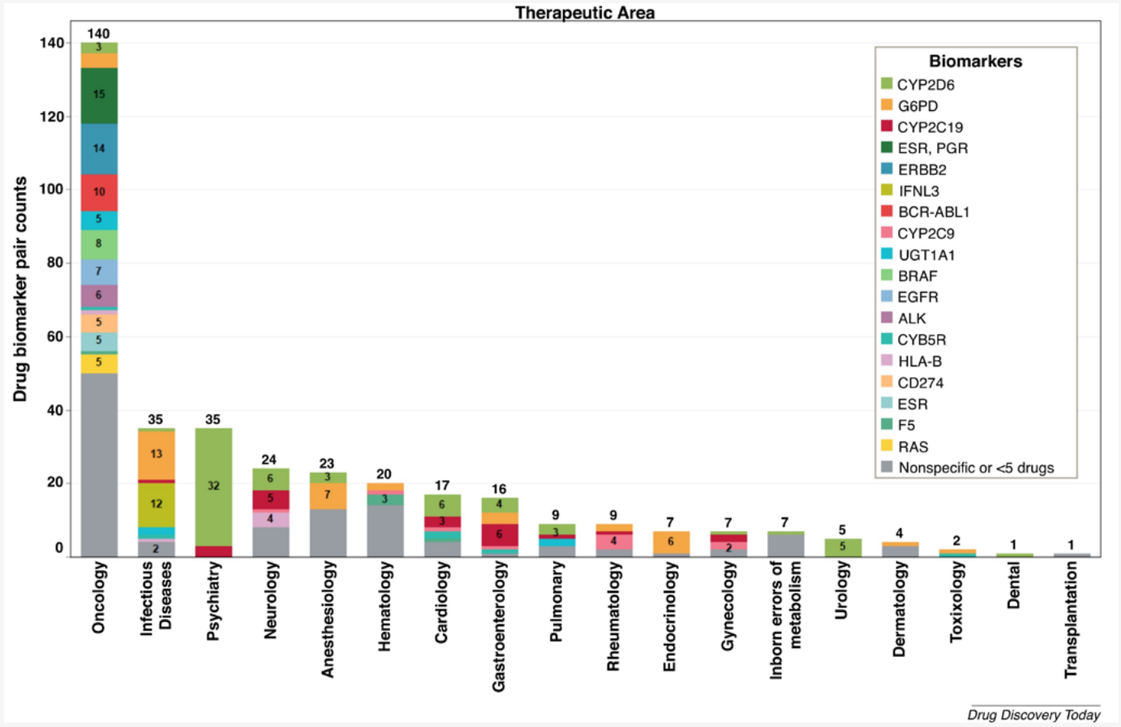
Over 250 drugs that contain PGx information in their labels have been approved by the US Food and Drugs Administration (FDA), with a similar number approved by the European Medicines Agency (EMA)[50,52] (Figure 1). Each year, the proportion of newly approved drugs that contain genomic biomarker information on their label is increasing[52,53].

Three initiatives that provide peer-reviewed, evidence based guidelines on how PGx can be used to optimise patient care and treatment success include:

- Pharmacogenomics Knowledge Base (PharmGKB)[54]
- Clinical Pharmacogenetics Implementation Consortium (CPIC)[55]
- Royal Dutch Association for the Advancement of Pharmacy Pharmacogenetics Working Group (DPWG)[56]

Pharmacogenomics is one application of 'omics that could be key to the success of personalised medicine

Figure 1: FDA-approved drug labelling by therapeutic area and the drug biomarker (gene) pair counts. Top of the bar is the number of drug-biomarker pairs in that therapeutic area there are. Colour is in the key on the top right and represents gene in the label. In brackets is the number of times the gene is present: CYP2D6 (66), G6PD (39), CYP2C19 (22), ESR PGR (15), ERBB2 (14), IFNL3 (12), BCR-ABL1 (10), CYP2C9 (10), UGT1A1 (9), BRAF (8), EGFR (7), ALK (6), CYB5R (6), HLA-B (6), CD274 (5), ESR (5), F5 (5), RAS (5).[57]



Why is pharmacogenomics useful?

PGx has the potential to strengthen personalisation by selecting therapies based in part on the genetic profile of individual patients, making it possible to:

Select targeted therapies appropriately. Analysis of germline, somatic and pathogen genomes can provide more precise information on the causative mechanism of diseases or refine a clinical diagnosis and identification of the appropriate therapy.

For instance, gain-of-function mutations in the *PCSK9* gene are a known underlying cause in some cases of familial hypercholesterolaemia, for which PCSK9 inhibitors are available[58-59]. In another example, *BRAF*^{V600} genetic mutations are present in approximately 50% of cases of metastatic malignant melanoma, and can be targeted using BRAF inhibitors to increase overall and progression-free survival of those affected[60-61].

Molecular monitoring of response to therapy can be used to determine if drug resistance is developing, suggesting that a switch to second line therapies or an alternative treatment strategy should be considered. For example molecular response milestones are part of tyrosine kinase inhibitor treatment regimens of chronic myeloid leukaemia[62].

Alleviate or avoid adverse drug reactions (ADRs). ADRs are a healthcare safety issue as they can be fatal or cause major health complications. The incidence of ADRs has remained relatively unchanged over time, with research suggesting that between 5% and 10% of patients may suffer from an ADR at admission, during admission or at discharge, despite various preventative efforts[63]. ADRs cost the NHS approximately £1 billion annually, and could be prevented by pre-emptive PGx testing in an estimated 20-30% of cases[64]. Some gene-drug pairs have been associated with serious ADRs (sADR), which could be avoided with PGx testing for selection of therapies that are more suitable or dosage adjustment to avoid toxicity associated with the medication.

> adverse drug reactions cost the NHS approximately £1bn annually, and could be prevented by pre-emptive PGx testing

One example is the association between the HLA-B*5701 variant and a significantly increased risk of severe and potentially fatal hypersensitivity reactions to the HIV antiretroviral drug abacavir[65]. National and international drug guidelines specify that variant screening should be performed before therapy starts[66]. This type of testing is available in the NHS.

Apply more accurate dosing strategies. Precision dosing through PGx testing can be used to help prevent ADRs but also can have an impact through improving efficacy of therapies. PGx information indicative of poor metabolism can inform the avoidance of drugs that would be ineffective, or be used to change a drug dose in a patient who is a high-metaboliser. Fewer ADRs, improved efficacy and minimising side effects also brings patient benefit through individuals being more likely to comply with their treatment regimens, thus improving clinical outcomes.

These approaches can allow the health system to:

- Maximise drug efficacy
- Improve the patient's clinical experience through optimal prescribing (e.g. through minimising side-effects)
- Reduce healthcare costs – through avoiding unnecessary drug use, and avoidance of ADRs

Disease areas that have had success with PGx testing include cancer, asthma, infectious diseases, cardiovascular disease and psychiatry (figure 1). Some economic evaluations of specific PGx-guided treatment scenarios are largely favourable and are considered to be cost-effective or cost-saving[67-69].

Pharmacogenomics testing methods

Numerous PGx genetic assay types exist ranging from simple PCR tests that look at a small number of variants in a single gene, targeted genotyping, microarrays that can capture most known PGx variants in a single test, to Sanger sequencing, panels and whole genome sequencing (WGS). The majority require a blood or tumour sample to carry out the test, and as discussed in chapter 1, liquid biopsy using ctDNA extracted from blood is becoming established for some forms of cancer companion diagnostic testing.

There are a number of analytical considerations for PGx testing. This includes the identification of appropriate platforms for analysis which can include laboratory developed tests or commercial assays[70]. The complexity of some of the PGx genomic regions under investigation can make interpretation challenging[71]. In certain instances technology currently not available in a clinical setting, such as long read sequencing, will be more appropriate for intricate analyses[72]. Examples of complexity include the *CYP2D6* gene (section 3.5) and human leukocyte antigen (HLA) regions, which code for the major histocompatibility complex proteins that regulate the immune system.

For these complex genetic regions, correct interpretation is important as different variants within the same gene can have different implications. For example, in the *CYP2D6* gene, some variants will result in sADR to certain drugs, while other variants in the gene will have a dosing recommendation. Correct interpretation is vital in determining which clinical decision is required.

Recommendation:
With pharmacogenomic information interpretation of results is not always straightforward and requires careful consideration. The most up do date evidence from databases and recent guidelines should be used to support clinical decision making.

Timing of testing

There are various time points when an individual could have their PGx status determined: either reactively or pre-emptively.

A **reactive test** would be done in response to a specific clinical situation. For example, a patient diagnosed with a condition for which there is a treatment available with a known gene-drug pair would benefit from a PGx test. In some clinical circumstances this decision might need to be made quickly – as such, there is research ongoing into rapid point of care testing to offer quick turnaround times. Reactive testing can also be done to determine the most appropriate therapy or dosage of a drug, which includes companion diagnostic for selection of a targeted therapy. Should an ADR occur, testing might be appropriate to determine why this has happened, Testing could also support monitoring of drug responses, and determining reasons for treatment failure. One proposed use of reactive testing is that when a patient requires a specific PGx test for a particular clinical reason, a comprehensive PGx panel (or WGS) is done to analyse all known gene-drug pairs. This would meet the current clinical need, but also make the PGx information available for potential future needs.

Pre-emptive testing would likely occur in healthy patients, before there is a known clinical need, or could be done at an individual's first health care contact[73]. It would need to be comprehensive through either WGS, a panel or genotyping microarray for all known gene-drug pairs. The resulting PGx genotype information of an individual would need to be available in their electronic health records (EHR), or as a report that can be reviewed for drug suitability when required.

Recommendation:
Further work is needed to determine for which situations reactive or pre-emptive pharmacogenomics testing will best meet the needs of patients and the health system and how such approaches could be delivered.

the timing of testing could have significant influence on what type of test is utilised

GPs should use caution when presented with DTC findings

Direct to Consumer pharmacogenomic tests

In October 2018, 23andMe was granted the first FDA authorization for direct-to-consumer (DTC) PGx reporting[74]. Eight pharmacogenes that affect the metabolism of some 50 drugs (all under CPIC guidelines) are included in the PGx testing done by 23andMe. As of October 2019 they remained the only DTC with FDA approval to offer PGx testing[75]. With DTC marketing of PGx tests, the fear is that individuals will modify their drug use on their own. The Royal College of General Practitioners and the British Society for Genetic Medicine released a position statement (November 2019) on DTC Genetic Testing, recommending that GPs should use caution when presented with DTC findings. They recommend that patients should be offered the NHS care which would otherwise have been offered (e.g. family history and risk assessment, healthy lifestyle advice, or referral to specialist care) regardless of their DTC result[76].

Current implementation in the NHS

Despite demonstrable clinical utility of specific examples of PGx testing (e.g. targeted cancer therapies) the translation to clinical care has been slow. At present the use of PGx in the NHS is limited, and testing for very few pharmacogenes are available to clinicians. Some genes of relevance to drug responses are included as integral parts of disease specific gene panels where access to these panels is permitted when clinically indicated for diagnostics. The selection of targeted molecular therapies is the most advanced application of PGx, in most cases requiring companion diagnostic testing: for example, *EGFR* testing in patients with NSCLC[13]. Examples of targeted non-cancer therapies recommended for use by NICE include ivacaftor for cystic fibrosis[77] and PCSK9 inhibitors for familial hypocholesterolaemia[78].

Avoidance of ADRs through pre-treatment genetic testing is only mandated in British prescribing guidelines prior to prescribing the HIV drug abacavir and carbamazepine therapy for epilepsy. Some pre-treatment testing is carried out using phenotypic methods before patients are prescribed drugs that are metabolised by the thiopurine s-methyltransferase (TPMT) enzyme. Patients with low TPMT activity can suffer bone marrow damage when prescribed drugs such as the immunosuppressant drug azathioprine or chemotherapy agent mercaptopurine. UK pharmaceutical guidelines recommend that TPMT activity is assessed on a patient's blood sample before prescribing these drugs, and is currently available in the NHS[79]. It is not yet clear whether genetic *TPMT* testing offers significant clinical advantages over phenotypic tests, but there is a very high level of agreement between genetic and phenotypic tests, and genetic testing may be beneficial for patients who have recently received a blood transfusion or drugs that affect TPMT activity, such as aspirin[80-81].

Future uses of pharmacogenomics

one study found that three pharmacogenes accounted for over 95% of the drugs prescribed

To inform the design of pre-emptive PGx testing in the UK a study analysed the prescriptions of 63 drugs and 19 pharmacogenes identified in PharmaKGB guidelines in half a million English primary care patients over a 20 year period[82]. It was found that three pharmacogenes (*CYP2D6*, *CYP2C19* and *SLCO1B1*) accounted for >95% of the drugs prescribed and that within English primary care, multiple exposure to drugs associated with PGx is extremely common with 60% of patients being prescribed ≥2 and 18% ≥5 of these drugs[82]. This demonstrates that exposure to gene-drug pairs is extremely common, and also highlights the future potential benefits of PGx testing.

Implementation of PGx testing for genes involved in the metabolism of chemotherapy drugs is being investigated at the in cancer services at Guy's and St Thomas' NHS Foundation Trust. Dihydropyrimidine dehydrogenase *(DPYD)* gene variants are implicated in toxicity arising from chemotherapy drugs. Clinical implementation of *DPYD* genotyping for metastatic breast cancer is being successfully trialled in routine clinical practice and being used to reduce the risk of severe side effects of these drugs[83]. However, nationwide *DPYD* testing is currently only available as part of an unrelated NHS congenital cataract and lens malformation gene panel.

Early evidence has demonstrated that testing the genetic variants in two genes, *CYP2C9* and *VKORC1* to determine dosing of the anticoagulant warfarin is superior to standard dosing[84] and reduces incidence of ADRs such as internal or external bleeding. The ability to optimise anticoagulant treatment is important because of the very high numbers of patients involved (e.g. there are more than 1.3 million people in the UK with atrial fibrillation, many of whom will be on anticoagulant medication to prevent stroke), meaning that these drugs, including warfarin, are among the drugs responsible for the highest number of ADRs and sADRs reported in patients[85].

Point of care (POC) pharmacogenomics testing

There is increasing interest in POC testing[86-87] as it may be able to yield PGx data at the time of prescribing. POC devices would allow for reactive, immediate PGx testing allowing for quick turnaround times and focused/targeted molecular analysis for a gene or variant of interest. These would be particularly useful in emergency situations,

for example when anaesthetics or pain killers need to be administered or when an infection is expected. However, no such POC device has yet received regulatory approval for use in clinical settings for PGx testing.

POC genetic testing when prescribing warfarin was recently trialled in three UK clinics and is the first demonstration of the implementation of genotype-guided dosing[88, 89]. The researchers used the ParaDNA POC genotyping platform developed by LGC Limited that used buccal swabs and had a turnaround time of 45 minutes. Another demonstration of POC testing for PGx is being tested in the 'Pharmacogenetics to Avoid Loss of Hearing' trial. It has been designed to determine the feasibility of implementing PGx testing at admission to the neonatal intensive care unit and antibiotic prescribing will be tailored to the presence or absence the *RNR1* gene variant[90]. The POC device and assay, genedrive® MT-RNR1 test platform, will be provided through the company genedrive plc who claim it is the first CE-IVD validated POC variant test available. It uses a buccal swab and can identify the variant in 22 minutes[91]. The company plan a product launch for Autumn 2020[92].

Ongoing activity to support pharmacogenomics implementation

Currently in the UK there are a variety of ongoing trials and programmes ongoing that aim to quantify the impact of PGx testing on patient benefit, clinical implementation and costs for the NHS. In particular, with the launch of the Genomic Medicine Service (GMS) in England, and the associated National Genomic Test Directory, there are efforts underway to incorporate PGx testing into the service. There are currently no tests listed specifically as PGx tests on the test directory, some companion diagnostic tests for cancer are however listed. NHS England and Genomics England have established a working group on 'Implementing pharmacogenetics in the NHS'[73] to evaluate which drug-gene pairs – other than targeted therapies – could be offered. These will be piloted through the Genomic Laboratory Hubs (GLH), followed by addition to the National Genomic Test Directory if the pilot demonstrates evidence of clinical effectiveness. It is anticipated that between 40 and 50 gene-drug pairs could be of clinical relevance.

it is anticipated that between 40 to 50 gene-drug pairs could be of clinical relevance

Cytochrome P450 drug metabolising enzymes (*CYP2D6* and drug metabolism)

Cytochrome P450 is a family of enzymes that are required for metabolism, including drug metabolism, and include *CYP2D6*, *CYP2C19* and *CYP2C9*, which together account for 25% of the PGx biomarkers listed in FDA drug labels (Figure 1). Results from the 100,000 Genomes Project indicate that around 12.5% of people have a *CYP2C9* genotype that would impact the appropriate dose of warfarin[93].

CYP2D6 in particular is one of the most widely studied PGx genes due to its direct role in the metabolism of many commonly prescribed medications.

These can be divided into four phenotypic groups:

- Poor metaboliser (PM) with no *CYP2D6* activity
- Intermediate metaboliser (IM) with reduced activity
- Extensive metaboliser (EM) with normal activity
- Ultra-rapid metaboliser (UM) with greater than normal activity

Individuals with the more extreme UM or PM phenotypes are at higher risks for increased toxicity or reduced efficacy of therapies, due to the genetic variants they carry. Depending on the variants an individual has, they may have a UM phenotype for one drug, and a PM phenotype for another.

Accurate genotyping of the *CYP2D6* gene is difficult because of the complexity in its structure, which can include, for example, large insertions and/or deletions of genetic sequence, or rearrangements of chunks of sequence of the gene. The result of this complexity is that sequencing using next generation 'short-read' technology is challenging – in next generation sequencing DNA is broken into short fragments, for sequencing before bioinformatic techniques reassemble the fragments into a continuous sequence. The types of genetic complexity seen in the *CYP2D6* gene makes the bioinformatics step prone to error, resulting in incorrect assembly of sections of sequence.

In addition, great care has to be taken in determining what the clinical implications of the genetic variation in a gene are. The complexity also means that there are over 100 known versions of *CYP2D6* gene variants[94-96].

There is therefore a growing interest in the development of user-friendly *CYP2D6* genotyping platforms with sufficiently high throughput to characterise clinically relevant genetic variations in the *CYP2D6* gene[97]. Current techniques available can be time-consuming, error-prone and characterise only a limited number of alleles, although there have been technology developments in this area.

great care must be taken because the complexity of the *CYP2DG* gene means that sequencing is challenging and prone to error

There are a number of commercially available tests to determine *CYP2D6* status, both reactive and pre-emptive. These include high-throughput microarray technology, such as GeneChip CYP450, Amplichip CYP450 and the DMET microarray (Table 3). These have good performance and allele coverage, but can also be technically difficult and costly to implement – it is likely that more development is needed in this area.

Table 3: Examples of commercially available *CYP2D6* genotyping and sequencing tests. *FDA commercially approved.[95, 98.]

Test Name	Manufacturer
AmpliChip CYP450 microarray*	Roche Molecular Systems
xTAG CYP2D6 Kit v3*	Luminex
Ion AmpliSeq Pharmacogenomics Research Panel	ThermoFisher/ Ion Torrent
GeneChip ® CYP450 assay	Affymetrix
DMET Plus micorarray	ThermoFisher/ Affymetrix
PharmacoScan	ThermoFisher/Affymetrix
iPLEX CYP2D6 Panel	Agena Bioscience
iPLEX PGx Pro Panel	Agena Bioscience
GenoChip Tamox	Akabiotech
INFINITI CYP450 2D6I	AutoGenomics
VeraCode ADME Core Panel	Illumina
GenoChip CYP2D6	PharmGenomics
Genelex genotyping CYP2D6	Genelex
GeneSight Psychotropic	Myriad Genetics

Targeted long-amplicon sequencing using the PacBio single molecule real-time sequencing platform (PacBio RSII sequencing) or the Nanopore sequencing MinION platform offers many advantages over these assays and could resolve many issues[94-95]. The main advantage of these techniques comes from their ability to handle and read much longer segments of DNA than short read sequencing[99]. This means that there are far fewer pieces of the genomic 'jigsaw' to put together which results in fewer errors. These technologies are however not currently available in clinical practice for this purpose.

While *CYP2D6* is known to be involved in the metabolism of numerous medications, of 385 FDA drug labels with PGx information, 66 have labelling naming the *CYP2D6* gene (Table 4, Figure 1)[100]. Considering some are commonly used medications, such as for pain treatment, infections and psychiatric disorders, such as depression, knowledge of *CYP2D6* genotype would be valuable information to support prescription of these medications. CPIC clinical guidelines are available for over 15 drugs, and DPWG have guidelines available for over 40 drugs (Table 5).

Table 4: List of approved drugs that includes *CYP2D6* information in drug label (PharmGKB accessed 20 Jan 2020)[101].

US- FDA (US Food and Drug Administration)	Amitriptyline, aripiprazole, atomoxetine, brexpiprazole, carvedilol, celecoxib, cevimeline, clomipramine, clozapine, codeine, darifenacin, desipramine, donepezil, doxepin, duloxetine, eliglustat*, fesoterodine, flibanserin, flibanserin, fluoxetine, flurbiprofen, fluvoxamine, galantamine, iloperidone, imipramine, lesinurad, metoprolol, modafinil, nortriptyline, ondansetron, palonosetron, paroxetine, perphenazine, phenytoin, pimozide*, pitolisant, propafenone, propranolol, protriptyline, quinidine, quinine, risperidone, siponimod*, tamoxifen, tamsulosin, terbinafine, tetrabenazine*, thioridazine, tiotropium, tolterodine, tramadol, trimipramine, venlafaxine, vortioxetine, warfarin
EMA (European Medicines Agency)	Aripiprazole, brexpiprazole, darifenacin, dextromethorphan / quinidine, dronedarone, duloxetine, eliglustat*, fesoterodine, gefitinib, mirabegron, olanzapine, palonosetron, propranolol, ranolazine, ritonavir, rucaparib, timolol, umeclidinium, vortioxetine
HCSC (Health Canada Santé Canada)	Acetaminophen & tramadol, aripiprazole, atomoxetine, carvedilol, codeine, darifenacin, fesoterodine, galantamine, metoprolol, nortriptyline, propafenone, risperidone, tamoxifen*, tetrabenazine, tolterodine, vortioxetine
PMDA (Pharmaceuticals and Medical Devices Agency)	Atomoxetine, codeine, eliglustat*, escitalopram, fesoterodine, gefitinib*, perphenazine, tetrabenazine, tolterodine

*Testing required: the label states or implies that some sort of gene, protein or chromosomal testing, including genetic testing, functional protein assays, cytogenetic studies, etc. should be conducted before using this drug.

Table 5: List of drugs for which treatment guidelines are available for *CYP2D6* (PharmGKB accessed 20 Jan 2020)[101]

CPIC Guidelines (Clinical Pharmacogenomics Implementation Consortium)	Amitriptyline, atomoxetine, clomipramine, codeine, desipramine, doxepin, fluvoxamine, imipramine, nortriptyline, ondansetron, paroxetine, phenytoin, tamoxifen, trimipramine, tropisetron, warfarin
DPWG Guidelines (Dutch Pharmacogenetics Working Group)	Amiodarone, amitriptyline, aripiprazole, atenolol, atomoxetine, bisoprolol, brexpiprazole, carvedilol, citalopram & escitalopram, clomipramine, clonidine, clozapine, codeine, disopyramide, doxepin, duloxetine, eliglustat, flecainide, fluoxetine, flupenthixol, fluphenazine, fluvoxamine, gefitinib, haloperidol, imipramine, methylphenidate, metoprolol, mirtazapine, nortriptyline, olanzapine, oxycodone, paroxetine, pimozide, propafenone, quetiapine, quinidine, risperidone, sertraline, sotalol, tamoxifen, tramadol, venlafaxine, zuclopenthixol

General considerations

Implementation

Although some access to PGx testing is available in many countries worldwide, this is fragmented and largely based upon local policies or proximity to hospitals where clinical research is conducted[102]. Despite international availability of PGx guidelines there is no nationwide pre-emptive PGx testing programmes currently in place in any healthcare system.

A number of international consortia (Table 4) are involved in promoting implementation by considering the incorporation of PGx data into EHRs and clinical pathways, as well as conducting some basic research[71-72]. A number of recent publications have investigated the processes, barriers and solutions around clinical implementation of PGx[71-72,102-103], some UK specific[89,104].

Implementation of PGx is currently under investigation by the GMS with pilot projects planned. It is anticipated that PGx testing will be a 'core' genomic test that is deliverable by all GLHs. More broadly, determining which tests to include is not straightforward and there is limited evidence on the clinical utility of PGx testing. However as outlined above projects are ongoing to gather data on improved health outcomes and cost effectiveness. In addition evidence gathering is ongoing to determine the frequency of the various PGx related variants within the UK Biobank. This would assist in determining the potential impact of PGx testing within the UK. Other areas where more data are needed is to determine which patient groups would most benefit from testing, at what stage testing should be implemented, and whether testing should be offered on a pre-emptive or reactive basis.

Demonstrating the clinical utility of PGx testing is further complicated by time lags in drug reactions, where some have immediate drug hypersensitivity while others may have delayed hypersensitivity or toxicity. Within clinical care questions of utility around polypharmacy management with individuals that have multi-morbidity is another area that requires further investigation[105]. For example, there are a number of drugs that are known to inhibit *CYP2D6* activity, meaning that an individual that has normal activity for *CYP2D6* may be inactivated through the use of a medication that would inhibit its activity. This *CYP2D6* drug-drug interaction alters effectiveness[106]. The age of individuals within the existing evidence and ongoing trials also do not always match the populations using the medication, such as the elderly.

Table 6: Examples of international pharmacogenomics implementation initiatives and institutes (adapted from[71])

Organisation	Activities
African American Pharmacogenomic Consortium Network (ACCOuNT)	Move studies of African American PGx from discovery to implementation; guidance for developing recommendations that consider ethnic background
CLIPMERGE PGx	Develop best-practice infrastructure for PGx implementation, real-time clinical decision support (CDS); the utility of genomic information in optimizing medication efficacy and safety
Electronic Medical Records and Genomics (eMERGE) network, collaboration with Pharmacogenomics Research Network (PGRN)	US-based network which works to combine DNA biorepositories with EHR systems in support of implementing genomic medicine. The process and clinical outcomes of integrating PGx data into EHRs and clinical decision support tools is being evaluated. PGRN also assess implementation of routine evidence-based PGx testing; templates for reporting results with CDS; educational materials for clinicians; gene–drug pair clinical guidelines
European Pharmacogenetics Implementation Consortium (Eu-PIC)	Aims to improve patient care in Europe by integrating PGx data into clinical care and facilitating PGx-guided personalisation of drug therapy[107]
Genomic and outcomes database for pharmacogenomics and implementation studies (Go-PGx)	Genomics-based precision health strategies to reduce the most common and serious ADRs; incorporate tests into clinical practice; study barriers; economic implications of testing in clinical practice
Implementing GeNomics In pracTicE (IGNITE) network	Comprises six genomic medicine research sites tasked with finding ways to incorporate genomic information into EHRs and CDS tools. Evaluate the feasibility of incorporating genomic information into clinical care; define, share and disseminate the best practices of implementation; contribute to the evidence base of outcomes of the use of genomic information in clinical practice

Organisation	Activities
INdiana GENomics Implementation: an Opportunity for the UnderServed (INGENIOUS)	Evaluate ADR incidence and annual healthcare cost, integration of results through the EHR and CDS
PG4KDS	Establish processes for using PGx tests in the EHR to pre-emptively guide prescription; develop interruptive CDS alerts; educational efforts for both patients and clinicians
The Pharmacogenomics Resource for Enhanced Decisions in Care and Treatment (PREDICT)	Develop infrastructure and a framework for incorporating PGx results into the EHR and making these available to clinicians at the time of prescription
RIGHT (Right drug, right dose, right time)	Develop best practice protocol for implementing genetic sequence data; point of care CDS
South East Asian Pharmacogenomics Research Network (SEAPharm)	Studies of ADR and developing guidelines adapted for the Asian population
The 1200 Patients Project	Establish a model system for eliminating practical barriers to implementing PGx; Interactive consultation portal for physicians; Clinical relevance of PGx and cost
Ubiquitous Pharmacogenomics (U-PGx) - Leading the 'PREemptive Pharmacogenomic testing for prevention of Adverse drug REactions' (PREPARE) study	Collaboration of experts across 16 different organisations in 10 European countries, PREPARE will assess the clinical value of pre-emptive testing of 13 important pharmacogenes, with the potential to guide the dose and drug selection of over 40 commonly prescribed medications, including some of those most involved in ADRs and fatal ADRs[85]. It is anticipated that PGx testing will particularly benefit older patients for whom simultaneous use of multiple medications (polypharmacy) is common. Results are expected in 2020.

Test-drug co-development

The UK Prescription Medicines Code of Practice Authority (PMCPA) states that companies can provide genetic testing or other biomarkers/ specific testing in relation to the rational use of one of its medicines. This means that where the use of a medicine requires specific testing prior to prescription, companies can arrange to provide such testing as a package deal even when the outcome of the testing does not support the use of the medicine in some of those tested[108]. One area of potential impact is in the support of the development of genetic tests that optimise the use of an approved medicine – one example is in the genetic companion diagnostic tests required to accurately prescribe targeted cancer drugs such as *EGFR* testing in NSCLC to support the prescription of targeted tyrosine kinase inhibitor therapy[13].

While co-development of a companion diagnostic or other PGx test with drug development is a preferred scenario, this is not always possible. International guidance on this process is available: in 2016, the FDA released draft guidance on the 'Principles for codevelopment of an in vitro companion diagnostic device with a therapeutic product'. This guides pharmaceutical companies that are developing treatments that rely on a biomarker/genetic test for their use.

Guidelines

Guidelines for PGx testing are available internationally: EMA and FDA guidelines share some level of agreement for most gene-drug pairs and also with the information available between CPIC and DPWG[109-110]. Current challenges with guideline development is that the evidence base used as the basis for inclusion in guidelines is inconsistent and variable – for example, see differences in the drugs listed in tables 4 and 5. A comprehensive review comparing PGx guidance for 505 different drugs by CPIC, DPWG, FDA, and other European agencies found discrepancies between the various organisations. Issues such as different formulations of the same active ingredient and variability in the information for different PGx biomarkers were identified[110]. Implementation of PGx drug labels into the clinics would strongly gain from a higher extent of consensus between agencies. Standardisation of nomenclature, labelling and guidelines are being addressed through large consortia (Table 6).

PGx drug labeling would strongly benefit from greater international consensus, and large consortia have already begun standardisation of nomenclature, labelling and guidelines

Recommendation:
Consideration needs to be given to the evidence requirements to support implementation of pharmacogenomics testing, and to supporting test developers to understand these requirements.

Information networks

The importance of the occurrence and consequence of a variant when considering the use of a therapy requires the correct interpretation of relevant information relating to drug-gene pairs. Data management networks that will interface with laboratories, medical records, interpretation datasets, and guidelines should ensure correct prescribing. Additional data management between basic research, clinical research, and pharmaceutical companies to design clinical trials and develop new drugs will advance the evidence base for PGx. This requires the transfer of information between multidisciplinary collaborations.

Recommendation:
Support for collaborations that facilitate sharing of resources and data are needed to underpin the information networks required to enable correct prescribing.

Incorporating PGx data into institutional Clinical Decision Support (CDS) systems has been shown to improve prescribing patterns aimed at reducing patient risk, and to significantly reduce emergency department visits and hospital readmissions in older patients requiring numerous concurrent medications necessitating consideration for drug-drug interactions[87,105,111]. An alternative strategy is for patients to carry their own PGx information in the form of a "safety-code card" that can be scanned to retrieve PGx-based dosing recommendations, this is being trialled in the PREPARE study (Table 6)[112].

Despite the currently limited availability of PGx testing, therapeutic recommendations formulated by DPWG have been incorporated into all electronic prescribing and medication surveillance systems in the Netherlands. The system has readily available pop-up alerts during drug prescribing and dispensing. The estimated nationwide impact of pre-emptive testing with actionable gene drug interaction is a dose adjustment or a switch to another drug in 5.4% of all new prescriptions[113].

incorporating PGx data into institutional Clinical Decision support systems has been shown to improve prescribing patterns

There are therefore likely to be significant opportunities for health technology companies focused on managing decision support around selection of appropriate tests, and utilising this information to improve medication-associated outcomes. Universal adoption of EHRs and electronic prescribing systems, improvement of data storage infrastructure and development of CDS tools would greatly improve accessibility and utility of PGx data in clinical settings. The CDS that is integrated within the Dutch EHR is a demonstration that this is possible.

Recommendation:
Clinical decision support systems for pharmacogenomics testing are a key area that requires further development and support.

Conclusions

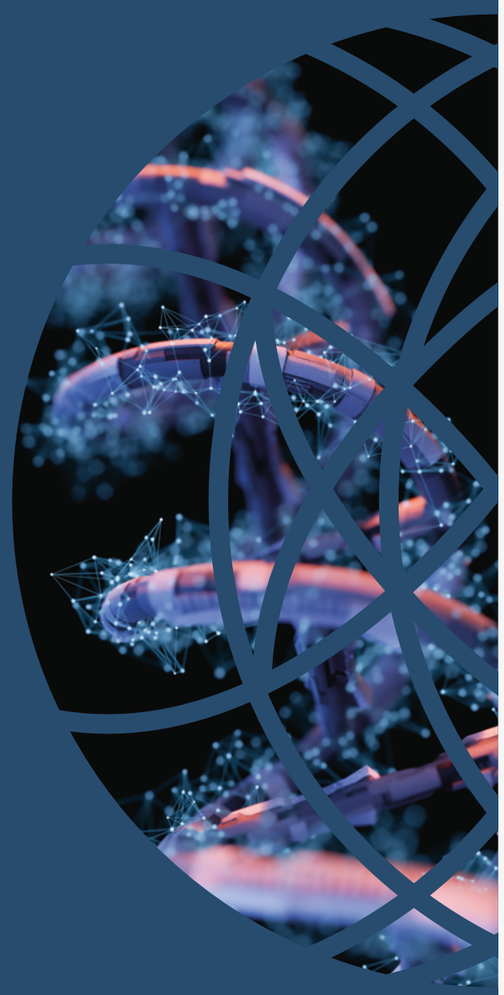
PGx information has the potential to significantly improve personalisation of medicine by enabling selection of targeted therapies, avoidance of drugs which may contribute to sADRs, and improve efficacy by informing more precise drug dosing for patients based upon their individual genetic make-up.

PGx testing is technically viable and testing for variants/genes with established clinical validity is feasible and currently being incorporated into the provision of genetic testing within the GMS. Understanding the genetic influences of drug effectiveness combined with robust clinical decision support tools and evidence based guidelines can aid the successful prescribing and monitoring of drug regimens. Once the PGx pilot projects within the GMS are complete, there is an opportunity to support further implementation through evidence gathering, particularly around different technological approaches to delivering PGx testing and interpretation.

PGx testing is technically viable, and tests with established clinical validity are currently being incorporated into the NHS England Genomic Medicine Service

3. Transcriptomics

Transcriptomics is another promising ‘omics technology for which three tests are already available for supporting clinical decision making in women with breast cancer



Transcriptomics is the study of RNA, which is a molecule produced from DNA. Messenger, or coding, RNA (mRNA) produced from protein-coding genes is used as a template for the production of proteins. The measurement of mRNA can be used to determine which genes are being expressed in a cell or tissue sample at a given time. The majority of transcribed RNAs do not code for proteins. Some of these non-coding RNAs, such as micro RNA (miRNA) or long non-coding RNA (lncRNA), can affect gene expression and cell function but are not directly translated into proteins.

The transcriptome is the set of all RNA molecules present in a sample (e.g. cell, tissue or organ) at a given time. Transcriptomics is an umbrella term for the study of mRNA plus non-coding RNAs. In the clinic, transcriptomics may refer to the measurement of the whole transcriptome or pre-defined subsets of it - this is also known as gene expression profiling/panel (GEP) testing.

measurement of mRNA can be used to determine which genes are being expressed in a cell or tissue sample at any given time

Why is transcriptomics useful?

Patterns of gene expression have been linked to different clinical outcomes in diseases such as cancer. GEP tests are being used to aid the personalisation of treatment by providing additional information in clinical decision making for:

- Treatment decisions, for example around potentially life-saving but toxic chemotherapy regimes
- Patient stratification to inform clinical management
- Understanding prognosis and risk of cancer recurrence

GEP tests could also provide improved cost effectiveness by helping to determine which treatments are likely to be more or less effective and allowing clinicians and patients to select optimal next steps.

Transcriptome analysis for rare disease may provide additional diagnostic power beyond that provided by whole genome sequencing (WGS), whole exome sequencing (WES), and other conventional diagnostics. It may help in narrowing down potential gene candidates or reveal where a gene seems to be functioning normally but is actually abnormally expressed, potentially leading to disease. This could provide more accurate diagnosis and tailoring of therapies for rare disease patients.

Methods used for transcriptome analysis

the methods employed can be broadly split into gene expression profiling tests and transcriptome analysis

There are several methods employed for examining parts of or the whole transcriptome relevant to health. These can be broadly split into gene expression profiling tests and transcriptome analysis.

Gene expression profiling/panel (GEP) tests: GEP tests examine levels of RNA transcripts (primarily mRNA) being expressed from pre-determined regions of the genome. GEP tests commonly use either quantitative reverse transcription-PCR, microarray or RNA sequencing to examine gene expression of a pre-determined panel of genes.

Transcriptome analysis: Transcriptome analysis is being used in research to determine whether it could provide additional benefits in the diagnosis of rare disease, beyond WGS and WES. The primary technique used is RNA-sequencing using next generation sequencing (NGS) technologies.

Several different techniques can be used to examine either the whole, or part of, the transcriptome.

Quantitative reverse transcription polymerase chain reaction (RT-qPCR) can be used to quantify expression levels of individual genes. This is a simple, low-cost method, but limits the number of genes that can be examined at once.

Microarray: Target sequences of complementary DNA (cDNA) are converted from RNA in the sample. These specifically bind to DNA probes attached to a microarray plate, which produces a signal which can then be measured to quantify gene activity. This technique will only detect pre-defined sequences and cannot be used for investigating novel RNA sequences.

RNA sequencing (RNA-seq): The RNA sequence is read (as cDNA), not just detected. RNA-seq can be applied to examine across the entire transcriptome and can be used to detect novel sequences. The computational burden and overall cost is greater than for simpler techniques.

Long read RNA-seq: Long read technologies are a developing field of sequencing technologies able to sequence much longer strands of nucleic acids. Currently applied in research, the techniques can be used to read long sections of DNA. Some platforms allow for direct sequencing of RNA molecules, rather than cDNA.

The current state of implementation

some relatively simple RNA tests have been used within the NHS for some time, and RNA-based tests are routinely used in the detection of gene-fusions in blood cancers

The application of transcriptomics and associated technologies varies across different areas of healthcare and with different types of test. Some relatively simple tests have been used for many years within the NHS: for example, single-marker RNA tests (primarily using RT-qPCR) are used in UK healthcare across several diseases, and RNA-based tests are routinely used in the detection of gene-fusions in blood cancers. These tests are listed in the National Genomic Test Directory for cancer[114]. RNA testing is also used in the detection or identification of pathogens – this is not covered in this chapter but is touched upon in chapter 5.

A small number of GEP tests are being used within healthcare and are available through the NHS. The use of GEPs in clinical settings is variable. GEPs can be developed in-house by NHS laboratory services and individual services may choose to validate 'research' commercial panels and make them available for clinical use. This means that availability of RNA panels can vary between laboratory services. Although many GEP tests exist and are being developed across diverse disease areas, commercially developed tests currently in use in UK healthcare are for cancer prognosis, treatment stratification and for assessment of recurrence risk.

Several multi-gene prognostic or predictive applications have been developed, though many such applications remain restricted to research. Whole-transcriptome diagnostics are not currently being utilised within the NHS, but are being investigated in research. The Test Directory lists RNA analysis for further investigation of candidate splice variants – different forms of RNA from the same gene that may have implications for disease - and RNA storage where RNA testing is likely to be required in the future[114].

Gene expression profiling tests for oncology

In use in the NHS or UK healthcare

GEP tests are available or are being developed for use as an adjunct in cancer treatment. Many of these aim to inform prognosis, estimate recurrence risk or inform treatment decisions. The majority of these tests have been developed for use in breast cancer. In 2018, NICE examined several GEP tests for use in breast cancer prognosis. Three tests – Oncotype DX, Endopredict, and Prosigna – were subsequently recommended for use in determining risk of breast cancer recurrence and/or guiding chemotherapy decisions in breast cancer patients with oestrogen receptor positive (ER+), human epidermal growth factor receptor 2 negative (HER2-), lymph node negative (LN-) breast cancer (Table 7).

Table 7: GEP tests recommended by NICE in its most recent review (December 2018) for guiding adjuvant chemotherapy decisions under specific conditions. Tests funded by the NHS where appropriate, patients can also buy the test privately[115-117].

Test	Purpose/aim	Further details
Oncotype Dx Genomic Health RT-qPCR	Prognostic: Measures breast cancer recurrence risk in patients with ER+/Her2- cancer, post-surgery. Patients with a low risk score can be spared chemotherapy, receiving hormone therapy only.	Expression of 16 genes plus 5 reference genes on RT-qPCR panel Samples are sent to laboratory in the US.
Endopredict Myriad Genetics RT-qPCR	Prognostic: CE-marked assay designed to predict the likelihood of metastases within 10 years of an initial breast cancer diagnosis.	Expression of 8 genes is measured, plus 4 reference or control genes, using RT-qPCR and considered alongside other clinical data to produce an 'EPclin score'. Samples can be processed in a local laboratory or at the company laboratory in Germany. A reduced testing price may be offered where analysis is not performed at the manufacturer's site, but in the clients own laboratory.
Prosigna Veracyte, previously NanoString Technologies mRNA counting	Prognostic: CE-marked assay designed to provide information on breast cancer subtype and predict distant recurrence-free survival at 10 years.	Measures the expression of 50 genes for subtype classification, plus 22 reference genes using mRNA counting.[118] Three company sites in the UK provide laboratory services for this test.

The RD-100i one-step nucleic acid amplification (OSNA) system is a gene expression test. Unlike other systems included in this chapter, the OSNA system examines RNA from one gene only. The OSNA system can be used to detect sentinel lymph node metastases in breast cancer patients who have had a sentinel lymph node biopsy. This information helps inform clinicians as to whether removal of the lymph node(s) would be most appropriate[119]. In 2013, NICE recommended that a national registry be developed to collect data on the use of RD-100i OSNA system in the UK[120].

As per clinical laboratory standards, some of these tests can be performed on formalin-fixed paraffin-embedded (FFPE) tumour biopsy samples. This means that if a patient has had surgery, an additional biopsy is not required, since tumour tissue removed during surgery can be used.

Assessment or further development of cancer GEP tests

Beyond breast cancer, GEP tests, some of which use RNA plus additional 'omic biomarkers such as DNA or proteins (referred to as 'multi-omic') e.g. Caris Molecular Intelligence, have also been developed for prostate cancer, cancers of unknown origin, and several other cancers. Examples demonstrating some of the variety of commercial GEP tests that have been developed internationally are given in Table 8.

Amongst others, Mammatyper, PredictSure-IBD and Prolaris have been the subject of med-tech innovation briefings by NICE[124, 127]. Metasin and Mammaprint have been reviewed and have not received recommendations. In addition, in guidance from 2010, NICE has explicitly stated that gene-expression based profiling should not be used to identify primary tumours in patients provisionally diagnosed with cancer of unknown primary (CUP) or when deciding which treatment to offer patients with confirmed CUP[128]; this guidance is now due for review[129]. In many cases, an improved evidence base is recommended or sought before reliable evaluations of clinical utility can be made.

Table 8: Examples of available gene expression tests

Test		Purpose of test and what it measures	Status	
Name:	Mammatyper	Cancer subtyping: classifies breast cancers into 4 subtypes for which there are different treatments.	CE Marked	Yes
Disease area:	Breast cancer		Available in UK?	Yes
Technology:	RT-qPCR	Measures expression of four genes: *ERBB2*, *ESR1*, *PGR*, *MKI67*, in human breast cancer tissue	FDA	Not approved
			NICE	Not recommended Guidance published: not recommended
Company:	BioNTech Diagnostics	This test would replace IHC testing in people with early-stage breast cancer		
Name:	Oncomine Focus Assay[121]	Treatment selection	CE Marked	No†
Disease area:	Cancer - solid tumours	Detection of variants in 52 genes using DNA and RNA	Available in UK?	Yes, undergoing validation in some NHS services[122]
Technology:	RNA and DNA sequencing using Thermo Fisher's Ion system	Measures RNA to detect rearrangements in four genes associated with solid tumours	FDA	Not approved
			NICE	Not assessed
Company:	Thermo Fisher Scientific			

	Test	Purpose of test and what it measures		Status
Name:	Oncomine Dx Target Test	Diagnostic and treatment selection	CE Marked	Yes
Disease area:	Lung cancer	Examines RNA for expression/ fusions of 21 genes	Available in UK?	Yes
Technology:	RNA and DNA sequencing using Thermo Fisher's Ion system	Examines DNA for mutations in 35 genes	FDA	Approved[123]
			NICE	Not assessed
Company:	Thermo Fisher Scientific			
Name:	Prolaris	Prognosis: Generates a score, which aims to provide prediction of patient's 10-year risk of mortality or 10-year risk of biochemical recurrence.	CE Marked	Yes
Disease area:	Prostate cancer		Available in UK?	Yes, available through trials (see Table 9)
Technology:	Unknown	Measures expression levels of 31 genes and 15 control or reference genes	FDA	Approved
			NICE	Not assessed, subject of MIB65[124]
Company:	Myriad Genetics			
Name:	Oncotype Dx Prostate	Prognostic: Predicts likelihood of adverse pathology, mortality, and metastasis at 10 years	CE Marked	No, Provided 'as a service'[†]
Disease area:	Prostate cancer	Measures expression of 12 cancer-related genes and 5 reference genes	Available in UK?	No
Technology:	RT-PCR	The expression levels are algorithmically combined to calculate the Genomic Prostate Score (GPS)	FDA	Not approved
			NICE	Not assessed
Company:	Genomic Health			
Name:	CancerTYPE ID	Cancer origin and subtyping: Determines tumour origin and primary site of metastatic cancer.	CE Marked	No[†]
Disease area:	Cancers of unknown primary	Classification of tumour into subtypes, up to 50 tumour types	Available in UK?	No
Technology:	RT-PCR	Measures the expression of 92-genes	FDA	Not approved
			NICE	Not recommended Guidance published: cancer origin tests not recommended
Company:	bioTheranostics			

	Test	Purpose of test and what it measures		Status
Name:	Tissue of Origin	Cancer origin test	CE Marked	No[†]
Disease area:	Cancers of unknown primary	Measures the expression of around 2000 genes	Available in UK?	No
Technology:	Microarray	Determines tumour origin and primary site of metastatic cancer	FDA	510k clearance
			NICE	Not recommended Guidance published: cancer origin tests not recommended
Company:	Cancer Genetics			
Name:	Caris Molecular Intelligence	Future management: helps guide future management of cancer	CE Marked	Yes
Disease area:	Locally advanced or metastatic cancer	CMI measures the expression of 53 genes	Available in UK?	Yes
Technology:	IHC* (proteins) ISH* (gene deletions/ amplifications) RNA sequencing in 53 genes DNA sequencing of hundreds of genes.	It also measures protein expression and specific DNA mutations. A variety of techniques are used	FDA	Not approved
			NICE	Not assessed, subject of MIB120[125]
Company:	Caris Life Sciences			
Name:	MI Transcriptome	Guiding treatment decisions: assists clinicians in determining best treatment options for each patient	CE Marked	No, Provided 'as a service'[†]
Disease area:	Solid tumours	Examines expression across the transcriptome for the detection of gene fusions, mRNA variant detection and variable gene expression in solid tumours	Available in UK?	No
Technology:	RNA-sequencing		FDA	Approved, breakthrough device designation (2019)
			NICE	Not assessed
Company:	Caris Life Sciences			
Name:	PredictSure-IBD	Prognosis and guiding treatment decisions: predicts long-term disease outcomes and guides the choice of treatment for a patient newly diagnosed with Crohn's disease or ulcerative colitis	CE Marked	Yes
Disease area:	Crohn's disease and ulcerative colitis		Available in UK?	Yes, available through a trial
Technology:	RT-PCR		FDA	Not approved
		Measures expression of 17 genes	NICE	Not assessed, subject of MIB178[126]
Company:	Predict Immune			

* IHC = Immunohistochemistry, ISH = *in situ* hybridisation

Test		Purpose of test and what it measures	Status	
Name:	miR Scientific Sentinel™ PCa Test and miR Scientific Sentinel™ CS or HG Test	Diagnostic: PCa test provides a binary result of cancer or no cancer based on expression profiles	CE Marked	No†
Disease area:	Prostate cancer	Stratification: Test classifies individuals as having clinically significant versus clinically insignificant disease	Available in UK?	No
Technology:	miR Scientific Disease Management Platform™ Uncertain	Measures the expression of 280 small-non-coding RNAs (sncRNAs) in a urine sample	FDA	Not approved
Company:	miR Scientific		NICE	Not assessed
Name:	RD-100i one-step nucleic acid amplification (OSNA)	Detection of metastases: detects sentinel lymph node metastases in breast cancer patients who have had a sentinel lymph node biopsy.	CE Marked	Yes
Disease area:	Breast cancer	Measures expression of the cytokeratin-19 (*CK19)* gene	Available in UK?	Yes
Technology:	Reverse transcription loop mediated isothermal amplification (RT-LAMP)	Benchtop platform that incorporates all stages of analysis	FDA	Not approved
Company:	Sysmex UK		NICE	Recommended Guidance published, recommended (guidance under review)[119]
Name:	Metasin	Detection of metastases: detects sentinel lymph node metastases in breast cancer patients who have had a sentinel lymph node biopsy.	CE Marked	Yes
Disease area:	Breast cancer		Available in UK?	Yes
Technology:	RT-qPCR	Measures two RNA biomarkers from *CK19* and mammaglobin (*SCGB2A2)*	FDA	No
Company:	Developed within the NHS		NICE	Not recommended, Guidance published: not recommended (guidance under review)[119]
Name:	Mammaprint	Recurrence risk and guiding treatment decisions: assess the risk of recurrence within 5 and 10 years and whether a person would benefit from chemotherapy	CE Marked	Yes
Disease area:	Breast cancer		Available in UK?	Yes
Technology:	Microarray	Measures the expression of 70 genes	FDA	Approved
Company:	Agendia		NICE	Not recommended Guidance published: not recommended (guidance under review)[118]

† Where CE marking is not evident, this may be due to the test being provided as a service (or other) rather than a medical device

Questions remain as to the impact of GEP tests on patient outcomes. Many GEP tests are being examined in clinical trials, examples of which are given in Table 9. Trials include those involving relatively established GEP products which may focus on the impact of implementing the technology, and those investigating the validity of less established panels.

Recommendation:
Further support is needed for evidence-gathering of clinical utility of gene expression profiling tests, particularly in terms of patient outcomes.

Research and development of gene expression profiling tests

GEP tests in use in cancer treatment currently rely on tumour biopsy, whether frozen, fresh or FFPE. RNAs are also being investigated as cancer biomarkers to be detected in liquid biopsy (see chapter 1 for more on liquid biopsy), making testing less invasive, especially where multiple samples may be required. Liquid biopsy is a liquid substitute for a solid tumour biopsy, measuring biomarkers in bodily fluids e.g. blood or urine. Below are examples of uses being investigated for cancer prognosis and diagnosis.

Table 9: Examples of clinical trials investigating the use and development of GEP tests (source: ClinicalTrials.gov)

GEP test	Trial	Aim (taken from relevant clinical trials page)
Endopredict (Breast cancer)	EndoPredict® Extended Endocrine Trial (EXET) NCT04016935	"...to evaluate how EndoPredict® is used clinically to inform treatment decisions for extended endocrine therapy"
Oncotype DX Prostate Cancer Assay (Prostate cancer)	Engaging Newly Diagnosed Men About Cancer Treatment Options (ENACT) NCT02668276	"...to better understand how a new laboratory test called the Oncotype DX Prostate Cancer Assay may impact what treatment men decide to get and how they feel and think about their choice of treatment"
Prolaris (Prostate cancer)	Prolaris Enhanced Risk Stratification - an ecONomic and clinicAL Evaluation (PERSONAL) NCT03851211	"...find out if the Prolaris® test score helps patients with newly diagnosed prostate cancer and their clinical team make better informed treatment choices that are tailored to the individual patient."

RNAs are also being investigated as cancer biomarkers for liquid biopsy, making testing less invasive

Examples from oncology

Prostate cancer: The miR Scientific Sentinel™ PCa Test and miR Scientific Sentinel™ CS Test aim to use RNA in the urine to provide an alternative to invasive solid core needle biopsies in individuals with suspected prostate cancer. The assay is currently being investigated in a clinical trial in the US, due to complete in 2022[130].

Glioblastoma: Early stage research at the University of Sussex is investigating a liquid biopsy technique that combines RNA and protein information to look for specific 'signatures' associated with the disease. RNA contained in extracellular vesicles are being investigated as a marker of glioblastoma, and as a potential indicator of tumour aggressiveness. Publication of results from a validation study is expected in early 2020[131].

Thyroid cancer: Two lncRNAs are being investigated as biomarkers in thyroid cancer using liquid biopsy, with the aim of improving early detection and better prediction of cancer progression[132].

Examples from other disease fields

Outside of cancer treatment stratification, GEP tests are being developed for use in a few disorders, for example in the diagnosis of eosinophilic esophagitis[133] and inflammatory bowel disease (IBD). PredictSure-IBD is a CE-marked blood-based 17-gene qPCR-based test for IBD prognosis developed by PredictImmune based in the East Midlands and East of England Genomic Laboratory Hub in Cambridge, UK. A UK-based clinical trial entitled 'PROFILE' aims to examine the use of PredictSure-IBD and has been provided funding through a Wellcome Trust translational award; the trial is currently recruiting and expected to complete in 2022[126, 134].

Gene expression panels are readily available for use in research - companies such as nanoString and Affymetrix (based in the US) have developed a large array of gene expression panels for the study of many different disease or biological states.

Transcriptomics for rare disease

Evidence is beginning to emerge demonstrating that examining the transcriptome could help provide a diagnosis for patients with rare disease who do not receive a diagnosis from DNA-based investigations alone.

Why analyse the transcriptome in rare disease?

Rare diseases are collectively common – while individual diseases tend to occur at rates of fewer than 5 in 10,000 people, taken together it is estimated that 1 in 17 individuals in the UK has a rare disease[135]. The rare diseases arm of the 100,000 genomes project was one of several worldwide initiatives to explore the genetic basis of rare disease, with the goal of increasing diagnoses and thus contributing to efforts to reduce the diagnostic odyssey that many rare disease patients and their families face.

The use of genomics in this context has been successful in terms of increasing diagnostic yield and building a repository of knowledge about the genetics of rare disease, however it is clear that the answers cannot always be found in the gene-coding DNA sequence alone. Additional investigation of the RNA may show over-, under-, or erroneous gene expression, or reveal alternative splicing of RNA, which can result in disease, or provide clues about non-coding regions which in turn have an impact on the expression of protein-coding genes[136].

What is the evidence?

There have been several studies investigating transcriptomics for rare disease using biopsy samples, and recently using less intrusive sample collection techniques e.g. blood samples. Additional diagnostic yield varies, ranging from around 5 to 40% diagnosis beyond that gained using WES or WGS. In combination, these can provide additional information required to narrow down a range of candidate genes[137]. A recent study using RNA-seq from whole blood for the diagnosis of rare disease yielded a diagnostic rate of 7.5% beyond that provided by exome sequencing alone, and narrowing down of gene candidates in an additional 16.7% of cases[138]. In an another small study, researchers in the US used whole RNA-seq to provide a genetic diagnosis in 36% of participants who were undiagnosed following WES or DNA gene panel analysis[136].

RNA may provide answers to our questions about rare diseases, that cannot be found in DNA

Ongoing research

As part of the Clinical Interpretation Partnerships (GeCIPs), Genomics England lists a research subdomain entitled: 'transcriptomics and RNA splicing' which is focused on the use of transcriptomics for diagnosis and further profiling in rare disease and cancer. During sample collection for WGS, the 100,000 genomes project also collected RNA from both rare disease and cancer patients. Whilst these samples were not intended for use in the main 100K genomes project, they, alongside some protein samples, were collected with

the intention of being used for future research should additional funding be obtained[139]. Analysis of these samples is now taking place, with a small number being assessed so far.

There are several research groups investigating the potential use of transcriptomics for the diagnosis of rare disease. Particular attention is being paid to errors in splicing, which is the way in which RNA is processed prior to being translated into protein. Dysregulation of this process has been linked to some human diseases.

the 100,000 genomes project also collected RNA from both rare disease and cancer patients for future research

Because of the tissue- and time-specific nature of gene expression, RNA is not present consistently throughout tissues. Transcriptomic analysis must therefore be applied appropriately for the disease in question. A key limitation is that it is difficult to apply in diseases which are organ-specific and for which the organ is relatively inaccessible e.g. brain disorders, especially where a tissues biopsy is required. Research is also underway to find more readily accessible tissues and samples that are representative of disease-affected organs.

Recommendation:
Ongoing research is required to identify the most promising applications for transcriptomics in rare disease, and these should be supported in terms of gathering evidence of clinical effectiveness.

Pipelines for the analysis of whole genome sequencing (WGS) often focus on coding regions of DNA – that is areas that code for proteins – however, disease can also occur as a result of variation in non-coding regions of the genome. Although they do not produce proteins, these regions may still produce RNA, some types of which may in turn impact upon the production of proteins and other molecules which can lead to disease. This RNA can be investigated using appropriate transcriptomic analysis.

Some expertise and infrastructure for RNA sequencing and analysis exists within the NHS and associated institutions, e.g. Sheffield Children's Hospital NGS facility lists RNA-sequencing as one of its capabilities including provision of bioinformatics support. Applications are listed as limited to research.

Recommendation:
Consideration should be given to how current DNA sequencing pipelines and infrastructure can be utilised and altered to support RNA sequencing efforts, should further evidence for its use arise.

Other research areas of interest

The relationship between gene expression and disease is also being investigated across many other diseases. Examples include:

Parkinson's disease: In a recent paper examining the genetics of Parkinson's disease[140], researchers implemented a transcriptome-wide association study to identify novel genes and mechanisms (beyond those identified in GWAS) that may be associated with risk of developing Parkinson's disease or disease progression.

Lung cancer: The TRACERx study being run by Cancer Research UK launched in 2014 and includes nearly 850 lung cancer patients. It aims to examine tumour heterogeneity in relation to clinical outcome. 164 samples of RNA-seq data were collected from 64 patients as part of this study. One of the outcomes of the study was the suggestion that immune response alters the evolutionary trajectory of the tumour[141].

Foetal monitoring: Research studies have explored the use of the amniotic fluid transcriptome to monitor the activity of genes involved in the growth and development of organs and the nervous system. This kind of approach, if shown to be effective, could be used to monitor high-risk pregnancies, however amniocentesis is required which carries additional risks.

RNA diseases: 'RNA disease' is a term used to describe disease that originates from errors in the processing of RNA (e.g. splicing, translation or degradation errors)[142]. These include some neuro-muscular diseases and cancers. Transcriptomics is helping to identify these diseases and to examine the underlying cause.

The 78 active trials (recruiting, enrolling by invitation, active but not recruiting) currently listed on ClinicalTrials.gov under the term 'transcriptomics' (as of January 2020), cover a wide range of conditions. In the majority of these studies, researchers are using transcriptomics to compare different healthy/disease states, in response to certain conditions and interventions, some with a view to developing gene expression signatures for clinical use.

the relationship between gene expression and disease is potentially relevant to many diseases, including Parkinson's, lung cancer and in foetal monitoring

beyond GEP tests, transcriptomics remains primarily in the research area, but it is likely that it will become much more widely used

General considerations

A number of GEP tests have shown potential for the stratification of treatment for various forms of cancer. However, questions around evidence and cost effectiveness mean that few of these have received approval for use in the UK, although a relatively small number of those available have been formally assessed by NICE (Table 8).

Beyond GEP tests, transcriptomics remains primarily in the research arena. However, it seems likely that it will become more widely used to provide information for diagnosis of rare disease and informing understanding of disease mechanisms in the clinic in the years to come, primarily in combination with other 'omics for diagnosis of disease. Publications reporting on transcriptomics and RNAseq as a potential complementary diagnostic tool to both WES and WGS indicates that this is an advancing area of research.

What enablers and barriers exist to achieving health impact?

Analysis of comprehensive transcriptome data is complex, time- and resource- consuming. Specific equipment and skills are needed to obtain and utilise transcriptomic data effectively.

There is also a lack of standardisation in methods, which can affect the ability to assess reproducibility, accuracy and precision. For now, widespread use of RNA-seq and transcriptomics is taking place in the research arena, and is limited to a relatively small number of patient samples.

For several applications of GEP tests e.g. for prognosis of different types of cancer, evidence relating to patient outcomes is lacking. This is being addressed to some extent (in a few of the panels available) through data collection alongside application in the clinic.

Recommendation:
Support for research into standardisation of RNA analysis methods is key to ensuring that evidence gathered is reproducible, accurate and reliable.

Implementation of transcriptomics for rare disease and cancer

Transcriptomics for rare disease could be ready for implementation in the near future. Studies have shown some additional diagnostic yield to using transcriptomic data alongside examination of the genome using WGS, WES or panels – although expanding, data is currently quite limited and further evidence will be required before resources are expended on further implementing this technology. Issues include:

- Bioinformatics pipelines for RNA sequencing involve extra layers of complexity above whole genome or exome analysis
- Appropriate collection, storage and utilisation of samples is required. RNA is unstable and samples do eventually degrade, even when stored in optimal conditions
- Knowledge linking variation to disease is lacking, but is building
- Ideally analysis would be performed using blood samples or other sample types that are less invasive than a tissue biopsy

Delivery of GEP tests

Knowledge of the purpose and potential outcomes of testing for both patients and clinicians is an important consideration. Clinicians will need to adequately explain test results to individual patients and be appropriately equipped to do so.

GEP tests currently available on the NHS require the collection and shipment of samples to off-site locations for testing and analysis. This requires additional consideration by clinicians and pathologists to ensure that appropriate processes are followed during sample collection that allow for timely sample delivery, minimising delays and ensuring prompt return of results to patients. However, some GEPs offer the option for on-site analysis at reduced cost.

Due to the time required for sample collection, shipment and analysis – often to international locations – time to return results from GEP tests may be substantial. Some qualitative evidence following the public funding of GEP tests in Canada has suggested that clinicians are wary of the potential of GEP testing to incur delays for patients awaiting treatment, lengthen consultations or induce anxiety[143, 144]. However, in some circumstances - where panels may facilitate testing of multiple targets that would otherwise be assessed independently or using alternative methods - time may be saved by implementing panels. This should be assessed on an individual panel and associated application basis.

transcriptomics for rare disease could be ready for implementation in the near future

there is a lack of evidence of clinical benefit and cost effectiveness, and continued uncertainty around long-term outcomes

Collection and assessment of appropriate evidence

One of the most notable concerns around the use of GEP tests is whether they provide substantial patient benefit. Trials have focused on the ability of GEP tests to affect clinical decision making and stratify patients, rather than on long-term patient outcomes. Some tests have not received recommendation following assessment by NICE on the basis of a lack of evidence of clinical benefit and cost effectiveness. There is continued uncertainty around long-term patient outcomes.

As part of the positive recommendation of Oncotype DX, Endopredict and Prosignia from NICE, a data collection agreement – to include clinical outcomes – was stipulated as a requirement, and is to be performed through the national database, National Cancer Registration and Analysis Service to 'ensure evidence is available that can be considered in future updates of this guidance'[118]. Therefore, support for data collection agreements which include clinical outcomes and are performed through national databases should be considered for supporting the evidence gathering required for implementation.

Understanding of the transcriptome and implications for disease

Scientific understanding of RNA and the implications of gene expression for health have been steadily expanding for some time; however the ability to draw clinically-relevant conclusions from gene expression information is still limited by knowledge of how this relates to disease. Gene expression can be highly variable and for some conditions it may not be possible to find reliable and consistent indicators of disease for diagnosis or prognosis across a population.

Conclusions

Transcriptomics is a promising field for both rare and more common but serious disease for which it is beginning to have an impact on patients and clinical practise through the availability of GEP tests for prognosis and treatment stratification in cancer. Theoretically, there is no limit to the potential range of panels that can be constructed to assess gene expression profiles linked to disease, and this is somewhat reflected in the number and range of such tests being developed in the commercial sector. Suitability of tests continues to be reviewed as questions surrounding patient outcomes and cost-effectiveness are examined through further data collection. There are opportunities to support evidence gathering around technologies that are closer to clinical implementation.

The evidence base surrounding the use of transcriptomics for rare disease diagnosis is growing and it seems likely that transcriptomics could provide additional diagnostic yield alongside WES and WGS. However, the use of these techniques has so far been scattered across research and techniques are not standardised, making it difficult to assess usefulness of transcriptomics for rare disease under the guise of a single technique.

The health system should be ready to respond to evidence as and when it emerges, and consider how existing laboratory genomics infrastructure can be developed to support the timely implementation of testing when appropriate.

the health system should be ready to respond to the evidence, and consider how existing laboratory genomics infrastructure can be developed to support timely implementation of testing

4.

Testing to support antimicrobial stewardship

Near patient testing to support antimicrobial stewardship – rapid diagnostic testing for infectious disease – has particular potential to contribute to global efforts to mitigate antimicrobial resistance

The global challenge of antimicrobial resistance (AMR) has increased the need to develop and implement rapid and sensitive near-patient diagnostic tests for the detection and identification of pathogens in patients with infectious disease.

Antimicrobials are a broad group of drugs that treat infections caused by a range of pathogens. These include antivirals, which treat viral infections such as influenza, and antibiotics, which are used to treat bacterial infections. While the development of antibiotic resistance is a growing health concern, antimicrobial stewardship approaches consider optimal use of all antimicrobial drugs, not just antibiotics.

The importance of diagnostic tests was recognised in Lord O'Neill's Independent review of AMR in 2016, which advocated that by 2020 antibiotics should not be prescribed without use of a diagnostic test if one was available[145]. The government responded by committing to investment in diagnostics[146], and outlining plans in the NHS Long Term Plan to optimise antibiotic use, support development of new antimicrobials and improve antimicrobial stewardship[12]. The recent UK five year action plan for tackling AMR includes rapid and accurate tests as a key area in helping to reduce unnecessary use of antibiotics[147].

Such diagnostic tests can improve antimicrobial stewardship by reducing unnecessary prescription and overuse of antibiotics, whilst helping support better clinical decision making and improving outcomes for patients.

use of rapid and sensitive diagnostic tests can reduce unecessary prescription and overuse of antibiotics

These tests can be used in patients where an infectious disease is suspected for a number of purposes.

Distinguishing between bacterial and viral infections

If a patient has symptoms that suggest an infection but the clinician is uncertain if the cause is bacterial or viral, a diagnostic test could be used to help determine the pathogen and support clinical decision making as to whether antibiotics should be prescribed.

Identifying resistance and susceptibility to antimicrobials

Tests to identify AMR can determine which antimicrobials should be avoided, whilst susceptibility tests reveal which antimicrobials are most suitable for use. This is important for ensuring patients receive antimicrobials that are effective and can be used to reduce the time spent on broad spectrum antibiotics for severe bacterial infections.

quick and accurate diagnosis of patients with infectious disease is neccesary to reduce the risk of outbreaks

Monitoring patient response to antibiotics

Monitoring patient response to antimicrobials, either by tracking levels of pathogens or the patient's own biological response to infection, can reveal if antibiotics are effective or not and reduce unnecessarily long periods of use.

Rapid tests are needed to provide information in a suitable timeframe for clinical care pathways. Being able to quickly and accurately diagnose patients with infectious disease can also help manage patients faster to reduce the risk of outbreaks. Here, rapid near-patient tests will be defined as diagnostic tests performed at or near the place of a patient's treatment (as opposed to sending samples to a clinical laboratory) to allow quicker and/or improved clinical decision making. This includes but is not limited to point of care testing (POCT), a term typically used for tests that can deliver results within a single patient encounter with a clinician, often requiring a turnaround time of 15 minutes or less[148].

Role of AHSNs

The five year plan states that tackling AMR will be a priority area for the AHSNs[147] – it also emphasises the need to make more use of Patient Safety Collaboratives (PSCs) a network of 15 regional organisations that support safety, continuous learning and improvement, and which are organised and delivered locally by AHSNs.

Recommendation:
With the use of their existing networks and by close collaboration with patient safety collaboratives AHSNs are well placed to support the identification, implementation and dissemination of new diagnostic tests as part of their work programme on AMR.

Types and choice of test

There are multiple types of diagnostic test already available and more are in development. Each comes with different advantages and disadvantages making them suited to different purposes.

Tests can broadly be divided into those that measure properties of pathogens directly; and those that measure host response to infection.

Tests that measure the properties of pathogens fall into three different categories:

1. Culture based phenotypic tests such as those that measure bacterial growth
2. Molecular phenotypic tests for example to detect proteins produced by pathogens
3. Molecular genotypic tests that measure genetic properties such as resistance mutations

Test types and examples of different technologies are described in Table 10.

In many instances a range of different testing types and methods are available and it has been recognised by NHS England that different settings might need different technical solutions[149]. The choice of test may depend not only on test parameters such as speed and sensitivity but also on the resources and equipment available in a particular clinical environment.

Table 10: Types of diagnostic testing methods

Pathogen based phenotypic analysis

Growth based test

Description	Clinical Uses
Methods that can quantify growth of bacteria faster than conventional culture based methods	▪ Antibiotic susceptibility and resistance testing ▪ Identifying type of pathogen
Advantages	**Disadvantages**
▪ Can be relatively inexpensive ▪ Resistance/susceptibility can be definitely measured and quantified ▪ Faster than conventional culture methods, sometimes by several days	▪ Current methods still rely on initial culture – too slow for POCT and some cultures fail ▪ May not be sensitive enough to detect emerging types of resistance e.g. bacteria that tolerate antibiotics but do not grow ▪ Current methods may require high initial investment in equipment

Pathogen based phenotypic analysis

Molecular test

Description	Clinical Uses
Methods that measure phenotypic biomarkers of pathogens, such as antigens or enzymes. Often make use of antibody based methods (immunochemistry)	▪ Antibiotic susceptibility and resistance testing ▪ Identifying type of pathogen

Advantages	Disadvantages
▪ Capable of fairly high sensitivity ▪ Capable of very high specificity ▪ Can be used directly on patient sample - suitable for rapid point of care use ▪ Some methods allow several samples to be analysed at the same time ▪ Tests can be affordable	▪ Some tests and initial investment required can be expensive ▪ Sensitivity not high enough for some applications ▪ Not capable of identifying resistance apart from in a few specific cases

Pathogen based genotypic analysis

Amplification based test

Description	Clinical Uses
Methods based on amplification of specific short DNA sequences from pathogens in a patient sample, based on the polymerase chain reaction (PCR) method. Common methods are qPCR and LAMP	▪ Antibiotic susceptibility and resistance testing ▪ Identifying type of pathogen ▪ Tracking specific resistance mutations

Advantages	Disadvantages
▪ Capable of very high sensitivity ▪ Capable of very high specificity ▪ Can be quantitative, helping predict level of resistance and ruling out background DNA contamination ▪ Some methods allow several samples to be analysed at the same time ▪ Can be used to track resistance mutations ▪ Can be used directly on patient sample and fairly fast, suitable for point of care in some cases	• For some tests, initial investment required can be expensive • Sensitivity not high enough for some applications • Not capable of identifying resistance apart from in a few specific cases

Pathogen based genotypic analysis

Sequencing based test

Description	Clinical Uses
Methods capable of sequencing whole regions of the genome, ranging from targeted sequencing of specific genes to whole genome sequencing. More rapid long read Nanopore techniques are showing promise for use in the clinic	▪ Antibiotic susceptibility and resistance testing ▪ Identifying type of pathogen ▪ Tracking specific resistance mutations

Advantages	Disadvantages
▪ Do not have to rely on preselected panel of mutations, highly useful for some bacteria which are associated with hundreds of resistance mutations ▪ Can be used to identify new resistance mutations arising and track their spread	▪ Current techniques too costly and time consuming for most clinics ▪ Identification of new mutations with uncertain impact on resistance may lead to uncertainty over clinical decision making ▪ Can be difficult to distinguish between pathogenic and normal commensal bacteria leading to false positives

Human response analysis

Biomarkers in response to pathogens test

Description	Clinical Uses
Methods that measure human biomarkers of infections, such as specific inflammatory proteins and hormones. Markers such as C-reactive protein, lactate and procalcitonin are all in clinical use	▪ Identification of bacterial infection

Advantages	Disadvantages
▪ Can be very rapid, suitable for POCT ▪ Some methods very cheap	▪ Tend to have low specificity ▪ Cannot be used to identify pathogen strains or presence or resistance

rapid near patient tests for certain infections are already widely used across the NHS

What is the current state of implementation for near-patient tests?

Rapid near patient testing for certain infections is already widely used across the NHS. For example POCT for HIV diagnosis is available for use in both community and home settings[150], and rapid 'dipstick' tests to detect human biomarkers of bacterial infection directly from urine are frequently used in primary care settings to help diagnose urinary tract infections[151]. Other POC tests based on detection of the human based biomarker C-reactive protein (CRP) are commonly used to help diagnose the presence of bacterial infections in a range of different clinical scenarios[152, 153].

However for many infectious diseases where near-patient diagnostic tests are already in use there is still an urgent need for development and implementation of better tests. Most current tests are based on non-specific biomarkers and are unable to accurately diagnose infection and/or have low sensitivity, limiting their usefulness. In addition there is a lack of tests that can identify specific pathogen species and even fewer that can rapidly detect drug resistance. There are many infectious diseases which would benefit from rapid diagnosis where no tests are currently available or in widespread use. In these cases there is often a need to develop and implement new testing technologies, with repurposing of existing tests used for other applications also a possibility in some cases.

There are several commercial CE marked tests available designed to help address these needs, with various levels of evidence supporting their clinical utility. In addition promising new testing methods to meet unmet need are in research and development phases. Over the last few years some Trusts have implemented new tests in certain clinical scenarios, through trials, pilot studies and in some cases for routine use. Implementation has not taken place as part of a coordinated national approach, and a wide variety of methods and testing strategies have been used, even when different NHS hospital trusts have used diagnostic testing for the same application.

There are some practical reasons for this range of approaches to the use of such diagnostics. Varying demand can produce different levels of need for rapid diagnostic testing across the country. For example, healthcare providers serving regions containing population groups at high risk of acquiring a particular infection may have more need of a near-patient test to help manage added pressure on the health service. The use of different types of test for the same application can arise due to different resources and facilities available to different care providers. Different near-patient tests and test applications have various levels of evidence supporting their use from clinical studies, and there is often a lack of data available on clinical effectiveness and utility from randomised controlled trials. Therefore, the evidence available for different near-patient tests and test applications should be considered carefully before choosing to run an evaluation study for local implementation.

Applications for which near-patient tests are ready for or approaching implementation

There are several applications where use of new rapid diagnostics has shown benefit in clinical trials and/or through pilots by some NHS trusts, and are expected to have a positive impact on clinical care.

Some areas of particular promise are:

- Diagnosis of influenza in primary and secondary healthcare
- Diagnosis of sexually transmitted infections (STIs) in primary healthcare
- Diagnosis of gastrointestinal infection in secondary healthcare
- Diagnosis of pneumonia in secondary healthcare
- Diagnosis and management of patients with sepsis

The most robust evidence available for the benefits of near patient diagnostic testing is for influenza detection in secondary health care settings. Although some promising tests are being trialled for the other applications, there is currently limited evidence of their clinical efficacy and utility which is required to recommend routine adoption outside of clinical studies. Some local NHS trusts have decided to launch pilot studies to evaluate test use in local care settings.

Point of care testing to diagnose influenza

There is robust evidence from randomised trials and pilot studies to support implementation of POCT to diagnose influenza in secondary care settings. These approaches show promise for use in other healthcare settings but there is limited evidence available to support implementation.

The clinical challenge is that it can be hard to distinguish symptoms of influenza and other respiratory viruses from those of bacterial infections, which can lead to patients being prescribed unnecessary

it can be hard to distinguish symptoms of influenza and other respiratory viruses from those of bacterial infections

antibiotics. This can contribute to the development of AMR, whilst patients who do have a virus may not receive the most appropriate treatment. Respiratory viruses are a particular problem in secondary care settings in winter, when hospitals have to cope with a large influx of cases and clinicians have to make treatment decisions rapidly to manage potentially severe illness and prevent outbreaks. Rapid tests are needed to better aid identification of respiratory viruses and allow decisions to be made in a timely manner. These tests could also be useful in community settings such as care homes and primary healthcare settings such as GP practices, to prevent patients having to make unnecessary visits to hospitals.

There are two main types of technology available for influenza testing, PCR based nucleic acid tests to detect viral genes (for both single Influenza A and B viruses or for multiple viral targets) and viral antigen detection tests. Both types of tests are capable of very high (95-99%) specificity, but antigen based tests have lower sensitivity than nucleic acid based tests. Therefore DNA based tests have been the focus of most clinical trials and are likely to replace the use of antigen based tests in future in secondary care settings[54]. For examples of tests see Table 11.

Evidence for use in secondary care settings: There have been several randomised trials carried out to assess rapid near-patient testing for flu using genetic based molecular tests, with evidence that tests:

- Have high positive predictive values[155, 156]
- Lead to more appropriate treatment with antivirals[156]
- Allow better management of patients to reduce the risk of outbreaks[156]
- Reduce use of antibiotics (unless patients have concurrent bacterial infections requiring antibiotics[156])
- Reduce patient stay in hospitals[156]
- Are likely to produce significant cost savings[155, 157, 158]

In addition positive results supporting the use of POCT for influenza in acute care settings are soon expected to be published from the ongoing FluPOC trial, a pragmatic multicentre randomised controlled trial evaluating a POC test strategy for influenza in adults hospitalised with acute respiratory illness[159].

Trials by NHS Trusts

Several NHS trusts have already trialled use of influenza tests either as part of large randomised controlled trials or through smaller observational trials and implementation studies. Public Health

Table 11: Examples of near-patient tests for diagnosis of influenza

Test	Type	Time	CE marked	FDA approved	Available in UK
Pathogen based genetic tests					
ID NOW A & B2 assay (Abbot)	PCR based test for detection of Influenza A&B	13 minutes	✓	✓	✓
BIOFIRE FILMARRAY respiratory panel 2 plus (Biomeriux)	Multiplex PCR test for 18 viruses and 4 bacteria causing respiratory infections.	45 minutes	✓	✓	✓
GeneXpert flu test (Cepheid)	PCR based test for detection of Influenza A&B	20-30 minutes	✓	✓	✓
cobas® Influenza A/B & RSV Assay (Roche)	PCR based test for detection of Influenza A&B, can also detect respiratory syncytial virus (RSV)	20 minutes	✓	✓	✓
Silaris Influenza A&B test (Sekisui Diagnostics)	Portable PCR based test for detection of Influenza A&B	30 minutes	X	✓	X
Pathogen based molecular phenotypic tests					
Fugi DRI-Chem immune AG FluAB (Fujifilm)	Antigen based test (Immunochromatography) for detection of Influenza A&B	15 minutes	✓	✓	✓

England have compiled a document containing details of these ‘pioneers’ in flu testing, and the results they have achieved[160]. For example, in Sheffield Teaching Hospitals NHS Trust Department of Infectious Diseases, influenza POCT is now standard of care following five years’ use as part of a prospective multicentre study[160]. Buckinghamshire Healthcare are in the process of commissioning a POC test produced by Fujifilm after a winter evaluation in the Acute Medical Unit at Stoke Mandeville Hospital, which was facilitated by Oxford AHSN. Use of the test produced cost savings of around £200 per patient tested, in line with savings seen by other studies, relieved pressures on healthcare resources and improved patient management[161]. In this case the test did not have an impact on antibiotic prescribing, but this was likely because it was used in critically ill patients who required antibiotics for other concurrent infections. Notably this test is not DNA based but uses a new method of antigen detection, suggesting that in some scenarios antigen based tests, though less sensitive, may still be useful. Oxford AHSN

also helped implement a genetic based POC test for influenza in the emergency department of the Royal Berkshire in Reading, with similar benefits[162].

Evidence for use in primary care settings: There is currently no evidence from randomised controlled trials of the clinical benefit of using influenza tests in primary or community healthcare settings. However the European multicountry, randomised controlled ALIC^{4}E trial is currently underway to determine whether antivirals should be routinely prescribed in primary care, which will provide important information on clinical utility[163]. This trial is sponsored by the University of Oxford and although its primary aim is not to evaluate POCT, as part of the study a PCR based POC test for influenza diagnosis will be used[164]. WHO guidance published in 2017 states that POCT in long-term care facilities can be a useful aid in managing outbreaks, but has to be confirmed with laboratory testing due to poor sensitivity [165]. Oxford AHSN is in the process of launching pilots to introduce influenza testing into out of hour GP services and to community settings such as care homes.

Evidence Gaps: There are still uncertainties over the type of test method to use as well as the best way to carry out near-patient testing for influenza. For the latter there are different possibilities, such as clinicians carrying out tests themselves or having a dedicated team of technicians within the hospital who can perform testing[157]. There is also a lack of evidence of the use of influenza testing in primary and community healthcare settings, with uncertainties including the effectiveness of tests in such settings, who would pay for and carry out testing.

the AHSNs could identify the most successful examples of implementation and help other hospitals trial and implement their own systems

The AHSNs could identify the most successful examples of implementation and help communicate these to other hospitals to help them trial and implement their own systems. By working with other organisations involved in generating evidence for new diagnostics, such as the NIHR Medtech and In Vitro diagnostics cooperatives (MICs), the AHSNs could also identify and facilitate the research needed to fill current evidence gaps, especially surrounding use of influenza testing in primary care.

Recommendation:
The AHSNs could help support further implementation and broader use of influenza point of care tests, through supporting dissemination and implementation of tests with sufficient evidence, and helping generate new evidence where needed.

Near-patient tests to help diagnose and monitor sepsis in critical care

Evidence is growing from clinical trials to support implementation of some tests for sepsis management in the near future. Sepsis (or deterioration) is a condition which occurs when the body's immune response to infection goes out of control, and if not treated fast enough can be fatal. NHS England is actively working to improve the identification, diagnosis and management of sepsis through the creation of the Cross-System Sepsis Programme Board; and the roll out of the NEWS2 scoring system is helping to identify and track sepsis[166].

These are important developments, but sepsis remains a non-specific condition making it hard to diagnose accurately. This, combined with the pressure that has been placed on hospitals to start antibiotics within one hour of presumed bacterial sepsis diagnosis, has led to the doubling of antibiotic use in England hospitals since 2015 when the one hour rule was introduced. Many patients treated with antibiotics would not have developed sepsis[167].

since the introduction of the one hour rule in 2015, the use of antibiotics in English hospitals has doubled

A single rapid diagnostic test for sepsis that allows decisions to be made within the recommended one hour time frame does not currently exist, and due to the non-specific nature of sepsis may not be possible[168].

However a combination of rapid diagnostic tools used alongside clinical judgement could:

- Help better identify those at highest risk of sepsis and start treatment
- Identify those at lower risk who do not need immediate antibiotics
- Monitor all patients to see if those at higher risk respond and to see if those at lower risk deteriorate and treatment should be started

faster more accurate diagnosis of sepsis would improve patient outcomes and help reduce the overuse of broad spectrum antibiotics

This would help improve patient outcomes by faster, more accurate diagnosis of sepsis, and help reduce overuse of broad spectrum antibiotics as the default option for those with lower risk of sepsis. There is also a need to more rapidly identify the bacteria that are causing the infection and the presence of any resistance, to allow the most appropriate antibiotics to be prescribed.

Near-patient tests fall into two categories:

1. Tests for biomarkers that allow rapid diagnosis of sepsis to support patient triage and monitoring
2. Tests that identify pathogens including those associated with resistance, to allow correct antibiotic or other treatment to be prescribed.

Tests in category 2 tend to take longer to perform (days), so only the more rapid initial diagnostic tests will be described here.

Rapid diagnostic tests for initial diagnosis are at a range of developmental stages. Current CE marked tests rely on detection of non-specific biomarkers of infection such as levels of CRP, lactate, and more recently the hormone procalcitonin (PCT). These can be used alongside clinical judgement to aid sepsis diagnosis and monitor for disease progression. Future tests are being developed that aim to more specifically diagnose sepsis by measuring more specific biomarkers of human response, such as changes in human gene expression. See Table 12 for examples of test technologies.

Evidence: Evidence accumulated over several years indicates that PCT can be more useful in aiding clinical judgement decisions than nonspecific markers of bacterial infection such as CRP and lactate. PCT testing can be useful both for predicting sepsis severity, and monitoring patients for signs of recovery or deterioration and stopping or starting antibiotics accordingly[169]. Use of PCT tests for monitoring has shown particular promise in improving patient outcome and reducing antibiotic use in several large international clinical trials[169, 170]. However most trials have not been based on newer rapid near-patient PCT testing, and more evidence is needed to evaluate the clinical utility of these PCT tests.

New multimarker POC tests are currently in development. A test by UK based Mologic is being evaluated as part of a University College London Hospitals study which aims to more specifically detect signs of infection and early signs of sepsis within 10 minutes using a panel of six biomarkers[171]. They are planning to acquire CE marking and launch the test in Europe in 2020.

Table 12: Examples of near-patient tests for rapid diagnosis of sepsis

Test	Type	Time	CE marked	FDA approved	Available in UK
Already on the market					
VIDAS BRAHMS PCT assay (bioMerieux UK)	Fluorescent Immunoassay for procalcitonin in human serum and plasma	20 minutes	✓	✓	✓
ADVIA Centaur BRAHMS PCT assay (Siemens Healthcare Diagnostics)	Chemilluminescent Immunoassay for procalcitonin in human serum and plasma	26-29 minutes	✓	✓	✓
AQT90 FLEX procalcitonin (PCT) assay (Radiometer)	Immunoassay	21 minutes	✓	**?**	✓
Still in early research					
Mologic sepsis test	Immunassay based test for 6 host biomarkers. Using algorithm to predict sepsis (in early stage trials)	10 minutes	**X**	**X**	**X**
HostDx sepsis (Inflammatix)	Test based on mRNA expression of immune response markers, using machine learning	45 minutes	**X**	**X**	**X**

In terms of use in the NHS, NICE assessed use of PCT POC tests in 2016, concluding there was not enough evidence for use of PCT for diagnosis and monitoring of sepsis[172]. However work is ongoing to evaluate rapid POC PCT tests, which are currently used for a few specific applications in NHS trusts, both for routine use and as part of studies. A six month study at Winchester and Eastleigh Trust found that 94 courses of antibiotics were avoided using PCT POC tests[173].

Evidence Gaps: There is currently limited evidence on the utility of POC PCT tests, although use of laboratory based PCT testing has been shown to be useful. The AHSNs could support the development of evidence on how such tests could be incorporated into the clinical care pathway, working with other research organisations to help carry out clinical trials when appropriate. In addition, new and better tests for rapid detection of sepsis are expected to become available in the next few years. Monitoring developments in this area will enable AHSNs to be ready to help generate implementation evidence for these tests in the NHS.

Recommendation:
In order to support the timely and effective implementation of point of care tests, test developers should work with the health system to understand evidence requirements early in the development process. This will require not only understanding the test performance evidence required, but also consideration of the health economic impact and changes to service models.

Promising applications and technologies on the horizon

Technologies for better diagnosis of Urinary Tract Infections

UTIs are often diagnosed in primary care, which means there is a particular need for rapid tests that can be performed within the time frame of a GP appointment

Urinary tract infections (UTIs) are very common, with approximately 50% of females experiencing a UTI in their lifetime, and 10% of females suffering from one in any given year[174]. Almost half of all gram-negative bloodstream infections originate from UTIs, and the Department of Health and Social Care has pledged to halve Gram-negative bloodstream infections by 2021. These were thought to have contributed to 5,500 deaths in the UK in 2015[175]. Rapid diagnostic tests already exist in primary care, typically in the form of dipstick tests, which detect human biomarkers that indicate a bacterial infection, typically white blood cells in the urine or high levels of nitrite. However, these tests have a very low level of sensitivity and do not indicate the type of bacteria causing the UTI, or if it is resistant to antibiotics. This results in clinicians often prescribing broad spectrum antibiotics based on uncertain test results, whilst awaiting results from samples sent to laboratories for culture based antibiotic susceptibility testing, a process which can take several days. New tests are urgently needed that can be used directly on a urine sample and can better confirm or rule out the presence of a UTI, ideally indicating the type of bacteria present and the presence of resistance in cases where a UTI is confirmed. As UTIs are often diagnosed in primary care settings, there is a particular need for rapid tests that can be performed within the time frame of a GP appointment.

Technologies including biosensors and microfluidics are being developed that can address these problems but they are in a relatively early stage of development. There is also the potential to adapt genetic analysis based molecular platforms for other disease applications such as FilmArray (bioMérieux) and GeneXpert (Cepheid) to detect bacterial species and resistance, although there are questions over whether these methods are fast enough for use in primary care settings[176].

Technologies based on human response to infection

Technologies that can more sensitively and specifically quantify biomarkers associated with the human response to infections are in development. A team from Imperial College London recently won a an EU grant worth €22.5m over five years, to develop technologies based on analysis of different RNA patterns that result from specific genes being switched on and off in response to infection. This could be used to more accurately diagnose the presence of a range of infections, including pneumonia, tuberculosis, sepsis, meningitis, and inflammatory and immune diseases, in under two hours. The researchers aim to conduct the first pilot trials in UK and European hospitals in 2023 and 2024[177].

General considerations

Implementing tests on a local versus national scale

There is currently a varied landscape of test use for different applications, which is especially apparent in influenza testing but also in other applications. Some NHS hospital trusts are carrying out evaluation studies, whilst others are already implementing routine use of diagnostic tests. This has created a wide variety of approaches to rapid testing and many different testing methods are in use. Some tests are piloted in care settings with varying levels of evidence for their clinical utility (e.g. POCT in primary care for Strep A infections are not recommended by NICE 178).

There is an opportunity for AHSNs to collaborate across the AHSN network, as well as work with other organisations such as NIHR, to identify which tests have already been piloted across the country. This could help coordinate approaches to generating additional evidence needed for test implementation on a larger scale across the country, and allow details of the most successful methods to be disseminated to help with standardisation of testing methods.

Tackling uncertainty over test choice and parameters

There are an increasing number of POC tests available for different applications, however very few studies have directly compared test performance. This can lead to uncertainty when deciding which test to use, as well as contributing to a lack of standardisation. Even tests with fairly high sensitivity and specificity for pathogen detection may not have sufficient evidence to influence clinical decision making. This is especially the case when identifying the presence of specific microorganisms or the presence of resistance mutations, when the absence of a mutation may be due to the test not detecting its presence. For life threatening conditions such as severe influenza or sepsis, clinicians may be unwilling to use these results and change their prescribing practice. This could limit the clinical effectiveness of these tests and make them less likely to be commissioned and successfully implemented.

Clinical implementation studies of new tests and evaluating these against traditional testing methods would generate data to support decision making around clinical utility of testing, and evidence of cost-effectiveness.

Distinguishing between healthy bacterial flora and pathogenic bacteria

An ongoing challenge with most technologies is being able to distinguish between healthy bacterial flora and pathogenic bacteria, especially as the same strains of bacteria that are pathogenic in some situations can be commensal in others. This can lead to false positive results for identification of infections. There should be continued identification and support of innovations that can solve this problem, from investing in research through to commercialisation.

Tests have to be suitable for real-world use

For rapid near-patient test applications in particular, tests often have to meet stringent criteria to make them suitable for use in the real world. For example they may have to be performed and provide results within minutes, not require specialist expertise, be affordable and have high positive predictive value.

By working with research organisations to identify the most promising new technologies, the AHSNs can ensure tests are designed in a way that meet the requirements of NHS clinical practice.

Change in clinical prescribing

Antibiotics can have side effects, but the risk of complications from infections may mean clinicians still prescribe them to prevent infections worsening, as the lowest risk option. In addition, some patients may have an expectation of receiving antibiotics, so clinicians may prescribe them to avoid losing the trust and engagement of patients. With the rise of AMR there are efforts ongoing to address these factors, but this requires robust evidence to support the use of new tools that could inform clinical decisions on antibiotic prescribing.

Engagement of both clinicians and patients is required to allow them to understand the importance of appropriate antibiotic prescribing. High quality evidence is needed to inform clinicians of the potential benefits of new diagnostic tests.

Wider issues surrounding uptake of new POC tests

As with all new diagnostic implementation, care providers can face significant upfront costs in purchasing new equipment and training. Some testing methods and applications mentioned in this chapter require expensive technologies that many care settings may not have access to currently. With near-patient tests in particular there may be challenges for existing laboratory practice. It is important to ensure all stakeholders are engaged and involved with deciding upon the implementation of a new technology, to make the most of everyone's expertise and increase the likelihood of success.

> robust evidence is required to support the use of new tools that could inform clinical decisions on antibiotic prescribing

Conclusions

There are a number of key areas in which rapid and near patient diagnostic testing has the potential to improve management of AMR, whilst providing better treatment for patients and allowing hospitals to more efficiently use their resources and save money on ineffective treatments. A range of commercial tests are already available to help improve upon current methods of distinguishing between bacterial and viral infections, identifying the species of pathogen and determining resistance and sensitivity to antimicrobials. In particular, new rapid tests for influenza have substantial evidence supporting their clinical utility, and are already in use by some NHS hospital trusts. Other test applications are showing promise in clinical studies and pilots.

Once a commercial test is available, tests are often implemented on a case by case basis to help meet a particular local need, through small implementation studies. These studies can be useful in generating real-world evidence for test use and in providing benefits for local trusts. However the evidence generated from these studies is often not suitable for informing wider implementation on a national level. For broader implementation, evidence of clinical utility is often required from randomised, controlled and multicentre trials.

The AHSNs could play a key role in helping facilitate real-world studies to meet local NHS needs for applications where there is sufficient evidence to begin implementation. In addition, by working with other organisations such as the NIHR, AHSNs could help generate additional evidence needed to inform use of tests on a broader national scale. As there is a constant requirement for new and better diagnostic tests for infectious diseases, the AHSNs could also consider identifying promising technologies at an earlier stage. This could enable them to assist with designing tests that are suitable for their intended application, for example by supporting test developers through the evidence gathering process, increasing the chances of successful implementation into the healthcare system.

5. Genetically modified regenerative medicines

Genetically modified regenerative medicines are a technically complex subset of regenerative medicines. There are a number of GMRMs that have been approved for use in the NHS, including innovative CAR-T therapies for blood cancers, and a gene therapy for a rare immuno-deficiency disorder, ADA-SCID

Regenerative medicines (RM) are treatments that seek to replace, repair or regenerate the body's cells, tissues and organs. RM can be split into several different categories, and there is little consensus on how they are split. Common categories include: gene therapies (including genome editing), cell therapies and tissue-engineered products.

In several cases, multiple complex techniques have been used to develop a RM therapy, for example cells used in RM therapies may have undergone genetic modification, meaning it is often difficult to draw a true distinction between the different therapy types.

In this chapter, RM therapies that include a genetic modification component are examined – genetically modified regenerative medicines (GMRM) – thereby focusing on gene therapies (including genome editing) and genetically modified cell therapies, which are key areas of RM.

Table 13 clarifies the terms we use to describe different aspects of RM. The terms gene and genome are often used interchangeably in the context of gene/genome therapy and editing; in recent years, the term 'genome editing' has been adopted to describe DNA editing technologies, alongside 'gene therapy' to describe the broader umbrella of technologies that alter the DNA content of cells. In this report we use the terms 'gene therapy' and 'genome editing'.

What are GMRMs?

Regenerative medicines are a collection of highly complex, targeted treatments which involve the use, manipulation or regeneration of cells or tissue as part of the therapy. To produce a GMRM, often the patient's own cells or tissues are extracted, modified and reintroduced to treat the condition in question (autologous treatment) or donor cells may be collected and specifically customised for the patient, to achieve clinical improvement with reduced risk of tissue rejection (allogeneic treatment).

Several GMRM therapies use one or more innovative approaches – combining, for example, cell population enrichment and gene therapy. Genetic modification may either be the therapy itself e.g. *in-vivo* genome editing, or help to create the therapy e.g. *ex-vivo* genetically modified immune therapies.

The modification of genetic material either to directly treat or to enhance treatment means that these therapies are often personal, designed for the individual being treated or for a group of individuals with specific genetic variants. The inherent complexity

Table 13: Definitions of common regenerative medicine terms

Term	Description	Examples
Gene therapy	The insertion of genetic information into one or more cells, often by viral vector. Also used as an umbrella term which includes genome editing.	▪ Luxturna for Leber Congenital Amaurosis ▪ Strimvelis for ADA-SCID (gene therapy applied to multipotent cells) ▪ Used to modify CAR-T-cells in Kymriah and Yescarta for cancer treatment
Genome editing	Alteration of an individual's genome in one or more cells through the delivery of genome editing tools e.g. CRISPR. Can include deletion, alteration or insertion of genetic material.	▪ CTX001 CRISPR-based treatment in trials for β-thalassemia and sickle cell disease ▪ Small trial of *in vivo* gene editing for treatment of Hunter Syndrome in the US
In vivo or *ex vivo*	Refers to whether the procedure or treatment (primarily genetic modification) is conducted inside or outside of the patient's body. *In vivo* = inside the body *Ex vivo* = outside the body	▪ *In vivo*: Luxturna gene therapy which involves injection of viral vector into the retina ▪ *Ex vivo*: During CAR-T therapy for cancer, cells are extracted from the patient, modified to express antigen receptors on their surface, and then re-introduced
Autologous or allogeneic	Refers to whether the cells of a healthy donor or the patient's own cells are used for the treatment Autologous = patient's cells Allogeneic = donor cells	▪ Autologous: Patient's own cells used in cancer treatments Kymriah and Yescarta ▪ Allogeneic: Donor cells used in one-off instances of modified CAR-T treatments used to treat two infants in the UK with leukaemia

and personalisation of these therapies means that they face specific challenges for delivery through the healthcare system, and there are ongoing issues around their classification and regulation.

Cell therapies utilise selectively collected cell populations administered to the patient to treat disease. Several different cell types can be used in therapy; this may include stem cells, which can develop into a number of specialised body cells e.g. blood or muscle cells, and have the ability to produce new stem cells. Cell therapies included in this chapter include an element of genetic modification.

Gene therapies aim to alter the genetic content (the DNA) of cells, whether by adding, deleting or altering DNA, in order to change gene function. The term 'gene therapy' is used as an umbrella term which includes 'genome editing' treatments. Therapies may establish, alter or stop gene function depending upon the gene target and the disease being treated. Therapies may utilise for example: viruses, transposons, one of several genome editing tools, or a combination of these. Viral

gene therapies utilise a viral vector to insert a synthetic DNA either into the patient genome – with the aim of establishing or interrupting gene function – or into the cell in a process called gene addition, which does not alter the cell's genome but instead deposits new DNA into the cell allowing it to be used by the cellular machinery[179].

Genome editing therapies involve the deliberate alteration of a cell's genome through cutting, inserting or otherwise altering DNA using one of several genome editing tools. Genome editing is considered part of the gene therapy family, but carries different safety implications and scope for application to viral and other gene therapies. Genome editing techniques are becoming more commonly used in both research and the development of therapies for genetic disease. Genome editing techniques can provide more effective targeting and precision, a greater range of DNA alterations, and increased longevity of effects. In some circumstances, they are also easier to produce than other gene therapies, however they come with their own technical limitations.

Why are GRMs useful?

GMRM approaches potentially offer treatments for conditions for which no other treatment is available, or may offer significant improvement on current treatment approaches. Areas of interest are rare genetic disease and cancers

There are several advantages of GMRM over conventional treatments, including:

- The development of treatments for genetic conditions for which there is currently no other available treatment – sometimes treating the cause (genetic) rather than symptoms of disease
- The replacement of expensive long-term treatment plans with one-off or infrequent interventions leading to long-term cost saving for health services and relief/respite from onerous regimes for patients. In some cases a permanent cure could be possible
- Autologous cell and gene therapies utilise cells or tissues from the patient, avoiding many of the potentially significant adverse events associated with immune rejection following transplant e.g. graft vs host disease
- Allogeneic treatments may be genetically modified to achieve much the same, and present other benefits such as potential 'off the shelf' treatments which are able to treat a wide range of patients and do not need be individualised for the treatment of each patient

> GMRMs have the potential to provide treatments for conditions for which there are currently none

GMRM therapies also come with their own limitations and challenges; these are outlined later in the chapter.

Trends in the development of cell and gene therapies

Partly due to the rarity of many of the diseases targeted by RM approaches and the substantial investment and expertise required to develop them, GMRM therapy development happens globally, with multi-national clinical trials a common occurrence.

According to figures from the UK Cell and Gene Therapy Catapult, the number of cell and gene therapy clinical trials being conducted in the UK has continued to grow, and has increased from 27 in 2013 to 127 in 2019 (from 2018 to 2019 alone, the increase was around 45%)[180-181].

Of ongoing trials, roughly 74% involve some form of genetic modification (41% *in vivo*, 59% *ex vivo*). The majority of these trials are phase I and II. Autologous therapies are more prevalent than allogeneic therapies, at roughly a 2:1 ratio[180].

the UK is a recognised leader in genetically modified regenerative medicine

There has been a consistent upward trend in trial sponsorship by commercial entities, rising from around 24% in 2013 to around 77% in 2019.

The Alliance for Regenerative Medicines reports that of 1,059 RM clinical trials that were ongoing internationally between July and September 2019, 788 involved some form of genetic modification (gene therapies and gene-modified cell therapies).

The UK is recognised as a leader in research and innovation in these areas.

GMRM applications in cancer

Much of the early focus of RM research was on rare disease, but in recent years cancer applications have gained prominence. Two genetically modified cell therapy treatments for cancer are now available on the NHS. Though significant investment has been made into the production of GMRM over the last decade, so far, only a handful of therapies have been approved for use. It is likely that over the next few years the number of FDA/EMA therapies will expand rapidly.

Genetically-modified immunotherapies

Few therapies are effective in late stage cancers. Genetically modified immunotherapies are particularly exciting because they may be able to treat cancers that have not responded to other treatments or have relapsed following treatment. In theory these therapies can be modified to target a range of cancers.

Chimeric antigen receptor T-cell (CAR-T) therapies have elements of both cell and gene therapies as they involve the extraction and genetic alteration of T-cells from the patient or a donor – to induce expression of specific antigen receptors on the cell surface. The receptors allow the T-cells to better detect and/or attack cancerous cells. CAR-T and other modified immune cell therapies in healthcare are currently limited to the treatment of specific blood cancers. In October 2018, two CAR-T cell therapies were made available on the NHS for specific indications:

- Kymriah, also known as tisagenlecleucel, for acute lymphoblastic leukaemia in children and young adults (up to 25 years old)[182]
- Yescarta, also known as axicabtagene-ciloleucel, for the treatment of diffuse large B-cell lymphoma (DLBCL) in adults[183]

two genetically modified cell therapy treatments for cancer are now available on the NHS

As of March 2019, Kymriah is also available for treating relapsed or refractory DLBCL in adults through the Cancer Drugs Fund. Following additional evidence collection and a review due in 2023, it may be recommended for use on the NHS[184]. These treatments are delivered in a number of centres across the UK (Table 14)[185], with around 200 Kymriah or Yescarta treatments delivered in England in the past year.

The delivery of CAR-T treatments is complex, involving international collaboration with laboratories in both Europe and the US, and though much of the research and clinical expertise is established in the UK, many commercial manufacturing sites are established in continental Europe and the US. The CGTC manufacturing site in Stevenage is now producing some of these products.

Clinical trials are also underway (recruiting) to evaluate the use of Kymriah for the treatment of other cancers. Many other CAR-T and similar therapies are in production and under investigation internationally, UK companies developing such products include: Autolous, a University College London spin-out, who are developing T-cell and T-cell receptor (TCR) therapies for cancer; Vaccitech, a University of Oxford spin out, developing not only vaccines and treatments for cancer, but also for infectious diseases such as hepatitis B[186]; Achilles Therapeutics, another UK company, is using sequencing data collected from the TRACERx project to generate

Table 14: NHS centres delivering CAR-T therapies in the UK

Centre	CAR-T availability (*planned)	
	Kymriah[+]	Yescarta
Cambridge University Hospitals*	✓	✓
Great Northern Children's Hospital (Newcastle)	✓	
Great Ormond Street Hospital	✓	
King' College Hospital	✓	✓
Leeds Teaching Hospitals NHS Trust*	✓	✓
Manchester Royal Infirmary	✓	✓
Newcastle Hospitals NHS Foundation Trust		✓
Queen Elizabeth Hospital (Birmingham)	✓	✓
Royal Manchester Children's Hospital	✓	
Royal Marsden NHS Foundation Trust*	✓	✓
The Christie NHS Foundation Trust*	✓	✓
University College London Hospital	✓	✓
University Hospitals Bristol NHS Trust	✓	✓

[+] Some centres may provide adult or paediatric only services

highly tailored personalised cancer therapeutics[187]. All four have products in early-phase clinical trials.

patients in trials have responsed at a higher rate than to some other therapies

Imlygic, also known as talimogene laherparepvec and developed by US-based company Amgen, is a virus-based immunotherapy used in the treatment of inoperable melanoma. Unlike CAR-T therapeutics, genetic modification is performed on the subsequently injected herpes simplex virus, not on host cells. Trials of this treatment have shown that patients respond at a higher rate than to some other therapies and it has received approval in the EU (where it is categorised as a 'gene therapy product') and subsequent recommendation by NICE in 2016[188].

In order to encourage safe use, a controlled distribution programme has been implemented to ensure appropriate education of clinical professionals and that the storage and distribution requirements of Imlygic are met e.g. specialist requirements of working with viruses, especially alongside immune-compromised patients. Trials are still underway to better understand the risks and benefits of this therapy.

Future uses

There are several other types of modified immune cell therapies alongside CAR-Ts that are under investigation. These include: T-cell receptor (TCR) therapies, tumour infiltrating lymphocytes, and other modified cell types e.g. natural killer cell therapies, marrow derived lymphocytes, and gammadelta T-cells[189].

TCR therapies are similar to CAR-Ts in that T-cells are extracted from the body and genetically modified to express a receptor. These therapies target proteins normally located on the inside of the cancer cell, rather than explicitly expressed on the cell surface. Companies developing TCR therapies (with therapies in trials) include Adaptimmune, Kite Pharma, and Medigene, with a range of cancer targets [190].

The majority of modified immune therapies for the treatment of cancers are autologous, however an ongoing goal of research is to generate allogeneic therapies that can be developed from healthy donors and modified to be immune-compatible with the patient. This provides several advantages, including:

- Providing treatment faster by having therapies ready ahead of time as an 'off-the-shelf' product allows a stock of treatments to be held
- Cells can be collected from healthy individuals rather than patients who may already be significantly weakened by disease

It has been proposed that genome editing will be key to the wider development of such allogeneic treatments. Examples of such genome edited treatments can now be seen in trials: a clinically-led implementation in collaboration with multiple UK-based institutions and commercial providers (details in Qasim *et al*. 2017[191]) was used successfully to achieve molecular remission in two patients with advanced leukaemia in the UK[191-193]. Allogene Therapeutics (US-based), CRISPR Therapeutics (Swiss/US based), and others are developing allogeneic treatments. One potential therapy for acute B-cell lymphoblastic leukaemia (B-ALL), called UCART19, is undergoing clinical trials in multiple locations internationally, including at King's College London and The Christie Hospital in Manchester[194,195].

genome editing will be key to the wider development of allogenic treatments, and examples of such treatments can now be seen in trials

Companies such as US-based Tmunity are working on several modified immune cell therapies for targeting solid tumours e.g. prostate cancer and melanoma, some of which are in early stage clinical trials (trial identifiers: NCT04227275, NCT04025216). Examples of modified immune cell therapies employing genome editing techniques are provided in Table 15.

Table 15: Examples of CAR-T and other cancer immune therapies using genome editing

Company	Treatment	Condition	Trials*
CRISPR Therapeutics	CTX120	Multiple myeloma	Phase I/II NCT04244656
	CTX110	Non-Hodgkin lymphoma	Phase I/II NCT04035434
Tmunity Therapeutics (and collaborators)	NYCE T Cells	Multiple myeloma	Phase I NCT03399448
Allogene Therapeutics	Allo-715	Multiple myeloma	Phase I NCT04093596
	UCART19	Acute lymphoblastic leukaemia	Phase I NCT02746952
	Allo-501	Non-Hodgkin lymphoma	Phase I NCT02735083
Precision Bioscience	PBCAR0191	Non-Hodgkin lymphoma and acute lymphoblastic leukaemia	Phase I NCT03666000
Cellectis	UCART123	Acute myeloid leukaemia	Phase I NCT03190278
	UCART22	Acute lymphoblastic leukaemia	Phase I NCT04150497

* One trial is listed for each potential therapy. In some cases, more trials are simultaneously underway. Trial information is available on clinicaltrials.gov

As the types of modification performed are highly flexible, modified immune cells could also be used to treat diseases other than cancer. A trial investigating the use of genome edited CAR T-cells for the treatment of HIV (based in the US) is currently recruiting (NCT03617198), and others are underway.

Genetically modified immune therapies for solid tumours

T4 Immunotherapy for Head and Neck Cancer

A phase I clinical trial taking place in the UK is investigating the use of modified immune cells for the treatment of locally advanced or relapsed squamous cell cancer of the head and neck (SCCHN). GM immune cell therapies are currently only available for specific types of blood cancer – a long term goal for immune therapies is to build towards the treatment of solid tumours.

The treatment, in development by King's College London and collaborators Guy's and St Thomas' NHS Foundation Trust, is autologous and involves the collection, alteration and re-administration of a patient's T-cells. SCCHN is a solid tumour for which the treatment is injected into the tumour directly.

T-cells are modified to express proteins which selectively attack tumour cells. Current treatments for SCCHN include chemotherapy, radiotherapy and/or additional cancer drugs, with five year survival at 50%; the primary cause of mortality is locally advanced disease rather than metastasis [196].

The immunotherapy aims to improve management of locally advanced (non-metastatic) disease and improve five year survival. It is expected that up to 30 patients will be recruited to the study. The trial is primarily to assess safety and is expected to complete in April 2020[197].

GMRM in other diseases

Current NHS use in rare disease

There are few gene therapies available on the NHS to treat rare diseases. Strimvelis is an autologous cell therapy that utilises genetically modified immune cells for the treatment of the rare and life-threatening condition adenosine deaminase severe combined immunodeficiency (ADA-SCID). Strimvelis is an *ex vivo* viral-based gene addition therapy that targets immune cells. NICE notes in its evidence review of Strimvelis that all patients receiving Strimvelis have survived (18 patients over 15 years), 83% of whom did not require further intervention, whereas overall survival following conventional treatment (haematopoietic stem cell transplantation) was between 67-71% of patients [198].

In 2019, NICE also approved Luxturna (voretigene neparvovec) an *in vivo* gene therapy for people with vision loss and inherited retinal dystrophy caused by specific *RPE65* gene variants. Conventional treatments are supportive only. The list price per patient is £613,410, however a managed access agreement has meant it is now available to NHS patients[199]. As is the case with many RM therapies, small patient numbers are a notable limitation on evidence collection for both therapies.

Case study: Oligonucleotide therapies for spinal muscular atrophy

In the past few years, notable developments have occurred in treatments of spinal muscular atrophy (SMA). SMA types 1 and 2 are rare and life-limiting genetic diseases which result in muscle weakness and wasting from a young age. It is caused by an absence of fully functional survival motor neuron protein, caused by mutations in the survival motor neuron 1 (*SMN1*) gene. Type 1 SMA is a genetic cause of infant mortality, usually before age five, and symptom management is the only therapeutic option available for all types of SMA.

Therapeutic approaches aim to restore functional SMN1 protein. Zolgensma, a gene therapy for SMA is discussed below under 'Gene therapy in rare disease'. Another therapy for SMA, Spinraza (Nusinursen), is an antisense oligonucleotide therapy (ASO), a type of oligonucleotide therapy (OT). OTs are not gene therapies in the strictest sense, as they target RNA rather than DNA, but are a genomic medicine approach that could be applied to the treatment of a range of genetic diseases. Spinraza was recommended for use in England by NICE in July 2019 through a managed access agreement[200]. It has been available for the treatment of SMA type 1 patients in Scotland since May 2018, and this is expected to be extended to type 2 and type 3 patients in the near future. Unlike many gene therapies, the effects of this therapy are not intended to be permanent, and repeated applications will be necessary.

How do oligonucleotide therapies work?

Oligonucleotide therapies (OTs) work by interrupting the formation of disease-causing proteins by interacting with RNA. When the DNA sequence of a gene is read, it is transcribed into an RNA molecule which is then translated into the protein. OTs function by targeting the RNA mediator between DNA and proteins and causing it to be broken down or by altering it in some way.

There are three major forms of oligonucleotide therapies currently under development, these are known as:

1. RNA-mediated interference through small interfering RNAs (siRNAs)
2. Antisense oligonucleotides (ASOs)
3. RNA blocking agents

These therapies can be highly specific, having a defined molecular target.

ASOs have been under investigation as disease therapeutics since the 1970s [201], and siRNAs and RNA blocking agents have emerged since then. In 1998, the FDA approved its first ASO therapy, formiversen, for the treatment of cytomegalovirus retinitis in immunocompromised patients (the therapy has since been withdrawn). Since then, there have been steady advances in OT development, with recent flurry of approvals refocussing attention on their therapeutic potential.

In 2019, NICE recommended two OTs for the treatment of rare disorders in the NHS. Patisiran is recommended for the treatment of hereditary (familial) transthyretin amyloidosis, a debilitating late onset genetic disorder that affects multiple organs and the nervous system and is life-limiting, for which no cure is available[202]. Spinraza for SMA was recommended by NICE in July 2019[200].

Most recently, the NHS has agreed to trial the siRNA Inclisiran developed by Novartis for the preventative silencing of a gene associated with the build-up of 'bad' cholesterol[203]. This twice-yearly injection is the first preventative genetic therapy available in the UK, and is hoped to reduce the incidence of cardiac events including heart attack and stroke. It may reduce healthcare's demand for statins. Inclisiran has not yet received approval from either the FDA or EMA.

Unlike genome editing therapies, OTs do not permanently disrupt the production of proteins from DNA and repeat dosing is required. OTs aren't necessarily difficult to synthesise, however there have been challenges surrounding molecule longevity as they can be quickly degraded *in vivo*. As with other genomic therapies, therapy delivery has also been an issue.

Disorders of the eye

NICE has recommended the use of rare disorder treatment Luxturna, developed by Novartis, for the treatment of Leber congenital amaurosis (LCA) *RPE65*-mediated vision loss. It is expected that 86 patients will be eligible for treatment in England[199]. The eye is a suitable target for many novel gene therapy approaches since it is an immunologically-isolated organ that is relatively accessible for the delivery of gene therapies, and the genetic cause of some eye diseases are well understood. In the UK, researchers based at Moorfields Eye Hospital and University College London Institute of Ophthalmology were amongst the first in the world to deliver Luxturna [204].

Other uses of gene therapy in rare disease

Research and clinical trials for gene therapies are ongoing in several promising clinical areas, including further cancer treatment, blood disorders and rare metabolic diseases.

A gene therapy for SMA, Zolgensma, was approved by the FDA in May 2019. Zolgensma is developed by AveXis (a Novartis company). An ongoing trial investigating the effectiveness and safety of Zolgensma has so far shown that treated infants have a substantially improved ability to reach physical milestones such as sitting without support; another showed patient survival in those treated to be improved well beyond expected for the condition and independence from mechanical ventilation to be common[205]. A number of trials are underway or planned to further assess patient outcomes and the effectiveness of Zolgensma. The SPR1NT study[206] aims to assess the safety and efficacy of the treatment; subsequent follow-up studies will examine long-term effectiveness. The therapy is under review by regulators in Europe.

Blood disorders

Gene therapy approaches are being explored for the treatment of several genetic blood disorders such as β-thalassemia and sickle cell disease. Many of these are at an advanced stage, and undergoing late phase trials or regulatory assessment (Table 16).

an ongoing trial of Zolgensma has demonstrated improved patient survival beyond expected for SMA and independence from mechanical ventilation to be common

Conditions such as haemophilia are controlled using frequent, expensive and invasive treatments that do not provide and long-term solution. Gene and cell therapies could offer patients the chance to avoid such treatments and deliver potentially longer term solutions which also help to avoid tissue damage as a result of the disease. Bluebird Bio's *ex vivo* gene therapy for β-thalassemia, 'Zynteglo', gained conditional market approval from the EMA in 2019[207]. It is designed for use in patients with all but the most serious forms of the condition who are over 12 years old and with no other treatment options. Following treatment, 15 out of 19 patients treated became transfusion-independent, although follow up has so far been relatively short. Zynteglo is currently undergoing single technology appraisal by NICE [208]. Although a one-off treatment course, this therapy, like many RM therapies, carries a substantial price tag - estimated at around €1.6 million per patient [207]. Orchard Therapeutics is also developing a gene therapy for β-thalassemia which aims to treat all forms of the disease and is now in long-term follow-up, having posted positive results in 2019 showing reduction in transfusion dependency (trial indentifier: NCT03275051).

Disorders of the eye

Several genetic conditions relating to eye function are being investigated as potential targets for GMRM.

In addition to the already approved Luxturna, a number of other gene therapies are in development for the treatment of eye disorders, including: Rentinostat, a therapy developed by Oxford Biomedica, has reached clinical trials and is undergoing long-term follow-up for the treatment of wet age-related macular degeneration (AMD) (trial identifier: NCT01678872), and RGX-314 - also for wet AMD - which is expected by its developer to move into phase II clinical

Table 16. Examples of gene therapy and genome editing approaches in development for the treatment of blood disorders. Primary source: American Society of Gene and Cell Therapy

Company	Treatment	Condition	Trials
Sangamo and Sanofi Genzyme	ST-400	Beta thalassemia	Phase I/II
	BIVV003	Sickle cell disease	Phase I/II
Aruvant Sciences	RVT-1801	Sickle cell disease and Beta thalassemia	Phase I/II
Bluebird Bio	LentiGlobin	Sickle cell disease and Beta thalassemia	Phase III and EMA designated OMP
Orchard therapeutics	OTL-300	Beta thalassemia	Phase I/II
Genethon (non-profit, part of EuroFancoLen)	-	Fanconi anaemia	Phase III
Rocket Pharmaceuticals	RP-L102	Fanconi anaemia	Phase I/II
CRISPR therapeutics and Vertex pharmaceuticals	CTX001	Sickle cell disease and Beta thalassemia	Phase I/II
Uniqure	AMT-061	Haemophilia	Phase III
Sangamo therapeutics	SB-525	Haemophilia A	Phase I/II
	SB-FIX	Haemophilia B	Phase I/II
BioMarin	BMN 270	Haemophilia A	Phase III

trials in 2020[209]. These therapies aim to address unmet needs in genetic degenerative eye disorders for which current treatments are either unavailable, or only aim to slow down decline or ameliorate discomfort.

Neurodegenerative and metabolic disease

There are gene therapy trials underway for several neurodegenerative or metabolic disorders, including:

- Spinal muscular atrophy – AveXis (US) conducting several trials of its gene therapy Zolgensma
- Huntington's disease – Uniqure Biopharma (Netherlands) are conducting phase I/II clinical trials of AMT-130 a single-dose gene therapy (trial indentifier: NCT04120493)
- Duchenne Muscular Dystrophy – a gene therapy developed by Sarepta Therapeutics (US) has been shown to be safe in mice and provides some pre-cursor indicators of affecting disease symptoms. A human clinical trial is now underway[210]
- Phenylketonuria – phase I/II clinical trials of HMI-102, a gene therapy developed by Homology Medicines Inc., are taking place to determine safety and effectiveness (trial identifier: NCT03952156)
- Fabry disease – ST-920, a gene therapy in development by Sangamo Therapeutics is undergoing clinical trials in the US

Genome editing

Genome editing techniques for the treatment of rare diseases are under investigation and development, in particular for eye and blood disorders.

Genome editing works on the basis of genome modification rather than gene addition. Changes are made to genomic DNA with the aim of inducing permanent changes to the targeted sequence in selected cells or tissues. DNA may be disrupted or additional synthetic DNA inserted at targeted sites. Unlike many other gene therapies, genome editing can achieve highly targeted insertion or disruption of DNA, a greater range of modifications, multiplexing of targets, permanency of effects (compared to gene addition), and can be easier to produce.

As research continues, new techniques for editing the genome are being developed and technical hurdles in more established techniques are being overcome. This is a rapidly advancing field which is attracting significant investment.

Genome editing is not yet frequently used in patients. A small number of isolated applications have taken place and the number of clinical trials involving genome editing techniques is increasing quickly. The first *in vivo* application of genome editing occurred in 2017, when a small number of patients received ZFN-based treatments for Hunter or Hurler syndrome (developed by Sangamo Therapeutics, trial identifiers: NCT03041324 and NCT02702115). The first trial established safety, and testing is now extending into larger trials but so far has not shown any major impact on disease.

A treatment utilising genome editing, designed to treat both β-thalassemia and sickle cell disease, is being trialled by Swiss company CRISPR Therapeutics and US collaborators Vertex. CTX001 has now entered phase I/II clinical trials where interim reports on 2 patients have suggested safe outcomes[211]. Examples of therapies in clinical trials are listed in table 17.

Other organisations such as Intellia Therapeutics and Precision Biosciences, have further therapies which are at different stages of development (research/pre-clinical) expected to reach clinical development in the near future.

> the first *in vivo* application of genome editing ocurred in 2017, and the number of clinical trials involving genome editing techniques has increased quickly since

General considerations

The term regenerative medicine covers a wide variety of treatment types across a broad range of diseases: they are diverse and complex. Many of the challenges facing RM treatment development and application are specific to the condition, therapy type, healthcare system or regulatory system. RM is an ongoing policy priority and expanding therapeutic area, and much work has already been done to consider how the implementation of RM can be supported. In a 2019 interview, the CEO of the Alliance for Regenerative Medicine (ARM), an international advocate for regenerative medicines stated that "Both the FDA and the EMA have said that they anticipate there being 10 to 20 therapies in this category being approved every year by 2025." [212]

Rare genetic diseases have always presented a challenge for treatment development – there are few patients spread over a wide geographical area, and the conditions often have varied symptoms requiring complex treatments for which it is difficult to make a return on investment.

The Cell and Gene Therapy Catapult has published findings of workshops held to discuss the challenges facing RM in the UK, most recently focussing on digital issues. Several recommendations were made to support real-world evidence collection addressing the needs of innovative therapies [213,214].

Table 17. Examples of potential therapies utilising genome editing that are currently in clinical trials

Company	Treatment	Condition	Trials
CRISPR therapeutics and Vertex pharmaceuticals	CTX001	Sickle cell disease and Beta thalassemia	Phase I/II in US NCT-4244656*
Editas Medicine and Allergan	EDIT-101	Leber Congenital Amaurosis	Phase I/II NCT03872479
Sangamo therapeutics	SB-913	Mucopolysaccharidosis type II (Hunter syndrome)	Phase I/II NCT03041324*
	SB-318	Mucopolysaccharidosis type I (Hurler syndrome)	Phase I/II NCT02702115*
	SB-FIX	Haemophilia B	Phase I/II NCT02695160*

* Study sites include centres in the UK (Source: Cell and Gene Therapy Catapult clinical trials database 2019)

These included:

- The identification of evidence gaps to target efforts and avoid repetition in evidence collection, and make best use of data that already exists
- Improving the understanding of data collection from routine clinical practice
- Review and build on current digital systems and identify mechanisms for interoperability
- Build strong collaborative relationships between industry and the NHS

Industry-NHS collaborations are vital to aid the development of RM therapies through improving industry understanding of clinical issues and needs. Such collaborative efforts should be encouraged at the earliest opportunity in the therapy development process.

Support for RM development

The inherent nature of RM as highly personalised therapies (with a few exceptions) aligns well with the government's aims for advancing personalised medicine. In 2017, the House of Commons Science and Technology Committee published a report on regenerative medicine (Fifteenth Report of Session 2016–17) recognising the UK's strengths in research in this field and the great potential of regenerative medicines for a range of disorders. It pointed to the need for a flexible regulatory environment (including long-term considerations following EU exit), appropriate financial incentives to stimulate innovation in this area, and collaboration between the NHS and other stakeholders to determine the most appropriate reimbursement strategies and best adoptive practises [215].

Several organisations have been set up either to encourage or oversee the development of regenerative medicines within the UK. These include:

- Cell and Gene Therapy Catapult (CGTC) – established in 2012 to encourage growth of the UK cell and gene therapy sector
- UK Regenerative Medicine Platform – established through UK research councils to address issues associated with translation of RM research into practice
- Knowledge Transfer Network (KTN) Regenerative Medicine Priority Area – the goal of the KTNs is to foster collaboration and stimulate innovation in key technology areas

Progress has been made in several areas, including investment of funds committed to manufacturing of RM and development of specialised treatment centres (see below), however challenges remain.

Current regulation of regenerative medicines

Regenerative medicines are subject to a range of regulatory pathways that differ depending on the techniques and materials used, and how many patients might benefit from the therapy. The regulatory landscape in this area is complex and will have an impact on implementation and spread of RM technologies.

Several bodies are involved in the regulation of RM in the UK, these include:

- Medicines and Healthcare Products Regulatory Agency (MHRA)
- Human Tissue Authority
- Health Research Authority
- Human Fertilisation and Embryology Authority
- European Medicines Agency (EMA)

Their role is somewhat dependent upon the type and potential application of the RM in question. The Gene Therapy Advisory Committee provides ethics review on clinical trials involving gene therapy based regenerative medicines in the UK.

The Regulatory Advice Service for Regenerative Medicine is described as a 'One Stop Shop' for professionals in academia, industry and the NHS seeking expert responses to queries about the regulation of RM. It collects guidance from the above listed regulatory authorities and others with the aim of providing a "single point of access" for this information, making it easier for individuals to navigate the complex regulatory landscape surrounding RM therapies.

Regulation of RM will face currently unknown impacts from EU exit. There is notable interaction between UK and EU regulation relating to RM; all advanced therapy medicinal products (ATMPs), which many GMRMs are classed as, must have marketing authorisation from the EMA in order to be distributed within the EU and are regulated through the centralised authorisation procedure, which is co-ordinated through the EMA[216].

Access to regenerative medicines can be provided via several routes in the UK:

- Managed Access Agreements
- Orphan Medicinal Product
- Peer Approved Clinical System – Tier One system for individual requests submitted by clinicians
- Conditional marketing authorisation (from the EMA)
- One-off uses for novel/unlicensed therapies may be permitted under the Hospital Exemption (EU) and Specials Exemption (UK)

Frequent re-examination of the regulatory frameworks surrounding the development and use of RM in the UK is required given the fast-pace of development and expected increase in the number and availability of these therapies.

Establishment of treatment centres in the UK

In 2017, Innovate UK announced a £30 million funding competition for the establishment or development of three Advanced Therapy Treatment Centres (ATTCs) across the UK as part of the government's Industrial Strategy Challenge Fund. The ATTC Network Programme is a funded three-year UK based initiative, comprised of a network of ATTCs.

The three centres are:

- Innovate Manchester Advanced Therapy Centre Hub in Manchester
- Midlands-Wales Advanced Therapy Treatment Centre comprising Birmingham, Wales and Nottingham
- Northern Alliance Advanced Therapy Treatment Centre, comprising Edinburgh, Glasgow, Newcastle and Leeds

The network is coordinated by the CGTC and operates within the NHS framework. The aim of the network is to expedite patient access to complex therapies. Additional funding has also been allocated for the manufacture of enabling technologies such as viral vectors for the delivery of gene therapies to cells.

Following the approval of Kymriah and Yescarta in 2018, several centres offering these treatments have been established across England, several of which are based in London – Table 14 provides the full list of centres.

Specialised delivery and manufacturing

The requirement for clean handling of 'live' biological materials such as cells and viruses requires both expertise and specialised infrastructure. Safe and appropriate genetic modification also requires specialist knowledge and processes. The provision of centres capable of delivering these treatments to patients (often with specialised needs), and being appropriately equipped and staffed to deal with the potentially serious side effects of administration is essential for the delivery of some RM. Several therapies list potentially serious side effects following treatment, which requires patient monitoring e.g. Imlygic (immune reaction), Zolgensma (liver issues), CAR-Ts (cytokine release syndrome). Some treatments, such as Strimvelis, require the patient to travel outside of the UK for treatment. The NHS has expertise in stem cell transplants and treatment of blood disorders which provide some overlapping infrastructure and skills relevant to the delivery of RM. Infrastructure should be flexible and able to respond to the emergence of new technologies and demand for products, ensuring that there is not a delay in research into RM or in the delivery of RM therapies to the health system.

Deciding what level of evidence base is needed and building an appropriate evidence base

Many RM medicines have been in development for many years. Several factors, including small patient numbers, may mean that many years of evidence collection are required before adequate

> there is notable interaction between UK and EU regulation relating to RM, and the impacts on the field related to EU exit are currently unknown

evidence has been gathered for the treatment to receive approvals, e.g. Strimvelis (for ADA-SCID) had been under investigation for 15 years when it received recommendation from NICE. There are inherent difficulties in building a sufficient evidence base for the efficacy of many regenerative medicines, especially given their biologically variable and patient-specific nature, and in many cases low patient numbers. Due to the potential life-saving or life-extending nature of many of these treatments, some may be provided to patients under one of the exemptions previously listed whilst additional evidence is gathered and long-term follow-up conducted.

many years of collection are required before adequate evidence has been gathered for treatment to receive approval

Recommendation:
Consideration needs to be given to the levels of evidence required on the clinical effectiveness of therapies that treat diseases with low patient numbers and how that evidence can support specialised commissioning of these therapies.

Technical issues in genetic modification

There are still important unknowns surrounding the application of genome-modifying technologies. For some, there remains debate about the existence and significance of off-target effects – modifications to parts of the genome not intentionally targeted – especially within genome editing and integrating gene therapies. There are also uncertainties around the longevity of benefits derived from gene addition therapies, where synthesised DNA is not integrated directly into the genome, as synthesised DNA may be lost as cells divide or die.

Cancer relapse

A significant issue in the application of CAR-T therapies is cancer relapse. Although the initial response can be dramatically positive, CAR-Ts targeting CD19 (a common CAR-T form) have a relapse rate of around 30-60% of patients, owing in part to immune escape and lack of CAR-T persistence [217,218]. Research, both close to and removed from the clinic is aiming to address some of these issues.

Reimbursement strategies

In a 2019 interview, the CEO of ARM identified the biggest challenge to RM as “trying to fit a new kind of medicine into an old kind of reimbursement system” [212]. As demonstrated in this chapter, RM therapies are often extremely expensive, single-use treatments designed specifically for very low patient numbers; this is exacerbated by issues in evidence collection meaning funding can be perceived as risky or harder to justify by healthcare agencies. This presents problems for reimbursement to developers and securing payment for the patient’s timely treatment. Many RM products approved by the EMA under the label advanced therapy medicinal product (ATMP) have subsequently been withdrawn due to issues with commercialisation or poor uptake.

Conclusions

Regenerative medicine is an international cross-sector endeavour, requiring closely-linked input from research, industry and healthcare organisations. Commercial interest and investment in genetically-modified RM therapies are growing, and primary research is highly active in this area – especially within genome editing. Several gene therapies are now available to patients for the treatment of cancers and rare disease internationally, with many more in trials, and a select number available to patients on the NHS.

RM can offer potentially long-lasting treatments to diseases for which a cure or treatment is not currently available. However, there are challenges associated with its implementation and patient access, most notably in reimbursement. These challenges are not necessarily unique to RM, but are exacerbated by several inherent factors including the personalised nature of therapies, small patient numbers, and the need for specialised infrastructure and expertise.

there are uncertainties arounds the longevity of benefits derived from gene addition therapies, as synthesised DNA may be lost as cells divide or die

Conclusions

The technology applications highlighted in this report demonstrate significant progress in a range of clinical areas compared to the previous reviews in *The Personalised Medicine Technology Landscape* **report.**

As described, there is potential in each of these for the AHSN Network to support innovation adoption and spread.

Circulating tumour DNA testing for cancer is a fast moving technology area and one type of companion diagnostic testing is already available for lung cancer treatment via the National Genomic Test Directory. In the next three years there is potential for implementation of further companion diagnostic testing in other cancers and the use of ctDNA testing as a monitoring tool is showing great promise.

Pharmacogenomics testing will be included in the National Genomic Test Directory in the near future and as such there are a number of pilot projects to explore which gene-drug pairs are most ready for clinical implementation. The opportunities in this area will come once the pilot projects are complete and there is a need for the implementation of pharmacogenomics to be realised in the NHS.

Transcriptomics is another 'omics technology where three tests are already available to support clinical decision making in women with breast cancer. There are a number of other clinical areas where further support and evidence gathering is needed, for example in the area of rare disease diagnosis.

Near patient testing to support antimicrobial stewardship including rapid diagnostic testing for infectious disease – is an area of varied and intense activity in terms of technology development and application. In particular, technologies that support antimicrobial stewardship have the potential to contribute to global efforts to mitigate antimicrobial resistance. Disease areas where there is already potential to support innovation and implementation efforts include influenza, urinary tract infections and sepsis.

Genetically modified regenerative medicines are a subset of regenerative medicines that involve an element of genetic modification - they are complex and technical innovations. There are a number of GMRMs that have been approved for use on the NHS, including innovative CAR-T therapies for blood cancers, and a gene therapy for a rare immuno-deficiency disorder, ADA-SCID. The opportunities to support innovation include further developments in gene therapies, and longer term, in genome editing approaches. Due to the rare nature of many of the diseases treated with regenerative medicine, consideration needs to be given to evidence requirements and collection, which can take time with small patient numbers. This will also have an impact on specialised commissioning approaches for these rare therapies.

the health system should have oversight and consider the implications on how care is delivered as personalised medicine is implemented across the system

Understanding the challenges ahead and building on opportunities

Personalised medicine will continue to develop and drive change from the 'one size fits all' delivery of care. The rate of development of healthcare innovation is increasing, as is the cost and the expectations of the public for improvements in NHS services. Supporting the implementation of the technologies that can deliver more personalised medicine will require a coordinated approach across the NHS including NHS England, the AHSNs and a number of stakeholders within and outside the health system. Each technology not only has to be considered on its own merits, but also as part of an integrated healthcare system. The desire to use an increasing range of new technologies and interventions to improve population health means that the health system should have oversight and consider the implications on how care is delivered when these changes are implemented across the system. Formal programmes of activities will be required to implement these new innovations into practice when appropriate. These efforts will focus on specific care pathways but there is also a requirement to consider the system impacts and the necessary infrastructure and resources that will be required to deliver the changes.

Transformation arising from the implementation of novel technologies brings opportunity, but also raises challenges in terms of understanding evidence requirements, engagement of commissioners, health system structures and preparing the workforce.

Evidence requirements

Industry should work more closely with the NHS in order to develop interventions and applications which best meet specific NHS needs. In addition, NHS and NICE evidence requirements will need to be addressed in order to facilitate effective and distributed health system adoption of innovation. This will require working with services to understand the clinical problem which the technology has been designed to address, and understanding the types of evidence required to demonstrate clinical utility and cost-effectiveness. By considering health system needs when planning studies and evidence gathering, more effective implementation of new technologies can be realised, for the benefit of patients and the health system. NICE, AHSNs, Academic Health Science Centres, the NIHR Community Healthcare MedTech and *In Vitro* Diagnostics Co-operatives, and other bodies such as the MHRA, already provide support for commercial developers in terms of study design and how to obtain the necessary evidence. However, NHS commissioners of services are key to the widespread implementation of innovation within the health service and should assist in defining the nature and level of evidence they require for their decision making. There will be different requirements depending on the nature of the intervention, the target population, expected costs and benefits and impact on certain care pathways. This will provide clarity for all stakeholders including those involved in evidence generation and evaluation.

Engagement of commissioners

Ongoing engagement of commissioners is an essential part of supporting implementation of new technologies into clinical services, however consideration needs to be given as to how to best to achieve this. Informing commissioners about innovations will help to ensure that opportunities for implementation are not missed. Commissioners should also be provided with the specific details and requirements of new technologies in the form of implementation support in order to ensure successful use and avoid unintended consequences for patients and health services. As some new innovations require complex infrastructure change to support their use, the implementation support needed will also be greater. The NHS digital infrastructure is increasingly unable to support the innovations being considered for implementation and if not addressed in a timely manner, will become a barrier to the uptake of certain new evidence-based interventions.

NHS commisioners of services are key to the widespread implementation of innovation within the health system, and should assist in defining the nature and level of evidence they require

Pathway transformation

Many innovations provide an opportunity to transform clinical pathway design and make changes to referral pathways, rather than being added into current pathways as an additional step. One example of where this could occur is where technologies are moved out of specialised services and into more mainstream secondary and primary care. Examples could include point of care testing or monitoring e.g. for cardiac conditions, carried out in a GP surgery. However, the resources and leadership required to implement such system changes should not be underestimated.

Engagement of workforce

There have been a number of efforts ongoing in terms of educating the workforce – in the area of genomics, the Health Education England genomics education programme has a number of online courses and a Master's in genomic medicine. Efforts such as these,

greater effort is needed to ensure that a balance is struck in terms of pre-implementation knowledge and on the job training

which aim to embed genomic literacy in the health workforce, are vital in terms of ensuring that genomic medicine is integrated into clinical practice, and will include equipping clinicians with the skills needed to interpret and act upon the outputs of genomics technologies. For the other technologies highlighted in this report, further efforts are required on how best to support the workforce and keep them informed about new innovations. In particular, as plans are made to transform care, greater effort is needed to ensure that a balance is struck in terms of pre-implementation knowledge and on the job training. As new interventions and diagnostics are deployed out of specialist services and into secondary and primary care, the roles of clinical scientists and other healthcare providers will need to be considered and appropriate provision made to ensure that they can utilise new technologies appropriately in different healthcare environments.

Delivering on the promise

The changes outlined above will be occurring in a health system that is undergoing technological transformation and infrastructural change. They will be underpinned by developments in digital services and infrastructure, which are vital to ensure their successful implementation. The key objectives remain in ensuring equitable access to these new health services across the NHS in England and to improve health inequalities. There is a valuable opportunity for the AHSN Network to play a central role in supporting the clinically appropriate, systematic implementation and spread of personalised medicine technologies in this new landscape, and to help realise the benefits to patients and the NHS.

Appendices

Appendix 1: Acknowledgements

We would like to gratefully acknowledge the following individuals for their insight into the various subject areas analysed within this report. While their contribution has been invaluable, all responsibility for the final content of the report rests with the PHG Foundation authors.

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Appendix 2: Acronyms, abbreviations and glossary

ADA-SCID	Adenosine deaminase specific severe combined immunodeficiency disorder.
ADR	Adverse drug reaction
ALL	Acute lymphoblastic leukaemia
Allogeneic	In the context of regenerative medicine, where cells from a donor are used to develop and implement treatment for another individual
AMR	Antimicrobial resistance. The ability of disease-causing microbes – including bacteria, viruses and fungi – to resist the effects of therapeutics used to treat them
ARM	Alliance for Regenerative Medicine
ASO	Antisense oligonucleotide. Class of oligonucleotide therapy. Single-stranded fragments of DNA or RNA used to target intermediary mRNA molecules produced by disease-causing genes, effectively switching them 'off' and mitigating symptoms of the disease
ATTC	Advanced Therapy Treatment Centre
ATMP	Advanced therapeutic medicinal product
Autologous	In the context of regenerative medicine, where the patient's own cells are used to develop and implement the treatment
B-ALL	Acute B-cell Lymphoblastic Leukaemia
Bioinformatics	Field of study involving the collection, curation and computational analysis of data relating to biology e.g. DNA sequencing data
CAD	Coronary artery disease
CAR-T	Chimeric antigen receptor T-cells. A type of immune therapy for the treatment of cancer in which immune cells have been intentionally modified to express a particular antigen which enhances targeting or destruction of cancer cells
CDS	Clinical decision support
cfDNA	Cell free DNA. DNA that is released from body cells, most commonly into the blood stream, but also into other bodily fluids such as urine
CGTC	Cell and Gene Therapy Catapult
CPIC	Clinical Pharmacogenetics Implementation Consortium
CRISPR	Clustered regularly interspaced short palindromic repeats. Detection machinery that, along with associated cutting proteins, forms part of a class of genome editing tools. Originally developed from bacterial DNA sequences and capable of making specifically targeted cuts in DNA
ctDNA	Circulating tumour DNA. DNA that is released from cells in tumour, most commonly into the circulation, but also into other bodily fluids such as urine
CUP	Cancer of unknown primary
DLBCL	Diffuse large B-cell lymphoma
DNA	Deoxyribonucleic acid
DPWG	Royal Dutch Association for the Advancement of Pharmacy Pharmacogenetics Working Group
DPYD	Dihydropyrimidine dehydrogenase (gene name)
DTC	Direct to consumer
EHR	Electronic health records
EM	Extensive metaboliser
EMA	European Medicines Agency
EQA	External Quality Assessment
ER	Oestrogen receptor (gene name)
FDA	US Food and Drugs Administration
FFPE	Formalin-fixed paraffin-embedded. A technique used to prepare and preserve tissue specimens in formalin so tissue morphology can be examined. Embedding the sample in paraffin facilitates preparation of the sample into thin slices that can be mounted on a glass slide and examined under a microscope
Gene therapy	Treatments for disease that involve the alteration of genetic material either of the patient's cells or of cells used for treatment. Umbrella term that also includes genome editing
Genome editing	Alteration of an individual's genome in one or more cells, or of other cells used for treatment, through the delivery of genome editing tools e.g. CRISPR
Genotypic	Relating to the genetic background of an organism e.g. a genotypic test may detect the presence of specific genes.
GEP	Gene expression profiling/panel. Collection of specific genes included on a physical or bioinformatic panel for the examination of gene expression
GLH	Genomic Laboratory Hub
GMRM	Genetically modified regenerative medicines. Treatments that replace, regenerate or engineer cells or tissues for the treatment of disease that also include an element of genetic modification through causing or having been subject of alterations to genetic content of cells e.g. gene therapy
GMS	Genomic Medicine Service
HD	Huntington's disease
HER	Human epidermal growth factor receptor (gene name)
HIV	Human immunodeficiency virus
HLA	Human leucocyte antigen (gene name)
IBD	Inflammatory bowel disease

IHC	Immunohistochemistry. Method for the identification of specific proteins in a tissue section through binding of complementary molecules to provide a visual signal
IM	Intermediate metaboliser
Immunotherapy	Biological therapies that help the body's immune system fight disease through the stimulation or targeting of the immune response
ISH	In situ hybridisation. Method for the identification of specific sections of DNA or RNA in a tissue section through binding of complementary molecules to provide a visual signal
IVD	In vitro diagnostic. A diagnostic test performed on samples that have been taken from the human body, such as blood and tissue
KTN	Knowledge Transfer Network
LCA	Leber congenital amaurosis
Liquid biopsy	A sample of bodily fluid such as blood or urine that contains material for analysis, such as protein or DNA, from a tissue of interest elsewhere in the body. This is in contrast to a solid biopsy where a sample is taken directly from the tissue in question for analysis
lncRNA	Long non-coding ribonucleic acid. Class of RNAs which include those that do not code for proteins and are of more than 200 bases in length
MHRA	Medicines and Healthcare products Regulatory Agency
MIB	Medtech innovation briefing
Microarray	Tool or technique used for the examination of RNA abundance primarily in a laboratory setting. Uses many thousands of short nucleotide chains of pre-defined sequence to hybridise and detect the presence of corresponding RNAs. Can be used to determine relative abundance of specific nucleic acid sequences
miRNA	Micro ribonucleic acid. Class of RNAs which include those that do not code for proteins and are very small, normally less than 30 bases in length
mRNA	Messenger RNA. Class of RNAs produced from protein-coding regions of DNA and can be translated to produce proteins
Multimorbidity	The simultaneous existence of multiple health conditions in one individual, often defined as two or more conditions or diseases
NGS	Next Generation Sequencing. Most prominent method used for examining the sequence of genetic material at scale. Provides higher throughput than original 'first generation sequencing' and is capable of reading huge numbers of DNA/RNA sections in parallel, producing millions of small reads
NHS	National Health Service
NICE	National Institute for Health and Care Excellence
NIHR	National Institute of Health Research
NSCLC	Non-small cell lung cancer
OMP	Orphan medicinal product. EU designation which may be applied to medicines or therapies which are developed to treat very serious and rare conditions (affecting 5 in 10,000 people or fewer in the EU). Market incentives such as limited-term market exclusivity are attached to the designation
OSNA	One-step nucleic acid amplification
OT	Oligonucleotide therapy. Class of genetic therapies developed for the treatment of some rare diseases. Comprised of a short-chain of nucleotides which specifically target and interact with other sequences, resulting in gene silencing or altered production of proteins
PCR	Polymerase chain reaction. A method for amplifying the quantity of DNA, by making multiple identical copies of a DNA molecule from a small of amount of template DNA, such as a sample of DNA from a patient
Phenotypic	Relating to the observable traits of an organism that arise from the interaction of its environment with its genes
PCT	Procalcitonin
PGx	Pharmacogenomics. The study of the role of the genome in drug response and the application of this information in clinical practice
Pharmacogene	A gene that is associated with a drug response
Polypharmacy	Simultaneous use of multiple medications
PharmGKB	Pharmacogenomics Knowledge Base
PM	Poor metaboliser
PMCPA	UK Prescription Medicines Code of Practice Authority
PMTL	Personalised Medicine Technology Landscape
POC	Point of care
POCT	Point of care testing
PSC	Patient Safety Collaborative
qPCR	Quantitative polymerase chain reaction. The same principle of PCR but performed in a way that allows the DNA product to be quantified, in order to understand the amount of DNA originally in a sample. Often performed in real time, using fluorescently labelled molecules to allow DNA quantification
RM	Regenerative medicine. Group of treatments that replace, regenerate or engineer cells or tissues for the treatment of disease
RNA	Ribonucleic acid. Class of nucleic acids, similar to DNA and often single-stranded. Transcribed from DNA and can be subsequently translated into proteins or perform a variety of other non-coding functions
RT-qPCR	Reverse transcription quantitative polymerase chain reaction. Method for the amplification and quantification of specific sections of RNA. Devised of two stages – converting RNA into cDNA (reverse transcription) followed by quantification
sADR	Serious adverse drug reaction

SCCHN	Squamous cell cancer of the head and neck
siRNA	Small interfering ribonucleic acid. Type of double stranded RNAs that can be engineered to alter the expression and levels of protein produced from specific genes through interaction with and degradation of the RNA. Class of oligonucleotide therapy
SMA	Spinal muscular atrophy
sncRNA	Small non-coding ribonucleic acid. Broad class of non-coding RNAs that are typically fewer than 100 bases in length and may be functionally important as regulators of gene expression or broader cell function. Micro RNAs are included within this group
Splice variants	Alterations in the DNA sequence at exon/intron boundaries which may affect the inclusion of these in RNA produced from that region
TCR	T-cell receptor. Complex of proteins found on the surface of T cells that are able to interact with a variety of antigen fragments - proteins presented by foreign bodies or tumours - and elicit an immune response
TKI	Tyrosine kinase inhibitor. A drug often used in cancer treatments to inhibit the function of a type of protein molecule called a tyrosine kinase
TPMT	Thiopurine s-methyltransferase (gene name)
Transcriptomics	Field of study examining all RNA (the transcriptome) in a sample or multiple samples at a given time. Term may also be applied to the study of smaller subsets of RNA.
UM	Ultra-rapid metaboliser
WES	Whole exome sequencing. Sequencing of the protein coding genes in the genome only
WGS	Whole genome sequencing. Sequencing of the entire genetic sequence of an organism, including both the protein-coding and non-protein coding regions of the genome
ZFN	Zinc finger nucleases. Synthesised enzymes that have been developed as a class of genome editing tools capable of targeting and cleaving at specific sequences of DNA

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