**How 'personalised' is personalised medicine? What are the Challenges for pathology in delivering this for patients?**

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**What is personalised medicine?**

Personalised medicine, in its broadest sense, is the tailoring of healthcare to the individual patient [[1](#_ENREF_1)]. It is essential to allow a major step in medicine from populations to individuals. Patients are classified according to their genotype and phenotype, allowing for their treatment to be targeted appropriately [[2](#_ENREF_2)]. The primary aim of personalised medicine is to maximise response from treatment and minimise harmful side effects for those that may gain no benefit. As a result, personalised medicine has the potential to reduce the cost of patient care [[3](#_ENREF_3)]. It helps to inform clinicians and commissioners which patients may not receive benefit from highly expensive treatments. It can be argued that personalised medicine emerged as a direct result of huge advances in medical genetics, in particular the completion of the human genome project (HGP). Sequencing the whole genome has allowed for us to develop a much greater understanding of the role genetic variance provides in disease [[4](#_ENREF_4)]. Once the genetics behind disease is established, it can be used to investigate the effected molecular pathways and then ultimately phenotype.

One of the key applications of personalised medicine, highly dependent on the pathologist, is the development and administration of chemotherapeutic agents. Molecular pathology allows for patients’ tumours to be characterised according to their protein expression or mutational profile and a suitable chemotherapy to target the affected cellular pathway is selected. One of the earliest examples of personalised medicine was seen with the development of tratuzumab (Herceptin) for the treatment of breast cancer. There is over-expression of the Human Epidermal Growth Factor Receptor (HER2) gene in 25-30% of breast cancers. HER2 positive breast cancers are associated with more aggressive disease and poorer disease-free and overall survival. Treatment of these cancers with murine monoclonal antibodies such as tratuzumab have shown to improve overall survival by 25% [[5](#_ENREF_5)]. HER2 amplification is detected in the pathology laboratory with either immunohistochemistry (IHC) or fluorescent in-situ hybridisation analysis (FISH) [[6](#_ENREF_6)]. Ascertaining the patient’s HER2 classification is essential in order to determine whether treatment with tratuzumab is beneficial. Therefore the pathologist is key in the delivery of personalised medicine.

Another example where the pathologist plays a vital role in determining targeted therapies is seen in the treatment of metastatic colorectal cancer. Anti-Epidermal Growth Factor Receptor (anti- EGFR) antibodies, such as Cetuximab and Panitunumab, are delivered to block EGFR and its signaling pathways [[7](#_ENREF_7)]. However, if the genes that code for proteins downstream in this pathway such as KRAS , NRAS and BRAF are mutated, patients will be resistant to Cetuximab and have a worse prognosis [[8](#_ENREF_8)]. Therefore treating these patients with such an agent would be futile and expose the patient to unnecessary side effects. Patients with mutations are identified through genetic sequencing, a key molecular pathology technique. In order to maximise the sensitivity of these genetic testing technologies, areas of highest tumour cell density should be tested, which are first identified by the pathologist. This will allow for any mutations present to be enriched and increase the likelihood of their detection.

**How personalised is personalised medicine?**

These are examples of personalised medicine in its infancy. Its application in clinical practice is currently somewhat simple and limited. Individual tests are conducted to identify individual molecular lesions, which then broadly categorise patients and direct treatment. Personalised medicine at this level is isolating a specific cellular pathway and treating appropriately. However many diseases and especially cancer are far more complex than this. Pathways do not act in isolation; there are numerous downstream signals that interact. The cancer cell itself is part of a far more complex dynamic process with other cancer cells and other cell types. We are not currently at the stage of sequencing each patient’s genome and choosing treatment on a genome-by genome basis. Personalised medicine in its current application could be more accurately described as group-personalisation. However, the advancement in genetics since the early 1990’s has been far more than we could ever have anticipated. There are a number of large-scale personalised medicine delivery initiatives such as the Cancer Research UK Stratified Medicine Programme [[9](#_ENREF_9)]. These have the potential to expand our knowledge and understanding and allow personalised medicine to develop further, yielding an even greater effect for patients

**Challenges for the pathologist**

There are a number of challenges the pathologist faces with the deliverance of personalised medicine as it continues to develop. These can be broadly summarised into the following: the role of the pathologist, laboratory techniques, data processing, analysis and interpretation from whole genomes, future pathology education and understanding the use of targeted therapies.

**From morphology to mutations**

The role of the pathologist as defined by the Royal College of Pathologists is someone who “explains why and how people fall ill and reveal the targets for their treatment” [[10](#_ENREF_10)]. This definition indicates that the role of pathologist has already begun to adapt to the emergence of personalised medicine. Traditionally, the role of pathologist in relation to cancer was to provide definitions based on anatomy and morphology [[2](#_ENREF_2)]. This was suitable where therapy was directed purely by morphology. However, targets for patient treatment are becoming increasingly molecular. This indicates that the emphasis of pathology in the future may lean more in the direction of molecular pathology than it currently does. It is therefore vital that pathologists are currently learning and adapt their knowledge and skills set to be able to identify the best targets for patient treatment.

**Tissue processing techniques**

Current tissue processing methods have been developed for the purposes of easily characterising tissue morphology. Although suitable for visualising tissue, it may not be optimal for downstream molecular technologies, in particular genetic sequencing. The most common tissue processing method that is routinely practiced by histopathology departments throughout the UK and worldwide is tissue fixation followed by embedding in paraffin wax (formalin fixed paraffin embedded, FFPE) [[11](#_ENREF_11)]. The formalin fixation step varies greatly between departments, as there are no official recommendations for the type of fixative or the duration of fixation [[12](#_ENREF_12)]. This has previously not interfered with basic morphometry analysis as fixation has little effect on the ability to visualise haematoxylin and eosin (H&E) stained tissue. For IHC analysis, poor fixation can have a substantial effect of the quality of staining. Antigens must be sufficiently mobilized whilst still maintain their immunoreactivity to allow for effective IHC and inadequate fixation can greatly affect this [[13](#_ENREF_13)].

Perhaps an even greater challenge with fixed tissue is faced with the increase in genetic sequencing for personalised medicine. It is reported that tissue fixation results in the de-amination of cytosine bases, thereby causing C:G>T:A sequence artefacts [[11](#_ENREF_11), [14](#_ENREF_14)]. These artefacts may not be a problem for highly targeted sequencing of oncogenes, where only specific hotspots are interrogated. However, for the purposes of whole genome sequencing and large gene panels current tissue processing methods may not be sufficient. In order to address this issue, samples may have to be tested in duplicate or triplicate. Another alternative is for pathology tissue processing techniques to be standardized and adapted to better allow downstream genetic testing. Samples could be frozen alongside formalin fixation as is common in research settings. This would allow for the move of research tools to be integrated better into pathology diagnostics.

**Data processing, interpreting and management**

The human genome project took 13 years to complete at a cost of around $3 billion [[1](#_ENREF_1)]. With the emergence of next-generation sequencing technologies, a whole genome can be sequenced at around $5000 and whole exomes for $1000 in a number of days [[15](#_ENREF_15)]. The price of whole-genome sequencing is continuing to fall and it is thought that 2013 will be the year of the “$1000-genome”[[16](#_ENREF_16)]. As whole-genome sequencing becomes ever increasingly economically feasible we will be able to capture a full picture of an individual’s genetic variants. However, the future challenge will fall with the interpretation of these variants and fully understanding their relationships to phenotype. Our ability to identify variants is far greater than our understanding of their functional affects [[17](#_ENREF_17)]. Therefore it is important that tomorrow’s pathologist is at the forefront of molecular biology advances and can provide an interpretation of genetic data so that it can be directly applied to clinical practice.

The practical aspects of implementing sequencing technologies into clinical practice will be a challenge [[4](#_ENREF_4)]. The main considerations that have to be made for implementing next-generation sequencing into the clinic are: the sequencing platform used, computational resources for analysis , data storage and interpretation of data [[18](#_ENREF_18)]. Clinical bioinformatics allows for this gap between genetic data and functional clinical information to be bridged. Deciding what bioinformatic approaches should be used to make sense of genetic data can have a huge impact on the resulting information [[19](#_ENREF_19)]. In the future, bioinformatics should become an integrated part of pathological practice. This could be done by making it part of molecular pathology training so that pathologists can develop a basic understanding of its application. It is also important to have increased collaboration and communications from other disciplines such as bioinformatics and statistics to allow for analysis pipelines to be developed that can be used by pathologists and clinicians [[18](#_ENREF_18)]. If pathologists can understand the language and tools of genetics they will be much better equipped to understand future advances and communicate these to the multi-disciplinary team. In this way, pathologists will ensure that future developments in genetics will result in the maximum patient benefit.

An associated challenge with the advancement of next generation sequencing is the amount of data storage and management required. Each sequencing run generates hundreds of giga-bytes of data [[15](#_ENREF_15)]. Storing and managing such large data amounts would put a lot of stress on current information technology (IT) systems. However, as these continue to develop, pathologists should work alongside experts in IT to allow for future systems to be developed with the pathologist’s requirements considered. With the generation of such large data amounts, an open and sharing environment is required to allow for the most clinically useful information to be obtained. The development of biobanks that are linked to clinical data may allow for easier discovery of genotype-phenotype relationships [[20](#_ENREF_20)].

There are a number of factors that must be considered as the amount of genetic data continues to expand such as data ownership and privacy. Is patient genetic data owned by the patient or the hospital/laboratory? If the latter, can data be transferred to third parties? Also should the pathologist report other genetic findings that may not directly relate to the requested investigated? [[18](#_ENREF_18)] Some of these issues can be temporarily addressed by anonymising data; however regulations and laws surrounding these issues have yet to be clarified. It is therefore important that pathologists can play a role in determining these laws. Data usage has to be restricted so that the balance can be made between making clinically relevant discoveries whilst still protecting the patient’s privacy.

 **Future education**

A key strategy to ensure the success of pathology as a specialty for the future in the age of personalised medicine is to educate and inspire the new generation of pathologists. Personalised medicine education in the current undergraduate medical curriculum is limited [[21](#_ENREF_21), [22](#_ENREF_22)]. Delivering this teaching as a component of pathology education would allow for personalised medicine to become synonymous with pathology. This would allow better integration of personalised medicine into pathology as a specialty. Similarly, post-graduate pathology education should be modified to have a stronger emphasis on molecular pathology and genetics. This would allow the future pathologists to have a better understanding of basic science, genetics and bioinformatics [[18](#_ENREF_18), [22](#_ENREF_22)]. As we continue to discover more about the genotype and how this relates to phenotype, molecular pathology will only continue to increase its presence within the pathology specialty.

**Understanding the use of targeted agents**

A challenge that emerges with the increased implementation of personalised medicine is the understanding of the appropriate use of targeted therapies [[23](#_ENREF_23)]. Therapies that are designed to act on particular molecular targets are only effective for those patients who carry certain molecular lesions [[24](#_ENREF_24)]. However, for those patients characterised by their molecular lesion, treatment can still be uneffective.For example the PICCOLO clinical trial illustrated that there is still a population of colorectal cancer patients, wild-type for KRAS, that when treated with anti-EGFR agents do not respond better [[25](#_ENREF_25)]. This highlights that our understanding of the molecular mechanisms behind the actions of personalised medicine is still relatively basic and requires further research.

Similarly, it is observed that after treatment with targeted agents, drug resistance can develop [[26](#_ENREF_26)]. We are beginning to further understand that cancers contain multiple clones. It has been shown that in colorectal cancer patients, those who are wild-type for KRAS and BRAF and are treated with anti-EGFR antibodies, will develop resistance after 12-18months [[24](#_ENREF_24)]. This is evidence that cancer is an evolutionary process and that anti-EGFR therapy selects for resistant clones. By destroying the drug-resistance clones you allow for the resistance sub-clones to grow and develop into a drug-resistant tumour [[27](#_ENREF_27), [28](#_ENREF_28)]. It is therefore thought that in the future, multiple targeted therapies may be administered in order to target the multiple clones that may be present within the cancer and therefore manage drug resistance [[27](#_ENREF_27)].

It is hoped that the increased implementation of next generation sequencing technologies will help to improve our understanding so we may address these issues. The PICCOLO trial indicated that other molecular targets alongside KRAS and BRAF may provide useful predictive and prognostic biomarkers [[25](#_ENREF_25)] and the future use of next generation sequencing may allow these to be fully evaluated. A more recent clinical trial has already demonstrated this, using next generation sequencing for mutation detection to a mutant allele frequency of 5% [[29](#_ENREF_29)]. As sequencing technologies and bioinformatic pipelines develop further, it may be possible to detect mutations down to an even lower level. It is currently unknown what the significance of a 1% mutation allele frequency holds for patient outcome or response to therapy. However, the use of these technologies may help to better understand the implementation of targeted agents for those will low levels of predictive and prognostic biomarkers.

Pathologists can learn from the targeted therapies of a certain disease type to implement them in a different setting for a population that may have similar molecular characteristics. For example, the use of tratuzumab was initially developed as personalised medicine for breast cancer [[5](#_ENREF_5)]. HER2 amplification has been observed in a proportion of gastric cancers and therefore the usefulness of HER2 antibodies as a therapeutic agent in gastric cancer is being evaluated by a number of clinical trials [[30](#_ENREF_30), [31](#_ENREF_31)]. This could prove to be an effective method to best deliver personalised medicine. In this way, targeted agents would be delivered according to molecular characteristics as opposed to cancer type.

**Conclusions: the future of the pathologist and personalised medicine**

“Personalised medicine” may not be currently as personalised as one may hope and there may be a number of challenges for pathology to effectively deliver it to patients. However, this can be seen as a great opportunity for pathology to expand as a specialty and provide an even greater benefit for patients. The future of personalised medicine is very bright and holds much promise. All of medicine as a whole, not just pathology, will have to adapt with the advent of personalised medicine. At the start of the personalised medicine revolution, pathology should embrace this challenge and be the leading example to other specialties.

It has taken a great deal of research to get to the current position and personalised medicine is still very much in its infancy. As we continue to learn more about how genomics relates to disease it creates further questions and adds further layers of complexity to the model. However, advances have been huge. The human genome project illustrates that whole-genome sequencing was once thought of as uneconomical and unobtainable. However, whole-genome sequencing will perhaps one day be an integral part of future clinical practice. Although there are numerous challenges facing current and future pathologists, it is the perfect time to begin to address these so that pathology can grow in the same direction as personalised medicine and delivery can be most effective.

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