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Genetics in Medicine

3. Precision Medicine

By

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Introduction

This is the third Galton Institute booklet in a series about genetics in modern medicine. In the first (*Genetics in Medicine 1. Conception and Early Life* (GIM1)) and second (*Genetics in Medicine 2. Adult Life* (GIM2)), we described the significant advances made in understanding the role of **genes** in human health and disease over the last 50 years. We also touched on our understanding of gene-encoded proteins and their role in the vast, complex networks of metabolic and signalling pathways. We outlined:

- The nature, size and function of the human **genome**
- The mitochondrial genome
- The role of **DNA** variants in determining inherited differences between individuals
- Patterns of inheritance of genetic conditions
- Simple versus **complex inheritance**
- Genetic risk and common diseases of adulthood
- The role of genes in the way we respond to medicines
- The development of treatments for genetic conditions
- Some of the main advances in molecular technologies

In this third booklet, we shall further explore the revolution in medicine built on this growing understanding of molecular mechanisms and the extraordinary advances in DNA sequencing technology.

What is precision medicine?

During most of the 20th century a patient was allocated treatment based on disease symptoms and routine clinical investigations. This approach was very successful for some patients, but not for others. In the early years of this century, it became clear that information embedded in patients' genomes could – along with other factors – contribute to more accurate 'precision' diagnosis and targeted treatment of some illnesses.

There has been recognition across the world of the importance of these developments: in 2014 the British Prime Minister David Cameron announced a four-year, £300 million investment in an ambitious programme – The 100,000 Genomes Project – to 'transform how diseases are diagnosed and treated' by

sequencing the genomes of patients with rare diseases or cancer. Then in 2015 President Barack Obama announced his Precision Medicine initiative, a similar \$215 million research undertaking. Other countries have analogous plans.

Precision medicine is driven by a technological revolution

Precision – sometimes called ‘personalised’ – medicine depends on the ability to ‘read’ a person’s whole genome, quickly and cheaply. New techniques for sequencing DNA, first introduced in 2005, were the start of a technological revolution that is still far from over. While the Human Genome Project took 13 years and cost \$3,000 million, the equivalent today might take a few days and cost \$1,000 – well below the cost of many medical investigations. The challenge facing precision medicine is to turn the technical triumph of ‘next-generation sequencing’ (NGS) into reliable cost-effective clinical services. It is beyond the scope of this booklet to describe and explain the technology, which is still evolving rapidly. For suitable sources of more detail see *Further Information*.

Clinical diagnosis with next-generation sequencing

Many genetic conditions are caused by a variant in a single gene. Identifying the gene and variant confirms the diagnosis. It may also inform management and treatment of the patient, enable reproductive choices and counselling, and testing of any family members who want it. For some conditions, the clinical features of the patient reliably point to the likely gene, and for such conditions the sequencing revolution has made little difference: it has been possible for many years to sequence a single gene to check for variants. However, for many conditions, the variant could be in any one of tens or even hundreds of genes. Deafness, blindness and intellectual disability are typical examples. The sequencing revolution – for the first time – makes it possible to check every relevant gene on a reasonable timescale and at a reasonable cost. This is transforming services for affected patients and families.

There are three possible ways of using the new technology in such cases:

- **Sequencing the whole genome.** With rapidly falling costs, in the future it might be most efficient to sequence the entire genome for all patients, with ‘filters’ applied so that only the regions of interest are analysed. But this is

currently a relatively expensive alternative, particularly in the requirement for analysing the data. A patient's genome would typically have four million variants compared to the Reference Human Genome; the majority of these have little or no effect. Sorting through to identify a single variant that causes a condition requires large amounts of computing power – as well as time and expertise – that is not yet feasible in the diagnostic setting.

- **Sequencing just the protein-coding DNA (the exome).** The great majority of variants that cause single-gene conditions are in protein-coding sequences which, remarkably, comprise well under 2% of our total genome sequence (see GIM1, pages 8-9); scientists have a better understanding of the possible effects of variants in these sequences. Restricting the sequencing to this so-called **exome** reduces the list of variants to maybe 20,000, making the burden of data analysis more manageable for a well set-up laboratory.
- **Sequencing a panel of candidate genes.** Instead of sequencing all 20,000 protein-coding genes, a laboratory could select up to a few hundred genes thought to be likely sites of variants that could cause a patient's condition. This approach generates clear diagnostic results in many, but not all patients. In 2016, 28 **gene panels** (comprising between 4 and 250 genes) used at the Manchester Centre for Genomic Medicine helped diagnose patients with learning disability, heart disease, metabolic conditions (enzyme deficiencies) and eye disorders in cases where the cause is judged to be genetic. A further gene panel is used to test tumours in patients with various cancers. In addition to these, there are numerous commercial **genotyping** kits available.

The choice between these alternatives depends at least as much on the difficulty of analysing the raw data as on the direct cost of generating it. Ethical considerations are also important, as discussed below. Sequencing the whole genome has an attractive finality, and it also avoids the processes needed to isolate just the DNA of interest from a mouthwash or blood sample. For some research, sequencing whole genomes is the obvious strategy. But for clinical diagnostic work the advantages are less clear. The great majority of variants in non-coding sequences (over 98% of the genome) are uninterpretable with present knowledge. Most would be expected to have no effect, but the general

level of uncertainty means that almost none of the data could be used in clinical reports, so one may question the utility of generating it in the first place. With increasing knowledge and ever-falling costs this may change, but currently most diagnostic laboratories sequence gene panels or exomes rather than whole genomes.

Filtering the list

Whichever strategy is used, the result is a list (longer for exomes, shorter for gene panels) of variants that must then be filtered to identify the one responsible for the patient's condition. Two aspects must be considered: the likely effect of a variant on the gene product, and then the likely effect of the resulting change in the gene product on the health of the patient.

- For understanding the effect of a variant on the encoded protein, we consider the way a gene sequence determines the structure of a protein. This was briefly set out in GIM1 (pages 8-9). Any variant that changes the **triplet** reading frame, alters the way the **exons** and **introns** of the primary transcript are spliced, or introduces a premature stop codon, is likely to wreck the protein. Changes that just replace one **amino acid** with another are assessed by looking at related proteins (in humans and other organisms). If many related proteins all have a certain amino acid at the corresponding position, then it is probably doing something important, and changing it is likely to be deleterious. If the related proteins show a range of different amino acids at that position, a change is less likely to matter (depending on the particular amino acid introduced). Where available, knowledge of the three-dimensional structure of the protein can provide important extra insight.
- Even if a DNA variant alters or inactivates a protein, it won't necessarily cause disease. We can get by with reduced amounts or complete absence of some proteins. Thus an essential second enquiry is to ask whether the same variant can be found in unaffected, healthy people. The very large (currently 60,000 exomes) and rapidly growing Exome Aggregation Consortium (ExAC) database makes this fairly easy. For any variant and gene of interest, one can quickly check how often the variant has been seen in healthy individuals and, more generally, how much variation the gene seems to tolerate without

causing disease. Finally, if a variant does seem likely to cause a disease, we must ask whether that disease will be the one from which the patient suffers.

The end result of all this analysis is to classify each variant on a five-point scale:

1. Not pathogenic
2. Unlikely to be pathogenic
3. A variant of unknown significance (VUS)
4. Likely to be pathogenic
5. Clearly pathogenic

The laboratory will base its classification on best current knowledge, but this is a developing field and judgements may need to be revised, particularly for variants in category 3. Finding variants in category 4 or 5 provides individual patients with extremely valuable information: firstly, the patient knows the exact cause of the disease in the family, understanding which, even on its own, can bring relief. Sometimes, it will direct treatment and interventions for the patient. Then, the patient and family are empowered to make decisions about wider relative testing, as well as about partnership and reproductive choices. But the consequences of getting it wrong are serious, especially when a healthy person is being tested for risk of a late-onset serious disease – currently mainly rare familial cancers, but also other late-onset conditions. Not only will the diagnosis and prognosis for the patient be incorrect, but other members of the family may be told that they are predicted to get, or not, the same disease in the future. Individuals informed in error that they carry a disease-causing variant may opt to have inappropriate interventions or treatments, and those informed they are not at risk may be given false reassurance.

What should be in the laboratory report?

Decisions on which variants to report depend both on judgements of the importance of each variant and on what the patient is expecting. In terms of the importance of variants, it is generally agreed that variants in categories 1 and 2 should not be reported. Category 3, variants of unknown significance, raise major problems. These are variants that might or might not be important: current knowledge does not allow a decision. Unfortunately, in the present state of knowledge they can be numerous. Reporting VUSs presents the doctor

with an almost impossible task of explanation, and the patient with information whose significance it is impossible to assess. Not reporting VUSs risks not mentioning something that later knowledge might show was highly significant. Variants in categories 4 and 5 would clearly be reported if they are relevant to the patient's condition – but what if a variant is found that is clearly pathogenic, but that would cause an entirely different condition? This is the problem of **incidental findings**. One of the arguments for using gene panels rather than exomes is that it reduces their number. The problem is not unique to genetics. Every time a physician asks for a chest X-ray there is a possibility it will show an unexpected tumour. A first step is to divide incidental findings into those that are actionable, where something can be done to avert or mitigate the harm, and those that are not actionable. But there is still much debate about what should be reported, and to whom (the patient or the referring clinician). A litigious climate, especially in the USA, makes doctors nervous about what and how much to withhold or disclose.

Case study: a cautionary tale

The challenge of dealing with a VUS in clinical practice is well illustrated by an article in the *New York Times* in March 2016: a blood sample sent for genetic analysis from Angie Watts – who had a lumpectomy for breast cancer and was expecting follow-up radiotherapy – generated a VUS in one of the genes known to be involved in the rarer forms of familial breast cancer. On the basis of this, her doctor advised her to have a

preventative double mastectomy saying, “I am not a betting man”. When Ms Watts later asked a geneticist, she was advised this variant was not known to be harmful, so she should go ahead with the radiotherapy and ignore the advice to have radical surgery. In the end Ms Watts said “they left it up to me to decide”. She decided to go ahead with just the radiotherapy but has to live with the uncertainty of her genetic test result. The article sums up the problem: “The ability to understand and interpret genetic tests will surely improve. But for now, what sounds like a simple test can leave patients with frightening information but no clear options or guidance for treatment decisions”.



Ethical Considerations

Consent

A prerequisite for any kind of diagnostic or research testing is the patient's 'fully-informed consent'. But it is unrealistic to expect patients to be able to consider all the possible outcomes of testing, especially as the doctor taking consent will not necessarily know what they are (the 'unknown unknowns'). Ideally patients or research subjects should be able to say what classes of result they would wish to know: every variant, every potentially pathogenic variant, actionable pathogenic variants only, or nothing except for any directly diagnostic finding. Consent must not be over-burdensome to the patient or the doctor, but it must be realistic about the uncertainties and should build in mechanisms for revised interpretation in the light of new knowledge. Currently a variety of protocols are being debated and explored in trials. Part of consent for any whole genome sequencing (WGS) research study must consider that the principle of anonymisation or de-identification of samples – a basic principle for using samples for research – is no longer truly possible. Apart from identical twins, WGS information will be unique to one single individual, so complete genetic privacy can never be guaranteed.

Testing children

When a child is ill, genetic tests may be carried out to confirm a diagnosis. Under these circumstances, testing is regarded as being in the best interests of the child as it may directly influence clinical management and treatment. However, a principle of genetic testing in children has been to avoid testing for conditions that do not have any implications in childhood, as most people agree that children should have the right to choose whether to be tested when they grow up. This would include testing for autosomal dominant, late-onset conditions which would affect an individual only after reaching adulthood, or for carrier status for autosomal recessive conditions such as cystic fibrosis which would have no effect on the carrier's health at any stage, but could have consequences for their future offspring.

However, some people think WGS for babies is likely to be an inevitable part of future newborn screening programmes, in which case a protocol for delivering

this information would be needed. One US study – *BabySeq* – looking at the potential of WGS in newborn babies found that over three-quarters of parents of healthy babies declined to let their babies be tested; in the long run public response might limit such a development.

Testing for predisposition to common complex conditions

In the 1990s, some enthusiasts claimed that advances in genetics put medicine on the edge of a transition from a ‘diagnose and treat’ to a ‘predict and prevent’ model. Geneticists who disputed this were accused of ‘sleepwalking into the 21st century’. How likely is this? The major targets for prediction, as a starting point for attempts at prevention, must be the common diseases of later life. As described in GIM2 (pages 10-14), these often have a degree of genetic predisposition, but genetics does not determine who will and who will not develop them. Research has indeed identified many genetic susceptibility factors, but even when taken in combination they seldom allow a useful prediction of whether or not an individual will develop one of these diseases. They just change the risk a bit, up or down. By and large, hopes for a general ‘predict and prevent’ strategy have been wound down or relegated to a distant future. This doesn’t stop some commercial organisations offering direct-to-consumer (DTC) ‘lifestyle’ genetic testing to healthy individuals, analysing some risk factors for common diseases. However, the regulators – concerned about adverse effects – are monitoring these companies closely, and in 2013, the US FDA ordered one company, 23andMe, to stop marketing its ‘Personal Genome Service’.

The main achievements of the new technologies are in improved diagnosis, and the main hope of precision medicine, at least for the foreseeable future, is for better targeted treatment. As we will document in the rest of this booklet, rare diseases and cancer are the main areas where there has been substantial progress to date.

Precision medicine treatments for rare diseases

In the past, patients with genetic conditions often had few options when it came to treatment. Most genetic diseases are individually rare, although some 3,200 genes are known to be linked to rare diseases, and in total they affect at least 1 in 50 of the population. Drug companies had little incentive to spend millions of dollars developing new drugs for tiny numbers of patients (so-called 'orphan' drugs), and the clinical expertise relevant to any particular condition was likely to be concentrated in one or two centres, often far away from where affected patients and their families lived.

Today, governments internationally have recognised that these patients – like those with more common disorders – are entitled to expect medical treatment. Expanding knowledge of the underlying biology raises the possibility of designing treatments targeted at individual gene defects. Because these disorders are rare, there is a need for strengthened international collaboration so that knowledge can be pooled, accessed and applied in new treatments. The International Rare Diseases Research Consortium (IRDiRC), launched in 2011, now has over 40 member organisations from four continents.

Precision medicine for rare diseases uses knowledge of the underlying mutations and resulting protein defects to design individual therapies that aim to correct the specific problem in the patient. These therapies are diverse: drugs and molecules that target specific mutations or defects, applying old drugs to new problems, novel IVF techniques, gene therapy and gene editing. We show a number of typical examples below, of which the first three are from the world of metabolic medicine, illustrating that some of the principles of precision medicine have been around for some time.

Dietary management of genetic conditions

There are some genetic conditions for which successful dietary management has been available for decades. The best known of these is phenylketonuria (PKU), where affected individuals cannot break down phenylalanine derived from protein in their diets. If undiagnosed, these children develop significant intellectual impairment, caused by toxic build-up of phenylalanine in the blood and brain. Since the 1960s, PKU is tested for in all newborn infants in the UK (by

the heel-prick test), and PKU babies are 'treated' by a lifelong regime of a diet of virtually protein-free foods to which is added a special formula containing all the amino acids – except phenylalanine – in protein. This is a challenging regime, but, if adhered to, will support completely normal development.

Enzyme replacement therapy

Type I Gaucher disease is an example of an **enzyme** deficiency that has been treated successfully by replacement therapy since 1991. A person with Gaucher type I can have an enlarged liver and spleen, bone and blood abnormalities, fatigue and intestinal complaints. Patients with debilitating symptoms receive regular infusions of replacement enzyme (beta-glucosidase).

Drug treatments

Drugs targeting the effects of enzyme deficiencies

Nitisinone (Orfadin®) is a drug that has been used to treat tyrosinaemia type I since 1991. Left untreated, tyrosine and its by-products build up in tissues and organs, leading to serious health problems, and children with tyrosinemia type I often do not survive beyond 10 years. Previously the only treatment was liver transplantation. Now, nitisinone treatment combined with dietary restriction of the amino acid tyrosine is used to prevent build-up of the damaging toxin.

Drugs targeting specific mutant proteins

Cystic fibrosis (CF) is a life-limiting genetic condition leading to severe respiratory symptoms. It is caused by mutations in the CF transmembrane conductance regulator gene (*CFTR*), that codes for a chloride **ion** channel in the membrane of specialised cells, in particular in the lungs. Absence of the functional CFTR protein results in the accumulation of mucus, promoting airway obstruction, chronic infections and ultimately lung failure. Advances in treating CF have significantly increased survival, so that in the UK about half of all people born with cystic fibrosis will live past 40. To further increase life expectancy, doctors and scientists are working to find treatments that address the specific defect associated with each individual *CFTR* gene mutation.

There are many different mutations in the *CFTR* gene that can cause CF. The most common one – F508del (**deletion** of the DNA **codon** coding for the amino

acid phenylalanine at position 508 in the protein) – stops the protein moving to the cell membrane. A new drug, lumacaftor, brings the CFTR to the cell surface, while another, ivacaftor, increases chloride transport by binding to CFTR to open the channel. The combination has been approved for patients with the F508del mutation – even though clinical trials showed a modest effect – because of a lack of other options. Meanwhile ivacaftor on its own brings significant improvement in symptoms for CF patients with some other specific CFTR mutations that affect the ion channel function.

Drug ‘repurposing’ – new applications for old drugs

Recent advances in the understanding of the biological basis of many rare conditions have led researchers to infer that some established drugs, previously used for a quite different purpose, might be effective treatments.

mTOR inhibitors in Tuberous Sclerosis Complex. Tuberous sclerosis complex (TSC) is a serious genetic condition that affects various parts of the body, causing benign tumours in the brain, lungs, heart and kidneys, as well as neurodevelopmental problems, including seizures and learning difficulties. Understanding the underlying biology of TSC has resulted in the identification of existing drugs which can be used as a treatment for TSC.

Mutations in either of the two main genes that cause TSC cause changes to proteins (hamartin and tuberin) that normally inhibit the activity of an important signalling pathway known as mTOR, which in turn affects many different cell processes. mTOR inhibitors are a group of drugs used in advanced cancers and in **immunosuppression** following transplantation; they act by inhibiting the same pathway that is hyperactive in TSC. Clinical trials have shown that these drugs – whilst not curing the condition – shrink the size of some tumours in TSC, and are now used as part of the treatment regime.

Anti-sense therapy

Spinal muscular atrophy (SMA) is a lethal autosomal recessive progressive motor neurone disorder, caused by a deletion or mutation in the survival of motor neuron 1 (*SMN1*) gene. A nearly identical ‘backup’ gene, *SMN2*, has a single **base-pair** change that causes the exons of around 90% of mRNA molecules to be spliced in a way that results in a non-functional SMN protein.

Research is concentrating on how to reduce that 90% figure.

Nusinersen is a short length of synthetic DNA, manufactured to be complementary and bind to a specific part of the *SMN2* mRNA (an anti-sense oligonucleotide or ASO) to increase the proportion of molecules that are correctly spliced. The treatment is radical, as the drug is delivered to the patient by direct injection into the spinal canal. However, the results are promising and so nusinersen is currently in advanced clinical trials. As mentioned in GIM1, similar trials have been conducted in boys with Duchenne muscular dystrophy (DMD).

Gene therapy

The goal of gene therapy for genetic conditions is to replace faulty genes with working ones. In the 1980s and 1990s, at the height of the gene-hunting bonanza, the discovery of disease gene variants raised hopes that it was only a matter of time before we would be able to correct those genes in a patient. It soon became clear that the reality was different. The practical obstacles to putting genes into patients safely, efficiently, and effectively, were too great in any but a small number of conditions.

To date the greatest success of gene therapy has been in treating immunodeficiencies – genetic disorders of white blood cells – where cells can be easily removed, treated and replaced. But there is now renewed optimism that gene therapy, perhaps in conjunction with the gene editing and stem-cell therapies described below, will be a useful therapy for a limited number of conditions where the tissue of interest is relatively accessible.

Gene therapy for cystic fibrosis

In 2015 a clinical trial in 136 CF patients demonstrated a modest, but significant, improvement in lung function. The functioning *CFTR* gene was carried in a **lipid** bubble, which was incorporated – albeit inefficiently – into the cells on the surface of the lungs. It was delivered using a nebuliser, which creates a mist inhaled into the lungs. A second trial is planned for 2017, using a virus to deliver the working gene into lung cells. This method has the advantage that a virus will deliver the working gene more effectively by infecting the lung cells; but the virus will cause the gene to be inserted into

the cells' DNA, with the risk that it might disrupt key genes and create a malignancy.

Gene therapy for Leber's congenital amaurosis

Leber's congenital amaurosis is a progressive disease of the **retina** that severely impairs sight in children. One form is caused by variants in the *RPE65* gene. In 2015, the results of a trial were published in which doctors used a virus to deliver a working gene by injection – under anaesthetic – to the retina of the eyes of 12 patients whose visual function was then checked over the course of three years: six patients showed some temporary improvement; three patients showed inflammation in the eye, and two showed significant deterioration. Whilst any improvement was modest and marginal, this study provided a basis for a second trial using a new, more powerful gene delivery mechanism.

Gene therapy for severe combined immunodeficiency

Babies with severe combined immunodeficiencies (SCID) are unable to produce T- and B-cells – essential cells of the immune response – because of mutations in one or other essential gene. SCID patients are susceptible to recurrent infections and without treatment often die within the first year of life. One form, ADA-SCID, is caused by absence of the enzyme adenosine deaminase. In 2016 the European Medicines Agency (EMA) approved a gene therapy treatment for ADA-SCID patients for whom no suitable matched human stem cell donor (for a bone marrow transplant) could be found. The patient's own bone marrow cells – including blood **stem cells** – are removed and genetically modified to insert a working copy of the *ADA* gene. The modified cells are infused back into the patient, and some find their way back into the bone marrow where the stem cells give rise to functioning T- and B- cells. A similar technique to treat X-linked adrenoleukodystrophy (ALD) is undergoing trials.

Gene editing

Gene editing is an exciting new technology that has potential applications across all branches of biological science from medicine to bacteriology, agriculture and plant science. Rather than attempting to insert genes into a cell, as in gene therapy, it enables the existing DNA to be modified in specific ways.

A number of gene editing techniques have been used in research for some years, but the recent enthusiasm comes from a new technique, CRISPR/Cas, that for the first time provides a simple and efficient way to introduce specific sequence changes into the genome of living cells. Based on enzymes from bacteria whose normal function is to cut up the DNA of invading viruses, it has unleashed an explosion of innovation that has many possible applications.

In medicine, gene editing could pave the way to the development of new more accurate gene therapy treatments for rare genetic conditions by correcting the gene mutation in the relevant tissue directly. CRISPR/Cas has already been used to create **transgenic** animals, and in 2015 scientists reported its successful use to correct muscular dystrophy in mice. In April 2015, a Chinese group reported its first application to early human **embryos**.

Case study: Layla Richards – a wonder of gene editing

In 2015, one-year-old Layla Richards was suffering from advanced leukaemia that was unresponsive to chemotherapy and bone marrow transplantation. As a final attempt to save Layla, doctors requested a special licence to use a novel immunotherapy. The therapy was based on gene-edited T-cells, which play an important role in identifying and killing cancer cells. The T-cells from a healthy donor were engineered to express a synthetic protein that recognised, targeted and killed the cancer cells in Layla. Most immunotherapies require a personalised immunotherapy for each patient; this therapy used T-cells from a healthy donor which were gene edited to inactivate the genes for proteins that would identify them as foreign. So the donor T-cells escaped recognition by antibodies in Layla's system. Three months following treatment Layla was well with no sign of cancer. However, she will not be regarded as cured until she has been cancer-free for five years. Further trials are being conducted to find out whether this miracle cure was due to the T-cell treatment, or some other factor.

There is a vigorous debate about the potential for this technology to be used in early human embryos, with the possibility of altering the **germ line** (the eggs and sperm cells) such that future generations of offspring will also carry the 'corrected' gene. In 2016 the UK Human Fertilisation and Embryology Authority (HFEA) authorised researchers to modify human embryos up to 14 days using CRISPR/Cas – but none of these experiments, in the UK or elsewhere, has involved re-implanting a modified embryo to produce a modified baby. There is

universal agreement that this technology should be thoroughly tested before any application in human patients, whether adults or embryos. This is likely to take many years of research. Additionally, there is the ethical question about the extent to which we should be allowed to create so-called 'designer' babies.

Three-parent embryos – preventing mitochondrial disease

As mentioned in GIM2 (pages 5 & 32), mitochondria (the structures in cells that produce energy) contain a tiny genome (16,569 base-pairs of mtDNA encoding 37 genes, compared to the six billion base-pairs of DNA and 20,000 genes in the nucleus). Mutations in mtDNA can cause a range of rare, serious diseases. All the mitochondria in an embryo come from the egg, and none from the sperm. So, if a woman has a pathogenic mtDNA mutation, all her children will inherit it. Her only current way of avoiding this is to use donated eggs or to adopt a child.

New reproductive technologies might help women with mitochondrial disorders have genetically related children free from mtDNA disease. One method is to take the nucleus from the would-be mother's egg – leaving the abnormal mitochondria behind – and to transfer it to a donated egg with healthy mitochondria from which the nucleus has been removed and discarded. The egg is then fertilised with the father's sperm. So the resulting baby would receive nearly all its DNA from its mother and father, with a tiny contribution from the donor 'mother'; hence the rather misleading term 'three-parent embryos'.

The UK 2008 Human Fertilisation and Embryology Act was amended in 2015 to allow this type of technology, but a licence is still awaited from the HFEA as it requires further safety assurances; until then it cannot be carried out in the clinic. If licensed, it will be the first technique to authorise genetic changes that will be carried over from generation to generation. Therefore, there is great concern to ensure that the technique is safe; those who argue against it say it is far too early to be confident. Furthermore, some object on moral or religious grounds, asserting that this technique is part of a 'slippery slope' towards 'designer' babies. Nevertheless, it was reported in September 2016 that this technique was used by a US team in Mexico to enable a couple to have a baby free of Leigh syndrome, a serious and fatal mitochondrial disease.

Progress in stem-cell therapies

Stem cells are unspecialised cells that have the remarkable potential to generate many different types of cells in the body. When a stem cell divides, each new cell can either become another stem cell or a different type of cell with a more specialised function, such as a muscle cell, a blood cell or a brain cell. In many tissues stem cells serve as an internal repair system, dividing to replenish other cells. In the era of precision medicine, it is these characteristics of stem cells that offer the prospect of new treatments for many serious medical conditions. Although some precision stem cell treatments have reached the clinic (such as the gene therapy in stem cells from SCID patients in the previous section), many are at the research stage.

It is important to note, too, that stem cell transplantation in the form of bone marrow from live, often related, donors has been used for many years to treat individuals with genetic conditions and blood cancers (eg: SCID, Hurler **syndrome** and acute myeloid leukaemia). In these cases, treatment will include lifelong immunosuppression to prevent rejection; precision stem-cell treatments – using the patient's own stem cells, together with editing of the deleterious gene – offer the prospect of a personalised cure.



Human blastocyst

Embryonic stem cells

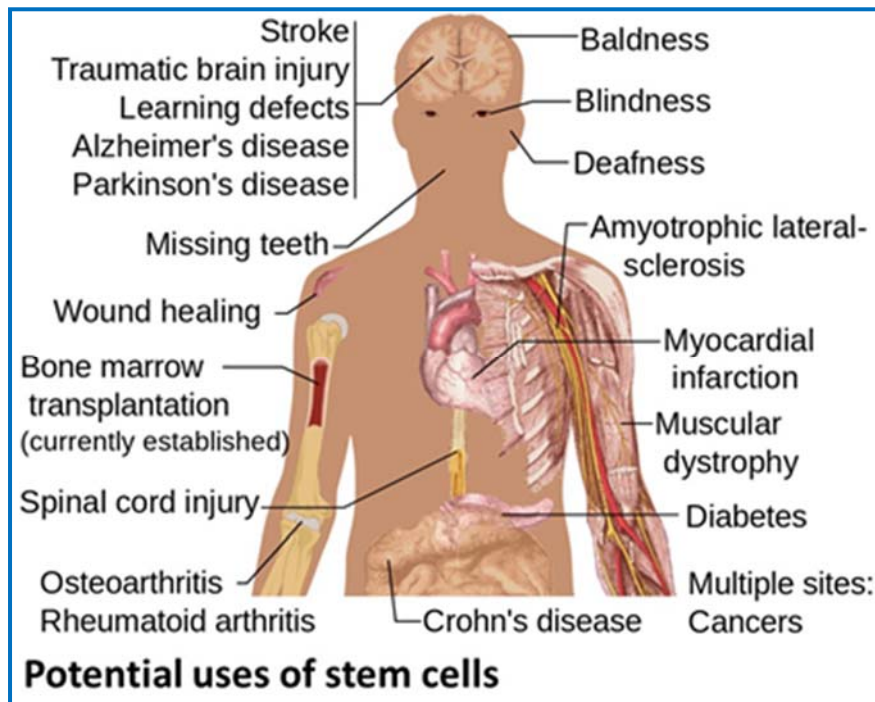
Human embryonic stem (ES) cells are found in the blastocyst (left), the stage of embryo development at 5-7 days. ES cells are pluripotent and can differentiate into *any* of the different specialised cells of the body. Adult stem cells are tissue-specific. In some adult tissues, such as the gut and bone marrow, stem cells regularly divide to repair and replace worn out or damaged tissues. In other organs, however, such as the pancreas and the heart, stem cells only divide under special conditions. A more detailed description of the types of stem cells can be found in the Galton Institute Occasional Paper: *An Introduction to Stem Cells*.

Stem cells - key developments

- 1950s – Successful transfer of nuclei from frog embryos, later by somatic cell nuclear transfer (SCNT) from an adult frog, into unfertilised frog egg cells, showing that cell nuclei could be reprogrammed to make cells pluripotent.
- 1996 – creation of Dolly the sheep by using SCNT from an udder cell of an adult sheep into an unfertilised sheep egg cell that had its nucleus removed; the manipulated egg was able to develop into Dolly.
- 1998 – stem cells from early human embryos grown successfully in the laboratory.
- 2007 – successful reprogramming of specialised cells to the pluripotent state (induced pluripotent stem cells – iPS). Hailed as 2008 Breakthrough of the Year. But eight years later there were still significant problems with this method coupled with safety concerns that have so far prevented it being a serious contender for treatment.
- 2014 – The first cloned human embryonic stem cell lines created using SCNT from adult human cells.

In the UK, research on human embryos is allowed by law up to 14 days post-fertilisation. In spite of the early promise of iPS cells, somatic cell nuclear transfer (SCNT) from the patient's cells into human ES cells is seen as the best potential source of error-free pluripotent cells for medical use, creating a source of cells that are a perfect genetic match to the patient, thus overcoming the challenges of rejection and immune suppression. Such personalised treatments will depend on the development of reliable methods for creating matched tissues, which, in 2016, was still some years away.

Embryos used in such research are 'spare', donated by couples undergoing IVF; they would otherwise be destroyed. In 2016 – for the first time – a human embryo was grown in the laboratory up to the day before the legal limit of 14 days; at this stage the embryo is smaller than a grain of rice, the organs have not yet formed but the appearance of the 'primitive streak' is the first sign of the nervous system developing, and is the point at which it can no longer split to form identical twins. This event was greeted with dismay by people who have religious or ethical objections to the use of human embryos for research, as they consider it wrong to create a human embryo that is not intended eventually to develop into a baby.



Teams of scientists and doctors are investigating how regenerative medicine and stem cell science could be used to treat many different diseases; we do not have room to examine them all here, but have selected examples showing recent research progress in diabetes and multiple sclerosis.

Type I diabetes

Type I diabetes is a complex condition with a strong genetic component; it is the **autoimmune** form of diabetes, caused by the patient's own immune system turning on the insulin-producing cells in the pancreas. Progress in the treatment of type I diabetes has included transplantation of the pancreas, or of kidneys for patients with kidney disease. Such transplant recipients will have to undergo lifelong immune suppression, but are released from constant blood sugar monitoring and regular insulin injections. In 2014, there were two exciting developments in research into precision treatments that brought hope to people with Type I diabetes. One team successfully derived

pluripotent stem cells by nuclear transfer from **somatic cells** of a woman with Type I diabetes into an enucleated human egg cell. This was the same 'cloning' technique that had been used to create Dolly, and one of the first successful attempts to produce **cloned** human cells. Since the cells produced are pluripotent, they could be induced to differentiate to form insulin-producing pancreatic cells; the therapeutic aim will be to transplant these back into the patient's pancreas, curing the diabetes without risk of rejection and the need for immunosuppression. A second team successfully converted human embryonic stem cells into insulin-secreting cells that were shown to reverse diabetes in mice. This work is immensely promising; however, years of further research and testing will be required to refine these techniques and to guarantee safety for use in human patients.

Multiple sclerosis

In 2016 a highly experimental stem cell therapy for multiple sclerosis (MS) was hailed as a breakthrough. MS is another autoimmune disease with an hereditary component, in which the immune system attacks the **myelin** sheath that surrounds the nerve cells of the **central nervous system** (CNS). Blood stem cells were collected from 24 patients with severe disease and a poor prognosis; they were then given strong chemotherapy to kill off the cells of the immune system, followed by transplantation of their own blood stem cells. The results were remarkable: nearly all patients had no further progression of the disease, whilst one third showed sustained improvement. However, one patient died as a result of transplantation-related complications, so this is a very risky procedure which is undergoing further clinical trials.

Pharmacogenetics

In GIM2 we described briefly how people respond differently to drugs. How:

- a drug that is effective in one person might not work in another, or require a much higher dose to work;
- a drug that is well tolerated in most may trigger a side effect in some;
- ineffective drugs waste time and money and delay relief for the patient;
- adverse drug reactions are a major cause of sickness in patients and also a drain on NHS resources.

Adverse drug reactions (ADRs) are a major drain on NHS finances and resources	Response rates of patients to a major drug for a selected group of therapeutic areas	
<p>A study of 18,820 consecutive admissions to two large UK general hospitals in 2001-2 concluded that:</p> <ul style="list-style-type: none"> • 6.5% of admissions were related to ADRs • 2% of patients admitted for ADRs died • The projected annual cost to the NHS was up to £466 million <p>Pirmohamed <i>et al</i>, <i>BMJ</i> 329: 15-19; 2004</p>	Therapeutic area	Efficacy rate (%)
<p>Variable drug responses – a problem for the NHS</p>	Alzheimer	30
	Analgesics	80
	Asthma	60
	Cardiac Arrhythmias	60
	Depression	62
	Diabetes	57
	Incontinence	40
	Migraine (acute)	52
	Migraine (prophylaxis)	50
	Oncology	25
	Osteoporosis	48
	Rheumatoid arthritis	50
	Schizophrenia	60
Physician's Desk Reference 54 th edition 2000		

A variety of factors contribute to the variable responses of patients to a drug. These include a patient's general health, other diseases they may have, and other drugs they may be using, their age, sex and weight, lifestyle factors (smoking, drinking, diet etc), but a major influence is genetics. The study of the role of genetic variation on the safety and efficacy of drugs is called pharmacogenetics. In some cases, as with some of the cancer drugs described later in this booklet, acquired mutations can affect the function of a critical protein or pathway, and that then becomes the target of a drug's action, determining its response. Often, however, the difference in response is the result of variation in our **constitutional genomes**, and these variants influence all aspects of the way drugs work. The hope for pharmacogenetic studies is

that they will help avoid adverse drug reactions by identifying susceptible patients before treatment and improve drug efficacy by tailoring treatment to the patient's genotype.

How does genetic variation influence drug action?

Our bodies possess elaborate sets of enzymes whose job is to detoxify and eliminate potentially harmful compounds present in our diet. The same enzymes act to metabolise drugs. People vary quite widely in the efficiency of the different enzymes, and so they also vary quite widely in how rapidly they eliminate particular drugs.

Genetic variation can affect how a person reacts to a drug in two ways: it can affect the absorption, distribution, **metabolism** and elimination of a drug (ADME), or it can affect the way a drug acts on its target (a cellular enzyme or receptor, for example).

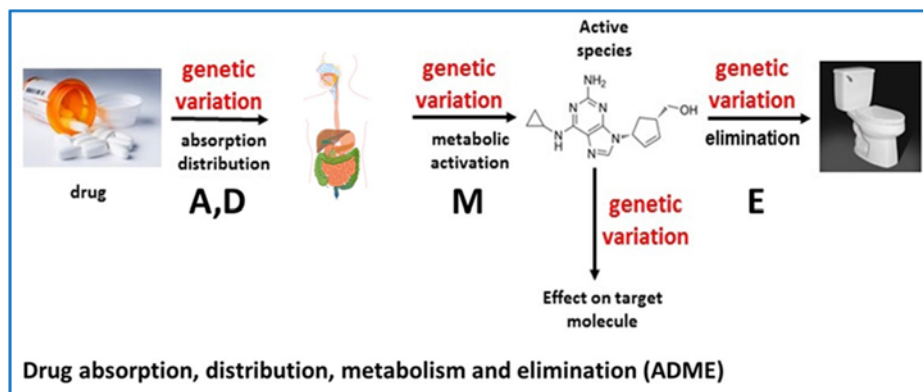
Absorption, distribution, metabolism and elimination (ADME)

Variations in **Absorption** – membrane proteins are important for transporting drugs taken by mouth from the stomach and intestines into the bloodstream.

Variations in **Distribution** – after entering the bloodstream, the drug needs to reach its target organ. This may be an active process involving specific protein transporters.

Variations in **Metabolism** – many drugs must undergo enzymic conversion to the active molecule. For example, codeine is converted into the active drug morphine by the enzyme CYP2D6. Some people perform this conversion more efficiently than others. People with a very low activity variant of CYP2D6 gain no benefit from codeine.

Variations in **Elimination** – drugs are not usually simply flushed out of the system. Eliminating a drug usually involves one or more enzymic reactions to convert it into a form that can be eliminated from the body. Somebody who eliminates a drug rapidly will need a higher dose of the drug to be effective, while somebody who eliminates it slowly will be exposed to the drug much longer, and may suffer overdose effects when given the standard dose.



In GIM2, we used the common anti-coagulant warfarin to illustrate how variants in the enzymes CYP2C9 and the warfarin target VKORC1 can affect the requirement for the drug from person to person.

P450 cytochromes

There are many different proteins that contribute to ADME of different drugs. One of the largest families is the P450 cytochrome group of about 60 iron-containing enzymes, found in the liver where they add an oxygen atom to some molecules, including some drugs. They are responsible for the initial stage in metabolism of an estimated 60% of all drugs.

Perhaps as a result of strong selective pressures operating at particular times during evolution, many of these enzymes vary considerably between individuals. To take one example as illustration: CYP2D6 is highly variable, with over 90 known genetic variants, among the first pharmacogenetic variants to be recognised. CYP2D6 alone is involved in metabolism of about 25% of commonly used drugs, including: antidepressants (eg, amitriptyline, citalopram), antipsychotics (eg, chlorpromazine, haloperidol), anti-arrhythmics (eg, flecainide, mexiletine), beta-blockers (eg, metoprolol, timolol), opioid analgesics (eg, codeine, morphine) and anti-cancer agents (eg, gefitinib, tamoxifen).

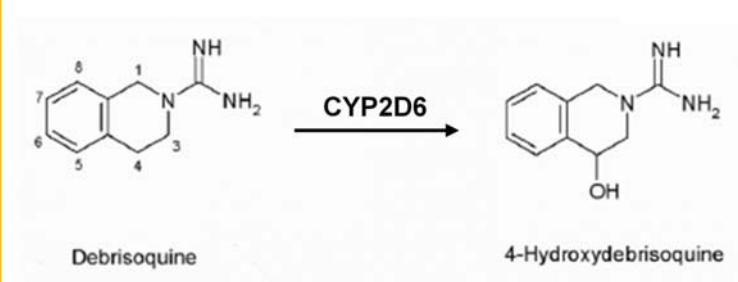
For practical purposes, copies of the gene can be classified into those with increased, normal, reduced or absent function.

Case study: Professor Robert L Smith and debrisoquine

Debrisoquine was a drug that was used to control high blood pressure. It is no longer in use but it was the subject of an important historical episode.

In 1975, acting in the tradition of the times when scientists experimented on themselves, Bob Smith, a laboratory director at St Mary's Hospital Medical School in London, ingested 32 mg of debrisoquine, as did some of his co-workers. His later account of his adverse response to the drug went: "Within two hours severe orthostatic hypotension [low blood pressure] set in with blood pressure dropping to 70/50 mm Hg. Hypotensive symptoms persisted for up to two days after the dose...". His colleagues, who had taken a similar dose, had no significant effects.

The first step in elimination of debrisoquine is a reaction that is mediated by the CYP2D6 enzyme, producing 4-hydroxydebrisoquine.



Analysis of this metabolite in the urine of the volunteers revealed that the extreme sensitivity was associated with a greatly decreased ability to carry out this reaction.

A later study of a larger number of participants led to the description of a genetic **polymorphism**, and eventually individuals were divided into four classes of 'ultra-rapid', 'extensive', 'intermediate' and 'poor' metabolisers, which reflect the variation in the activity of CYP2D6. Professor Smith was, of course, a 'poor' metaboliser.

The variable effect of a drug on its target molecule

The most dramatic effects of this type are seen in anti-cancer drugs that are designed to inhibit one specific mutant form of the target (see pages 36-39). A prime non-cancer example is the effect of variants of VKORC1 on warfarin requirement as described in GIM2. Another non-cancer example is the ACE inhibitors.

ACE inhibitors: example to illustrate the variable effect of a drug on its target

Captopril and enalapril are used for treating hypertension and heart failure; they act by inhibiting the activity of the angiotensin-converting enzyme (ACE). The ACE enzyme converts the inactive peptide hormone angiotensin I into angiotensin II, which regulates blood pressure. A common variant in the *ACE* gene affects the quantity of the enzyme that the gene produces. One version (known as D) produces more enzyme than the other (I). Individuals with two copies of the D version have about twice the level of circulating ACE as people with two copies of the I version. So, ACE inhibitors may be more effective in lowering blood pressure in DD patients than in II patients.

Angiotensin converting enzyme genotype	Effect of genotype on levels of ACE	Effect of genotype on blood pressure	Effect of ACE inhibitor drugs on lowering blood pressure
II	lower	lower	lesser
ID	intermediate	intermediate	intermediate
DD	higher	higher	greater

Adverse drug reactions

Adverse drug reactions (ADRs) are classified into two types:

Type A reactions increase or diminish the normal effect of a drug. They are generally dose-dependent and predictable, and usually the result of DNA variants that are common in a population. The case of debrisoquine causing a dangerous drop in blood pressure in CYP2D6 poor metabolisers is an example. Such cases account for 80-95% of adverse drug reactions.

Type B reactions cause idiosyncratic rare adverse effects that are dose-independent but unpredictable from the normal action of the drug; they are more likely to result from rare variants in genes whose products are only peripherally related to the main metabolism and action of a drug.

Carbamazepine is one such case and is described in GIM2. A further case is brought about by the deficiency of the enzyme Glucose-6-phosphate dehydrogenase (G6PD).

Case study: a Type B adverse drug reaction – a problem with anti-malarial drugs

History: Some African-Americans suffer a haemolytic crisis (resulting from the destruction of red blood cells in the circulation) after receiving the anti-malarial drug primaquine. It was after it caused problems during the Second World War among GIs sent into malarial environments that the US army was prompted to fund investigation into its cause.



Experiments in the early 1950s showed that the problem was an abnormality of red cells. In 1956 the abnormality was shown to be a relative deficiency of the enzyme G6PD. As recently as 2008, the anti-malarial drug LapDap® was withdrawn from the market due to ADRs in individuals with G6PD deficiency.

Biochemistry: G6PD is an important enzyme in red cells. G6PD-deficient cells are more sensitive to damage, and although most G6PD-deficient people are completely asymptomatic, many triggers – including primaquine and other drugs – can induce haemolysis. The commonest trigger is infection; another cause is eating broad beans. This reaction, known as favism, has been known since ancient times.

G6PD deficiency is the commonest of all human enzyme deficiencies, affecting over 400 million people worldwide. The global distribution closely parallels the distribution of malaria. As in sickle cell disease, there has been selection in favour of G6PD deficiency because carriers are relatively resistant to malaria.

Genetics: The *G6PD* gene is on the **X-chromosome**. Thus females have two copies but males have only a single copy, so the deficiency is mostly seen in males, whilst females can be carriers.

A further complication is that many drugs themselves activate or repress certain drug-metabolising enzymes. As a result, one drug can affect the metabolism of another, so that certain combinations of drugs are either ineffective or dangerous. As we age some of us require more drugs to keep us going, so these problems are particular concerns for older adults. Details of all these effects can be found in the PharmGKB database (<https://www.pharmgkb.org>).

Companion diagnostics

An increasing number of drugs are prescribed with a 'companion diagnostic', a genetic test to determine how the patient will respond. Most of these tests are for tumour characteristics in the treatment of cancer (see page 40). But it is likely that these will become more widespread, particularly as pharmaceutical companies try to maximise return on investment in the drug development pipeline. It can take 15 years and cost \$1 billion to bring a new drug to market; most of this cost is in the later phases. Yet only 11% of drugs that pass through the early stages of safety testing actually make it through to the market. Furthermore, rare adverse reactions may only become apparent after 100,000 or more patient exposures. This can force an

otherwise promising drug to be withdrawn post-marketing – a major financial, reputational and legal disaster for the manufacturer. In the future, better understanding of the basis of these rare ADRs could lead to a licence for a companion diagnostic to ensure the drug is prescribed only to those who are least likely to suffer harm. The main factor holding back wider application of such tests is the time delay in getting the test result: both patients and doctors want a quick consultation leading to an immediate prescription. The development of bedside genetic testing devices promises to eliminate this obstacle.



Bedside genotyping

New gadgets such as this – the size of a mobile phone – promise to deliver immediate 'point-of-care' testing and quick results.

Image courtesy QuantuMDx Group Ltd

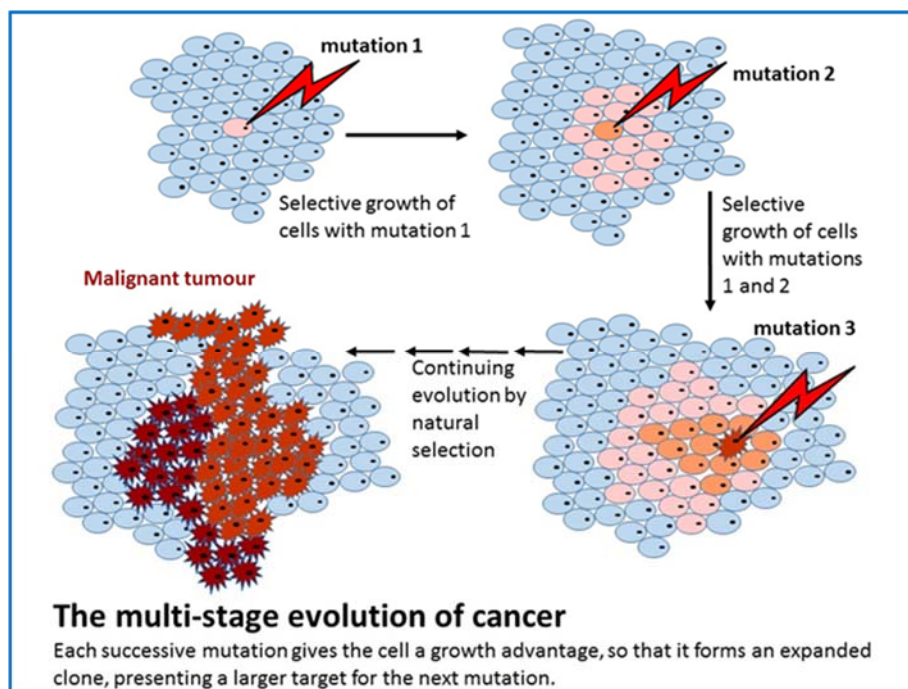
Cancer

What is cancer?

Cancer is the name given to a number of related diseases which are a major cause of morbidity and mortality in the UK. Cancer occurs when the usual controls on cell growth and division are lost and cells grow into **tumours** with the potential to invade tissues and spread to other parts of the body.

Cancer – a product of evolution

In GIM2 we explained how natural selection acts among the cells of our body just as it acts in populations of whole organisms. If one cell acquires a **somatic mutation** that allows it to divide faster than the surrounding cells, then we would expect the progeny of this cell to outgrow the other cells in the tissue. Therefore, multicellular organisms have a natural tendency to develop tumours. To resist this, all the processes involved in cell proliferation are very tightly controlled.

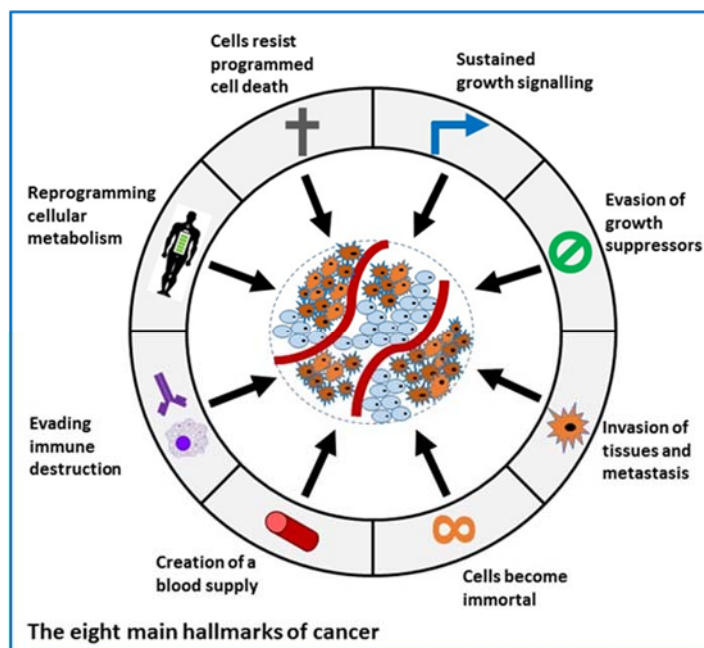


Whilst cancer is not a genetic disease in the sense that it is seldom inherited, it *is* a genetic disease in that it is caused by **mutations** in genes responsible for cell growth and division. Changes to every cell's genome can occur during the lifetime of an individual, but their accumulation in specific critical genes can cause cells to grow and divide in an uncontrolled manner, resulting in cancer. This is why cancer is, most often, a disease of old age. Mutation occurs as a result of many different processes, including chance errors in the process of **DNA replication** during cell division; damaging **radiation** leading directly to DNA breakage, or chemicals (carcinogens) modifying the structure of **nucleotides** in the DNA sequence.

Mutations in a number of different genes are required in the process of **oncogenesis**. This results ultimately in the eight main hallmarks of cancer as defined by Douglas Hanahan and Robert Weinberg in 2011.

The multiple mutations that are needed to

escape all the different controls on cell division can involve any of a large number of genes. Cancer cells usually have destabilised genomes and high mutation rates, spawning innumerable random irrelevant 'passenger' mutations in addition to the so called 'driver' mutations that actively promote cancer development.

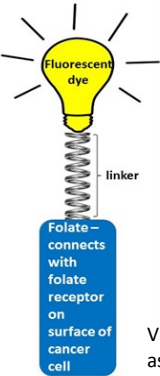


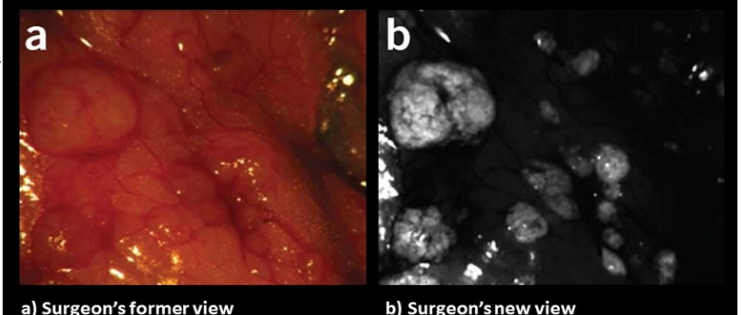
Defining the cancer genome

No two tumours are genetically identical. Large international projects – such as the Cancer Genome Atlas project, started in 2005 – aim to understand the cellular pathways disrupted in cancer, documenting the changes in patients by comparing the genomes of their tumour cells with their healthy cells. The results allow classification of cancers by molecular mechanisms instead of traditional methods, which rely on their tissue of origin and appearance under the microscope. For example, the UK Cancer Genome Project reported in 2016 that they had found probable ‘driver’ mutations in 93 individual protein-coding genes in breast tumours from 560 patients. Not all 93 genes were mutated in every tumour, but specific patterns were found that will help characterise tumours, define the prognosis and identify the best treatment.

Treatments in cancer

Treatment and survival rates for many cancers have improved dramatically over the latter part of the 20th century, with overall 10-year survival now at 50%. The most successful treatment remains the surgical removal of the





a) Surgeon's former view **b) Surgeon's new view**

View of part of the peritoneal cavity (in the abdomen) in a patient with ovarian cancer as seen with the naked eye (a) or with the aid of a tumour-targeted fluorescent dye (b)

Reproduced by permission from Macmillan Publishers Ltd: Nature Medicine, 2011; 17 1315-1319, copyright (2011)

Image guided biopsy and surgery

An example of a new development that brings precision medicine to surgery is the visualisation of a protein, called the folate receptor, that is over-represented on the surface of some cancer cells. The protein is responsible for the uptake of the B vitamin folic acid. A folate molecule is attached to a fluorescent dye via a linker and administered to the patient so that, under fluorescent light, the surgeon can clearly see the cancerous cells and remove them.

primary tumour. Surgeons are guided by X-rays, scans and by new targeted imaging technologies that show the exact extent of the tumour.

Patients have also benefited from advances in precision radiotherapy, including stereotactic radiotherapy, which uses precise measuring instruments to deliver highly focussed radiation; and proton beam therapy, which uses a precise beam of sub-atomic particles. Both methods kill the tumour cells without straying into and damaging surrounding tissues.

Chemotherapy drug treatment for cancer has also improved over the years, with new clinical trials for more specific treatments for individual cancers, more effective drug combinations, improved cancer symptom management and better control of side effects. But the older chemotherapy drugs are highly toxic as they kill not just cancer cells, but all rapidly dividing cells. This causes a long list of side effects, which includes hair loss, nausea, tiredness, weakness, immune suppression, constipation and diarrhoea. Furthermore, in the majority of patients, older chemotherapies have limited success. It is in the field of drug treatments for cancer that genetics is now playing a major role.

Precision drug treatments for cancer

During the early years of the 21st century we have seen gradual progression from generic treatments to precision treatments. Traditionally, a solid tissue tumour would be excised and subjected to classification according to its appearance under the microscope, and the treatment selected accordingly. Now that it is possible to identify the genetic changes that drive development of a particular tumour, it is becoming possible to design treatments that target these specific changes. Greater understanding of the many molecules and pathways involved in cancer means that new targeted cancer therapies are currently the focus of much anticancer drug development. Although only a minority of patients can currently benefit from this new approach, already over eighty targeted therapies have been developed for treatment of about thirty different cancers. Here we will look at how some of these molecules block the growth of tumours by interfering with target proteins.

Identification of targets for therapy

The development of targeted therapies requires the identification of a good

target; that is, one that plays a key role in the growth and survival of a cancer cell. It is for this reason that targeted therapies are sometimes referred to as the products of 'rational' drug design. Targets can be identified by studying proteins, mRNAs or the DNA of cancer cells.

- Proteins that are known to be part of pathways involved in cell growth or survival are potential targets when present – or are more abundant – in cancer cells but not normal cells. For example, some breast cancers over-produce the human epidermal growth factor receptor 2 (HER-2). This is a protein that makes cells grow and divide. Several targeted therapies are directed against such 'HER-2 positive' cancers. Herceptin® is a **monoclonal antibody** that attaches to HER-2 on cancer cells, blocking growth signals; it is approved for treating a number of aggressive cancers, sometimes in conjunction with other types of chemotherapy. Because it will have no effect on a cancer that does not express HER-2, has some rare but serious side effects, and is also very expensive, it is important to give the drug *only* to patients whose cancer is likely to respond.
- Cancer progression is often driven by abnormal proteins, the products of genes carrying 'driver' mutations. For example, the BRAF protein, which is part of a cell growth signalling pathway, is present in an altered form known as BRAF V600E in a wide range of tumours, including many melanomas. In the V600E variant the normal valine at amino acid 600 of the BRAF protein is replaced by glutamic acid. This leads to the BRAF signalling pathway being 'switched on' permanently. Zelboraf® targets this mutant form of the BRAF protein specifically, and is approved to treat patients with advanced metastatic tumours that express V600E (about 60% of melanomas). The drug initiates programmed cell death (**apoptosis**) in melanoma cells. Melanoma cells without these mutations are not inhibited; in this case the drug *stimulates* normal BRAF and may actually promote tumour growth. Again, this shows how essential it is to characterise the tumour in every patient to ensure not only that the treatment will be effective, but also to ensure that it does no harm.

- Some abnormal proteins are the result of chromosomal rearrangements present in cancer cells but not in normal cells. Sometimes particular **translocations** are typical of a certain type of cancer. They result in the creation of a novel gene out of parts of two different normal genes, and the novel gene encodes a 'fusion protein' that drives cancer development. Such fusion proteins are potential targets for cancer therapies. For example, Glivec® targets the BCR-ABL fusion protein, made from components of two genes, *BCR* and *ABL*, that get joined together in the cells of some types of leukaemia and permanently switch on the growth promoting pathway.

Developing targeted therapies

Once a candidate target has been identified, the next step is to develop a therapy that interferes with its ability to promote cancer cell growth or survival. A targeted therapy might reduce the activity of the mutant protein, prevent it from binding to a receptor that it normally activates, or interfere with other molecules in the same pathway. Most targeted therapies are either small molecules or monoclonal antibodies (MAbs). Small-molecule compounds are typically developed for targets that are located inside the cell because they are able to enter cells relatively easily. MAbs are relatively large and generally cannot enter cells, so they are used only for targets that are on the cell surface.

Small molecule drugs

Candidate drugs are usually identified by high-throughput screens, in which the effects of thousands of small molecules on a specific target protein are examined. Compounds that affect the target are then chemically modified to produce numerous closely related versions, which are then tested to determine which are most effective with the fewest unwanted effects on other molecules.

Monoclonal antibodies (MAbs)

MAbs are made by injecting animals (usually mice) with purified target proteins. This stimulates the animal to make many different types of antibodies against the target. Individual B-cells, each producing just one antibody, are converted into immortal cells called **hybridomas** that can be grown in quantity, then screened to identify ones that produce an antibody with the desired specificity

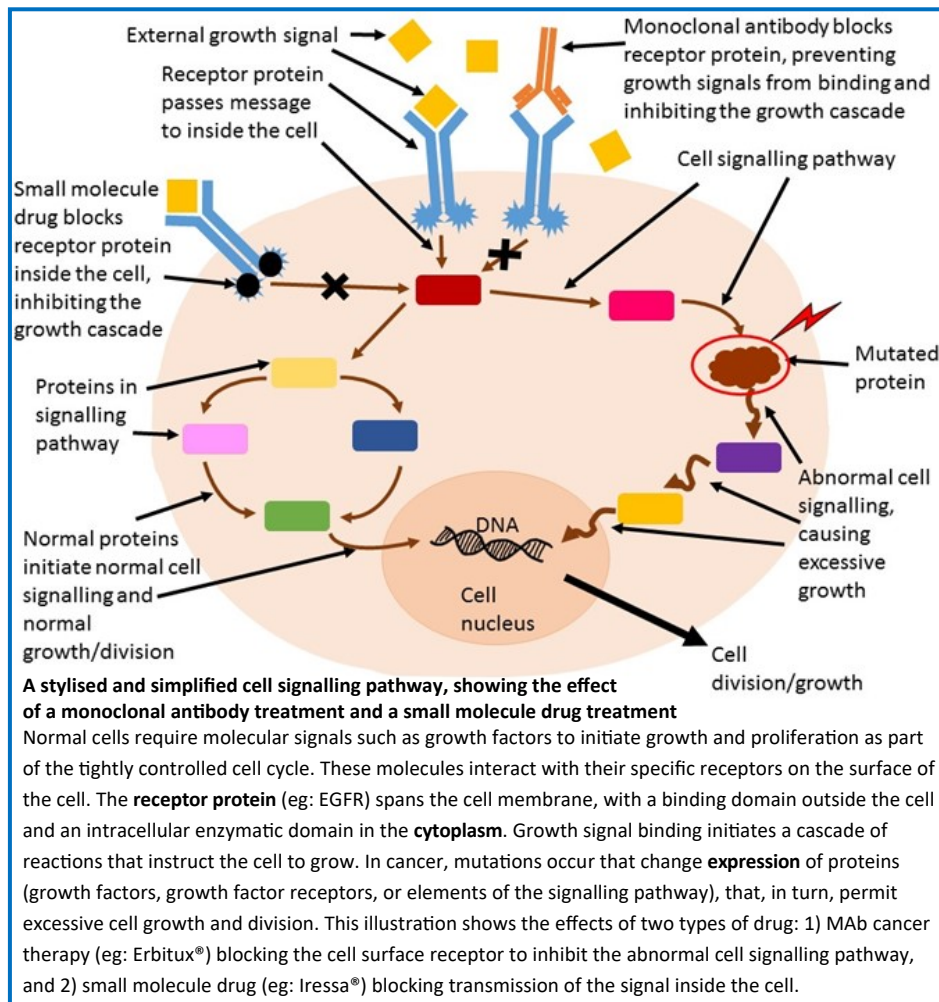
and effect. For cancer therapy, MAbs are sought that recognise single specific regions of proteins that are sometimes found in large numbers on the surfaces of cancer cells.

Therapeutic MAbs may work in a number of different ways. They may block signals instructing cancer cells to divide, trigger the immune system to attack cancer cells or carry drugs or radiation to cancer cells. Before MAbs are used in humans, the relevant genes in the hybridoma are modified by replacing as much as possible of the mouse sequence with corresponding human sequence. Humanising is necessary to prevent the human immune system from recognising the antibody as foreign and destroying it before it has a chance to bind to its target protein. This is not an issue for small-molecule compounds because they are typically not recognised by the immune system.

Current targeted therapies for cancer

Many different targeted therapies have been approved or are under development for use in cancer. The following list is not all-inclusive but covers the major types. Sometimes a therapy will fall into more than one category.

- ***Hormone therapies.*** Hormones carried in the bloodstream act as chemical messengers. They have several effects and one of these is controlling the growth and activity of certain cells and organs. Some tumours are hormone-dependent and need these signals to grow. Preventing production of the hormone or blocking its activity can slow down or stop the growth of the tumour. A well-known hormone therapy is tamoxifen, used to reduce growth signals in breast cancers with oestrogen receptors (see page 39).
- ***Inhibitors of cell signalling.*** When a cell receives a specific signal from its environment – eg, a growth-promoting signal – the signal is relayed within the cell through a series of biochemical reactions that ultimately produce the appropriate response. In some cancers, the malignant cells are stimulated to divide even in the absence of external growth factors. For example, the epidermal growth factor receptor (EGFR) is stimulated by epidermal growth factor (EGF) to initiate a raft of different processes within cells (especially skin, breast, colon and lung cells), including proliferation and survival. In some cancers EGFR is present in increased amounts or in



mutant forms that trigger cell proliferation. Signal transduction inhibitors (eg, Iressa® and Erbitux®) interfere with this inappropriate signalling.

- **Inducers of apoptosis.** Programmed cell death (apoptosis) is one method the body uses to get rid of unwanted or abnormal cells, but cancer cells have developed ways of avoiding apoptosis. Apoptosis inducers can get around these strategies, causing cancer cells to die. As mentioned above, the use of Zelboraf® in advanced melanoma ultimately acts by inducing apoptosis.

- ***Inhibitors of angiogenesis.*** A tumour needs a blood supply to grow beyond a certain size, as blood provides the oxygen and nutrients it needs for continued growth. The development of new blood vessels is called angiogenesis; treatments (eg, Avastin®) that interfere with this process help block tumour growth.
- ***Immunotherapies.*** Immunotherapies trigger the immune system to destroy cancer cells. Although cancer cells are abnormal, they develop from normal cells so they can be difficult for the immune system to recognise. Cancer treatment vaccines are designed to activate the immune system to recognise and attack specific types of cancer. An antibody that binds to specific molecules on the surface of cancer cells can trigger immune destruction of cells bearing that target molecule. This is one mechanism by which Herceptin® has its effect. In addition, the antibody itself may trigger cell death via apoptosis and other mechanisms. Other MAbs are designed to bind to molecules that inhibit the action of certain immune system cells, lifting the inhibition and making them more effective at killing cancer cells.
- ***Toxic monoclonal antibodies.*** So-called conjugated MAbs are linked to toxic drug molecules or radioisotopes. Once the antibody has bound to its target cell, its toxic payload is taken up by, then kills, the cell. The toxin will not affect normal cells that lack the target for the antibody.
- ***PARP inhibitors.*** Cells have two main mechanisms for repairing breaks in their DNA. DNA repair proteins such as BRCA1, BRCA2 and PALB2 are part of a particular repair pathway. PARP1 is an enzyme important for an alternative repair pathway. If either pathway is non-functional, the other can take over. Cancer cells often have mutations in genes for proteins such as BRCA1, BRCA2 or PALB2 that inactivate one pathway. Unlike normal cells they are therefore completely reliant on the PARP1 pathway, and so are vulnerable to drugs that inhibit PARP (eg, Lynparza®). The way that a combination of two defects, each individually survivable, causes the cell to die is called 'synthetic lethality'.

A table with examples of targeted cancer treatments and their mode of action is shown opposite.

Some examples of precision medicines for cancer and their action

Tissue/cancer	Drug brand name (generic name in brackets)	Type of therapy	Protein target	Mode of action
Breast	Many brands (Tamoxifen)	Hormone therapy (small molecule drug)	Oestrogen receptor (ER) inside breast cancer cells	Blocks oestrogen hormone receptor, preventing growth signals.
Breast	Herceptin® (Trastuzumab)	Monoclonal antibody immunotherapy	Human epidermal growth factor receptor 2 (HER-2)	Attaches to receptor on breast cancer cells; identifies them as targets for the immune system.
Ovarian (advanced)	Lynparza® (Olaparib)	PARP inhibitor	PARP1 enzyme	Blocks enzyme, preventing it from repairing single-stranded 'nicks' in DNA. Results in lethality in cancer cells.
Prostate (advanced cancer)	Provenge® (Sipuleucel-T)	Cancer vaccine immunotherapy	Prostate acid phosphatase (PAP)	Patient's own blood cells mixed with a manufactured molecule incorporating PAP to create immune active cells which are reinfused into the patient, stimulating a T-cell response to the cancer cells.
Skin/melanoma	Zelboraf® (Vemurafenib)	Small molecule drug	BRAF protein with V600E	Inhibits the enzyme function in the B-Raf protein only when it contains a V600E mutation; brings about programmed cell death (apoptosis).
Skin/melanoma	Yervoy® (Ipilimumab)	Monoclonal antibody immunotherapy	CTLA-4 – an inhibitor of T-cells	Binds to CTLA-4, an inhibitor of T-cells, enabling T-cells to attack and kill cancer cells.
White blood cells/leukaemia	Mabthera® (Rituximab)	Monoclonal antibody immunotherapy	CD20 – a protein on the surface of B-cells	Binds to CD20 on the surface of leukaemic cells, identifying them as targets for the natural killer cells of the immune system.
White blood cells/leukaemia	Glivec® (Imatinib)	Small molecule drug	BCR-ABL fusion protein	Targets BCR-ABL fusion protein and turns off its enzyme activity; inhibits cell signalling; blocks growth pathway.
Lymphatic system/ Lymphoma	Zevalin® (Ibritumomab)	Monoclonal antibody – linked to radio isotope	CD20 – a protein on the surface of mature B-cells, but not stem cells	Binds to CD20 on B-cells, allowing radiation from conjugated isotope to kill cells; healthy stem cells take over.
Colon, lung, head and neck cancer	Erbix® (Cetuximab)	Monoclonal antibody therapy	Epidermal growth factor receptor (EGFR)	Binds to EGFR on the outside of cells, preventing binding of EGF and blocking growth signals.
Lung/various cancer	Iressa® (Gefitinib)	Small molecule drug	Epidermal growth factor receptor (EGFR)	Blocks enzyme action of EGFR on the inside of cells, blocking growth signals.
Various advanced cancers	Tagrisso® (Osimertinib)	Small molecule drug	EGFR T790M positive tumours	Blocks enzyme action of EGFR with drug resistant T790M mutations, blocking growth signals.
Various advanced cancers	Avastin® (Becacizumab)	Monoclonal antibody therapy	Vascular endothelial growth factor (VEGF) on cell surface	Blocks action of VEGF, stopping growth of blood vessels (anti-angiogenesis) to tumour.

Combating acquired resistance

Targeted treatments can have a dramatic immediate effect on a patient's condition, but remission is followed by relapse and overall survival is only usually increased by months rather than years. Just as prolonged use of antibiotics eventually results in the evolution of strains of resistant bacteria, eventually a tumour will evolve a clone of cells that is not susceptible to the original treatment. For example, most EGFR⁺ tumours treated with Iressa® will eventually develop resistance; of those, two thirds will carry a particular mutation, T790M, in which the amino acid methionine replaces threonine at position 790 in the EGFR protein. T790M blocks the insertion of the Iressa® molecule into the enzymatic domain of EGFR, rendering the drug ineffective. A second-line – and eventually third-line – therapy will be needed.

For these more aggressive tumours, a new molecule – osimertinib (Tagrisso®) was specifically engineered to overcome the barrier created by the T790M resistance mutation. After early clinical trials, the EU gave approval in February 2016 for use of this drug in metastatic EGFR T790M-positive non-small cell lung cancer that had progressed from the earlier therapy. Osimertinib was the first new medicine to be approved under the European Commission's expedited process: it took under three years from the start of clinical trials to approval, as compared to 12-15 years in a traditional drug development programme.

Combination therapies

In 2016 it was reported that melanoma patients showed a dramatic improvement in survival when given a cocktail of two drugs (Yervoy® and Opdivo®) that block different inhibitors of T-cells. Some tumours showed complete regression. Future precision approaches will include multiple agents and drugs that target different points in the pathways that get disrupted, in order to kill cancer cells before resistance has a chance to develop.

Companion diagnostics in cancer treatment

As targeted treatments only work for tumours with specific characteristics, and are usually ineffective and sometimes harmful in tumours without those characteristics, it is important to have quick and reliable tests that let doctors know the exact make-up of a tumour. Many companies are working to develop

so-called ‘companion diagnostics’ that test for the mutation that indicates a particular proposed treatment. For example, in 2009, the US Food and Drug Administration (FDA) approved cetuximab – which blocks the EGF receptor (EGFR) – for treatment of colon cancer, but only those with **wild type** (normal) KRAS (a protein coded for by another gene often mutated in cancer), since the drug had little or no effect in colorectal tumours harbouring a *KRAS* mutation. This was the first genetic test to guide treatment of cancer. In 2012, the FDA approved a companion diagnostic test for *KRAS*. Since then a number of others have been developed, including one to test for different activating mutations in *EGFR* to tell doctors whether Iressa® is the right treatment for the patient.

Non-surgical testing – ‘liquid biopsies’

Assessing the prognosis for a cancer patient normally requires a surgically removed sample of the tumour for analysis of its appearance under the microscope and identification of potential treatment targets, either by **histochemical** or DNA-based techniques. After this, the patient has to recover from surgery, then must endure any post-operative treatment. It is clearly not desirable to have further invasive intervention unless absolutely necessary, and so until recently it has generally not been possible to monitor the genetic evolution of a tumour at the molecular level.

Scientists have discovered that not only can modern technologies detect circulating tumour cells in the bloodstream, but also that minute quantities of circulating tumour DNA – shed by tumour cells as they die – is found in **plasma**. Using ultra-sensitive methods, this DNA can be analysed to identify the particular mutations present in the tumour.

This technology of ‘liquid biopsies’ may radically alter the future management of cancer by enabling monitoring of tumour progression without invasive biopsies. It is particularly useful for early identification of resistance mutations such as T790M in *EGFR*, so that appropriate action can be taken before the tumour progresses too far. Furthermore, surgical biopsy can sample only one or two sites at once; a liquid biopsy would potentially include all the information from different clones within the tumour, and from

all sites of **metastasis**. Such a technique might even be adapted as a pre-symptomatic screening test for various cancers.

The world's first registration of a companion diagnostic based on liquid biopsy was made in 2015 to assess *EGFR* mutation status in plasma samples from patients with non-small cell lung cancer. In the future this technique will be used for simultaneous detection of multiple drug resistance mechanisms.

Cancer therapy – a positive outlook

Full genetic analysis of a patient's tumour offers the opportunity to truly personalise therapy. An individual treatment would be a bespoke multi-drug cocktail, simultaneously targeting several cancer-specific proteins. It's early days, but such an approach offers the tantalising future possibility of a precision medicine therapy that would finally offer a cure for cancer, rather than an extension of life-span, often amounting to just a few months of extra life.

The future of precision medicine

Precision medicine has the power to revolutionise how we diagnose and treat disease. Knowledge of individual genomes accelerates biomedical discovery and helps explain susceptibility to illness and routes to recovery. Doctors have new tools to understand better the complex mechanisms underlying a patient's condition and to predict better which treatments will be most effective. From the use of next generation sequencing in the diagnosis of genetic disorders, to the identification of the right dose of a medicine, the editing of a deleterious gene or the selection of the appropriate therapy for cancer, genomic analysis is now important for treatment of a raft of different conditions. More detailed understanding of gene interactions and networks is also helping to develop new therapeutic approaches, including repurposing existing drugs, as well as developing new compounds. The potential of precision medicine has only just started to be realised. Whilst recognising the ethical and practical challenges, it offers the future prospect of new personalised treatments for patients with conditions previously regarded as incurable, and renewed optimism to the supporters and beneficiaries of biomedical research.

Glossary

Allele – one of two or more alternative forms of a gene that arise by mutation.

Amino acids – organic compounds that are the building blocks of proteins.

Apoptosis – cell death occurring as a part of an organism's normal growth/development.

Autoimmune – caused by antibodies or lymphocytes produced by a person's own immune system against substances naturally present in the body.

Autosomal – of any chromosome except the sex chromosomes (X and Y).

Autosomal dominant – inheritance pattern of a character that can be seen when a person has a single copy of the variant allele.

Autosomal recessive – inheritance pattern of a character that is only seen when both copies of a gene have the variant allele.

Base-pair – A single letter of DNA sequence, one base paired with its complement base on the opposite strand.

Central nervous system – the nerve cells of the brain and spinal cord.

Chromosome – one of the DNA-protein packages into which the human genome is packed into the cell nucleus.

Codon – sequence of three bases which form a unit of genetic code in DNA or RNA.

Clone – a DNA sequence, cell or whole organism that is an exact genetic copy of another.

Coding DNA – DNA containing the **genetic code** for a protein.

Complex inheritance – where a condition that can have different causes, or combinations of causes so that no single genetic model or mode of inheritance can fit it.

Constitutional genome – a person's genomes as inherited from the parents.

Cytoplasm – the substance of a cell between the cell membrane and the nucleus.

DNA – deoxyribonucleic acid, the ultimate repository of genetic information.

DNA replication – the synthesis of new DNA strands from the template of the existing DNA.

Deletion – a missing segment of a gene or chromosome.

Electrocardiogram (ECG) – A recording of the electrical activity of the heart.

Embryo – the earliest stages of development in the womb up to ~9 weeks post-fertilisation.

Enzyme – a protein catalyst, acting to bring about a specific biochemical reaction.

Exon – the DNA of a gene that is represented in the mature messenger RNA (**cf intron**).

Exome – the totality of exons in the genome.

Expressed gene – a gene that is 'switched on' to produce the protein it encodes.

Gene – the unit of heredity, a sequence of DNA, transferred from a parent to child.

Gene panel – a set of genes chosen for simultaneous next-generation sequencing.

Genome – the totality of genes or genetic material of an individual.

Genetic code – the information encoded within the DNA of the genome that is translated into proteins by living cells.

Genotype – the genetic constitution of an individual (at one or more loci, or over the whole genome) Cf. **phenotype**.

Germ cells – the gametes (the sperm and egg cells).

Germ line – the line of cells that produce the gametes.

Histochemistry – the microscopic identification of constituents of tissues.

Hybridoma – cell produced by fusion of a tumor cell with a normal antibody-producing

cell, which then proliferates, yielding large amounts of a single 'monoclonal' antibody.

Immunosuppression – the suppression of a person's immune response.

Incidental finding – an unintentional discovery of genetic testing for unrelated issues.

Intron – a section of the DNA of a gene that is present in the primary RNA transcript, but which is removed during processing of the messenger RNA (cf. exon).

Ion – an atom or molecule with a positive or negative electrical charge.

Lipid – fat-like molecules; cell membranes comprise a double lipid layer.

Metabolism – the chemical processes that occur in a living cell or organism.

Metastasis – the spread of cancer to a new part of the body.

Monoclonal antibody – an antibody produced by a single clone of cells or cell line and consisting of identical antibody molecules.

Mutation – (that which causes) a change in the DNA sequence in a genome.

Myelin – a mixture of protein and lipid forming an insulating sheath around many nerve fibres, increasing the speed of nerve impulses.

Natural killer cells – cells of the immune system circulating in the bloodstream.

Non-coding DNA – DNA that does not code for protein. It may however still have important functions, encoding functional RNAs or controlling gene expression.

Nucleotide – the basic unit of DNA or RNA, consisting of a base (normally adenine, guanine, cytosine or thymine in DNA; adenine, guanine, cytosine or uracil in RNA), a sugar (deoxyribose in DNA, ribose in RNA) and a phosphate.

Oncogenesis – the process by which cancerous tumours develop.

Phenotype – the observable properties or behaviour of an organism. Cf. **genotype**.

Plasma – the fluid part of blood, in which the white and red cells are suspended.

Polymorphism – (of a gene) the existence of two or more forms of a gene in a population.

Primary tumour – the first occurrence of a tumour, prior to spreading (metastasis).

RNA – ribonucleic acid – closely related to DNA, deoxyribonucleic acid. RNA molecules are very heterogeneous and have many different functions in the cell.

Radiation – emission of energy as electromagnetic waves or high-energy subatomic particles.

Receptor protein – a molecule, often in a cell membrane, which responds specifically to a particular neurotransmitter, hormone, antigen, or other substance.

Retina – a layer of cells at the back of the eye that is sensitive to light.

Somatic cell – a body cell, as distinct from a **germ-line** cell. The genotype of somatic cells is not transmitted to the next generation.

Somatic mutation – a mutation that takes place in a somatic cell.

Stem cell – an undifferentiated cell of a multicellular organism, capable of giving rise to cells of the same type, or – by differentiation – other kinds of cell.

Syndrome – a characteristic combination of clinical features occurring together.

Transgenic animal – one with a gene from a different species inserted into its genome.

Translocation – a chromosomal rearrangement in which two chromosomes swap segments.

Triplet – of DNA, a codon.

Tumour – an abnormal growth creating a mass of tissue; benign or malignant (cancerous).

Wild type – original characteristic or allele, distinct from an atypical variant or mutant type.

X chromosome, Y chromosome – the sex chromosomes. Males have one X and one Y, females have two Xs.

Further Information

(All websites accessed 26 September 2016)

The Sanger Centre - educational resources

<http://www.yourgenome.org/>

Next generation sequencing technique

<http://www.yourgenome.org/video/sequencing-at-speed>

CRISPR-Cas9

<http://www.yourgenome.org/facts/what-is-crispr-cas9>

Exome Aggregation Consortium (ExAC) database

<http://exac.broadinstitute.org/>

The International Rare Diseases Research Consortium (IRDiRC)

<http://ec.europa.eu/research/health/index.cfm?pg=area&areaname=rare>

RDCRN Clinical research

<https://www.rarediseasesnetwork.org/>

The pharmacogenomics knowledge base

<https://www.pharmgkb.org/>

PHRMA – Medicines in development

<http://www.phrma.org/science/meds%E2%80%90in%E2%80%90development>

Cancer Genome Atlas

<https://cancergenome.nih.gov/>

Genetics in Medicine 3. Precision Medicine

This is the third booklet in a series on genetics in medicine, published by The Galton Institute. This booklet is about the growing field of precision – or ‘personalised’ – medicine, and is aimed at non-specialists with an interest in the area.

During most of the 20th century, medical treatment was based on disease symptoms and routine clinical investigations. This approach was very successful for some patients, but not for others. In the early years of this century, it became clear that information embedded in patients’ genomes could help more accurate diagnosis and targeted treatment of some illnesses. This booklet describes some of the main advances.

