Understanding recent advances in genomic testing within paediatric oncology

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Abstract

Advancements in genomics have had a significant impact on our ability to diagnose and treat several health conditions, notably cancer. In this article, we discuss the evolution of genomic methods from the early first- generation genome sequencing methods such as Sanger sequencing to the latest technology. We also discuss how clinicians can decide which test to use depending on what type of result is required. We discuss the clinical significance of genomic testing including identification of cancer predispositions syndromes, the role of genomics in accurately diagnosing a number of cancers, as well as the utility of genomic data to predict response to targeted therapies. Finally, we detail some of the targeted therapies currently available to treat the various types of childhood cancers. The expansion of genomic testing within the NHS and the essential nature of genomic data to accurately diagnose and optimally treat childhood cancers also poses challenges to the healthcare system both in terms of infrastructure and personnel.

Introduction

The WHO estimates that worldwide there are 400,000 new diagnoses of cancer in children and adolescents aged 0-19 each year (WHO). Traditionally, chemotherapy, radiotherapy and surgery have been the mainstays of paediatric cancer treatment, but recent advances in the understanding of the genetic basis of tumours has led to the development of highly targetable, precision therapies, that have dramatically increased survival for paediatric cancer patients.

An introduction to genomics & the evolution of genomic sequencing

Every single cell in our bodies contains the genetic information required for life and the combination of all the DNA in our cells is known as our genome. Our genome is comprised of sequences of bases or nucleotides Adenine (A), Cytosine (C), Guanine (G), Thymine (T) and Uracil (U) that form genes. DNA sequencing involves the use of various methods to decipher the exact order of the nucleotides that make up an organism's DNA.

If the DNA in a cell is mutated, it can disrupt the usual processes of the body and lead to diseases such as cancer (NHS England). There are now a variety of technologies available that allow us to sequence the whole genome or look at just parts of it – these technologies look for mutations or variations within the genome that explain why some people have rare or inherited diseases and some cancers. These can involve single gene mutations, or a complex combination of genetic and environmental factors (NHS England). In addition to sequencing an individual's genome, we can sequence the genome of a tumour to identify the mutations that have led to the development of malignant cells. Knowledge of the specific mutations within a tumour has resulted in the development of treatments directed at the specific genetic error.

Historically, multiple key scientific discoveries paved the way for the development of DNA sequencing, genomics and ultimately precision medicine in use in paediatric oncology today (Figure 1).

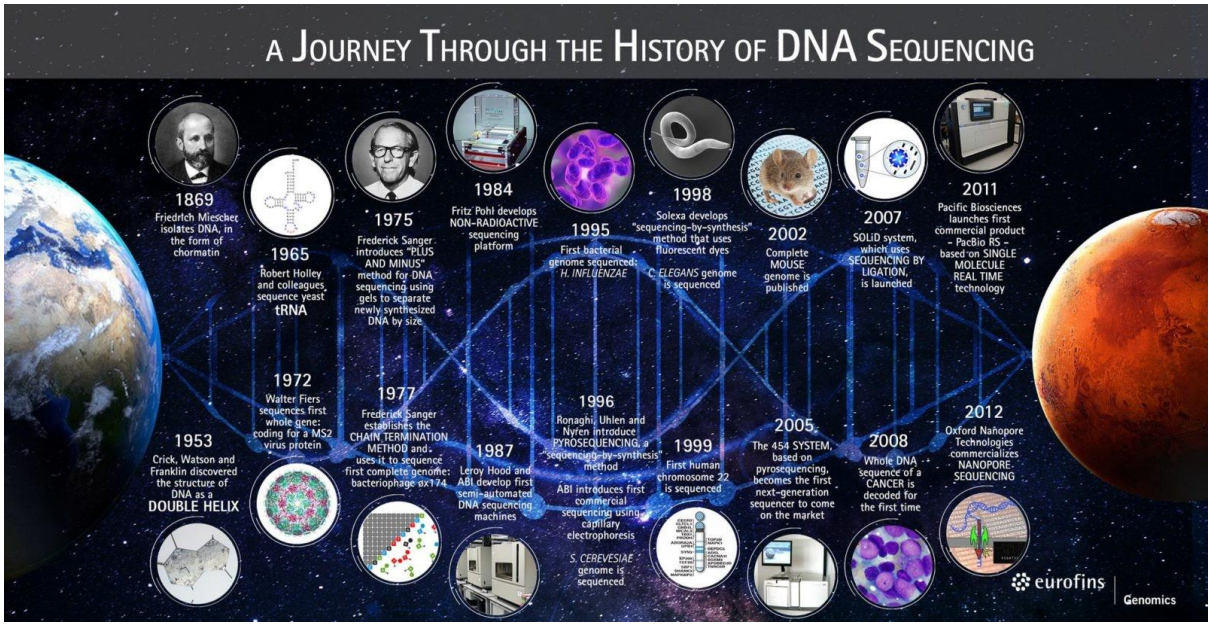

Figure 1: The History of DNA Sequencing through the years from the discovery of DNA to the development of multiple next generation sequencing technologies. Reproduced from Eurofins Genomics available at https://the-dna-universe.com/2020/11/02/a-journey-through-the-history-of-dna-sequencing/. With permission from the copyright holder (TBC).

Friedrich Miescher identified the presence of nuclein and other nuclear proteins as far back as 1869. In 1910, Albrecht Kossel discovered the 5 nucleotide bases and in 1950, Erwin Chargaff identified the pairings of A with T and C with G. In 1953, Watson & Crick discovered the double helix structure of DNA and subsequently, the first natural polynucleotide sequence was identified in 1965. Since then, Maxam & Gilbert devised their chemical degradation method of DNA sequencing, which was slow, laborious, and expensive. This was soon surpassed by automated Sanger 'first generation sequencing in 1977, which was used to sequence the first full genome. The Human Genome Project (1990–2003) provided vast amounts of data and the first public documentation of a complete human genome. Newer techniques have been developed which are able to provide results in a shorter time period, but this is usually at the expense of accuracy. The increased knowledge of genomics has led to the identification of an array of genes associated with paediatric cancers that now play a hugely important role in diagnosis, prognosis, patient stratification, participation in clinical trials and evaluation of response to treatment.

Which Test?

Depending on what we are trying to find out there are a range of different tests we can use. We can choose how much of a genome we want to analyse, whether to sequence the DNA, or quantify changes in the amount of genetic information, or which genes are on which chromosome. Different tests are more suited to certain circumstances than others.

Quantification of Genetic Material

These are methods of looking at the genome to see if there is the expected number of chromosomes or genes, and whether it is packaged in the way we would expect.

Karyotype – are there 46 chromosomes? Do the chromosomes look the expected size?

FISH – Fluorescent in-situ hybridisation. Using specific probes to attach to parts of a chromosome or gene, we can identify duplications, deletions or translocations.

Microarray (array CGH) - a high resolution view of the whole genome looking for copy number variants (either deletions or duplications). There are two different types of microarray:

- Array Comparative Genomic Hybridisation (aCGH) is a method of comparing a patient's DNA with a reference DNA and looking for areas where there are differences in the amount of genetic material. It will not identify copy number neutral loss of heterozygosity.
- Single Nucleotide Polymorphisms (SNP array) can have a higher resolution than aCGH and targets areas of the genome that are known to have differences between individuals. It can detect copy number neutral loss of heterozygosity.

Sequencing of Genetic Material

Targeted variant – analysis to look for a specific variant, or small number of common variants.

Single /targeted gene – sequencing of all the protein coding regions of a specific gene. This may identify several variants within this specific gene.

Panel testing – analysis of specific genes known to be implicated in a clinical condition. This may involve looking at less than 10 to several hundred different genes depending on the suspected condition. The rest of the genome is not sequenced.

Whole exome sequencing (WES)- This involves sequencing only coding parts of DNA and RNA that contain coding instructions (exons), as these are where many genetic mutations occur, and therefore can be extremely useful, despite not sequencing the entire genome.

Whole Genome Sequencing (WGS) – the most comprehensive method by which to sequence the entirety of an organism's genome. WGS can identify variants in any part of the genome as it includes chromosomal DNA, mitochondrial DNA, and sequences both coding (exon) and non-coding (intron) regions – this is a huge amount of data, approximately 3 billion base pairs.

Sequencing methods

A variety of different methods of obtaining genomic information have been developed over time. Each has its advantages and disadvantages, and sometimes a variety of different techniques are used together. Some techniques were developed in parallel while others have evolved from previous iterations. Most platforms use either a ligation reaction or a synthesis reaction to read sequences of varying lengths. The terminology used for the different processes can be confusing. Next Generation Sequencing (NGS) generally refers to 2nd generation sequencing techniques. Each of these techniques can be used to sequence all or some of the genome.

Commonly Used Techniques in Sequencing

Sanger (1st gen)

This was one of the first methods of DNA sequencing to be developed. It is a fast and cost-effective way of reading small, targeted regions of a genome. It is used in the diagnostic sequencing of a single gene, or in testing for a known familial sequence variant. It can confirm variants identified by NGS and fill in gaps in the data produced by NGS.

Illumina (2nd gen)

In this second generation technique the DNA is broken into strands 200-600 base pairs in length. Fluorescently labelled markers are added to the stands of DNA. Each base is read in turn, making it a highly accurate method. This is currently the most commonly used sequencer, in part due to the high precision and low cost, although it only has a short read length.

Roche 454 (2nd gen)

This second generation technique is faster, and therefore cheaper, than other methods, but is also less accurate. It can sequence the equivalent of a third of the human genome in one day. It uses polymerase chain reactions (PCR) to create identical copies of segments of the DNA sequence to aid the reading.

SOLiD (2nd gen)

Sequencing by Oligonucleotide Ligation and Detection (SOLiD) is similar to the Sanger technique, but it reads 2 bases at a time. Although very accurate, this method is only able to produce short reads and is a slow process.

SMRT (3rd gen)

Single-Molecule Real-Time (SMRT) sequencing is a 3rd generation sequencing technique that uses nanotechnology to read much longer read lengths (up to 20,000 base pairs). It is also able to provide some functional information as it shows methylated bases.

Ion torrent (3rd gen)

This method again uses PCR but instead of using fluorescence to enable the DNA to be read, computer chips are used measure tiny changes in the pH from each nucleotide.

Nanopore (4thgen)

Instead of breaking the genome into multiple small strands of DNA this technique is able to produce ultra-long reads. A single stand of DNA is passed through a tiny nanopore. The signals produced are converted to read the base nucleotides. This is a rapid process that enables the study of long stretches of the genome. This technique has been used to sequence the genome of viruses in recent outbreaks such as Zika, Ebola and SARS-CoV-2. In addition to its use in microbes, it has also been used to sequence human genomes opening up the potential for its use in cancer research and diagnosis.

Methylation

In addition to looking at mutations in the genes of tumour cells, methylation allows us to look at reversible DNA alterations. The presence or absence of methyl marks impacts the activity of the underlying genes, with the potential to either increase or decrease their activity. Using this technique has been shown to more accurately classify brain tumours than using traditional histopathology methods alone.

Clinical genomics

Clinical genomics is a rapidly evolving specialty in which doctors (clinical geneticists) and genetic counsellors work with an MDT to diagnose genetic conditions, evaluate patients' risk of developing genetic conditions and provide coordinated treatment, counselling and emotional support for patients and their families (Genomics England).

The assessment of an individual's genome can be done in a variety of ways depending on the level of detail required. Each method has its advantages and disadvantages. When we know someone has a specific variant, it can be helpful to understand whether this is an inherited or a de novo mutation, or if siblings are affected. In this situation, we can test for a specific variant without needing to sequence the whole genome. This can also be used when we are looking for a small number of common variants in a known condition.

In those patients with a specific clinical condition such as cystic fibrosis genetic testing can look for the mutations known to cause it. Specific panels are used to look for a range of known variants. Identifying the specific genetic variant is helpful for genetic counselling, but can also give an indication of the expected clinical course. Within oncology, panels are used to identify known genetic variants within the tumours that either have prognostic value, or the potential for treatment opportunities.

We also look for genetic variants that are known to impact how an individual responds or metabolises specific drugs, enabling us to form pharmacogenomic profiles and adjust dosing more accurately.
Since 2021, all children in the UK with a new diagnosis of a malignancy have been eligible for WGS. The testing and analysis of results is organised within geographically defined Genomic Hubs.

Following a diagnosis of cancer, patients and their families are given information about WGS and are consented using a nationally standardised record of discussion (RoD) form (Genomics Education Programme, England) and have the option to participate in the National Genomics Research Library (NGRL). In WGS for cancer, the genome of the tumour is compared to the patient's own genome (germline). Tumour sample is collected from blood, bone marrow or a biopsy (depending on the type of cancer) and is compared with a germline sample from blood,

saliva or skin. The samples are sent for genetic analysis which involves DNA sequencing, mapping of the small read sequences using bioinformatics software, with subsequent identification and filtering of variants identified. The sequences from the tumour and the patient's own cells are compared and the changes specific to the tumour (somatic variants) can then be analysed to look for mutations that resulted in tumour development. These somatic variants can be used to aid the diagnosis of specific tumours as well as identify possible mechanisms to target treatments at. Results are discussed in a multidisciplinary Genomic Tumour Advisory Board (GTAB) before being shared with the patient and their family. If indicated, a referral to a clinical geneticist is made to help the family understand the significance of the results. A summary of the current NHS Genomic Service Infrastructure is provided in Figure 2.

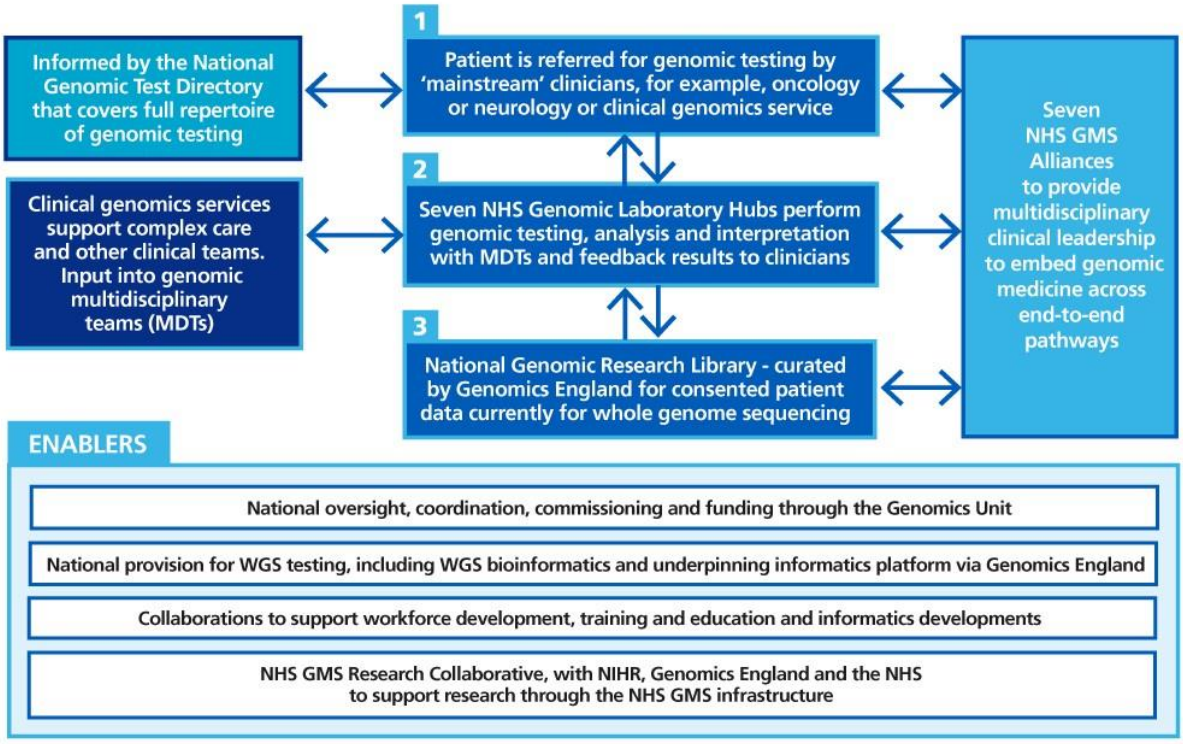

Figure 2: The infrastructure that makes up the NHS Genomic Medicine Service, including the enablers of the service. Reproduced from NHS England]available at https://www.england.nhs.uk/long-read/accelerating-genomic-medicine-in-the-nhs/]. With permission from the copyright holder (TBC).

The Clinical Application of Genomic Information within Oncology

Whole genome sequencing can be offered to patients with cancer and their families to find out if there are any genetic changes which have made the patient more likely to develop cancer. These can either be 'de novo' (new) or inherited, and may have implications for the wider family.

WGS can help us understand why patients develop cancer, what type of cancer they have, which treatments may work best, the risk of developing other cancers and whether there is an increased familial risk for developing cancer (NHS England).

Identification of Germline Cancer Pre-disposition Syndromes (CPS)

Germline genetic factors play a significant role in childhood cancer. Traditionally, the diagnosis of CPSs in children with cancer is based on clinical suspicion prompting referral to a clinical geneticist. However, the diagnostic approach is increasingly shifting toward a genotype-first approach. The incidence of CPS in unselected large cohorts is reported to be between 7-12% . Identifying CPS in children enables surveillance for early detection of second cancers and genetic counselling and testing of relatives.

Prognostication & Treatment Stratification

For many years we have been able to identify specific genetic changes within tumours that have identified them as having a worse outcome, allowing us to intensify treatment at an early stage. An example of this is MYC-N amplification in neuroblastoma. More recently, methylation techniques have enabled us to more accurately classify paediatric brain tumours than we were able to using histopathology techniques alone. In paediatric leukaemia, molecular characteristics of tumour cells is enabling the identification of those with high and low risk disease, enabling some patients to have their treatment de-escalated and preventing some of the long term toxicities associated with the treatment of childhood cancer.

Targeted Therapies

With continued advances in DNA research and genomics in, new drugs have been developed that can bind to pivotal regions of cancers cells, resulting in highly specific, targeted therapies that offer attractive alternatives to traditional chemotherapy, or additional treatments that

complement established treatment regimens. The main categories of targeted therapies considered here are

- immunotherapies
    - monoclonal antibodies
    - immune checkpoint inhibitors
    - vaccines
- cancer growth blockers, anti-angiogenics, and poly-ADP ribose polymerase (PARP) inhibitors

but the list is continuously evolving as new therapies are developed. Table 1 shows some of the more commonly used targeted treatments.

Table 1: List of commonly used targeted therapies

Class	Drug Name		Tumour Group
Monoclonal antibody	Rituximab		NHL
Monoclonal antibody	Blinatumamab		B-ALL
HER2 Monoclonal antibody	Trastuzumab	Herceptin	Breast cancer
Antibody – drug conjugate	Gemtuzumab ozogamicin	Mylotarg	AML
Chimeric monoclonal antibody	Dinutuximab beta		Neuroblastoma
Chimeric antigen receptor T-cell	CAR-T		B-ALL
PD-1 inhibitor	Nivolumab		Hodgkin's
CTLA-4 inhibitor	Ipilimumab		Melanoma, RCC
TKI	Imatinib		Leukaemia
mTOR inhibitor	Everolimus		Neuroendocrine tumours & RCC
BRAF inhibitor	Dabrafenib		LGG, LCH
MEK inhibitor	Trametinib		LGG, LCH
Anti-VEGF	Bevacizumab	Avastin	HCC, Colorectal tumours
2nd gen FLT3 inhibitor	Regorafenib		Liver & GI tumours
1st gen FLT3 inhibitor	Sorafenib		HCC & thyroid tumours
PARP inhibitors	Oloparib		BCRA+ breast & ovarian tumours
PARP inhibitors	Niraparib		BCRA+ breast & ovarian tumours
	Thalidomide		

<u>Immunotherapies</u>

Monoclonal antibodies are synthetic immune proteins that can be used to target cancer cells in a variety of ways. *Rituximab* was the first monoclonal antibody approved for clinical use in oncology patients – it is an anti-CD20 monoclonal antibody used in the treatment of non-

Hodgkin's lymphoma (NHL). Rituximab attaches to the CD20 antigen on the surface of B-lymphocytes and can cause cell death by complement activation, cell-mediated cytotoxicity or by directly signaling apoptosis.

Gemtuzumab ozogamicin (or *Mylotarg*) is another targeted therapy used primarily to treat acute myeloid leukaemia (AML). It is an antibody-drug conjugate (ADC) - a combination of an anti-CD33 monoclonal antibody and a cytotoxic antibiotic (Calicheamicin) that can selectively bind to cells expressing CD33 (most AML cells), into which it then delivers the cytotoxic drug, blocking cell growth and causing apoptosis.

Trastuzumab (Herceptin) targets the human epidermal growth factor receptor protein (HER2) that is often overexpressed in several paediatric solid tumours, most commonly medulloblastoma, nephroblastoma and osteosarcoma. Trastuzumab prevents cellular proliferation by inhibiting the activation of intracellular tyrosine kinase.

Dinutuximab beta is a chimeric anti-GD2 monoclonal antibody that targets the GD2 ganglioside cell surface marker commonly expressed on neuroblastoma and osteosarcoma cells, causing cellular lysis (Balaguer 2023).

Adoptive cell therapy is also known as T-cell transfer therapy is when T-cells are collected from a patient and grown in a lab under conditions that train them to fight cancer cells. Chimeric antigen receptor T-cell (CAR-T-cell) therapy is the best-known of this type of therapy, and is perhaps the most important breakthrough in cellular immunotherapy for pediatric malignancies. The process involves harvesting a patient's own T-cells and fusing them with the antigen binding portion of a monoclonal antibody targeting CD19 receptors which are present on the surface of most B-cells. This is then multiplied and returned to the patient, where they are able to specifically recognise and destroy cancerous cells. Although the initial side effects can be severe, it can be highly effective against relapsed leukaemia or lymphoma. This is currently being used for some patients with B-ALL, and the possibility of using CAR-T for other malignancies is under investigation.

The concept of using a vaccine to train the immune system to identify & destroy malignant cells has been hypothesised as a potential development in cancer immunotherapy. A novel

vaccine for neuroblastoma has been developed to identify and destroy any neuroblastoma cells lurking in the body after current standard treatment. More than 250 children have currently received the vaccine in a clinical trial, it is a bivalent vaccine because it targets two proteins found on neuroblastoma cells: GD2L and GD3L. It helps train the body to do the work of naxitamab (an anti-GD2 monoclonal antibody). This treatment is designed to help people who have no evidence of disease remain clear of neuroblastoma without needing ongoing naxitamab therapy.

Immune Check Point Inhibitors

This group of drugs are a form of immunotherapy which block specific checkpoint proteins, and may be named after the specific checkpoint proteins. Checkpoint proteins exist to prevent T cells from attacking healthy cells by turning the immune system off. Some tumours produce large numbers of these proteins, stopping the immune system from killing the cancer cells. Immune checkpoint inhibitors reverse this action. *Nivolumab* is a PD-1 inhibitor (programmed cell death protein 1) which is used in Hodgkin lymphoma and some renal tumours. *Pembrolizumab and cemiplimab* are also PD-1 inhibitors. Other immune checkpoint inhibitors target the CTLA-4 (cytotoxic T lymphocyte associated protein 4) or PD-L1 (programmed cell death ligand 1) checkpoints. The CTLA-4 and PD-1 checkpoints are found on T cells, whereas the PD-L1 checkpoints are on the tumour cells themselves.

CTLA-4 inhibitors such as *Ipilimumab* are used in melanoma and renal cell carcinoma, and PD-L1 inhibitors such as *Atezolizumab, avelumab, durvalumab* have been used in both solid tumour and haematological malignancies.

Cancer growth blockers (also referred to as kinase inhibitors)

Many of the genetic changes within tumour cells promote cell proliferation or prevent the usual cell death by encoding for an abnormal protein. Various therapies have been developed to block the abnormal pathway that tumour cells are using. If similar pathways are identified in other tumours, it raises the possibility that the same treatments may be useful. There are various different categories of growth blockers which each target different changes within the tumour's genes.

Patients with chronic myeloid leukaemia have an abnormal chromosome 22 known as the Philadelphia chromosome that has a gene mutation known as BCR-ABL. This mutation

encodes the abnormal BCR-ABL fusion protein (a tyrosine kinase enzyme) that promotes growth and prevents apoptosis (programmed cell death), which ultimately leads to tumour growth. *Imatinib* (Gleevec) is a tyrosine kinase inhibitor (TKI) that blocks this pathway. It has also been used in patients with acute lymphoblastic leukaemia who have either the Philadelphia chromosome or other BCR-ABL mutations.

Another pathway that has been identified within tumour cells is the mammalian target of rapamycin (mTOR), a protein kinase that regulates cell growth and proliferation. Inhibition of this pathway has shown tumour response in various tumours. *Everolimus* is used in renal cell carcinomas and neuroendocrine tumours.

There are a number of changes to the BRAF gene which can result in increased cell proliferation. The most common is the BRAF V600E. BRAF mutations occur in a variety of different tumours in various parts of the body. They may cause tumours to grow more quickly. In paediatric oncology, BRAF mutations are seen in gliomas, Langerhans' Cell Histiocytosis (LCH) and Non-Hodgkin Lymphoma. BRAF inhibitors such as *Dabrafenib* are used to prevent tumour growth. The BRAF protein is part of a larger mechanism called the PAS-RAF-MEK-ERK pathway, and so BRAF inhibitors may be used in combination with MEK inhibitors such as *Trametinib.*

The importance of angiogenesis (the production of new blood vessels) for tumour growth was identified back in the mid 20th century (Folkman, 1971). Since then various pathways have been identified and agents developed to prevent a tumour from utilising them. Agents such as *Bevacizumab (Avastin)* block the VEGF pathway for new blood vessel growth, whereas *Regorafenib* and *Sorafenib* are specific tyrosine kinase inhibitors (TKI) which block cell signaling within the cell, known as FLT3 inhibitors (fms-like tyrosine kinase 3 inhibitors). Sorafenib is a first generation FLT3 inhibitor, and Regorafenib is a second generation.

Tumour cells use a protein known as poly (ADP-ribose) polymerase (PARP) to repair their damaged DNA. Without this ability the damage would result in cell death. Drugs known as PARP inhibitors (*Olaparib & Niraparib)* have been developed to block the protein. The BRCA1 and BRCA2 genes are involved in cell repair, and hence a faulty BRCA gene increases the cancer risk. These drugs have been used in breast and ovarian tumours.

Not all targeted treatments are new drugs. Thalidomide is no longer a new drug, but it's action in blocking the signaling between cells has meant it has found a new role from that which it was originally intended. It has both an anti-angiogenic and immunomodulatory action.

Advantages and challenges to progress in genomics

There are many advantages to progress in genomics. Technical advances will result in faster methods, more accurate sequencing, lower error rates, lower amounts of input DNA/RNA, less reagents, and lower costs. The benefits of genome sequencing/genomic medicine include

1)identification of predisposing genetic markers

2) earlier identification of cancers

3) better identification of the underlying cause (in some cases)

4) improved diagnostic accuracy

5) better targeted treatment selection and therefore increased effectiveness with fewer side effects and adverse reactions (NHS England).

The main challenge posed by progress in genomics is the vast amounts of data generated, which requires storage, the time needed to generate and process this data and personnel with sufficient expertise to bring together the integrated diagnosis. The added cost is a significant consideration.

Conclusions

Genomics can help to identify cancer-specific proteins used to produce effective / personalised anti-cancer vaccines to ultimately reduce side effects / toxicity and to improve patient outcomes and survival rates. The implementation of the genomics strategy across the NHS signals a massive step forward for paediatric oncology.

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Figures