Review

Personalisation of Therapy – clinical impact and relevance of genetic mutations in tumours

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Abstract

As technological advances in genetic sequencing and the parallel reduction in costs of sequencing make testing more accessible, genomic profiling of tumours is increasingly becoming integrated into routine clinical care. This personalisation of medical care is especially relevant in the area of oncology, where interest in tumour testing as part of standard care has dramatically increased. Tumour genomic profiles are particularly interesting, as they harbour mutations acquired temporally as somatic events, and less commonly, may reveal defects that have been inherited through the germline. Numerous techniques can be utilised to interrogate the tumour genomic landscape, ranging from tried and tested techniques, such as karyotyping, to full mutational analysis using more modern next and third generation technologies. The challenge for the clinician is no longer predominantly in accessing genomic technologies, but rather in interpreting complex reports, and separating relevant clinically actionable mutations from incidental mutations reflective of the damaged DNA repair mechanisms that are intrinsic to the neoplastic process. This may be especially difficult if the mutational spectrum includes variants in less well-studied genes, or in genes not commonly implicated as drivers of the cancer under investigation. Increasing utilisation of genomic profiling of cancers has informed our understanding that the timing of a mutation in a particular gene is as relevant as the gene in which it occurs in determining the neoplastic course. Once the key driver mutations in a cancer have been identified, the next challenge is to find and utilise an appropriate agent that targets the specific defect. Many hundreds of targeted agents have been designed and put through rigorous in vitro and phase 1 or even phase 2 trials, but few have made it into routine clinical use to date. In this review, we discuss the underlying mechanisms of genomic changes and mutational signatures that can be potentially targeted for therapeutic benefit, and some successful targeted agents that have been developed to date.

Keywords: Precision Medicine; Mutational Signature; Driver Mutations; inherited predisposition

Background

Over the last decade, there has been a huge increase in the applications of genetic testing for personalising patient treatment. The improvements in the available genetic sequencing techniques and expansion in access to testing has occurred in parallel with a substantial decrease in the cost of testing. Ten years ago, the cost of whole genome sequencing was in the order of $20,000,000; falling to less than $2000 by end

of 2015 (1). Unsurprisingly, "genetic testing" is therefore becoming increasingly incorporated into mainstream medical care, particularly as information about the patient's and their cancer's genetic profile can provide improved prognostic information (2, 3); identify suitable patients for specific therapeutic agents (4, 5); and facilitate sub-classification (6), or identification of tumours of unknown origin (7). Several hundreds of "paradigm shifts" in medicine have been proposed in recent times, none as profound as the move towards precision medicine, and the n-of-1 clinical trial (8). Targeted therapy in cancer is not a new concept, but the drive to develop agents specific to the individual has gained significant momentum with the announcement of President Obama's Precision Medicine Initiative in 2015 (9), heralding an "era of Precision Medicine" (10). This move is greatly supported by research funding agencies (11-13), and by the public (14). Surveys of public attitudes towards "genetic testing" suggest strong support for testing that can positively influence selection of targeted therapy, but reticence to participate in testing that might have implications for the wider family, or that might predict disease in the future (15, 16).

In this overview, we aim to discuss:

1. The role of tumour suppressor genes and proto-oncogenes
2. Driver and passenger mutations
3. Chromosomal aberrations in cancer
4. Techniques for molecular profiling in cancer
5. Examples of targetable mutations and targeted therapies
6. Inherited Tumour Predisposition
7. Tumour mutational signatures
8. Challenges in variant interpretation

1. *Tumour Suppressor Genes and Proto-oncogenes*

The hallmarks of cancer include loss of response to normal controls of cell growth and programmed cell death, as well as immortality, self-sustained angiogenesis, invasion, and metastasis. These hallmarks can reflect defects in the genes involved in pathways of cell growth and division. Two broad classes of genes are implicated in tumourigenesis; tumour suppressor genes, and proto-oncogenes [table 1]. Most proto-oncogenes (e.g. *Ras*) function in control of the cell cycle; without them, normal cell growth and development could not occur. Conversion of proto-oncogenes to oncogenes leads to abnormal over-activity or over-expression of the protein product. Such conversion may occur in a number of ways; including mutations in the gene sequence or in a gene regulatory region; gene amplification by localised DNA segmental duplication or multiplication (copy number variation); or by chromosomal translocation leading either to production of an abnormally and continually active fusion protein (e.g. BCR-ABL). These mechanisms may have a dominant effect, meaning that they may trigger oncogenesis even if only one allele is affected. Furthermore, epigenetic influences can lead to hyper- or hypo- methylation of cytosine residues, leading to over- or under-activity of a gene. These epigenetic influences may be somatic, but rarely can be inherited, leading to constitutional hyper- or hypo-methylation of a gene (e.g. constitutional *MLH1* epimutations).

Tumour Suppressor genes (TSGs) (e.g. *BRCA1*, *TP53*) generally function to inhibit replication and proliferation of damaged cells, either by DNA repair or by control of cell cycle checkpoints or apoptosis. Loss-of-function mutations in such genes are oncogenic. Generally, the presence of one normal allele affords sufficient cellular control; but a "second hit" affecting this normal allele (loss of heterozygosity) disrupts control, leading to tumourigenesis. Knockout of the DNA repair function of one or more TSGs leads to sequential acquisition of more mutations, ultimately leading to dysplasia/neoplasia. Tumours arising in patients as a result of inherited defects in tumour suppressor genes, therefore, tend to have a very high mutational load. Pathways of which control is lost by virtue of loss of function of TSGs represent putative targets for therapies (17).

2. *Drivers and passengers*

Cancer cells may acquire 10s to 100,000s of mutations depending on the cell turnover rate of the organ in which they occur, which can be classified as driver or passenger mutations depending on whether they confer an advantage to the cell in terms of growth

Table 1: Definitions of Genetic concepts discussed in this review

Concept	Definitions
Cancer Susceptibility genes	*Tumour suppressor Genes* (TSG) – These genes encode proteins that act in DNA damage repair or in inhibition of inappropriate proliferation of cells. Generally, one copy of a TSG is functionally sufficient. Loss of function mutations in TSGs may be dominantly inherited, but they act recessively at a cellular level, and a second somatic hit is required for oncogenesis. *Proto-oncogenes*: Proto-oncogenes may be converted to oncogenes by a number of gain-of-function mechanisms, including mutation, amplification or chromosomal translocation resulting in a fusion product. Most proto-oncogenes function in cell growth and proliferation, a function which can be pathogenic if constitutionally activated. Gain-of-function mutations act dominantly, and a mutation of only one allele is sufficient to contribute to the neoplastic process.
Drivers and Passengers	*Driver Mutations*: This is a genetic mutation involved in initiation and often continuation of neoplastic transformation; conferring selective growth advantage on the cell in which it occurs, resulting in clonal expansion. *Passenger mutations*: These mutations, which are functionally irrelevant, are common in cancer, as a direct consequence of impaired DNA repair mechanisms. They do not confer growth advantage and are not a necessary component of cancer development.
Germline Predisposition	*Germline mutation* – These mutations are usually inherited from a parent and can be transmitted to the next generation. Such mutations occur in cells in the "germline" – i.e. cells destined to produce gametes. Mutations in cells in the paternal germline occur more commonly with increasing paternal age, which can manifest as apparently de novo paternally-derived mutations in subsequent generations (e.g. *RET*) *Somatic mutation* – This is a genetic mutation which occurs in a single cell in tissue of the soma. Cells in the clonal population derived from this mutated cell will all carry the same variant, but this cannot be transmitted to progeny of the individual.
Mutational signatures	*Mutational signature*: different tumour types harbour mutations in different genes that occur in response to different underlying genetic or environmental aetiologies; for example, as a consequence of defective mismatch repair or in response to UV radiation and aberrant nucleotide excision repair. The unique combination and proportion of different mutations is referred to as the mutational signature of a tumour.
Gene expression analysis	*Gene expression*: This is a two-step process by which the nucleotide sequence of a gene encodes for a particular sequence of amino acids, and therefore a particular protein, through transcription to mRNA, with subsequent translation to protein. This can be followed by post-translational modification of the protein to produce the final protein product. *Transcription*: production of mRNA from DNA by RNA polymerase *Transcriptomics:* the study of RNA transcripts *Translation*: synthesis of protein using mRNA template
Chromothripsis	Localised clustering of chromosomal rearrangements as a consequence of defective DNA repair or replication mechanisms
Kataegis	Localised hypermutation

and/or survival (18). Separating driver mutations that contribute to progression and growth of the cancer from inconsequential passenger mutations can often be extremely challenging. Most somatic mutations are passengers, and will not confer a growth or selection advantage; and designing or applying therapies to exploit such mutations is of little value. It had been thought that several driver mutations are required to

promote neoplasia. However, more recent evidence suggests that only three of these key driver mutations are required in certain cancer subtypes (19, 20), suggesting that a combination of a few appropriately targeted agents may be sufficient to combat the driving forces in these cancers.

Increased utility of somatic testing in tumours has implicated mutations in well-known TSGs and oncogenes as drivers of disease, but expanded gene panels have also led to detection of putative driver mutations in less well-characterized genes. Such genes can be classified as TSGs or oncogenes depending on a number of factors; whether the mutations identified confer loss- or gain-of function; and if one or both alleles are lost. Allelic status is not always informative, as wild-type alleles can be silenced in a number of ways, including by epigenetic mechanisms or silencing by a dominant-negative mutant allele. In certain instances, particularly in non-hereditary cases, bi-allelic loss of a TSG is not sufficient to promote oncogenesis, and other driver mutations are required. Depending on the context, a gene may function as either an oncogene or a tumour suppressor gene (e.g. *NOTCH1*) (21). Therefore, the allelic status of mutations in such genes must be considered in light of the clinical context to help classify the mutation as a driver or passenger. Analysis of both tumour and normal DNA is required to optimally classify mutations in TSGs (22).

3. *Chromosomal aberrations in cancer*

Cancer cells display high rates of chromosomal copy number and structural instability compared to normal cells. The genetic aberrations that occur in cancer may involve whole chromosomes, limited segments of chromosomes, single genes, or related genes within a single pathway, or specific hotspot mutations. As such, "genetic testing" of tumours can be targeted to individual genetic loci or extensive across the entire tumour genome, depending on the clinical context, and the specific tumour type (23, 24). It has been shown that a limited number of specific chromosomes in a cancer genome may harbour many hundreds of clustered chromosomal rearrangements as a consequence of defective DNA repair or replication mechanisms, a phenomenon termed *chromothripsis*. This characteristic is commonly identified in cells with defective p53, permitting replication of cells with chromosomal mis-segregation (25). Foci of localised hypermutation (*kataegis*), with enrichment of C>T or C>G mutations, may also be found in association with certain genomic rearrangements; and may reflect aberrant activity of the APOBEC family of enzymes on dsDNA breaks (26). It has been shown that induction of di-centric chromosomes during telomeric crises induce chromothripsis and kataegis (27).

4. Molecular Profiling of cancers

Transcriptomics

Gene expression analysis can be performed in a number of methods, ranging from low to high-throughput, depending on the clinical indication. Low throughput techniques include utilisation of a reporter gene assay (e.g. Luciferase); Northern or Western blots to measure RNA and protein levels respectively; Fluorescence in situ Hybridisation (FISH) to assess copy number variation; and Reverse-Transcription Polymerase Chain Reaction (RT-PCR), to detect and quantify mRNA from small volume samples. Higher throughput techniques include SAGE (serial analysis of gene expression); DNA microarray and RNA-Seq. SAGE and DNA microarray technologies serve to quantify expression of different isoforms of a large number of genes simultaneously. Different cancers have different gene expression profiles, and thus such methods may be used to sub-classify tumours. SAGE is more expensive, and more technically challenging than DNA microarray based studies, but provides more sensitive quantification of transcript expression, and can be used in discovery of novel mRNA transcripts, while DNA microarray probes must be designed based on known mRNA sequences. RNA-seq (*whole transcriptome sequencing*) also facilitates accurate quantification of gene expression; as well as detection of alternatively spliced or novel transcripts; post-transcriptional modifications; gene fusion, and mutations. Different approaches may be used in the same patient depending on the clinical context. For example, in breast cancer subtyping, gene expression analysis by DNA microarray is used to differentiate between luminal, non-luminal and normal-like

subtypes, while gene expression analysis by RNA expression analysis using RT-PCR as part of the *Oncotype DX* test can predict those ER-positive/HER2-negative early stage tumours at highest risk of relapse (2).

Immunophenotyping

Immunohistochemistry (IHC) is routinely used to characterise tumours based on their immunophenotype. IHC can detect expression of particular antigens by virtue of binding with applied corresponding antibodies. Specific markers may be characteristic of different cellular processes, such as ki67 (proliferation) or CASP3 (apoptosis); or different tumour types. IHC is a simple, routine method that can be particularly useful in clarifying origin of tumours, particularly where morphological appearances are atypical, or if there is difficulty in differentiating between a primary tumour or metastatic deposit; for example, in the ovary, by analysis of the expression of proteins such as CK7, CK20, ER, WT1(28); or in the lung by IHC of CK7, CK30, B-catenin (29).

IHC can be used in the diagnostic work-up of patients with high-risk personal or familial cancer history. Loss of expression of mismatch repair proteins MLH1 and PMS2 in colorectal cancers, for example, may reflect germline mutations in *MLH1*, and more rarely germline missense mutations in *PMS2*; but such pattern of loss also occurs in tumours in association with somatic *BRAF* V600E mutation. Colorectal tumours displaying loss of MLH1 and PMS2 on IHC should routinely be tested for this variant before embarking on germline testing. Loss of MLH1/PMS2 can also be seen in endometrial cancers, particularly in the context of Lynch syndrome. *BRAF* mutations occur infrequently in endometrial cancers however, and the utility of BRAF testing in these tumours in predicting germline MMR defects is limited (30).

Cytogenetics

Molecular profiling techniques may include specific mutation testing, sequencing or expression analysis of specific genes; more extensive analysis of coding regions of all cancer genes as part of a next generation panel; analysis of coding regions of all genes as part of a whole exome analysis; or interrogation of coding and non-coding regions of all genes by whole genome analysis. Gross analysis of the genome can be undertaken by cytogenetic analysis using array CGH; or karyotype with or without Fluorescent in situ Hybridization (FISH). Karyotyping with FISH for recurrent genetic abnormalities remains a critical and recommended component of the diagnostic work-up of certain rapidly progressing haematological malignancies, such as acute myeloid (AML) (31)or acute lymphoblastic (ALL)(32) leukaemia and myelodysplastic syndrome (MDS) (33) supplemented by RT-PCR testing for fusion genes of interest, such as BCR-ABL in ALL; or sequencing of specific genes of interests using next generation multi-gene panels, e.g. *KIT*, *NPM1* and *CEBPA* in AML; *TP53*, *ASXL1*, *ETV6*, *RUNX1* and *EZH2* in MDS. Low rates of cellular proliferation in other haematological malignancies, including Multiple Myeloma, limit the utility of karyotyping, and interphase FISH using probes specific to regions of interest is the preferred method of cytogenetic analysis of such cancers. Recurrent chromosomal aberrations can be recognised among different cancer types or in the organ in which they occur, for example; breast cancers often display gains of chromosomes 1q, 8q, 17q and 20, and losses of chromosomes 8p, 16q, 17p; gastric cancers display copy number gains of 8q and 20; and gliomas recurrently demonstrate gain of chromosome 7 and losses of 1p and 19q (34). Cytogenetic analysis of solid tumours by karyotyping has been eschewed in recent years in favour of more advanced technologies such as oligo- or SNP-arrays, in combination with specific gene testing or investigation for specific fusion genes of interest by RT-PCR or FISH. Cytogenetic studies do still have utility in certain solid cancers, for example, testing of somatic deletions of 1p19q for prognostication in oligodendroglioma (23) and for diagnosis and sub-classification of soft tissue sarcomas (24).

Microarray-based comparative genomic hybridisation (Array CGH) can detect copy number variation at a much higher resolution than standard karyotype (~80kB-v-5MB); but requires significant volumes of

differentially labelled patient/tumour and reference DNA to co-hybridize to the array chip. The sensitivity of array-CGH is highly dependent on the proportion of the sample that is representative of the tumour compared to control; degree of tumour heterogeneity; and resolution of the selected microarray platform (number of probes).

Neither array-CGH nor SNP array detect balanced rearrangements, so other methods (PCR/karyotype/FISH) must be relied on for this purpose. SNP arrays are particularly useful in assessing copy number variation, loss of heterozygosity, and in detection of uniparental disomy (UPD); but rely on utilisation of well-described DNA sequences with known SNPs as probes.

Mutation analysis

Molecular genetic testing of solid organ tumours can be performed in a host of methods depending on the clinical question. Interrogation of tumour DNA can be performed by RT-PCR for specific recurrent mutations in particular genes, by sequencing of entire genes of interest by Sanger sequencing, MLPA or multi-gene panels, by sequencing of coding regions of all genes using exome sequencing, or by interrogation of introns and exons of all known genes using whole genome sequencing. More extensive sequencing increases the likelihood of detecting one/more pathogenic mutations; but inevitably increases the probability of detecting variants of uncertain significance; variants in poorly characterised genes; or heritable pathogenic variants arising from the germline. Molecular genetic analysis of tumours is challenging, because most methods require significant amounts of relatively good quality DNA. Tumour testing by next-generation sequencing, using multi-gene panels is increasingly being used in a diagnostic setting. Genomic DNA from a tumour sample is extracted, quantified and qualified, and amplified using PCR. These amplified products (DNA library) are then sequenced in parallel using fluorescently labelled nucleotides. Hybridisation of probes to the complementary strand is assessed by fluorescence. These synthetic sequences are then analysed in parallel using complex bio-informatics pathways. It is interpretation of results that is often the most complex step in the pathway (35), and robust reporting and classification of variants is essential.

5. Targeted agents in Cancer

A comprehensive overview of the targeted agents currently available in all cancer types is beyond the scope of this article, but here we outline the application of targeted therapy in a common (Breast) and rare (Gastro-intestinal stromal tumours) cancer. Some common agents and the genes they target are outlined in table 2.

Breast Cancer

The concept of personalised therapy in breast cancer is not a new one (15). The treatment of breast cancer, for example, has become increasingly personalised over the last several decades, with shifts towards less radical breast and axillary surgeries wherever possible, minimisation of systemic chemotherapy in patients at lowest risk, and utilisation of several targeted agents according to the molecular profile of the tumour. Tamoxifen, which targets oestrogen and progesterone receptors, was first developed in the 1960s during an attempt to develop new contraceptive agents (36) but is now a standard weapon in the armamentarium of breast cancer therapies for ER-positive disease. Increasing refinement of molecular profiling of tumours has led to the development of more and more targeted agents, in a more deliberate and considered manner than the Tamoxifen discovery effort. Other selective oestrogen receptor modulators include Raloxifene and Toremifene, the latter being currently utilised only in the metastatic context. Several lines of endocrine therapies have now been developed and incorporated into the routine management of patients with ER/PR-positive breast cancer; other endocrine agents include aromatase inhibitors (AIs), including non-steroidal AIs; Letrozole and Anastrozole; and steroidal aromatase inactivator, Exemestane; and luteinising hormone-releasing hormone (LHRH) analogs (Goserelin, Leuprolide). Application of these agents is dependent on menopausal status, response, or acquisition of resistance. Less commonly, agents such as Megestrol acetate, ethinyl estradiol or fluoxymestrone may also have a role.

In the metastatic setting, emergence of resistance to endocrine therapy can prove a significant clinical challenge. The agent Palbociclib alongside endocrine agents in ER-positive, HER2-negative advanced breast cancer has been supported by the results of the Phase II PALOMA-1 (37) and subsequent Phase III PALOMA-2 (38) trials. Palbociclib works to inhibit cyclin-dependent kinases-4 and -6; kinases implicated in the development of Endocrine resistance (39). Palbociclib preferentially inhibits proliferation of ER-positive breast cancer cells (40). The utility of Palbociclib was further evaluated in PALOMA-3, which compared combination Palbociclib and Fulvestrant to Fulvestrant monotherapy in endocrine-resistant ER-positive, HER2-negative metastatic breast cancer. This study was halted early because of efficacy (41).

Another mechanism of endocrine resistance is by aberrant signalling through the *PI3K-AKT-mTOR* pathway. This pathway can become overactive by virtue of loss-of-function mutations in genes such as PTEN, TSC1/TSC2 or STK11 (17), or by loss of the negative regulators PTEN or AKT. Over-activation of the same pathway can arise as a consequence of gain-of-function mutations in genes such as PI3KCA (17), which have been commonly identified in all types of breast cancer (42), particularly in ER/PR-positive subtypes. Agents that target the PI3K/AKT/mTOR pathway have therefore been of particular interest in cancers with such molecular aberrations, in different combinations with endocrine or other therapies; as part of a number of Phase I, Phase II, and Phase III trials (43). Everolimus, originally designed as an anti-fungal agent, has been utilised with in a variety of cancers with mutations in mTOR-signalling pathway, with proven efficacy in subependymal giant cell astrocytomas (SEGAs) occurring as part of Tuberous Sclerosis and in pancreatic neuroendocrine tumours(44, 45). A large Phase 3 study (BOLERO-2) provided robust data to support the use of Everolimus alongside endocrine therapy to reduce or reverse endocrine resistance in breast cancer (46); and current NCCN guidelines now recommend consideration of Everolimus in combination with Exemestane in patients with Stage IV disease fulfilling BOLERO-2 inclusion criteria (47). Other studies are ongoing investigating the utility of PI3K inhibition; including the FERGI phase II study (48), investigating the role of PI3K inhibitor Pictilisib in post-menopausal ER-positive/HER-2-negative advanced breast cancer in combination with Fulvestrant versus Fulvestrant monotherapy, although interestingly PI3K mutational status was not associated with differential response. BELLE-2 (49) and BELLE-3 studies similarly looked at Fulvestrant monotherapy versus Fulvestrant/PI3Ki combination, using Buparlisib (50).

The *ERRB2* gene, encoding the ErbB2 (HER2/neu) protein, was discovered in the 1980s (51, 52). HER2 amplification is reported in 20-30% of breast tumours, and is a poor prognostic indicator. Over-expression of HER2 leads to ligand-independent activation and dimerization of HER2; inducing signalling through mTOR/PI3K and Raf/MEK pathways (53). Trastuzumab (Herceptin), a monoclonal antibody targeting HER2/neu was deliberately designed in 1990, with a view to blocking the increased proliferative activity conferred by amplified HER2, by obstructing activation of the receptor and inducing antibody-medicated cytotoxicity in cancer cells expressing the HER2 antigen (54). This agent is now routinely used as part of combination treatment of HER2-overexpressing breast, gastric and lung tumours. The use of Trastuzumab, alone or as part of the drug conjugate, Ado-trastuzumab emtansine, has been shown to improve patient disease-free and overall survival; but only in ~70% of patients in whom *HER2*-expression is over-amplified, a reflection of the highly intelligent nature of cancer cells in developing complex networks to promote growth, cell survival and resistance to therapy. Other monoclonal antibodies targeting HER2 have since been developed, including Pertuzumab (Perjeta), which inhibits dimerisation of HER2.

As of 2016, 28 small molecule kinase inhibitors were licenced by the FDA (55), of which two; Lapatinib and Palbocib, are utilised in trastuzumab-exposed HER2-overexpressing; and post-menopausal, ER-positive, HER2-negative breast cancer, respectively. Lapatinib is a tyrosine kinase inhibitor (TKI) which has targets both ErbB1 (encoded by *EGFR)* and ErbB2. Combination Lapatinib and Capecitabine therapy has been shown to halt disease progression more effectively, and improve survival compared to Capecitabine monotherapy (56, 57) in patients with advanced pre-treated HER2-overexpressing breast cancers. The

Table 2: Examples of Targeted therapies, and the gene they target

Target	Agent	References
ALK	Crizotinib	Kwak et al (117)
BCR-ABL	Imatinib Dasatinib Nilotinib	Heinrich et al (71) Talpaz et al (118) Kantarjian et al (119)
B-raf	Vemurafenib	Chapman et al (120) Hyman et al (121)
EGFR	Gefitinib Erlotinib	Mok et al (122) ; Lynch et al (123) Shepherd et al (124)
HER-2	Trastuzumab Lapatinib Pertuzumab Neratinib	Piccart-Gebhart et al (125) Johnston et al (126) Swain et al (127) Chan et al (128)
mTOR	Everolimus	Motzer et al (129) ; Beaver et al (130)
PARP	Olaparib Niraparib	Ledermann et al (65) Mirza et al (131)
PIK3CA	Pictilisib	Schmid et al (132)
PTCH1	Vismodegib	Basset-Seguin et al (133)
RET	Vandetanib	Wells et al (134)
VEGF	Abflibercept Bevacizumab	Van Cutsem et al (135) Saltz et al (136) ; Perren et al (137)
Multi-targeted agents	Cabozantinib Sorafenib Sunitinib	Choueiri et al (138) ; Viola et al (139) Llovet et al (140) Motzer et al (141)

combination of trastuzumab and lapatinib has been shown to be superior to lapatinib monotherapy in patients with advanced HER2-positive breast cancer, even in the context of heavy pre-treatment with Trastuzumab (58). A significant proportion of sporadic HER2-overexpressing breast cancers harbour *TP53* mutations. Lapatinib has been shown to downregulate mutant p53 in HER2-overexpressing cells, suggesting that TP53 mutational status may be predictive of response to this targeted agent (59). Trastuzumab is also licenced for, and has been shown to have efficacy as an adjunct to chemotherapy in, advanced HER2-expressing gastric cancer (60), although other HER2-targeted agents such as Ado-trastuzumab emtansine and Lapatinib have not been shown to be improve overall survival for these patients (61, 62).

Consequences of inherited single gene mutations can also be exploited by targeted agents. PARP (Poly-ADP Ribose Polymerase) inhibitors, for example, work by inhibiting alternative pathways of DNA repair in BRCA-deficient cells; leading to increasing mutational burden to such a point as to induce cellular apoptosis (63) (synthetic lethality). Neighbouring BRCA-proficient cells are spared; by virtue of their intact BRCA-mediated DNA repair mechanisms. PARP inhibitors have been shown to significantly extend progression-free survival in patients with ovarian, breast and prostate cancers with germline or somatic BRCA deficiency (64-67).

Gastro-intestinal Stromal Tumours

GIST tumours are mesenchymal tumours of the gastro-intestinal tract. Most GISTs occur sporadically, but a small proportion arises as a result of germline susceptibility. Inherited predisposition to GIST can be in part explained by mutations in *c-kit* and *PDGFRa*; and to a lesser extent as part of a wider genetic syndrome, such as Neurofibromatosis type I (*NF1*) or Carney-Stratakis Dyad (*SDH* family). Sporadic GISTs harbour somatic events in these and other genes.

Approximately 85-90% of sporadic GISTs harbour primary somatic mutations in *c-kit*, and, similar to germline events, these occur most commonly in exon 11, encoding for the juxta-membrane domain, less commonly in exons 9, and rarely in exons 13 and 17(68). Small molecule kinase inhibitors, such as Imatinib, a tyrosine kinase inhibitor (TKI), has been associated with increased response rates in patients Gastro-intestinal stromal tumours harbouring *c-kit* activating mutations, as well as in CML associated with the BCR-ABL fusion protein as a consequence of somatic chromosomal rearrangements (69, 70). GISTs can go on to acquire secondary kinase mutations that can influence the development of resistance to therapy. It is has been shown that the response of GISTs to TKIs such as Imatinib and Sunitinib is dependent on the primary, and secondary *c-kit* mutational status of the tumour, with increased disease-free and overall survival post-Imatinib in patients with tumours with primary exon 11 mutations compared to tumours with exon 9 mutations (71). Conversely, progression-free and overall survival post-Sunitinib in Imatinib-resistant patients was noted to be greater in patients with tumours harbouring primary *c-kit* mutations in exon 9 versus those with mutations in exon 11 (72, 73). Between 5-7% of *c-kit* negative GISTs harbour mutations in the kit-homologous gene *PDGFRa,* predominantly in exons 12 and 18; encoding juxta-membrane domains and kinase activation loops, respectively. Most studies investigating the impact of mutational status of GISTs on treatment response include small numbers of patients with *PDGFRa* mutations, and so the evidence for exon-specific responses is scant. However, a recurrent mutation in exon 18 of *PDGFRa*, substitution D842V, which accounts for the majority of all *PDGFRa* mutations, is associated with Imatinib resistance (74, 75).

Approximately 10-15% of GISTs do not harbour mutations in *PDGFRa* or *c-kit.* Tumours without these mutations ("wild type" tumours) can be further subdivided into type I and type II wild-type GISTs depending on expression of SDHB (76). Type I GISTs, with in-tact SDHB include tumours arising in NF1 (77) as well as sporadic wild type tumours. Lack of SDHB expression in type II tumours can occur as a consequence of inactivating mutations in the SDH-family of genes, as part of the Carney-Stratakis Dyad (78). "Quadruple" wild-type tumours are those tumours without mutations in *c-kit*, *PDGFRa*, *SDHx*, or *NF1* or other *Ras* family genes.

A proportion of wild-type GISTs harbour the recurrent *BRAF* V600E hotspot mutation (79, 80) that is rarely identified in tumours with *c-kit* or *PDGFRa* over-expression. A number of BRAF-inhibitors have been designed to target common *BRAF* mutations V600E and V600K in melanoma, e.g. Dabrafenib and Vemurafenib, and have been applied with some success in patients with melanoma harbouring these hotspot mutations (81, 82). Sorafenib, a non-selective *BRAF* inhibitor, has had limited efficacy in melanoma (83), but proven efficacy in certain types of renal cancer (84). A single case report suggests that Dabrafenib may have a role in *BRAF*-mutated GIST (85). The results of a Phase II trial investigating the utility of

Vemurafenib in BRAF-mutated malignancies is awaited (86). Numerous phase I and phase II trials are ongoing looking at the utility of other targeted agents in GIST including known agents such as Palbociclib and Buparlisib, and numerous novel such as KIT/PDGFRa inhibitors Ponatinib and Dasatinib, an comprehensive overview of which is provided by Szucz et al (87).

6. Inherited Tumour Predisposition

Knowledge of an individual's inherited predisposition to cancer is critical, as it facilitates strategies for prevention and may influence treatment, not just in the proband, but also in members of the wider family. It may also have implications for family planning, as most cancer predisposition syndromes are inherited in an autosomal dominant fashion. Genetic testing of both tumour and patient DNA is becoming more and more mainstream, a practice that has obvious merits in terms of prognostication and treatment. However, it is critical that this testing be performed with some insight into the relevance and potential implications of the result for the wider family. Tumour testing, in particular, may be difficult to interpret, as some apparently somatic mutations may be inherited or may reflect germline mutations in other genes. Several authors have noted that a significant proportion of tumours have such mutations (88-91). Other authors have noted that the follow up and onward referral of these patients to a Clinical Genetics professional is poor (92).

While testing of tumour DNA by next generation sequencing is a relatively new procedure, tumour testing by other methods has been in place in routine clinical practice for many years. However, it has been shown that, even routine tests such as IHC for MMR defects in patients with colorectal cancer is not uniform across centres, even within countries (93). Similarly, it has been shown that there may not be a standard procedure of referral for patients with abnormal IHC for further genetic assessment (94, 95). The new guideline from the National Institute for Health and Care Excellence provides a standardised framework to ensure investigation in a broader cohort of patients, and onward referral of individuals in whom MMR defects are detected (96).

7. *Tumour mutational signatures*

Germline mutations can be associated with increased somatic mutational load as a consequence of impaired DNA repair mechanisms. The unique combination of mutations in any given tumour is referred to as a "mutational signature". Twenty-one distinct recurrent somatic mutational signatures have been defined by Alexandrov et al (97), which may reflect the underlying tumourigenic defect, as well as the cancer aetiology. Such "signatures" can be differentiated by proportional representation of 96 different substitution types based on the six substitution types (C>A; C>G; C>T; T>A; T>G; T>C), and possible permutations and combinations of the bases immediately 5' and 3' of the mutation locus. Signature 6, for example, occurs primarily in cancer arising as a result of defective mismatch repair; with enrichment of C>T transitions at NpCpG sites. Comparatively, the mutational signature of tumours associated with germline pathogenic variants in *BRCA1* and *BRCA2* (signature 3) demonstrates an increased frequency of indels (signature 3), reflecting reduced efficacy in homologous recombination.

Other signatures can reflect exposure to environmental carcinogens, such as signature 7 in cancers associated with UV-exposure (melanoma, SCC); which has an over-representation of C>T transitions with bias for the non-transcribed strand, and signature 4, which is observed in cancers associated with smoking (cancers of the lung, head and neck, liver), and is likely to reflect the generation, and subsequent nucleotide excision repair of bulky adducts by toxins in smoke. Transversions of C>A are predominantly found in smokers, as carcinogens in cigarettes tend to form adducts on guanine (97). The most common of the mutational signatures, Signature 1A and 1B, reflect endogenous spontaneous deamination of methylated cytosine residues. These signatures occur commonly in both the germline and somatic cells, and correlate with the age of the individual in which the tumour arises. The relationships between cancer types, mutational processes and mutational signatures are complex. Certain mutational signatures are found only in specific classes of cancer; and similarly different

tumours within certain classes of cancer express a limited number of different mutational signatures. However, different tumours within other classes of cancer can be heterogeneous in the mutational signatures they express (97). Considering mutagen-related signatures, signature-specific mutational burden can be correlated with the amount of exposure to the mutagen; heavy smokers have a higher burden of signature-4 specific mutations than light smokers; and melanomas in sun-exposed areas have higher burdens of UV-related signature-specific mutations (98).

Germline mutations in certain genes can also cause specific somatic mutations in other genes. For example, the *MUTYH* gene encodes a highly conserved post-replicative DNA glycosylase, which has dual adenine and 2-OH-A DNA glycosylase functions. Removal of adenine from in the DNA backbone by MUTYH at sites of mis-pairs with 8-oxoG is followed by base excision repair (BER) to form C:8-oxoG base pairs (99). OGG1 DNA glycosylase then acts to remove 8-oxoG paired opposite cytosine from DNA, restoring C:G pairing. Deficiency of these enzymes predispose to failure of repair of mutated DNA, which may then confer a risk of carcinogenesis. *MUTYH* mutations in particular fail to repair G → T transversions in other genes, namely *APC* (100-102) and *KRAS* (103) with a special predilection for guanine residues in particular hotspots. A particular somatic mutation c.34G>T in *KRAS* can reflect the presence of germline biallelic *MUTYH* mutations, which cause *MUTYH*-associated polyposis (MAP) (103). Because of the recessive nature of inheritance of this condition, patients with MAP often do not have a strong family history of cancer, and clinicians may therefore not always be alert to the possibility of an inherited cancer predisposition. Furthermore, patients with MAP can present with a colorectal cancer without preceding polyposis (104, 105). The *KRAS* proto-oncogene is one of the most commonly somatically mutated genes in cancer. Assessment of the *KRAS* gene, along with *NRAS* and *BRAF*, is performed routinely in colorectal cancers, to help determine application of anti-EGFR therapeutic agents. Generally, tumours mutations in codons 12 and 13 of *KRAS* will respond to this therapy, while tumours with wild-type *KRAS* are less likely to do so (106).

There are numerous methods by which *KRAS* testing can be performed, some of which target only common mutations, others that involve sequencing of the entire *KRAS* gene. Therefore, clinicians performing *KRAS* sequencing need to be cautious in interpreting *KRAS* sequencing data, as this subtle indicator of inherited cancer predisposition may easily be overlooked (107).

Gene expression profiles may also be modified by germline mutations. Germline mutations in *BRCA1* or *BRCA2* may not only have specific mutational signatures, but may also have an impact on the RNA expression levels of certain genes as measured by RT-PCR. The Oncotype DX assay is one of a number of multi-gene expression assays that have been developed in an attempt to identify patients at greatest risk of tumour recurrence, and therefore those in whom the need for systemic treatment is greatest (108). The Oncotype DX assay for breast cancer generates a recurrence score between 1 and 100, which correlates with 10-year risk of recurrence of disease (2, 109, 110). The use of this assay has been incorporated into the routine clinical assessment of patients with ER-positive, node negative breast cancer (2, 47). Tumours in carriers of *BRCA1* and *BRCA2* gene mutations are more likely to have intermediate- or high-risk recurrence score compared with tumours of similar molecular phenotype in age-matched controls with wild-type *BRCA1* and *BRCA2* (111, 112), suggesting that patients with *BRCA1* and *BRCA2* germline mutations have tumours of more aggressive biology, even if nodal disease and ER-negativity is not present.

8. Challenges in tumour testing

Genomic profiling of tumours can be challenging for a number of reasons. Firstly, different methods of preservation can greatly impact the utility of different genomic testing techniques. Formalin fixation, for

example, increases DNA cross-linking; and DNA extracted from such tissues is often significantly degraded. The optimum specimen on which to perform DNA testing is a fresh, or fresh frozen sample, but such samples are rarely available routinely. Furthermore, it is well documented that the genomic landscape of a cancer is dynamic, changing as the tumour evolves and acquires more and more DNA damage; meaning that there is significant tumour heterogeneity over space and time; within the main body of the tumour, and between the primary tumour and its metastases. Small samples of tumour provide only a snapshot of the landscape of mutations in that part of the tumour, at that point in time. Certain sub-clonal populations of tumour cells may prove more resistant to treatment than others. Patients in whom mixed response to therapy is observed may merit re-biopsy of areas with minimal response or progressive disease, to facilitate further genetic analysis.

Interpretation of somatic mutations requires consideration of a number of factors. The first challenge is to determine whether or not the mutation is pathogenic or not. Guidelines for the interpretation of sequence variants have been derived by consensus recommendations from The American college of Medical Genetics and Genomics, and the Association for Molecular Pathology (113), outlining the evidence, and strength of evidence, required to classify a variant into one of five categories of pathogenicity. Factors to be considered in determining pathogenicity of a germline mutation include population minor allele frequency, segregation and inheritance data, as well as computational and predictive data about the variant, functional data, and frequencies of the variant in different databases (114). Some of these factors must also be considered in interpretation of somatic variants. Interrogation of databases such as IARC or COSMIC will provide useful information regarding frequency of the variant in question in different cancer types. However, in somatic tumour testing, functional consequence must often be predicted from structural information rather than proven by biochemical analysis (115). Interpretation of somatic testing reports is becoming more and more challenging as panels expand to include more novel and less well-described genes. The expansion of panels certainly increases the likelihood of detecting a clinically actionable mutation, but also increases the likelihood of detecting variants of uncertain significance; which in turn increases the workload of the testing laboratories before a report can be generated. It is likely that the proportion of patients in whom clinically actionable mutations are detected by larger panels is outweighed by the proportion of patients in whom VUS, or pathogenic mutations to which targeted agents do not yet exist, are identified. Controversy regarding the use of large panels for germline testing exists, and as large scale tumour testing becomes part of the routine diagnostic workup, it is likely that similar controversies will emerge in that context.

Pathogenicity is not the only important factor to consider. The gene in which the mutation occurs may be an indicator as to whether it is a driver mutation or not, but is complicated by virtue of the fact that passenger mutations can occur in typical “driver” genes in different clinical contexts (21). The order in which mutations occur is also important, and may be dependent on the cancer type – for example mutations in genes in the *RAS* pathway are important drivers of melanoma (e.g. in *BRAF*), but arise in colorectal cancer (in *KRAS*) at a later stage than mutations in *APC* and in the *APC* pathway, which happen in early adenomatous development (116). It is important therefore to be mindful of the “cellular context” in applying targeted therapies; therapies directed against *BRAF* mutations may have high response rates in melanoma but not in colorectal cancers, even if the tumours are noted to have identical mutations (19).

Conclusions

Personalisation of therapy is an ambitious concept; but is becoming increasingly achievable given improvements in our understanding of tumour biology and the impact of different mutations in gene/genes in certain biological pathways, combined with rapidly improving technology and decreasing costs. Although our knowledge with respect to tumour mutational signatures, driver-passenger pathways, and the evolution of cancer phenotypes has improved dramatically over the past several decades, immense

difficulties still exist. Separating the wheat from the chaff in terms of defining the driver mutations among tumours of high mutational burden, differentiating germline from somatic events, and defining the "cellular context" in which these mutations occur in less well-studied cancers remain challenges; not to mention the "unknown unknowns" including epigenetic factors, gene-environment interactions, and adaptation mechanisms of cancer cells in response to therapy. Our knowledge is perhaps not increasing as such a rapid rate as genomic technology, and it is important to apply the technology only in circumstances in which we are confident we will be able to interpret and utilise the results for the benefit of the patient, and to apply targeted therapies without inflicting undue toxicity or at the expense of potential benefit from proven standard approaches.

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Abbreviations

AKT	V-Akt Murine Thymoma Viral Oncogene Homolog 1
ALK	Anaplastic Lymphoma Receptor Tyrosine Kinase gene
APC	Adenomatous Polyposis Coli gene
APOBEC	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like family of genes
ASXL1	Additional Sex Combs Like 1, Transcriptional Regulator
ATM	Ataxia Telangiectasia Mutated serine/threonine kinase gene
ATR	Ataxia Telangiectasia and Rad3-related serine/threonine kinase gene
BCR-ABL	fusion protein arising from chromosomal translocation approximating breakpoint cluster region protein gene/Abelson murine leukemia viral oncogene homolog 1 gene
BRAF	B-Raf proto-oncogene, serine/threonine kinase
BRCA1	Breast Cancer 1, Early Onset
BRCA2	Breast Cancer 2
CASP3	Caspase 3
CEBPA	CCAAT/Enhancer Binding Protein Alpha
CGH	Comparative Genomic Hybridisation
COSMIC	Catalogue Of Somatic Mutations In Cancer
EGFR	Epidermal Growth Factor Receptor
ETV6	ETS Translocation Variant 6
EZH2	Enhancer of Zeste 2 Polycomb Repressive Complex 2 Subunit
FAP	Familial Adenomatous Polyposis
HER-2/neu	Human Epidermal Growth Factor Receptor 2
IARC	International Agency for Research on Cancer
IHC	Immunohistochemistry
KIT	KIT Proto-Oncogene Receptor Tyrosine Kinase
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
MAP	MUTYH-associated Polyposis
MAPK	Mitogen-Activated Protein Kinase
mTOR	mammalian target of Rapamycin
MUTYH	MutY DNA Glycosylase
NF1	Neurofibromin 1
NOTCH1	Notch (Drosophila) Homolog 1 (Translocation-Associated)
NPM1	Nucleophosmin

PARP	poly ADP ribose polymerase
PCR	polymerase chain reaction
PDGFRa	Platelet Derived Growth Factor Receptor Alpha
PI3K	Phosphatidylinositol-4, 5-bisphosphate 3-kinase
PTEN	Phosphatase And Tensin Homolog
RT-PCR	Reverse transcription polymerase chain reaction
RUNX1	Runt Related Transcription Factor 1
SDHX	Succinate Dehydrogenase Complex family
SEGA	Subependymal giant cell astrocytoma
STK11	Serine/Threonine Kinase 11
TP53	Tumour Protein P53
TSC1	Tuberous Sclerosis 1
TSC2	Tuberous Sclerosis 2
TSG	Tumour Suppressor Gene
VUS	Variant of Uncertain Significance

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