Problem Solving Through Precision Oncology

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Preface

We have seen remarkable progress in the management of cancer. More than half of cancer patients can now expect to achieve long-term survival and cure in the UK, and slightly more in countries with the very best cancer outcomes. This progress, however, has been achieved at the cost of toxicity for cancer patients and financial cost to their healthcare economies. Oncology has historically been an imprecise medical discipline that has relied heavily on empirical evidence. We generally have not been able to predict with accuracy which patients will benefit and which have the best chance of cure. Treatments are associated with toxicity as well as efficacy because we cannot precisely target the cancer. Our choice of treatment has been determined by historical probabilities and clinical characteristics because we generally lacked the means to test a cancer to determine which treatments will work and which will not. This background makes the advent of precision medicine as precision oncology especially exciting for cancer patients and cancer professionals. The dramatic advances that we have seen in our knowledge of the fundamental biology of cancer, genomics, the transcriptome and other aspects of the phenotype are now genuinely informing the tests that tell us how a cancer is likely to behave, and the treatments that we can use to influence that behaviour.

Discussions of precision oncology are often couched in highly scientific terms, bringing molecular biology, molecular genetics, proteomics and sophisticated imaging to bear on the diagnosis, prognosis and selection of treatment for a cancer. The challenges to delivering precision oncology, however, lie not only at the cutting edge of modern science but also in the way we provide cancer care and how we organize ourselves to do so. We need to communicate effectively with patients in order to personalize their care and provide them with clear choices. Organization and logistics are important themes in precision oncology. We have a growing portfolio of molecular tests to determine the behaviour of a cancer and to predict its response to therapy. We need to look carefully, however, at how they can be deployed in a hard-pressed healthcare system to bring benefits to the maximum number of patients, in the quickest time, and in the most cost-effective way. We need to be careful that the intuitive appeal of molecular testing to guide therapy does not lead us to exaggerate the potential benefits, and keep a clear-eyed view of the evidence.

This most recent book in the Association of Cancer Physicians' prize-winning *Problem Solving* series seeks to bring out in an accessible way the potential of precision oncology and its challenges and pitfalls. Fifteen chapters are written by leading authorities in the field to give an overview of the development of precision oncology at a molecular, clinical and patient-centred level. The 21 individual case histories are then used to illustrate how precision oncology can and should be woven into the practice of cancer medicine and the organization of healthcare services. The approach is broad and inclusive and covers all currently topical aspects of precision oncology. This is a fast-moving field and the principles that are described will be enduring, although the individual tests and the individual treatments are likely to evolve rapidly in the coming decade.

Precision oncology offers to patients the prospect of more effective treatments and the avoidance of unnecessary toxicity from treatments that do not work. Patients and patient advocates see this as a vitally important goal for oncology. The potential for improving the well-being and outcomes of treatment for cancer patients through precision oncology, and the challenge of delivering it effectively and quickly, are immense.

Ellen R. Copson, Peter Hall, Ruth E. Board, Gordon Cook and Peter Selby, Editors
Johnathan Joffe, Chairman, Association of Cancer Physicians
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Ellen R. Copson, Peter Hall, Ruth E. Board, Gordon Cook and Peter Selby

Association of Cancer Physicians

The *Problem Solving* series of cancer-related books is developed and prepared by the Association of Cancer Physicians, often in partnership with one or more other specialist medical organizations. As the representative body for medical oncologists in the UK, the Association of Cancer Physicians has a broad set of aims, one of which is education of its own members and of non-members, including interested clinicians, healthcare professionals and the public. The *Problem Solving* series is a planned sequence of publications that derive from a programme of annual scientific workshops initiated in 2014 with 'Problem Solving in Acute Oncology', followed by 'Problem Solving in Older Cancer Patients', 'Problem Solving Through Precision Oncology' and, most recently, 'Problem Solving in Patient-Centred and Integrated Cancer Care'.

The publications involve considerable work from members and other contributors; this work has been done without remuneration, as an educational service. The books have been well received and we are delighted with their standard. *Problem Solving in Older Cancer Patients* was awarded the 2016 BMA prize for best oncology book of the year.

The Association of Cancer Physicians wishes to thank all the contributors to this and previous books, and to those yet to come.

Johnathan Joffe, Chairman, Association of Cancer Physicians

Abbreviations

ABC	Activated B cell
ABL	Abelson murine leukaemia viral oncogene homologue 1
AFP	Alpha-fetoprotein
AGO	Arbeitsgemeinschaft Gynäkologische Onkologie [German Gynaecological Oncology Working Group]
Akt	Protein kinase B
ALK	Anaplastic lymphoma kinase
ALL	Acute lymphoblastic leukaemia
ASCT	Autologous stem cell transplant
ATP	Adenosine triphosphate
BCL	B cell lymphoma protein
BCR	Breakpoint cluster region
BEP	Bleomycin, etoposide, cisplatin
BiTE	Bi-specific T cell engager
BRAF	Serine/threonine-protein kinase B-Raf
BSO	Bilateral salpingo-oophorectomy
BTK	Bruton's tyrosine kinase
CA9	Carbonic anhydrase 9
CD	Cluster of differentiation
CDF	Cancer Drugs Fund
CEA	Carcinoembryonic antigen
CK-7	Cytokeratin 7
CMI	Caris Molecular Intelligence
CODOX-M	Cyclophosphamide, vincristine, doxorubicin, methotrexate
CPET	Cardiopulmonary exercise test
CRAF	RAF proto-oncogene serine/threonine-protein kinase C-Raf
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CSG	Cancer susceptibility gene
ctDNA	Circulating tumour DNA
CTLA-4	Cytotoxic T lymphocyte-associated protein 4
CXCL12	C-X-C motif chemokine 12
CYP	Cytochrome P450
DCIS	Ductal carcinoma *in situ*
DEC	Diagnostic Evidence Co-operative
DLBCL	Diffuse large B cell lymphoma
DOG-1	Discovered on GIST-1
DRE	Digital rectal examination
ECX	Epirubicin, cisplatin, capecitabine
EFS	Event-free survival
EGFR	Epidermal growth factor receptor
ELF	Enhanced Liver Fibrosis
EPOCH-R	Rituximab, etoposide, prednisolone, vincristine, cyclophosphamide, doxorubicin
ER	Oestrogen receptor
ERK	Extracellular signal-regulated kinase
EZH2	Enhancer of zeste homologue 2
FDG	Fluorodeoxyglucose
FFPE	Formalin-fixed paraffin-embedded
FISH	Fluorescence *in situ* hybridization
FOLFIRI	Fluorouracil, folinic acid, irinotecan
FOLFOX	Fluorouracil, folinic acid, oxaliplatin
5-FU	Fluorouracil
GCB	Germinal centre B cell
GERCOR	Groupe Coopérateur Multidisciplinaire en Oncologie
GIST	Gastrointestinal stromal tumour
GOJ	Gastro-oesophageal junction
GWAS	Genome-wide association study
hCG	Human chorionic gonadotrophin
HER2	Human epidermal growth factor receptor 2

HGBL	High-grade B cell lymphoma
HGSOC	High-grade serous ovarian carcinoma
HIF	Hypoxia-inducible factor
HNPCC	Hereditary non-polyposis colorectal cancer
HPF	High-powered field
HPV	Human papillomavirus
IAP	Immunosuppressive acidic protein
IGF-1R	Insulin-like growth factor 1 receptor
IGFBP	Insulin-like growth factor binding protein
IgG	Immunoglobulin G
IPI	International Prognostic Index
IRS	Intergroup Rhabdomyosarcoma Study Group
ISH	*In situ* hybridization
ISS	International Staging System
IVA	Ifosfamide, vincristine, dactinomycin
IVAC	Ifosfamide, etoposide, cytarabine
IVD	*In vitro* diagnostics
JAK2	Janus kinase 2
KIM-1	Kidney injury molecule-1
KRAS	KRAS proto-oncogene
LDH	Lactate dehydrogenase
LFS	Li–Fraumeni syndrome
LS	Lynch syndrome
MAPK	Mitogen-activated protein kinase
MDT	Multidisciplinary team
MEK	Mitogen-activated protein kinase kinase
miRNA	MicroRNA
MLH	MutL protein homologue
MMP	Matrix metalloproteinase
MMR	Mismatch repair
mRCC	Metastatic renal cell carcinoma
MRD	Minimal residual disease
MSH	MutS protein homologue
MSI	Microsatellite instability
mTOR	Mechanistic target of rapamycin
NAC	Neoadjuvant chemotherapy
NACRT	Neoadjuvant chemoradiotherapy
NCI	National Cancer Institute
NFκB	Nuclear factor kappa B
NGS	Next generation sequencing
NIHR	National Institute for Health Research
NLR	Nucleotide-binding domain and leucine-rich repeat containing receptor
NMP	Nuclear matrix protein
NOS	Not otherwise specified
NRAS	NRAS proto-oncogene
NSCLC	Non-small-cell lung carcinoma
NSTGCT	Non-seminomatous testicular germ cell tumour
NT-proBNP	N-terminal prohormone of brain natriuretic peptide
OPSCC	Oropharyngeal squamous cell carcinoma
OS	Overall survival
p53	Tumour protein p53
PARP	Poly (adenosine diphosphate-ribose) polymerase
pCR	Pathological complete response
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein 1
PDGF	Platelet-derived growth factor
PDGFR	Platelet-derived growth factor receptor
PD-L1	Programmed death-ligand 1
PFS	Progression-free survival
PI3K	Phosphatidylinositol 3-kinase
PI3KCA	Phosphatidylinositol 3-kinase catalytic subunit alpha
PKC	Protein kinase C
PMBL	Primary mediastinal B cell lymphoma
PMS2	Postmeiotic segregation 1 homologue 2
POG	Personalized Oncogenomics

PR	Progesterone receptor
PS	Performance status
PSA	Prostate-specific antigen
PTEN	Phosphatase and tensin homologue
QALY	Quality-adjusted life year
QOL	Quality of life
QTc	Corrected QT interval
RAF	Rapidly accelerated fibrosarcoma
RAS	Rat sarcoma
RCC	Renal cell carcinoma
R-CHOP	Rituximab, cyclophosphamide, doxorubicin, vincristine, prednisolone
R-CODOX-M	Cyclophosphamide, vincristine, doxorubicin, methotrexate, cytarabine, rituximab
RCT	Randomized controlled trial
RFS	Recurrence-free survival
R-IPI	Revised International Prognostic Index
R-IVAC	Rituximab, ifosfamide, etoposide, cytarabine
RTK	Receptor tyrosine kinase
SAA	Serum amyloid A
SCC	Squamous cell carcinoma
SHH	Sonic hedgehog
SLAMF7	Signalling lymphocytic activation molecule F7
SNP	Single nucleotide polymorphism
STAT3	Signal transducer and activator of transcription 3
SYK	Spleen tyrosine kinase
TB	Tuberculosis
TdT	Terminal deoxynucleotidyl transferase
TGCT	Testicular germ cell tumour
TGF	Transforming growth factor
TKI	Tyrosine kinase inhibitor
TNF	Tumour necrosis factor
TNT	Triple-negative tumour
TTF-1	Thyroid transcription factor 1
VAF	Variant allele frequency
VEGF	Vascular endothelial growth factor
VEGFR	Vascular endothelial growth factor receptor
VHL	von Hippel–Lindau
VUS	Variant of unknown significance
Wnt	Wingless/Int-1

PERSPECTIVE

01 An Introduction to Precision Oncology

Ellen R. Copson, Peter Hall, Ruth E. Board, Gordon Cook, Peter Selby

Background

The principle of personalized medicine, which aims to deliver a management schedule based on the attributes of the individual rather than on the whole population with the same diagnosis, has always been particularly attractive in oncology. Identification of patients who will receive maximum benefit from aggressive treatment regimens as well as those who will not benefit from standard therapies has the potential to improve cure rates in early disease, reduce the risk of toxicity and improve quality of life in advanced cancer. Historically, however, cancer has largely been treated simply on the basis of the anatomical site and extent of the disease using cytotoxic drugs that cause significant collateral damage through a tendency to identify malignant cells only by their rapid movement through the cell cycle. Prior to 1990, drugs that recognized cancer cells in a more targeted fashion did exist, but their use was largely unselected; for example, the anti-oestrogen tamoxifen was originally given to all breast cancer patients. Its use was only limited to oestrogen receptor-positive patients after retrospective data published some 30 years later showed clear evidence of effectiveness only in this subgroup.

Use of biomarkers to measure disease course or treatment responsiveness has also largely relied on circulating protein 'tumour markers', which vary significantly from one patient to another in terms of their specificity and clinical utility. Important exceptions are alpha-fetoprotein (AFP) and human chorionic gonadotrophin (hCG), which have gained clinical acceptability as useful prognostic markers of germ cell cancer. The production of trastuzumab, a humanized monoclonal antibody specifically designed to bind to a protein overexpressed on the cell surface in an aggressive subgroup of breast cancers was a pivotal development in oncology. The success of this drug, which combines clinical effectiveness with a preferable toxicity profile to conventional cytotoxics, has been followed by the development of many other antibodies and small molecules designed to match newly identified tumour features.

Recent developments

The completion of the Human Genome Project in 2000 heralded a new era in cancer biology. We now know that there are typically between 1000 and 10,000 somatic genetic changes in the genomes of most adult cancers. The mutational landscapes of many tumours have been made publicly available through projects such as The Cancer Genome Atlas project. Identification of the key driver mutations in some tumour types has permitted the development of a number of therapeutic agents that specifically target the aberrant protein product. In current clinical practice the abnormal protein or genetic fault is usually detected using tests developed to detect that specific tumour-associated change. More recently, however, with massive advances in genomic technologies over the last decade, it is now feasible and potentially cost-effective to directly examine the whole DNA sequence of an individual tumour specimen in a timely fashion in order both to predict response to novel targeted therapies and to increase our prognostic accuracy by categorizing disease subtypes at a molecular level.[1] The feasibility of large-scale

tumour genomic testing in the NHS has been demonstrated by the success of the first phase of the Cancer Research UK Stratified Medicine Programme in which >40,000 genetic tests were performed on over 9000 patient tumour samples at three central laboratories during a 2 year pilot study.[2]

Provision of tumour genomic profiling in a timely fashion has resulted in a wave of new clinical trial designs, in which patients, often from a broad spectrum of solid tumour types, are admitted to a trial on the basis of broad clinical eligibility criteria, and subsequent treatment allocations or randomizations are contingent on the presence or absence of specific somatic mutations. Such trials are increasingly offering a 'basket' of targeted agents as treatment arms, frequently supplied by more than one pharmaceutical company.

In addition, use of the same technology on germline DNA samples has permitted the advent of fast track testing for inherited mutations in cancer predisposition genes.[3] This, together with advances in our understanding of the biology of certain cancer susceptibility syndromes and the development of drugs which specifically aim to exploit the inherited genetic variation, is increasingly bringing genetic testing out of specialist clinics and into mainstream oncology. Identification of rare DNA variants associated with enhanced toxicity to specific chemotherapeutic agents has also become possible and is starting to enter routine clinical practice.

In parallel with the advances in genomics, other tools to analyse changes in cell biology and the proteomics of cancers and patients are becoming more powerful, faster and cheaper.[4] They are slowly but clearly adding to our ability to identify new markers to support precision oncology and to evaluate them thoroughly. Clinical research to evaluate the effectiveness of precision oncology strategies will increasingly benefit from developments in methodologies in informatics and applied health research.

The rewards

The introduction of novel anticancer drugs designed to target aberrant proteins specific to the malignant tumour has already resulted in some impressive advances in certain solid tumours. Metastatic melanoma has traditionally been associated with extremely poor survival due to a relative lack of chemosensitivity. Identification of the driver gene mutation *BRAF* V600E in approximately half of patients with metastatic melanoma led to trials of serine/threonine-protein kinase B-Raf (BRAF) kinase inhibitors in this patient group. A phase III trial comparing use of the BRAF inhibitor vemurafenib in melanoma patients with standard chemotherapy dacarbazine reported a significant increase in progression-free survival (PFS) among those who received vemurafenib.[5] Similar results have subsequently been reported with the alternative BRAF inhibitor dabrafenib.

Advances in our understanding of the mutational landscape of non-small-cell lung carcinoma (NSCLC) have already resulted in changes in the standard management of this group of patients.[6] Several tyrosine kinase inhibitors are now approved for use in the 10–40% of NSCLC patients whose tumours harbour mutations in *EGFR*, the epidermal growth factor receptor gene, following clinical trials reporting response rates of up to 80% and higher median survival than with conventional chemotherapies. Rearrangements of the *ALK* gene are also now being tested routinely in selected NSCLC patients following licensing of the anaplastic lymphoma kinase (ALK) inhibitor crizotinib as a second line therapy for this patient group.

A recent multivariable analysis of clinical trial data from 641 studies reported that personalized therapy compared with non-personalized therapy was associated with a higher response rate

(31% vs 10.5%), prolonged PFS (5.9 vs 2.7 months) and longer overall survival (13.7 vs 8.9 months), whilst another meta-analysis including over 38,000 patients also demonstrated improved outcomes with personalized treatment approaches.[7]

Use of genomic tumour profiling to predict benefit from traditional chemotherapy has also now been successfully introduced into the clinic. In 2013, NICE approved the funding of Oncotype DX (Genomic Health, London, UK), a commercial genomic assay that compares expression of 16 genes implicated in breast cancer prognosis with five reference genes to produce a 'recurrence score'. This provides selected breast cancer patients with enhanced information about their individual benefit from adjuvant chemotherapy. A number of similar tests are also now under evaluation in clinical trials.

The challenges

Despite the encouraging results of meta-analyses reviewing the benefit of personalized anticancer therapies, the only prospective trial to date randomizing patients with advanced cancer to either a targeted treatment based on molecular tumour profiling or treatment of physician's choice has recently reported disappointing results. The Randomized Phase II Trial Comparing Therapy Based on Tumor Molecular Profiling versus Conventional Therapy in Patients with Refractory Cancer (SHIVA) trial[8] found no significant difference in PFS between the two treatment arms and more toxicity with the targeted therapies. The full trial report demonstrates many of the difficulties now being recognized as challenges to fulfilment of the potential of precision oncology.

The first practical issue is the need to provide high-quality DNA in sufficient quantities for successful sequencing; thus, the ideal source of tumour DNA is a fresh-frozen tumour sample of sufficient size.[4] This may therefore require patients to have additional biopsies beyond those normally required for diagnostic purposes. Secondly, our rapidly developing appreciation of the heterogeneity of solid tumours suggests that a single site biopsy at a single time point will provide only limited information. Traditionally genomic testing is done on the primary tumour biopsy, which may not reflect the true DNA sequence of a metastatic deposit. Thirdly, the vast amount of data produced particularly by whole genome sequencing and uncertainties over the reporting of variants as well as difficulties in identifying driver and passenger mutations mean that producing a genomic profile report in a timescale where it can be of benefit to a patient is a significant challenge. Fourthly, introduction of routine genomic testing and targeted therapies will require significant financial investment. Finally, there are unique ethical questions raised by this new availability of heritable genetic data.

The future

Most clinical trials to date have used parallel tests of a panel of frequently mutated genes relevant to cancer to demonstrate significant germline or somatic DNA variations. The rapid reduction in sequencing costs has led to increasing use of whole genome sequencing as both an exploratory research tool and as a clinical test. There is also increasing interest in the concept of liquid biopsies,[9] which use repeated peripheral blood tests to capture tumour genomic data via circulating tumour DNA in order to provide a longitudinal picture of metastatic cancer as it evolves. The use of high-throughput large data technologies ('omics') is already extending beyond DNA and into the analysis of RNA, protein and metabolites with the aim of identifying highly sensitive and specific novel biomarkers that will permit identification and monitoring of cancers in a previously unknown manner. This is all leading to a paradigm shift in the perception

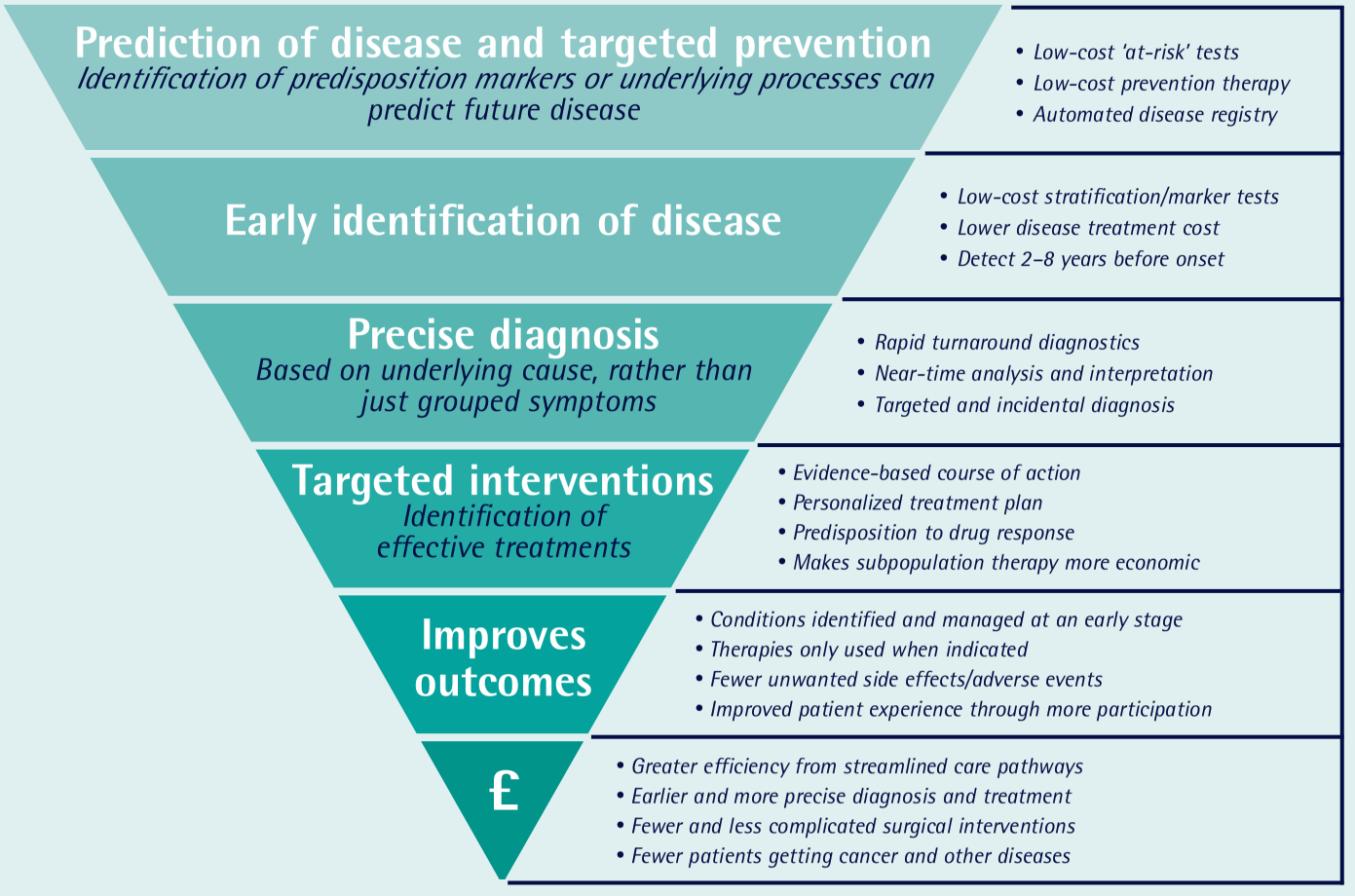

Figure 1.1 Personalized medicine strategy, as presented by Sir Bruce Keogh in a Board Paper to NHS England, 24 September 2015.[10]

of personalized cancer care with the potential use of germline and somatic genomic, transcriptomic, proteinomic and metabolomic profiles to facilitate diagnosis and treatment selection for cancer patients as well as to inform cancer prevention and screening strategies for at-risk individuals, thus providing a precision oncology approach.

The role of precision medicine as a means to improve the cost-effectiveness of modern healthcare has recently been highlighted in an NHS White Paper, presented by Sir Bruce Keogh (Figure 1.1).[10] Support from the Department of Health for the concept of precision medicine has resulted in government funding of the 100,000 Genomes Project, a highly ambitious project which is aiming to perform whole genome sequencing on 50,000 genomes from cancer patients (germline and tumour) in addition to genomes from families with rare diseases, and pathogens. This project is jointly managed by NHS England and Genomics England and has been envisaged as a transformative project that will embed genomic medicine into routine NHS patient care whilst also promoting the UK as a world leader in genomic technologies and research. NHS patients are currently being recruited into the cancer arm via regional genomic medicine centres in England, but it is anticipated that Scotland and Wales will be involved as the project progresses. A novel aspect of this project is that genomic medicine centres are obliged to inform participating patients about any 'actionable' genomic results, and patients can also opt to receive information about additional genetic findings that are not related to their primary cancer but that might benefit their long-term health. At present, few patients will receive somatic tumour information in a timeframe that would affect management of their primary cancer, but the project is aiming to achieve this within its lifetime.

The successful implementation of precision oncology will require attention to the following issues:

- Robust data collection linking clinical and pathological phenotypes to tumour and germline genotypes and treatment response data.
- Establishment of agreed standards in molecular pathology including processing methods, reporting formats, turnaround times and integration of molecular and histopathological results.
- Training of relevant multidisciplinary teams and individual professionals in genomics, in order to understand report results and their implications for management, as well as the relative merits and limitations of different techniques used for genomic analysis.
- The need to ensure that molecular testing and consequent clinical recommendations are rigorously assessed with high-quality study designs[11,12] and are appropriately funded at national levels.

Conclusion

Precision oncology offers a hugely exciting opportunity to personalize cancer treatments for the benefit of the individual, but it also presents great challenges, both scientific and ethical.[13] It will remain essential to pursue evidence-based medicine; whether it is appropriate to prescribe off-licence on the basis of genomic profiling without clear evidence of benefit outside a clinical trial remains a matter of debate. Delivery of this new era of cancer care will require a new type of multidisciplinary model involving oncologists, geneticists, cellular and molecular pathologists, information technology experts and bioinformaticians.

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PERSPECTIVE

02 Testicular Cancer: a Successful Model for Biomarker-Guided Precision Cancer Care

Johnathan Joffe

Introduction

Testicular germ cell tumours (TGCTs), regarded as rare cancers, are increasing in frequency: over the last 30 years, their incidence in the UK has approximately doubled. Similar trends have been observed in multiple geographical locations.[1] It has been suggested, however, that in some countries the number of cases may have plateaued in recent years and the incidence of the two main histological subtypes of TGCTs may be starting to diverge. With the development of platinum-based chemotherapy regimens in the last 30–40 years, this once fatal disease has become a model for curability in cancer, as disease-specific survival exceeds 98%.

As a curable malignancy, much effort in clinical research in TGCTs is directed towards identifying those patients in early-stage disease who need therapies that will prevent recurrence, sparing the burden of treatment toxicities for those who will not relapse, and tailoring treatments in advanced disease to minimize toxicities. Most patients may then survive TGCTs and live long and productive lives.

In the development of management strategies, a number of histological and biochemical factors have been identified that support these aims. They can be used to advise patients with different stages of disease on the most suitable management strategy for their disease that best suits their own physical and psychosocial characteristics.

A biomarker has been defined as: 'A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention'[2] and 'Any substance, structure or process that can be measured in the body or its products and influences or predicts the incidence or outcome of disease.'[3]

This chapter outlines how biomarkers have revolutionized the management of TGCTs, making it a prime example of precision oncology. It explores how the traditional medical biomarkers derived from histological analysis and serum protein expression are being augmented, and potentially superseded, by novel molecular and genetic markers that will allow even greater precision in treatment selection and development.

Current biomarker-directed management of TGCTs

Early-stage disease

TGCTs are biologically complex malignancies that can demonstrate a number of histological patterns within both the primary tumour and the metastatic deposits that occur at distant sites in advanced disease. A full description of this classification is beyond the scope of this chapter. All TGCTs, however, are understood to derive from a common primordial germ cell.[4] The clinical management of early-stage TGCTs is directed by the separation of seminomas from all other

TGCTs (including those comprising seminomas mixed with other histological subtypes): these are referred to as non-seminomatous TGCTs (NSTGCTs). Seminomas have a different sensitivity to treatment with radiotherapy and chemotherapy compared with NSTGCTs. Different histological characteristics appear to predict relapse in early-stage seminomas compared with NSTGCTs following orchidectomy.

In early-stage NSTGCTs, tumour invasion of intratesticular blood and lymphatic vessels increases the likelihood of relapse in patients managed by surveillance without therapy following orchidectomy.[5] This categorical biomarker identifies a group of patients with a risk of relapse with distant disease of between 40% and 50% compared with patients lacking this feature in whom relapses are seen in fewer than 20% of cases. Studies have demonstrated that adjuvant (postoperative) chemotherapy in high-risk patients reduces the risk of recurrence from 40–50% to 2% with two cycles of low-dose bleomycin, etoposide and cisplatin (BEP) chemotherapy.[6] This spares those patients destined to relapse from at least three cycles of higher dose and more toxic BEP chemotherapy. Potentially, current studies will refine this adjuvant therapy to a single high-dose cycle of BEP. In that situation the total use of BEP in 100 patients with high-risk stage I disease would be 100 cycles, compared with surveillance of this group in which 50 patients would receive three cycles of BEP with far greater short- and long-term toxicities, including death (<1%), infertility, and cardiovascular, kidney, lung and neurological (auditory and sensory) damage.

In seminomas, lymphovascular invasion does not appear to be as predictive of relapse in stage I disease. Tumour size, however, is predictive of relapse, and in some studies tumour infiltration of a central testicular structure called the rete testis is also predictive.

Advanced-stage disease and previously treated patients

Two specific serum 'tumour markers' have been identified that are useful in assessing disease activity in TGCTs, both in patients with active disease and in the detection of relapse in patients on follow-up after previous treatment. The beta-subunit of human chorionic gonadotrophin (hCG) is raised in patients with active TGCTs: in 60% of NSTGCTs and in <40% of seminomas. It is produced in the syncytiotrophoblastic cells of seminomas and in choriocarcinoma cells within NSTGCTs. If it is produced by a primary testicular tumour without metastatic disease, following orchidectomy the serum levels of β-hCG fall with a predictable half-life of approximately 24–30 h. Alpha-fetoprotein (AFP) is produced, characteristically, from the yolk-sac component of approximately 50% of NSTGCTs. Production can also be seen in non-seminomatous tumours containing an embryonal cancer component. Following complete removal of a secreting tumour, the protein level falls to a half-life of approximately 5 days.

Following orchidectomy, in patients without radiological or clinical evidence of secondary cancers, knowledge of the half-life of these two biomarkers allows clinicians to confirm stage I disease by following serum levels, usually at weekly intervals, and confirming that the expected half-lives are followed and that the levels fall into the normal reference ranges. In patients in whom the markers do not normalize, it is recognized that this leads to an eventual rise and identification of metastatic disease requiring therapy. The specificity of this phenomenon is such that failure of normalization of postorchidectomy is recognized as identification of a unique stage of TGCTs called stage 1M, which in the TNM staging system requires a specific 'S' stage (serum), such that stage 1M disease is classified as T1N0M0S1.

The specificity and sensitivity of β-hCG and AFP allow these proteins to be measured in the follow-up of TGCTs and, particularly in NSTGCTs, they enable a reduction in the need for surveillance CTs,[7] which carry a significant risk of radiation-induced second malignancies.

In advanced NSTGCTs, AFP and β-hCG have been combined with an anatomical classification of distribution of secondary deposits and the levels of a third serum protein, lactate dehydrogenase (LDH), which is not specific for TGCTs but in the context of advanced disease has similar predictive value to β-hCG and AFP with respect to prognosis.[8]

The International Germ Cell Cancer Collaborative Group classification (Table 2.1) allows the identification of patients with advanced TGCTs who have a good prognosis and are likely to be cured by a standard schedule of three cycles of BEP chemotherapy. It also identifies two further groups of patients with intermediate and poor prognosis who are less likely to be cured by standard therapies (Figure 2.1) and who are selected for inclusion in trials of more intense or novel mechanistic therapies which can be compared with standard BEP.

New directions in biomarker-directed management of TGCTs

The current use of biomarkers in testicular cancer may be seen as a model for how conventional histological and serum markers can be used in prognostic, predictive and management protocols. Moreover, the rapid gain in understanding of the molecular biology and genetics of tumours provides opportunities to further develop the use of biomarkers in TGCTs.

In early-stage NSTGCTs, retrospective analysis of patients included in Medical Research

Table 2.1 Summary of International Germ Cell Cancer Collaborative Group classification of advanced germ cell tumours of the testis and mediastinum in adult males (adapted from International Germ Cell Cancer Collaborative Group[8]).

Prognostic classification	NSTGCT	Seminoma
Good prognosis	Testis/retroperitoneal primary and No non-pulmonary visceral metastases and Good markers 56% of NSTGCT 5 year PFS 89% Survival 92%	Any primary site and No non-pulmonary visceral metastases and Normal AFP, any β-hCG, any LDH 90% of seminomas 5 year PFS 82% Survival 86%
Intermediate prognosis	Testis/retroperitoneal primary and No non-pulmonary visceral metastases and Intermediate markers 28% of NSTGCT 5 year PFS 75% Survival 80%	Any primary site and No non-pulmonary visceral metastases and Normal AFP, any β-hCG, any LDH 90% of seminomas 5 year PFS 67% Survival 72%
Poor prognosis	Mediastinal primary or No non-pulmonary visceral metastases or Poor markers 16% of NSTGCT 5 year PFS 41% Survival 48%	No seminomas classified as poor prognosis

PFS, progression-free survival.

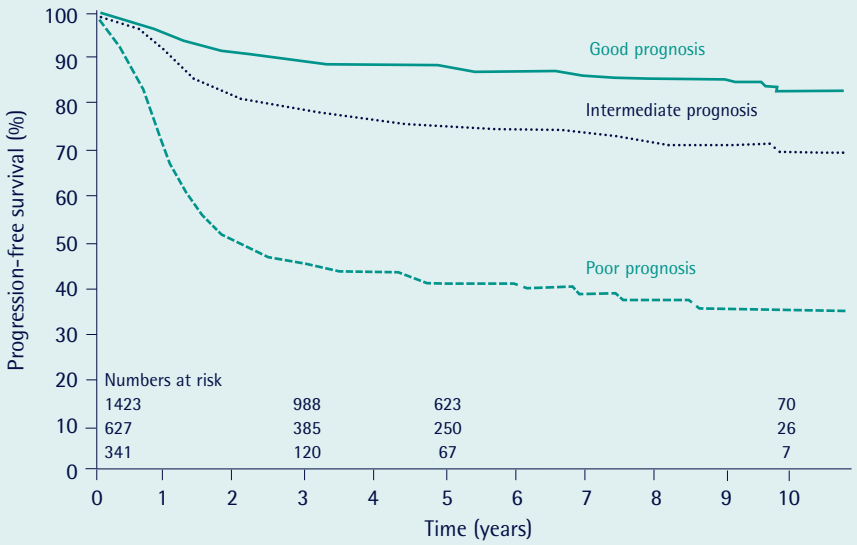

Figure 2.1 International Germ Cell Cancer Collaborative Group prognostic classification of advanced TGCTs.[8]

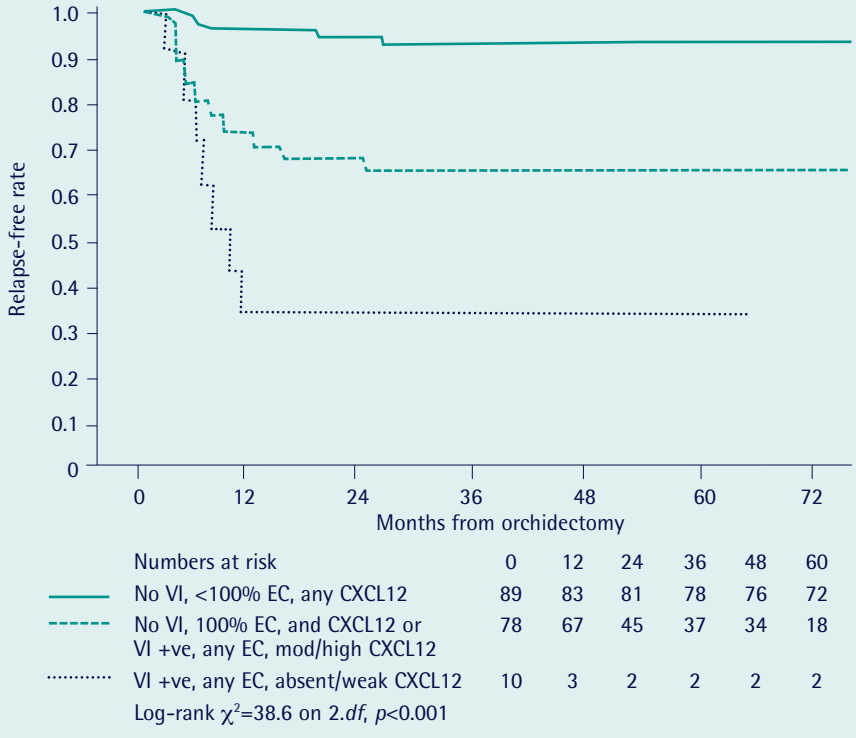

Figure 2.2 New potential prognostic classification of stage I NSTGCTs (adapted from Gilbert *et al.*[9]). CXCL12, expression of CXCL12 chemokine; EC, expression of embryonal carcinoma; +ve, positive; VI, vascular invasion.

Council surveillance and imaging studies[9] have identified a new predictive marker for relapse, C-X-C motif chemokine 12 (CXCL12), which is expressed in a proportion of TGCTs, is involved in maintenance of the spermatogonial stem cell niche, and in embryogenesis is involved in survival and migration of primordial germ cells to the adult gonadal sites.[10]

In combination with lymphovascular invasion and the histological manifestation of embryonal carcinoma in non-seminomatous tumours, the CXCL12 level has recently been shown to have potential in predicting relapse in stage I disease, and may have greater sensitivity than the use of lymphovascular invasion alone (Figure 2.2). A prospective study evaluating this novel combination of predictive biomarkers is in planning in the UK.

A further area of current interest and development is in the growing understanding of the comparative biology of different germ cell tumours that can arise at different anatomical sites and in different age groups. Although TGCTs are by far the largest group of such tumours, germ cell tumours also occur in infancy, in childhood and in females at sites that include the sacrococcygeum, CNS, ovaries and midline particularly the anterior mediastinum. At all of these sites the histological appearances of germ cell tumours can be very similar or identical to those of adult TGCTs. However, there is clear evidence that the tumours may behave differently from adult germ cell tumours and may have different requirements for treatment with curative intent.[11]

A number of microRNAs (miRNAs) have been identified that are detectable in serum and may offer similar biomarker utility to AFP and β-hCG in terms of monitoring disease.[11] Of interest is

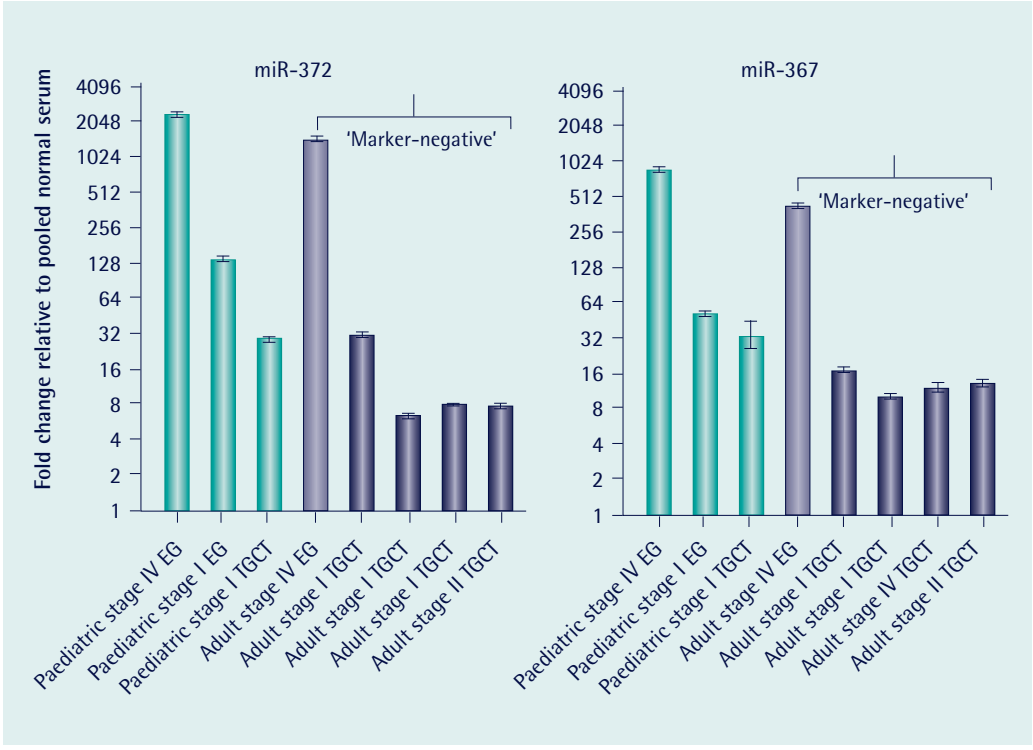

Figure 2.3 Expression of two specific miRNAs, miR-372 and miR-367, in different types of germ cell tumours, and correlation with expression of standard tumour markers (adapted from Murray and Coleman[11]). EG, extragonadal; 'Marker-negative', no serum expression of AFP or β-hCG.

that, collectively, these miRNA fragments are common to a wide group of germ cell tumours that occur at different anatomical sites and at different ages. Specific miRNAs may, however, be differentially expressed by different groups of germ cell tumours. Early analysis of these novel biomarkers suggests that they may have value in determining the likely biology of a germ cell tumour with respect to its classification as a paediatric or adult type of malignancy. This may have specific utility in determining the optimum management strategy, particularly in teenagers and young adults where there is uncertainty as to whether paediatric or adult management strategies are more effective (Figure 2.3).

Conclusion

Germ cell tumours, specifically adult testicular cancers, have been a model for the use of biomarkers in diagnosis, prognostication and prediction of outcome, and in monitoring disease activity. They have supported the development of globally accepted management policies and standardization of curative treatments. The utility of conventional biomarkers, however, leaves a number of questions unanswered. They may lack precision in determining optimum personalization of management. New molecular biomarkers under investigation will complement existing tumour markers and histological classifications and lead to a higher level of understanding of individual cancers. This will drive increasing personalization of care and higher rates of cure, with even less need for use of toxic therapies in those patients who are unlikely to require them.

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CASE STUDY

01 A Breast Cancer Patient with a *BRCA1* Mutation

M.H. Ruhe Chowdhury, Ellen R. Copson

Case history

A 32-year-old woman presented with a 6 week history of a lump in her right breast. Her medical history consisted only of an appendectomy performed 10 years previously. She was otherwise well and had no systemic symptoms. She did not take any regular medications and had no known drug allergies.

She had a significant family history of malignant disease: her sister was diagnosed with breast cancer aged 39, a paternal aunt was diagnosed with breast cancer aged 43, and her paternal grandmother was diagnosed with ovarian cancer aged 55.

She was a non-smoker and drank fewer than 10 units of alcohol per week. She lived with her partner and 2-year-old daughter. She had one younger sister and an older sister and brother.

On physical examination the patient had a palpable mass in the right breast. There was no palpable lymphadenopathy and the rest of the examination was unremarkable.

Mammography and ultrasound imaging of the right breast revealed a suspicious 26 mm mass in the upper outer quadrant. A core biopsy confirmed a grade 3 invasive ductal carcinoma, oestrogen receptor (ER)-negative (quick score 0/8), progesterone receptor (PR)-negative (0/8) and human epidermal growth factor receptor 2 (HER2)-negative (1+ immunohistochemistry). Breast MRI confirmed unifocal disease in the right breast. There was no radiological evidence of involvement of the axillary lymph nodes.

The multidisciplinary team (MDT) recommended neoadjuvant chemotherapy, followed by surgical resection of the tumour. Fast-track genetic testing identified a mutation in the *BRCA1* gene that was considered to be pathogenic.

After six cycles of neoadjuvant chemotherapy the patient had a radiological complete response. She underwent mastectomy and sentinel node biopsy. Histology revealed a pathological complete response (pCR).

What are the indications for referring this patient for *BRCA1*/*BRCA2* mutation testing?

What is the optimum systemic therapy regimen for this patient?

What should be taken into consideration when recommending breast surgery for this patient?

What is the role of prophylactic bilateral salpingo-oophorectomy (BSO) in this patient?

What are the implications of the *BRCA1* mutation finding for other family members?

What are the indications for referring this patient for *BRCA1/BRCA2* mutation testing?

Approximately 5% of all breast cancers are associated with an underlying mutation in the high penetrance cancer susceptibility genes *BRCA1* (chromosome 17) or *BRCA2* (chromosome 13). The estimated population frequency of *BRCA1/BRCA2* mutations in the UK is 1 in 500–10,000; the estimated prevalence varies between ethnic groups. *BRCA1* carriers have a cumulative risk of breast cancer of 50–80% by the age of 70, and a lifetime risk of ovarian cancer of 40–60% (Figure 1.1).

This patient met the NICE 2013 familial breast cancer guidance conditions for referral to a specialist genetics clinic, based on her family history but also on the onset of triple-negative breast cancer before 40 years of age.[1] Referrals for *BRCA* testing are based on an individual's probability of carrying a *BRCA* mutation; this can be calculated from the family history and tumour pathology using recognized assessment tools such as BOADICEA (http://ccge.medschl.cam.ac.uk/boadicea) and the Manchester scoring system. The 2013 NICE guidance recommends that genetic testing should be offered if the estimated probability of carrying the *BRCA1/BRCA2* mutation exceeds 10%.[1]

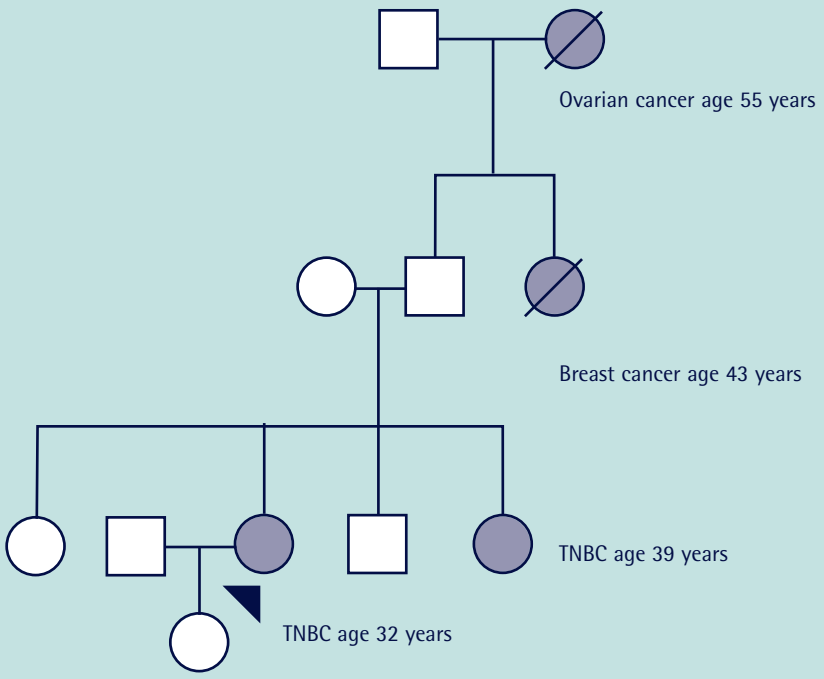

Figure 1.1 Family tree of the patient. TNBC, triple-negative breast cancer.

Approximately 80% of breast cancers in *BRCA* mutation carriers are ER-negative, PR-negative and HER2-negative: termed triple-negative tumour (TNT). Even with no family history of breast or ovarian malignancy, 15% of women diagnosed with a TNT aged <40 are *BRCA1*/*BRCA2* mutation carriers. This group of patients now meets the threshold for *BRCA* testing.[2]

Although the 2013 NICE guidance does not advocate accelerated *BRCA* testing (within 4 weeks of cancer diagnosis) outside clinical trials, the pathway has been adopted by some specialist centres in order to inform treatment options. All patients undergoing *BRCA* mutation testing require counselling regarding the potential for ambiguous (DNA variant of unknown significance [VUS]) as well as clear positive or negative results, and all patients found to carry a pathogenic *BRCA* mutation or a DNA VUS need to be seen by a clinical genetics team to discuss the full implications of these results.

What is the optimum systemic therapy regimen for this patient?

Until recently, *BRCA* mutation carriers with early breast cancer routinely received the same systemic treatments as non-mutation carriers, in view of both a lack of good-quality evidence that a germline *BRCA* mutation affects response to treatment and the difficulty in obtaining *BRCA* mutation screening results in time to alter treatment decisions. There is, however, increasing interest in the role of platinum chemotherapy agents in the treatment of *BRCA1* mutation carriers. Pathological *BRCA* mutations result in deficiencies of homologous recombination repair of DNA. Platinum chemotherapy causes inter-strand DNA crosslinks; it is therefore hypothesized that tumours with *BRCA* mutations are more sensitive than wild-type tumours to platinum chemotherapy agents. The TNT trial reported evidence of greater benefit from carboplatin in metastatic *BRCA* mutation carriers than in wild-type TNT patients.[3] Recent trial evidence in early breast cancer is, however, conflicting.[4]

The Addition of Carboplatin to Neoadjuvant Therapy for Triple-Negative and HER2-Positive Early Breast Cancer (GeparSixto) randomized phase II trial examined the addition of carboplatin to anthracycline, taxane and bevacizumab chemotherapy in the neoadjuvant setting.[5] In the TNT subgroup (n=315), pCR rates improved from 36.9% to 53% with carboplatin (p=0.005), and disease-free survival improved from 76.1% to 85.8% (p=0.0350). Compared with controls, in *BRCA* wild-type patients the odds ratio for pCR was 2.09 in favour of carboplatin, whereas in the *BRCA*-mutant patients it was 1.6 in favour of carboplatin; the difference was not statistically significant.[6] Paclitaxel with or without Carboplatin and/or Bevacizumab Followed by Doxorubicin and Cyclophosphamide in Treating Patients with Breast Cancer That Can Be Removed by Surgery (CALGB 40603) examined 443 patients with stage II and III triple-negative breast cancer in the neoadjuvant setting.[7] Patients were randomized to receive either carboplatin or bevacizumab, or both, concurrently with paclitaxel, followed by anthracycline chemotherapy. The trial reported a pCR rate (breast and axilla) of 54% in those receiving carboplatin compared with 41% (p<0.0029) in those not receiving carboplatin; however, the addition of carboplatin had no impact on either event-free survival or overall survival. Data on *BRCA* status are pending.

To date, studies examining the role of platinum chemotherapy agents in *BRCA* mutation-positive early TNT have suffered from small numbers and lack of power to fully assess the link between *BRCA* status and platinum sensitivity. The use of pCR as a surrogate of long-term outcome also remains controversial. Further trials to clarify the role of platinum chemotherapy agents in *BRCA* mutation and triple-negative breast cancer are in progress.

Poly (adenosine diphosphate-ribose) polymerase (PARP) inhibitors are a group of drugs that inhibit a protein involved in DNA repair. Cells with *BRCA* mutations are less able to facilitate efficient DNA repair, and PARP inhibitors can exploit this DNA repair deficiency leading to

cellular death. PARP inhibitors have been shown to have efficacy in the treatment of ovarian cancers in *BRCA* mutation carriers. A number of clinical trials are currently investigating PARP inhibitors in breast cancer.

What should be taken into consideration when recommending breast surgery for this patient?

The surgical plan should be fully discussed at an MDT meeting. The final decision should be determined by the degree of clinical response, whilst aiming for cure and respecting patient choice. In known *BRCA* mutation carriers there are some additional issues that need consideration.

- The risks of a new primary in the ipsilateral breast as well as the risk of recurrent disease in the ipsilateral breast if breast-conserving surgery is performed.
- The risks of a new primary in the contralateral breast.
- The risk of a future primary cancer at a non-breast site.

BRCA mutation carriers have a higher risk of second primary breast cancers compared with the general breast cancer population.[8] The 10 year risk of contralateral breast cancer in women with a *BRCA* mutation diagnosed before the age of 50 is approximately 24% for *BRCA1* compared with 5.1% for non-carriers. The risk of distant recurrence in young, unscreened breast cancer patients is overall higher than the risk of contralateral or ipsilateral new primary breast cancer. Therefore, although consideration of the prevention of local recurrence and new primary disease is an important aspect of the management of *BRCA* mutation-positive breast cancer patients, it is secondary to management of the primary disease and must be approached with an appropriately balanced discussion.[9]

In this case study, the patient had a radiological complete response, and in a non-*BRCA* mutation carrier the MDT would be expected to offer breast-conserving surgery. Given the lifetime risk of recurrence and second primary tumours, however, the benefits of immediate unilateral or bilateral mastectomy and associated risks may be discussed with the patient and compared with the alternative option of breast-conserving surgery followed by radiotherapy and annual screening with MRI plus mammography, with the potential to proceed to mastectomies at a later date if desired. An underlying *BRCA1* mutation is not a contraindication for breast irradiation but may have an impact on options for delayed reconstructions. The implications of mastectomy and reconstructive surgery in a young woman should also be discussed. Women who receive breast implants may require multiple reconstructions in their lifetime, whilst autologous implants are complex surgeries with a higher risk of complications.[9,10] The risk of distant metastases in patients with TNT is greatest within the first 3 years of diagnosis; therefore, for patients with a high risk of recurrence it may be appropriate to delay prophylactic surgery until after this time.

What is the role of prophylactic BSO in this patient?

Studies examining BSO in *BRCA* mutation carriers have reported a risk reduction in ovarian and breast cancer in premenopausal women and a decrease in death related to ovarian and breast cancer. Lifetime risk of ovarian cancer is usually accrued after the age of 40 for *BRCA1* carriers, with an approximate 10 year risk of 10–15% over the remaining lifetime. Ovarian cancer is associated with a later presentation and a higher mortality than breast cancer. Prophylactic BSO reduces ovarian cancer risk in *BRCA* carriers by up to 96%.[11,12] *BRCA* mutation carriers are therefore generally recommended to undergo BSO when they have completed their family. Those

with a strong family history may wish to proceed with this surgery at an earlier age; decisions regarding this should be made with the patient in a multidisciplinary setting that includes discussion with a clinical geneticist, an oncological gynaecologist and an oncologist. All patients need to be counselled regarding management of a surgically induced menopause. In this case, delaying BSO until about 40 years of age would be sensible, since she will have lived through much of the risk of recurrence from her initial primary tumour and at that stage use of oestrogen-only replacement therapy (with either a hysterectomy or local progesterone therapy) can be offered.

What are the implications of the *BRCA1* mutation finding for other family members?

BRCA mutations are inherited in an autosomal dominant fashion; therefore, the risk of carrying a *BRCA* mutation is 50% for each of the patient's full siblings and for her daughter. The patient should be seen by the clinical genetics service as early as possible to discuss sharing information with relevant family members. In this family, siblings and any children of the paternal aunt should have access to the information about the mutation so they can discuss the option of genetic testing with their regional genetics service.

Conclusion and learning points

- Management of patients with a *BRCA* mutation is a complex clinical dilemma and should involve full, wide-ranging MDT discussions. The patient should also be involved in all decisions and given full information.
- Treatments for patients with a *BRCA* mutation are rapidly evolving: targeted therapies such as PARP inhibitors offer further development of the treatment spectrum for these patients.
- The surgical plan in this patient group is multifactorial, taking into account treatment of the primary tumour, prophylactic surgery, contralateral surgery, risk of recurrence and patient choice.
- Treatment should be offered with curative intent; options should be clearly discussed (type and timing) for both therapeutic and risk-reducing breast surgery.
- Surgical plans can be complex and require multidisciplinary input.
- BSO should be discussed with the involvement of an oncological gynaecologist, and due consideration given to appropriate timing.
- Further studies are needed to establish the role of platinum chemotherapy in *BRCA* mutation-positive patients.
- Genetic counselling is required for the patient at an early stage and to help in communicating risk and testing options with family members.

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CASE STUDY

02 A Patient with DNA Mismatch Repair-Deficient Colorectal Cancer

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Case history

A 54-year-old man with a 3 week history of a change in bowel habit and abdominal pain was referred for urgent assessment. Investigations identified a 3.5 cm non-obstructing tumour in the ascending colon with no signs of significant lymphadenopathy or metastatic spread. Colonoscopy and biopsy demonstrated a moderately differentiated adenocarcinoma. His medical history included type 1 diabetes mellitus, complicated by mild sensory neuropathy. He was a teacher, married with two children and an ex-smoker of 20 years. His father was diagnosed with colorectal cancer at the age of 64.

The colorectal multidisciplinary team recommended primary resection of the tumour, and he proceeded to have a right hemicolectomy. Final pathological stage was pT3pN0 (0/21) M0V1R0 poorly differentiated adenocarcinoma of the ascending colon. Immunohistochemistry demonstrated a loss of expression of mutL protein homologue (MLH)-1 consistent with defective DNA mismatch repair (MMR). He was referred to the clinical genetics service and subsequently found to have a germline mutation in the MMR gene *MLH1.*

In view of the patient's pre-existing diabetic peripheral neuropathy he was considered unsuitable for standard adjuvant fluorouracil (5-FU), folinic acid and oxaliplatin (FOLFOX) chemotherapy. After considering his young age, poorly differentiated histology and DNA MMR status, however, his oncologist recommended 6 months of adjuvant 5-FU, folinic acid and irinotecan (FOLFIRI) combination chemotherapy. This was given, and he remains disease free at 5 years.

What is DNA MMR, and how does MMR deficiency lead to cancer?

What are the clinical features of inherited MMR gene mutation (Lynch syndrome [LS]), and how can we recognize and confirm this condition?

How does sporadic MMR deficiency occur in bowel cancer, and how does it differ from LS?

What is the impact of MMR deficiency, inherited or sporadic, on the prognosis and treatment choices for bowel cancer?

What primary and secondary cancer prevention strategies are advocated in individuals with LS?

What is DNA MMR, and how does MMR deficiency lead to cancer?

DNA MMR is a biological pathway that plays a key role in maintaining the stability of the genome. It is primarily concerned with the repair of base–base mismatches and small insertions or deletions acquired during DNA replication processes. Effective MMR therefore reduces the rate of spontaneous mutations. Mutations accumulate in cells with a deficiency of MMR activity, increasing the likelihood of carcinogenesis.

Replication errors most frequently occur in regions of microsatellite DNA (parts of the genome that have repetitive base sequences, normally 1–6 base pairs), as the replicative machinery more frequently 'slips' on repetitive sequences than on non-repetitive sequences.

Microsatellite instability (MSI) describes tumour cells with microsatellite regions that have changed in length, compared with the patient's normal tissue DNA, due to nucleotide insertions or deletions of the repeated motif. MSI is characteristic of an underlying MMR deficiency and can be identified using a panel of microsatellite markers targeted at mononucleotide repeats. They are classified as high, low or stable according to shifts in alleles. Assessment of MSI is sensitive for deficiencies in MMR, although it is not specific to inherited genetic defects in MMR, as only 20–25% of high MSI tumours are associated with germline mutations. The use of immunohistochemistry screening and MMR mutation detection is discussed later.

What are the clinical features of inherited MMR gene mutation (LS), and how can we recognize and confirm this condition?

LS, also known as hereditary non-polyposis colorectal cancer (HNPCC), is a hereditary cancer predisposition syndrome with an autosomal-dominant pattern, characterized by a high risk of early-onset colorectal cancer and an increased frequency of endometrial, ovarian, gastric, duodenal and urological malignancies. Pancreatic and brain tumours and sebaceous adenomas/carcinomas are also associated. LS has an incidence of 1 in 1000 of the general population and 1 in 100 patients with colorectal cancer, accounting for 2–3% of cases of colorectal cancer.[1] Individuals with LS have up to an 80% lifetime risk of colorectal cancer and typically present at a young age (45 vs 69 years in sporadic colorectal cancer). Characteristically, the colonic tumours are right sided (70%) and there is an increased rate of synchronous and metachronous tumours. Typical pathological features are Crohn's-like lymphocytic reaction, tumour-infiltrating lymphocytes and mucinous/signet ring histology. LS tumours demonstrate an accelerated adenoma/carcinoma progression but have a better prognosis than sporadic colorectal cancer.

In up to 70% of patients with LS the underlying genetic defect is a germline mutation in one of the DNA MMR genes *MLH1*, *MSH2*, *MSH6* and *PMS2*. In addition, mutations in the *EPCAM* gene can lead to hypermethylation and silencing of *MSH2*. Immunohistochemistry usually shows loss of the relevant protein and its binding partner; tumour DNA analysis shows high MSI in over 90% of individuals with LS-associated colorectal tumours.

The Bethesda guidelines use clinical and histological features to identify patients who should be investigated for LS (Table 2.1).[2] The criteria, however, fail to identify some individuals with LS; therefore, UK units are increasingly adopting immunohistochemistry to screen a wider group of patients. Screening with immunohistochemistry is performed by staining for the MMR proteins MLH1, mutS protein homologue (MSH)-2, MSH6, and postmeiotic segregation 1 homologue 2 (PMS2). Seventy-five percent of tumours that have no *MLH1* expression will be a sporadic cancer; therefore, second-stage tumour testing for epigenetic silencing of *MHL1* promoter (either by testing for hypermethylation of *MHL1* promoter or for V600E *BRAF* mutation) is advised. Tumours that fail second-stage testing should then be tested for germline mutations.

Table 2.1 Revised Bethesda guidelines (adapted from Umar *et al.*[2]).

One of the following criteria must be met:
• Diagnosed with colorectal cancer before the age of 50 years
• Synchronous or metachronous colorectal or other HNPCC-related tumours (endometrial, gastric and ovarian cancer), regardless of age
• Colorectal cancer in patients under 60 years showing high MSI morphology (Crohn's-like lymphocytic reaction, mucinous/signet ring differentiation, medullary growth pattern and poorly differentiated)
• Colorectal cancer and one or more first-degree relatives with colorectal cancer or other HNPCC-related tumour. One of the cancers must have been diagnosed before the age of 50 years (including adenoma, which must have been diagnosed before the age of 40 years)
• Colorectal cancer and two or more relatives with colorectal cancer or other HNPCC-related tumour, regardless of age

This case of colorectal cancer was in a 54 year old with a poorly differentiated proximal tumour. His father was over 50 when he was diagnosed with colorectal cancer; however, his pathological features dictated that he should be screened for MMR deficiency. A germline mutation in the *MLH1* gene was identified after immunohistochemistry screening detected loss of MLH1 protein expression.

How does sporadic MMR deficiency occur in bowel cancer, and how does it differ from LS?

Two to three percent of individuals with colorectal cancer have MSI as a consequence of a germline mutation in MMR genes (LS). Up to 15% of patients with sporadic colorectal cancer, however, also have MSI. The majority of these tumours have lost expression of *MLH1* and *PMS2* through hypermethylation of their promoter regions. They characteristically do not have familial clustering; exhibit absence of MLH1 and PMS2 proteins; show bi-allelic methylation of the *MLH1* promoter; and frequently have mutations in *BRAF*. Compared with LS patients with colorectal cancer, patients with sporadic colorectal cancer with MSI are older; the loss of *MHL1* expression increases with age, rising to 50% in patients with colorectal cancer older than 90 years.

MSI associated with sporadic colorectal cancer arises from hypermethylation in a CpG island methylator phenotype. Cytosines in promoter regions are methylated through methyltransferases, which leads to the silencing of corresponding genes. When a tumour suppressor gene such as *MLH1* is silenced it contributes to carcinogenesis. Methylation increases with age, and in response to chronic inflammation is accelerated in the colon.

While *BRAF* mutations are more frequent in sporadic colorectal cancer with MSI, they are rarely seen in patients with LS and confer a more favourable outcome. Conversely, *KRAS* mutations are more frequent in patients with LS, and both are involved in pathways that lead to epithelial proliferation. Colorectal cancer patients with MSI share loss of the MMR proteins through gene inactivation. In LS it results from inactivating germline mutations, while in sporadic colorectal cancer it is from silencing of MMR genes in the CpG island methylator phenotype. The result is a hypermutable state that leads to accelerated carcinogenesis.

What is the impact of MMR deficiency, inherited or sporadic, on the prognosis and treatment choices for bowel cancer?

Two factors – prognostic and predictive – may affect the decision to use adjuvant cytotoxic chemotherapy in MMR-deficient colorectal cancer.

Prognostically, colorectal cancer in patients with DNA MMR has long been known to confer improved survival. Compared with sporadic colorectal cancer, patients with LS have a lower stage at diagnosis, reduced incidence of distant metastases and improved overall survival (HR 0.67; $p<0.0012$).[3] The survival benefit extends beyond those just with LS but to all patients with MSI.[4]

As a predictive factor, preclinical data from the 1990s suggested that DNA MMR confers resistance to cytotoxic drugs via the DNA MMR system, including 5-FU.[5] Clinical studies have subsequently demonstrated that patients with MMR-deficient colorectal cancer may derive no benefit, or even harm, from single-agent adjuvant 5-FU.[6] By contrast, MMR-deficient cells may show increased sensitivity to oxaliplatin and irinotecan. Retrospective analysis of the Multicenter International Study of Oxaliplatin/5-FU–Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) clinical trial suggests that oxaliplatin and 5-FU, the standard adjuvant therapy for moderate- or high-risk colon cancer, is also effective in MMR-deficient cancers;[7] however, the patient in our case study had diabetic neuropathy, which is a relative contraindication to oxaliplatin. Irinotecan is not a validated adjuvant therapy for colon cancer, but retrospective analysis of the Combination Chemotherapy in Treating Patients with Stage III Colon Cancer (Cancer and Leukemia Group B 89803) trial found that patients with MMR-deficient tumours benefited markedly from 5-FU and irinotecan,[8] providing the basis for its use in this case.

Immunotherapies have changed the treatment paradigm for some cancers. Those with a high mutagenic burden are more susceptible to checkpoint blockade; one reason could be the increased expression of 'non-self' immunogenic antigens. There is accumulating evidence that patients with high MSI colorectal cancer may benefit from these checkpoint inhibitors.

What primary and secondary cancer prevention strategies are advocated in individuals with LS?

Patients who have colorectal cancer in the context of LS have a standardized incidence rate of a second colorectal cancer of 28% over 10 years.[9] Options to reduce risk of a second colorectal cancer are either extended colectomy at primary diagnosis or intensive postoperative surveillance. Three yearly colonoscopy reduces colorectal cancer incidence and mortality. Patients with LS, however, advance through carcinogenesis at an increased rate and more intensive surveillance every 1–2 years may have an increased survival benefit (no trials directly compare surveillance frequency).

A long-term intention-to-treat analysis found that use of 600 mg aspirin as a chemo-preventative agent for at least 2 years reduced the risk of developing colorectal cancer by 60% in patients with LS (with no significant difference in adverse events).[10] There are, however, no long-term data on the impact on mortality, and the dosing and timing have yet to be established. There was also a trend towards a reduced incidence of other HNPCC-associated cancers.

Annual colonoscopy screening from the age of 20–25 years is recommended for carriers of HNPCC-associated gene mutations. Regular upper endoscopy screening should also be considered for HNPCC families with a history of gastric cancer, and urine cytology for patients with a family history of urological tumours. The role of prophylactic colectomy in the management of patients with HNPCC remains controversial. Female patients with LS should be screened for ovarian and endometrial tumours by pelvic ultrasonography, with annual transvaginal measurements of endometrial thickness and/or endometrial aspirates. Some specialists recommend prophylactic hysterectomy and bilateral salpingo-oophorectomy on completion of childbearing for female carriers of HNPCC-associated gene mutations.

Conclusion and learning points

- LS is an inherited cancer predisposition syndrome associated with germline mutations in MMR genes.
- Patients diagnosed with colorectal cancer under the age of 70 should be screened for high MSI or deficiency in MMR proteins, and immunohistochemistry used to direct second-stage testing for germline mutations in *MSH2*, *MLH1*, *HSH6*, *PMS2* and *EPCAM*.
- Tumours with high MSI confer improved outcomes, but there is no evidence for use of single-agent 5-FU in patients with LS.
- Sporadic colorectal cancer with MSI most frequently results from the silencing of *MHL1* through the hypermethylation of its promoter region. It typically occurs in an older population and is associated with improved outcomes in comparison with stable MSI colorectal cancer.
- Secondary cancer prevention strategies can include regular surveillance programmes or extended colectomy at diagnosis.
- MSI tumours exhibit a good response to checkpoint blockade and provide a good biomarker for responses to immunotherapy

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