RESEARCH

REVIEW ARTICLE

The precision medicine approach to cancer therapy: part 2

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Abstract: The era of a one-size-fits-all approach to the treatment of many different diseases was dominant in the twentieth century but is rapidly receding and being replaced with an increasingly personalised 'precision medicine' (PM) approach. Biomarkers (also known as a 'molecular markers' or 'signature molecules') are now being used for pre-disposition, diagnostic, predictive, prognostic, toxicological or monitoring purposes, and patients are benefiting from more individualised treatments. Oncology has led the field in introducing precision therapies, with a similar approach now being developed in areas such as neurology and endocrinology. In this new genomic era of medicine, healthcare professionals need to have a sound working knowledge of not only the PM available and used in practice, but also of the biomarkers targeted and the pharmacogenomic tests available to detect them.

This review focuses on predictive biomarkers, with examples of pre-disposition, diagnostic and prognostic biomarkers. It aims to bring together anticancer agents that have been approved for use in the UK (at the time of publication) as part of a PM approach, along with information relating to the relevant biomarkers and biomarker assays available. Although this should prove useful to practicing clinicians and pharmacists, it can only represent a snapshot of the current therapeutic landscape owing to the increasingly rapid movement of this field. This review is in two parts and presented in sections relating to cancer type, although some biomarkers are relevant to more than one.

Part 1 focuses on solid tumours and includes an introduction to the technologies used for companion diagnostic tests, along with discussions of the limitations of biomarker testing, and the role of regulatory bodies in validating biomarkers and companion diagnostic tests. PM-based clinical trials, tumour agnostic anticancer agents, supportive therapies, and funding challenges for the NHS in relation to the growing introduction of novel PM agents and biomarker assays are also covered. This review focuses on haematological cancers, and includes a summary of potential future applications of the PM approach in oncology.

Keywords: Biomarkers, cancer, clinicians, oncology, personalised medicine, pharmacists, precision medicine.

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Introduction

Everyone has their own unique genome, which includes small, single nucleotide polymorphisms (SNPs) or large changes in DNA base-pair sequence (e.g. translocation mutations). These can be inherited or introduced during a person's lifetime owing to the effect of external agents, such as carcinogenic chemicals or radiation. Although usually harmless, these changes can affect the way an individual responds to a therapeutic agent either through differences in the drug target, or through changes to ADME parameters (i.e. absorption, distribution, metabolism and excretion).

Precision medicine, sometimes known as 'personalised medicine' and abbreviated to 'PM'[1,2], is a term that is increasingly being used to describe treatments tailored to individual patients or groups of patients[3]. The overall goal of the PM approach is to match therapies to individual patients, thus ensuring they receive effective treatment with minimal toxicity. This is particularly important for patients with cancer who may have a limited life expectancy. Furthermore, there has been an ongoing decrease in the cost of sequencing the human genome, which has led to the widespread adoption of integrative sequencing strategies for the study and treatment of cancer[4].

Within the field of oncology, most aspects of a PM approach involve the identification of 'biomarkers' associated with a particular cancer type. A biomarker is a mutated nucleic acid sequence, protein or group of proteins, expressed uniquely by the tumour cells (e.g. mutated *BRCA1* and *BRCA2*), or a non-mutated protein or receptor (e.g. HER2 or VEGF) upregulated in tumour cells compared with healthy cells[1].

This review is presented in sections relating to cancer type, although some biomarkers are relevant to more than one. Part 1, (bit.ly/PJPrecisionMedicinePart1) which focuses on solid tumours, includes an introduction to the technologies used for companion diagnostic (CDx) tests, along with discussions of the limitations of biomarker testing and the role of regulatory bodies in validating biomarkers and CDx tests. PM-based clinical trials, tumour agnostic anticancer agents, supportive therapies, and funding challenges for the NHS in relation to the growing introduction of novel PM agents and biomarker assays are also covered. Part 2 focuses on haematological cancers, and includes a summary of potential future applications of the PM approach in oncology.

Biomarkers, companion diagnostic tests and precision anticancer agents for haematological malignancies

Haematological malignancies are a group of blood-forming cancers that develop in either the bone marrow or the cells of the immune system. Therefore, they are sometimes referred

to as blood or liquid tumours[5]. The World Health Organization (WHO) has developed a unified classification system for haematological malignancies, dividing them primarily into neoplastic diseases of the haematopoietic or lymphoid tissues, which are then further divided into sub-categories according to the cells from which they originate[6].

Cancer-relevant biomarkers, the assays to detect and evaluate them, and the associated anticancer drugs have been reviewed based on the primary and patent literature, and information available from conferences and the websites of pharmaceutical companies. The information has been grouped below in sections relating to the type of malignancy. However, although some biomarkers are highly specific for a particular cancer type (e.g. BCR-ABL for chronic myeloid leukaemia [CML])[7], others are relevant to more than one different type of cancer (e.g. PD-L1 for both myeloma and lymphomas).

Leukaemias: chronic lymphocytic, chronic myeloid, acute lymphoblastic and acute myeloid leukaemias

The signal transduction pathways of leukaemias such as CML are activated by the BCR-ABL translocation gene mutation[7], and patients with this mutation can be selected for treatment with kinase inhibitors such as imatinib (Glivec, Novartis), ponatinib (Iclusig, Incyte Biosciences UK Ltd), bosutinib (Bosulif, Pfizer), dasatinib (Sprycel, Bristol-Meyers Squibb) and nilotinib (Tasigna, Novartis).

When patients become resistant to the first agent used for treatment (i.e. typically imatinib) owing to mutations of the amino acid residues within the ATP-binding pocket where these agents interact, treatment can be swapped to one of the second-generation agents that fit with a different orientation in the ATP-binding pocket, thus restoring therapeutic activity (see Figure 1)[8].

The biomarker CD20 is expressed by B-lymphocytes. Expression increases when these cells are grown in the laboratory and treated with interleukins 2, 4 and 7[9], suggesting that it is a marker of activation and DNA synthesis. It is expressed or over-expressed in acute lymphoblastic leukaemia (ALL) and other haematological malignancies, and can be used to select patients for treatment with agents such as rituximab (MabThera, Roche), obinutuzumab (Gazyva, Roche) or ofatumumab (Azerra, Novartis). In January 2018, Novartis announced the withdrawal of ofatumumab for chronic lymphocytic leukaemia (CLL) from markets outside the United States owing to a low uptake by clinicians, although the company has set up compassionate-use programmes. For example, Denmark's Genmab has received a US$50m compensation payment so that current patients can continue treatment[10,11].

CD33 is a biomarker present on the surface of acute myeloid leukaemia (AML) blasts (present in 85–90% AML patients) and leukaemic stem cells. It is also present at high levels in patients carrying the nucleophosmin mutation NPM1. Therefore, it has gained clinical importance as a target for antibody-based therapies, which can ablate disease-relevant CD33+ cells[12,13]. A study by Propris *et al.* demonstrated that AML patients carrying the NPM1 mutation typically have a high level of CD33 expression, suggesting that this correlation may be disease-associated and potentially important for managing this patient subgroup[13]. Gemtuzumab ozogamicin (Mylotarg, Pfizer) is an anti-CD33 antibody-drug conjugate (ADC) containing the highly cytotoxic DNA-cleaving calicheamicin payload, which is released within the tumour cells after internalisation of the ADC. Although it improves the survival rate of AML patients when used in conjunction with chemotherapy[12], it is widely agreed that CD33 is not an ideal biomarker, as many non-malignant haematological cells can express it as well. Walter *et al.* have hypothesised that CD33-targeting agents, such as gemtuzumab ozogamicin, are more beneficial for patients with mature leukaemic stem cells[14].

CD22 is an inhibitory receptor of B-cell signaling expressed in more than 90% of patients with leukaemia[15]. Therefore, it can be used as a biomarker for drug therapy in patients over-expressing it (15). Inotuzumab ozogamicin (Besponsa, Pfizer) is a CD22-targeted ADC also based on the DNA-cleaving agent calicheamicin. It is used to treat B-lymphoid malignancies[16], and works by binding to its target receptor with a sub-nanomolar affinity, followed by cellular internalisation followed by release of its highly cytotoxic payload[15].

P53 plays a crucial role in cell cycle regulation and apoptosis following DNA damage. Its role in tumorigenesis is well established in both solid and hematologic malignancies, particularly in AML and CLL, in which its deregulation represents an important predictor of poor outcome[17]. Although idelasib (Zydelig, Gilead Sciences) and ibrutinib (Imbruvica, Janssen-Cilag) were not developed alongside their own CDx tests, patients can be selected for treatment with these agents using Abbott Molecular's Vysis CLL FISH Probe Kit which was developed for use with venetoclax (Venclexta, AbbVie Inc) for the treatment of CLL[18].

Bruton's tyrosine kinase (BTK) has been shown to be critical for the development and function of normal B-lymphocytes, and expression of this protein appears to be necessary for CLL cells to survive and proliferate[19]. However, the precise role of BTK in the initial development of CLL, as well as in the disease expansion phase, is unclear[19]. In addition, it is known that leukaemic cell lines may express FLT3 and FLT3 ligand (see below) simultaneously, suggesting that autocrine or paracrine stimulation may cause proliferation of these cells along with BTK[20].

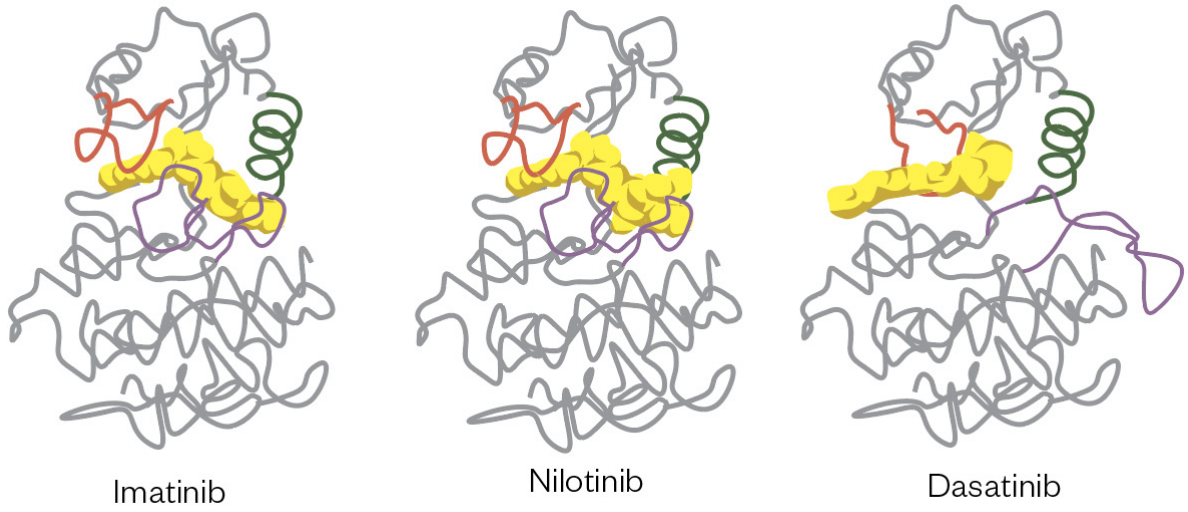
FLT3 is a receptor tyrosine kinase expressed by haematopoietic cells, and is important for the development of stem cells[21,22]. Mutations in the corresponding gene are seen in 30% of patients with AML, and in a small population of patients with ALL[21,22]. The presence of this mutated gene and its expression is linked to a poor prognosis[21,22]. FLT3 inhibitors targeted to the mutation in the kinase are in development[21,22]. These agents can inhibit the transformation of primary AML cells harboring this mutation, and inhibit the growth of those that have already been transformed, such as haematopoietic and leukaemic cell lines[21,22]. Midostaurin (Rydapt, Novartis) is the only FLT3 inhibitor approved by the National Institute for Health and Care Excellence (NICE). It is indicated, in combination with standard daunorubicin and cytarabine induction and high-dose cytarabine consolidation chemotherapy, and for patients in complete response followed by Rydapt single agent maintenance therapy, and for adult patients with newly diagnosed acute myeloid leukaemia (AML) harboring FLT3 mutations[23].

A novel diagnostic/therapeutic biomarker has recently been discovered for AML[16]. Isocitrate dehydrogenase 1 and 2 (IDH1 and IDH2) are key metabolic enzymes that convert isocitrate to alpha-ketoglutarate[24], with neomorphic mutation leading to accumulation of hydroxyglutarate. Hypermethylation is the dominant feature of IDH2 mutation in AML patients, and it is this mutation that leads to decreased expression of the enzyme, thus limiting the conversion of isocitrate[24]. Parker *et al.* discovered that point mutations in both IDH1 and IDH2 occur in a variety of cancers including AML and gliomas[25]. These mutations inactivate the wild-type enzymatic activity of IDH, which results in a novel molecular function for the enzyme, the production of D2HG (i.e. D-2-hydroxyglutarate), which accumulates at millimolar concentration levels in the tumour cells[25]. This disturbs various molecular pathways in certain cancer cells including AML[25]. The US Food and Drug Administration (FDA) has recently approved the use of enasidenib (Idhifa, Agios Pharmaceuticals Inc), together with a CDx test, to treat patients whose tumour cells carry IDH1 and IDH2 mutations[26]. In the laboratory, IHC is used to investigate prototypical gain of function mutations (e.g. R132H), and sequencing is used for less common neomorphic IDH1 and IDH2 mutations.

FIGURE 1

Structures of imatinib, nilotinib and dasatinib interacting in the ATP-binding pocket of the BCR-ABL protein

These interactions result in small but significant changes in binding mode, meaning that nilotinib and dasatinib can be used to treat patients who have become resistant to imatinib owing to small changes in structure within the ATP-binding pocket.



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Another biomarker, phosphoinositide 3-kinase (PI3K), has a role in both leukaemia and lymphoma. PI3K is a multigene family comprising of at least eight different isoforms of the catalytic subunit and seven isoforms of the regulatory subunit[27]. This set of genes encodes proteins with multiple functions, especially some important in the activation/differentiation pathways of haematopoietic cells, such as the PI3K/PTEN/AkT/mTOR pathway (see Figure 2) in leukaemia[27]. For example, it is known that the PI3K/AkT pathway is frequently activated in AML together with the mTORC1 pathway[28]. Both pathways are responsible for the proliferation of blast cells and for control over the clonogenicity of leukaemic progenitor cells[28], and inhibition induces apoptosis[28]. Two agents have been developed to target the PI3K biomarker, idelalisib (Zydelig, Gilead Sciences) and duvelisib (Copiktra, Verastem Inc)[29,30]. Idelalisib is an orally active ATP-competitive kinase inhibitor that targets the PI3K p110 δ isoform (PI3Kδ) with high potency and selectivity[29]. PI3Kδ is predominantly expressed in B-cell malignancies, acting as a key oncogenic driver[29]. Duvelisib (IPI-145) is a dual inhibitor of PI3Kδ and PI3Kγ, both known to support the growth and survival of malignant B-cells and T-cells[30], and to have a role in the formation and maintenance of the supportive tumour microenvironment[30]. The FDA accepted a new drug application (NDA) in early 2018 for duvelisib for the treatment of relapsed or refractory chronic lymphocytic leukaemia/small lymphocytic lymphoma (CLL/SLL), and for accelerated approval for the treatment of relapsed or refractory follicular lymphoma (FL).

Lymphomas: Hodgkin and non-Hodgkin lymphoma

Lymphomas are a group of haematological malignancies that derive from lymphocytes and occur predominantly in lymph nodes and other lymphoid organs[36]. There are two main types of lymphomas: Hodgkin and non-Hodgkin, each with their own cancer subtypes. Classical Hodgkin lymphomas are characterised by the presence of Reed–Sternberg cells (mature malignant B cells), whereas non-Hodgkin lymphomas are derived from B- and T-cells, but can arise in the lymph nodes or other surrounding organs[37].

CD20 is used as a biomarker and drug target in B-cell non-Hodgkin lymphoma for targeted radiotherapy and immunotherapy, respectively, for previously untreated relapsed or refractory disease, or in the treatment of relapsed CLL and follicular lymphoma[38]. The agents used to target this biomarker include ibritumomab tiuxetan (Zevalin, Acrotech Biopharma), rituximab (Rituxan, Genentech) and obinutuzumab (Gazyva, Roche)[38].

CD30 is a membrane protein of the tumour necrosis factor receptor and is associated with the Reed–Sternberg cells of classical Hodgkin lymphoma. It plays a role in normal lymphoid interactions, as suggested by its histological detection on the surface of lymphoid cells in reactive lymph nodes, and by its induced expression by purified T- and B-cells following lectin activation[39]. Together with PD-L1, CD30 is one of the biomarkers used in Hodgkin lymphoma, and can be used to select patients for treatment with brentuximab vedotin (Adcetris, Seattle Genetics/Millennium Pharmaceuticals/Takeda).

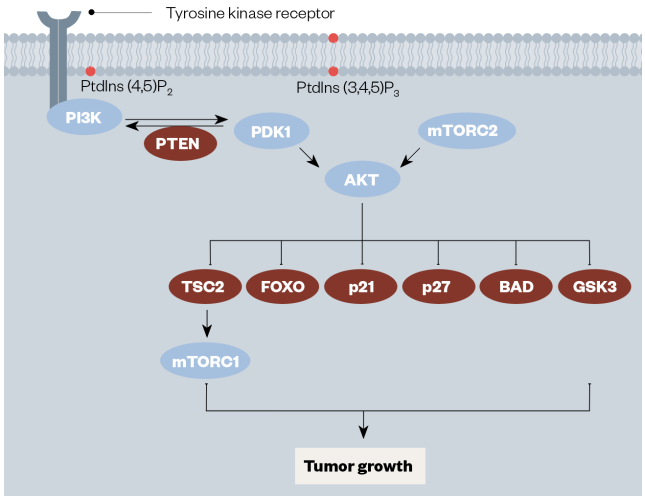
The tumour suppressor protein 53 (TP53) mutation, despite its low frequency in haematological cancers, is considered to be a prognostic biomarker in patients with diffuse large B-cell (DLBCL), follicular and mantle cell lymphomas[36]. For example, TP53 mutation status is associated with poor prognosis in patients with DLBCL[36]. However, Nair *et al.* have reported that idelalisib (Zydelig, Gilead Sciences) promotes caspase-dependent apoptosis independent of CLL prognostic factors such as mutated TP53[40]. Both TP53 and CD30 are used as part of a second-line diagnosis in Hodgkin lymphoma and cutaneous T-cell lymphoma, as well as in peripheral T-Cell lymphoma (PTCL) patients non-responsive to the CHOP chemotherapy regimen (Cyclophosphamide, Hydroxydaunorubicin [doxorubicin], Oncovin [vincristine] and Prednisolone) used in the treatment of non-Hodgkin lymphoma.

Bruton's tyrosine kinase (BTK) is a biomarker associated with B-cell maturation and is considered to be the major oncogenic driver in some B-cell lymphoid tumour malignancies[41] including non-Hodgkin lymphoma and diffuse large cell B-lymphoma (DLBCL)[41]. The kinase inhibitor ibrutinib (Imbruvica, Janssen-Cilag) works by inhibiting BTK, and has demonstrated efficacy in the activated B-cell-like subtype of diffuse large-cell B-lymphoma but not in the germinal centre (a specialised microstructure that is formed within the follicles of secondary lymphoid tissues such as the spleen and lymph nodes) of B-cell-like subtypes[41].

The PD-1 biomarker is associated with an inferior survival

FIGURE 2

Diagram of the PI3K/PTEN/AkT/mTOR signaling pathway important in leukaemia, with key drug targets highlighted



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rate for Hodgkin lymphoma regardless of disease status[36], and its presence is characteristic of nodular lymphocyte-predominant Hodgkin lymphoma. The T-follicular helper lymphocyte cells that rosette around the PD-L1-expressing malignant cells express PD-1, and it is this PD-1/PD-L1 expression that has proven to be of both prognostic and disease-monitoring value[36]. Therefore, anti-PD-1 agents could be useful to treat patients with this type of lymphoma.

PI3K is also important in DLBCL[42]. The activity of the PI3K/AkT pathway acts as a prognostic marker[42], with constitutive activation of the pathway playing a prominent role in regulating the growth and survival of DLBCL cells[43]. There is currently interest in exploiting this biomarker for the treatment of lymphomas.

The biomarkers used in both types of lymphomas, the tests used to identify them, and the use of this information in therapy are summarised in Table 2.

Myeloproliferative neoplasms

These are a group of rare disorders of the bone marrow (including CML) that cause an increase in the number of blood cells[45]. Interest in the Janus family of kinase proteins (JAKs) as potential biomarkers in myeloproliferative neoplasms (MPNs)[46] has shifted from their placement at the centre of multiple point mutation activations in various tumour types, to their production in response to an increase in inflammatory cytokines in the tumour microenvironment produced by infiltrating innate immune cells[47], to the point where they are now viewed as a biomarker for myeloproliferative diseases. Oncogenic JAKs 1, 2 and 3 are all associated with both lymphoid and myeloid neoplasms[48]. Of particular importance is JAK2V617F, which is prevalent in BCR-ABL1-negative MPN[48]. This mutation affects the non-catalytic pseudo-kinase domain of the Janus family JAK2, thereby diverting its kinase activity[48]. Inhibition of the JAK/STAT3 pathway offers a significant therapeutic benefit due to the capacity of JAK/STAT3 signalling to promote cancer 'hallmarks', such as proliferation, survival, angiogenesis and tumour metabolism while suppressing antitumour immunity[47].

Steensma *et al.* have described the clinical use of JAK2 V616F mutation diagnostic tests in myeloid disorders[49]. They noted that this missense mutation plays an important role in normal haematopoietic growth factor signalling, and is present in patients who are BCR-ABL-negative but have chronic MPN disorder[49]. This mutation causes constitutive activation of the kinase, leading to deregulation of signalling during haematopoietic growth factor stimulation[49]. There are a variety of tools that can detect this mutation, from direct DNA sequencing using allele-specific PCR in either real-time mode or through an amplification refractory mutation system (ARMS), to pyrosequencing[49]. Ruxolitinib (Jakavi, Novartis) was developed to target both JAK1 and JAK2, but is only used for symptom control, reducing both splenomegaly and anaemia.

Akpinar *et al.* sequenced the MPL gene coding the thrombopoietin receptor, and uncovered a new molecular abnormality in JAK2 mutation-negative MPN patients[50]. The most common of these were W515L (tryptophan to leucine) and W515K (tryptophan to lysine) substitutions[50]. These account for only 10% of mutations found in MPN but are present in patients with essential thrombocythemia (ET) and primary idiopathic myelofibrosis (PMF)[50]. Often, patients with W515K and W515L mutations display specific signs and symptoms, including low haemoglobin levels and higher platelet counts at diagnosis as compared with patients with a JAK2 V617F mutation[50].

Another biomarker that has been studied since the 2014 revision of the WHO diagnostic criteria for BCR-ABL1-negative MPN patients is the calreticulin (CALR) mutation[51]. The *CALR* gene is located on the short arm of chromosome 19, and can contain two different types of mutations, either a 52-bp deletion mutation or a 5-bp TTGTC insertion[51]. Currently, the role of *CALR* mutations in the molecular pathogenesis of MPN is not entirely clear, but they seem to affect the disease phenotypes of patients with JAK2 mutations and those presenting with ET[51,52].

The biomarkers used in MPNs, the tests used to identify them, and the use of this information in therapy are summarised in Table 3.

Tefferi *et al.* have described novel biomarkers found in myeloma patients[48]. One of these, the myeloproliferative leukaemia (MPL) virus[16], is the key growth and survival factor in megakaryocytes[48]. MPL induces oncogenesis through JAK-STAT activation, and may require the presence of mutant specific variants and receptor variants, such as MPL515 (a stem cell-derived event that involves both myeloid and lymphoid progenitors), although it is not clear at present how these mutations contribute to disease initiation, clonal evolution or blast transformation[48].

Multiple myeloma

This is a malignant disorder originating in bone marrow plasma cells, and accounting for 13% of all haematological cancers[53]. In multiple myeloma (MM), malignant plasma cells undergo a massive clonal expansion resulting in the production of a high level of monoclonal immunoglobulin[54]. These cells proliferate rapidly and infiltrate the bone marrow, interfering with cell signalling pathways involved in osteoblast formation. Novel therapies used to treat this malignancy have been reviewed, together with the role of biomarkers as tools for imaging and diagnosis[55]. Treatment starts with the identification of symptoms and co-morbidities, leading to either an autograft or the start of one to three lines of therapeutics.

Cereblon (CRBN) is a protein encoded by the *CRBN* gene, and is a target for immunomodulatory drugs (IMDs) such as lenalidomide (Revlimid, Celgene Ltd) and pomalidomide (Imnovid, Celgene Ltd)[56,57], key agents within the various treatment strategies for multiple haematologic malignancies including MM. It has been suggested that over-expression of cereblon could serve as a predictive biomarker for resistance to IMDs, which could be useful to establish prior to initiating therapy, although this remains controversial[56]. The *CRBN* -associated transcription factors ikaros and aiolos are considered by some researchers to have more potential as functional biomarkers since they appear as downstream substrates in the transcription of this protein[56].

HR23B is another potential biomarker for MM that has an important role in transporting ubiquitinated cargo proteins to the

TABLE 1
Biomarkers associated with the leukaemias, the biomarker tests used to identify them, and the use of this information for therapy

Biomarkers	Agents used[1]	Examples of companion diagnostic tests[2]	Use of test results
BCR-ABL1 Ph+	Imatinib (Glivec) Ponatinib (Iclusig)	Quantidex BCR-ABLNHS (Asuragen Inc)	Confirms the presence and expression of the BCR-ABL biomarker, allowing patients to be selected for treatment with imatinib or ponatinib.
	Nilotinib (Tasigna)	MRDx BCR-ABLnNHS and the MolecularMD Test (Genoptix Inc)	Identifies patients who are Ph+ to select them for chronic treatment with Nilotinib[32]. Not considered to be cost-effective by NHS.
	Bosutinib (Bosulif) Dasatinib (Sprycel)	Ph+CML TestNHS (Novartis Oncology Inc)	Identifies patients who are Ph+ and suitable for treatment with bosutinib or dasatinib[33].
CD20	Rituximab (MabThera)	None	—
	Obinutuzumab (Gazyva)	None	—
CD33	Gemtuzumab Ozogamicin (Mylotarg)	None	—
CD22	Inotuzumab Ozogamicin (Besponsa)	None	—
TP53 Tumor Suppressor Protein Mutation, 17p	Idelalisib (Zydelig)	None	—
Deletions (marker of MDR, aggressiveness and progression) Bruton's Tyrosine Kinase (BTK)	Ibrutinib (Imbruvica) Venetoclax (Venclyxto)	Vysis CLL FISH Probe KitNHS (AbbVie/Genentech Inc)	Identifies CLL patients with 17p TP53 deletions suitable for treatment with ibrutinib and venetoclax.
FLT3 Mutation (prognostic marker only)	Gilteritinib** (ASP2215)[1]	None	—
	Midostaurin (Rydapt)	LeukoStrat CDx FLT3 Mutation Assay[2] NHS (Invivoscribe Technologies Inc)	Used to detect the FLT3 mutation in AML patients, suggesting their suitability for treatment with midostaurin.
IDH2 Mutation (seen in glioblastoma and acute leukaemia)	Enasidenib** (Idhifa)[3]	Real Time IDH2 AssaynNHS (Abbott Inc)	Detects IDH2 mutations in blood or bone marrow samples, allowing patients to be selected for treatment with enasidenib. Not considered to be cost-effective by NHS.
PI3K (Phosphoinositide 3-kinase)	Duvelisib** (IPI-145, INK1197)	None	—

Source: Genome Web[32,33]
* agents likely to be approved by the National Institute for Health and Care Excellence (NICE) in the near future, but that may not necessarily be used in the NHS[29,30]
** agents that are unlicensed in the UK at the time of writing
*** agents that NICE has rejected
[2] NHS and nNHS denote companion diagnostic tests that are, or are not, used in the NHS, respectively[34,35]
[3] Agents of increasing interest, but not yet established in practice

proteasome[58]. Khan *et al.* have reported that HR23B governs the sensitivity of tumour cells to histone deacetylase inhibitors such as vorinostat (Zolinza, MSD) and panobinostat (Farydak, Novartis)[58]. Thus, it can be used as a predictive biomarker in this clinical setting.

Using novel technologies such as 2D-DIGE and mass spectroscopy (MS)[59], Rajpal *et al.* have described biomarkers found only in MM patients responding to thalidomide (Thalidomide, Celgene). These biomarkers, which include ZAG, VDB, SAA, B2M and Hp, are found at levels statistically different compared with non-responders[59]. They comprise a combination of serum protein biomarkers (e.g. ZAG, VDB and SAA) present on the surface of many cells including lymphocytes, a highly prognostic factor in combination with albumin, and an apolipoprotein synthesised in response to inflammatory biomarkers secreted by activated monocytes and macrophages[59,60].

The interactions between chemokines such as stromal cell-derived factor-1 and its receptor (chemokine C-X-X motif receptor-4) play a central role in MM including effects on

TABLE 2
The biomarkers used in both types of lymphoma the tests used to identify them and the use of this information in therapy

Biomarkers	Agents used[1]	Examples of companion diagnostic tests[2]	Use of test results
CD20	Rituximab (Rituxan)	None	—
CD30	Brentuximab vedotin (Adcetris)	None	—
TP53 Tumor Suppressor Protein Mutation (17p Deletions)	Idelalisib** (Zydelig)	Vysis CLL FISH Probe KitNHS (Abbott Molecular/Genentech Inc)	Identification of CLL patients with TP53 17p deletions suitable for treatment with idelalisib and ibrutinib[44].
Bruton's Tyrosine Kinase (BTK)	Ibrutinib (Imbruvica)		
PD-L1	Nivolumab (Opdivo)	PD-L1 IHC 28-8 PharmDx TestNHS (Agilent Technologies/Dako Inc)	Used in the selection of patients who may benefit from treatment with nivolumab.
	Pembrolizumab (Keytruda) [only for refractory Hodgkin lymphoma].	PD-L1 IHC 22C3 PharmDx TestNHS (Agilent Technologies/Dako Inc)	Used in the selection of patients who may benefit from treatment with pembrolizumab.
PI3K (Phosphoinositide 3-kinase)	Idelalisib* (Zydelig)	Vysis CLL FISH Probe KitNHS (Abbott Molecular/Genentech Inc)	Identification of anaplastic large cell lymphoma patients with 17p TP53 deletions suitable for treatment with idelalisib.
	Duvelisib** (IPI-145, INK1197)	None	—

Source: Genome Web[44]

* agents likely to be approved by the National Institute for Health and Care Excellence (NICE) in the near future, but that may not necessarily be used in the NHS[29,30]

** agents that are unlicensed in the UK at the time of writing

*** agents that NICE has rejected NHS companion diagnostic tests that are used in the NHS[34,35]

nNHS companion diagnostic tests that are not used in the NHS[34,35]

† agents of increasing interest, but not yet established in practice

cellular proliferation, phosphorylation of mitogen-activated protein kinase1/2 (MAPK) and p42/44 MAPK, induction of interleukin-6 and VEGF secretion[61]. Therefore, this interaction can be used as a potential biomarker for diagnosing MM. It has also been used to develop proteasome inhibitors such as bortezomib (Velcade, Janssen-Cilag Ltd) and carfilzomib (Kyprolis, Amgen Ltd) which work by inhibiting the interaction between stromal cell-derived factor-1 and its receptor C-X-X motif[62]. The sensitivity to these inhibitors is increased through the mobilisation of MM cells from the bone marrow microenvironment[63]. The reduction in overall expression of CXCR4 in bortezomib-resistant cells has led to the conclusion that CXCR4 could be used as a potential diagnostic biomarker for predicting patient survival in relation to treatment with these drugs[62,63].

CD38 is a 46kDa transmembrane glycoprotein with a role in adhesion, signalling events and bifunctional ectoenzymatic activities contributing to the mobilisation of intracellular calcium[64]. It is present in low levels in myeloid cells under normal circumstances but is over-expressed when the cells become malignant[64]. Therefore, CD38 has potential use as both a diagnostic biomarker and as a therapeutic target for this disease[64]. Daratumumab (Darzalex, Janssen-Cilag) is a human IgG1k monoclonal antibody that binds to CD38[65], and pre-clinical studies have shown that it induces target-cell killing through a number of mechanisms including complement-mediated and antibody-dependent cell-mediated cytotoxicity (ADCC) events, antibody-dependent cellular phagocytosis, apoptosis and inhibition of the enzymatic activity of CD38[65], all of which occur at very low concentrations and induce a potent cytotoxic response[64].

Elotuzumab (Empliciti, Bristol-Meyers Squibb) is a humanised IgG1k monoclonal antibody that has been approved by the FDA for the treatment of MM[66]. It targets the signalling lymphocyte activation molecule family member 7 (SLAMF7), and works by enhancing the cytotoxic activity of natural killer cells (NKCs) (see Figure 3)[67]. SLAMF7 is expressed by a number of cell types including MM cells, NKCs, leukocytes and plasma cells[66,67]. Studies by Wang *et al.* have shown that SLAMF7-mediated signalling is important for the adhesive interaction between myeloma cells and bone marrow stromal cells (BMSC), a process that activates the ERK1/2, STAT3 and AKT signalling pathways[66]. Therefore, SLAMF7 is considered to be an ideal therapeutic target for MM[66].

As a complication of their disease, many MM patients experience skeletal problems such as bone pain, hypercalcaemia and pathological fractures resulting from secondary or lytic bone lesions[68]. Studies by Roux *et al.* have suggested that these problems may be exacerbated by excessive production of osteoclast-activating factors produced either by the bone marrow itself or by the surrounding microenvironment[68,69]. The main factor expressed by developing osteoclasts is receptor activator of nuclear factor kappa-B ligand (RANKL)[68,69]. Since MM is characterised by a high rate of bone resorption, it is useful for clinicians to evaluate and monitor RANKL expression. The human monoclonal antibody denosumab (Xgeva, Amgen) is targeted to RANKL, and so is used to treat patients with bone disease[70].

TABLE 3
Biomarkers used in myeloproliferative neoplasms the tests used to identify them and the use of this information in therapy

Biomarkers	Agents used[1]	Examples of companion diagnostic tests[2]	Use of test results
Mutated JAK1/JAK2	Ruxolitinib (Jakavi)	JAK2 Mutation AssayNHS	Indicates the prevalence of the JAK2 V617F missense mutation in tumour DNA, suggesting that patients may benefit from treatment with ruxolitinib.
BCR-ABL1 Ph+	Dasatinib** (Sprycel)	Ph+CML TestNHS (Novartis Oncology Inc)	Identifies patients with the BCR-ABL1 Ph+ mutation, who may benefit from treatment with dasatinib.
	Nilotinib** (Tasigna)	MRDx BCR-ABLnNHS (MolecularMD)	Identifies patients with the BCR-ABL1 Ph+ mutation, who may benefit from treatment with nilotinib[32]. Not considered to be cost-effective by NHS.

* agents likely to be approved by the National Institute for Health and Care Excellence (NICE) in the near future, but that may not necessarily be used in the NHS[29,30]

** agents that are unlicensed in the UK at the time of writing

*** agents that NICE has rejected NHS companion diagnostic tests that are used in the NHS[34,35]

nNHS companion diagnostic tests that are not used in the NHS[34,35]

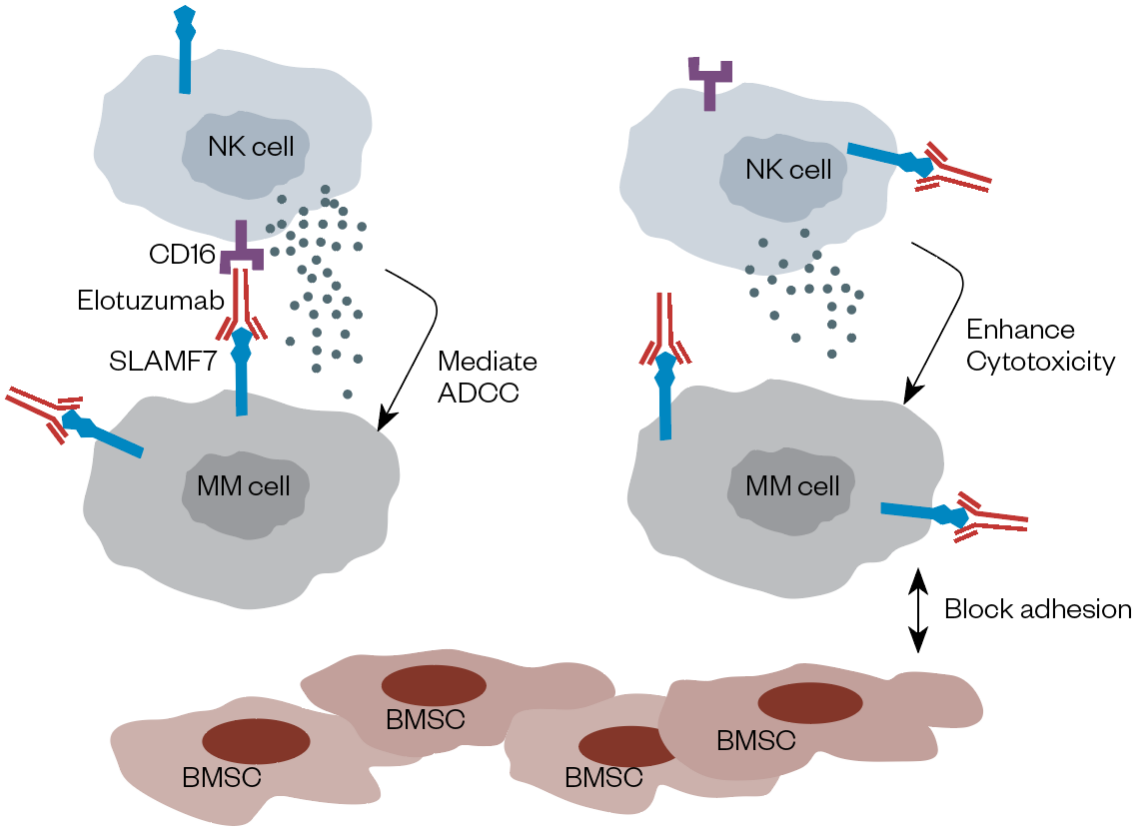
† agents of increasing interest, but not yet established in practice

The biomarkers used in myeloma, the tests used to identify them, and the use of this information in therapy are summarised in Table 4.

Supportive therapies

Supportive therapies are used to offset the side effects frequently experienced with most types of anticancer therapies, including PMs, and this was discussed in detail in relation to solid tumours in part 1 of this review. Although most of this previous discussion is relevant, with haematological cancers immunosuppression can result from the immune defects of the underlying disease. In addition, the chemotherapy regimens used for haematological cancers are often very intensive, further increasing the risk of infection[72]. Therefore, supportive therapies for these patients can include platelets, blood infusions, immunoglobulins and drug therapies including antibiotics[72]. As with solid tumour patients, anti-emetics are often required pre- and post-treatment to reduce nausea and vomiting[73]. Other medicines used specifically for haematology patients include allopurinol to prevent tumour-lysis syndrome,

FIGURE 3
Diagram of the mechanism of action of Elotuzuma b (Empliciti)



Reproduced with permission from *J Haematol Oncol*[66]

TABLE 4

The biomarkers used in both types of lymphoma the tests used to identify them and the use of this information in therapy

Biomarkers	Agents used[11]	Examples of companion diagnostic tests[2]	Use of test results
CRBN (Cereblon)	Lenalidomide (Revlimid) used as first, second and third line treatment	None	—
	Pomalidomide (Imnovid)	None	—
HR23B	Vorinostat** (Zolinza)	None	—
	Panobinostat (Farydak)	None	—
Panel of biomarkers including ZAG, VDB, SAA, B2M and Hp	Thalidomide (Celgene) used as first-, second- and third-line treatment	None	—
CXCR4 (Chemokine C-X-C Motif Receptor 4)	Bortezomib (Velcade Carfilzomib (Kyprolis) Ixazomib (Ninlaro)	None	—
CD38	Daratumumab (Darzalex)	Hydrashift 2/4 Test for use with Sebia's FDA-approved CE-marked Hydrasys 2 Agarose Gel PlatformnNHS [71]	Humanised mAb treatments for MM can interfere with the patient's native antibodies in immunofixation tests, thus misleading the pathologist in interpreting the response to treatment. HYDRASHIFT 2/4 daratumumab is a reagent used on Sebia's HYDRAGEL (agarose gel) immunofixation test to mitigate interference by daratumumab[71]. Not considered to be cost-effective by NHS.
		CD38 Antibody Flow Cytometry TestNHS	Measures the co-expression of CD8 and CD38, and predicts likely responders to daratumumab.
SLAMF7 (Signaling Lymphocyte Activation Molecule Family Member 7) (used in research setting only)	Elotuzumab*** (Empliciti)	None	—
RANKL (Tumour Necrosis Factor Receptor-κβ) (used in research setting only)	Denosumab** (Xgeva)	None	Given for skeletal related events; not directly related to the treatment of myeloma.

Source: Sebia, Janssen Biotech Ink multiple myeloma IVD test agreement[71]

* agents likely to be approved by the National Institute for Health and Care Excellence (NICE) in the near future, but that may not necessarily be used in the NHS[29,30]

** agents that are unlicensed in the UK at the time of writing

*** agents that NICE has rejected NHS companion diagnostic tests that are used in the NHS[34,35]

nNHS companion diagnostic tests that are not used in the NHS[34,35]

† agents of increasing interest, but not yet established in practice

co-trimoxazole to prevent infection with pneumocystis pneumonia, and aspirin or a low molecular weight heparin to reduce the risk of thrombosis[73].

Future strategies in the precision medicine approach

There are several visions for the PM approach in the longer term. First, the development of accurate and selective non-invasive tumour biomarker screens, and their widespread and regular use by healthy individuals (e.g. the identification of biomarkers in urine or finger-prick blood samples), should lead to the very early diagnosis of most cancers along which effective treatments (hopefully 'cures' in many cases). Another vision is that, for cancers that do develop, new generations of PM-based agents might be used to treat most cancer types as chronic diseases, thus allowing effective treatment and long-term survival through simple oral dosing, a precedent set with agents such as imatinib.

Ultimately, whole genome sequencing (WGS) could be carried out at birth (potentially even in *utero*) for the entire population, and genetic biomarkers used to predict cancer risk for everyone, with prophylactic intervention if appropriate. However, the ethical implications of this approach are not insignificant and may be considered more of a barrier than the practicalities. Although space does not permit a detailed discussion of recent advances in the development of techniques to identify

and quantify biomarkers in blood or urine, some examples of the development of methods to detect and measure tumour miRNAs and DNA in blood samples are briefly described below. Methods are also being developed to isolate tumour cells from blood and urine samples, which should not only allow identification of the cancer type, but may also produce biomarker information from their DNA and cellular contents, thus allowing early PM drug selection and treatment.

A significant research effort is underway to develop methodologies to detect cancer-relevant biomarkers in small samples of blood or urine so that various cancer types might be diagnosed early through a simple, minimally invasive test. For example, microRNAs (miRNAs) are being investigated as a new class of small non-coding RNAs that regulate gene expression at a post-transcriptional level by either blocking or degrading the translation of messenger RNA targets[74]. MiRNAs have been identified in the blood of cancer patients, and this discovery has led to the use of miRNAs as important cancer biomarkers. MiRNAs appear to have multifunctional roles, and can be used to help distinguish between healthy and malignant cells[74], in identifying the origin of secondary (i.e. metastatic) tumours[74] and in distinguishing between different subtypes of cells from the same tumour[74]. Two recent studies have focused on the detection of circulating miRNAs in blood as specific methods for cancer detection[53,75]. These dysregulated miRNAs were found in the blood of patients who had monoclonal gammopathy of unetermined significance (MGUS) and myeloma[53,74]. In addition, researchers in Japan have recently developed a new approach to non-invasive liquid biopsies using nanowires to extract thousands of urinary miRNAs from just 1 milliliter of urine[76]. This approach was used to successfully discriminate between samples from patients with pancreatic, prostate and bladder cancer and those collected from healthy individuals[76], although further work is required to fully validate this approach.

Another rapidly advancing technology involves the detection of circulating DNA in blood. For example, a study in 2017 demonstrated the possibility of using circulating tumour DNA to detect MM. In this approach the use of liquid-biopsy sequencing (LB-Seq) helped to uncover a large set of MM mutations[77,78], and it was established that 96% of the mutations could be accurately detected by the genetic profiling of matched bone-marrow-derived tumour DNA with more than 98% specificity[78]. These results suggest that LB-Seq might be used in the future as a complementary method to invasive bone marrow aspiration-based testing, perhaps eventually replacing it.

More recently, technologies have been developed to detect a combination of circulating proteins and mutated cell-free DNA in blood[79] as a means to detect cancer as early as possible through a simple finger-prick test[80]. For example, researchers at Johns Hopkins (US) have developed a test, known as CancerSEEK[80] capable of analysing circulating tumour DNA and proteins together for the detection of eight common surgically resectable, non-metastatic cancer types including ovary, liver, stomach, pancreas, oesophagus, colorectal, lung and breast[81,82]. In a study involving 1,005 patients, the results were positive in a median of 70% of the eight cancer types[82]. The sensitivities ranged from 69% to 98% for the detection of five cancer types (i.e. ovary, liver, stomach, pancreas and esophagus) for which there are presently no screening tests available for average-risk individuals. The specificity of CancerSEEK was >99%, and only 7 out of 812 control samples from healthy individuals generated a false positive result. In addition, CancerSEEK localised the cancer to a small number of anatomic sites in a median of 83% of the patients.

Other areas of research are focusing on the development of advanced (e.g. multiplexed) assays to analyse multiple genes in biopsied tumour tissue for purposes including therapy selection and prognosis. For example, the FoundationOne CDx (F1CDx) test utilises next-generation sequencing (NGS) to detect variants in up to 324 genes, also identifying two key signatures, microsatellite instability[65] and tumour mutational burden (TMB) across solid tumours of many different types[83]. In addition to its use in NSCLC, melanoma, breast, colorectal and ovarian cancers, it has been used as a companion diagnostic test for 17 FDA-approved targeted treatments[83] including the anti-PD 1 immuno-oncology agent pembrolizumab (Keytruda, MSD)[84], which is discussed in more detail in part 1 of this review.

Conclusion

In the past three decades the PM approach to cancer therapy has progressed from the concept phase to practical utility. This has significant implications for healthcare systems such as the NHS, in that it creates the opportunity to provide anticancer therapies for patients with a higher degree of certainty of providing clinical benefit[79]. However, these new therapies are expensive and require CDx tests to select patients, which has cost implications. Although pharmaceutical companies are adjusting to this new paradigm by setting higher prices for novel PM-based anticancer agents that will sell in lower volume, government-funded healthcare organisations such as the NHS will come under increasing pressure to fund these new therapies because of their clinical effectiveness.

For these new PM approaches, the growing burden on clinicians, pharmacists and other healthcare professionals will become increasingly significant owing to the additional requirements for the selection of patients using CDx tests, followed by appropriate drug selection and dose scheduling based on the test results[80]. The complexity of this new treatment paradigm demands that healthcare professionals not only have the technical expertise and knowledge to deliver it, but also have the necessary communication skills to explain diagnostic test results and drug selection decisions clearly and confidently to patients and other healthcare professionals.

This review provides concise information on the current status of the PM approach in the diagnosis and therapy of haematological malignancies, and should be of use to clinicians, pharmacists and other healthcare professionals. Although this part 2 of the review has focused on haematological cancers, part 1 reviewed the PM approach to the treatment of solid tumours.

Finally, it should be noted that the accuracy and coverage of this review (and Part 1 which covered solid tumours) will be short-lived owing to the rapid progress being made in this area, with new agents and CDx tests entering the clinic at a remarkable rate. By necessity, each paragraph of this review represents only a brief summary of what ideally should be a systematic review of thousands of publications. The reader should be aware of this, and is encouraged to look further into the literature in their area of interest.

Key points

- Introduction of the 'precision medicine' (PM) approach has seen its most significant advances in the field of oncology.
- The key scientific principle behind this approach is to identify predictive biomarkers that can be used to select the most suitable therapeutic agents.
- Biomarkers can be used for diagnostic, prognostic, toxicological and treatment-monitoring purposes.
- Many pharmacogenomic assays have been developed to identify biomarkers.

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Disclaimer
The treatment strategies described in this review are for educational purposes only and should not be used to guide the treatment of patients. Readers are referred to NICE or SIGN guidance in the UK, and relevant medical texts and specialist journals, for information about prescribing and treatment regimens. The authors alone are responsible for the views expressed in this article which do not necessarily represent the views, decisions or policies of the institutions with which they are affiliated.

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