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Pharmacogenetic biomarkers for the prediction of response to antiangiogenic treatment

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Abstract

Antiangiogenic treatments have shown activity across multiple tumour types and in various settings. Despite having been approved on the basis of efficacy, the therapeutic index varies substantially in different settings for many of these agents. A major limitation is the current inability to personalise treatment a priori according to findings on measurement of a predictive biomarker. The roles of germline single-nucleotide polymorphisms have been investigated as potential biomarkers for antiangiogenic treatments. The rationale is founded on the understanding that the drugs target the vasculature rather than the tumour, which could mean that much of the variability is regulated by the host. Several single-nucleotide polymorphisms have been associated with differential outcomes and toxic effects in clinical trials. In this Review we provide an overview of available data with particular attention paid to the pitfalls and strengths of potential biomarkers. We also highlight continuing work and plans for confirmatory studies.

Introduction

The blocking of tumour angiogenesis as an anticancer strategy originated in the laboratory of Judah Folkman more than three decades ago.[1] The approach was successfully tested in rodent tumour models and led to pivotal clinical trials of several drugs that have been approved by regulatory agencies in the USA and Europe. Many strategies to block or disrupt tumour angiogenesis are possible, but, so far, the humanised monoclonal antibody against VEGFA and the small-molecule receptor-tyrosine-kinase inhibitors (RTKIs) of *VEGFA* receptors have proven most effective[2] and are indicated for use in various malignant diseases.

The monoclonal antibody to VEGFA, bevacizumab, is approved for several cancer types, which reflects the broad activity of this drug. It was approved by the US Food and Drug Administration (FDA) in 2004, and by the European Medicines Agency in 2005, for the treatment of metastatic colorectal cancer. Shortly thereafter, the FDA also approved it for the treatment of non-squamous-cell, non-small-cell lung cancer. Metastatic renal-cell carcinoma is very sensitive to angiogenic blockade, and treatment with bevacizumab for this disease was approved in the European Union in 2007, and in the USA in 2009. Additionally, this drug was approved by the FDA in 2009 for use in patients with glioblastoma multiforme. For metastatic breast cancer, however, the route to approval was less

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straightforward.[3] Bevacizumab was approved as first-line treatment for metastatic breast cancer in the European Union in 2007, and achieved accelerated approval by the FDA in 2008 for administration in combination with weekly paclitaxel. Approval in both regions was based largely on the positive results of the E2100 trial.[4] Marginal benefit in subsequent trials (AVADO[5] and RIBBON-1[6]), however, led the US Oncology Drug Advisory Committee to recommend that approval be withdrawn. In a landmark decision by the FDA, the approval was withdrawn despite all trials having met the primary endpoint of improved progression-free survival (PFS). By contrast, the European Commission reviewed the same data and maintained approval.

Several small-molecule RTKIs have received approval for various cancers. Sorafenib was approved for the treatment of metastatic renal-cell carcinoma by the FDA in 2005, and received marketing authorisation in the European Union in 2006. In the USA, sorafenib has also been approved for the treatment of advanced hepatocellular carcinoma; it was also granted marketing authorisation for hepatocellular carcinoma in Europe, except for in the UK, where the National Institute of Clinical Excellence and the Scottish Medicines Consortium deemed it to have low benefit and high cost. Sunitinib is approved in the USA and Europe for metastatic renal-cell carcinoma, imatinib-refractory gastrointestinal stromal tumours (GIST), and progressive, well differentiated pancreatic neuro-endocrine tumours. Pazopanib has also been approved by the FDA for renal-cell carcinoma. Axitinib was approved in the USA for use in patients with metastatic renal-cell carcinoma who have not responded to a previous systemic therapy, on the basis of its activity compared with sorafenib in a phase 3 study.[7]

Despite clear activity in many disease types, the vacillation or discordance seen for bevacizumab and sorafenib has highlighted the marginal therapeutic benefit in some studies. The debate has crossed disease types, therapeutic classes, and continents and might have been fuelled by unrealistic forecasts that these drugs would cure all cancers with few or no toxic effects.[8] Therapeutic index is ambiguous for several reasons. First, risks and benefits of drugs cannot be generalised at the antiangiogenic class level because of differences in mechanisms of action (affinities for targets and the promiscuity of targeted receptors), for example between monoclonal antibodies and small-molecule RTKIs.[9] Furthermore, there is heterogeneity across disease types, with some being highly susceptible and others showing marginal benefit from only specific agents. The second confounder is that each agent has a unique toxicity profile. Unlike conventional cytotoxic drugs, for which side-effect profiles are fairly similar, the side-effects of antiangiogenic agents are novel and often unpredictable. Headache is a dose-limiting adverse event for bevacizumab[10] and hypertension is the most frequent grade 3 or higher toxic effect.[11,12] Other rare and unpredictable but life-threatening adverse effects include thromboembolic events, pulmonary haemorrhage, and gastrointestinal perforations.[11] The small-molecule RTKIs have toxic effects, including hand-foot syndrome, mouth pain, rash, and fatigue.[13–16] Which patients are likely to experience drug-induced toxic effects is difficult to predict. Third, antiangiogenic drugs are expensive. The consideration of pharmacoeconomics in the clinical decision-making process is fraught with controversy, but it becomes important when benefit is marginal and resources are limited.

A major frustration with antiangiogenic drugs centres around the use of the term targeted therapy when no unique targets have been identified to guide which patients should receive which drugs. Much of the controversy might be mitigated if the subgroup of patients who gained most benefit and had fewest toxic effects could be identified before treatment. Treatment selection based on an established target has been a model for many of the highly successful anticancer agents. The high therapeutic index of these agents (eg, trastuzumab for *HER2* [*ERBB2*]-amplified breast cancer or imatinib for *KIT*-positive GIST) almost makes

concerns related to expense or toxic effects irrelevant. Thus, much effort has been expended to identify biomarkers that can predict outcomes and to help individualise antiangiogenic therapy.

Areas of research have included tumour expression, concentrations of growth factors in plasma or serum, and radiographic predictors.[17] The early markers in tissue, serum, and plasma did not consistently predict outcome,[18,19] but potentially predictive effects for bevacizumab have been reported.[17,20] Measurement of cytokines or growth factors in serum or plasma is limited by sequestration, time-specific variability, and uncertain correlations with concentrations in the tumour microenvironment. Biomarkers associated with many anticancer therapies have capitalised on variability in gene or protein expression, or both, in tumours, as these features are stable and easily tested in routine tissue samples, but similar success has not been seen for antiangiogenic therapies. One potential explanation for poor results is that antiangiogenic therapies affect the tumour vasculature. The dynamic nature of the vasculature and its response to proangiogenic stimuli are controlled by the host. Thus, we have hypothesised that host-specific variability (single-nucleotide polymorph isms [SNPs]) might be useful biomarkers for response to antiangiogenic therapy.

The goal of personalised medicine is to better understand the benefits and toxic effects of a given therapy in a specific patient. Much of the variation in response is associated with differences between tumours and between hosts. The most common differences are SNPs, which can be non-synonymous or synonymous. Non-synonymous SNPs result in changes in the coding for an aminoacid and are viewed as high-yield candidates for altered outcomes. Synonymous SNPs do not change aminoacids but can substantially affect responses to drugs through gene-expression alterations or post-transcriptional modifications. SNPs can affect the efficiency of drug metabolism and excretion, and might have effects at the level of the target. In this Review we discuss the role of inherited (ie, germline or host) variability in response to antiangiogenic drugs.

Bevacizumab

The SNPs selected for pharmacogenetic studies of bevacizumab have been diverse. Some studies have included a few SNPs in one gene, whereas others have assessed many SNPs across multiple genes. The most widely assessed gene is *VEGFA*. High, although not 100%, linkage disequilibrium has been reported between the *VEGFA* –2578 A, –1498 C, –1154 A, and –634 G alleles.[21] Linkage disequilibrium is a higher frequency than expected of carriers of a combination of two or more alleles in a specific population. Thus, although the alleles are not interchangeable, correlations with one might represent a signal of uniformity with the others. Unfortunately, no causative SNPs have yet been identified, which could limit some of the congruence between trials. Studies have also involved a wide range of disease types and settings, efficacy phenotypes, and antiangio-genic drugs, and have varied in size. Comparisons of studies must, therefore, be viewed with caution. Despite these limitations, strengths of pharmacogenetic studies include that the genotype is constant and, therefore, independent of the time of collection, and that assays are technically simple and highly reproducible. Significant efficacy markers identified in pharmacogenetic correlative studies are shown in table 1.

Candidate SNPs were studied in the clinical E2100 phase 3 trial (table 1).[4] Patients received weekly paclitaxel with or without bevacizumab as first-line therapy for metastatic breast cancer. The addition of bevacizumab was associated with improved response rate and PFS (the primary endpoint), but not with improved overall survival. Common SNPs in the *VEGFA* gene and its receptor, VEGFR2, were retrospectively studied.[22,43] The *VEGFA* SNPs were all in regulatory regions (no common non-synonymous polymorphisms have

been found in *VEGFA*) and all had a high minor allele frequency. *VEGFA* –2578 AA and –1154 AA genotypes were associated with better median overall survival than other genotypes. Similar effects were seen for PFS but were non-significant. No such effect was seen in the control groups, which suggests that these SNPs had predictive value.

In the AVADO trial,[5] docetaxel alone or with 7·5 mg/kg or 15·0 mg/kg bevacizumab were assessed as first-line treatments for metastatic breast cancer (table 1). The AVADO investigators studied 26 SNPs across 13 genes important for regulation of the *VEGFA* pathway, hypertension, and inflammation.[23] Median PFS was improved in carriers of the *VEGFA* –2578 A allele who received docetaxel plus 7·5 mg/kg bevacizumab, but not in those who received docetaxel alone or with 15·0 mg/kg bevacizumab. PFS was also improved in patients with the *VEGFA* –634 CC genotype who received docetaxel alone, but not in those who received bevacizumab, which suggests a prognostic effect. No correlation was seen between overall survival and any of the SNPs tested.

The Hellenic Cooperative Oncology Group did a correlative study on their phase 3 trial findings for bevacizumab with either FOLFIRI (leucovorin, fluorouracil, and irinotecan) or XELIRI (irinotecan and capecitabine).[24] The genotypes *VEGFA* –2578 CC and –1154 GG correlated with shortened overall survival (table 1).[25] This finding is similar in direction to that in the E2100 trial where the alternate genotypes (*VEGFA* –2578 AA and –1154 AA) were associated with improved overall survival. A marginal improvement in PFS was also associated with the *VEGFA* –1154 AA genotype, and the greater effect on overall survival than on PFS supports the findings of the E2100 trial. Although the exaggerated effect on overall survival (compared with that for PFS) could clearly be due to chance, it might reflect biological changes that occur after disease progression, as has been seen in some preclinical models.[44,45] Another small cohort study assessed FOLFIRI with bevacizumab as first-line therapy in 40 patients with metastatic colorectal cancer[26] and investigated correlations between median PFS and *VEGFA* SNPs. Improved PFS correlated with the *VEGFA* –1154 AA genotype. A correlation was also seen between the *VEGFA* –634 GG genotype and improved response rate (table 1).

The E4599 trial[27] was a phase 3 study that assessed bevacizumab in the treatment of metastatic lung cancer. 878 patients were randomly assigned paclitaxel and carboplatin alone or with bevacizumab. Median overall survival and PFS were better in the bevacizumab group than in the group that received paclitaxel and carboplatin alone. In a correlative substudy in 133 patients,[28] SNPs in nine genes that regulate angiogenesis and inflammation were assessed. An SNP profile or signature (as opposed to an individual SNP) of *VEGFA* –634GG, *ICAM1* 469T/C, and *IL8* –251T/A was the best predictor of overall survival and PFS (table 1). Of note, the *VEGFA* –634 G allele, which was seen in the profile associated with improved outcomes, is in linkage disequilibrium with the *VEGFA* –1154 A and –2578 A alleles that correlated with improved outcome in E2100.

Similar pathway approaches were implemented in a phase 2 study of low-dose cyclophosphamide plus bevacizumab to treat metastatic ovarian cancer.[29] 53 (76%) of 70 patients had SNP biomarkers assessed. The investigators selected SNPs across 30 genes. For *VEGFA*, only the –634 C/G and the 936 C/T SNPs were assessed. Decreased response was correlated with the *IL8* –251 A allele and improved PFS with the *CXCR2* 785 CC or CT and *VEGFA* 936 CT genotypes (table 1). This trial, however, was limited by small sample size and multiple comparisons, although the selections of genes and SNPs were extensive.

The AViTA trial[30] was a phase 3 trial done in patients with metastatic pancreatic cancer who were randomly assigned gemcitabine and erlotinib, alone or with bevacizumab. Of the 607 patients enrolled, 154 (25%) had samples available for SNP analysis.[31] 157 SNPs in the

angiogenesis pathway were assessed. Correlations were seen between a *VEGFR1* (also known as *FLT1*) SNP (rs9582036) and improved overall survival and PFS in patients who received bevacizumab (table 1). The investigators subsequently studied another *VEGFR1* SNP (rs7993418) that had functional implications and was in full linkage disequilibrium with rs9582036 in the AVOREN trial. In AVOREN, an association was found with improved PFS but not overall survival. No correlation was seen between the *VEGFA* –2578 AA genotype and outcome.

The similar findings from two independent phase 3 studies (the Hellenic Cooperative Oncology Group and E2100 studies) provide strong evidence that SNPs in *VEGFA* have predictive value as biomarkers for response to bevacizumab. The strength of the relation is tempered by the lack of correlation in the AVADO study,[5] despite the disease type (breast cancer) and setting being similar to those in E2100. Docetaxel in AVADO was associated with less benefit with bevacizumab than was paclitaxel in E2100. The lack of concordance in the AViTA trial[31] is less concerning, as patients in that study had pancreatic cancer and the addition of bevacizumab added no benefit. Although in a negative trial a biomarker can identify a subgroup of patients who will benefit, if the agent being assessed has little or no effect, the likelihood of a biomarker being useful diminishes. The discovery of a *VEGFR1* SNP, however is provocative, and if replicated will deserve attention. The results from the E4599 trial[28] provide some additional support for the correlations seen with *VEGFA*, but the findings are inadequate to draw firm conclusions. Additionally, the development of a multigene SNP signature makes comparison with the findings of other studies difficult. The level of evidence for the use of *VEGFA* SNPs in the clinical setting is inadequate, but further study is clearly warranted.

Small-molecule receptor-tyrosine-kinase inhibitors

Multiple studies have been done to assess the effects of small-molecule RTKIs. A pharmacogenetic correlative study done in 397 (68%) of 585 patients with metastatic renal-cell carcinoma who received pazopanib assessed 27 SNPs across 13 candidate genes (table 1).[32–34] A correlation was seen between decreased PFS and the *IL8* 2767 TT and –251 AA and the *HIF1A* 1790 AG genotypes, as well as between poor response rates and the *HIF1A* 1790 AG, *NR1I2* –25385 TT, and the *VEGFA* –1498 CC, –634 GG, and –2578 AA genotypes.

Several correlative studies been done for sunitinib. One was part of a prospective observational study of 101 patients with metastatic clear-cell renal-cell carcinoma (table 1).[35] Patients were enrolled across 15 institutions through the Spanish Oncology Genitourinary Group. 16 polymorphisms were assessed across nine genes. Two *VEGFR3* (also known as *FLT4*) missense polymorphisms (rs307826 and rs307821) were associated with decreased PFS. None of the *VEGFA* SNPs correlated with improved outcome. Another study was a retrospective, multicentre, pharmacogenetic association study that included 136 patients with metastatic clear-cell renal-cell carcinoma (table 1).[36] 30 SNPs across 11 candidate genes were assessed. Improved PFS was seen for patients with the *CYP3A5* 6986 AA genotype, or who had a CAT copy absent in the *NR1I3* haplotype or a TCG copy present in the *ABCB1* haplotype. *VEGFA* SNPs were not assessed. In a third cohort study of 63 patients with metastatic clear-cell renal-cell carcinoma, candidate SNPs from *VEGFA* and *VEGFR2* (also known as *KDR*) were assessed (table 1).[37] No individual SNPs correlated with improved outcome, but overall survival was inferior for patients who had the combined *VEGFA* 936 CC and *VEGFR2* 889 GG genotypes, even after adjustment for clinical and pathological risk factors.

SNP biomarkers for sorafenib have been assessed in two small correlative trials (table 1). One was a substudy of the E2501 trial,[38] which was a randomised, discontinuation, phase 2 study of sorafenib versus placebo in patients with metastatic non-small-cell lung cancer in whom disease had progressed after at least two chemotherapy regimens. DNA was available from 88 plasma samples.[39] The *VEGFA* –1498 CC and –634 CC genotypes correlated with improved PFS. In a small phase 1 trial of sorafenib with gemcitabine and radiotherapy in 27 patients with unresectable pancreatic cancer, 19 (70%) underwent pharmacogenetic assessment.[43] *VEGFA* –2578 AA and –1154 AA genotypes were associated with improved overall survival, which reflects the findings of the E2100 trial.[22] The *VEGFA* –1498 CC genotype, which is in linkage disequilibrium with the *VEGFA* –2578 AA and –1154 AA genotypes, also correlated with improved overall survival, as did the *VEGFR2* 272 GG and 889 GG genotypes (table 1).

The AXIS study[41] was a phase 3 trial of 723 patients with metastatic renal-cell carcinoma who were randomly assigned axitinib or sorafenib. SNPs were assessed in 249 white patients (table 1).[42] Improved PFS correlated significantly with the *VEGFA* –2578 AA and –1498 TT genotypes before adjustment for multiple comparisons, but only marginally so after adjustment. More importantly, however, was the correlation between the *VEGFA* –2578 AA genotype and benefit in the axitinib group, but not in the sorafenib group.

The associations between SNPs and response to small-molecule RTKIs are harder to interpret than those between SNPs and bevacizumab. Small-molecule RTKIs are promiscuous in terms of targets. Thus, although inhibition of angiogenesis is a likely mechanism for the antitumour effects of some of these drugs, it is unlikely to be the only cause. The optimum biomarker will, therefore, need to incorporate several different pathways or a more elusive common pathway. The number and variety of genes assessed are higher than for monoclonal antibodies, which further indicate the range of effects with small-molecule RTKIs. Thus, these two drug types are not interchangeable for use in biomarker studies. The evidence so far does not support a specific SNP or gene as a reliable biomarker of response to small-molecule RTKIs, and further exploratory work is needed.

Toxic effects

Hypertension is the most common grade 3–4 toxic effect for bevacizumab.[12] In the E2100 trial[4,22] and other studies,[7,46–48] severe hypertension has been correlated with improved overall survival. This relation could reflect a biological association between toxic effects and outcomes. However, a large meta-analysis showed no such relation.[49] A possible limitation of the meta-analysis, though, was that it included several trials that showed no significant benefits with the addition of anti-VEGF therapy to standard therapy. This contrast in findings suggests that, in the absence of benefit, toxic effects might not be useful as biomarkers. Nonetheless, if a biomarker for hypertension were identified, it could lend insight into the likelihood of efficacy.

The discovery and validation of biomarkers for hypertension face several challenges. Hypertension has widely varied phenotypes, all with inherent technical challenges. Additionally, most trials have not collected or reported hypertension as a raw value, but rather according to the National Cancer Institute common toxicity criteria. Although blood pressure is measured objectively, the treating physician's response to the result can introduce bias and variability into the definition of the phenotype. The criterion for hypertension has also changed over time, which means that it is imperative to determine which was used in trials before comparison.

Bevacizumab causes other toxic effects, including headaches, proteinuria, stroke, thrombosis, perforations, and bleeding.[11] Headaches are difficult to quantify and to attribute

to treatment. Even when side-effects are clearly related to antiangiogenic therapy, the cause might be multifactorial. Fortunately, many of the toxic effects are rare, although the small number of events yields inadequate statistical power for them to be useful as biomarkers. The small-molecule RTKIs share some toxic effects with bevacizumab, but do also have unique side-effects. Table 2 summarises the data of SNP biomarkers for toxic effects.

The most common grade 3–4 non-haematological toxic effects in the E2100 trial was hypertension.[4] No patients with the *VEGFA* –634 CC genotype developed grade 3–4 hypertension, compared with 19–22% of those with other genotypes (p=0·005).[22] Similarly, grade 3–4 hypertension was seen less frequently in patients with the *VEGFA* –1498 TT genotype than in those with other genotypes (8% *vs* 22–23%; table 2). In a study of 63 patients who received sunitinib, the prevalence and duration of hypertension were assessed.[37] Both features were decreased on univariate and multivariate analysis in patients with the *VEGFA* –634 CC genotype when adjustments were made for baseline blood pressure and use of antihypertension medication. In the univariate analysis, the *VEGFA* –634 CC, –1498 TT, and –2578 CC genotypes had protective effects against hypertension. In the AXIS trial,[42] which compared axitinib with sorafenib, no association was seen between hypertension and genotype, although the frequency of hypertension was not reported for the subgroup analysed (table 2).

Garcia-Donas and colleagues[35] reported genetic associations with toxic effects in their study of 101 patients with renal-cell carcinoma who received sunitinib. *VEGFA* –2578 A or –1154 A alleles or *VEGFR2* 1416 TT genotype were associated with risk of hyper tension (table 2). The *VEGFA* –2578 A and –1154 A alleles are in linkage disequilibrium with the *VEGFA* –1498 C and –634 G alleles and, therefore, the associations with SNPs in *VEGFA* would be congruent with those seen in the E2100 trial.[4,22] However, significance was lost for the correlations with these SNPs after correction was made for multiple comparisons. The researchers did note a significant association between carriers of the *CYP3A5*1* high metabolising allele and an increased risk of dose reduction due to toxic effects.

Van Erp and colleagues[50] assessed several toxic effects caused by sunitinib by use of a broad candidate approach. 19 polymorphisms in seven genes involved in the pharmacokinetics and 12 polymorphisms in five genes involved in the pharmacodynamics of sunitinib were assessed in 219 patients with renal-cell carcinoma, GIST, and other malignant diseases (table 2). They found an association between increased risk of leucopenia and the *CYP1A1* 2455 G allele, the *FLT3* 738 T allele, or absence of CAG in the *NR1I3* haplotype. The risk of mucosal inflammation was also raised in carriers of the *CYP1A1* 2455 G allele, whereas those with a copy of TTT in the *ABCB1* haplotype were at increased risk of hand-foot syndrome. Patients with the *VEGFR2* 1191 T allele or who had a copy of TT in the *ABCG2* haplotype had an increased risk of toxic effects more severe than grade 2.

The data strongly suggest that variability in the *VEGFA* gene correlates with hypertension induced by blockade of angiogenesis. A common biological pathway for hypertension induced by small-molecule RTKIs and monoclonal antibodies seems likely. Furthermore, the variability in the candidate pathway is much more likely to be secondary to host genomics than to that in the tumour. Thus, biomarkers for hypertension are likely to be uniform irrespective of the class of drug. An additional layer of complexity, however, is that metabolism (and thus exposure) might still play a part in variability for the small-molecule RTKIs. The major concern in assessment of consistent associations between genotype and hypertension centres on the accurate definition of the phenotype. Other toxic effects associated mainly with small-molecule RTKIs (eg, hand-foot syndrome and leucopenia) are probably unrelated to inhibition of the *VEGFA* pathway. Thus, SNPs from other metabolic or target pathways are more likely to be useful biomarkers.

Explanation for discordance

A successful, validated biomarker to predict which patients will have the best therapeutic index would be valuable. Despite interest, however, the results are incomplete and non-uniform. Several reasons are possible for the inconsistencies. The associations could be false positive or false negative. If a real biological connection is assumed, the efficacy of the drug might be crucial. Identification of a biomarker for a drug in a disease or setting where the agent simply had little to no effect would be difficult, if not impossible. Whether congruence across therapeutic drug classes should be expected needs to be taken into account. The small-molecule RTKIs have a different mechanism of action and clearly have more targets than other antiangiogenics. Identification of biomarkers is, therefore, difficult, as some patients might gain benefit from blockade of one pathway, whereas others would do so through blockade of different pathways. These underlying differences and the available data strongly suggest that monoclonal antibodies and the small-molecule RTKIs should be studied separately. Several trial-specific variables must be considered. First, the setting and endpoint of the trial could be important. The selected efficacy endpoints vary greatly across studies of first-line therapy versus those of later therapy in refractory populations. Furthermore, although PFS and response rate are generally deemed reasonable surrogate outcome markers for cytotoxic therapies, how reliable they are for antiangiogenic agents remains unclear. Finally, the comparison of candidate variants across different disease types can be difficult. This principle, termed divergent phenotypes, implies that causative genomic variability results in different phenotypes in different environments.[51]

Another reason for inconsistencies is small study sample sizes. Biomarker studies are typically done in a subset of patients enrolled in a parent trial and, therefore, are frequently not statistically adequate to answer clinical questions. Analysis is hampered further by multiple comparisons in correlative studies. Although this approach is crucial to sound statistical methodology, correction for multiple comparisons (or the failure to do so) has probably led to heterogeneity. A major statistical flaw is the potential for false-positive associations because of assessment of multiple SNPs. The opposite is a concern too; biologically important associations frequently cannot be detected after stringent correction because the selection of SNPs is too broad. Study power might also be inadequate if SNPs with excessively rare minor allele frequency are selected. Finally, racial heterogeneity within the trial is important to take into account, and proper correction or analysis of patients in subgroups by ethnic origin must be done.

Signal of promise

Despite less-than-perfect congruence, several of the large clinical trials have shown consistent findings, mainly where notable drug effects have been seen in the parent trial. The *VEGFA* SNPs assessed in the E2100 trial, the Hellenic Oncology Group Study, and the E4599 trial have provided the most compelling replications, and the findings from other phase 3 trials, such as the *VEGFR1* SNP in the AViTA trial, are provocative but would benefit from additional validation.[31] Several factors support the positive findings. Most of the SNPs mentioned in this Review were selected for study on the basis of solid biological rationale. Second, some parent studies have been large, randomised, phase 3 trials that revealed both statistically and clinically meaningful signals. The inclusion of placebo arms has enabled testing for interactions by treatment groups. With regards to toxic effects, the theme is even more consistent and almost certainly described by host-specific variation. Variability in *VEGFA* seems to be a predictive biomarker for hypertension, although the optimum definition of phenotype and clinical relevance is yet to be fully elucidated. Other toxic effects, such as headache, hand-foot syndrome, and leucopenia, are more likely to be explained by variability in other metabolic and target pathways.

We believe that biomarkers should not be used for clinical decision making related to antiangiogenic therapy at this time because of the retrospective nature of the findings and the lack of complete congruence. The findings are sufficient, however, to warrant additional investigation and to make this area a research priority.

Moving forward

Clinical applicability of biomarkers represents the final and most challenging hurdle for translational research. Excellent guidelines to define clinically useful biomarkers have been established.[52] Simon and colleagues[53] established a refined system for biomarker study design that used archived specimens. This approach was typically used in the studies we have discussed. The prospective-retrospective design and use of archived specimens can optimise the level of evidence and lessen some of the cost and other logistical burdens that are commonly encountered with correlative studies. Nevertheless, some elements must be carefully planned. An adequate supply of archived specimens that are sufficiently comprehensive to mirror the makeup of the parent trial in covariates and outcomes must be available. The optimum statistical plan for a correlative study would be to assess a larger number of samples or patients than are in the parent trial, but, typically, markedly fewer samples are available for correlative studies. A high percentage of samples has, however, been achieved in some studies. The patients assessed in the E2100 correlative study had similar clinical covariates and outcomes to the population in the parent trial. Unfortunately, many studies simply do not report these data, although this limitation can be easily remedied. The a-priori hypothesis and statistical plan frequently require that several hypotheses are assessed simultaneously and, therefore, the statistical plan is often directed by the total number of available samples rather than biological parameters, which weaken the power of the study. An advantage in antiangiogenic biomarker studies is that the assays to assess SNPs are reliable, fast, reproducible, and can be clinically implemented without difficulty.

Beyond the recommendations outlined above, a major limitation of completed studies has been the heterogeneous selection of candidate genes and SNPs. Although there is no master blueprint for the selection of SNPs within a given gene, the use of those that tag for the common haplotypes is a rational approach. We have reported the resequencing of the promoter and 5′-UTR of the *VEGF* gene.[21] These tag SNPs are biologically important as they represent the inherited variation in a given population. Furthermore, the actual causative SNP or SNPs must be determined if complete congruence is to be achieved. Although SNPs that have high linkage disequilibrium will allow for some degree of success, if the linkage disequilibrium is incomplete the validations will be inconsistent. The number of SNPs selected, their minor allele frequencies, and the expected number of events must be prospectively calculated to ensure statistical power is achievable even after correction for multiple comparisons. As described above, many biomarkers are destined to fail not because of biology but because the statistical expectations are unrealistic.

The genes to select—from simple target genes to complex networks that encompass angiogenesis, inflammation, and so on—raise similar concerns. The use of candidate and pathway approaches is being overtaken by high-throughput approaches that help to remove bias, but they increase the statistical complexity and require larger numbers of patients to study. High-throughput studies could provide insight into the importance of tumour-specific variability and clarify whether tumour-specific and host-specific variation play parts in defining heterogeneous outcomes. Such a finding would in turn present further challenges, as few biomarkers identified so far reflect both features.

Trial design is equally important to biomarker selection. McGuire and colleagues[54] described three types of biomarker trials: pilot, definitive, and confirmatory. Pilot studies gather evidence and assess whether biomarkers warrant further investigation. Definitive studies aim to confirm hypotheses. They are typically retrospective studies that investigate biomarkers in a subgroup of patients from a parent trial. The use of a subgroup is often necessary because of the available biological samples or data pertinent to the biomarker being tested. Selection of patients is, therefore, random to some degree, which has the potential to introduce unintended bias. Ideally, confirmatory studies should be designed to prospectively validate or refute the hypothesis and as many patients from the parent trial as possible should be included. Many experts feel that definitive study designs, including those for anti angiogenic biomarkers, have been overused and that confirmatory study designs have been underused. The overuse of definitive trial approaches is likely to lead to heterogeneous results that make discernment of the real usefulness (or lack thereof) of a biomarker difficult.

We have embarked on a prospective evaluation of the clinical trial E5103 to explore this issue further.[55] This study is an FDA trial of bevacizumab in the adjuvant setting for the treatment of breast cancer (NCT00433511). Around 5000 patients have been assigned to standard chemotherapy alone or with concurrent or concurrent plus sequential bevacizumab. We did a genome-wide association study on more than 3300 samples from patients in the study to confirm or refute preliminary findings from the E2100 trial and for biomarker discovery.[55]

We have identified several promising markers for grade 3–4 hypertension, and work is continuing into other markers for toxic effects related to bevacizumab and other chemotherapeutic agents (eg, taxane-induced peripheral neuropathy). Study of biomarkers for efficacy, which requires maturation of the clinical trial, will follow. The primary efficacy endpoint of our biomarker study will be 5-year PFS, which is also the primary endpoint of the parent trial, and the secondary endpoint will be overall survival. We have used the study design recommended by Simon and colleagues.[53]

Search strategy and selection criteria

We included studies that involved patients with malignancy, assessed agents with inhibition of angiogenesis as the main proposed mechanism of action, and outcomes (efficacy or toxic effects) in relation to at least one single-nucleotide polymorphism, and had been published in peer-reviewed journals or presented at international meetings. We searched Medline, Current Contents, PubMed, and references from relevant articles with the search terms “anti-angiogenic”, “anti-VEGF”, “pharmacogenetic”, “single nucleotide polymorphism/SNP”, “biomarker”, and “cancer/malignancy”. Only articles published in English between January, 1971, and December, 2011, were included. Abstracts and reports from international meetings in 2011 were also included.

A crucial component to our pharmacogenomic study is collaboration of experts and patient advocates in multiple institutions. This approach has enabled us to formally analyse quality of life and quality-adjusted life-years at several time points for 500 patients enrolled in E5103. We hope the results will lead to development of formal decision-making tools with input from treating oncologists and patients that will help assessment of the incremental risk-to-benefit ratio and make the biomarkers clinically relevant. Additionally, we will develop educational tools for oncologists and patients to aid counselling about and understanding of risks.

Conclusions

The use of biomarkers to identify the risk-to-benefit ratio for a specific drug in an individual patient has the potential to substantially improve the therapeutic index. The road to identification of clinically relevant biomarkers is long and winding, and is littered with signs of promise and misdirection. Validation is often lacking but remains important. Clinical implementation is even more challenging and more crucial. Germline SNPs are gaining ground as biomarkers for antiangiogenic therapy, but clear direction is needed, particularly in view of the heterogeneity in benefit and toxicity in this class of drugs. Identification of successful biomarkers could lead to the success that other targeted agents, such as trastuzumab, have experienced.

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Table 1

Efficacy markers in pharmacogenetic correlative substudies of antiangiogenic agents

	Cancer	Number of patients in substudy/ parent trial	Genotypes and SNPs assessed	Findings
Bevacizumab				
E2100: phase 3, paclitaxel *vs* paclitaxel +bevacizumab[4,22]	Breast	363/722	*VEGFA*: –2578 C/A (rs699947), –1498 C/T (rs 833061), –1154 G/A (rs1570360), –634 G/C (rs2010963), 936 C/T (rs3025039); *VEGFR3*[*]: 1416 A/T (rs1870377), 889 G/A (rs2305948)	Improved OS with paclitaxel +bevacizumab in carriers of *VEGFA* –2578 AA and –1154 AA
AVADO: phase 3, docetaxel *vs* docetaxel +bevacizumab 7·5 mg/kg or 15·0 mg/kg[5,23]	Breast	336/736	*VEGFA*: –2578 C/A, –1498 C/T, –1154 G/A, –634 G/C, 936 C/T; *VEGFR3*[†]; *VEGFR3*[*]; *ADM; EGF; ERCC3; eNOS*[‡]; *IL3; IL3; CXCR3; ICAM3; TP33; WNK3*	Improved PFS with docetaxel alone in carriers of *VEGFA* –634 CC; improved PFS with docetaxel +bevacizumab 7·5 mg/kg in carriers of *VEGFA* –2578 AA
Hellenic Cooperative Oncology Group: phase 3, FOLFIRI +bevacizumab *vs* XELIRI +bevacizumab[24,25]	Colon	209/285	*VEGFA*: –2578 C/A, –1154 G/A, –634 G/C, 936 C/T	Decreased OS with bevacizumab in carriers of *VEGFA* –2578 CC, –1154 GG
Formica et al: observational study, first-line FOLFIRI +bevacizumab[26]	Colon	40/40	*VEGFA*: –2578 C/A, –1512 18 bp ins/del (rs35569394), –1498 C/T, –1451 T/C (rs1005230), –1411 4G–5G (rs35864111), –1154 G/A	Improved PFS with bevacizumab for carriers of *VEGFA* –1154 AA, and improved response rate in carriers of *VEGFA* –634 GG
E4599: phase 3, paclitaxel/carboplatin *vs* paclitaxel/carboplatin +bevacizumab[27,28]	Lung	133/878	*VEGFA*: –1498 C/T, –1154 G/A, –634 G/C, –936 C/T; *VEGFR3*[*]: 3′ UTR T/A; *CXCR3*: –785 C/T; *COX3*[‡]: –765G/C; *EGF*: –61A/G; *EGFR*: 497G/A; *FGFR3*: 388G/A; *ICAM3*: 469T/C; *IL3*: –251T/A	Improved OS and PFS with bevacizumab in carriers of SNP profile *VEGFA* –634*G*, *ICAM3* 469T⁄C, *IL3* –251T⁄A
Schultheis et al: phase 2, low-dose cyclophosphamide +bevacizumab[29]	Ovarian	53/70	*VEGFA, CXCR3, CXCR3, COX3*[§]*, ADM, ARNT, EGF, EGFR, FGFR3, HIF3A, ICAM3, IGF3, IGF3R, IL3, IL3, IL3B, IL3R3, VEGFR3*[*]*, LEP, MDM3, MMP3, MMP3, MMP3, NFKB3, NRP3, PGF, TP33, CXCL33, TF, TNF*	Decreased response rate with bevacizumab in carriers of *IL3* A allele; improved PFS for *CXCR3* 785 C, and *VEGFA* CT genotype
AVITA: phase 3, gemcitabine/erlotinib *vs* gemcitabine/erlotinib+ bevacizumab[30,31]	Pancreatic	154/607	Total 157 SNPs in *VEGFA*, *VEGFB*, *VEGFC*, *VEGFD*[¶], *VEGFR3*[†], *VEGFR3*[*], inducers of VEGF ligand, *PlGF*	Superior OS with bevacizumab in carriers of *VEGFR3*[†] rs9582036
Pazopanib				
Combined trials phase 3 VEG105192 and phase 2 VEG102616, and open-label rollover VEG107769[32–34]	Renal	397/585	*VEGFA*: –2578 C/A, –1498 C/T, –1154 G/A, –634 C/G, 936 C/T; *VEGFR3*[*]: –604 A/G, 889 G/A, 1416 A/T; *VEGFR3*[//]: 1480 A/G (rs307826); *ABCB3*: 1236 T/C (rs1128503), 2677 G/T, (rs2032582), 3435 C/T (rs1045642); *ABCG3*: 34 G/A (rs2231137), 421 C/A (rs2231142), 869 C/T (rs72552713); *CYP3A3*: –392 A/G (rs2740574); *CYP3A3*: 6986 A/G (rs776746); *FGF3*: 224 C/T (rs1449683); *FGFR3*: IVS2+906 C/T (rs2981582); *HIF3A*: 1772 C/T, 1790 G/A; *IL3*: –251 T/A, 2767 A/T (rs1126647); *NR3I3*: –25385 C/T (rs3814055), 10620 C/T (rs1054190), 7635 A/G (rs6785049); *PDGFRA:* –573 G/T (rs1800812)	Decreased response rate with pazopanib in carriers of *VEGFA* –2578 AA, –634 GG, –1498 CC, *HIF3A* 1790 AG, *NR3I3* –25385 TT and decreased PFS in carriers of *IL3* 2767 TT, –251AA, *HIF3A* 1790 AG
Sunitinib				

	Cancer	Number of patients in substudy/ parent trial	Genotypes and SNPs assessed	Findings
Garcia-Donas et al: observational study[35]	Renal	89/101	*VEGFA*: –2578 C/A, –1154 G/A, –634 C/G; *VEGFR3*[*]: 889 G/A, 1416 A/T; *VEGFR3*[//]: 330+31 T/G (rs448012); 1480 A/G (rs307826), 3971 G/T (rs307821); *ABCB3*, 1236C/T, 2677 G/T, 3435 C/T; *ABCG3*: 421 C/A; *CYP3A3*: –392 A/G; *CYP3A3*: 6989 A/G; *IL3*: 2767 A/T; *PDGFRA*: 1580 T/C (rs35597368)	Decreased PFS with for carriers of SNPs in *VEGFR3*[//]: –1480 A→G, 3971 G→T
van der Veldt et al: retrospective, multicentre study[36]	Renal	136/136	*ABCB3*: 1236C/T, 2677G/T, 3435C/T; *ABCG3*: –15622C/T 34 G/A, 421 C/A, 1143 C/T; *VEGFR3*[*]: –604 A/G, –92 G/A (rs1531289), 54 T/C (rs7692791), 889 G/A, 1416 A/T; *VEGFR3*[//]: 1480 A/G; *CYP3A3*: 2455 A/G (rs1048943); *CYP3A3*: –163 A/C (rs762551); *CYP3A3*: 6986 A/G (rs776746); *FLT3*: 738 T/C (rs1933437); *NR3I3*: –25385 C/T, –24113 G/A (rs2276706), 10620 C/T, 10799 G/A (rs1054191), 7635 A/G, 8055 C/T (rs2276707); *NR3I3*: 5719 C/T (rs2307424), 7738 A/C (rs2307418), 7837 T/G (rs4073054); *PDGFRA*: –1171 C/G (rs1800810), –735 G/A (rs1800813), –573 G/T (rs1800812), 1580 T/C	Improved PFS with sunitinib in carriers of *CYP3A3* 6986 AA, CAT copy absent in the *NR3I3* haplotype, TCG copy present in the *ABCB3* haplotype
Kim et al: retrospective cohort study[37]	Renal	63/NA	*VEGFA*: –2578 C/A, –22459 ins/del, –1498 C/T, –1154 G/A, –634 G/C, 936 C/T; *VEGFR3*[*]: 889 A/G, 1416 A/T	Decreased OS with sunitinib in carriers of combined *VEGFA* 936 CC and *VEGFR3*[*] 889 GG
Sorafenib				
E2501: randomised, placebo- controlled, phase 2, chemotherapy-resistant patients[38,39]	Lung	88/333	*VEGFA*, *EGF*, *EGFR*, *IL3*, *CXCR3*, *COX3*[‡], *VEGFR3*[*]; *ICAM3*, *FGFR3*	Improved PFS with sorefenib in carriers of *VEGFA* –1498 CC, –634 CC
Anderson et al: phase 1, gemcitabine +radiotherapy+ sorafenib[40]	Pancreatic	19/27	*VEGFA*: –2578 C/A, –1498 C/T, –1154 G/A, –634 G/C, 936 C/T; *VEGFR3*[*]: 889 G/A, 1046 G/A, 1174 G/A, 1416 A/T, 2341 G/A, 2359 C/G, 2505 G/C, 2530del G, 2854 G/A, 3628 C/G, 4039 T/A	Improved OS with sorefenib in carriers of *VEGFA* –2578 AA, –1498 CC, –1154 AA, *VEGFR3*[*] 272 GG, 889 GG; all responders had *VEGFA* –7 CC, *VEGFR3*[*] 1416 AA
Axitinib				
AXIS: phase 2, axitinib *vs* sorafenib[41,42]	Renal	249/723	*VEGFA*: –2578 C/A, –1498 C/T, –1154 G/A, –634 C/G, 936 C/T; *VEGFR3*[†]: rs9513070, rs9554316, rs9554320, rs9582036, rs111458691; *VEGFR3*[*]: –604 A/G (rs2071559), 889 G/A, 1416 A/T; *HIF3A*: 1772 C/T (rs11549465), 1790 G/T (rs11549467)	Improved PFS with axitinib over sorafenib for carriers of *VEGFA* –2578 AA

SNPs=single-nucleotide polymorphisms. OS=overall survival. PFS=progression-free survival. FOLFIRI=leucovorin, fluorouracil, and irinotecan. XELIRI=irinotecan and capecitabine. ins/del=insertion-deletion.

[*] Approved symbol *KDR*.

[†] Approved symbol *FLT3*.

[‡] Approved symbol *NOS3*.

[§] Approved symbol *PTGS3*.

[¶] Approved symbol *FIGF*.

[//] Approved symbol *FLT3*.

Table 2

Markers of toxic effects in pharmacogenetic correlative substudies of antiangiogenic agents

	Toxic effect	Cancer	Number of patients in substudy/ parent trial	Genotypes and SNPs assessed	Findings
Bevacizumab					
E2100[4,22]	Hypertension ≥grade 3–4	Breast	363/722	*VEGFA*: –2578 C/A, –1154 G/A, –1498 C/T, –634 G/C, 936 C/T; *VEGFR3*[*]: 889 G/A, 1416 A/T	*VEGFA* –1498 TT, –634 CC associated with decreased risk of hypertension
Sunitinib					
Kim et al[37]	Hypertension (SBP >150 mm Hg and/or DBP ≥90 mm Hg)	Renal	63/NA	*VEGFA*: –2578 C/A, –22459 ins/del, –1498 C/T, –1154 G/A, –634 G/C, 936 C/T; *VEGFR3*[*]; 889 G/A, 1416 A/T	*VEGFA* –2578 CC, –1498 TT, –634 CC associated with decreased risk of hypertension
van Erp et al[50]	Thrombocytopenia, leucopenia, hand-foot syndrome, mucosal inflammation, any toxic effect >grade 2	Renal, GIST, other	219/NA	*VEGFR3*[*]: –604 A/G, –92 G/A, 54 T/C, 889 G/A, 1416 A/T; *VEGFR3*[†]: 1480 A/G; *ABCB3*: 1236 T/C, 2677 G/T, 3435 C/T; *ABCG3*: –15622 C/T, 34 G/A, 421 C/A, 1143 C/T (rs2622604); *CYP3A3*: 2455 A/G; *CYP3A3*: –163 A/G: *CYP3A3*: 6986 A/G; *FLT3*: 738 T/C; *PDGFRA*: 1580 T/C, –1171 C/G, –735 G/A, –573 G/T; *NR3I3*: –24113 G/A, 7635 A/G, 8055 C/T, 10620 C/T, 10799 G/A, 25385 C/T, *NR3I3*: 5719 C/T, 7738 A/C, 7837 T/G; *RET* 2251 G/A (rs1799939)	*CYP3A3* 2455 GG, *FLT3* 738 TT, CAG absent in *NR3I3* haplotype associated with increased risk of leucopenia; *VEGFR3*[*] 1191 TT, copy of TT in *ABCG3* haplotype associated with increased risk of toxic effects; *CYP3A* 2455 GG associated with increased risk of mucosal inflammation; copy of TTT in *ABCB3* haplotype associated with increased risk of hand-foot syndrome
Garcia-Donas et al[35]	Mucositis, hand-foot syndrome, hypertension, anaemia, thrombocytopenia, any toxic effect ≥grade 3–4, adverse events leading to dose reductions	Renal	95/101	*VEGFA*: –634 C/G, –2578C/A, –1154 G/A; *VEGFR3*[*]: 889 G/A, 1416 A/T; *VEGFR3*[†]: 1480 A/G, 330+31 T/G, 3971 G/T; *ABCB3*: 1236 T/C, 2677 G/T, 3435 C/T; *ABCG3*: 421 C/A; *CYP3A3:* –392 A/G; *CYP3A3*: 6986 A/G; *IL3*: –251 T/A; *PDGFRA*: 1580 T/C	Dose reductions associated with *CYP3A3*[*]*3* (6986 GG) high-metabolising allele; *VEGFA* –2578 AA, –1154 AA, *VEGFR3*[*] 1416 TT[‡] associated with increased risk of hypertension
Axitinib					
AXIS[41,42]	Hypertension ≥grade 3–4	Renal	249/723	*VEGFA*: –2578 C/A, –1498 C/T, –1154 G/A, –634 C/G, 936 C/T; *VEGFR3*[§]: rs9513070, rs9554316, rs9554320, rs9582036, rs111458691; *VEGFR3*[*]: 889 G/A, 1416 A/T, –604 A/G; *HIF3A*: 1772 C/T, 1790 G/A	None; rate of hypertension in subgroup not reported

SNPs=single-nucleotide polymorphisms. SBP=systolic blood pressure. DBP=diastolic blood pressure. GIST=gastrointestinal stromal tumour.

[*]Approved symbol *KDR*.

† Approved symbol *FLT3*.

‡ Significant before adjustment; none significant after adjustment for multiple comparisons.

§ Approved symbol *FLT3*.