Chapter 24
The Prognostic and Predictive Value of VEGF Across Various Tumor Types

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24.1 Introduction

Angiogenesis, the formation of blood vessels, is a complex process involving numerous pathways and receptors that are essential for tumor growth and vascular metastasis. Tumors release a spectrum of proangiogenic cytokines, driven by metabolic and acidic environmental effects and hypoxia. Of them vascular endothelial growth factors (VEGFs) are essential regulators of tumor angiogenesis (Fig. 24.1) (Hicklin and Ellis 2005) (PlGF). The VEGF family consists of VEGF-A to VEGF-E and placental growth factors 1 and 2 which bind to three structurally related receptor tyrosine kinases, VEGFR-1, VEGFR-2, and VEGFR-3 (Fig. 24.2) (Takahashi and Shibuya 2005).

The best characterized member of the VEGF ligand family is VEGF-A. Several isoforms of VEGF-A exist; they differ chiefly according to the presence or absence of heparan sulfate (HS)-binding domains. In larger isoforms (e.g., VEGF-A_{165} and VEGF-A_{189}), the HS-binding domains engage HS in the extracellular matrix (Krilleke et al. 2009; Poltorak et al. 2000). However, lower molecular weight VEGF-A, such as VEGF-A_{110} (a plasmin cleavage fragment of longer isoforms) and the VEGF-A_{121} isoform, lack this motif and are freely soluble. Extracellular matrix-bound and soluble VEGF-A isoforms have differing effects on vascular morphogenesis: (Lee et al. 2005) soluble VEGF-A is associated with large, tortuous, unbranched vessels, whereas matrix-bound VEGF-A is associated with thinner, more branched vessels.

Several anti-angiogenesis strategies have been developed and shown preclinical promises, and some have translated into clinical success. Of these, the development of inhibitors such as the monoclonal anti-VEGF antibody bevacizumab has

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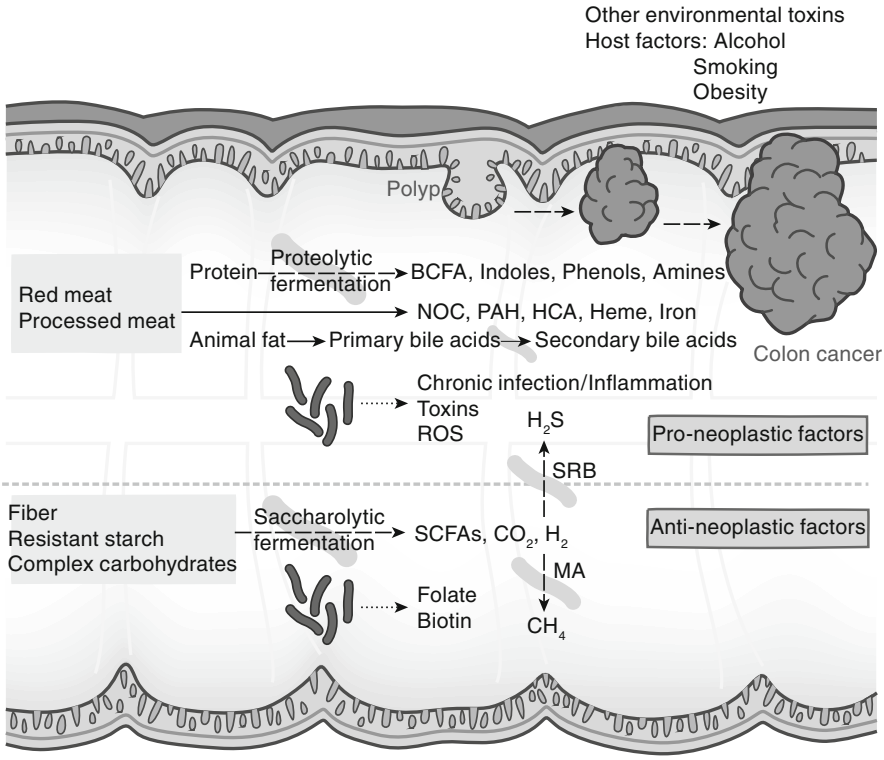


Fig. 24.1 The role of VEGF/VEGFR in tumor angiogenesis (*Source*: Reprinted with permission from Hicklin and Ellis 2005) *EPC* endothelial progenitor cell, *VEGF* vascular endothelial growth factor

progressed the furthest by demonstrating significantly improved efficacy when combined with standard therapy compared to standard therapy alone across a range of tumor types: in advanced colorectal, breast, non-small cell lung, renal, gastric, pancreatic, and ovarian cancers in terms of progression-free survival (PFS) (Hurwitz et al. 2004; Saltz et al. 2008; Giantonio et al. 2007; Arnold et al. 2012; Miller et al. 2007; Miles et al. 2010; Robert et al. 2011; Brufsky et al. 2011; Reck et al. 2009; Sandler et al. 2006; Escudier et al. 2007; Escudier et al. 2010; Rini et al. 2008; Ohtsu et al. 2011; Van Cutsem et al. 2009; Burger et al. 2011; Perren et al. 2011; Aghajanian et al. 2012; Pujade-Lauraine et al. 2012) and, in some cases, overall survival (OS) (Hurwitz et al. 2004; Giantonio et al. 2007; Arnold et al. 2012; Sandler et al. 2006) (Table 24.1). Subgroup analyses in these trials suggested that bevacizumab provides a significant but relatively modest benefit in almost all clinically the identification of who subsets of patients. Who will obtain the greater benefit from this therapy or for how long they should be administered in the treatment algorithm are major open questions for clinicians and challenges for present and future research.

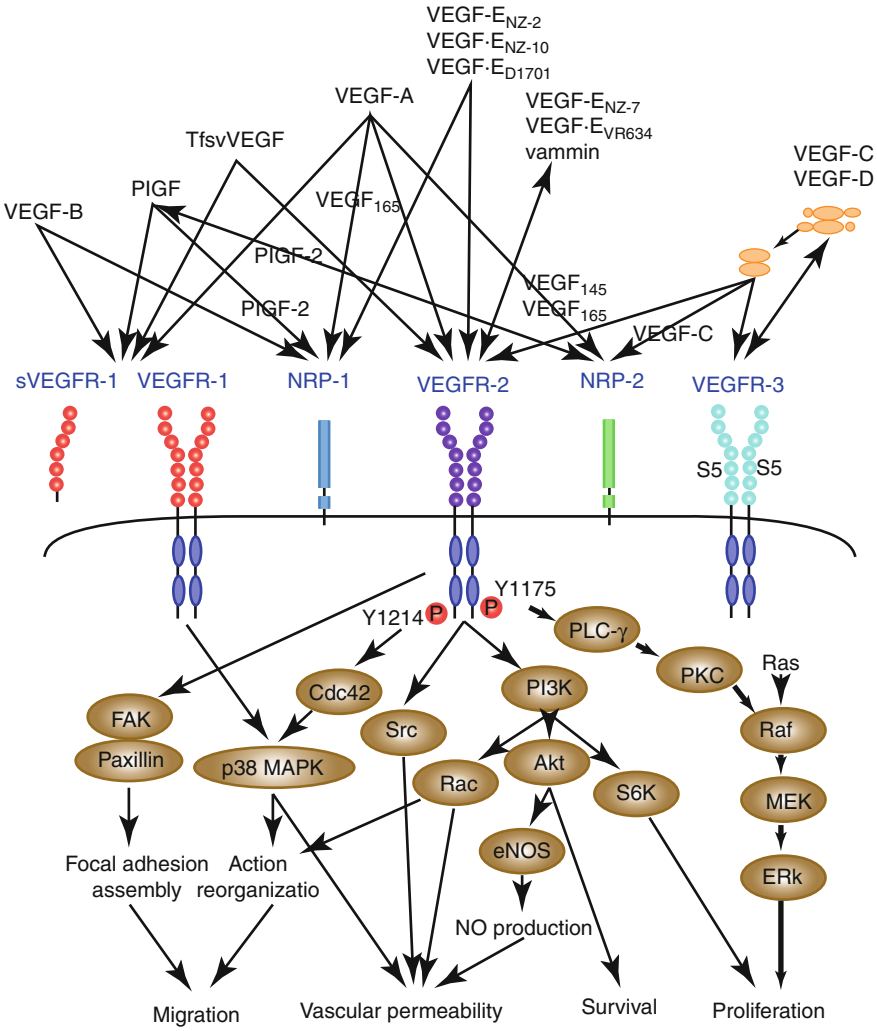
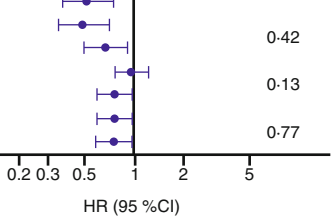


Fig. 24.2 Downstream signaling of the VEGF receptors (*Source*: Takahashi and Shibuya 2005). The VEGF family of ligands and their receptor-binding patterns are shown at the *top*. Downstream VEGFR signaling pathways focusing on VEGFR-2 are shown at the bottom. Tyr^{1175} (Y1175) and Tyr^{1214} (Y1214) are the two major autophosphorylation sites in VEGFR-2. PLC-g binds to Y1175, leading to the phosphorylation and activation of this protein. Y1214 appears to be required to trigger the sequential activation of Cdc42 and p38 MAPK. Many proteins are activated by VEGFR-2 through an unknown mechanism, including FAK, PI3K, and Src. The activation of downstream signal transduction molecules leads to several different endothelial cell functions such as migration, vascular permeability, survival, and proliferation. *NRP* neuropilin, *VEGF* vascular endothelial growth factor, *PlGF* placenta growth factor, *PLC* phospholipase C, *FAK* focal adhesion kinase, *ERK* extracellular signal-regulated kinase, *Akt* cytosolic protein kinase, *eNOS* endothelial nitric oxide synthase

Table 24.1 Treatment effect according to cutoff selected for plasma VEGF-A: AVADO trial (a) bevacizumab 7.5 mg/kg dose; (b) bevacizumab 15 mg/kg dose

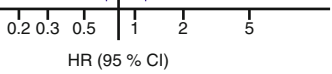
a

Trial	Plasma VEGF-A	Median PFS, months BEV	Control	HR	95 % CI	Interaction *p* value
AVADO (15 mg/kg)	Low	8·5	8·0	0·86	0·56–1·32	0.0808
	High	8·8	6·6	0·49	0·31–0·76	
AVADO (7.5 mglkg)	Low	8·8	8·0	0·96	0·62–1·48	0·0136
	High	8·5	6·6	0·52	0·33–0·81	
A VITA	Low	5·3	4·6	0·77	0·53–1·13	0·06
	High	5·1	3·3	0·52	0·35–0·78	
AVAGAST (In)	Low	7·0	5·7	0·86	0·67–1·10	0·11
	High	6·8	4·9	0·66	0·52–0·85	
AVAGAST (non-Asia/Pacific)	Low	6·8	5·2	0·85	0·57–1·26	0·06
	High	8·1	4·4	0·54	0·39–0·76	
AVEREL	Low	16·5	13·6	0·83	0·50–1·36	0·79
	High	16·6	8·5	0·70	0·43–1·14	
AVF2107g	Low	9·8	6·9	0·64	0·45–0·92	0·61
	High	10·6	5·6	0·52	0·37–0·74	
AVOREN	Low	12·9	7·2	0·49	0·35–0·70	0·42
	High	7·7	3·7	0·67	0·49–0·91	
AVAIL (15 mglkg)	Low	6·9	6·6	0·96	0·76–1·23	0·13
	High	6·5	6·0	0·76	0·60–0·97	
AVAIL (7.5 mg/kg)	Low	7·1	6·6	0·77	0·60–0·99	0·77
	High	6·6	6·0	0·75	0·59–0·95	



b

Trial	Plasma VEGF-A	Median OS, months BEV	Control	HR	95 %CI	Interaction *p* value
AVADO (15 mg/kg)	Low	33·1	33·4	1·07	0·64–1·77	0.55
	High	25·5	29·8	1·02	0·63–1.67	
AVADO (7.5 mglkg)	Low	32·8	33·4	1·34	0·80–2·24	0·044
	High	33·7	29·8	0·87	0·53–1·43	
AViTA	Low	7·0	6·9	1·02	0·69–1·50	0·03
	High	7·4	4·8	0·56	0·38–0·83	
AVAGAST (ITT)	Low	13·7	12·9	1·01	0·77–1·31	0·07
	High	11·7	8·3	0·72	0·57–0·93	
AVAGAST (non-Asia/Pacific)	Low	12·4	10·8	1·01	0·68–1·51	0·04
	High	11·3	7·1	0·59	0·43–0·82	
AVF2107g	Low	24.5	18·7	0·70	0·46–1·05	0·95
	High	16·9	13·1	0·68	0·48–0·95	
AVOREN	Low	NR	NR	0·62	0·31–1·23	0·55
	High	18·2	14·5	0·86	0·57–1·30	
AVAIL (15 mglkg)	Low	15·8	15·8	0·97	0·72–1·31	0·67
	High	13·5	11·2	0·98	0·75–1·28	
AVAIL (7.5 mg/kg)	Low	15·1	15·8	0·92	0·68–1·25	0·99
	High	12·6	11·2	0·89	0·68–1·15	



CI confidence interval, *PFS* progression-free survival, *VEGF* vascular endothelial growth factor

To date, validated predictive markers to select patients who will obtain the greater benefit from this therapy are still lacking. The value of VEGF as prognostic and predictive marker across tumor types for the anti-VEGF agent bevacizumab has been examined.

24.2 Biomarker: Definition of Prognostic Versus Predictive Marker

The NCI define a biomarker as "a biological molecule found in blood, other body fluids, or tissues that is a sign of normal or abnormal process, or of a condition or disease. A biomarker may be used to see how well the body responds to a treatment for a disease or condition" (http://www.cancer.gov/dictionary?cdrid=45618). Biomarkers that are associated with the risk of developing a disease, the risk of spread, aggressiveness, or survival rates independent of treatment are termed "prognostic," while those that predict the rate of response to a particular therapy are termed "predictive." For example, estrogen and progesterone are weak prognostic biomarkers in breast cancer but are strong predictive factors for response to hormonal therapy. Oncological biomarkers are biological substances whose concentration or level of expression can be measured in the blood or other biospecimens such as tumor tissue. Although concentrations or expression levels of a biomarker are continuous variables, analysis of their association with disease is often easier if they are transformed into binary variables. This entails setting a threshold level and grouping patient data as high (above the threshold) or low (below it). Other markers, such as gene mutations, are binary variables, since a mutation is either present or not present in a given patient. Biomarkers are only valuable if the information they provide supplements or improves that already available from other measurable factors. It should be demonstrated that biomarker-"positive" patient populations derive clinically meaningful benefit from specific treatment compared to those who are biomarker "negative" (Working Group 2009). The potential for biomarker use should be validated in controlled, phase III clinical trials. Ideally, biomarkers should be measurable in an easily obtainable sample, and the method for quantifying them should be reliable and reproducible, have a high specificity and selectivity, and be widely available (Cummings et al. 2010).

24.3 Prognostic Value of VEGF Across Tumor Types

A number of studies have shown that VEGF tumor expression is associated with poor prognosis across various tumor types.

Farhat et al. (2012) reported findings from 11 studies showing a consistent negative prognostic effect of VEGF tumor expression in non-small cell lung cancer (NSCLC) patients. Another systematic literature review from 11 studies (total 767

patients) supports that tumor expression of VEGF represents a significant and reproducible marker of adverse prognosis in resected pancreatic cancer (PaC) (Smith et al. 2011). A meta-analysis (Des Guetz et al. 2006) of 18 studies with 2,050 colorectal cancer (CRC) patients reported that VEGF expression significantly predicted poor relapse-free survival (RR = 2.84; 95 % CI 1.95–4.16) and OS (RR = 1.65; 95 % CI 1.27–2.14). Similarly, Liu et al. (2012) reported from a meta-analysis on 44 published studies with 4,794 resected gastric cancer patients that positive expression of tissue VEGF, VEGF-C, VEGF-D, and circulating VEGF was all associated with poor prognosis in resected gastric cancer. However, authors hypothesized that circulating VEGF may be better than tissue VEGF in predicting prognosis.

A lesser number of studies than those assessing tumor VEGF expression have investigated the role of VEGF circulating levels as prognostic marker of patient outcomes in gastric and other tumor types.

In the AVAGAST study (bevacizumab in combination with chemotherapy as first-line therapy in advanced gastric cancer: a randomized, double-blind, placebo-controlled phase III study), retrospective analysis of pretreatment plasma VEGF-A levels has also been shown to be prognostic for PFS and OS patients. In the placebo group with high baseline plasma, VEGF-A levels had a shorter median overall survival (8.3 months) than patients with low levels (12.9 months) (Van Cutsem et al. 2012).

In patients with previously untreated NSCLC, who receive regimens without anti-VEGF therapy, significant correlation between high pretreatment serum VEGF levels of isoform 189VEGF-A and poor survival ($p = 0.0002$) has been observed (Yuan et al. 2001). In the AVAiL study (randomized, controlled study of bevacizumab in combination with platinum-based chemotherapy in NSCLC), retrospective analyses of pretreatment plasma VEGF-A levels have been shown to be prognostic for PFS: pVEGF-A low HR = 0.96 versus pVEGF-A high HR = 0.76, $p = 0.13$ (Fig. 24.3a) (Jayson et al. 2011); however, the prognostic value of VEGF-A was not observed for overall survival in E4599 in advanced non-squamous NSCLC patients randomized to chemotherapy +/− bevacizumab (Dowlati et al. 2008).

In patients with mCRC, baseline VEGF levels were treatment-independent prognostic biomarkers for PFS and OS in two randomized phase III studies HORIZON II ($n = 860$; FOLFOX/XELOX plus cediranib 20 mg ($n = 502$) or placebo ($n = 358$)) and HORIZON III ($n = 1{,}422$; mFOLFOX6 plus cediranib 20 mg ($n = 709$) or bevacizumab ($n = 713$)) (Jürgensmeier et al. 2013). The prognostic effect of circulating VEGF-A in CRC is consistent with the observation of Hurwitz et al. (2004) from the AVF2107 trial.

In AVITA phase III randomized study of bevacizumab with gemcitabine-erlotinib in patients with mPaC, pretreatment plasma concentration of VEGF-A showed prognostic effect. Patients in the control (non-bevacizumab-treated) groups who had high plasma VEGF-A concentrations had shorter PFS and OS than patients with low concentrations (Table 24.2). Similar prognostic value for baseline plasma VEGF-A has been observed from other randomized Ph3 trials of bevacizumab in breast cancer (AVADO, AVEREL) and renal cell carcinoma (AVOREN).

In summary, most research supports the clinical prognostic value of VEGF tumor expression in chemotherapy and anti-VEGF-naïve patients across tumor types.

In most of these studies, VEGF overexpression was associated with poor prognosis. Although the data on the prognostic implication of circulating VEGF in blood samples of patients across tumor types is more heterogeneous, the evidence is persuasive but needs further investigation and validation. Different assays and storage techniques could explain the inconsistent results seen as these factors are known to affect the reliability of biomarker measurement.

24.4 Predictive Value of VEGF Across Tumor Types

The assessment of plasma and serum levels of VEGF as potential predictive marker of anti-VEGF therapies has been reported in a number of studies (Jayson et al. 2011).

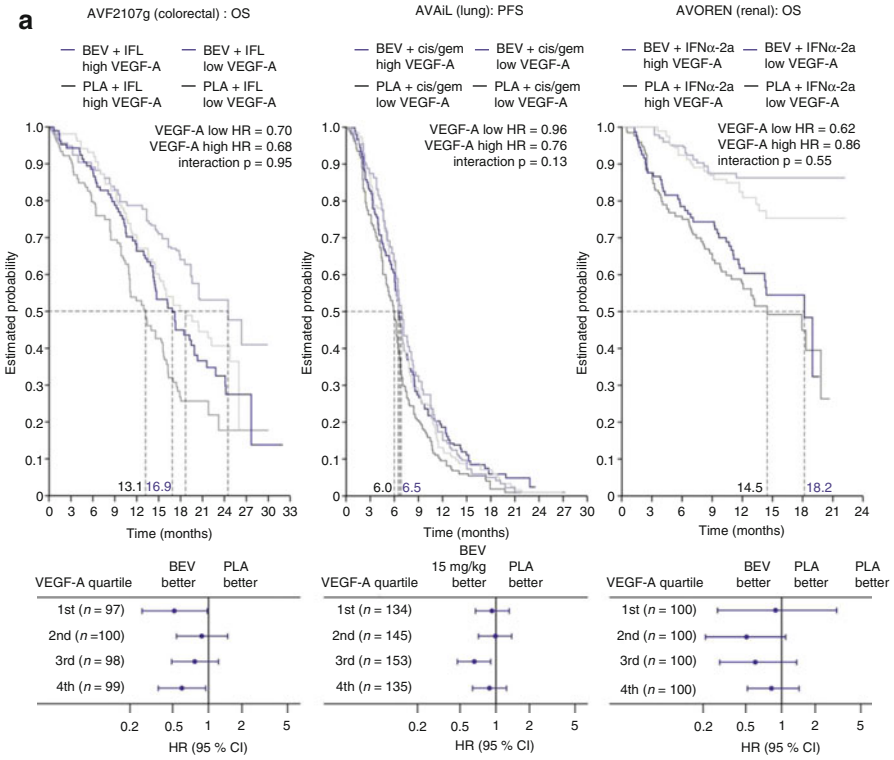


Fig. 24.3 (**a**) Hazard ratios and survival data showing prognostic value of VEGF-A levels in CRC, NSCLC, and RCC. (**b**) Hazard ratios and survival data showing prognostic and predictive value of VEGF-A levels in PaC, Ga, and mBC (*Source*: Jayson et al. 2011). *BEV* bevacizumab, *CI* confidence interval, *cis* cisplatin, *doc* docetaxel, *erlot* erlotinib, *gem* gemcitabine, *H* trastuzumab, *HR* hazard ratio, *IFL* irinotecan + 5-fluorouracil + leucovorin, *IFN* interferon, *OS* overall survival, *mBC* metastatic breast cancer, *PLA* placebo, *PFS* progression-free survival, *VEGF* vascular endothelial growth factor

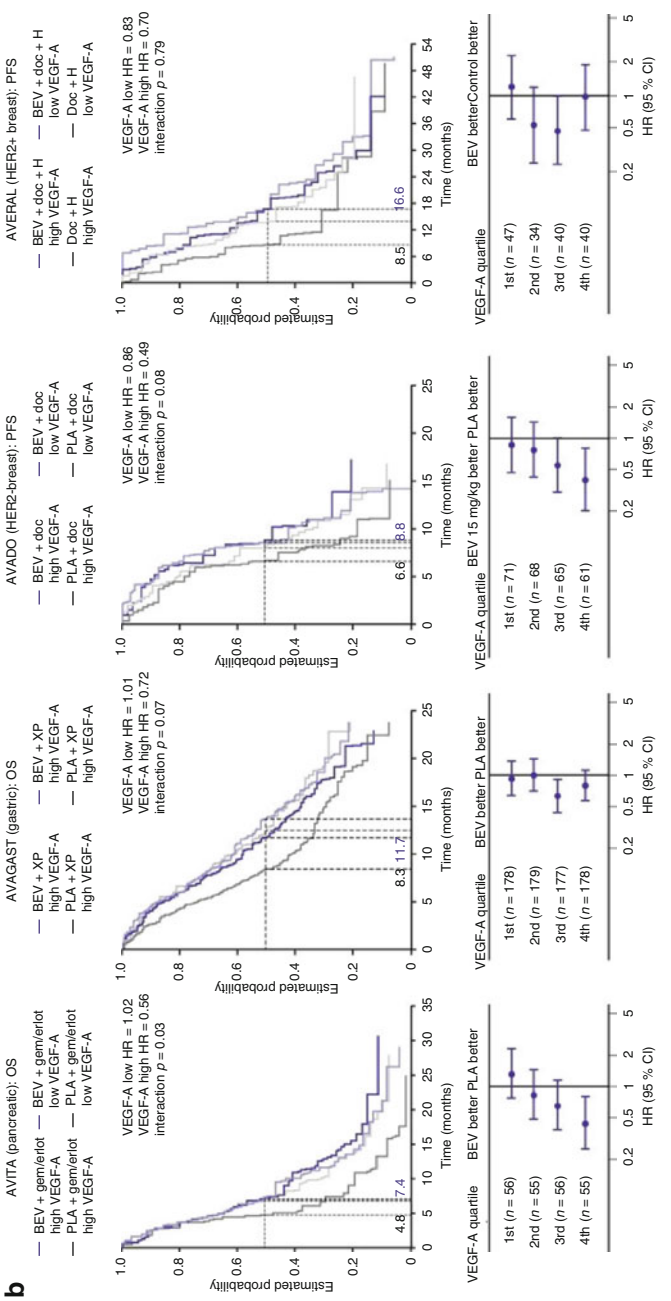


Fig. 24.3 (continued)

Table 24.2 Summary of findings for plasma VEGF-A using the impact assay

		Prognostic		Potentially predictive		
Tumour	Trial	PFS	OS	PFS	OS	Anticoagulant
Gastric	AVAGAST	✓	✓	✓	✓	EDTA
Pancreatic	AViTA	✓	✓	✓	✓	EDTA
Breast	AVADO	✓	✓	✓	✓[a]	EDTA
Breast	AVEREL	✓	NR	✓	NR	EDTA and citrate
Colorectal	AVF2107	×	✓	×	×	Citrate
Non-small-cell lung	AVAiL	✓	✓	×	×[a]	Citrate
Renal cell	AVOREN	✓	✓	×	×[a]	Citrate

Source: Jayson et al. (2011)
IMPACT immunological multi-parametric chip technique, *NR* not reported, *OS* overall survival, *PFS* progression-free survival, *VEGF* vascular endothelial growth factor
[a]Results may be confounded by crossover

Low baseline plasma VEGF levels were also associated with superior PFS in studies of vandetanib (a multi-kinase inhibitor) versus gefitinib (HR 0.55 [95 % CI 0.35–0.86], $p=0.01$) and in docetaxel plus vandetanib or placebo (HR 0.25 [95 % CI 0.09–0.68], $p=0.01$) (Hanrahan et al. 2009). High plasma VEGF-A levels were also shown to be predictive of increased response to bevacizumab plus carboplatin/paclitaxel (BCP) compared with carboplatin/paclitaxel alone (CP), $p=0.004$ in advanced non-squamous NSCLC patients in a study by Dowlati et al. (2008).

Plasma VEGF-A levels have shown potential predictive value in trials of bevacizumab in gastric, pancreatic, and breast cancer (Jayson et al. 2011). High baseline plasma VEGF-A concentrations correlated with greater PFS benefit and, in some cases, OS benefit in patients receiving bevacizumab-containing therapy compared with those treated without bevacizumab. Results from the AVEREL trial in HER2-positive metastatic breast cancer corroborated these findings despite a limited sample size (Gianni et al. 2011).

Consequently an attempt to investigate and hopefully replicate these results in NSCLC, RCC, and CRC was undertaken. Plasma samples from trials in mCRC, NSCLC, and RCC were reanalyzed using a novel ELISA-based assay which has a better sensitivity for short VEGF-A isoforms that might be diverse in different tumor types (Fig. 24.4) (Jayson et al. 2011). However, the predictive value of plasma VEGF-A levels was not reproduced in the AVF2107g (mCRC), AVOREN (metastatic RCC), and AVAiL (NSCLC) trials (Table 24.2) (Jayson et al. 2011).

Interpretation of these apparently differing results is complex. Confounding factors such as variations of pre-analytical and analytical could have contributed to the conflicting intertrial findings, although true negative results in colorectal, renal, and non-small-cell lung cancers cannot be excluded; thus, VEGF-A may be predictive for bevacizumab efficacy in some but not all tumor types. There was also a suggestion of potential predictive value for VEGFR-2, at least for PFS in breast cancer in which high VEGFR-2 concentrations were associated with a greater bevacizumab effect, indicating potential predictive value (Carmeliet et al. 2012) (beatrice, AVADO, AVEREL).

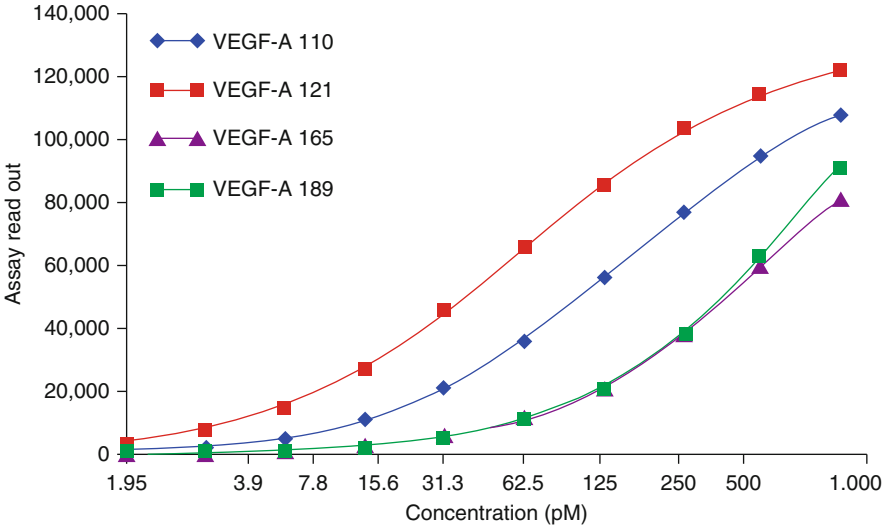


Fig. 24.4 VEGF-A ELISA assay better sensitivity for short isoforms (*Source*: Jayson et al. 2011)

Although a pan-tumor effect of VEGF-A was not confirmed in the collective data from these bevacizumab trials in six tumor types, the demonstration of predictive potential of both VEGF-A and VEGFR-2 in two trials in breast cancer, supported by a suggestion of a predictive effect of VEGFR-2 in a third trial, is noteworthy.

24.5 Issues with Interpreting Results of VEGF as a Predictive Biomarker Across Tumor Types

There is controversy regarding whether plasma, serum, or whole blood will provide the best reflection of the situation at the tumor site (Webb et al. 1998; Banks et al. 1998; Vermeulen et al. 1999). From the trials mentioned above, it has been hypothesized that differences in the way samples were handled, known to influence plasma VEGF-A levels, could affect the results. Significant amounts of VEGF can be released from platelets and leukocytes during sampling and handling. The choice of anticoagulant is of importance. Serum VEGF levels may reflect blood platelet counts rather than VEGF synthesis in peripheral tissues. Some authors advise the use of a citrate rather than an EDTA buffer in AVF2107g, AVOREN, and AVAiL could theoretically have changed the observed, measured levels of VEGF-A. Some authors advise the use of plasma (citrated, EDTA treated, or heparinized) in glass tubes for this reason. It has also been demonstrated that VEGF levels further increase with clotting duration and temperature. The biosamples were stored for prolonged periods in AVF2107g and AVOREN, and there were more than two freeze/thaw

cycles in a subset of samples in AVF2107g, AVOREN, and AVAiL that could all contribute to the inconsistent predictive values observed between studies.

Another possibility for the apparent disparities is that a median baseline biomarker cutoff value might not be a uniformly appropriate cutoff value across tumor types. The median plasma concentration of VEGF-A appears to be a reasonable cutoff in the AVADO (breast), AVAGAST (gastric), and AViTA (pancreatic) trials; for example, in the AVADO trial (7.5 mg/kg arm), a median cutoff gives the greater hazard ratio difference when comparing the "low" plasma VEGF-A cohort (i.e., no treatment effect) and the high plasma VEGF-A cohort, representing one of the most substantial treatment effects when comparing different cutoffs. The threshold for defining high versus low baseline plasma VEGF-A concentrations was lower in the AVF2107g and AVAiL trials than in the other trials.

Perhaps the most obvious explanation for the apparent discrepancies between the biomarker results of the different trials is that the potential predictive value of pretreatment plasma VEGF-A is tumor specific. Thus, VEGF-A may be predictive for bevacizumab efficacy in some but not all tumor types. The presence of shorter isoforms (VEGF-A121 and VEGF-A110), which are detected with greater sensitivity than longer isoforms by the novel ELISA, may vary between tumor types and contribute to heterogeneity of predictive value across tumor type. It has been reported by Oshika et al. that VEGF-A isoform VEGF189 was more frequently expressed in NSCLC (90.5 %) than in extraneoplastic lung tissue (57.6 %, $p = 0.00004$) (Oshika et al. 1998).

It would seem to be important for the research community to reach a consensus with regard to the preferred biospecimen, optimal collection, handling, analytical method, and storage, as well as the most appropriate approach to define cutoff, in order to facilitate data interpretation and cross-trial comparisons.

24.6 Summary and Conclusion

Cancer is a genetic disease that involves a number of biological pathways at each step of its progression. The nature of the tumor microenvironment is complex and transient, which can lead to challenges in certain areas of oncology biomarker discovery. In the era of personalized medicine, there is a growing interest in novel screening tools and a more individualized approach to the treatment of cancer depending on the tumor type. VEGF expression has been observed across multiple and various tumor types. Presently, there is evidence that high blood and tumor levels of VEGF across tumor types are negative prognostic indicators for survival. While VEGF-A has been potentially shown as a predictive marker in NSCLC and other tumor types for bevacizumab efficacy, it has yet to be validated. Thus, further large prospective studies are still needed to define the role of VEGF and other markers in different tumor types and to define their utility.

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