[haematologica reports]
2005;1(9):43-46

Commentary on thrombotic and bleeding complications of clinical trials of growth factor inhibitors

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A number of naturally occurring and pathologic growth factors have been identified in recent years to play pivotal roles in the growth and metastatic potential of various cancers. As such, they have emerged as interesting targets for the development of novel therapeutic inhibitory agents and intervention strategies in the cancer patient. Extensive preclinical and clinical trial results with growth factor inhibitors have indicated that there is close interplay between hemostasis, humoral anticoagulation, and fibrinolytic mechanisms and the interference of these integral growth factors in cancer cells.[1] These interactions may provide explanations for some of the thrombotic and hemorrhagic complications associated with their administration.

Only a few of the many potential growth factor inhibitors in pharmaceutical pipelines have entered the clinical arena and they will be the focus of this commentary. These are predominantly agents, which inhibit vascular endothelial growth factor (VEGF) and epidermal growth factor (EGF). The concept that tumor growth depends on production of angiogenic growth factors to facilitate its own proliferation was validated initially by the murine tumor explant xenograft models of Folkman *et al.*[2] and subsequently by transgenic mouse oncogene bone marrow transplantation experiments.[3,4] Perhaps, VEGF is the most exploited of the malignant tumor growth factors to date, resulting in the development of a variety of therapeutic products intended to inhibit angiogenesis. VEGF has been shown to be overexpressed in a variety of malignancies, including cancers of the breast, colon, kidney, and esophagus, and clinical trials have been initiated to determine the usefulness of VEGF inhibitors. These are intended to diminish VEGF's biologic properties as proangiogenic, anti-apoptotic (mediating endothelial cell proliferation and mitogenesis), and pro-inflammatory (inducing vascular permeability, leukocyte adhesion, etc).

The biologic effects of extracellular VEGF require interactions with transmembrane receptor tyrosine kinases for subsequent signal transduction activities. VEGFR-2 is believed to be most critical to solid tumor proliferation.[5] Inhibitors to angiogenesis in general, and to VEGFR, specifically, are not cytotoxic and thus, unlike chemotherapy agents, are not expected to cure malignancies. Rather they are somewhat cytostatic agents in that they are anti-proliferative, block tumor growth, and establish a type of tumor dormancy.[6] Inhibitors of angiogenesis are classified according to whether their inhibitory properties are achieved via a direct or indirect mechanism. The former block the proangiogenesis activities of VEGF, EGF, and basic fibroblast growth factor (bFGF) on endothelial cells via a systemic mechanism, such as p53 regulation. The latter category of inhibitors impede angiogenesis indirectly through such mechanisms as neutralization of growth factor activity, receptor blockade on the surface of endothelial cells, or prevention of the synthesis of endogenous proangiogenic proteins by the tumor cells.[1] Agents with indirect antiangiogenesis properties have been most commonly used in clinical trials, including bevacizumab, SU5416, thalidomide, and trastuzumab. Thrombotic complications associated with the use of antitangiogenic agents were initially appreciated when an unexpectedly high incidence of venous thromboembolic episodes (VTE) was noted with thalidomide administered in combination with multiple chemotherapy agents for the treatment of multiple myeloma.[7] The pathological mechanism for VTE in these myeloma patients remains obscure although there was a definite association

with concurrent administration of doxorubicin as part of the chemotherapy cocktail and a background incidence of chromosome 11 abnormalities in the myeloma tumor cells was apparent.[7,8] These episodes occurred mostly around the first cycle of chemotherapy and did not correlate with tumor load or degree of tumor aggressiveness. Single agent thalidomide for the treatment of multiple myeloma did not precipitate hypercoagulable events.[9] In a more recent open label prospective study, this same group observed a significant risk of VTE associated with thalidomide administration with doxorubicin (30%) (hazard ratio of 4.53) compared to no thalidomide (9%).[10] Most of the VTE occurred in the first 3 months of therapy and fortunately were significantly reduced by prophylaxis with low molecular weight heparin (enoxaparin 40 mg subcutaneously daily) to the same incidence of VTE observed in the no thalidomide intervention cohort. Low dose warfarin (1 mg/day) had no mitigating effect on VTE incidence in cohorts receiving thalidomide with doxorubicin chemotherapy.[10] In this same study, thalidomide combined with a non-doxorubicin containing chemotherapy regimen during the consolidation phase of treatment was associated with only a 2% incidence of VTE in the cohort with no prior VTE and 10% incidence of rethrombosis in individuals who had experienced VTE during the induction phase, again implicating the contributory role of doxorubicin to the thrombogenic potential of thalidomide use in multiple myeloma. No excessive bleeding complications were noted in any of the patients, even within the anticoagulant cohorts. The pathophysiologic mechanism whereby hypercoagulability is precipitated with the combination of doxorubicin and anti-VEGF agent remains obscure. Acquired deficiency of activated protein C resistance has been observed but no cause-effect relationship has been established.

Thalidomide has also been administered in the treatment of other solid tumor malignancies. In 22 individuals with progressive metastatic renal cell carcinoma, a cancer known to be associated with a high incidence of VTE, thalidomide (200 mg/day) was combined with prolonged use of interleukin-2 and concurrent radiotherapy. Symptomatic VTE was noted in 4 patients (18%).[11] Four other published studies have reported deep vein thrombosis and/or pulmonary embolism at rates ranging from 3 to 23% when thalidomide was used alone or in combination with chemotherapy[12] and a particularly high rate of VTE of 43% (3 pulmonary emboli, 5 DVT, and 1 cardiac arrest out of 21 patients) was observed when thalidomide was combined with gemcitabine and 5-FU.[13] Gemcitabine may provoke a procoagulant state as a single chemotherapeutic agent and may be potentiated by an antiangiogenic medication.[14]

When temozolomide was combined with thalidomide for treatment of 26 patients with metastatic melanoma to the brain, treatment was terminated early for intracranial hemorrhage in 7, symptomatic pulmonary embolism in 2, and deep vein thrombosis in 1 other patient.[15] In a prior publication, this same regimen was associated with a significant GI hemorrhage.[16] This brings up the possibility that inhibition of VEGF can also induce bleeding problems.

The question of whether thalidomide by itself is intrinsically thrombogenic was tested again when a phase II trial was conducted to explore the efficacy and tolerability of combining thalidomide (100 mg/d p.o.) with an erythropoietic growth factor (darbepoietin-alpha 2·25 μg/kg/d s.c.) in patients with low-to-intermediate-risk myelodysplastic syndromes (MDS).[17] The trial was terminated prematurely due an unexpectedly high incidence of VTE in a disease state not associated with thrombophilic complications. Of the first seven patients enrolled into this study, 2 developed DVT and 1 suffered a fatal massive PE. The authors concluded that thalidomide significantly increased the hypercoagulable risk of recombinant erythropoietin[17] since either thalidomide or recombinant erythropoietin used alone in MDS has been associated with a very low rate of VTE complications (1.5%). Lenalidomide, a 4-amino-glutarimide analogue of thalidomide, does not appear to have the same thrombogenic potential of thalidomide in the treatment of MDS although the pivotal study was conducted in individuals who were refractory to recombinant erythropoietin.[18]

Bevacizumab (Avastin, Genentech, South San Francisco, CA) is a recombinant humanized monoclonal antibody which targets VEGF. The considerable in vitro data indicating the importance of angiogenesis in the development and metastasis of various cancers, many of which overexpress VEGF, provided reasonable rationale to pursue clinical trials. A phase I study with bevacizumab doses ranging from 0.1 to 10 mg/kg revealed no thrombotic complications; however, 2 patients experienced severe bleeding episodes at the 3.0 mg/kg Avastin dose (intratumor bleeds into an intracerebral metastatic lesion from a hepatocellular carcinoma primary and into an intramuscular metastatic lesion from an epitheloid sarcoma primary) and another 2 had self-limited hemoptysis from pulmonary metastases. These were all attributed to tumor necrosis resulting from the antiangiogenic effectiveness of bevacizumab.[19] In those who continued bevacizumab for over a year, 5 developed VTE and 2 experienced GI bleeds associated with their colon cancers.

Phase II studies of dose escalations of single agent bevacizumab or in combination with vinorelbine in

metastatic breast cancer and a phase III study of bevacizumab (15 mg/kg every 3 weeks) and capecitabine revealed no thromboses and approximately 25% minor bleeding consisting mainly of epistaxis.[20] A recent ECOG study of 722 chemotherapy naïve women with advanced breast cancer randomized patients to receive paclitaxel with or without bevacizumab.[21] The authors reported a 49% improved progression-free survival and 28% response rate for the combination regimen versus paclitaxel alone (14% response rate). They also noted no increased bleeding or thrombotic complications with the combination; however, all patients with hypercoagulable histories or those on anticoagulants were excluded from the study.[21]

Promising results for angiogenic inhibition with bevacizumab have also been observed in colorectal cancer but there are somewhat confusing data as to the increased thrombogenicity and bleeding complication rate associated with the combination of bevacizumab with chemotherapy. In a phase II randomized trial comparing bevacizumab plus 5-FU/leucovorin versus 5-FU/leucovorin alone in 104 patients with metastatic colorectal cancer, 10.4% of those receiving bevacizumab developed VTE complications (1 fatality) versus 2.9% VTE in the control group.[22] In addition, there was transient epistaxis in almost 50% of the bevacizumab recipients versus 11% incidence in the control group. Three grade 3 or 4 GI hemorrhages occurred in the 10 mg/kg bevacizumab cohort.[23] Larger trials[24] and a meta-analysis[25] were unable to show any significant differences in VTE rates between regimens with (13.8%) and without (19.4%) bevacizumab; however, there still appeared to be a greater risk of mild epistaxis with the VEGF inhibitor (32.1% vs 10.2%).

Bevacizumab has also been administered in combination with other solid tumors, such as advanced non-small cell lung cancer. Data are too sparse to determine if there are increased risks of bleeding or VTE. It is apparent that any chemotherapy regimen which in itself is potentially thrombogenic, eg. cisplatin, etc., should be used with anti-VEGF agents with care. A phase II trial, however, suggests that paclitaxel/carboplatin based regimens with bevaciazumab may provoke an increased incidence of adverse thrombotic and bleeding events (4/6 hemorrhagic events fatal; 17.6% at 15 mg/kg dose vs 9.4% VTE) compared to the control group.[26]

A similar theme also applies to SU5416 (semaxanib, Sugen, South San Francisco, CA), an experimental small molecule which inhibits VEGF receptor-2 and KIT receptor tyrosine kinases. In a phase II study of 15 patients with advanced soft tissue sarcomas, SU5416 was administered as a single agent and was associated with a significant incidence of VTE (2/13 evaluable patients) despite the routine use of VTE prophylaxis with low dose coumadin or low molecular weight heparin.[27] The VTE prophylaxis was prompted by the high rate of chronic venous access thromboses noted in phase I studies with SU5416.[27] In another study designed to compare chemotherapy with cisplatin/gemcitabine with or without SI5416 in advanced NSCLC patients with a low risk of thrombophilia, an unexpectedly high incidence of thromboembolic events was observed. In 19 treated patients, 8 developed 9 thromboembolic events (three transient ischemic attacks, two cerebrovascular accidents, and four deep venous thromboses). Because this exceeds the incidence observed with this type of chemotherapy alone (0%) and SU5416 (2.2%) alone, thrombogenicity was attributed to the combination of a procoagulant chemotherapy regimen exacerbated by anti-VEGF properties of the experimental molecule.[28] No hemorrhagic complications were described in any of these studies.

The final prototype of a VEGF inhibitor is PTK787/ZK222584 (PTK/ZK), a VEGF receptor tyrosine kinase inhibitor. This experimental agent has been assessed in phase I studies of advanced colorectal and other advanced cancers. No increased incidence of thrombogenic or hemorrhagic adverse events has been reported.[29-31]

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