# Therapeutic implications of intrinsic or induced angiogenic growth factor redundancy in tumors revealed

**There is a large family of known proangiogenic growth factors, many of which can be expressed by a single tumor, especially in advanced stages of disease. Such redundancy, which can be amplified by hypoxia, has long been suspected as a potential cause of acquired resistance when tumors are treated with highly specific targeted antiangiogenic drugs. Definitive preclinical evidence for antiangiogenic drug evasion by alternate pathways of angiogenesis in tumor cells, likely induced by antiangiogenic drug-mediated increases in tumor hypoxia, is reported in this issue of *Cancer Cell* (Casanovas et al., 2005); it has major implications for the development of strategies to prolong the effectiveness of antiangiogenic drugs as monotherapies, and for their use as chemosensitizing agents in combination treatment strategies.**

The approval and subsequent widespread clinical application of a diverse array of new molecularly targeted anticancer drugs over the last several years has served to intensify the spotlight on one of the most significant problems in clinical oncology: acquired drug resistance. This is especially true for tyrosine kinase inhibitors such as imatinib (Gleevec), which can induce striking tumor responses in patients with chronic myelogenous leukemia harboring the *bcr-abl* translocation or in patients with gastrointestinal sarcoma containing *c-kit* mutations (Sawyers, 2005). Eventually, however, this is followed by relapses where the tumors are no longer responsive to the drug. Such acquired resistance is frequently the consequence of the genetic instability of tumor cells and hence their mutational prowess (Sawyers, 2005).

It is the cancer cell hallmark of genetic instability that led to an often-cited theoretical advantage for developing and using antiangiogenic drugs: lack of drug resistance (Kerbel, 1991). Because the drug target is a genetically stable normal host cell, namely, the "activated" vascular endothelium of a tumor's growing neovasculature, the initial assumption was that such cells might not develop acquired drug resistance in the way tumor cells can. While a few early preclinical studies seemed to support this hypothesis, others suggested (Klement

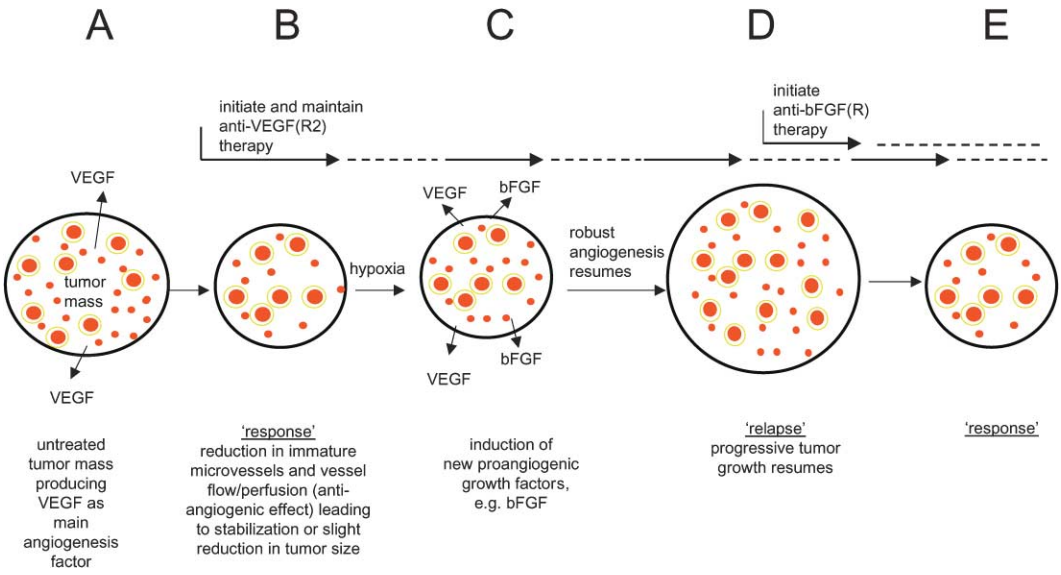


**Figure 1.** A model for acquired resistance to a targeted antiangiogenic therapy

Schematic representation of some of the ways resistance can develop, in principle, to a targeted antiangiogenic drug in tumors which initially respond to the drug, e.g., anti-VEGF or anti-VEGFR-2 antibody, as exemplified by the results of Casanovas et al. (2005). Angiogenesis in untreated tumors (**A**) is driven mainly, for example, by VEGF. Upon repetitive treatment with an agent such as an anti-VEGFR-2 antibody (**B**), some regression of newly formed immature tumor neovasculature (small red circles) occurs, and further angiogenesis is halted along with reduced perfusion/flow in some remaining vessels, many of which are more mature, pericyte-covered vessels (larger red circles with a yellow border to symbolize pericyte coverage), leading to a tumor "response," e.g., a small reduction in tumor mass or no new growth ("stable disease"). The aforementioned effects on the tumor vasculature lead to an overall increase in the levels of tumor hypoxia, which in turn leads to induction of expression of new hypoxia-regulated proangiogenic growth factors, such as bFGF (**C**). The induction of bFGF induces angiogenesis in the face of ongoing anti-VEGFR-2 therapy, leading to tumor "relapse," i.e., resumption of angiogenesis and robust expansion of tumor mass (**D**). Initiation of bFGF(R)-directed antiangiogenic therapy at this point could lead to angiogenesis inhibition once again and a tumor response (**E**). Eventually, such a therapy could also fail over time due to such factors as: (1) induction of another functional proangiogenic growth factor, (2) increased proportion of remodeled and mature (pericyte-covered) vessels, which are less responsive to antiangiogenic drugs in general, or (3) selection of hypoxia-resistant tumor cell variants, which have a reduced dependence on tumor angiogenesis.

et al., 2000) or showed (Yu et al., 2002) otherwise, findings now ostensibly supported by clinical experience with the anti-VEGF monoclonal antibody bevacizumab, the first approved antiangiogenic drug for the treatment of cancer (Hurwitz et al., 2004). This has spawned a number of theories about the diverse routes tumors can take to eventually evade the effects of an antiangiogenic drug treatment. These include mutation-driven selection for hypoxia-resistant tumor subpopulations that have a reduced dependence on blood vessels and oxygen for survival (Yu et al., 2002), selection for more mature "remodeled" stabilized blood vessels that are intrinsically less responsive to antiangiogenic therapies (Glade Bender et al., 2004), or redundancy of proangiogenic growth factors. This last cited mechanism is a consequence of the well-known plethora of proangiogenic growth factors expressed by tumor cells (Kerbel and Folkman, 2002). A partial list of some of the better-known ones include VEGF, bFGF (FGF-2), angiopoietin-1, and probably angiopoietin-2, IL-6, IL-8, placental growth factor (PLGF), and PDGF, among others. Consequently, the concern was expressed many years ago that targeting a single tumor cell-associated growth factor, or pathway, would likely lead to the selection and outgrowth of subpopulations producing alternative angiogenic stimulators or pathways (See, for example, Relf et al., 1997). This would be especially true in late stage, advanced cancers where the degree of such redundancy appears to be substantially greater than early stage disease (Relf et al., 1997). Given the nature of cancer, the logic of this type of drug resistance mechanism is compelling and probably accounts for it being the most cited of all theoretical mechanisms for acquired resistance to antiangiogenic drugs despite the fact that there was never any direct definitive evidence for it – until now.

In this issue of *Cancer Cell*, Casanovas et al. (2005) report a series of elegant experiments to show that specifically targeting the function of tumor-associated VEGF leads to significant tumor responses eventually followed by tumor relapses in which angiogenesis is apparently no longer driven by VEGF, but by an alternate pathway (Figure 1). Mice with advanced primary islet cell pancreatic carcinomas were repeatedly treated with a monoclonal antibody to the major endothelial cell signaling receptor tyrosine kinase receptor for VEGF, called VEGFR-2 or flk-1. VEGF is considered to be the major proangiogenic factor for most forms of pathologic angiogenesis, including tumor angiogenesis (Ferrara et al., 2003). Indeed, it was previously reported to be indispensable for growth of the aforementioned tumor model based on gene knockout studies (Inoue et al., 2002). Thus, as expected, the tumors responded and significant growth delays ("stabilization"), or even regressions, were observed. Eventually, however, the tumors relapsed and started to grow robustly, despite continued therapy during which the ability of the drug to block phosphorylation of VEGFR-2 was not impaired, along with the maintenance of VEGF expression in the tumors. This was taken as evidence that another proangiogenic pathway must be taking over, thus making the VEGF/VEGFR-2 pathway dispensable. Indeed, a profiling analysis revealed that several new proangiogenic growth factors had popped up in the relapsing tumors, including bFGF and angiopoietin-1, and even VEGF itself was elevated—all likely consequences of increased levels of tumor hypoxia induced by the initially successful anti-VEGFR-2 treatment. Evidence that bFGF was the main culprit driving angiogenesis in the relapsing tumors was shown by the fact that they responded well to a therapy specifically designed to target only bFGF receptors. These results nicely complement a very recent report by Mizukami et al. (2005) who found that hypoxia-induced IL-8 tumor cell can compensate for partial loss of VEGF and complete loss of HIF-1, normally considered a major driver of both VEGF and angiogenesis. Taken together, these two studies suggest that there are likely many alternative angiogenic pathways that emerge after one specific pathway is initially targeted in tumors. The (first) one that arises will likely depend on many factors, such as tumor type, stage of disease, and the nature of the therapy.

Aside from highlighting, in principle, a mechanism for eventual evasion (resistance) to a therapy directed at the function of a specific proangiogenic factor—in this case VEGF—and an approach to discover alternative targets for antiangiogenic therapy, the results would appear to bolster the rationale for using drugs which selectively target hypoxic tumor cells (Brown and Wilson, 2004) with antiangiogenic drugs, or alternatively, using multi-targeted antiangiogenic receptor tyrosine kinase inhibitors (RTKIs). With respect to the latter, multi-targeted RTKIs, to varying degrees, block phosphorylation of several different tumor or endothelial cell associated RTKs involved in promoting angiogenesis (Kerbel and Folkman, 2002). This strategy, however, highlights several ironies. First, the subject of the study by Casanovas et al. concerns the development of resistance to a targeted antiangiogenic drug administered as a *monotherapy*. But the major use and successes of the antiangiogenic drug approach in the clinic (up to this point at least) have been when they are used *in combination* with standard chemotherapy regimens. This was first rigorously established using a bevacizumab plus standard chemotherapy combination in a large randomized phase III clinical trials of patient with advanced metastatic colorectal cancer (Hurwitz et al., 2004), but has been duplicated more recently in patients with advanced stage non-small cell lung cancer, advanced metastatic breast cancer, and refractory (second line) colorectal cancer, as reported at this year's American Society for Clinical Oncology (ASCO) annual meeting (Marx, 2005). Strong signals indicating the likelihood of similar combination treatment successes to come in other types of cancer, e.g., pancreatic cancer, are evident from a variety of phase II trials testing bevacizumab and chemotherapy. So, it would appear that at least one antiangiogenic drug, bevacizumab, may turn out to be the most broadly generic and effective chemosensitizing agent available to delay acquired resistance to chemotherapy. This is doubly ironic given that antiangiogenics are usually considered to be "tumor starving" drugs that compromise tumor blood flow and perfusion—effects which would intuitively be expected to diminish, rather than enhance, the efficacy of chemotherapy—all of which leads to the next irony, namely, that so far, small molecule antiangiogenic multi-targeted RTKIs such as PTK787 have failed to demonstrate the chemosensitizing ability of antibodies, such as bevacizumab, in clinical trials (Marx, 2005). Instead, the main clinical successes of RTKIs developed to inhibit angiogenesis have been as monotherapies for a single cancer type, renal cell carcinoma (Marx, 2005), where the striking response rate results report-

ed may be due to a variety of mechanisms, not just inhibition of angiogenesis.

Given the fact that antiangiogenic drugs are mainly used in combination with chemotherapy, an obvious question is whether relapses observed in patients who are treated with a targeted drug such as bevacizumab with chemotherapy occur mainly as a consequence of development of resistance to the targeted antiangiogenic drug in a manner similar to that described by Casanovas et al., or alternatively, to the chemotherapy component of the regimen. If it is the former, the need for discontinuation of the antiangiogenic drug therapy is obvious, along with its possible replacement by another drug that has a good chance of showing resurrecting efficacy. If it is the latter, continued use of the original antiangiogenic drug and a change in chemotherapy would be called for. Considering the therapeutic consequences of making the right (or wrong) decision, as well as the enormous economic implications, given the huge cost of many new anti-cancer drugs, distinguishing between the two will obviously assume an obvious degree of future importance. Viewed from this perspective, the results of Casanovas et al., and also of Mizukami et al. (2005) firmly establish the multiplicity of compensatory angiogenic pathways available to cancer cells as a likely cause of resistance to specific targeted antiangiogenic drugs, and suggest potential strategies to delay such resistance, thus facilitating not only their intrinsic antiangiogenic properties, but their chemosensitizing effects as well.

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**Selected reading**

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# A mouse model for studying therapy-induced cancers

**As more pediatric cancer patients survive for longer periods following treatment with cytotoxic agents, therapy-induced second malignant neoplasms (SMNs) have become a major concern. In this issue of *Cancer Cell*, Chao et al. report that mice carrying a mutation in *Nf1*, the gene responsible for neurofibromatosis type 1, treated with radiation and/or cyclophosphamide, developed tumors similar to human SMNs at a significantly higher rate than did wild-type controls treated similarly. This model provides efficient and rational means for testing procedures and agents that could inform clinicians regarding second cancer risks associated with treatment and, perhaps, reducing them.**

This issue of *Cancer Cell* carries a report of experiments with a mouse model of the most common hereditary condition facing the pediatric oncologist, neurofibromatosis type 1 (NF1) (Chao et al., 2005). Shannon and colleagues used adolescent and young adult mice that carry a mutation in *Nf1*, the gene responsible for NF1, to study cancer resulting from the effects of cytotoxic therapy in genetically susceptible individuals. Mice heterozygous for that mutation developed second malignant neoplasms (SMNs) with or without the administration of radiation (RAD) and/or chemotherapy with an alkylating agent (cyclophosphamide [CY]) at a significantly higher rate than wild-type controls who were similarly treated (51/81 compared to 17/100).

It was inevitable that I would be excited about this report, since the interaction of therapy and genetic predisposition in the etiology of SMNs has preoccupied me during the past 30 years of my work with survivors of childhood cancer. During the decade of the 1970s, as it became evident that children with cancer would be able to survive for many years after receiving treatment, concerns began to be expressed regarding the long-term effects of the RAD and chemotherapy responsible for cure. With colleagues at the National Cancer Institute and three major pediatric oncology centers, I embarked on a study of late effects, especially SMNs, in survivors of childhood cancers.

Our report published in 1985 on the spectrum of 308 SMNs in 292 childhood cancer survivors seen at 13 institutions in the United States, Canada, and Europe noted that 68% had developed in a tissue that had been exposed to RAD, that bone and soft tissue sarcomas were the most