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How can we manage resistance to antiangiogenic drugs?

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The scope of the problem: defining resistance to angiogenesis inhibitors

Angiogenesis is well recognized as an important process in tumor growth and progression. It can be an extremely complex process, although the preponderance of drivers and mediators permits opportunities in drug development to inhibit this process. Key signaling molecules in angiogenesis include the VEGF, FGF2, angiopoietins 1 and 2, HGF and ephrin [1]. The prototypical example of an angiogenesis inhibitor in routine clinical use is bevacizumab, a first-generation recombinant humanized monoclonal antibody against VEGF-A [1]. In disrupting ligand–receptor interactions the signaling cascade within the cell that eventually promotes angiogenesis is inhibited predominantly at an upstream signaling event. A second major class of antiangiogenesis agents are tyrosine kinase inhibitors. Tyrosine kinase inhibitors, in contrast with monoclonal antibodies, are small molecules that can cross lipid bilayers and interact directly with the intracellular domains of cell surface receptors and/or intracellular signaling proteins. Unfortunately, angiogenesis inhibitors are subject to the same limitation of other antineoplastic drugs of the eventual observed resistance that results after prolonged exposure to these agents.

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To develop strategies to overcome resistance to angiogenesis inhibitors, one must first be able to properly recognize the complexity of defining drug resistance in the clinic. In a retrospective analysis of the observational BRiTE registry, patients with metastatic colorectal cancer whose treating physicians elected to continue bevacizumab with a differing cytotoxic chemotherapy regimen after progression on first-line chemotherapy and bevacizumab demonstrated a dramatically improved median survival of 31.6 versus 19.9 months [2]. This hypothesis of continuing bevacizumab beyond progression was validated in the prospective Phase III TML (ML18147) clinical trial, although this demonstrated a more modest 1.4-month benefit in median overall survival [3]. One can speculate that development of resistance mechanisms to cytotoxic chemotherapy drugs and VEGF pathway inhibitors are independent. However, the TML trial provides a valid argument that resistance to VEGF pathway inhibition in the first-line setting may only be partial given that bevacizumab can still provide additional clinical benefit when simply partnered with differing chemotherapy drugs. The Phase III VELOUR trial of second- line FOLFIRI with aflibercept (VEGF Trap) in metastatic colorectal cancer patients who progressed on first-line oxaliplatin-based chemotherapy also supports this strategy [4]. Aflibercept differs from bevacizumab in being a fusion protein VEGF receptor decoy and in addition to VEGF-A also recognizes and inhibits VEGF-B and PlGF, thereby theoretically inhibiting more stimulators of angiogenesis. Uncannily, median overall survival improved by 1.4 months with aflibercept versus placebo, similar in magnitude to the TML trial, although the study populations differed in that only a minority (30%) of patients in VELOUR had exposure to first-line bevacizumab as opposed to all patients enrolled onto the TML study. Whether continued inhibition of the VEGF-A signaling axis alone or additional inhibition of VEGF-B and PlGF is superior remains unknown as there are no trials to date to compare the two strategies head to head. Regardless, the preponderance of clinical data illustrates the complexity of defining what comprises ‘resistance’ to angiogenesis inhibitors. Tissue- and/or blood-based biomarkers still need to be developed that can be routinely utilized in the clinic that may hopefully address this conundrum.

Novel preclinical models describing mechanisms of resistance to angiogenesis inhibitors

Much investigation has been carried out by various laboratories to better define preclinically the resistance pathways that arise in tumor cells that allow them to overcome angiogenesis targeting strategies. One such example are integrins, which are major mediators in the interactions between cancer cells and the tumor microenvironment [5]. Specifically, β1 integrin signaling in tumor cells has been shown to promote resistance to radiotherapy and chemotherapy, and acts through downstream signaling pathways, such as FAK, ERK/MAP kinase, Src, Akt and Ras. Microarray gene expression analyses and immunohistochemistry have demonstrated increased β1 integrin levels in bevacizumab- resistant glioblastoma patient samples in comparison to their matched prebevacizumab-treated specimens [6]. The same investigators were able to duplicate findings *in vitro* of increased β1 integrin expression in the U87MG cell line-derived xenograft model of bevacizumab resistance. This mechanism of β1 integrin upregulation may not be unique to glioblastoma, as two non-CNS human cancer cell lines, HT-1080 (fibrosarcoma) and MDA-MB-231 (breast

adenocarcinoma), also displayed significantly increased β1 integrin expression when cells were exposed to hypoxic conditions. Preclinical development has been promising for OS2966, a humanized monoclonal antibody against β1 integrin, with evidence of tumor shrinkage in bevacizumab-resistant glioblastoma-derived xenograft models [5].

Further recent preclinical evidence of the tumor microenvironment mediating resistance to angiogenesis inhibitors was discovered by Chung *et al.* [7]. In an elaborate series of experiments, they found the proinflammatory cytokine, IL-17, secreted by tumor-infiltrating T helper type 17 (T_H17) cells, initiated a paracrine signaling network that resulted in resistance to anti-VEGF therapy. Analysis of 23 advanced stage human colorectal adenocarcinoma specimens demonstrated positive correlation of IL-17 immunopositive tumor-infiltrating T_H17 lymphocytes with the number of tumor-associated Bv8-expressing neutrophils, in which the latter has been previously demonstrated to be associated with poor outcome in colorectal, hepatocellular and lung carcinomas [8]. Chung *et al.* also demonstrated dual treatment with inhibitory antibodies against IL-17 and VEGF led to a greater decrease in tumor burden in lung and colorectal tumor-bearing mice than treatment with inhibitory antibody against VEGF alone. Anti-IL-17 antibodies, such as ixekizumab and secukinumab, are already in Phase III trials for psoriasis [9], although likely the safety of these agents in combination with angiogenesis inhibitors will need to be established prior to exploiting this potential in human cancers.

Strategies to overcome resistance

With the recognition of novel molecular mechanisms of resistance, it becomes apparent that the identification of biomarkers that may predict for sensitivity or resistance to angiogenesis inhibitors would be vital for developing effective strategies to overcome resistance. To date, in spite of multiple analyses of clinical trials utilizing antiangiogenic agents, there are no validated biomarkers in routine clinical use. However, a mandatory biomarker analysis of the Phase III AVAGAST trial that investigated the addition of bevacizumab to chemotherapy in advanced gastric cancer revealed that high baseline circulating VEGF-A levels and low tumor NRP1 expression correlated with bevacizumab benefit in non-Asian patients [10]. NRP1 has thus far been found to be a coreceptor with VEGFR1 and VEGFR2, although the exact molecular mechanisms in which it mediates VEGF signaling and angiogenesis still remain under investigation [11]. Bevacizumab, however, has not been approved for gastric cancer given that the primary end point of improving median overall survival was not met in the AVAGAST trial. Interestingly, an alternative antiangiogenic monoclonal antibody, ramucirumab, which targets VEGFR2, did improve survival both as a single agent and when added to chemotherapy as demonstrated in the Phase III REGARD and RAINBOW trials, respectively [12,13]. The biomarker analyses from these studies are ongoing, although hopefully they may help elucidate where angiogenesis inhibitors may succeed or fail in the clinic. One may postulate that for patients with high tumor NRP1 expression, dual targeting of NRP1 and VEGF may provide beneficial synergy. Unfortunately, a Phase Ib study of a novel NRP1 monoclonal antibody inhibitor, MNRP1685A, in combination with bevacizumab and paclitaxel led to a higher than expected rate of clinically significant proteinuria [14].

The development of novel nanopharmaceutical delivery vehicles to improve the therapeutic index of cytotoxic chemotherapy drugs has also led the way to potential strategies that may overcome resistance to angiogenesis inhibitors. CRLX101 is a 20–30-nm diameter dynamically tumor-targeted nanopharmaceutical containing the payload camptothecin, which accumulates in tumors after intravenous injection and slowly releases camptothecin in cancer mouse models [15]. Analyses of protein expression levels in animal tumor xenografts reveal that CRLX101 decreases expression of the proangiogenic transcription factor HIF-1α and its downstream gene targets encoding for CAIX and VEGF [15,16]. In addition, in the A2780 ovarian tumor xenograft model, treatment with VEGF pathway targeting agents, including bevacizumab, aflibercept, and pazopanib, leads to increased HIF-1α protein levels that are subsequently inhibited when these agents are given in combination with CRLX101 [17]. The safety profile of CRLX101 observed to date appears favorable, and the combination of CRLX101 and bevacizumab is currently being tested in Phase II trials in renal cell carcinoma (NCT01625936) and recurrent platinum-resistant ovarian cancer (NCT01652079).

Conclusions & future directions

It is becoming increasingly apparent that many different pathways of resistance to angiogenesis inhibitors exist. However, a better understanding of these mechanisms such as elucidation of the cytokine milieu in the tumor microenvironment permits the development of strategies to circumvent these barriers to effective antiangiogenic therapy. The thorough identification of candidate biomarkers will also allow for better selection of patients to maximize currently available drugs. The future generation of clinical trials should intelligently and prospectively incorporate these predictive biomarkers in the investigation of novel antiangiogenic agents.

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