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VEGF targeting increases the cystostatic effect of docetaxel on prostate and breast tumor cells; a new interpretation of the therapeutic effect

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Background: Bevacizumab is a VEGF targeted humanized monoclonal antibody preventing tumor neoangiogenesis. Several studies demonstrated the benefit of bevacizumab combined with taxane, a class of microtubule stabilizing agents. As VEGF signaling pathway has been described as a survival pathway for tumor cells, we hypothesized that bevacizumab could also directly targets tumor cells.
Materials and Methods: We studied the effect of docetaxel (a taxane class drug) and bevacizumab on two tumor cell lines with metastatic potential: MDA 231 (breast cancer) and PC3 (prostate cancer). Cell growth was evaluated using automatic cell counter. VEGF and VEGF receptor (VEGF-R) promoter activity was determined using luciferase reporter assays; mRNA levels were evaluated using quantitative real time PCR. Intracellular and extracellular concentrations of growth factors were measured by ELISA assay; intracellular and membrane expression of VEGF-R was determined by western blot.
Results: Bevacizumab alone has no significant effect on cell growth, but increases the cytostatic activity of docetaxel. To explore this finding, we studied the action of docetaxel on VEGF signaling pathway. Docetaxel induces a dose dependent activation of the VEGF and VEGF-R promoters, and increases both VEGF and VEGF-R mRNA levels. However, extracellular amounts of VEGF and membrane VEGF-R expression are reduced by docetaxel whereas cytoplasmic amounts of both VEGF and VEGF-R increased. The inhibitory effect of docetaxel on the expression of VEGF-R at the plasma membrane is further increased by bevacizumab VEGF trap. Extracellular quantity of others growth factors, such as EGF and PDGF are also diminished by docetaxel treatment.
Conclusion: The combination of bevacizumab plus docetaxel strongly locks the VEGF pathway. VEGF was described to maintain the expression of its receptors at the plasma membrane. Thus, any down regulation of external VEGF amounts would also alter normal VEGF-R expression. Here we demonstrated that docetaxel, by its highly documented action on microtubule, prevents normal secretion of a lot of growth/pro survival factors including VEGF, EGF and PDGF. Hence, trapping VEGF by bevacizumab suppress a growth/pro survival pathway increasing the cytostatic effect of docetaxel. These results described for the first time that inhibitors of VEGF target the endothelial cells participating in tumor neoangiogenesis but also tumor cells themselves. This mechanism could explain why the combination of bevacizumab and taxane is strongly efficient on patients and increases clinical outcome.

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VEGF receptor expression in human tumours: VEGFR-2 and -3 are confined predominantly to tumour vasculature

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Background: Vascular endothelial growth factor (VEGF) signaling is key to the angiogenic process required to support solid tumour growth and as such is an important target in the development of anti-cancer drugs. Approaches that inhibit VEGF signaling are currently under evaluation in the clinic e.g. cediranib (Recentin; AZD2171), vandetanib (Zactima; ZD6474), and bevacizumab (Avastin). There is no clear consensus from the literature on the location (vasculature and/or cancer cells) or extent of expression of VEGF receptors in human malignancies. Our aim was to develop a better understanding of the VEGF receptor (VEGFR) status of human cancers to potentially identify patients who may benefit most from treatment with VEGF signaling inhibitors.
Materials and Methods: A range of commercial antibodies, raised to human VEGFRs were assessed for specificity and sensitivity using several platforms. Only antibodies, 55B11 (VEGFR-2, CST) and AF349 (VEGFR-3, R+D systems) were validated as suitable for immunohistochemical analysis (IHC) of archival formalin-fixed paraffin-embedded human tissue. No VEGFR-1 antibody tested passed validation. Archival human tumour tissue was assessed for tissue quality and extent of antigen preservation to select samples suitable for VEGFR expression analyses.
Results: Both VEGFR-2 and VEGFR-3 were expressed on vascular endothelial cells in all human colorectal (27/27), lung (24/24) and breast (21/21) cancer samples tested. VEGFR-2 and VEGFR-3 signal intensity was greater in vessels associated with malignant lung and breast tissues compared with adjacent non-tumour tissue. In addition, VEGFR-3, but not VEGFR-2, signal intensity was greater in colorectal cancer tumour vessels. Expression of both receptors was absent from tumour cells in the majority of samples evaluated. However, VEGFR-2 or VEGFR-3 were expressed in 7/24 lung cancer samples; VEGFR-2 in 2/12 squamous cell carcinomas (SCC) and 2/12 adenocarcinomas, and VEGFR-3 in 4/12 SCC. Determination of the mechanisms responsible for up-regulation in lung cancer cells are now underway. In a supporting study, VEGFR-2 expression was confined to murine vasculature associated with 32 different human xenograft tumours (generally, 10−50% of vessels) and absent from tumour cells. Indeed by western blot analysis, VEGFR-2 protein was detected in xenografts lysates but was absent from the parental human cell lines.
Conclusions: By using qualified VEGFR-specific antibodies for IHC analysis of high quality human samples, we show that the frequency of VEGFR-2 and VEGFR-3 expression in human cancer cells is lower than previously reported and that expression of these receptors is largely confined to tumour vasculature.
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UNBS5162: A new naphthalimide derivative with radiosensitizing and anti-angiogenic activity entering phase I

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UNBS5162, a new naphthalimide designed to avoid the specific metabolism of amonafide that induces hematotoxicity has been evaluated for anti-cancer activity in vitro as well as in various in vivo models in tumor-bearing mice.
Biochemical and molecular biology investigations have also been conducted to determine UNBS5162's mechanism of action. In addition, UNBS5162 has been evaluated in broad ligand binding and enzyme assays and in toxicology and safety pharmacology studies.
In a NCI screen to assess anti-proliferative activity against 60 different human tumor cell lines, UNBS5162 revealed an activity profile markedly dissimilar to that of other compounds present in the database. Although, UNBS5162 is not pro-apoptotic and cannot be classified as a cytotoxic, it appears that rapidly dividing cells are especially sensitive to the toxic effects of the compound. Our in vitro data indicate marked down-regulation of pro-angiogenic chemokines (CXCL-1 and CXCL-8) and consequently an anti-angiogenic mechanism of action for UNBS5162 in human prostate and esophageal cancer and in glioma models. UNBS5162 treatment significantly reduced blood vessel surface area in vivo in human prostate cancer xenografts. In vivo studies in orthotopic models of prostate and lung cancer in nude mice and in a s.c. model of mammary cancer in syngeneic mice, demonstrated that UNBS5162 prolongs animal survival when administered i.v. either alone (at 10 mg/kg) or in combination with Taxol or radiation. The cardiac, central nervous and respiratory system components of the core battery of safety pharmacology studies conducted with UNBS5162 indicated no compound-related undesirable effects on physiological function. Following single or multiple i.v. injection, UNBS5162 was eliminated from plasma with a half-life of 1.6–3.9 h in all species investigated (mouse, rat and dog). UNBS5162 was highly cleared by the liver and highly distributed into body fluids. The no-observable-adverse-effect level (NOAEL) post i.v. was 30 mg/kg in rats and 15 mg/kg in dogs. The bone marrow, thymus and secondary lymphoid tissues, and the gastrointestinal tract have been identified as targets for UNBS5162.
The available non-clinical data indicate that UNBS5162 has considerable promise as an anti-cancer agent and is sufficiently well tolerated to be advanced into Phase I trials in cancer patients.

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sFLT01, an anti-angiogenic protein with antitumor activity

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Background: Vascular endothelial growth factor (VEGF) is essential for promoting the growth of blood vessels. Anti-angiogenic agents have shown clinical value in combination with chemotherapy by targeting VEGF or its receptors. In the current study we have used a novel engineered soluble hybrid form of VEGFR1, sFLT01, with VEGF binding affinities comparable