(i.e., VEGF, CM-GSF, bFGF, TGFbeta) was abrogated in the presence of trans-resveratrol and FAS21. Neither migration nor proliferation was altered by trans-resveratrol and FAS21 in basal medium-cultured hepatic cells. Consistent with in vitro inhibitory effects on liver sinusoidal cell-induced tumor cell growth, Ki67 staining of B16M cells decreased in metastasized livers from mice treated with trans-resveratrol and FAS21 as compared to controls. However, neither apoptosis nor toxicity was observed in tumor and host cells receiving above treatments under basal culture conditions.
Conclusions: These results demonstrate the potent antimetastatic effect of trans-resveratrol during hepatic melanoma metastasis formation, and suggest the potential therapeutic interest of the studied synthetic compound to target proangiogenic action of tumor-activated hepatic sinusoidal myofibroblasts.

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Inhibition of cytosolic superoxide dismutase (SOD1) in endothelial cells: a possible mechanism for the antiangiogenic properties of the copper depleting drug ATN-224

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Background: Copper has been demonstrated to be elevated in tumor tissue and plasma from patients with various malignancies. *In vitro* and *in vivo* animal studies have implicated copper in the process of angiogenesis and tumor progression, and the depletion of systemic copper using a copper binding agent such as tetrathiomolybdate has been demonstrated to inhibit tumor growth in animal models. However, the role of copper in mediating angiogenesis is poorly understood at the molecular level. Using a second generation tetrathiomolybdate analog (ATN-224, currently in Phase I clinical trials), we demonstrate the ability of this analog to inhibit SOD1 activity in endothelial cells by depleting copper from this enzyme. Further, we demonstrate that the inhibition of SOD1 activity by ATN-224 is sufficient to completely inhibit endothelial cell proliferation *in vitro* and angiogenesis *in vivo*.
Materials and Methods: Using a combination of *in vitro* cell proliferation assays and enzymatic assays we demonstrate that one of the anti-angiogenic targets for ATN-224 is intracellular CuZn-superoxide dismutase (SOD1). *In vivo* experiments extend these findings and show that SOD1 is a target for ATN-224 in animal models.
Results: Inhibition of angiogenesis by ATN-224 in the Matrigel plug takes place without changing the levels of copper in plasma or in tissue suggesting that depletion of systemic copper may not be required for the anti-angiogenic activity of the drug. ATN-224 is able to accumulate in proliferating human umbilical vein endothelial cells (HUVECs) and inhibits HUVEC SOD1 activity by removing the bound copper with an IC_{50} similar to that observed for the inhibition of HUVEC proliferation. The inhibition of HUVEC proliferation by ATN-224 *in vitro* can be substantially reversed using a synthetic porphyrin SOD mimetic (MnTBAP) that is not copper dependent. Similar results are observed *in vivo*, where the inhibition of angiogenesis by ATN-224 in a Matrigel plug model of angiogenesis is also reversed by MnTBAP. SOD1 inhibition by ATN-224 results in a concomitant increase in intracellular reactive oxygen species (ROS) in HUVECs. In pharmacodynamic studies, one dose of orally administered ATN-224 provides ~60% inhibition of SOD1 activity in red blood cells within 1 hour which is sustained for at least 6 hours. Inhibition of SOD1 activity in red blood cells was maintained at 30% even after 24 hours after a single dose of ATN-224.
Conclusions: These data suggest a molecular target for copper depletion therapy, demonstrate that SOD1 inhibition is achievable *in vivo* in cellular compartments after oral administration of drug and suggest that SOD1 may be a promising target for the inhibition of angiogenesis.

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The VEGFR-2 tyrosine kinase inhibitor, ZD6474, enhances the antitumor effect of radiation

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Background: Angiogenesis is essential for the growth and metastatic spread of solid tumors. Vascular endothelial growth factor and its receptors are crucial to this process and therefore represent key targets for antiangiogenic cancer therapy. ZD6474 is an orally available inhibitor of vascular endothelial growth factor receptor-2 (VEGFR-2) tyrosine kinase activity with additional activity against epidermal growth factor receptor tyrosine kinase. The antitumor effects of this novel agent have been evaluated in combination with fractionated radiation therapy (RT) in a human colorectal cancer xenograft model (HT29).
Methods: The effects of increasing doses of ZD6474, either alone or in combination with radiation, on endothelial cell (HMVEC-L) and HT29 cell proliferation and survival were initially evaluated *in vitro*. *In vivo* studies were performed in mice bearing HT29 tumors grown intramuscularly in one hind limb. When tumors reached approximately 200 mm^3 animals were randomly assigned to receive 2 weeks' treatment with RT (2 Gy per fraction, Monday–Friday), ZD6474 alone (25 mg/kg/day, p.o. Monday–Friday), a combination of RT plus ZD6474 according to three schedules, or no treatment. In the combination groups, 2 weeks' treatment with ZD6474 preceded, followed, or was given concurrently with 2 weeks' RT. In the concurrent group ZD6474 was given 1 hour after each radiation dose. Tumor response was assessed using a growth delay assay.
Results: *In vitro*, ZD6474 (50 nM) significantly inhibited the proliferation of stimulated endothelial cells, but no inhibitory effects on HT29 tumor cells were observed at doses up to 5 μM. ZD6474 did not affect cell survival and did not enhance the extent of radiation-induced cell killing. *In vivo*, ZD6474 or RT alone led to tumor growth delays of approximately 13 and 18 days, respectively. Tumor responses were significantly greater when RT and ZD6474 were combined. Tumor growth delays of 36.5, 36 and 32 days were observed when ZD6474 was administered before, after, or concurrently with RT. No statistically significant differences were seen between these regimens. ZD6474 treatment was well tolerated with no obvious toxicities.
Conclusions: These results suggest that inhibition of tyrosine kinase signaling pathways with ZD6474 may provide an effective means by which to enhance the efficacy of RT in the treatment of solid tumors. ZD6474 is currently in Phase II clinical development.

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PTK787/ZK 222584 (PTK/ZK), a potent orally active and highly selective inhibitor of VEGFR kinases, is highly efficacious in various experimental tumor models either as mono- or combination therapy

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Background: The recent approval of Avastin by the FDA was the starting point to introduce the concept of anti-angiogenesis therapy into clinical practice within the next years. PTK/ZK a small molecule which is currently in phase III clinical trials blocks in contrast to Avastin all known VEGFRs*. Here we summarize recent findings documenting its efficacy and good tolerability.
Material and Methods: PTK/ZK inhibitory activity was assayed *in vitro* with GST-fusion constructs of the various VEGFR-kinases or in the case of VEGFR 3 as inhibition of cellular receptor autophosphorylation in MVEC. For *in vivo* analyses various tumor cell lines of different tumor origin were xenografted onto nude mice. PTK/ZK was applied mostly with a dose of 50 mg/kg daily p.o. Cytostatic compounds were applied based on literature data or in-house experience. During the course of the experiments tumor areas and body weights were recorded, and following experimentation the animals were sacrificed and tumors weights were determined. Calculation of tumor concentrations of cytostatic agents after PTK/ZK treatment was done either by HPLC (oxaliplatin) or by radioisotope analysis (5-FU).
Results: To test the specificity of PTK/ZK as a tyrosine kinase inhibitor, almost 100 different kinases were analyzed (10 μM). In accordance with earlier results the VEGFR kinases 1 and 2 were found to be inhibited with an IC_{50} of 20–50 nM and VEGFR 3 in the cellular auophosphorylation assay with an IC_{50} of 20–30 nM. Other kinases inhibited were c-fms (IC_{50}-40 nM), c-kit (IC_{50}-364 nM), PDGFRß (IC_{50}-567 nM), lyn (IC_{50} – 1 μM) and c-raf (IC_{50} – 5 μM). All other kinases tested exhibited no inhibition (^{IC50} >10 μM). *In vivo* studies of PTK/ZK as a monotherapy revealed in most cases a tumor growth inhibition of ~50%. When PTK/ZK was used in combination studies generally additive effects were observed. In all cases the mice showed no acute signs of toxicity due to PTK/ZK treatment. In combination studies only body weight decreases due to the administration of cytostatic compounds were observed. The results of the cytostatic tumor concentrations analyses revealed no significant differences of the concentrations either with or without additional PTK/ZK treatment.
Conclusion: Since PTK/ZK is inhibiting only VEGFRs and a few other kinases the observed efficacy *in vivo* is mainly due to an inhibition of tumor angiogenesis. Thus, a concept is provided that efficaeous treatment of cancer may be possible without harmful side effects. However, since the majority of cancers is treated by a combination of various cytostatic compounds, our data show that the additional use of PTK/ZK in combination therapy may also yield additional benefit.
*PTK/ZK is co-developed by Schering AG, Berlin and Novartis.