240 Beyond maximum tolerated doses: COMBATing poor prognosis pediatric cancer.

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Although the past 30 years have seen remarkable progress in the treatment of childhood malignancies, not all types of cancer have achieved this improvement in prognosis. The outcome for children with metastatic sarcomas, and some other high risk, or relapsed malignancies continues to be poor despite the use of dose-intensified chemotherapy and/or high dose chemotherapy. Intensification of therapy by dose escalation beyond the standard, MTD chemotherapy regimes has not yielded definitive evidence of increased activity so far, and additional treatment strategies are needed. Novel treatment approaches are being evaluated, including immunotherapy, radionuclide therapy, and the use of novel apoptosis and/or differentiation inducers. Among novel cancer treatment strategies is the inhibition of angiogenesis one of the most promising. Angiogenesis is fundamental in many biological processes, and it is tightly regulated under normal condition. In cancer tissues, compelling data suggest that inhibition of angiogenesis cannot only prevent tumor-associated neovascularization but also affects tumor growth and spread. An anticancer approach in which the tumor-induced new blood vessels are targeted is particularly appealing for several reasons. First, despite the extreme molecular and phenotypic heterogeneity of human cancer, it is likely that most, if not all, tumor types require neovascularization to achieve their full malignant phenotype. Second, the endothelial cells, although rapidly proliferating, are inherently normal with a very low rate of mutation. Therefore, they are unlikely to evolve an angiogenesis inhibitor-insensitive phenotype. This is in distinction to the rapidly proliferating tumor cells that do undergo a high rate of spontaneous mutation and therefore can readily generate drug-resistant clones.

The use of angiogenesis as a target for novel approaches to cancer therapy is based on the hypothesis of *Folkman J.* (1971) that tumor growth is dependent on the induction of new blood vessel formation by the tumor itself. Nowadays, low-dose continuous chemotherapy is extensively utilized as a standard part in the treatment for acute lymphoblastic leukemia. Of note is that in solid tumors, 40% of patients with non-small cell lung cancer who showed no response to standard, MTDs of intravenous etoposide administered intermittently responded to the same drug given orally at a lower dose using a more frequent (e.g. every day or every other day) and protracted schedule. The same appears to be the case for other drugs such as taxol, or cyclophosphamide and methotrexate in the treatment of advanced metastatic breast cancer patients, etoposide in relapsed drug resistant non-Hodgkins lymphoma (NHL), and/or vinblastine in Hodgkin's disease. Thus, it seems that the use of a low-dose, prolonged course of chemotherapy – metronomic chemotherapy – produces clinical responses, which are distinct from those produced by standard doses of cytotoxic agents. Of interest is the observation regarding synergy of metronomic chemotherapy with angiogenesis inhibitors via CD95 death receptor up-regulation by the use of conventional chemotherapy drugs at low doses. Metronomic chemotherapy also targets angiogenesis by affecting proliferating endothelial cells and circulating endothelial cell precursors. It may be that the rest periods between traditionally administered chemotherapy doses (e.g. every three weeks) provide the endothelial cell compartment of a tumor the opportunity to repair some of the damage caused by chemotherapy. This repair process could be partially inhibited by administering lower doses of a chemotherapeutic agent more frequently. For example, the use of extremely low (e.g. 10^{-12}) doses of vinblastine, which are devoid of endothelial cytotoxicity, can still block angiogenesis in vitro or in vivo.

Recent preclinical studies suggest that cyclooxygenase-2 enzyme (COX-2) is important in angiogenesis and that commercially available drugs such as celecoxib, a selective COX-2 inhibitor, demonstrate anti-angiogenic activity both after oral or intraperitoneal administration. Various studies have additionally shown that COX-2 is up-regulated in the activated endothelial cells of tumor vessels as well as in the stromal and tumor cells of virtually all types of human carcinoma and gliomas. Thus, COX-2 inhibitors offer the potential to block tumor growth both by direct effects such as the induction of tumor cell apoptosis as well as indirectly, by inhibiting angiogenesis and it was documented that the latter mechanism may be the more important.

Celecoxib is an oral anti-angiogenic agent that has acceptable side effects reported in children and there are published even similar clinical studies already, with very mild toxicity profiles. A greater risk of heart attacks, strokes and/or deaths resulting from heart or blood vessel disease in adults taking celecoxib has been reported and thus, taking celecoxib might increase a child's risk for heart and blood vessel damage as well. However, in studies with children and adolescents, no increased risk of heart and blood vessel damage has been seen to date.

Induced differentiation of transformed cells into mature phenotypes accompanied by apoptosis induction represents another promising strategy of novel cancer therapy. Retinoids are the most frequently used inductors of differentiation. The mechanism of retinoids activity is based on their affinity to nuclear receptors RAR and/or RXR, which function as transcriptional transregulators controlling the expression of specific subsets of genes in a ligand-dependent manner. As all-trans retinoic acid (ATRA, tretinoin) had been clinically used in therapy of acute promyelocytic leukemia, translational research was focused mainly on leukemic cells in the past. Recently, increasing number of reports concerning biological activity of retinoid has appeared also for solid tumors. In neuroblastoma, the most frequent extracranial solid tumor in children, improved survival with acceptable toxicity was found in children with neuroblastoma treated after autologous bone marrow transplant with the maximum tolerated dose of 160 mg/m^2/day of oral 13-cis RA (2 weeks on/off). Retinoids have been also proven effective against brain tumor cell lines by

inhibiting cell growth and inducing apoptosis. Observation by revealed apoptosis induction in medulloblastoma/primitive neuroectodermal tumor cells treated with 13-cis-retinoic acid. Caspase activation and induction of apoptosis have been demonstrated *in vitro* in glioma cell lines treated with synthetic retinoids. In clinical trial, substantial responses were noticed among adults with recurrent malignant glioma treated for more than 4 weeks with 60-100 mg/m^2/day of 13-cis-retinoic acid, suggesting some degree of activity of 13-cis-retinoic acid against high-grade gliomas even in patients with bulky disease, and trials combining 13-cis-RA with temozolomide have been reported in adults as well. Moreover, preclinical data have shown that Ewing's sarcoma cells can be induced to differentiate toward a neuronal phenotype by exposure to retinoic acid. and similar data exist for other sarcomas. Interestingly, intracellular isomerization of 13-cis RA into all-trans retinoic acid (ATRA, tretinoin) was experimentally confirmed both *in vivo and in vitro* and seems to be the main mechanism of biological as well as therapeutical effect of 13-cis RA.

Thus, recent reports demonstrate that there are possibilities to develop differentiation-based approaches to therapy of human cancer. Biologically active form of vitamin D(3), 1,25-dihydroxyvitamin D(3) and its chemically modified derivatives (deltanoids) are among the most promising agents for differentiation therapy. The attention has been paid to possibilities of combined induction of differentiation and/or enhancement of differentiation effects. The compounds involved include those that have differentiation-inducing activity of their own such as retinoids, deltanoids and transforming growth factor-beta, or agents modifying their intracellular metabolism and/or signalling. For example, additive differentiation effect was displayed when combining ATRA and vitamin D3. Moreover, differentiation induced by the use of vitamin D3 was increased by combined application of antioxidants and nonsteroidal anti-inflammatory drugs in myeloid leukemia cells. Recently, combination treatment with synthetic retinoid fenretinide (4-HPR) and vitamin D3 resulted in synergistic growth suppression. Inhibitors of enzymes that participate in intracellular degradation pathway of retinoids were demonstrated to enhance ATRA-induced differentiation in several *in vitro* model objects: noticeable results were achieved using cytochrome P-450 inhibitors and 5-lipoxygenase (5-LOX) inhibitors. Combined COX-2/5-LOX inhibition augmented growth arrest and death of malignant human lung cells.

Interestingly, 1,25-dihydroxyvitamin D(3) is not only differentiation-inducing agent, but it was also proven to inhibit angiogenesis *in vitro* and *in vivo.* Of interest is the direct evidence that treatment with 1,25-dihydroxyvitamin D3 (12.5 pmol/d for 8 weeks) produced xenografted tumors that were less vascularized than tumors affected with vehicle alone. The antiangiogenic effects of 1,25-dihydroxyvitamin D3 was revealed to be mediated by the hypoxia-inducible factor (HIF)-1 pathway. Thus, the clinical use of vitamin D3 derivates seems to be really promising because of combining both differentiation-inducing and antiangiogenic capability.

Designing drug combination for the COMBAT 2 regimen, several factors were considered, such as possibility of oral administration, different mechanisms of action, non-overlapping toxicity and availability of preclinical and human clinical data demonstrating activity in cancer.

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