



# PPARgamma agonists can be expected to potentiate the efficacy of metronomic chemotherapy through CD36 up-regulation

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**Summary** The ability of metronomic chemotherapy to induce endothelial apoptosis has been traced to increased endothelial expression of thrombospondin-1, which activates endothelial CD36 receptors, triggering the extrinsic apoptotic pathway. Endothelial expression of CD36 is variable. Recent studies show that PPARgamma agonists – previously shown to have angiostatic activity – can markedly boost endothelial expression of CD36, thereby potentiating the apoptotic response of endothelial cells to thrombospondin-1-mimetic peptides. Thus, concurrent administration of PPARgamma agonists would be expected to enhance the efficacy of metronomic chemotherapy. These considerations may help to rationalize recent reports that a regimen consisting of low-dose trofosamide, pioglitazone, and a cox-2 inhibitor achieves tumor regression or prolonged tumor stasis in a meaningful proportion of cancer patients. The angiostatic efficacy of metronomic chemotherapy complemented by PPARgamma agonist administration would likely be potentiated by ancillary measures that block the survival signals evoked by endothelial growth factors such as VEGF or angiopoietin-1.

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## Role of thrombospondin-1/CD36 interaction in metronomic chemotherapy

There is cogent evidence that increased production of thrombospondin 1 (TSP-1) is an obligate mediator of the endothelial apoptosis induced by metronomic therapy [1–3]. Thus, continual exposure of cultured endothelial cells to modest concentrations of genotoxic agents (too low to kill cancer

cells directly) increases their production and secretion of TSP-1, which can interact with the CD36 receptors typically expressed by endothelium to induce apoptosis [4–7]. The pro-apoptotic pathway triggered by CD36 activation appears to involve p59<sup>fyn</sup>-mediated stimulation of c-Jun NH2-terminal kinase and p38 MAP kinase, resulting in increased Fas ligand expression. This ligand then interacts with Fas – which metronomic chemotherapy also induces [8,9] – to trigger extrinsic apoptosis [5,8–10]. A monoclonal antibody that inhibits TSP-1 binding to CD36 largely abrogates the adverse impact of continuous genotoxin exposure on the proliferation and survival of endothelial cells,

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pointing to the TSP-1/CD36 interaction as key to the evoked apoptosis [1]. Moreover, metronomic chemotherapy with cyclophosphamide fails to impede tumor growth in mice that are TSP-1-null, whereas intermittent high-dose cyclophosphamide retains antitumor activity in these mice [1]. Why genotoxins provoke TSP-1 expression in endothelial cells remains unclear, though a likely possibility is induction via p53 activation [11]; p. 53 can likewise induce Fas expression [12].

## PPARgamma agonists Up-regulate CD36

The extent of expression of CD36 by endothelial cells varies as a function of their location, and this expression is substantially diminished in cultured human microvascular endothelial cells after multiple passages. Recently, Huang and colleagues have established that exposure to various PPARgamma agonists, including the drugs troglitazone and rosiglitazone, markedly boosts CD36 expression in late passage endothelial cells, restoring their sensitivity to TSP-1 [13]. Analogous induction of CD36 by PPARgamma had previously been observed in various hepatic cells [14]. This finding is of particular interest in light of multiple reports that PPARgamma agonists exert marked anti-angiogenic tumor-retardant activity in vivo in rodent xenograft models [15,16]; Whether CD36 induction contributes importantly to this effect is not clear. In vitro, PPARgamma agonists decrease VEGF receptor expression by cultured endothelial cells, and reduce their responsiveness to exogenous growth factors [17]. This latter effect may reflect, in part, an up-regulation of PTEN that down-regulates Akt activation [18]. The anti-angiogenic impact of PPARgamma is not contingent on PPARgamma expression by the tumor, though in some tumors which do express this receptor PPARgamma agonists can decrease production of angiogenic factors [19]. In any case, treatment with PPARgamma agonists has been shown to potentiate the anti-angiogenic impact of concurrent administration of ABT510 [13], a modified peptide derived from TSP-1 that has high affinity for CD36 and mimics the pro-apoptotic effects of TSP-1 on endothelial cells [20,21]. In particular, the extent of apoptosis in tumor endothelial cells was markedly higher in animals treated jointly with rosiglitazone and ABT510 than in animals receiving either agent alone; the effect appeared to be synergistic rather than merely additive [13].

Whether ABT510 – currently being assessed in phase I studies [22,23] – or other TSP-1 mimetics, ever become available for clinical use remains to

be seen; they require daily parenteral administration. However, as noted above, it is feasible to evoke autocrine TSP-1 expression in endothelial cells through use of metronomic chemotherapy, which is an established clinical technique – a regimen involving low-dose cyclophosphamide and methotrexate having shown very worthwhile efficacy in late-stage breast cancer [24–26]. Thus, it is proposed that treatment with PPARgamma agonist drugs (such as glitazones) will potentiate the tumor-retardant activity of metronomic chemotherapy by boosting endothelial expression of CD36. In addition, PPARgamma-mediated down-regulation of Akt activity might be expected to potentiate the apoptotic response to metronomic chemotherapy, inasmuch as Akt works in a number of complementary ways – most notably, BAD phosphorylation – to oppose apoptosis in endothelial cells and other tissues [27–31]. Testing the interaction of metronomic chemotherapy and PPARgamma agonists would make good sense in any case, as both of these modalities have demonstrated good angiostatic tumor-retardant activity in rodent xenograft models.

## Clinical experience

Indeed, Vogt and colleagues at the University of Regensburg have evaluated a combination angiostatic regimen – entailing daily administration of pioglitazone (45 mg), rofecoxib (25 mg) and trofosfamide (50 mg t.i.d.) – in patients with late-stage melanoma, soft-tissue sarcomas, and various vascular tumors (including kaposi sarcoma). A significant proportion of these patients achieved either objective responses or prolonged disease stabilization [32–35]. These findings evidently do not allow any conclusions regarding the contributions of the individual components of this regimen to the observed responses, but they are certainly consistent with the possibility that combining metronomic chemotherapy with PPARgamma agonists can achieve worthwhile angiostatic activity. (The inclusion of rofecoxib in the Regensburg regimen reflects the angiostatic utility of cox-2 inhibition; induction of cox-2 in plays a role in endothelial tube formation, pericyte recruitment, and endothelial cell survival during early angiogenesis [36,37]).

## Adjunctive potentiators of metronomic chemotherapy

Of related interest is a study by Pietras and Hanahan demonstrating that concurrent inhibition

of VEGFR-2 and PDGF receptors greatly boosts the angiostatic efficacy of metronomic chemotherapy in transgenic mice bearing autogenous pancreatic islet tumors [38]. Presumably, the metronomic chemotherapy provides a TSP-1-mediated apoptotic stimulus to tumor endothelial cells, while inhibition of VEGF and PDGF activity eliminates crucial survival signals that protect endothelial cells from apoptosis. (The effect of PDGFR inhibition appears to be indirect – it prevents the interaction of free endothelial cells with pericytes that can provide survival signals such as angiopoietin-1 [39,40]). Other researchers have demonstrated synergism between metronomic vinblastine and an antibody targeting VEGFR-2 in a neuroblastoma xenograft model [41]. These findings are particularly exciting in light of the fact that the drug sunitinib, which can inhibit both VEGFR-2 and PDGFR in low nanomolar concentrations achievable with acceptable toxicity [42], has recently become available for clinical use in the US [43,44]. Thus, one can envision an angiostatic regimen combining metronomic chemotherapy, sunitinib, and a PPARgamma agonist – possibly in conjunction with other well-tolerated oral agents such as fish oil, cox-2 inhibitors, silibinin, and salicylate that also have angiostatic potential [45]. Salicylate and silibinin may be of particular interest in this regard, as the endothelial survival signals provided by VEGFR-2 and various integrins are contingent on activation of NF- $\kappa$ B and its upstream activator IKK-2 [46–49] – which these agents target [50,51]; NF- $\kappa$ B also induces the Tie2 receptor and plays a key role in capillary tube formation [52,53]. There seems to be a broad consensus that an angiostatic therapy that is both effective and clinically tolerable will require use of multiple agents – preferably orally administrable ones – that address multiple targets in a complementary fashion.

In passing, it should be noted that, while PPARgamma agonists appear likely to potentiate metronomic chemotherapy, they also have the potential to increase the responsiveness of certain tumors to standard chemotherapy. In tumors which express PPARgamma and still carry at least one functional PTEN gene, PPARgamma agonists could be expected to down-regulate Akt activity by boosting PTEN expression, just as they do in endothelial cells [54–60]. Increased PTEN expression in cancer cells has been shown to exert a chemo- and radiosensitizing effect [61–64]. Furthermore, longterm down-regulation of tumor Akt activity would likely have a favorable impact on tumor aggressiveness [65].

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