Resistance to metronomic chemotherapy and ways to overcome it

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Abstract

Therapeutic resistance is amongst the major determinants of cancer mortality. Contrary to initial expectations, antivascular therapies are equally prone to inherent or acquired resistance as other cancer treatment modalities. However, studies into resistance to vascular endothelial growth factor pathway inhibitors revealed distinct mechanisms of resistance compared to conventional cytotoxic therapy. While some of these novel mechanisms of resistance also appear to be functional regarding metronomic chemotherapy, herein we summarize available evidence for mechanisms of resistance specifically described in the context of metronomic chemotherapy. Numerous preclinically identified molecular targets and pathways represent promising avenues to overcome resistance and enhance the benefits achieved with metronomic chemotherapy eventually. However, there are considerable challenges to clinically translate the preclinical findings.

Keywords

Therapeutic resistance; Chemoresistance; Metronomic chemotherapy; Antivascular tumor therapy

Introduction

Twenty years after the late Judah Folkman had described the conceptual framework of antivascular tumor therapy, in the 1990's vascular endothelial growth factor (VEGF) pathway inhibitors (VEGFi), notably the monoclonal anti-VEGF antibody bevacizumab, entered clinical development with very high expectations [1,2]. In fact, antivascular tumor therapy

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was heralded as a promising way to overcome inherent or acquired therapeutic resistance, a key characteristic of malignant growth, and as a treatment modality potentially 'resistant to resistance' [3,4]. It was thought that diploid, genetically stable tumor endothelial cells were less prone to acquire mutational resistance than genetically unstable tumor cells.

Although VEGFi have become important components of standard treatment regimens for advanced stages of numerous tumor types over the last 15 years, a number of shortcomings of anti-vascular tumor therapy came to the fore: (i) most tumors are inherently resistant to VEGFi and other anti-vascular therapies used alone; (ii) even when used in combinations that increase the initial response rate, responsive tumors typically develop acquired resistance within a few months; and (iii) as opposed to life-prolonging applications of antivascular tumor therapies in advanced disease stages, the adjuvant use of these agents did not increase cure rates [5].

Resistance to antivascular tumor therapies is thought to be largely distinct from resistance to conventional cytotoxic treatment [6]. Based on mainly preclinical studies a number of mechanisms of resistance to VEGFi have been proposed, including evasive resistance due to angiogenic growth factor redundancy or HIF1α mediated overexpression of angiogenic factors, vascular remodeling resulting in more mature and VEGFi resistant tumor blood vessels, preferential expansion of VEGFi resistant vessel subtypes, the selection of hypoxia-resistant tumor cell subpopulations with reduced vascular dependence, the integration of trans-differentiated tumor cells with endothelial cell properties into the tumor vasculature in a process named vasculogenic mimicry, vessel co-option by tumor cells capable of exploiting the abundant presence of pre-existing host vessels in organs such as liver and lungs, tumor infiltration by bone marrow derived leukocytes with proangiogenic properties, and stromal cell activation (Fig. 1) [5,7–9].

Research activities focusing on targeting the tumor vasculature revealed that many conventional chemotherapeutics and targeted agents exert collateral damage to tumor vessels [10]. In the case of standard, maximum tolerated dose (MTD) chemotherapy (i.e. the cyclical administration of high doses of chemotherapeutics with interspersed treatment-free breaks), the anti-vascular effects seen are similar in nature but also as short-lived as the vascular destruction inflicted by vascular disruptive agents [11,12]. On the other hand, the frequent and sustained use of low doses of conventional chemotherapeutics (i.e. low-dose metronomic chemotherapy; hereafter metronomic chemotherapy, MC) mimics the long-term antiangiogenic activities of VEGFi.

Two seminal preclinical publications described key characteristics of the MC concept, which have been refined over time and largely validated in numerous clinical trials [12–14]. First, MC may overcome resistance to MTD chemotherapy. In other words, the mechanisms of resistance to metronomic versus MTD chemotherapy are at least partially distinct [15]. Second, inducing endothelial cell apoptosis is a main mechanism of action of MC, but MC may also affect other endothelial cell processes such as proliferation, migration, tube formation and sprouting [12,16–18]. Third, the majority of tumors are inherently resistant to MC alone, and even initially responding tumors eventually acquire resistance to MC, similar to what is seen with VEGFi [14]. Fourth, high levels of proangiogenic factors may

contribute to resistance to MC, but such resistance may be overcome by combination with VEGFi amongst other strategies [13,19].

While initial publications on MC focused on the antiangiogenic activities of MC, there is emerging evidence that MC may also impair vasculogenesis [20,21], target tumor stem cells and their vascular niche [22,23], promote anti-tumor immunity [24,25], delay acquired chemoresistance compared to MTD chemotherapy [25,26], and may induce tumor dormancy [27]. This broad range of MC activities renders studies on mechanisms of resistance to MC challenging. Such studies also need to account for differential effects of distinct chemotherapeutics when used in metronomic manner [28]. Finally, MC is typically applied in combination with other treatment modalities that may affect the resistance phenotype and genotype seen [29].

Herein, we review current knowledge on mechanisms of resistance to MC, and discuss challenges with respect to how the mainly preclinical findings might be translated clinically in the future. Considering the complex anti-tumor activities of MC, an integral understanding of resistance to MC not only involves endothelial cell-intrinsic mechanisms, but also tumor cell and host traits, as outlined in Fig. 1.

Mechanisms of resistance to metronomic chemotherapy

Endothelial cell-driven resistance

Pro/anti-angiogenic balance—When compared *in vitro* to tumor cells, endothelial cells are ultra-sensitive to the pro-apoptotic and anti-proliferative effects of low-dose, sustained chemotherapy administration [16]. This differential sensitivity is mediated in part by MC-induced expression of the endogenous angiogenesis inhibitor thrombospondin 1 by endothelial, tumor and/or stromal cells [30,31]. As such, the preclinical use of the thrombospondin 1 peptide ABT-510 has been shown to amplify the anti-tumor effects of MC [32]. In contrast, proangiogenic factors such as VEGF and basic fibroblast growth factor impair the pro-apoptotic activities of chemotherapeutics towards endothelial cells [19]; and MC was successfully combined with VEGFi in numerous preclinical studies, either upfront or to counteract acquired resistance [13,33].

Vascular remodeling—In a Wilms' tumor model, Huang et al. identified remodeled blood vessels with increased diameter and mural cell proliferation as a mediator of resistance to metronomic topotecan chemotherapy [34]. The remodeling process was associated with platelet-derived growth factor B and ephrin B2 expression.

AKT pathway and stress-activated chemoprotective signaling—The AKT pathway, including the anti-apoptotic factor survivin downstream thereof, has been shown to mediate the chemo-protective effects of VEGF and basic fibroblast growth factor seen *in vitro* in human umbilical vein endothelial cells and human dermal microvascular endothelial cells [19]. Mavroeidis et al. further implicated the AKT pathway in resistance to MC [35]. Briefly, MC may induce severe tumor hypoxia, which in turn is thought to contribute to acquired therapeutic resistance by upregulating the expression of proangiogenic factors amongst others [18]. As such, severe hypoxia reduced the antiproliferative and pro-apoptotic

effects of metronomic vinorelbine on human umbilical vein endothelial cells, while sparing its inhibitory activities on migration, tube formation and sprouting [35]. The AKT inhibitor V was able to antagonize the chemoprotective effects of severe hypoxia. Finally, Meng et al. studied differential responses of normal versus tumor endothelial cells isolated from mouse liver tissues or diethylnitrosamine-induced liver tumors, respectively [36]. Gemcitabine chemotherapy induced VEGF in tumor endothelial cells via NFκB-dependent AKT activation and VEGF expression, resulting in enhanced endothelial cell survival and migration. In contrast, no such response was observed in normal endothelial cells.

Role of βIII-tubulin expression—Pasquier et al. used BMH29L immortalized, bone marrow-derived endothelial cells to study the differential impact of repeated MTD versus metronomic vinblastine therapy [26]. MTD vinblastine resulted in reduced endothelial cell proliferation and resistance to paclitaxel. By contrast, metronomic vinblastine (or etoposide) increased endothelial cell chemosensitivity, coinciding with decreased expression of βII- and βIII-tubulin. Since this MC-induced endothelial cell behavior could be phenocopied by siRNA against βIII-tubulin (but not by silencing of βII-tubulin), βIII-tubulin may represent a promising therapeutic target to amplify MC efficacy.

Chemoresistance—Although normal diploid endothelial cells are commonly considered genetically stable and hence less prone to therapeutic resistance than tumor cells, isolated tumor endothelial cells feature distinct functional properties when compared to normal endothelial cells, such as increased tolerance to serum starvation and resistance to chemotherapeutic drugs [37]. However, with a few exceptions chemoresistance of endothelial cells is poorly understood.

Several groups found tumor endothelial cells to harbor cytogenetic abnormalities suggestive of genetic instability, which in turn could result in conventional drug resistance [37]. Akiyama et al. showed that VEGF-induced AKT activation increases the expression of the P-glycoprotein drug efflux pump [38]. Hence, the addition of VEGF to tumor endothelial cells rendered them resistant to paclitaxel, a P-glycoprotein substrate, and the P-glycoprotein inhibitor verapamil enhanced the antiangiogenic effects of metronomic paclitaxel in the A375SM human melanoma xenograft model [39]. Of note, metronomic versus MTD chemotherapy administration may impact the expression of P-glycoprotein and other drug efflux pumps in endothelial cells in a complex manner, depending on the type of endothelial cells and chemotherapeutic agents used [17,26]. Overall, MTD chemotherapy seems to have a higher propensity to induce drug efflux pumps in both endothelial and tumor cells compared to MC [17,40].

Endothelial stem cells—The differential expression of drug efflux pumps has been used to identify a vascular stem/progenitor cell side-population by flow cytometry following staining with Hoechst 33342 DNA dye, a drug efflux pump substrate [41]. Side-population endothelial cells were found in treatment-naïve tumors of Lewis lung carcinoma and KLN205 squamous carcinoma cells grown in syngeneic mice [42]. Importantly, the frequency of such cells increased upon treatment with the VEGFi axitinib and vandetanib. In other words, drug efflux pump positive endothelial stem/progenitor cells may contribute both to inherent or acquired resistance to VEGFi, and possibly to MC using

chemotherapeutics that are efflux pump substrates. On the other hand, adding drug efflux pump inhibitors such as verapamil or cyclosporine A decreased the colony formation of tumor-derived side-population endothelial cells exposed to vandetanib.

Tumor cell-driven resistance

Vasculogenic mimicry—HUH-7 human hepatocellular carcinoma cells made resistant to metronomic cyclophosphamide *in vivo* (i.e. HUH-REISO cells), were found to be more sensitive to activated cyclophosphamide *in vitro* compared to their parental counterparts, or *in vivo* passaged HUH-7 control cells. However, HUH-REISO retained resistance to cyclophosphamide when grown *in vivo* [43]. In the HUH-REISO model, acquired resistance develops through two steps. First, metronomic cyclophosphamide induces the expression of the cyclophosphamide-detoxifying enzyme ALDH-1, and of an antiapoptotic program mediated by NOTCH-1. Thereafter, tumor cells acquire a pluripotent phenotype mediated by stemness markers such as THY-1, OCT-4, SOX-2 and NANOG. Such pluripotent tumor cells may transdifferentiate into endothelial cells and contribute to the treatment-resistant tumor vasculature via vasculogenic mimicry.

Reduced vascular dependence—Whilst reduced oxygenation is one of the mediators of the antitumor effects of MC, hypoxia may also simultaneously contribute to the selection of tumor cells less dependent on tumor vascularization. This has been demonstrated in metronomic cyclophosphamide resistant PC-3 tumor xenografts that progress despite sustained microvascular rarefaction [18]. To date, the molecular mechanisms driving resistance via reduced vascular dependence are poorly understood.

Stem cell properties—Aside from endothelial cell transdifferentiation resulting in vasculogenic mimicry, the pluripotent properties of the aforementioned HUH-REISO model may also directly contribute to resistance to metronomic cyclophosphamide [43]. This notion is supported by studies using the human C6 glioblastoma model. Stem cell high C6 cultures result in tumor xenografts with increased microvessel density, enhanced endothelial progenitor cell recruitment, and superior perfusion compared to tumors of C6 preparations with low cancer stem cell content [44]. The stem cell properties of C6 went along with increased expression of VEGF and stromal-derived factor 1. Although MC and other antiangiogenic therapies initially may reduce the number of tumor stem cells via disruption of the vascular stem cell niche, tumor stem cells are a potential source of therapeutic resistance [22]. In fact, Martin-Padura et al. showed that CD13 + dormant hepatocellular cancer stem cells contribute to acquired resistance to metronomic cyclophosphamide, which in turn can be counteracted by the CD13-targeting drug, bestatin [23].

Autophagy deficiency—Macroautophagy (hereafter referred to as autophagy) is an evolutionary conserved cellular mechanism involved amongst others in adaptation to stress [45]. In a highly context-dependent manner autophagy may contribute to both cell survival and cell death, including during carcinogenesis and cancer therapy [46]. With respect to metronomic cyclophosphamide therapy there are multiple lines of evidence that low autophagic activity contributes to therapeutic resistance [47]. First, metronomic cyclophosphamide resistant PC-3 cells feature a lower autophagic flux than their control

counterparts. Second, the autophagy inhibitor chloroquine impairs the response of PC-3 tumor xenografts to metronomic cyclophosphamide. Finally, tumors of immortalized baby mouse kidney cells rendered autophagy-deficient via engineered BECLIN 1 haploinsufficiency are less responsive to metronomic cyclophosphamide than autophagy-competent tumors. It remains to be seen whether these findings are applicable to other tumor models and metronomic regimens using drugs other than cyclophosphamide.

Gene expression analyses—Although PC-3 human prostate cancer xenografts are highly sensitive to metronomic cyclophosphamide, eventually they acquire stable, transplantable therapeutic resistance [15,48]. Therefore, PC-3/metronomic cyclophosphamide is the best-studied model of acquired resistance to MC to date. Using PC-3 variants made resistant *in vivo* to metronomic chemotherapy administered continuously in the drinking water compared to *in vivo* passaged control PC-3 tumors, Chow et al. identified 41 upregulated genes (e.g. Janus kinase 1 (JAK1) and the FLI1 proto-oncogene) and enrichment for genes involved in protein translation (e.g. EIF2B1, IMP3, PES1), by applying cDNA microarray analyses [28]. Of note, there were no differential expression changes of pro- or antiangiogenic factors such as VEGF or thrombospondin 1. In separate studies of PC-3 variants with acquired resistance to weekly intraperitoneal metronomic cyclophosphamide, Thoenes et al. used comparative proteome analyses to identify elevated thioredoxin containing protein 5 (TXNDC5), cathepsin B (CTSB) and annexin A3 (ANXA3) as possible mediators of therapeutic resistance [49]. Applying cDNA microarray technology to study the same PC-3 model suggested an association of therapeutic resistance with genes comprised in the gene ontology terms 'complement and coagulation cascade', 'axon guidance' and 'steroid biosynthesis' [50]. Overall, there was minimal overlap of the genes identified in these three studies. More efforts will be needed to validate the gene expression findings in view of potential clinical translation.

Cruz-Muñoz et al. studied SKOV-3-13 human ovarian cancer cells made resistant to metronomic topotecan plus the small molecule VEGFi pazopanib *in vivo* [51]. Oligo-microarray analysis of these variants revealed a number of upregulated genes implicated in resistance to various chemotherapeutic agents, namely alpha B crystallin (CRYAB), heat shock 27 kDa protein 2 (HSPB2), transketolase-like 1 (TKTL1), and the cytochrome P450 member CYP1B1. Since the SKOV-3-13 variants studied by Cruz-Muñoz et al. were made resistant to combined metronomic topotecan and pazopanib, it remains to be seen how the identified genes contribute to resistance to metronomic monotherapy.

Host-driven resistance

Pharmacokinetics—In the case of MTD chemotherapy, the infrequent administration of chemotherapeutics is unlikely to change the metabolism of subsequent drug doses. By contrast, long-term MC is oriented towards the lowest dose necessary, and small changes in drug metabolism may result in steady state drug levels below the threshold needed for optimal anti-tumor activity [52,53]. While certain chemotherapeutics may induce their own metabolism, steady state drug levels may also be affected by pharmacogenomic traits, concurrent medications, treatment adherence, or organ dysfunctions commonly seen in elderly patients.

There are only a few dedicated MC pharmacokinetic studies available, the major findings of which have been reviewed recently [53]. Preclinical studies are reassuring in a number of ways. As an example, metronomic cyclophosphamide administered to mice results in circulating drug levels that have been shown to have antiendothelial cell effects *in vitro* [54]. Furthermore, circulating drug levels were maintained over prolonged periods of time, and thus altered cyclophosphamide metabolism is an unlikely cause for acquired resistance to metronomic cyclophosphamide.

Pharmacokinetic studies in patients undergoing MC are challenging. Amongst others, commonly used drug detection methods are not always sensitive enough to determine very low drug levels [53]. Altogether, the few clinical pharmacokinetic studies of MC confirm the preclinical findings. In patients with metastatic gastrointestinal cancer undergoing metronomic tegafur-uracil (5-FU prodrug) and cyclophosphamide therapy combined with celecoxib administration, Allegrini et al. also showed a relationship between circulating 5-FU levels and patient outcome [55]. It remains to be seen whether pharmacokinetic studies might help circumventing inherent resistance to MC by guiding individualized drug dosing.

"Angiogenetics"—While individual pharmacogenetic traits may affect active drug levels, there are other patient factors that may alter the benefit achieved with MC. The response of tumors to antiangiogenic and conventional cytotoxic therapies is not only shaped by characteristics of the intratumoral vasculature, but also depends on the frequency of intratumoral, bone-marrow derived circulating endothelial progenitor cells [20,56]. The number of circulating endothelial progenitor cells varies in different inbred mouse strains and is positively correlated with the robustness of tumor angiogenesis [57]. Thus, it is fair to assume that similar genetic heterogeneity may contribute to differential baseline or treatment-induced levels of circulating endothelial progenitor cells in patients. Of note, in hepatocellular carcinoma patients undergoing treatment with the small molecule VEGFi sorafenib combined with metronomic tegafur-uracil, high baseline endothelial progenitor cell levels were associated with poor outcome in multivariate analysis [58]. Furthermore, in patients with heavily pre-treated advanced gastrointestinal malignancies who underwent treatment with metronomic tegafur-uracil and cyclophosphamide, combined with celecoxib, CD133 mRNA expression in peripheral blood mononuclear cells (a surrogate marker for circulating endothelial progenitor cells) increased in subjects with progressive disease [55]. Orlandi et al. analyzed VEGF single nucleotide polymorphisms in men with advanced castration-resistant prostate cancer patients treated with metronomic cyclophosphamide, plus celecoxib and dexamethasone [59]. The VEGF (-634CC) genotype was significantly associated with a shorter progression free survival. Genetic heterogeneity may not only affect the antivascular but also the immunomodulatory activities of MC [60].

Age-dependent vascular characteristics—Tumor growth studies are mainly performed in young or adolescent mice, whereas the typical cancer patient is elderly and may suffer from vascular co-morbidities such as atherosclerosis. By analyzing the vasculature of renal cell carcinomas in patients above versus below 65 years of age, Meehan et al. showed that the microvascular density was higher in patients >65 years [61]. There were also age-related differences in the expression of tumor endothelial markers such as

delta-like ligand 1. Such age-related structural and molecular vascular characteristics may affect the spontaneous growth of tumors and treatment responses. In fact, Lewis lung and B16F1 melanoma mouse cancer cells grow faster in young (4–8 weeks), non-atherosclerotic mice compared to their old (12–18 months) and/or atherosclerotic counterparts [62]. Furthermore, the antitumor effects of metronomic cyclophosphamide were diminished in old and/or atherosclerotic mice. A reverse differential response pattern was found with the small molecule VEGFi sunitinib, which was less efficacious in young mice [63].

Overcoming resistance to metronomic chemotherapy

The response rate of metronomic monotherapy is moderate, as is typically also the case with other antivascular monotherapies [5]. On the other hand, MC is associated with a comparably low risk of severe side effects [14,64]. Hence, MC can be easily combined with other treatment modalities.

Preclinical studies have analyzed a wide range of combination therapies. To name a few, MC was successfully combined with standard anticancer treatment modalities such as VEGFi [13,51], conventional MTD chemotherapy [65], targeted therapies [33,66], and radiation [67]. The immunomodulatory activities of MC were exploited to enhance different types of cancer immunotherapy [68]. Other studies have capitalized on MC-induced tumor hypoxia and acidification by utilizing the hypoxic cell cytotoxin tirapazamine or the proton pump inhibitor lansoprazole in conjunction with MC [18,69]. Finally, molecular mediators of resistance to MC discussed herein represent excellent therapeutic targets to enhance the antitumor effects of MC (Fig. 2).

With few exceptions, preclinical studies have focused on overcoming intrinsic rather than acquired resistance to MC. On the other hand, tumor xenografts of PC-3 variants made resistant to metronomic cyclophosphamide *in vivo* remained highly sensitive to MTD cyclophosphamide [15]. In addition, PC-3 tumor xenografts that progressed during metronomic cyclophosphamide therapy were responsive to MTD docetaxel, whereas parental PC-3 tumors were found to be largely resistant to MTD docetaxel [28]. These findings suggest that the use of below-MTD doses of conventional cyctotoxics does not necessarily promote acquired resistance to the same or other chemotherapeutic drugs used in MTD fashion. More importantly, by considering the seminal observations by Browder et al. (i.e. that MC can overcome resistance to MTD chemotherapy) [12] it becomes apparent that MC might be used to overcome resistance to MDT chemotherapy, as much as MTD chemotherapy might be able to conquer resistance to MC.

Numerous combination strategies have already been tested clinically, including in a number of phase III trials [14,70–76]. However, only a few randomized phase II trials were specifically designed to study the efficacy of metronomic monotherapy versus MC combined with other treatment modalities, by combining MC with the poly (ADP-ribose) polymerase inhibitor veliparib [77,78], the tumor stroma modulating agents rofecoxib and pioglitazone [79], the antiangiogenic and immunomodulatory compound thalidomide [80], and the VEGFi bevacizumab [81]. Study characteristics are summarized in Table 1. There is a numerically promising trend of improved overall survival of melanoma patients treated with

metronomic trofosfamide combined with rofecoxib and pioglitazone compared to metronomic trofosfamide alone. However, none of the reported randomized studies reveals both a clinical relevant and at the same time statistically significant benefit of any of the combination regimens over MC alone. Chi et al. reported a 40% response rate and a 84% disease control rate by simultaneously adding the autophagy inducer rapamycin and the autophagy inhibitor hydroxychloroquine in 25 patients with various tumor types presenting with intrinsic resistance to numerous MC regimens [82].

Summary and outlook

Therapeutic resistance is amongst the major determinants of cancer mortality. Although available phase III clinical trial findings position MC as a promising anticancer treatment strategy, especially when used as maintenance or adjuvant therapy, typically the response rates to MC are moderate, and acquired resistance ensues within months [70–76]. MC shares a number of mechanisms of resistance that are also functional in VEGFi therapy (Fig. 1). Herein we have summarized molecular data on a number of promising combination strategies aimed at increasing both response rates and duration of responses to MC (Fig. 2). However, none of these combination treatments have been tested in phase III trials to date.

Unfortunately, it is not unexpected that randomized trials comparing MC alone versus MC combined with other treatment modalities are rare. First, the most common MC regimens apply off-patent agents without associated commercial interest. Second, regulatory authorities are not expected to honor the metronomic use of conventional chemotherapeutics, which limits the interest of industry partners to conduct trials of MC combined with novel agents. Finally, partnering MC with other treatment modalities may compromise some of the advantages of MC used alone, such as a low costs and low rates of severe side effects [14,83]. Nonetheless, MC is a very attractive treatment concept, especially in low and middle-income countries [84,85]. Maybe, such countries will show us the way, including with trials that combine MC with off-patent and affordable repurposed drugs such as metformin [86].

Acknowledgments

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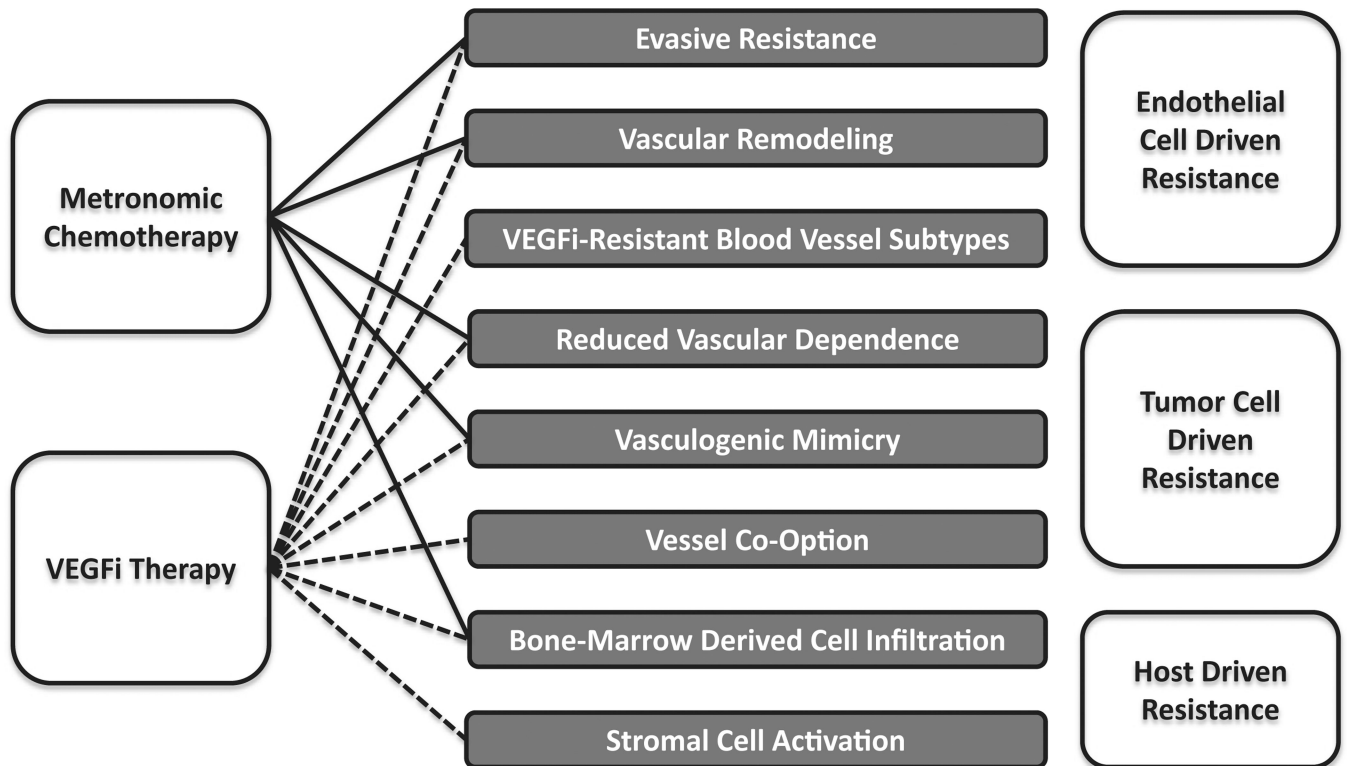
Fig. 1.
Mechanisms of resistance to metronomic chemotherapy or vascular endothelial growth factor pathway inhibitors (VEGFi) – concepts. Numerous mechanisms of resistance to VEGFi have been described, involving endothelial cell, tumor cell, and host-driven mechanisms. Many of these mechanisms were also found to be functional when it comes to resistance to metronomic chemotherapy.
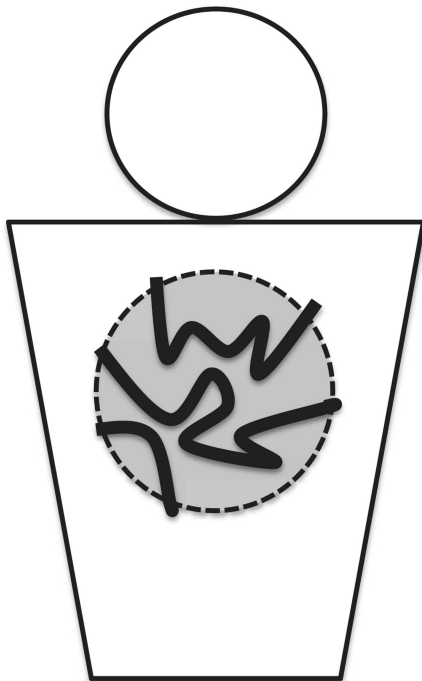
Endothelial Cell Directed Targets:

Vascular Endothelial Growth Factor ↘
Basic Fibroblast Growth Factor ↘
Thrombospondin-1 ↗
Platelet-Derived Growth Factor B ↘, Ephrin B2 ↘
AKT -> Survivin ↘
AKT -> P-Glycoprotein ↘
βIII-Tubulin ↘

Tumor Cell Directed Targets:

'Stemness' ↘, CD13 ↘
NOTCH-1 ↘
Aldehyde Dehydrogenase ↘
Autophagy ↗

Host Directed Targets:

Endothelial Progenitor Cells ↘

Fig. 2.
Targets to overcome resistance to metronomic chemotherapy. Preclinical analyses have revealed a number of targets to overcome resistance to metronomic chemotherapy, either by enhancing their activity (↗) or by impairing them (↘).

Table 1

Randomized phase II trials exploring ways to overcome resistance to metronomic chemotherapy.

Reference	Tumor type	Treatment arm A	Treatment arm B	Outcome (A/B)
Kummar et al., Investigational New Drugs 2016;34:355–63	Recurrent, advanced triple-negative breast cancer	Metronomic cyclophosphamide (18 patients)	Metronomic cyclophosphamide **plus veliparib** (21 patients)	Median progression free survival 1.9 vs 2.1 months (P = 0.034)
Kummar et al., Clinical Cancer Research 2015;21:1574–82	BRCA-mutant ovarian cancer; primary peritoneal, fallopian tube, or high-grade serous ovarian cancer	Metronomic cyclophosphamide (38 patients)	Metronomic cyclophosphamide **plus veliparib** (37 patients)	Median progression free survival 2.3 vs 2.1 months (P = 0.68)
Reichle et al., Melanoma Research 2007;17:360–4	Advanced melanoma	Metronomic trofosfamide (32 patients)	Metronomic trofosfamide **plus rofecoxib and pioglitazone** (35 patients)	Median progression free survival 1.2 vs 2.0 months (P = 0.003); median overall survival 8.2 vs 18.8 months (P = 0.086)
Colleoni et al., Annals of Oncology 2006;17:232–8	Advanced breast cancer	Metronomic cyclophosphamide/methotrexate (90 patients)	Metronomic cyclophosphamide and methotrexate **plus thalidomide** (88 patients)	Median time to progression 3.8 vs 4.1 months (P = 0.46); median overall survival 18.2 vs 17.1 months (P = 0.98)
Burstein et al., Breast Cancer Research and Treatment 2005;94:Supplement 1	Advanced breast cancer	Metronomic cyclophosphamide/methotrexate (21 patients)	Metronomic cyclophosphamide and methotrexate **plus bevacizumab** (34 patients)	Median time to progression 2.0 vs 5.5 months (P = not reported)