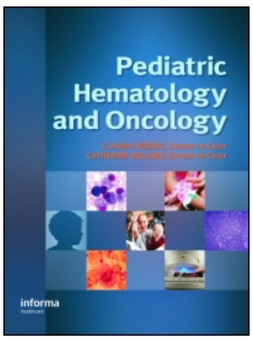

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Metronomic therapy has low toxicity and is as effective as current standard treatment for recurrent high-risk neuroblastoma

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ABSTRACT

The metronomic therapy concept uses low doses of continuously applied chemotherapeutic, anti-angiogenetic, and immunomodulating drugs. Twenty patients with recurrent and 3 with refractory high-risk neuroblastoma were treated by the metronomic concept using celecoxib, cyclophosphamide, vinblastine, and etoposide for up to 24 months. The outcome was compared to 274 matched patients with a first recurrence from stage 4 neuroblastoma using the variables time from diagnosis to first recurrence, number of organs involved, and MYCN amplification. All were treated with dose-intensive conventional chemotherapy. The study patients experienced 1–3 recurrences and had 1–3 sites involved (osteomedullary, primary tumor, central nervous system, lymph nodes, liver, lungs) before the metronomic therapy started. Two patients in complete remission and three with active refractory disease following recurrence treatment were excluded from the outcome analysis. The curves for secondary event-free and overall survival demonstrated no significant differences. The toxicity was minimal except for ≥3 grade thrombocytopenia and leukopenia (all heavily pretreated). The treatment was realized in an outpatient setting. The metronomic approach is similarly effective as standard treatment in recurrent high-risk neuroblastoma, has low toxicity, and is applicable in an outpatient setting. A prospective study including propranolol as a fifth drug is underway.

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Introduction

Neuroblastoma remains the second most frequent cause of cancer-related death in childhood in Germany (10.4%) [1], although increasingly complex and aggressive treatment protocols have been adopted during the recent decades. The overall 5-year survival rate of children with

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high-risk neuroblastoma has been reported to be between 29% and 50% [2] and therefore remains unsatisfactory. After relapse from high-risk disease, the outcome is even worse (5-year overall survival rate of approximately 10%). Only small subgroups have a better outlook [3].

Primary and acquired drug resistances are the likely reasons for tumor-related death during or after intensive therapy. The current maximum tolerated dose (MTD) concept aims to overcome drug resistances by using higher doses, but requires breaks between the cycles. Metronomic chemotherapy is characterized by frequent, continuous low dosing of cytotoxic and other repositioned drugs (i.e., originally used for other indications). From experience with metastatic breast [4], metastatic colorectal [5], and castration-resistant prostate [6] cancer, it has been recognized that metronomic treatment approaches may overcome drug resistances. The metronomic protocols were mainly designed to maintain a stable disease for as long as possible together with low toxicity but have demonstrated surprising responses. The experience of metronomic therapy in pediatric oncology has been recently reviewed [7,24]. The studies are limited in terms of the number of patients and included only a few of cases with high-risk neuroblastoma. More focused studies are therefore required.

Today, cancer is widely considered to be a multicomponent disease. This phase 1/2 pilot study adopted the concept of multi-targeting. The combination of treatments employed in this study targeted tumor cells and the tumor microenvironment by inhibiting neoangiogenesis as well as tumor-promoting immune cells. In neuroblastoma, high tumor vascularity is correlated with an aggressive phenotype: a strong correlation was demonstrated between vascular index (total number of vessels per mm^2 of neuroblastoma tissue), disease stage, MYCN amplification, and outcome [8]. Therefore, the inhibition of angiogenesis is an attractive therapeutic option. Human neuroblastoma xenografts (SK-N-MC and SK-N-AS) in SCID mice have shown reduced cell viability and a loss of preexisting tumor architecture after application of low-dose vinblastine [9]. This was particularly evident when combined with DC101—a monoclonal antibody targeting the flk-1/KDR (type 2) receptor of the vascular endothelial growth receptor (VEGF). Tumor perfusion experiments using intravascular fluorescence have demonstrated that vascular injury, rather than a direct anti-tumor effect, was the likely underlying primary mechanism [9]. Vinblastine [9], etoposide [10], and cyclophosphamide [11] are considered to act anti-angiogenetically in addition to their known cytostatic effect.

Several lines of evidence have demonstrated that metronomic treatment may also contribute to restoring immune response to cancer. Tumor-associated macrophages exert anti- as well as pro-tumor functions [12] depending on the cytokine milieu of the microenvironment [13]. Regulatory T cells can promote tolerance of tumor cells [14]. It has been shown that a higher expression of genes representing tumor-associated macrophages (CD33/CD16/IL6R IL10/FCGR3) was observed in metastatic neuroblastoma tissue compared to locoregional tumor specimen. Thus, activated macrophages can contribute to a more malignant subtype [15]. Since cyclooxygenase-2 (COX-2) is overexpressed in neuroblastoma tumor cells, COX-2 inhibitors like celecoxib may not only change the pro-tumor macrophage M2 into the anti-tumor phenotype M1 [16], but also directly suppress tumor cell growth [17]. Modulation of the immune function of macrophages, dendritic cells, and regulatory T cells has also been reported for cyclophosphamide [14] and vinblastine [18]. Thus, metronomic therapy could be characterized as a microenvironmental therapy which affects both endothelial and immune cells.

Nonetheless, metronomic therapy is still the exception and not the rule in cancer treatment, in particular in pediatric tumors. In this report, the first experience with a new combination

of well-known drugs (celecoxib, vinblastine, cyclophosphamide, and etoposide) is presented, demonstrating an encouraging anti-tumor effect together with low toxicity.

Patients and Methods

Inclusion criteria for this phase 1/2 pilot study were first to fourth recurrence from regional or metastatic (stage 4) neuroblastoma or refractory stage 4 disease. Diagnosis, staging, and response evaluation were done according to the International Neuroblastoma Staging System [19]. The sites of disease refer to organs, not to the number of metastases. A multitude of spots within the skeleton taking up Metaiodobenzylguanidine (mIBG) was counted as one. Bony and medullary metastases in the skeleton were summarized as osteomedullary metastasis. Cytological evidence of bone marrow infiltration was likewise categorized as osteomedullary. Metastatic involvement of the central nervous system (CNS) was defined as intracerebral lesions by magnetic resonance imaging and/or the presence of neuroblastoma cells in the cerebrospinal fluid. Only lesions taking up contrast medium from mIBG or magnetic resonance imaging (MRI) were considered to be an active disease. No evidence of an active tumor during the 4 weeks before the start of metronomic therapy was categorized as complete remission (CR, two cases). Progressive disease was defined as the detection of any new lesion, an increase in tumor volume by >25% of any measurable lesion, or if previously negative bone marrow became positive for tumor cells (>10%). Stable disease met the criteria neither for progressive disease nor for partial response [19].

In all cases, informed consent was obtained from the patients' parents for the treatment and for the collection of the data at the local treating institutions. The patients signed age-adapted assent forms when appropriate. The patients were treated on a compassionate use basis. The study was conducted in accordance with the published principles of the Guidelines for Good Clinical Practice (ICH-GCP), the applicable European and national legislation, and in accordance with the Declaration of Helsinki of the World Medical Association. The extended trial is registered under the EudraCT number 2011-004593-29 and was approved by the ethics committee of the University of Cologne (trial protocol code Uni-Koeln-1495). The patients were treated at one of the participating institutions: Universities of Cologne (Germany), St. Petersburg (Russia), Minsk (Belarus), and Brno (Czech Republic).

Preceding therapy (type, duration) was not defined. Study patients could have been in a palliative situation, but had to have a minimum life expectancy of more than 12 weeks.

The drugs of the metronomic therapy were intended to be administered for up to 24 months in an outpatient setting as long as no event occurred. It consisted of oral doses of 200 mg/m² celecoxib (anti-neuroblastic, immune modulating) twice daily, 25 mg/m² cyclophosphamide (anti-neuroblastic, anti-angiogenetic, immune modulating) daily, 25 mg/m² etoposide (anti-neuroblastic, anti-angiogenetic) daily on days 1–21 every second month (4 cycles only), and a 1× 3 mg/m² intravenous dose of vinblastine (anti-neuroblastic, anti-angiogenetic, immune modulating) every 14 days.

The group for comparison consisted of 274 matched patients with stage 4 neuroblastoma over 18 months of age at first diagnosis and diagnosed between 1990 and 2010. First line treatment of the control group was given according to the GPOH neuroblastoma trials NB90/97/04 [20]. The patients included in the control group experienced a first recurrence and underwent dose-intense cyclic chemotherapy with curative intent. Chemotherapy cycles and drug doses were not defined. Thirty percent of this group had additional myeloablative chemotherapy and autologous stem cell support. Children who were treated with palliative intent for the recurrence were excluded from the control group in order to avoid a selection bias. The decision

regarding curative intent versus palliative intent was made by the parents and the patients (if appropriate) following discussions with the treating physician. For survival analysis, only the study patients with newly actively progressing tumors were included (n = 18). Two patients in CR after preceding recurrence therapy and three patients with refractory active tumors were excluded. Response was assessed according to the international criteria [19].

Secondary event-free survival (secEFS) was calculated as the time from the first day of metronomic therapy following diagnosis of recurrence or refractory disease until an event or to the date of the last assessment in patients without an event. An event was defined as progressive disease or death for any reason. Secondary overall survival (secOS) was defined as the time from the first day of metronomic therapy following diagnosis of recurrence until death for any reason or until the last assessment in surviving patients. The time-to-event distributions were displayed according to the Kaplan-Meier method. Curves of the cases and variable-matched controls were compared using the stratified log rank test (Wald) as recommended for matched cohorts [21]. Survival times were analyzed using the Cox' proportional hazard model. Statistical analyses and matching were performed using the R software version 3.1.2. The data lock was August 1st, 2016.

The matching criteria to compare the study and the control group were MYCN amplification (yes vs. no), time from first diagnosis to first recurrence ($\leq$18 months vs. $>$ 18 months), and number of recurrence sites (1 vs. $\geq$2) [22]. Seven of the eight possible combinations were occupied by the study patients. Only the combination MYCN amplified, time from first diagnosis to first recurrence more than 18 months, and number of recurrence sites = 1 had no patient in the study group (Supplemental Figure 1, stratum 8). Strata 1–7 show that each study patient was matched to 6–25 (average 14) control patients.

All six patients with stage 2b or 3 at first diagnosis had metastatic disease at recurrence. Nine patients had the second to fourth recurrence at the start of metronomic therapy (Table 1). It is well-known that the prognosis decreases with each consecutive recurrence. Therefore, the clinico-biological characteristics of the study group were at least equal to if not worse than the control group.

Results

Twenty-three patients were treated according to the metronomic concept. Table 1 shows the characteristics and the outcome of the patients. Three children had refractory stage 4 disease and 20 had recurrent neuroblastoma. Six of the children who had recurrences from regional disease had metastatic recurrences. Of the 20 patients with recurrent disease, eleven patients had 1, seven had 2, and two had 3 recurrences before the start of metronomic therapy. Also, nine, ten, and two patients had 1, 2, and 3 involved organs, respectively. The sites were osteomedullary in 16, primary tumor in 11, CNS in 2, lymph nodes in 2, liver in 1, and lungs in 1 patient. Two patients were in CR with inactive residual tumor after pretreatment of the relapse. Of the 21 patients with active tumors (18 recurrent, 3 refractory disease), 2 achieved complete response (CR) and 9 partial response (PR) as the best response (table 1). In 8 patients the disease was only stabilized (SD), and in 2 patients no response was seen (PROG).

The median time to progression of the 18 patients with recurrences and not in CR at the start of metronomic therapy was 14 months (95% CI 4–15 months) for the metronomically treated patients and 9 months (95% CI 7–10 months) for the control group. The 1-year secEFS was 61.1% (95% CI 35.3%–79.2%) for the metronomic and 35.4% (95% CI 29.8%–41.1%) for the control group. The stratified log rank test showed no difference in secEFS between

Table 1. Characteristics of patients with recurrent high-risk neuroblastoma treated according to the metronomic pilot trial.

Patient number	Sex f/m	age	stage at 1st diagnosis	recurrence number1	age1	tumor sites1	MNA yes/no	time 1stdx →1st rec.2	best response to METRO	side effects to METRO	final outcome
01	m	7.1y	4	2nd	10.10y	OM soft tissue	no	25 mo	SD for 5mo	leukopenia grade 3 thrombopenia grade 3	death at 12.2y
02	f	1.7y	4	1st	2.7y	OM CNS	yes	11 mo	SD for 3 mo	leukopenia grade 2 thrombopenia grade 2	death at 2.11y
03	f	3.3y	3→4(1st rec.)	1st	6.3y	PT OM	no	14 mo	PROG	leukopenia grade 2 thrombopenia grade 2	death at 6.7y
04	m	7.0y	3→4(2nd rec.)	2nd	11.2y	OM	no	36 mo	CR for 15 mo	leukopenia grade 1 thrombopenia grade 1	
				4th 3	14.7y	OM			SD for 6 mo 3		death at 15.4y
05	m	5.0y	4	1st	8.0y	CR	no	24 mo	CR for 6 mo	leukopenia grade 3 thrombopenia grade 4	death at 9.2y
06	m	5.7y	4	2nd	8.2y	PT OM	unknown	29 mo	PR for 12 mo.	none	death at 9.2y
07	m	30y	3→4(1st rec.)	2nd	33.6y	OM PT	no	51 mo	SD for 13 mo	anemia grade 3 thrombopenia grade 4	death at 40.0y
08	m	5.1y	3→4(1st rec.)	1st	7.2y	PT LN liver	no	14 mo	PR for 14 mo	liver toxicity (ALT) grade 3	death at 8.8y
09	f	4.10y	2b→4(2nd rec.)	2nd	8.1y	PT lungs LN	no	8 mo	SD for 22 mo.	leukopenia grade 4	death at 13.11y
10	f	6.2y	3→4 (1st rec)	2nd	8.5y	OM CNS	no	17 mo	PROG 5 weeks	leukopenia grade 4	LFU at 8.7y
11	m	7.2y	4	3rd	14.5y	OM	no	33 mo	PR for 14 mo	leukopenia grade 4 oral mucositis grade 3	AWD 16.8y
12	f	2.8y	4	2nd	4.0y	PT	yes	9 mo	SD for 5 mo	neutropenic fever grade 2 leukopenia grade 4 thrombopenia grade 3	death at 4.6y
13	m	3.11y	4	3rd	7.8y	PT intraspinal	no	16 mo	SD for 2 mo	neutropenic fever grade 3 leukopenia grade 4 thrombopenia grade 4	death at 8.0y
14	m	2.9y	4	1st	6.3y	CR	no	29 mo	CR for 28 mo	none	AWD 10.3y
15	m	5.0y	4	1st	8.0y	OM PT	yes	7 mo	SD for 24 mo	anemia grade 2 leukopenia grade 2 trombocytopenia grade 1	death at 11.1y from sec. MPNST

(*continued on next page*)

Table 1. Continued

Patient number	Sex f/m	age	stage at 1st diagnosis	recurrence number1	age1	tumor sites1	MNA yes/no	time 1stdx →1st rec.2	best response to METRO	side effects to METRO	final outcome
16	f	1.7y	4	refr.	3.9y	PT OM	no	—	PR for 24 mo	anemia grade 2 leukopenia grade 2 hyponatremia grade 1	AWD at 7.3y
17	m	2.4y	4	1st	5.6y	OM PT	yes	22	SD for 24 mo	anemia grade 2 leukopenia grade 2	AWD at 7.9y
18	f	2.3y	4	refr.	4.1y	OM	yes	—	CR for 22 mo	anemia grade 1 leukopenia grade 2 alevated creatinine grade 2	NED at 6.2y
19	f	2.6y	4	1st	4.2y	PT	no	18	PR for 25 mo	anemia grade 3 leukopenia grade 4 trombopenia grade 4 elevated creatinine grade 2 hypokalemia grade 3	AWD at 6.4y
20	m	2.11y	4	1st	3.7y	OM	no	8 mo	PR for 8 mo	none	AWD at 4.3y
21	f	3.4y	4	1st	7.3y	OM	no	27 mo	PR for 4 mo	leukopenia grade 2 thrombopenia grade 3	death at 7.9y
22	m	1.6y	4	1st	4.0y	OM	no	27 mo	PR for 2 mo.	leukopenia grade 2 thrombopenia grade 3	death at 4.4y
23	f	1.2	4	refr.	3.5y	OM	no	—	SD for 25month.	anemia grade 3 leukopenia grade 2 elevated creatinine grade 2	AWD at 5.6y

[1]At start of METRO therapy.
[2]Time interval from first diagnosis to first recurrence in months.
[3]The 4th recurrence in this patient was retreated by METRO (2nd recurrence counted only).
Note. AWD = alive with disease; CNS = central nervous system metastases; CR = complete remission; dx = diagnosis; f = female; liver = liver metastasis; lungs = lung metaststasis; m = male; mo = months; METRO = metronomic therapy according to the study; MNA = MYCN amplification; NED = no evidence of disease; OM = osteomedullary metastases; PR = partial remission PROG = progression; PT = primary tumor; rec. = recurrence; refr. = refractory active disease; SD = stable disease; sec. MPNST = secondary malignant peripheral nerve sheet tumor; y = years.

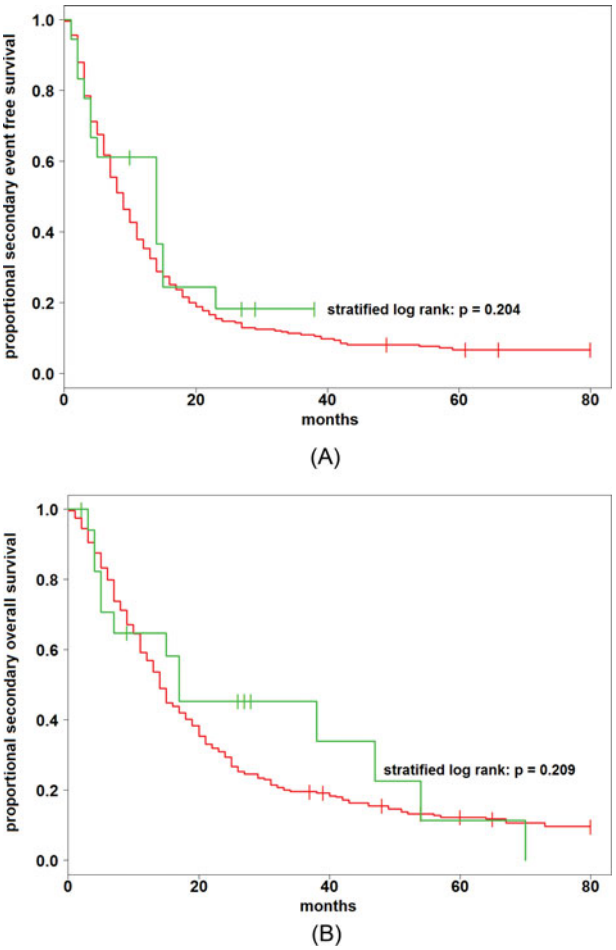

Figure 1. Kaplan–Meier estimates for secondary event-free survival (A) and secondary overall survival (B) for 18 metronomically treated patients (green curve) and for 274 matched control patients (red curve).

the groups ($p = .204$). The median time to death was 17 months (95% CI 5–47 months) for the metronomic and 14 months (95% CI 13–16 months) for the control group. The 1-year secOS was 64.7% (95% CI 37.7%−82.3%) for the metronomically treated children and 56.8% (95% CI 50.9%–62.6%) for the control group. The stratified log rank test demonstrated no significance of superiority for the metronomic patient group ($p = .209$). Figure 1 shows the Kaplan–Meier plots for the 18 study patients and the 274 control patients. The Supplemental Figure 1 displays the eight single strata for secEFS and secOS.

The side effects during metronomic therapy are shown in Table 1. All metronomically treated patients were pretreated by toxic cyclic chemotherapy including myeloablative therapy with stem cell support; almost all children showed hematologic toxicity up to grade 4 requiring some transient dose reduction or discontinuation of etoposide, cyclophosphamide and/or vinblastine at the discretion of the local physician. Elevated serum creatinine grade 2 was reported for 3 patients; neutropenic fever grade 2 and 3 was observed in 2 patients; stomatitis grade 3 was seen in 1 patient; and elevated liver enzyme (ALT grade 3) was noted in 1 patient. The metronomic therapy could be realized in an outpatient setting in all patients.

Discussion

An overview on pediatric clinical studies using various metronomic concepts has been published recently [23]. Table 2 shows a focused review confined to neuroblastoma patients. The main drugs used are cyclophosphamide, vinca alkaloids, and celecoxib in various doses and schedules. The number of neuroblastoma patients in these trials is limited (1–21), the treatment approaches differed, and the conclusions on efficacy are difficult to derive. However, all studies report a good to excellent tolerability of the treatment. This statement can be supported by the experience with the 23 neuroblastoma patients presented here. With the exception of leukopenia and thrombocytopenia ≥ grade 3 in these heavily pretreated patients, the number of other expected side effects like abdominal pain and constipation (celecoxib, vinblastine) and febrile episodes during neutropenic periods was either low or not observed at all. All patients could be treated in an outpatient setting.

Reliable quantitative data on side effects in the matched patients' cohort are lacking because there was no systematic collection. The reporting was restricted to sites of recurrence, response, treatment elements, and outcome. However, pediatric oncologists who are experienced in the second and higher lines of treatment of high-risk neuroblastoma patients, using the standard approaches with dose-intensive chemotherapy cycles ± other elements, will appreciate that most standard treatment approaches for recurrences require an inpatient setting and that it is associated with much higher proportions and grades of side effects, e.g. grade 4 neutropenic fever and blood product transfusions in almost all patients.

The metronomic approach was equally efficacious in comparison to matched patients treated with curative intention, but much less toxic. Patients of the control group who were treated with palliative intention were excluded from the analysis. All patients in the control group had first recurrences from high-risk neuroblastoma and underwent a second line treatment with toxic cyclic chemotherapy including second stem cell transplant. To avoid selection bias in the control group, the most powerful prognostic criteria identified in a large series of patients with recurrences were used for matching: time from first diagnosis to 1st recurrence (cut-off 18 months) and the number of organs involved at recurrence (1 vs. ≥2) [20]. In addition, MYCN amplification was included. This led to a study control ratio of 1 to 14, resulting in a robust estimation. A potential confounding variable was that the experimental group was dissimilar to the control group with respect to stage at first diagnosis. Six study patients had localized disease at first diagnosis, but all had metastatic disease at recurrence. Furthermore, nine children experienced two recurrences and two children three recurrences before starting metronomic therapy. Thus, we are convinced that this analysis is definitely not biased in favor of the study patients. The time to progression and the Kaplan–Meier curves for the study and the control groups were close to each other. The overall survival data were somewhat more favorable in the metronomically treated group, but overall survival data should be generally seen with caution because of the potential influence of further therapies consecutive to the metronomic treatment.

Meaningful comparisons are always difficult to perform if a study is not randomized. We tried to use a group of patients for comparison with equal or slightly better prognostic characteristics. The outcome is generally better for patients with first recurrences (control group) than for patients with >1 recurrence (metronomic group: 7 with two and 2 with three recurrences). Recurrences of stage 2b and 3 neuroblastoma are less unfavorable compared to only stage 4. Of the 6 patients with initial stages 2b and 3 in the metronomic group, all had metastatic recurrence at the start of metronomic therapy.

Table 2. Review of published data on metronomic therapy in neuroblastoma patients.

Patient population/disease setting	Phase of study	Regimen	Number of study patients	Number of neuroblastoma patients	Evidence of response in neuroblastoma patients	Reference
Neuroblastoma refractory or relapsed	Phase 1/2	Oral etoposide 50 mg/m² d1–21, 7days rest. Then repeat	20	20	Refractory: 1 /4 CR; 3 /4 SD Asymptomatic relapses: 2 /5 PR; 3 /5 SD Progression: 0 /11 responses	Kushner BH et al. 1999 J Clin Oncol 17:3221–5
Various recurrent pediatric tumors	Phase 1/2	Oral celecoxib 250 mg/m²xd, vinblastine 1 mg/m² 3x/wk i.v. or oral cyclophosphamide 30 mg/m²xd. Then repeat	33	3	Not reported	Stempak D et al. 2006 J Pediatr Hematol Oncol 28:720–8
Various recurrent pediatric tumors	Phase 1/2	Oral celecoxib 200 mg/m²xd d1–78, oral etoposide 25 mg/m²xd d1–21, oral temozolomide 60 mg/m²xd d36–77, isotretinoin 100 mg/m²xd d1–14, 29–42, 57–70. Then repeat	22	4	2 /4 VGPR, 1 /4 SD, 1 /4 PROG	Sterba J et al. 2006 Onkologie 29:308–13
Various recurrent pediatric tumors	Phase 1/2	Oral etoposide 25 mg/m²xd d1–14, oral cyclophosphamide 25 mg/m²xd d15–28, oral celecoxib 100–400 mg/d d1–28	17	1	1/1 PROG	André N et al. 2008 Clin Therapeutics 30: 1336–40
Various refractory pediatric tumors	Phase 1/2	Vincristine 1.5 mg/m² 1x/wk i.v. d1–22 cycle 1 and 2x/cycle following cycles, oral cyclophosphamide 25 mg/m²xd d1–21, oral methotrexate 15 mg/m² 2x/wk during d21–42	12	1	1/1 PROG	Fousseyni T et al. 2011 J Pediatr Hematol Oncol 33:31–4
Neuroblastoma refractory or relapsed	Phase 1/2	Zoledronic acid i.v. every 28 days in escalating doses, oral cyclophosphamide 25 mg/m²xd cont.	21	21	1 /21 PR, 9 /21 SD, 10 /21 PROG, 1 /21 not evaluable	Russell HV et al. 2011 Pediatr Blood Cancer 57:275–82
Various refractory pediatric tumors	Phase 1/2	Vinblastine 3 mg/m² 1x/wk wk 1–7, oral cyclophosphamide 30 mg/m²xd d1–21,oral methotrexate 10 mg/m² 2x/wk d21–42, oral celecoxib 100–400 mg/d d1–56	16	1	1/1 PROG	André N et al. 2011 Oncotarget 2:960–5

(*continued on next page*)

Table 2. Continued

Patient population/disease setting	Phase of study	Regimen	Number of study patients	Number of neuroblastoma patients	Evidence of response in neuroblastoma patients	Reference
Various recurrent pediatric tumors	Phase 2	COMBAT I-III schedules combining variously oral temozolomide 30–60 mg/m²xd d36–77, oral etoposide 25 mg/m²xd d1–21 or d1–35, oral celecoxib 200–400 mg/m²xd d1–77 or 1–78, oral cholecalciferol 300kU/m²xd d1, oral vitamin D3 1,500 U/m²xd d1–78, oral fenofibrate 100 mg/m²xd d1–78, isotretinoin 100 mg/m²xd d1–14, 29–42, 57–70, bevacizumab i.v. 10 mg/kg every 14 days	74	11	1/11 VGPR for >12 months, 1/11 PR for 63 months,8/11 temporary responses or SD, 1/11 PROG	Zapletalova D et al. 2012 Oncology 82:249–60.
Various relapsed or refractory pediatric tumors	Phase 2	Vinorelbine 25–30 mg/m²xd i.v. d1,8,15, oral cyclophosphamide 25 mg/m²xd d1–28. Then repeat without rest.	117	16	1/16 PR,1 /16 SD, 13/16 PROG	Minard-Colin V et al. 2012 Eur J Cancer 48:2409–16
Various refractory pediatric tumors	Phase 2	Oral methotrexate 15 mg/m² 2x/wk weeks 5–7, oral cyclophosphamide 30 mg/m²xd d1–21, vincristine 1.5 mg/m² i.v. 1x/wk weeks 1,2,3,4,9,13, oral valproic acid 200 mg/kgxd cont. wk1–15	7	1	1/1 PR	Traore F et al. 2013 Indian J Cancer 50:250–3
Various relapsed or refractory pediatric tumors and leukemias	Phase 2	Oral thalidomide 3 mg/kg start and weekly increase by 50 mg as tolerated to 24 mg/kg (maximum 1,000 mg/d), oral celecoxib <20kg 2x100 mg/d, 20–50kg 2x200 mg/d, >50kg 2x400 mg/d, oral fenofibrate 90 mg/m²xd,oral etopside 35–50 mg/mxd d1–21 alternated with oral cyclophosphamide 2,5 mg/kgxd (maximum 100 mg) d1–21. Repeat up to 27 wk	97	3	2 /3 SD, 1 /3 PROG	Robison NJ et al. 2014 Pediatr Blood Cancer 61:636–42

Note. CR = complete remission; d = day; i.v. = intravenously; PR = partial remission; PROG = progressive disease; SD = stable disease; VGPR = very good partial remission; wk = week.

Two patients (05, 15) were in complete remission with inactive tumors after treatment of recurrence. The metronomic therapy was given as consolidation for the complete remission in those two patients. Inclusion of the two patients and the 3 patients with active refractory, but not actively progressing tumors (total group of study patients n = 23), demonstrated a trend towards better EFS and OS curves compared to the total group of control patients (n = 274). This may look astonishing but should be viewed with great reservation in our opinion.

The control group consisted of patients who were clinically well enough to undergo second line treatment with curative intention. All children with a poor prognosis who were receiving palliative therapy were excluded. Altogether, the number of patients for comparison was high and had a 1: 14 (range 6–25) ratio of metronomic study patients to the control group. Thus, we believe that the comparison between the metronomic and the control group is well balanced, fair, and meaningful.

In conclusion, this pilot study of 23 patients with recurrent and refractory high-risk neuroblastoma demonstrates an encouraging response to metronomic therapy accompanied by low toxicity and realization in an outpatient setting. The brief median survival time (EFS and OS) is an important argument in favor of offering a low toxic regimen in the outpatient setting with an at least equal outcome. The approach is continued in a larger study with the addition of a fifth drug (oral propranolol).

Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

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References

[1] Annual Report 2013/14 German Childhood Cancer Registry. www.kinderkrebsregister.de p. 39. [Assessed 2016, August 9th]
[2] Simon T, Berthold F, Borkhardt A, et al. Treatment and outcomes of patients with relapsed, high-risk neuroblastoma: Results of German trials. *Pediatr Blood Cancer*. 2011;56(4):578–583.
[3] Pinto NR, Applebaum MA, Volchenboum SL, et al. Advances in risk classification and treatment strategies for neuroblastoma. *J Clin Oncol*. 2015;33:3008–17.
[4] Munzone E, Bertonini F. Colleoni M. Metronomic chemotherapy in breast cancer. In: Bocci G, Francia G, eds. *Metronomic Chemotherapy. Pharmacology and Clinical Applications*. Berlin Heidelberg: Springer-Verlag;2014:93–110.
[5] Salvatore L, Zoratto F, Loupakis F, et al. The role of metronomic chemotherapy in the treatment of metastatic colorectal cancer. In: Bocci G, Francia G, eds. *Metronomic Chemotherapy. Pharmacology and Clinical Applications*. Berlin Heidelberg: Springer-Verlag;2014:135–142.
[6] Delos Santos K, Sivanathan L, Lien K, et al. Clinical trials of low-dose metronomic chemotherapy in castration-resistant prostate cancer. In: Bocci G, Francia G, eds. *Metronomic Chemotherapy. Pharmacology and Clinical Applications*. Berlin Heidelberg: Springer-Verlag;2014:119–134.
[7] Meitar D, Crawford SE, Rademaker AW, et al. Tumor angiogenesis correlates with metastatic disease, N-myc amplification, and poor outcome in human neuroblastoma. *J Clin Oncol*. 1996;14:405–414.
[8] Klement G, Baruchel S, Rak J, et al. Continuous low-dose therapy with vinblastine and VEGF receptor-2 antibody induces sustained tumor regression without overt toxicity. *J Clin Invest*. 2000;105:R15–R24.

[9] Bruneel A, Labas V, Mailloux A, et al. Proteomics of human umbilical vein endothelial cells applied to etoposide-induced apoptosis. *Proteomics*. 2005;5:3876–3884.
[10] Bocci G, Francia G, Man S, et al. Thrombospondin 1, a mediator of the antiangiogenic effects of low-dose metronomic chemotherapy. *Proc Natl Acad Sci U S A*. 2003;100:12917–12922.
[11] Qian BZ, Pollard JW. Macrophage diversity enhances tumor progression and metastasis. *Cell*. 2010;100:39–51.
[12] Wang YC, He F, Feng F, et al. Notch signaling determines the M1 versus M2 polarization of macrophages in antitumor immune responses. *Cancer Res*. 2010;70:4840–4849.
[13] Ghiringhelli F, Larmonier N, Schmitt E, et al. CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. *Eur J Immunol*. 2004;34:336–344.
[14] Asgharzadeh S, Salo JA, Ji L, et al. Clinical significance of tumor-associated inflammatory cells in metastatic neuroblastoma. *J Clin Oncol*. 2012;30(28):3525–3532.
[15] Nakanishi Y, Nakatsuji M, Seno H, et al. COX-2 inhibition alters the phenotype of tumor-associated macrophages from M2 to M1 in ApcMin/+ mouse polyps. *Carcinogenesis*. 2011;32:1333–1339.
[16] Kaneko M, Kaneko S, Suzuki K. Prolonged low-dose administration of the cyclooxygenase-2 inhibitor celecoxib enhances the antitumor activity of irinotecan against neuroblastoma xenografts. *Cancer Sci*. 2009;100:2193–2201.
[17] Tanaka H, Matsushima H, Nishibu A, et al. A Dual therapeutic efficacy of vinblastine as a unique chemotherapeutic agent capable of inducing dendritic cell maturation. *Cancer Res*. 2009;69:6987–6994.
[18] Brodeur GM, Pritchard J, Berthold F, et al. Revisions of the international criteria for neuroblastoma diagnosis, staging, and response to treatment. *J Clin Oncol*. 1993;8:1466–1477.
[19] Berthold F, Spix C, Kaatsch P, et al. Incidence, Survival, and Treatment of Localized and Metastatic Neuroblastoma in Germany 1979–2015. Pediatr Drugs 2017. https://doi.org/10.1007/s40272-017-02513
[20] Klein JP, Moeschberger ML. *Survival Analysis: Techniques for Censored and Truncated Data*, 2nd ed. New York: Springer; 2003.
[21] Berthold F, Volland R, Simmon T, et al. Characteristics and risk factors of 517 patients with first recurrence from stage 4 neuroblastoma over 18 months. Advances in Neuroblastoma Research Congress, Cairns, Australia, 19th–23rd. June 2016;Abstract 157:135. www.ANR2016.org→Cairns2016ProgramsandAbstracts(pdf).
[22] André N, Carré, Pasquier E. Metronomics: towards personalized chemotherapy? *Nature Rev*. 2014;11:413–431.
[23] Baluch N, Kumar S, Mokhtari R, et al. Metronomic chemotherapy in pediatric malignancies. In: Bocci G, Francia G, eds. *Metronomic Chemotherapy. Pharmacology and Clinical Applications*. Berlin Heidelberg: Springer-Verlag; 2014:157–172.
[24] Pantziarka P, Hutchinson L, André N, et al. Next generation metronomic chemotherapy-report from the fifth biennial international metronomic and anti-angiogenic therapy meeting, 6–8 May 2016, Mumbai. *Ecancermedicalscience*. 2016;10:689. eCollection2016. PMID 27994645.