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Clinical trial

METRO-PD1: Phase 1 study of nivolumab in combination with metronomic chemotherapy in children and adolescents with relapsing/refractory solid tumors

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ABSTRACT

Background: This multicenter Phase I study (NCT03585465) evaluated nivolumab in combination with 3 metronomic chemotherapy (MC) regimens in children with refractory/relapsing solid tumors. **Objectives**: To evaluate the feasibility and safety of the three regimens

Methods: Patients aged < 18 years were enrolled. Nivolumab was combined with cyclophosphamide and vinblastine (arm A), capecitabine (arm B), or cyclophosphamide, vinblastine and capecitabine (arm C). Arm A and B were allocated sequentially. Arm C opened only if A and B were deemed safe. Dose-limiting toxicities (DLTs) were evaluated over the first two cycles. Patients were evaluable if they received > 2 cycles and > 70% of the planned dose.

Population: Sixteen patients were enrolled, 3 in arm A, 6 in arm B, and 7 in arm C. Median age was 11.5 years (range, 5–19). Patients previously received a median of 3.5 (range, 1–4) lines of systemic treatment, 14 patients had surgery and 11 had radiotherapy.

Results: Median number of cycles was 2 (1−24), median treatment duration was 56 days (18−714). In arm C, median number of cycles was 4 with median treatment duration of 95 days. No DLT was observed. Grade 3 adverse events (AE) and serious AE were observed in 8 patients (50%) and 1 patient (6%), respectively, over the first 2 cycles. No grade 4 AE occurred. The 6-month PFS and OS were 12% and 44%, respectively, in the whole population. Prolonged stable disease was observed in a high-grade glioma and an atypical teratoid rhabdoid tumor.

Conclusion: Arm C appears safe. A randomized phase II trial evaluating the addition of nivolumab to the triple MC is ongoing.

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1. Introduction

Nowadays, an overall survival (OS) of 80% is reached among children, adolescents, and young adults diagnosed with cancer in high income countries. However, this also implies that 20% still die from recurrent or refractory disease [1]. Unveiling new therapeutic approaches with better efficacy and low toxicity is crucial to further improve the survival of children with cancer.

Recently, immune checkpoint inhibitors (ICI), and more specifically those acting through the programmed cell death protein 1 (PD-1) pathway have yielded a considerable interest in pediatric oncology following ground breaking results obtained in some adult malignancies such as melanoma, lymphoma or lung carcinoma, and synergistic combinations based on immune checkpoint inhibitors are promising approaches to fight cancer [2]. However, single-agent ICIs in children have so far demonstrated limited activity in pediatric tumors with responses observed in Hodgkin lymphoma, hyper-mutated tumors, and few rare tumor types [3–9]. To find the right combinations to overcome intrinsic resistance to ICI in pediatric tumors is therefore a crucial issue but the number of possibilities to be tested is almost infinite [10].

Metronomic chemotherapy (MC) consists in giving low doses of anticancer agents on a daily/weekly basis [11–13]. MC has been showed to be both a safe and effective way to administer chemotherapy to obtain anti-cancer effects through new mechanisms of action. Indeed, changes in pharmacokinetics (PK) related to the higher frequency and lower dose induce changes in pharmacodynamics (PD) [12]. Consequently, MC targets distinct features of tumor biology and has been shown to inhibit tumor angiogenesis at least in part by increasing thrombospondin-1, decreasing vascular endothelial growth factor (VEGF) and circulating endothelial progenitor cells as well as killing endothelial cells and blocking their pro-angiogenic functions [13]. MC can also directly target cancer cells or cancer stem cells [12]. Lastly, the immune system can also be stimulated through multiple mechanisms (e.g. selective depletion in regulatory T cells, modulation of myeloid-derived suppressor cells or maturation of dendritic cells) [14].

More precisely, CD4+ CD25+ regulatory T cells (Tregs) have become an intense focus of cancer research [15] as the elimination of Tregs within the tumor microenvironment, is considered to be critical for successful immunotherapy. MC can lead to depletion or neutralization of Tregs [11,14–17]. For instance, oral metronomic cyclophosphamide in advanced cancer patients can induce a profound and selective reduction of circulating Tregs cells, associated with a suppression of their inhibitory functions on conventional T cells and natural killer (NK) cells leading to a restoration of peripheral T cell proliferation and innate killing activities [16].

Elsewhere, MC can also electively deplete myeloid-derived suppressor cells (MDSCs) while preserving T cells subset which may enhance latent tumor immunity [18]. Several groups have reported changes in MDSCs when using 5-FU/capecitabine in mice in different tumors models [19–21] and Peereboom and al. have shown that doses of capecitabine 300 or 450 mg BID could lead to a reduction in circulating MDSCs in adult patients with glioblastoma [22].

Dendritic cells (DC) are the most potent antigen-presenting cells in the induction of primary immune responses [23]. Accumulating data demonstrate that DC-based immunotherapy can induce strong anti-tumor immune responses in multiple pre-clinical and clinical trials [23]. Stimulation of DC functions is associated with the up-regulation of expression of antigen (Ag) processing machinery components and co-stimulatory molecules on DC, as well as increased interleukin-12 expression [24]. Some studies have demonstrated that DC maturation can be obtained with low-dose vinblastine [24–26].

Accordingly, combining MC to anti-PD1 shall not only prevent chemotherapy induced immunosuppression but can also paradoxically specifically deplete or mature cells from the immune system and may in turn strengthen the inhibition of the immune blockade obtained with anti-PD1.

We proposed here to target different cellular components of the immune system using different anticancer agents given in a metronomic manner and ultimately combine metronomic chemotherapy regimen and nivolumab to generate a multiple targeted restoration of the immune system. We report herein the results of the phase I part of the METRO-PD1 (NCT03585465) trial evaluating nivolumab in combination with MC chemotherapy in children and adolescents with relapsing/refractory solid tumors.

2. Materiel and methods

2.1. Study design

This is an international, multicentric, interventional, open-label, non-comparative, and non-randomized phase I study. The main objective of the study was to evaluate three MC regimens (arm A, B and C given in combination with nivolumab in children and adolescent with refractory/relapsing solid tumors. The three MC regimen were defined as vinblastine and cyclophosphamide (arm A), capecitabine alone (arm B) and vinblastine, cyclophosphamide and capecitabine (arm C). If arm C was evaluated as safe, it would be chosen as the RDP2-like arm given its broader potential for modulating the immune system. The secondary objective was to evaluate the safety profile of the different combinations of MC when given in combination with nivolumab in this population during the whole treatment duration.

2.2. Study population

Main eligibility criteria were: patients aged < 18 years (or above if diagnosis made before 18 years old) with a relapsed or refractory malignancy, evaluable or measurable disease, Lansky Play scale (for patients $\leq$16 years of age) or Karnofsky performance status at least 70%, adequate organ function and ability to comfortably swallow oral medications. Patients with a known partial deficiency of dihydropyrimidine-deshydrogenase (DPD) if uracilemia value of $\geq$ 16 ng/ml and $<$ 150 ng/ml; stable doses of corticosteroids ($<$ 0.25 mg/kg/d prednisolone or equivalent) during the 7 days prior to receiving study drugs; prior treatment with anti-PD1 or anti-PDL1 allowed if at least stable disease was obtained for 6 months. Main exclusion criteria were symptomatic central nervous system (CNS) metastases who are neurologically unstable; complete deficiency of DPD activity (uracilemia $\geq$150 ng/ml). Patients with CNS tumor were excluded in case of evidence of $>$ Grade 1 recent CNS hemorrhage and in case of bulky tumor defined as tumor with any evidence of severe midline shift, largest diameter $>$ 6 cm on contrast-enhanced MRI.

2.3. Ethic

Patients and/or their legal guardians gave written informed consent, and assent was obtained as appropriate at the time of enrollment. The protocol and amendments received regulatory approvals from independent ethics committees and complied with the French regulations and the declaration of Helsinki.

2.4. Treatment plan

Treatment was given in 28-day cycles. Nivolumab was given intravenously (IV) at the dose of 3 mg/kg on day 1 and day 15 of each cycle in all 3 arms (A, B and C). In arm A, vinblastine was administered at the dose of 2 mg/m^2 IV weekly, together with oral cyclophosphamide 30 mg/m^2/day on day 1 to 4, day 8 to day 11, day 15 to day 18, and day 22 to day 25. In Arm B, capecitabine was orally given as single agent at a dose ranging from 400 to 600 mg/m^{2}daily. In arm C, vinblastine was administered at the dose of 2 mg/m^2 IV weekly, together with oral cyclophosphamide at the dose of 30 mg/m^2/day from day 1 to day 4 and day 15 to day 18 alternating with oral capecitabine at the dose ranging

from 400 to 600 mg/m^2 from day 8 to day 11 and from day 22 to day 25. Treatment continued until progressive disease (PD), unacceptable toxicity, patient or legal representative withdrawal of consent, or investigator's decision, for a maximum of 2 years.

2.5. Statistical design

Three patients + /− 3 additional patients were planned to be sequentially enrolled in arm A and in arm B (A/B/A/B/A/B). In each arm, the second patient was not recruited before the first patient had been observed for a 28-day duration. Safety of Arm A and Arm B were deemed acceptable if the number of dose-limiting toxicities (DLT) (as defined in the subsection below) was 0/3 or 1/6. In case arm A and arm B were deemed feasible, next patients were then enrolled in arm C. Six patients were planned to be enrolled in arm C unless 2 DLTs are reported in the first patients. Safety of arm C was deemed acceptable if the number of DLT was 0 or 1/6 patients. A patient was deemed not evaluable for safety if he received less than 70% of the planned dose for at least one drug over the first 56 days after start of treatment for a reason other than toxicity (early stop for progression). Non evaluable patients were replaced. This algorithm was associated with a probability $\geq$ 67% of correct selection of the arm to be recommended in a future phase 2 study if the true probability is $\leq$ 15% in the "safe arm", compared to 40% in the unsafe arm. The probability of stopping the trial with the conclusion of unsafe combination in all arms was 48% if both arms A and B were associated with a 40% probability of DLT.

2.6. Safety evaluation

Safety of the study treatment was evaluated based on the clinical and biological evaluations, adverse events (AE) (type, grade) were graded according to the NCI-CTCAE v5.0 criteria per 28-day cycle and over the whole treatment duration. All AEs occurring during treatment or in the 28 days after end of treatment were reported, regardless of reported causal relationship, except symptoms unequivocally related to the underlying disease or its progression. DLT and serious adverse events (SAEs) were reported over the whole treatment duration plus 28 days, for all patients. After the end of the treatment, only SAE related to the study treatment have been recorded.

DLTs were evaluated over the first two 28-day cycles to explore the potential occurrence of slower occurring DLT related to the lower dosing used with metronomic. DLTs were defined as follows: grade 4 neutropenia for more than 7 days, grade 3 or 4 thrombocytopenia requiring transfusions for more than 7 days, febrile neutropenia with or without documented infection, grade 3 or 4 non-hematologic toxicities, or grade 2 toxicities that are considered not tolerable for the patient. Toxicity leading to significant dose reduction for at least one molecule (< 70% of planned dose over the first 56 days) was also considered as a DLT, even if the grade of toxicity did not in itself justify this classification. Toxicities not considered as DLT included < 72 h of grade 3 fatigue, grade 3 fever or infection without neutropenia and lasting < 5 days, grade 3 laboratory abnormalities that were responsive to oral supplementation or deemed by the investigator to be clinically insignificant and adverse events unequivocally related to the underlying disease or its progression. Descriptive analysis (types of adverse events, grades) have been performed to document safety over the whole treatment duration.

2.7. Efficacy evaluation

Response assessment was based on Magnetic Resonance Imaging, Computer Tomography, Scintigraphy according to disease and extension, evaluated every two cycles. Tumor response was evaluated by the local investigator using Response Assessment in Neuro-Oncology (RANO), International Neuroblastoma Response Criteria (INRC), WHO, or Response Evaluation Criteria In Solid Tumors (RECISTv1.1), for glioma, neuroblastoma, other brain tumors, and other solid tumors, respectively. Best response was evaluated over the whole duration of treatment. Progression-free (PFS) was defined as the time from study entry to the date of progression or death, whichever occurred first. Overall survival (OS) was defined as the time from study entry to the date of death of any cause. In the absence of any event, patients were censored at the date of the last follow-up.

The PFS and OS curves have been estimated using the Kaplan–Meier method, with their 95% confidence intervals (CIs).

We compared the distribution of the best response between the three arms using Fisher exact test (post hoc analysis).

2.8. Treatment feasibility evaluation

All dates and treatment doses were reported in the database with the reasons for temporary treatment discontinuation or dose reduction if any, as well as the reason for definitive treatment discontinuation. The relative dose-intensity of the different drugs was estimated for each drug as the ratio between the computed dose-intensity (cumulative dose expressed in mg/m^2 divided by the study duration and expressed in mg/m^2/week) and the protocol dose-intensity.

2.9. Sample analysis

Tumor cellularity in specimens from the sample used for nucleic acid extraction was determined by an experienced pathologist; those with $\geq$ 30% tumor cellularity were processed. Tumor DNA, RNA, and germline DNA from whole blood samples were extracted using the AllPrep DNA/RNA Mini Kit and DNeasy Blood and Tissue Kit. Then, *WES* and RNA-Seq & *Mutational Tumor Load* were performed as previously described [27].

3. Results

3.1. Patient and tumor characteristics

Sixteen patients were included in the phase I part of the trial between March 2019 and September 2020. Median age at study entry was 11.5 years (range 5–19) and 11 patients (69%) were male. Main diagnoses were neuroblastoma (n = 5), high-grade glioma (HGG) and diffuse midline glioma (DMG) (n = 2), Ewing sarcoma (n = 2), medulloblastoma (n = 2) and other non-brain tumors (n = 4). Details of the population, previous treatment and underlying malignancies are summarized in Table 1. Patient characteristics including molecular profiling data when performed through the MAPPYACTS trial [26] is given in Table 2.

Patients had all previously received a systemic treatment, with a median of 3.5 lines (range 1–4) including chemotherapy containing anthracyclines or other cardiotoxic treatments in 8 patients (50%). Eleven patients (69%) had received radiotherapy; local treatment also included surgery in 14 patients (88%) and radiofrequency in 1 patient. None of the patients had received previous treatment with anti-PD1 or anti-PDL1.

3.2. Treatment exposure

Three patients were treated in arm A, 6 patients in arm B and 7 patients in arm C (Fig. 1). The percent of dose received was < 70% over the first 2 cycles for at least one drug for 3 patients (2 in arm B, 1 in arm C) due to early tumor progression. These patients were deemed not evaluable for DLT and were replaced as planned in the study protocol. In addition, one patient was initially classified as not evaluable for DLT due to an early stop of treatment, and consequently replaced; however this patient had actually received> 70% of doses of all drugs over the first 2 cycles, and was finally reviewed as evaluable for the DLT, leading to a cohort of 4 patients in arm B. Median number of cycles was 2 (range, 1–24) and median treatment duration was 56 days (range, 18–714). Of note, for arm C median number of cycles was 4 (range, 1–24) and median treatment duration was 95 days (range, 18–714). Overall, 68 cycles

Table 1
Patient and tumor characteristics at study entry (n = 16).

Characteristics	Arm A n = 3	Arm B n = 6	Arm C n = 7	Total n = 16
Age at study entry (years)				
Median (Range)	15 (5-16)	10.5 (6-13)	13 (6-19)	11.5 (5-19)
Mean (SD)	12 (6.1)	10 (2.6)	12.4 (5.0)	11.4 (4.3)
Sex				
Male	1 (33.3%)	5 (83.3%)	5 (71.4%)	11 (68.8%)
Female	2 (66.7%)	1 (16.7%)	2 (28.6%)	5 (31.2%)
Time interval from initial diagnosis (months)				
Median (Range)	24.3 (22.0-36.7)	42.3 (7.2-102.1)	28.4 (6.5-117.5)	32.0 (6.5-117.5)
Mean (SD)	27.7 (7.9)	48.2 (36.5)	47.5 (42.1)	44.0 (35.0)
Histological type				
CNS tumors	1 (33.3%)	1 (16.7%)	3 (52.9%)	5 (31.3%)
DMG K27M-mutant	*0*	*0*	*1*	*1*
Anaplastic PXA	*0*	*0*	*1*	*1*
Medulloblastoma	*1*	*1*	*0*	*2*
ATRT	*0*	*0*	*1*	*1*
Non-CNS tumors	2 (66.6%)	5 (83.3%)	4 (57.2%)	11 (68.8%)
Neuroblastoma	1 (33.3%)	2 (33.3%)	2 (28.6%)	5 (31.3%)
RCC TFE3-fused	*0*	*1*	*0*	*1*
FL-HCC	*0*	*0*	*1*	*1*
Embryonal Rhabdomyosarcoma	*0*	*0*	*1*	*1*
Osteosarcoma	*0*	*1*	*0*	*1*
Ewing sarcoma	*1*	*1*	*0*	*2*
Metastatic disease at diagnosis	3 (100.0%)	3 (50.0%)	3 (42.9%)	9 (56.3%)
Disease status at study entry				
Relapsed disease	3 (100.0%)	6 (100.0%)	5 (71.4%)	14 (87.5%)
Refractory disease	0	0	2 (28.6%)	2 (12.5%)
Time from last progression/relapse (months)				
Median (Range)	1.3 (0.5-3.7)	0.7(0.2-3.5)	0.5 (0.3-1.4)	0.6 (0.2-3.7)
Mean (SD)	1.8 (1.7)	1.1 (1.2)	0.6 (0.4)	1.0 (1.1)

PXA: Pleomorphic Xanthoastrocytoma; ATRT: Atypical Teratoid and Rhabdoid Tumor; DMG: Diffuse Midline Glioma; RCC: Renal Cell Carcinoma; FL-HCC: Fibrolamellar Hepatocellular Carcinoma

were started in 16 patients. Interestingly, the relative dose-intensity over the treatment duration was ≥ 87% for all the drugs and all the patients. Fig. 2.

At time of analysis, all patients have stopped study treatment, 14 due to disease progression, 1 due to parent decision, and 1 after completion of the 24 cycles planned in the protocol.

3.3. Safety evaluation

Thirteen out of 16 patients were available for DLT. No DLT was observed during the DLT period observation corresponding to the first 2 treatment cycles. Overall, treatment was deemed safe and feasible. No grade 4 adverse event was reported over the whole treatment duration. One patient (6%) did not experience any adverse events related to study treatment during all duration of treatment. Details of adverse events are given in Tables 3 and 4.

The most frequent clinical grade 3 adverse event was asthenia. The most frequent grade 3 biological adverse event was lymphopenia. Considering all AEs reported over the whole treatment duration, a grade ≥ 3 adverse event was reported in 8 patients (see Table 4 for details).

Five SAEs were reported in four patients (25%) in the study (1 in arm A, 2 in arm B, 2in arm C). All occurred during cycle 1 or 2. All but one was related to disease progression. A patient included in arm A presented with cytokine release syndrome, considered as grade 2 and related to the study drugs, leading to vomiting (grade 1), abdominal pain (grade 1), fever (grade 2), shiver (grade 1) and tachycardia (grade unknown). Treatment was continued for another cycle without AE and then discontinued for disease progression. Two patients included in arm B experienced SAE: pleural effusion and pain, respectively. Both patients had lungs metastasis. One patient included in arm C experienced 2 SAEs due to pruritus. No immune related severe adverse events were reported (Tables 3 and 4).

3.4. Efficacy evaluation

The 3-month and 6-month progression-free survival rates were 37% (95%CI: 15–60) and 12% (95%CI: 2–33), respectively. The 6-month OS was 44% (95%CI: 20–66). Fifteen events were observed, all were disease progression. Best overall response was stable disease for 6 patients (37%) while a disease progression was reported at the first evaluation for 10 patients (63%). As detailed in Table 2, a stable disease was achieved in 0/3 patients (0%) of arm A, 1/ 6 patients (17%) of arm B and 5/ 7 patients (71%) of arm C (Fisher exact test, p-value=0.09). Interestingly, one patient with ATRT included in arm C was alive free of

Table 2
Patient characteristics and response to treatment, including molecular profiling of tumors in 11 patients with WES and RNA-seq performed previously.

Patient number	Age (yr)	Gender	Histology	Treatment Arm	Main molecular abnormalities	TMB Mut/Mb	Response to treatment
1	15	F	Medulloblastoma group WNT	A	APC mutation	0.1	PD
2	6	F	Neuroblastoma	B	Del 11q (ATM), FBX05 mutation	0.2	PD
3	5	M	Ewing sarcoma	A	-	ND	PD
4	13	M	Ewing sarcoma	B	EWS-FLI fusion	0.6	PD
5	16	F	Ewing sarcoma	A	TP53 mutation, STAG2 mutation, EWS_FLI fusion	0.7	PD
6	8	M	Renal cell carcinoma	B	PRCC-TFE3 fusion, FANC-D2 mutation, TP53 mutation	0.1	PD
7	12	M	Medulloblastoma group 4	B	None	0.5	SD
8	11	M	Neuroblastoma	B	Del 11q (ATM, CHK1)	1.4	PD
9	10	M	Osteosarcoma	B	Del 13q (RB), PIK3R1	0.4	PD
10	18	F	Fibrolamelar hepatic carcinoma	C	DNAJB1_PRKACA fusion	0.1	SD
11	13	M	Midline grade IV glioma	C	-	ND	SD
12	9	M	Embryonal Rhabdomyosarcoma	C	PI3K3CA mutation, MYOD1 mutation, GNAI2 mutation, del9p (CDKN2A/B)	0.8	PD
13	6	F	Neuroblastoma	C	-	ND	SD
14	8	M	Atypical Teratoid and Rhabdoid Tumor	C	-	ND	SD
15	14	M	Neuroblastoma	C	-	ND	PD
16	19	M	Anaplastic xanthoastrocytoma	C	-	ND	SD

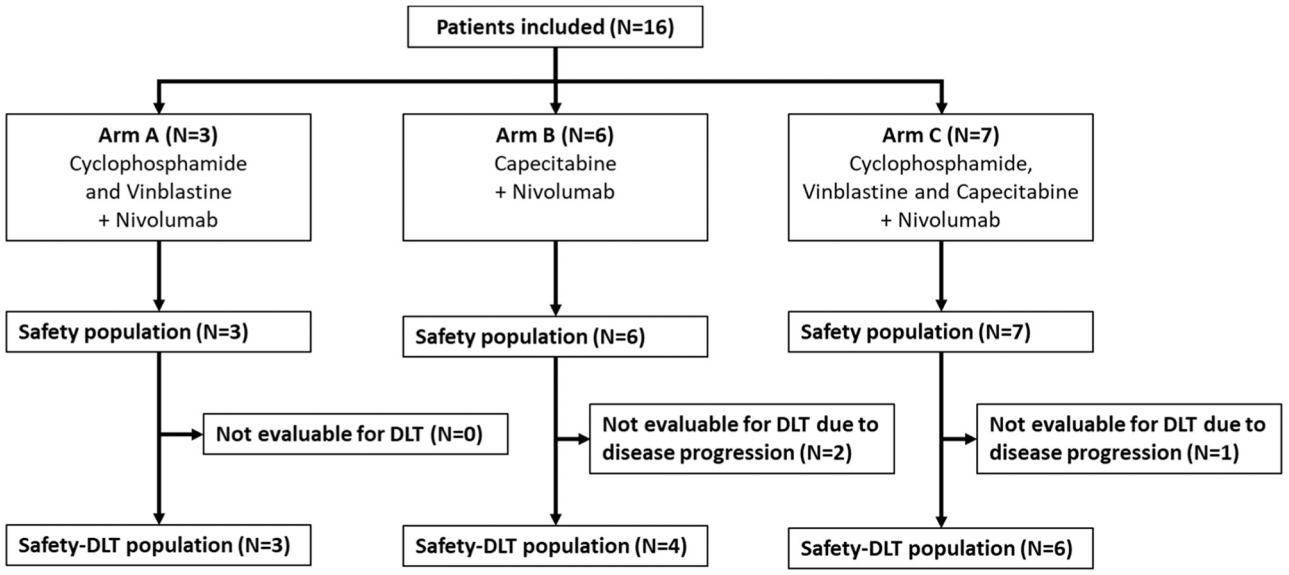

Fig. 1. Flow-chart of the study.

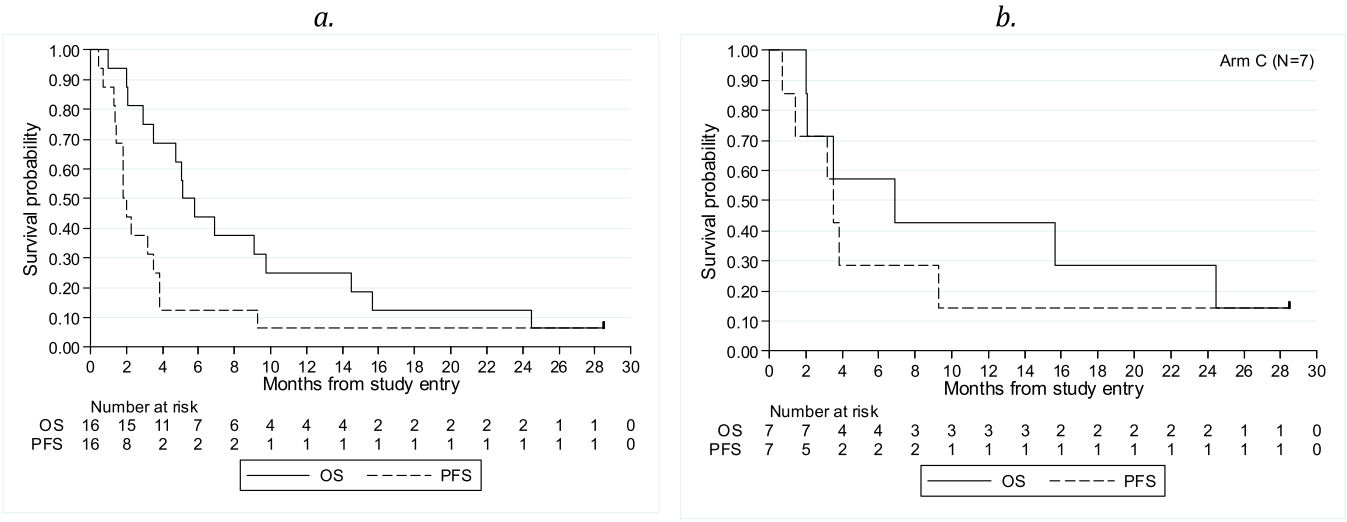

Fig. 2. Kaplan-Meier curves for overall survival (OS) and progression-free survival (PFS) of all patients (a, N = 16) and patients included in arm C (b, N = 7).

progression at the date of last news with a follow-up of 28.5 months. He received 24 cycles of treatment Another patient with an anaplastic xantho-astrocytoma had a prolonged disease stabilisation with a PFS duration of 9.3 months and an overall survival of 24.5 months.

4. Discussion

We report here the results of a phase 1 trial in children and adolescent with relapsing/refractory solid tumors evaluating the combination of three metronomic chemotherapies and an anti-PD1 inhibiting antibody. The trial was designed based on the pro-immune anti-cancer properties of metronomic chemotherapy [11,12,14,23]. We made the hypothesis that the immune effect of metronomic chemotherapy together with the lack of major haematological toxicity could leverage the activity of nivolumab.

All arms were well tolerated with no DLT being observed in any of the 3 arms. Furthermore, only 4 patients reporting at least one grade 3 lymphopenia were observed confirming the lack of overt toxicity on white blood cells of our regimen. For each metronomic agent, the dosing used in this study was extrapolated from available in vitro, in vivo and clinical data. [15–26]. Overall, it led to using lower doses than those usually used in the clinic [28] consistent with the metronomic paradigm. This has very likely contributed to the good safety profile of the combinations evaluated here, with for instance the lack of palmar-plantar erythrodysesthesia syndrome commonly observed with capecitabine. Nevertheless, one patient presented with a reversible minor Cytokine Release Syndrome. It has been possibly attributed to nivolumab since the association has already been reported [29] and that we could not find any report of its association with metronomic chemotherapy.

Both preclinical and clinical evaluations of the combination of metronomic chemotherapy and immune checkpoint inhibitors have confirmed both the safety as well as the potential activity of combining those two approaches in miscellaneous adult malignancies for instance with metronomic pemetrexed-oxaliplatin and antiPD1 for colon cancer [30], metronomic gemcitabine and antiPD1 for lung cancer [31] or metronomic navelbine with durvalumab for miscellaneous advanced solid tumors [32].

As the anti-tumoral activity of ICI in paediatric oncology has been so far very limited, many trials are now investigating combination of ICI with targeted agents, other ICI or other immune modulators to reverse

Table 3
Safety profile over the first two treatment cycles (patients with NCI-CTCAE grade ≥3).

Type of adverse events	Arm A (n = 3)		Arm B (n = 6)		Arm C (n = 7)		Overall (n = 16)	
Any type*	1	**33%**	3	**50%**	3	**43%**	7	**44%**
Blood disorders	**1**	**33%**	**2**	**33%**	**2**	**29%**	**5**	**31%**
Anaemia	0	0%	2	33%	1	14%	3	19%
Lymphopenia	1	33%	1	17%	1	14%	3	19%
Thrombocytopenia	0	0%	0	0%	1	14%	1	6%
Cardiac disorders	**0**	**0%**	**0**	**0%**	**1**	**14%**	**1**	**6%**
Presyncope	0	0%	0	0%	1	14%	1	6%
General disorders	**0**	**0%**	**2**	**33%**	**1**	**14%**	**3**	**19%**
Asthenia	0	0%	2	33%	1	14%	3	19%
Infections and infestations	**0**	**0%**	**1**	**17%**	**0**	**0%**	**1**	**6%**
Venous access device related infection	0	0%	1	17%	0	0%	1	6%
Metabolism and nutrition disorders	**0**	**0%**	**1**	**17%**		**0%**	**1**	**6%**
Hypokalaemia	0	0%	1	17%	0	0%	1	6%

*Considering the maximum grade of adverse event per patient, whatever the type
The table represents the number of patients experiencing adverse events of grade 3 per system organ class (SOC) and per preferred term (PTname) of the MEDDRA dictionary (N = 16). All AE observed during the 2 first cycles of treatment are described, related or not to the study treatment, except those classified as related to the underlying tumour or its progression.

Table 4
Safety profile over the whole treatment period (Patients with NCI-CTCAE grade >3).

	Arm A (n = 3)		Arm B (n = 6)		Arm C (n = 7)		Overall (n = 16)	
Any type*	1	**33%**	3	**50%**	4	**57%**	8	**50%**
Blood disorders	**1**	**33%**	**2**	**33%**	**3**	**43%**	**6**	**38%**
Anaemia	0	0%	2	33%	1	14%	3	19%
Lymphopenia	1	33%	1	17%	2	29%	4	25%
Thrombocytopenia	0	0%	0	0%	1	14%	1	6%
Cardiac disorders	**0**	**0%**	**0**	**0%**	**1**	**14%**	**1**	**6%**
Presyncope	0	0%	0	0%	1	14%	1	6%
General disorders	**0**	**0%**	**2**	**33%**	**1**	**14%**	**3**	**19%**
Asthenia	0	0%	2	33%	1	14%	3	19%
Infections and infestations	**0**	**0%**	**1**	**17%**	**0**	**0%**	**1**	**6%**
Venous access device related infection	0	0%	1	17%	0	0%	1	6%
Metabolism and nutrition disorders	**0**	**0%**	**1**	**17%**	**1**	**14%**	**2**	**13%**
Decreased appetite	0	0%	0	0%	1	14%	1	6%
Hypokalaemia	0	0%	1	17%	0	0%	1	6%

*Considering the maximum grade of adverse event per patient, whatever the type
The table represents the number of patients experiencing adverse events of grade 3 per system organ class (SOC) and per preferred term (PTname) of the MEDDRA dictionary (N = 16). All AE observed during the entire study are described, related or not to the study treatment, except those classified as related to the underlying tumour or its progression.

intrinsic resistance to ICI and turn cold microenvironment into hot microenvironment. Unfortunately, neither the molecular profiling nor the TMB performed in this cohort of patients allow to identify patients more likely to benefit from this treatment.

Interestingly, we report here that one patient treated in arm C with ATRT experienced a sustained response lasting over 24 cycles of treatment. In addition, 6 patients presented SD after 2 cycles, in one patient with anaplastic xantho-astrocytoma lasting for 9 months. While ICI have very limited activity on ATRT as reported with pembrolizumab [3], the long term anti-tumoral activity we observed here may be related to the metronomic activity previously reported for instance with the MEMMAT regimen [33–35].

Although formal comparison cannot be made, arm C also seems to display a better activity than the Arm G of AcSé -ESMART trial [36] which also relies on the combination of metronomic cyclophosphamide, and nivolumab (+/- radiotherapy). Thirteen patients were treated but only limited activity was observed with 2 patients with a desmoplastic round cell tumor and an ependymoma presenting unconfirmed partial response. An ancillary study showed a limited immune infiltrate in the primary tumors and a lack of circulating Tregs modulation upon treatment with metronomic cyclophosphamide. Moreover, a 6-month PFS of 7.7% was reported [36], versus 29% here. Definitive conclusions cannot be drawn based on the limited number of patients, but this shall be considered as an interesting signal of activity.

Besides, a phase I evaluating avelumab in 21 children has been recently reported and interestingly while no response were observed, 4 patients achieved stable disease including 2 sustained stable disease in 4 patients with low grade glioma [37]. As metronomic chemotherapy is an interesting alternative option for low grade glioma [38], our study has been amended to enrol patients with low grade glioma for the phase 2 part so that additional data regarding the use of PD1 inhibitors will be able in the future for this type of disease.

We made the initial hypothesis that the immune effect of metronomic chemotherapy together with the lack of major haematological toxicity could lead to an increase in the activity of nivolumab but the negative impact of an immune checkpoint inhibitor cannot be ruled out and whether the anti-tumoral activity seen in this limited number of patient is due to the strengthening of the nivolumab properties or due to the intrinsic multi-targeted activity of the metronomic combination cannot be answered with the data provided by the phase 1 trial. Therefore, a randomized phase 2 evaluating the addition of nivolumab to the triple metronomic combination is ongoing and shall help responding to these questions. Furthermore, ancillary study monitoring circulating immune cells as performed in the new trial will help to better understand the immune effect of metronomic chemotherapy in children with cancer.

CRediT authorship contribution statement

Conceptualization, NA and PL; methodology, NA, PL and MCLD.; validation, N.A., P.L. and MCLD; formal analysis, NA, PL, CL, MCLD and RT; investigation, NA, LM, IA, CFC, GRR, VM, BG, PC, NEW, PL.; resources, NA. PL and AP.; data curation, NA PL CL AP RT and MCLD; writing—original draft preparation, N.A. P.L MCLD; writing—review and editing, all authors; supervision, N.A. PL; project administration NA PL and AP. All authors have read and agreed to the submitted version of the manuscript.

Declaration of Competing Interest

Nivolumab and funding has been provided by BMS. NA has had an advisory role for Bayer and Partners Therapeutics and receives grants (institution) from Bristol Myers Squibb and drugs for a trial from Bristol Myers Squibb, Pierre Fabre, Merck, Pfizer, travel support from Roche; Speaker's Honoraria for Alection, he further has IDMC roles for Accord Healthcare. IA receives speaker's honoraria from Alection. BG has had an advisory role for AstraZeneca and IDMC roles for trials sponsored by Roche and Novartis. Speaker's Honoraria - Bayer. PC speaker's honoraria from Alection. NEW receives drug for a trial from Novartis, speaker's honoraria from Alection, Eusapharma & Novartis. PL receives drug from BMS for a trial and speaker's honoraria from Alection. MCLD, CL, AP, LW, CFC, GRV, VM and AB declare no conflict of interest.

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References

[1] Grabow D, Kaiser M, Hjorth L, Byrne J, Alessi D, Allodji RS, et al. The PanCareSurFup cohort of 83,333 five-year survivors of childhood cancer: a cohort from 12 European countries. Eur J Epidemiol 2018;33:335–49.
[2] Melero I, Berman DM, Aznar MA, Korman AJ, Gracia Pérez, Haanen J JL. Evolving synergistic combinations of targeted immunotherapies to combat cancer. Nat Rev Cancer 2015;15:457–72.
[3] Geoerger B, Kang HJ, Yalon-Oren M, Marshall LV, Vezina C, Pappo A, et al. Pembrolizumab in paediatric patients with advanced melanoma or a PD-L1-positive, advanced, relapsed, or refractory solid tumour or lymphoma (KEYNOTE-051): interim analysis of an open-label, single-arm, phase 1-2 trial 2020;21:121–33.
[4] Geoerger B, Bergeron C, Gore L, Sender L, Dunkel IJ, Herzog C, et al. Phase II study of ipilimumab in adolescents with unresectable stage III or IV malignant melanoma. Eur J Cancer 2017;86:358–63.
[5] Dunkel IJ, Doz F, Foreman NK, Hargrave D, Lassaletta A, et al. Nivolumab with or without ipilimumab in pediatric patients with high-grade CNS malignancies: Safety, efficacy, biomarker, and pharmacokinetics-CheckMate 908. Neuro Oncol 2023;25(8):1530–45.
[6] Geoerger B, Zwaan CM, Marshall LV, Michon J, Bourdeaut F, Casanova M, et al. Atezolizumab for children and young adults with previously treated solid tumours, non-Hodgkin lymphoma, and Hodgkin lymphoma (iMATRIX): a multicentre phase 1-2 study. Lancet Oncol 2020;21:134–44.
[7] Davis KL, Fox E, Merchant MS, Reid JM, Kudgus RA, Liu X, et al. Nivolumab in children and young adults with relapsed or refractory solid tumours or lymphoma (ADVL1412): a multicentre, open-label, single-arm, phase 1-2 trial. Lancet Oncol 2020;21(4):541–50.
[8] Bouffet E, Larouche V, Campbell BB. Immune checkpoint inhibition for hypermutant glioblastoma multiforme resulting from germline biallelic mismatch repair deficiency. J Clin Oncol 2016;34. 2206-2211.
[9] Davis KL, Fox E, Isikwei E, Reid JM, Liu X, Minard CG, et al. A phase I/II trial of nivolumab plus ipilimumab in children and young adults with relapsed/refractory solid tumors: a Children's Oncology Group Study ADVL1412. Clin Cancer Res 2022;28:5088–97.
[10] Ciccolini J, Barbolosi D, André N, Benzekry S, Barlesi F. Combinatorial immunotherapy strategies: most gods throw dice, but fate plays chess. Ann Oncol 2019;30:1690–1.
[11] Pasquier E, Kavallaris M, André N. Metronomic chemotherapy: new rationale for new directions. Nat Rev Clin Oncol 2010;7:455–65.
[12] André N, Tsai K, Carré M, Pasquier E. Metronomic chemotherapy: direct targeting of cancer cells after all? Trends Cancer 2017;3:319–25.
[13] Kerbel RS. The anti-angiogenic basis of metronomic chemotherapy. Nat Rev Cancer 2004;4:423–36.
[14] André N. Metronomics: towards personalized chemotherapy. Nat Rev Clin Oncol 2014;11:413–31.
[15] Sakaguchi S. Taking regulatory T cells into medicine. J Exp Med 2021 7;218(6): e20210831.
[16] Ghiringhelli F, Menard C, Puig PE, Ladoire S, Roux S, Martin F, et al. Metronomic cyclophosphamide regimen selectively depletes CD4+CD25+ regulatory T cells and restores T and NK effector functions in end stage cancer patients. Cancer Immunol Immunother 2007;56:641–8.
[17] Shevchenko S. Low-dose gemcitabine depletes regulatory T cells and improves survival in the orthotopic Panc02 model of pancreatic cancer. Int J Cancer 2013; 133:98–107.
[18] Park YJ. Tumor microenvironmental conversion of natural killer cells into myeloid-derived suppressor cells. Cancer Res 2013;73:5669–81.
[19] Otsubo D, et al. Early-phase treatment by low-dose 5-fluorouracil or primary tumor resection inhibits MDSC-mediated lung metastasis formation. Anticancer Res 2015; 35:4425–31.
[20] Vincent J, et al. 5-Fluorouracil selectively kills tumor-associated myeloid-derived suppressor cells resulting in enhanced T cell-dependent antitumor immunity. Cancer Res 2010;70:3052–61.
[21] Qu X. Antitumor effects of anti-CD40/CpG immunotherapy combined with gemcitabine or 5-fluorouracil chemotherapy in the B16 melanoma model. Int Immunopharmacol 2013;17:1141–7.
[22] Peereboom D, Alban T, Grabowski M, et al. Metronomic capecitabine as an immune modulator in glioblastoma patients reduces myeloid-derived suppressor cells. JCI Insight 2019;4:e130748.
[23] Galluzzi L. Immunological Effects of Conventional Chemotherapy and Targeted Anticancer Agents. Cancer Cell 2015;28:690–714.
[24] Kaneno R. Chemotherapeutic agents in low noncytotoxic concentrations increase immunogenicity of human colon cancer cells. Cell Oncol 2011;34:97–106.
[25] Tanaka H. Dual therapeutic efficacy of vinblastine as a unique chemotherapeutic agent capable of inducing dendritic cell maturation. Cancer Res 2009;69:6987–94.
[26] Shurin GV. Chemotherapeutic agents in noncytotoxic concentrations increase antigen presentation by dendritic cells via an IL-12-dependent mechanism. J Immunol 2009;183:137–44.
[27] Berlanga P, Pierron G, Lacroix L, Chicard M, Adam de Beaumais T, Marchais A. The European MAPPYACTS Trial: Precision Medicine Program in Pediatric and Adolescent Patients with Recurrent Malignancies. Cancer Discov 2022 2;12(5): 1266–81.
[28] Kilburn LB, Kocak M, Baxter P, Poussaint TY, Paulino AC, et al. A pediatric brain tumor consortium phase II trial of capecitabine rapidly disintegrating tablets with concomitant radiation therapy in children with newly diagnosed diffuse intrinsic pontine gliomas. Pedia Blood Cancer 2018;65(2). https://doi.org/10.1002/pbc.26832.
[29] Ceschi A, Noseda R, Palin K, Verhamme K. Immune checkpoint inhibitor-related cytokine release syndrome: analysis of WHO global pharmacovigilance database. Front Pharm 2020;11:557.
[30] Maharjan R, Choi JU, Kweon S, Pangeni R, Lee NK, Park SJ, et al. A novel oral metronomic chemotherapy provokes tumor specific immunity resulting in colon cancer eradication in combination with anti-PD-1 therapy. Biomaterials 2022;281: 121334.
[31] Skavatsou E, Semitekolou M, Morianos I, Karampelas T, Lougiakis N, Xanthou G, Tamvakopoulos C. Immunotherapy combined with metronomic dosing: an effective approach for the treatment of NSCLC. Cancers 2021;13(8):1901.
[32] Vicier C, Isambert N, Cropet C, Hamimed M, Osanno L, Legrand F, et al. MOVIE: a phase I, open-label, multicenter study to evaluate the safety and tolerability of metronomic vinorelbine combined with durvalumab plus tremelimumab in patients with advanced solid tumors. ESMO Open 2022;7:100646.
[33] Berland M, Padovani L, Rome A, Pech-Gourg G, Figarella-Branger D, André N. Sustained Complete Response to Metronomic Chemotherapy in a Child with Refractory Atypical Teratoid Rhabdoid Tumor: A Case Report. Front Pharm 2017; 8:792.
[34] Winnicki C, Leblond P, Bourdeaut F, Pagnier A, Paluenzela G, Chastagner P, et al. Retrospective National "Real Life" Experience of the SFCE with the Metronomic MEMMAT and MEMMAT-like Protocol. J Clin Med 2023;12:1415.
[35] Peyrl A, Chocholous M, Sabel M, Lassaletta A, Sterba J, et al. I. Sustained Survival Benefit in Recurrent Medulloblastoma by a Metronomic Antiangiogenic Regimen: A Nonrandomized Controlled Trial. JAMA Oncol 2023. Oct 26:e234437.
[36] Pasqualini C, Rubino J, Brard C, et al. Phase II and biomarker study of programmed cell death protein 1 inhibitor nivolumab and metronomic cyclophosphamide in paediatric relapsed/refractory solid tumours: Arm G of AcSé-ESMART, a trial of the European Innovative Therapies for Children With Cancer Consortium. Eur J Cancer 2021;150:53–62.
[37] Loeb DM, Lee JW, Morgenstern DA, Samson Y, Uyttebroeck A, Lyu CJ, Van Damme A, Nysom K, Macy ME, Zorzi AP, Xiong J, Pollert P, Joerg I, Vugmeyster Y, Ruisi M, Kang HJ. Avelumab in paediatric patients with refractory or relapsed solid tumours: dose-escalation results from an open-label, single-arm, phase 1/2 trial. Cancer Immunol Immunother 2022;71:2485–95.
[38] Verschuur A, Heng-Maillard MA, Dory-Lautrec P, Truillet R, Jouve E, Chastagner P, Leblond P, Aerts I, Honoré S, Entz-Werle N, Sirvent N, Gentet JC, Corradini N, André N. Metronomic Four-Drug Regimen Has Anti-tumor Activity in Pediatric Low-Grade Glioma; The Results of a Phase II Clinical Trial. Front Pharm 2018;9: 00950.