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Original Research

# 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

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**KEYWORDS**
Paediatric cancers;
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**Abstract** ***Purpose:*** AcSé-ESMART is a European multicentre, proof-of-concept multiarm phase I/II platform trial in paediatric patients with relapsed/refractory cancer. Arm G assessed the activity and safety of nivolumab in combination with metronomic cyclophosphamide +/- irradiation.

***Experimental design:*** Following a Phase II Simon two-stage design, nivolumab was administered intravenously at 3 mg/kg every 2 weeks of a 28-day cycle, oral cyclophosphamide at 25 mg/m$^2$ twice a day, 1 week on/1 week off. The primary endpoint was objective response rate. Irradiation/radioablation of primary tumour or metastasis could be administered as per physician's choice. Biomarker evaluation was performed by tumour immunohistochemistry, whole exome and RNA sequencing, and immunophenotyping of peripheral blood by flow cytometry.

***Results:*** Thirteen patients were treated with a median age of 15 years (range: 5.5–19.4). The main histologies were high-grade glioma, neuroblastoma, and desmoplastic small round cell tumour (DSRCT). The safety profile was similar to those of single-agent nivolumab, albeit haematologic toxicity, mainly lymphocytopenia, was commonly reported with the addition of cyclophosphamide +/- irradiation. Two patients with DSRCT and ependymoma presented unconfirmed partial response and prolonged disease stabilisation. Low mutational load with modest intratumour CD3+ T-cell infiltration and immunosuppressive tumour microenvironment were observed in the tumour samples. Under combined treatment, no positive modulation of circulating T cells was displayed, while derived neutrophil-to-lymphocyte ratio increased.

***Conclusions:*** Nivolumab in combination with cyclophosphamide was well tolerated but had limited activity in this paediatric setting. Metronomic cyclophosphamide did not modulate systemic immune response that could compensate limited T-cell infiltration and the immunosuppressive tumour microenvironment.

***ClinicalTrials.gov Identifier:*** NCT2813135.


## 1. Introduction

In spite of 80% of overall survival (OS) among children, adolescents, and young adults diagnosed with cancers, 20% still die from recurrent or refractory disease [1]. Widening the therapeutic approaches is of paramount importance, and defining new compounds or combinations is the next step to improve paediatric patient's survival. Immunotherapy with immune checkpoint inhibitors acting through the programmed cell death protein 1 (PD-1) pathway has shown efficacy in some chemotherapy-resistant adult cancers [2–6]. However, single-agent checkpoint inhibitors in children have demonstrated activity only in Hodgkin lymphoma, hypermutated tumours, and few rare tumour types [7–11].

A synergistic effect of metronomic cyclophosphamide with T-related immunotherapies had been suggested [12] through its immunomodulatory properties characterised by T regulatory (Treg) depletion and Natural Killer (NK) cell modulation, although limited activity has been observed in sarcoma [13,14]. Preclinical data and clinical trials in adults reported that irradiation is potentially capable of increasing the response to immune checkpoint inhibitors by inducing tumour-specific immunity [15,16].

Based on these data, we hypothesised that metronomic cyclophosphamide +/- irradiation of primary tumour or metastasis could enhance PD-1 inhibitor nivolumab activity in paediatric patients with relapsed/refractory malignancies. Potential biomarkers of response were studied using immunohistochemistry (IHC), bulk RNA sequencing (RNAseq), whole exome sequencing (WES), and peripheral white blood cell flow cytometry.

## 2. Methods

### 2.1. Study and patients

AcSé-ESMART (Secured Access—European proof-of-concept therapeutic Stratification trial of Molecular Anomalies in Relapsed or refractory Tumors; NCT02813135/EUDRACT N°:2016-000133-40, Innovative Therapies for Children with Cancer ITCC-057) is an international, multicentre, open-label, proof-of-concept phase I/II platform trial, in which each arm is separately implemented [17].

Arm G consisted of a combination of nivolumab, metronomic cyclophosphamide, and, according to physician's decision, irradiation of limited lesions. Patients aged less than 18 years at initial diagnosis with

recurrent/refractory evaluable or measurable malignancy were eligible.

Molecular profiling of the recurrent/refractory tumour by RNAseq and WES and access to raw data and tumour material was required before inclusion. Programmed cell death 1 ligand 1 (PD-L1) expression and high tumour mutation burden were enrichment criteria, albeit they were not mandatory for inclusion. Patients required a performance scale $\geq$70 and adequate haematological and organ function. Patients with active brain metastases, previous allogeneic transplantation, diagnosis of immunodeficiency, active autoimmune disease, those receiving systemic steroid therapy within 7 days before study treatment, or those with evidence of interstitial lung disease were excluded. All patients or legal representatives provided written informed consent. The trial complies with the Declaration of Helsinki, International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use, local laws, and regulations.

### 2.2. Study treatment and evaluations

Nivolumab was administered intravenously every 2 weeks of a 28-day cycle (Days 1 and 15) at 3 mg/kg/dose, and cyclophosphamide was given orally at 25 mg/m$^2$ twice a day, 1 week on/1week off. Irradiation, whenever appropriate, started at least 2 weeks after the first nivolumab injection.

Response assessment was performed according to Response Evaluation Criteria In Solid Tumors (version 1.1) [18], Response Assessment in Neuro-Oncology [19], and International Neuroblastoma Response Criteria [20] for solid tumours, glioma, and neuroblastoma, respectively, every two cycles. Objective responses (ORs), defined as complete response or partial response (PR), were to be confirmed 4–6 weeks after the first occurrence.

Adverse events were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events 4.03.

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.

### 2.3. Trial design

Based on previously reported data [7,8,10], only one dose level was explored in this Phase II study. A Simon two-stage minimax design [21] was implemented to test whether the true response rate was lower than 10%, with a 90% power if the objective response rate was higher than 30%, at the level of 10%. In Stage 1, 12 evaluable patients were expected. If one response or less was seen, the arm was interrupted for lack of activity. Otherwise, Stage 2 was initiated with 13 additional evaluable patients. The arm was declared positive if six or more ORs were observed.

The response rate based on radiological response after two cycles, best response over the whole duration of treatment, and progression-free survival (PFS) and OS rates calculated from the date of treatment initiation are reported. The PFS and OS curves have been estimated using the Kaplan–Meier method. The 95% confidence intervals (CIs) have been estimated using the Rothman method.

### 2.4. Tumour microenvironment and mutational load analysis

The evaluation of the density of tumour-infiltrating lymphocytes (TILs) was done by an experienced pathologist (J.Y.S.) in ten consecutive fields in haematoxylin and eosin–stained 4 μm-thick formalin-fixed paraffin-embedded tissue sections of tumour material used for the prior molecular profiling analysis. Their density was expressed as a percentage of all tumour and/or stromal cells present and not as a percentage of the stromal surface as, as expected in most cases, no or very little stroma was present in the tumour tissue samples available.

The PD-L1 (clone E1L3N; Cell Signalling Technology, Danvers, MA) and CD3 (clone 2GV6, Ventana) expression was analysed using an automated stainer (Ventana Benchmark Ultra, Tucson, AZ). PD-L1 expression was defined as positive if displayed on more than 1% of tumour cells. CD3+ cell density was expressed as the mean of CD3-positive cells in at least ten consecutive fields of 1 mm$^2$ in surface.

WES and RNAseq of the recurrent or refractory disease were performed in MOSCATO-01 and MAPPYACTS as previously described [22,23]. Tumour immune infiltrates were estimated using the absolute version of the CIBERSORT algorithm [24]. Somatic coding mutations were filtered according to their enrichment in the tumour samples compared with the paired normal samples using a Fisher test and $P$ value $<$0.001. Questionable somatic variants were observed in less than three reads or with an allele frequency lower than 0.05% or described in 1000 genomes and EXAC databases with a frequency higher than 0.05% or non-exonic variants were excluded. Mutational load was calculated as the number of non-synonymous somatic variants divided by the total length of targeted regions by the Exome capture kits with a minimum coverage of 10x.

### 2.5. Host immune response profiling

Heparinized blood samples (5–8 mL) were collected at Days 1 and 15 to monitor circulating immune system populations by flow cytometry. Fresh whole blood phenotyping of white cells (lymphocytes, granulocytes,

and monocytes) and T cells was performed using Numeration, T-cell-3, and Treg 2 panels as previously described [25]. For the main populations (lymphocytes, neutrophils, eosinophils, monocytes, and NK cells), absolute counts are reported. For the subpopulation of T cells (CD4, CD8, and Treg), their modulation is expressed as percentage to avoid the impact of the absolute count variation likely because of chemotherapy. Derived Neutrophil-to-Lymphocyte ratio (dNLR) was calculated as follows: dNLR = absolute neutrophil count/(white blood cell count − absolute neutrophil count) [26]. For immune parameters, statistical analyses were performed using Prism 6 software (GraphPad, San Diego, CA). *P* values $< 0.05$ were considered significant. Groups were compared using Mann–Whitney U test.

## 3. Results

### 3.1. Patient characteristics

Between August 2016 and July 2017, 13 patients were enrolled (Table 1). The median age at study entry was 15.9 years (range, 5.5–19.4), 10 (77%) were male. Histologies were high-grade glioma, neuroblastoma, desmoplastic small round cell tumour (DSRCT), three each, two alveolar rhabdomyosarcoma, one ependymoma, and one melanoma. The median delay from diagnosis to study entry was 2.0 years (range: 0.6–14.4). Ten (77%) had metastatic disease. Patients had received a median of 3.5 lines of treatment (range, 1–5). All but one had conventional chemotherapy, all three patients with neuroblastoma had high-dose chemotherapy, and the patient with melanoma had received anti-CTLA4 immunotherapy. Radiation therapy and surgery of the primary tumour had been performed in 9 (69%) and 11 (85%) patients, respectively.

### 3.2. Study treatment and toxicity

Overall, 39 cycles were administered in 13 patients (Table 1), with a median of two cycles (range: 1–8) per patient. Eight patients received irradiation (locoregional, n = 6; metastasis, n = 2) at doses of 20–40 Gy; all four brain tumours had reirradiation.

Seventy-two of 194 (37%) adverse events of any grade were possibly treatment related (Table 2); all non-haematologic events except one G3 vomiting were mild or moderate (G1-2). Five patients experienced possibly immune-mediated diarrhoea (G1, n = 3; G2, n = 2), all with favourable recovery.

### 3.3. Antitumor activity

No confirmed objective response was observed in the 12 evaluable patients (Table 1). One patient with melanoma discontinued because of clinical progression after the first cycle. Five patients experienced stable disease (SD)

Table 1
Patients and tumour characteristics (n = 13).

| Patient | Diagnosis | Gender | Age (years) | Site of biopsy | PDL1 | TILs | CD3 (cc/mm$^2$) | Mutational load (Mut/Mb) | RT | If RT Gy | N° cycles | Best response | Best response cycle | PFS (months) | OS (months) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Neuroblastoma | M | 5.8 | M | 1% | 1% | 90 | NA | No | | 1 | PD | 1 | 0.5 | 0.8 |
| 2 | Neuroblastoma | M | 19.4 | M | 0% | NA | NA | 1.52 | No | | 2 | PD[a] | 2 | 1.6 | 13.7 |
| 3 | Glioblastoma—midline (pineal, H3F3A) | M | 15.9 | M | NA | NA | NA | 0.26 | Brain | 25 | 2 | PD | 2 | 1.7 | 7.5 |
| 4 | Malignant glioma NOS | F | 18.0 | L | 0% | 1% | 0 | 0.62 | Brain | 20 | 3 | SD | 2 | 3.0 | 13.7 |
| 5 | DSRCT | M | 17.9 | L | 0% | 10% | 120 | 0.22 | LN | 24 | 8 | SD (uPR) | 6 | 8.2 | 16.4 |
| 6 | Melanoma | M | 5.5 | M LN | 0% | 1% | 0 | 0.32 | No | | 1 | PD | 1 | 0.7 | 1.3 |
| 7 | Ependymoma | M | 16.4 | L | 0% | 5% | 40 | 1.12 | Brain | UKN | 6 | SD (uPR) | 4 | 5.6 | 27.8 |
| 8 | High-grade glioma—thalamic ACVR1 | F | 7.2 | L | 0% | 1% | 0 | 0.04 | Brain | 25 | 4 | SD | 2 | 3.6 | 7.2 |
| 9 | Rhabdomyosarcoma—alveolar | F | 11.8 | M | NA | NA | NA | 0.36 | Extremity | 40 | 2 | PD | 2 | 1.7 | 2.7 |
| 10 | Neuroblastoma | M | 6.9 | M LN | 0% | 10% | 200 | 0.48 | LN | 25 | 2 | PD | 2 | 1.8 | 3.1 |
| 11 | DSRCT | M | 16.5 | L | 0% | 1% | 8 | 0.35 | No | | 4 | SD | 2 | 3.4 | 3.4 |
| 12 | Rhabdomyosarcoma—alveolar | M | 14.3 | M | 0% | 10% | 300 | 0.55 | Whole lung | 25 | 2 | PD | 2 | 1.7 | 3.5 |
| 13 | DSRCT | M | 17.3 | M | 0% | 1% | 30 | 0.70 | No | | 2 | PD | 2 | 1.3 | 2.2 |

DSRCT, desmoplastic small round cell tumour; NOS, not otherwise specified; M, male; F, female; M, metastasis; L, locoregional; M LN, lymph node metastasis; NA, not available; PD-L1, programmed death-ligand 1; TILs, tumour-infiltrating lymphocytes; SD, stable disease; uPR, unconfirmed partial response; PFS, progression-free survival; OS, overall survival.

[a] Patient had mIBG-positive disease only.

Table 2
All grades treatment-related adverse events (n = 72) in 13 patients receiving 39 treatment cycles during the whole treatment duration.

| AE term (CTCAE v4.0) | G1 | G2 | G3 | G4 |
|---|---|---|---|---|
| **Blood and lymphatic system disorders** | | | | |
| Anaemia | 1 | 8 | 4 | 0 |
| **Gastrointestinal disorders** | | | | |
| Abdominal pain | 1 | 0 | 0 | 0 |
| Nausea | 0 | 1 | 0 | 0 |
| Vomiting | 3 | 1 | 2 | 0 |
| Oral mucositis | 0 | 1 | 0 | 0 |
| Diarrhoea | 3 | 2 | 0 | 0 |
| **General disorders** | | | | |
| Fatigue | 4 | 1 | 0 | 0 |
| **Investigations** | | | | |
| Cholesterol high | 1 | 0 | 0 | 0 |
| Lymphocyte count decreased | 0 | 11 | 7 | 2 |
| Neutrophil count decreased | 1 | 1 | 3 | 1 |
| Platelet count decreased | 0 | 0 | 0 | 1 |
| White blood cell decreased | 2 | 1 | 1 | 1 |
| **Metabolism and nutrition disorders** | | | | |
| Anorexia | 1 | 0 | 0 | 0 |
| Hyperglycaemia | 0 | 1 | 0 | 0 |
| Hyponatraemia | 1 | 0 | 0 | 0 |
| **Respiratory, thoracic, and mediastinal disorders** | | | | |
| Cough | 1 | 0 | 0 | 0 |
| Dyspnoea | 1 | 0 | 0 | 0 |
| **Nervous system disorders** | | | | |
| Paraesthesia | 1 | 0 | 0 | 0 |
| **Skin and subcutaneous tissue disorders** | | | | |
| Skin pain | 1 | 0 | 0 | 0 |
| **Total** | 22 | 28 | 17 | 5 |

as best response. One patient with DSRCT experienced reduction of target lesions of −29% at Cycle 4 and −39% at Cycle 6. A reduction of −37% of target lesions was noted at Cycle 4 for the patient with ependymoma, although PR was not confirmed at the next assessment. They were treated during 8 and 6 months, respectively. Three additional patients had SD at Cycle 2 as best response and progressed at 3.0–3.6 months. Discontinuation was related to PD in all patients. Six-month PFS and OS rates were 7.7% (95% CI: 1.4–33.3) and 46.2% (95% CI: 23.2–70.9), respectively (Fig. 1). The median PFS was 1.7 months (95% CI: 1.3; 3.4), and the median OS was 3.4 months (95% CI: 2.2–13.5).

### *3.4. Biomarker analysis and treatment-related immune system modulation*

Eleven of 12 patients had available tumour material to perform IHC for tumour immune infiltrate (Table 1, Fig. 2A–D). Low PD-L1 expression (1%) on tumour cells was observed in one neuroblastoma, all other samples were PD-L1 negative. TILs were low in ten evaluable samples, 1% in six patients, 5% in one patient, and 10% in three patients. The median density of CD3-positive cells was 35 cells/mm$^2$ (range: 0–300).

Tumour mutational load was low in all 12 samples ranging from 0.04 to 1.52 mutation/Mb, with a median of 0.42 mutation/Mb (Table 1).

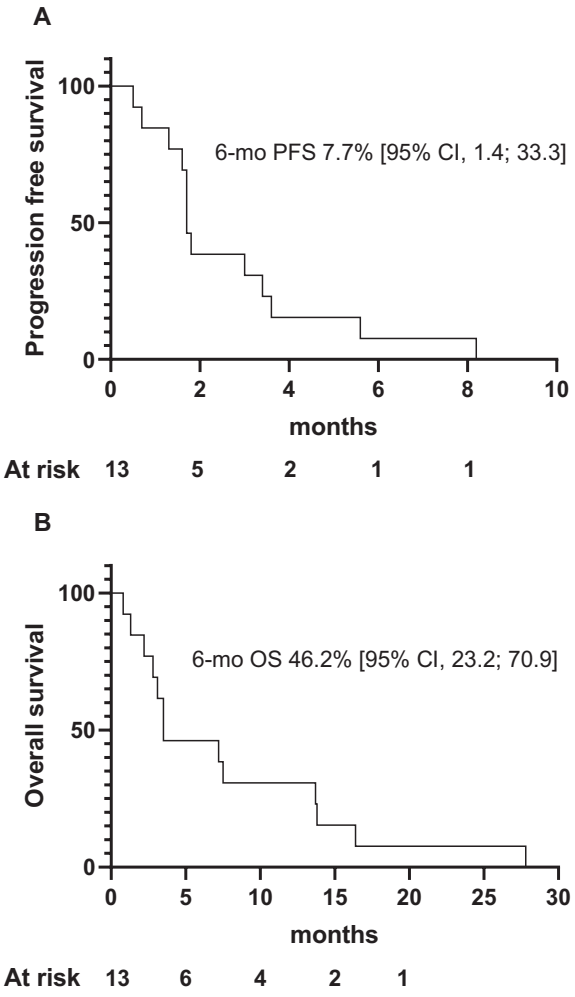


Fig. 1. Kaplan–Meier curves of progression-free survival (PFS) (A) and overall survival (OS) (B) in the population of all treated patients (n = 13).

Bulk RNAseq analysis in nine contributive relapsed samples confirmed a low T-cell signature, compared with a predominant protumoural macrophage infiltration (Fig. 2E).

We further analysed the circulating immune cell phenotypes to explore the peripheral immune modulation under treatment (Fig. 3; Supplementary Table 1). Paired analyses between Days 1 and 15 showed a decrease in absolute lymphocyte count ($P$ = 0.01) without a significant relative change of CD4+ and CD8+ T cells ($P$ = 0.2 and p = 0.4, respectively). Among CD4+ T cells, Treg percentage remained stable. The pretreatment level of PD-1+/CD8+ T cells varied from 0% to 61% of all CD8+ T cells, with no association with achieved disease control defined as prolonged SD. Study treatment exposure did not significantly modulate the percentage of CD4+ and/or CD8+ T cells expressing immune modulatory markers such as CD57, CD160, CD5, CD69, OX40, and LAG3. The absolute peripheral circulating monocyte count (CD14+CD16-cells) as well

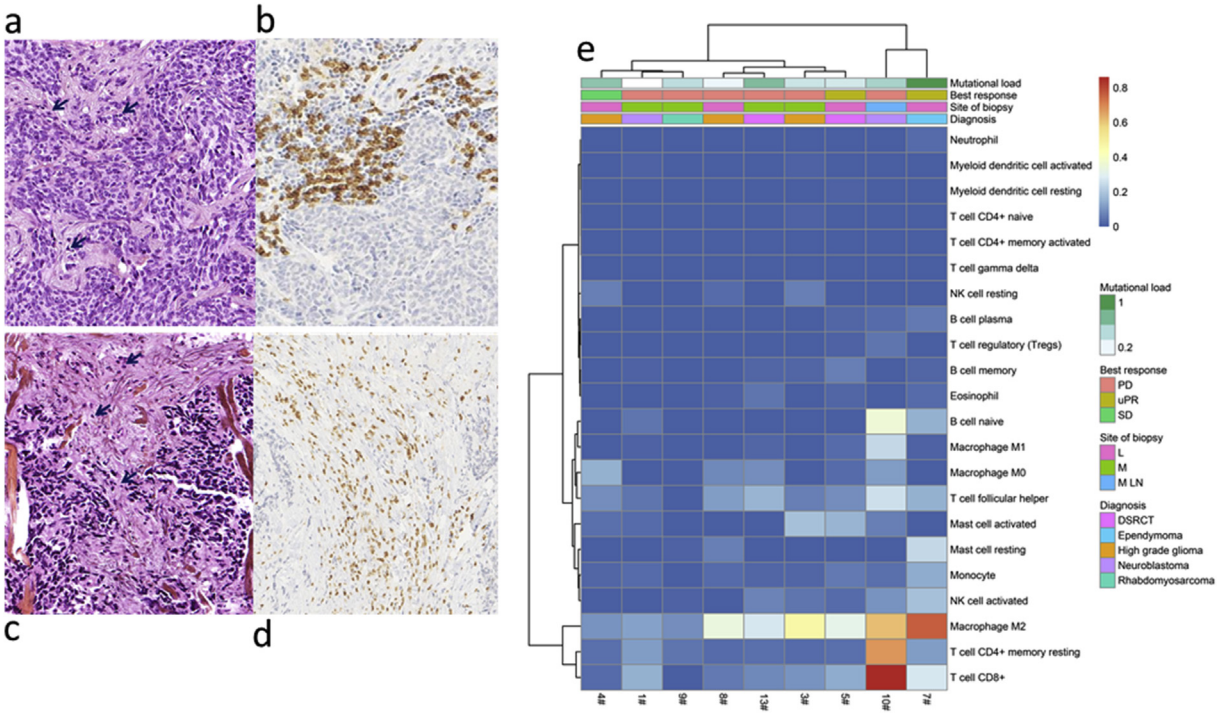


Fig. 2. **Tumour microenvironment features.** Tumour microenvironment features. (A–D) Representative sections of one case of DSRCT (a and b), and one case of alveolar rhabdomyosarcoma (c and d); haematoxylin-eosin-saffron (HES) staining (a and c) and CD3 immunostaining (b and d). In the case of the DSRCT, TILs are scattered in the stroma (a, arrows) and small aggregates of CD3+ T cells (b) are detected at the interface between stroma and tumour cells; no CD3+ cell is present within the tumour. In the case of alveolar rhabdomyosarcoma, TILs (c, arrows) and CD3+ T cells (d) are scattered in the stroma at distance from tumour cells. Original magnifications: (a) ×280; (b) ×220; (c) ×240; (d) ×180. (E) Heatmap of RNA expression of immune cell subtypes (raws) by CIBERSORT abs., according to diagnosis, site of biopsy, best response, and mutational load (n = 9, patients in columns). DSRCT, desmoplastic small round cell tumour; L, locoregional primary tumour; M, metastasis; M LN, lymph node metastasis; uPR, unconfirmed partial response; SD, stable disease; PD, progressive disease.

as transient (CD14+CD16+) or residual monocytes (CD14low/CD16+) did not significantly vary ($P$= 0.17). Conversely, the NK cell absolute count as well as low/neg NK cells (CD56-CD16+) decreased significantly between baseline and C1D15 ($P$ = 0.01 and $P$ = 0.007, respectively), whereas bright- ($CD56^{high}$CD16-), inter- ($CD56^{high}$CD16+) and dim- (CD56+CD16+) NK cells remained globally stable. No significant modification was displayed for neutrophils nor eosinophils ($P$ = 0.77 and $P$ = 0.47, respectively). Interestingly, dNLR was lower than three in all patients at baseline but increased significantly for all of them at Day 15 ($P$ = 0.007).

## 4. Discussion

This Phase II study explored the hypothesis that metronomic cyclophosphamide, alone or with radiation therapy, may modulate the immune response and enhance the activity of PD-1 inhibition in relapsed or refractory paediatric malignancies.

In our cohort of heavily pretreated patients, no patient experienced objective tumour response, five had SD as best response, two of them prolonged and with transient partial tumour regression. Based on these data, the study did not proceed to the second stage as per protocol design. These results are in line with previously reported data in adults on the modest activity of the PD-1 inhibitor pembrolizumab with metronomic cyclophosphamide in advanced osteosarcomas [13,14] and not different to single-agent activity [7–10]. The direct and 'abscopal' impact of irradiation on cancer immune surveillance has been suggested in preclinical and clinical studies, although optimal doses and schedules have not been defined [27]. Eight of our 13 patients received radiation therapy as per physician's decision. Although it is important to underline that among the three patients with DSRCT, the only patient who presented a prolonged SD had received concomitant irradiation, the study design does not allow drawing any conclusion on its efficacy.

The identification of potential biomarkers is critical to better select patients and monitor therapeutic effects. In adults, several biomarkers involving tumour features (tumour cells and TME (tumor microenvironment)), blood (circulating cells, chemokines, and cytokines), and stool microbiota have been associated to the activity of immune checkpoint inhibitors [28,29].

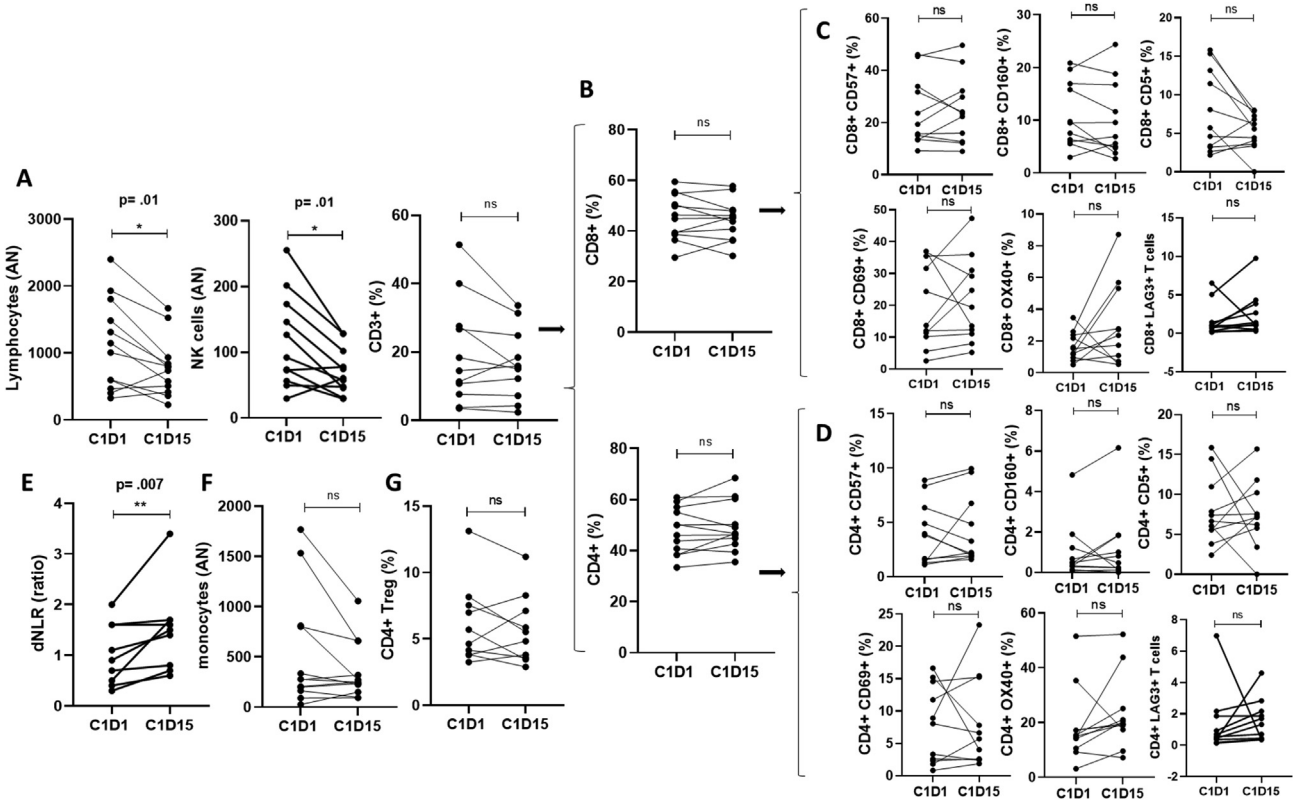


Fig. 3. **Modulation of circulating immune populations by flow cytometry, comparison of paired samples between C1D1 and C1D15.** (A) A decrease of the absolute number (AN) of lymphocytes and NK cells is displayed during treatment, whereas the percentage (%) of CD3+ cells is stable, n = 10; (B) Relative number of CD4+ and CD8+ cells among CD3+ cells does not vary over time, n = 8; (C and D) The expression of immune modulation markers, such as CD57, CD69, CD160, CD5, and OX40 on CD8+ (C) and CD4+ cells (D) is not significantly modified by the treatment, n = 10; (E) derived neutrophil-to-lymphocyte ratio (dNLR) significantly increases during treatment, n = 8; (F and G) The AN of monocytes (F, n = 8) and the percentage of regulatory T cells (Treg) among CD4+ T cells (G, n = 10) do not significantly vary over time, respectively.

Pre-existing T-cell antitumour immunity is an important prerequisite to the anti-PD-1/PD-L1 response [30]. In our cohort, IHC and RNAseq analyses confirmed the low density of tumour-infiltrating T cells compared with adult cancers, thus at least partially explaining the results of this study [31,32]. In a meta-analysis including 1475 adults treated with nivolumab, pembrolizumab, or atezolizumab, response rates were significantly higher in PD-L1 positive tumours (34% versus 19.9%), considering percentages of positivity higher than 5% [33]. Although PD-L1 expression in tumours has been associated with improved clinical response to PD-1/PD-L1 inhibitors in some adult tumour types, in paediatric cancers, this evidence is limited [7,8]. In our cohort, PD-L1 expression was found to be positive only in one patient and at low level, in agreement with previously reported studies [7,34,35]. Other reasons that contribute to this limited activity might be the overall low tumour mutational load, predictive of response to checkpoint inhibitors since responsible for a reduced antitumour T-cell response [29,36].

Finally, the presence of an immunosuppressive TME, mainly protumoural macrophages, is likely to be crucial to interpret these disappointing results. Indeed, the impact of tumour-associated macrophages (TAMs) on cancer progression has been described, although the involved mechanisms have not been fully characterised yet [37,38].

To monitor the treatment effects directly on the TME was found not reasonable, given the challenge to perform sequential tumour biopsies in children. In adults, the assessment of the prognostic and predictive value of circulating immune biomarkers in patients treated with immune checkpoint inhibitors is currently used, being an attractive tool because of the easy accessibility of blood samples and limited costs of the technique [26,39,40]. In our cohort, the flow cytometry analysis of circulating immune cells showed the lack of a significant phenotype modulation in T cells, including Treg, previously described with metronomic chemotherapy [41,42]. This could be explained by the early timing of the second sampling or be related to the chemotherapy schedule. Moreover, the potential counterproductive impact of the combination with a chemotherapy likely responsible for increased haematologic toxicity compared with immune checkpoint

inhibitor monotherapy cannot be excluded, and other drugs, doses, and schedules could be investigated [43].

Finally, although dNLR was low in comparison to adult cutoffs at baseline [26], noteworthy dNLR significantly increased after 2 weeks of treatment in all our patients, and its increase is a known negative predictive factor of response in adult cohorts [26].

To conclude, options for a more efficient development of checkpoint inhibitors in paediatrics could be the combination with modalities capable of bringing tumour-reactive effector lymphocytes into the tumour, such as engineered proteins (i.e. monoclonal antibodies and T-cell engaging agents), cellular products (i.e. CAR-T cells and T-cell receptor–engineered T cells), or peptide intratumour injection while improving, on the other hand, the reduced T-cell recognition [44]. In parallel, the exploration of new immunotherapies targeting the innate immunity, especially TAMs, appears to be a top priority.

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## Data sharing statement

Data available on request due to privacy/ethical restrictions.

## Conflict of interest statement

N.A., V.M.C., G.V., and B.G. declared consultancy or advisory role to BMS. All other authors declared no potential conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejca.2021.03.032.

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