Safety, Pharmacokinetics, and Immunomodulatory Effects of Lenalidomide in Children and Adolescents With Relapsed/Refractory Solid Tumors or Myelodysplastic Syndrome: A Children's Oncology Group Phase I Consortium Report

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ABSTRACT

Purpose

To determine the maximum-tolerated or recommended phase II dose, dose-limiting toxicities (DLTs), pharmacokinetics (PK), and immunomodulatory effects of lenalidomide in children with recurrent or refractory solid tumors or myelodysplastic syndrome (MDS).

Patients and Methods

Cohorts of children with solid tumors received lenalidomide once daily for 21 days, every 28 days at dose levels of 15 to 70 mg/m²/dose. Children with MDS received a fixed dose of 5 mg/m²/dose. Specimens for PK and immune modulation were obtained in the first cycle.

Results

Forty-nine patients (46 solid tumor, three MDS), median age 16 years (range, 1 to 21 years), were enrolled, and 42 were fully assessable for toxicity. One patient had a cerebrovascular ischemic event of uncertain relationship to lenalidomide. DLTs included hypercalcemia at 15 mg/m²; hypophosphatemia/hypokalemia, neutropenia, and somnolence at 40 mg/m²; and urticaria at 55 mg/m². At the highest dose level evaluated (70 mg/m²), zero of six patients had DLT. A maximum-tolerated dose was not reached. No objective responses were observed. PK studies (n = 29) showed that clearance is faster in children younger than 12 years of age. Immunomodulatory studies (n = 26) showed a significant increase in serum interleukin (IL) -2, IL-15, granulocyte-macrophage colony-stimulating factor, natural killer (NK) cells, NK cytotoxicity, and lymphokine activated killer (LAK) cytotoxicity, and a significant decrease in CD4⁺/CD25⁺ regulatory T cells.

Conclusion

Lenalidomide is well-tolerated at doses up to 70 mg/m²/d for 21 days in children with solid tumors. Drug clearance in children younger than 12 years is faster than in adolescents and young adults. Lenalidomide significantly upregulates cellular immunity, including NK and LAK activity.

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INTRODUCTION

Lenalidomide (CC-5013, Revlimid; Celgene, Summit, NJ), a structural analog of thalidomide with enhanced immunomodulatory potency and decreased sedative and neurotoxic properties,¹ is approved in the United States for use in adults with myelodysplastic syndromes (MDS) and in the United States, European Union, and Canada for multiple myeloma. Its mechanism of action is complex and includes inhibition of angiogenesis^{2,3}; downregulation of tumor necrosis factor- α in inflammatory states⁴; inhibition of cyclooxygenase-2⁵; enhanced activation of CD8⁺ T-cells^{3,6}

with increased production of interleukin (IL) -2⁷; and stimulation of natural killer (NK) and dendritic cell function.⁸⁻¹²

We conducted a pediatric phase I study to determine the maximum-tolerated dose (MTD) or recommended phase II dose (the dose level below the MTD) of lenalidomide administered orally once daily for 21 days, followed by a 1 week rest, in children with refractory solid tumors; to describe lenalidomide toxicities; and to characterize the pharmacokinetics (PK) of lenalidomide in children. Secondary aims included obtaining preliminary data on antitumor activity and determining changes in cellular immunity.

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PATIENTS AND METHODS

Patient Eligibility

Institutional review board approval and subject consent and assent were obtained according to federal and institutional guidelines. Eligible subjects were older than 12 months and ≤ 21 years of age at the time of study entry with: body-surface area higher than 0.4 m^2 (due to lenalidomide capsule size); diagnosis of solid tumor (excluding primary brain tumor) or MDS; no known curative therapy or therapy proven to prolong survival with an acceptable quality of life; Karnofsky or Lansky performance score ≥ 50 ; and recovery from toxicity of prior therapy. Adequate organ function was required (peripheral absolute neutrophil count $\geq 1,000/\mu\text{L}$; platelet count $\geq 100,000/\mu\text{L}$; hemoglobin $\geq 8.0 \text{ g/dL}$; creatinine normal for age or creatinine clearance or glomerular filtration rate $\geq 70 \text{ mL/min/1.73 m}^2$; total bilirubin $\leq 1.5\times$ upper limit of normal for age; ALT $\leq 110 \text{ U/L}$; serum albumin $\geq 2 \text{ g/dL}$). Patients with MDS were required to have transfusion supported platelet count $\geq 30,000/\mu\text{L}$ and hemoglobin $\geq 8.0 \text{ g/dL}$.

Drug Administration

Lenalidomide, provided by the Cancer Therapy Evaluation Program of the National Cancer Institute, was administered orally once daily for 21 days with a 1-week rest. The starting dose in patients with solid tumors was $15 \text{ mg/m}^2/\text{d}$ (approximately the 25 mg/d MTD described in adults), rounded to the nearest 5 mg. Patients with MDS received $5 \text{ mg/m}^2/\text{d}$ rounded to the nearest 5 mg; there was no intra- or interpatient dose escalation for patients with MDS. Courses could be repeated every 28 days if the patient had at least stable disease, did not have dose limiting toxicity (DLT), and met laboratory parameters as defined in the eligibility section.

Trial Design

Toxicities were graded according to the National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.¹³ DLT was defined as any of the following events occurring in the first course of therapy and at least possibly attributable to lenalidomide: grade 4 nonhematologic toxicity; grade 3 nonhematologic toxicity (except nausea and vomiting of < 5 days duration; transaminases that returned to grade 1 or better within 7 days of study drug interruption and that did not recur on rechallenge with study drug; and fever or infection < 5 days duration); any grade 2 nonhematologic toxicity that persisted for longer than 7 days and was considered sufficiently medically significant or intolerable by patients that it required treatment interruption; and any other adverse event that required interruption of lenalidomide for longer than 7 days or that recurred on rechallenge with lenalidomide. Hematologic DLT was defined as grade 4 neutropenia or thrombocytopenia that occurred during drug administration or lasted longer than 7 days; or grade 3 thrombocytopenia that required transfusion therapy on more than two occasions during a course, or any myelosuppression causing a delay of ≥ 7 days between treatment courses. Patients with MDS were not evaluated for hematologic DLTs.

Any patient who experienced DLT at any time during protocol therapy was considered evaluable for toxicity. Patients without DLT who received at least 85% of the prescribed dose during the first 28-day course were considered evaluable for toxicity as well as response. Dose escalation proceeded using a modified 3 + 3 cohort design in which three patients were initially studied at each dose level. If none of these three patients experienced DLT, the dose was escalated to the next higher level. If one of three patients experienced DLT, then up to three more patients were accrued at the same level. If none of these three additional patients experienced DLT, then the dose was escalated. If one or more of these three additional patients experienced DLT the MTD was exceeded, unless one of the DLTs did not appear to be related to dose or the DLTs were of different classes and the toxicities were readily reversible. In that circumstance, the cohort could be expanded to 12. The MTD was the maximum dose at which fewer than one third of patients experienced DLT.

Dose Modifications for Toxicity

If a patient with a solid tumor experienced grade 4 neutropenia or thrombocytopenia during the 21 days of drug administration, treatment was suspended for a minimum of 7 days, then resumed at the next lowest dose level

for subsequent courses. Patients with nonhematologic DLT who returned to baseline within 7 days received subsequent treatment at the next lower dose level. Patients with grade 3 or 4 nonhematologic toxicity that did not resolve to baseline by 7 days after the planned start of the next course were removed from protocol therapy. Any patient experiencing a thromboembolic event was removed from study.

Response and Pharmacokinetics

Responses in solid tumors were evaluated using the RECIST (Response Evaluation Criteria in Solid Tumors)¹⁴ and in MDS using the MDS International Working Group Response Criteria as modified by Cheson et al.¹⁵

Plasma samples were obtained before lenalidomide and at 30, 60, and 90 minutes and 2, 4, 6, 8, and 24 hours and day 7 ± 2 days and day 14 ± 2 days after the first dose. Participation in this portion of the study was optional. Samples were analyzed for S- and R-lenalidomide concentrations by a chiral liquid chromatography tandem mass spectrometry method as described previously.¹⁶ The lenalidomide concentration was calculated as the sum of S-lenalidomide and R-lenalidomide.

Plasma PK parameters including area under the plasma concentration-time curve from time 0 to infinity (AUC_{∞}); apparent total body clearance normalized to body-surface area (CL/F/BSA); apparent volume of distribution (V/F); and terminal elimination half-life ($t_{1/2}$) were estimated from the

Table 1. Patient Demographic and Clinical Characteristics (n = 49)

Characteristic	No.	%
Age, years		
Median	16	
Range	1-21	
Sex		
Male	26	53.1
Female	23	46.9
Race		
White	39	79.6
Asian	2	4.1
Black or African American	2	4.1
Unknown	6	12.2
Ethnicity		
Non-Hispanic	39	79.6
Hispanic	5	10.2
Unknown	5	10.2
Diagnosis		
Nonrhabdomyosarcoma soft tissue sarcoma	11	
Osteosarcoma	10	
Ewing's sarcoma	4	
Carcinoma	4	
Rhabdomyosarcoma	4	
Malignant peripheral nerve sheath tumor	3	
Myelodysplastic syndrome	3	
Hepatoblastoma	2	
Nephroblastoma	2	
Alveolar soft part sarcoma	1	
Atypical teratoid/rhabdoid tumor	1	
Hepatocellular carcinoma	1	
Hodgkin's disease	1	
Neuroblastoma	1	
Pleuropulmonary blastoma	1	
Prior therapy		
Chemotherapy regimens		
Median	2	
Range	0-7	
Radiation	24	

Table 2. Evaluable Patients and DLTs

Dose Level (mg/m ² /d)	No. Entered	No. Evaluable	No. With DLTs	Type of DLT
5*	3	3	0	
15	7	6	1	Hypercalcemia
20	3	3	0	
25	5	3	1	Cerebrovascular ischemia
30	3	3	0	
40	13	12	3	Hypophosphatemia/hypokalemia (n = 1); somnolence (n = 1); neutropenia (n = 1)
55	7	6	1	Urticaria
70	8	6	0	

Abbreviation: DLT, dose-limiting toxicity.

*Patients with myelodysplastic syndrome.

concentration-time data on day 1 by noncompartmental methods using WinNonlin professional version 5.1 (Pharsight Corporation, Mountain View, CA).

Immune Modulation

Peripheral blood samples were collected before and at day 21 $\pm$ 3 days after the start of lenalidomide. Samples were collected in ethylenediaminetetraacetate and in tubes containing no anticoagulants and shipped overnight at room temperature. Serum and mononuclear cells (MNCs) were obtained by standard methods and stored at -80°C until day of analysis.

MNCs were washed in phosphate buffered saline supplemented with 1% heat inactivated fetal bovine serum (Sigma-Aldrich, St Louis, MO) and 1% azide (Sigma-Aldrich). NK CD3⁺/CD56⁺ and regulatory T (T_{reg}; CD4⁺/CD25⁺) cells were characterized by flow cytometry as previously described.^{17,18} NK receptor (NKR) phenotype was further characterized in seven patients by flow cytometry including expression of inhibitory C-lectin NKRs (CD94, NKG2a), inhibitory NKRs (KIR3DL1), activating C-lectin receptor (CD94, NKG2D), activating NK natural cytotoxicity receptors (Nkp46), and activating NKRs (KIR2DS4) as previously described.¹⁷

Granzyme B and perforin were analyzed with fluorescein isothiocyanate antigranzyme B (BD Biosciences, San Jose, CA) and R-phycoerythrin antiperforin (BD Biosciences) –conjugated monoclonal antibodies, as previously described.¹⁷ Lysosomal-associated membrane protein-1 (LAMP-1) whose expression on the cell surface follows degranulation,¹⁹ was measured with fluorescein isothiocyanate antigranzyme B and R-phycoerythrin anti-CD107a (BD Biosciences).

NK and lymphokine activated killer (LAK) tumor cytotoxicity was determined by a europium release assay (Perkin Elmer, Waltham, MA).¹⁷ NK cytotoxicity was measured against an NK-sensitive human erythroleukemia cell line, K562 (ATCC, Manassas, VA) and LAK cytotoxicity against an NK-resistant cell line, Daudi (ATCC).

Serum IL-2, IL-8, IL-15, and granulocyte macrophage colony-stimulating factor (GM-CSF) were measured by enzyme-linked immunosorbent assay, as previously described.¹⁷ Samples were run in triplicate and data presented as mean $\pm$ standard deviation. The sensitivity of the assays were: IL-2, 2 pg/mL; IL-8, 1 pg/mL; IL-15, 15.6 pg/mL; and GM-CSF, 7.8 pg/mL.

Statistics

Analysis of variance was used to examine the difference between mean CL/F/BSA and V/F/BSA across the age groups 5 to 11, 12 to 17, and 18 to 21 years (representing children, adolescents, and young adults). Repeated measures analysis of variance was used to examine the change in mean immunologic functions from baseline to day 21. Analysis was performed with SAS (version 9.1.3, SAS Institute Inc, Cary, NC). All *P* values were two sided at a 5% significance level.

RESULTS

Forty-nine subjects, all eligible, were enrolled (Table 1). Three had a diagnosis of MDS. Of 46 patients with solid tumors, six were inevaluable for DLT or response, including four who did not receive 85% of the planned course and did not have DLT and two who withdrew before receiving any study drug; one with early disease progression was inevaluable for DLT.

No patient with MDS (5 mg/m² dose level) had DLT (Table 2). At 15 mg/m², one of six patients with solid tumors developed DLT (grade 3 hypercalcemia). One patient with synovial sarcoma at the 25 mg/m² dose level had a transient speech impairment and arm numbness that was considered consistent with cerebrovascular ischemia, but was not considered to be related to study; this subject had no evidence of thrombosis or embolism on imaging studies. No other thromboembolic events were observed. At 40 mg/m², two of six patients developed DLTs including one with grade 3 hypophosphatemia and hypokalemia and one with grade 4 neutropenia delaying the start of the next cycle for longer than 7 days. Therefore, the cohort was expanded to accrue an additional six patients, one of whom had grade 3 somnolence. At 55 mg/m², one of six patients had grade 3 urticaria. There were no DLTs in six patients enrolled at 70 mg/m². Non-DLTs were

Table 3. PK Results for 29 Subjects Consenting to Optional PK Studies

Dose Level (mg/m ² /d)	No. Patients	Half-Life (hours)		T _{max} (hours)		AUC _∞ (ng $\times$ h/mL)		CL/F/BSA (mL/min/m ²)		V/F/BSA (L/m ²)	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
5	1	1.4		0.5		543		202		24.2	
15	3	3.1	1.5	1.5	0.0	2,740	835	100	35	24.0	2.6
20	3	2.4	0.8	1.3	0.6	2,470	800	148	43	29.1	3.3
25	3	1.9	0.5	1.2	0.3	3,070	495	136	13	20.6	6.2
30	2	2.2	0.2	0.8	0.4	3,850	895	137	28	25.2	2.5
40	9	3.1	1.2	1.5	1.5	7,260	4,070	117	52	27.0	8.7
55	5	2.3	0.9	1.5	0.6	8,070	3,220	131	55	22.8	4.4
70	3	3.1	1.1	1.2	0.8	11,750	7,770	127	67	31.0	15.2
All doses		2.6	1.1	1.5	1.0			128	46	25.9	7.3

Abbreviations: PK, pharmacokinetics; T_{max}, time to reach maximum plasma lenalidomide concentration; AUC, area under the curve; CL/F/BSA, apparent total clearance normalized to body surface area; V/F/BSA, apparent volume of distribution during terminal phase normalized to body surface area; SD, standard deviation.

Fig 1. (A) Natural killer (NK; CD3/CD16⁺/CD56⁺) subset expression at baseline and day 21 post-lenalidomide administration as determined by flow cytometry. The bar graph and the associated dot plot/histogram represent the increase in NK subset expression at baseline and at day 21. Results represent mean $\pm$ standard deviation ($n = 26$ pairs; $P < .01$). The CD3 lymphocyte gate was used as a reference to determine the percentage of NK expression at baseline and day 21 after lenalidomide administration. (B) CD3/56⁺/KIR3DL1⁺ subset at baseline and day 21 post-lenalidomide administration. Peripheral blood (PB) NK KIR3DL1 subset increase in CD3/56⁺/CD158b⁺ expression at day 21 post-lenalidomide administration compared with baseline ($n = 7$ pairs; $P < .001$). Representative dot plot of CD3/56⁺/CD158b⁺ at day 21 compared with baseline. The lymphocyte population was gated and used as a reference to determine the specific subsets. (C) CD3/56⁺/KIR2DS4⁺ subset at baseline and day 21 post-lenalidomide administration. PB NK KIR2DS4 subset increase in CD3/56⁺/KIR2DS4⁺ expression at day 21 post-lenalidomide administration compared with baseline ($n = 7$ pairs; $P < .001$). Representative dot plot of CD3/56⁺/KIR2DS4⁺ at day 21 compared with baseline. The lymphocyte population was gated and used as a reference to determine the specific subsets. (D) Expression of NK cells expressing the natural cytotoxicity receptor CD3/56⁺/Nkp46⁺ at baseline and day 21 post-lenalidomide administration. Increased PB CD3/56⁺/Nkp46⁺ subset expression at day 21 post-lenalidomide administration compared with baseline ($n = 7$ pairs; $P < .01$). Representative dot plot of CD3/56⁺/Nkp46⁺ at day 21 compared with baseline. The lymphocyte population was gated and used as a reference to determine the specific subsets.

sporadic and not clearly dose related (Appendix Table A1, on-line only).

No objective responses were observed. The median number of cycles of therapy was 1 (range, 1 to 11). Of the three patients with MDS, all had had prior immuno- or chemotherapy; two had cytogenetics including 5q-; two received 1 cycle of lenalidomide then were removed from study for progressive disease or transformation to acute myeloid leukemia; and one was removed from study after 2 cycles of therapy without objective response. The subject who received 11 courses of therapy was a 19-year-old male with synovial sarcoma who was treated at the 30 mg/m²/d dose level. This patient required a dose reduction after course 8 for a grade 3 absolute neutrophil count causing a delay in the start of his next cycle. Seven other subjects received three or more courses: one each with liposarcoma (five courses), malignant hemangioendothelioma (five courses), atypical teratoid/rhabdoid tumor (four courses), and osteosarcoma, adrenal cortical carcinoma, Ewing's sarcoma, and hepatocellular carcinoma (three courses each).

PK analysis (Table 3) showed no evidence of nonlinear clearance. The mean terminal half-life was 2.6 ± 1.1 hours and the clearance was 128 ± 46 mL/min/m². Clearance was significantly higher in children younger than 12 years of age ($n = 10$; 160 ± 40 mL/min/m²) than in those in the 12- to 17- ($n = 9$; 120 ± 40 mL/min/m²) or 18- to 21-year old ($n = 10$; 105 ± 40 mL/min/m²) age ranges ($P < .05$); there was no difference in clearance between the latter two groups.

Immune Modulation

There was a significant increase in the percentage of NK (CD3⁺/16⁺/56⁺) cells (29.9 ± 17.2 v $9.3\% \pm 7.1\%$ $P < .01$; Fig 1A) and in the absolute NK cell number/ μ L at day 21 compared with baseline (770 ± 527 v 187 ± 162 / μ L; $P < .001$). A dose response effect was observed for both % NK cells (21%; $P = .003$) and absolute NK cell number (582/ μ L; $P = .003$). T_{reg} cells (CD4⁺/CD25⁺) significantly decreased at day 21 compared to baseline (12.1 ± 2.3 v $23.1\% \pm 2.6\%$; $P < .05$).

There was a significant increase in NK cells expressing the inhibitory KIR3DL1 (CD3⁺/56⁺/CD158b⁺) receptor (31 ± 4.3 v $2.9\% \pm 1.2\%$; $P < .001$; Fig 1B), the activating KIR2DS4 (CD3⁺/56⁺/KIR2DS4⁺) receptor (58.6 ± 6.9 v $2.3\% \pm 1.7\%$; $P < .001$; Fig 1C), and the natural cytotoxicity receptor Nkp46 (CD3⁺/56⁺/Nkp46⁺) (14.3 ± 3.1 v $2.3\% \pm 1.8\%$, $P < .01$; Fig 1D). There was no change in expression of inhibitory or activating c-lectin receptors CD94/NKG2a or CD94/NKG2D.

NK cytotoxicity against K562 tumor targets (55.2 ± 18.5 v $26.6\% \pm 10.6\%$; $P < .01$) and LAK cytotoxicity against Daudi (33 ± 13.8 v $15.5\% \pm 7.03\%$; $P < .01$) were both significantly increased at day 21 compared to baseline. Intracellular perforin was decreased (24 ± 3 v $7\% \pm 2\%$; $P < .01$).

Expression of intracellular granzyme B (19.4 ± 11.8 v $1.82\% \pm 2.45\%$; $P < .01$; Fig 2A) and LAMP-1 (19.6 ± 9.4 v $2.0\% \pm 1.7\%$, $P < .05$; Fig 2B) were significantly increased at day 21 compared to baseline.

Fig 2. (A) Expression of granzyme B, a protease of natural killer (NK) cells, at baseline and day 21 post-lenalidomide administration. Increased granzyme B expression day 21 post-lenalidomide administration compared with baseline ($P < .01$). Representative dot plot of granzyme B expression compared with baseline. Results represent 26 paired patients. (B) Expression of NK activation marker, CD107a, at baseline and day 21 post-lenalidomide administration. Increased CD107a expression day 21 post-lenalidomide administration compared with baseline ($P < .05$). Representative dot plot of CD107a expression compared with baseline. Results represent 26 paired patients.

Fig 3. (A) Interleukin (IL) -2 serum levels pre- and post-lenalidomide determined by enzyme-linked immunosorbent assay (ELISA) assay. Results are expressed as mean $\pm$ standard deviation (SD) and all 26 paired samples were run in triplicate. IL-2 levels were significantly increased at day 21 compared with baseline ($P < .01$). (B) IL-15 serum levels pre- and post-lenalidomide determined by ELISA assay. Results are expressed as mean $\pm$ SD and all 26 paired samples were run in triplicate. IL-15 levels were significantly increased at day 21 compared with baseline ($P < .001$). (C) Granulocyte macrophage-colony stimulating factor (GM-CSF) serum levels pre- and post-lenalidomide determined by ELISA assay. Results are expressed as mean $\pm$ SD and all 26 paired samples were run in triplicate. GM-CSF protein production was significantly increased at day 21 compared with baseline ($P < .01$).

IL-2 (138.1 ± 41.1 v 30.7 ± 31.1 pg/mL; $P < .01$; Fig 3A), IL-15 (381.5 ± 126.9 v 30.7 ± 31.1 pg/mL; Fig 3B) and GM-CSF levels (122.5 ± 117.6 v 17 ± 10.7 pg/mL; $P < .001$; Fig 3C) were all significantly increased at day 21. A dose response effect was noted for IL-15 (mean change, 360 pg/mL; $P < .001$) and GM-CSF (mean change, 105 pg/mL; $P < .01$) from the 15 to 70 mg/m² dose levels. There was no significant difference in IL-8 concentrations at day 21 compared to baseline.

DISCUSSION

Lenalidomide was well-tolerated by children with solid tumors at doses up to 70 mg/m²/d given daily for 21 days every 28 days. The 70 mg/m² dose level, equivalent to approximately 120 mg in an adult with a BSA of 1.7 m², is at least 5 times the usual adult dose of 10 to 25 mg/d. There is evidence from adult studies that the DLT of lenalidomide is cumulative myelosuppression, rather than acute myelosuppression in the first course of therapy.^{20,21} Thus, we did not pursue further dose

escalation, as we had little opportunity to evaluate cumulative toxicity since most patients received only a single course of therapy (although the subject who received > 5 courses required a dose reduction for neutropenia). Preliminary results of a study of lenalidomide in children with brain tumors also show myelosuppression as the primary toxicity.²² We did not observe venous thromboembolic events, as have been reported in adults especially when lenalidomide is given in combination with dexamethasone,²³⁻²⁵ or tumor flare, which has been reported in adults with B-cell malignancies.^{26,27}

Lenalidomide was rapidly absorbed and cleared, with a half-life of 2.6 ± 1.1 hours and clearance of 128 ± 46 mL/min/m², similar to adult parameters.^{21,28,29} Children younger than 12 years of age appeared to clear drug faster than those older than 12 years, although the absolute difference was small (160 v 120 mL/min/m²). Such small differences in drug disposition are unlikely to explain differences in tolerability between children and adults. Most studies of lenalidomide in adults were performed in patients with multiple myeloma, whose bone marrow reserve was likely compromised. Patients with MDS and

5q deletions may be especially sensitive to lenalidomide-induced myelosuppression.^{20,30} Adults with solid tumors appear to tolerate drug better than those with MDS,^{28,31-35} and doses up to 75 mg/d may be tolerated,³⁶ although this dose is rarely used. In our study, only three children with MDS were enrolled, and no dose escalation beyond 5 mg/m² was attempted in this population. Thus, although it is possible that a pharmacodynamic difference in susceptibility to myelosuppression exists between children and adults, the differences more likely result from variations in study population and definitions of MTD.

Lenalidomide significantly affected cytokine concentration and immune cell number and function in children in this study. We found increases in IL-2, IL-15, and GM-CSF after lenalidomide therapy, consistent with observations in adults.^{37,38} Lenalidomide strongly induces IL-2 production in MNCs,⁷ and IL-2 in turn enhances both NK number and function.^{39,40} IL-15 plays an essential role in NK cell development, survival and cytotoxicity.⁴¹⁻⁴³ GM-CSF stimulates monocytes, macrophages, and dendritic cells, potentially boosting the presentation of tumor antigens.^{37,44}

Both the percentage of NK cells and the absolute NK cell number/ μ L increased in a dose-responsive fashion in our study. Furthermore, in a small subset of patients, we demonstrated that the NK cell population after lenalidomide was characterized by increased expression of activating receptor KIR2DS4 and natural cytotoxicity receptor Nkp46, which may also have contributed to the increased NK and LAK cytotoxicity we observed.⁴⁵ Finally, we demonstrated a significant increase in granzyme B, which is critically important in the cytotoxicity of NK and cytotoxic T lymphocytes,⁴⁶ and an increase in the expression of LAMP-1, a general marker of NK activity.¹⁹ Perforin decreased rather than increased; in a previous study in adults, there was no change in perforin after lenalidomide.¹¹

In agreement with Galustian et al,⁴⁷ who demonstrated that agents like lenalidomide inhibit the proliferation and function of T_{reg} cells, we found a decrease in the percentage of T_{reg} cells at day 21. Decreasing T_{regs} may promote host antitumor immunity since T_{reg} cells are associated with tumor escape from immune surveillance.⁴⁸ Furthermore, many solid tumors contain infiltrating T_{reg} cells,^{49,50} and T_{reg} cells are increased in patients with multiple myeloma,⁵¹ non-Hodgkin's lymphoma,⁵² and chronic lymphocytic leukemia,⁵³ conditions that respond to lenalidomide treatment.⁴⁷ These observations suggest that inhibition of T_{regs} contributes to lenalidomide's antitumor activity.

In summary, lenalidomide in children was well-tolerated at doses up to 70 mg/m²/d in children with solid tumors, with PKs similar to those in adults. Although we did not observe single-agent anticancer activity of lenalidomide in this population of children with advanced solid tumors or in three children with MDS, the histologies varied

widely, and no conclusions can be drawn about the activity of lenalidomide in pediatric cancer. Our results, however, do suggest that lenalidomide may enhance antitumor immunity through a variety of mechanisms including increasing cytokines, NK, or LAK activity and decreasing T_{regs}. Patients with advanced refractory disease might be less likely to respond to immunomodulating agents than patients with lower disease burden. The combination of lenalidomide with other agents, such as proteasome inhibitors,⁵⁴ or with other immunomodulatory agents such as IL-2, IL-15, IL-18, GM-CSF, or tumor vaccines might be more likely to produce responses. In addition, the combination of adoptive cellular therapy (NK cells) with lenalidomide might enhance antitumor immunity. These combinations should be considered for future studies of lenalidomide in the treatment of children with relapsed/refractory solid tumors.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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