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Safety and immunogenicity of BNT162b2 vaccine in children with acute leukaemia: results and perspectives of an open-label, two-centre, phase 1/2 trial with dose finding study

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ABSTRACT

Background. COVID-19 can be severe in children with acute leukaemia (AL), significantly delaying chemotherapy. This is the first study to address the safety and immunogenicity of BNT162b2 in children with AL. **Methods:** The PACIFIC trial (NCT04969601) was a phase 1/2 dose-finding study in children aged <15 years with AL and their siblings. Two doses of BNT162b2 vaccine were administered 21 days apart. The co-primary endpoints were safety, assessed by dose-limiting toxicity, and humoral immunogenicity, defined by an anti-Spike IgG titer ≥260 BAU/ml one month after the second injection. A third dose of vaccine was administered to children with an anti-Spike IgG titer <260 BAU/ml. Humoral and cellular immune responses were assessed for 12 months after the first vaccine injection. **Results:** Sixty-one patients and 15 siblings were included. No toxicity was observed during dose escalation. Thus, 44/53 children received the 30 μg vaccine dose. Two months after the first injection, the humoral response was lower in patients than siblings (52 % vs 100 %, $p < 0.001$), whereas the T-cell response was similar in the two groups (80 % versus 100 %, $p = 0.1$). A significant humoral response was observed in 43 % of patients after the third dose. Both humoral and Covid-19-specific T-cell responses persisted for at least one year after vaccination. No severe Covid-19 occurred during the study. **Conclusions:** Vaccination of children with acute leukaemia with adult doses (30 μg) of BNT162b2 is well tolerated and results in significant T-cell response children with AL, even during chemotherapy. As doses of 10 μg is currently recommended for children, these results support the value of increasing vaccine doses in immunocompromised patients.

Abbreviation: AL, Acute Leukaemia; ALL, Acute Lymphoblastic Leukaemia; AML, Acute Myeloblastic Leukaemia; TEAEs, Treatment-Emergent Adverse Events; HM, Haematological Malignancies.

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1. Introduction

Because of chemotherapy-induced immunodeficiency, children with acute leukaemia (AL) are at risk of severe viral infections [1]. Among them, SARS-CoV-2 infections can be particularly severe. Indeed, the mortality rate associated with SARS-CoV-2 infections in pediatric cancer patients is high: 3.5 %, i.e., 10-fold lower than in adults with haematological malignancies [2,3] but 10-fold higher than in healthy children [4–8]. Faced with the potential problem of delaying chemotherapy in the event of SARS-CoV-2 infection, exposing patients to a higher risk of relapse, we turned to the only convincing preventive strategy, the timely administration of a SARS-CoV-2 vaccine.

Covid-19 mRNA vaccines, notably BNT162b2 (COMIRNATY®, Pfizer), swiftly received European authorization for use in adults [9]. Subsequently, based on immunogenicity and safety data, its authorization was extended to adolescents aged 12–16 [10]. The schedule was two 30-μg doses of vaccine 21 days apart. Phase 1 and 2–3 trials were then conducted in the United States between March and August 2021 in children under 12 years of age with lower doses (two 10-μg doses of vaccine 21 days apart), showing both good safety and immunogenicity in the 5–11 years age group [11]. This led to authorization for vaccination in this age group in France in November 2021, particularly for high-risk children, such as those with leukaemia [12].

At the beginning of our study in September 2021, BNT162b2 was approved in France for individuals aged 12 and above but there was only limited data concerning the immune response of immunocompromised patients to the vaccine, particularly those with haematological malignancies. Studies in adult patients with such conditions showed lower rates of immune responses to the vaccine than healthy individuals or patients with solid tumours [13]. In addition, data on long-term immune responses were lacking.

In this context, we conducted a Phase 1/2 trial with dose finding study to evaluate the safety and immunogenicity of BNT162b2 in children aged 2–15 with AL and their siblings, with follow-up extending up to 12 months post-vaccination.

2. Methods

2.1. Study design

The PACIFIC trial was a prospective, open-label, phase 1/2, clinical trial conducted at two tertiary-care centres in Paris (France) in children between 2 and 15 years of age with AL.

The primary objective was to assess vaccine safety and tolerability. In the initial part of the phase 1 of the trial, we aimed to determine the maximum tolerated dose (MTD) in children between 2 and 12 years of age with AL using the dose-limiting toxicity (DLT). According to the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials [14], DLT was defined as any local (pain or reaction at the site of injection) or general (allergic reactions or anaphylaxis, thromboembolism, non-documented fever, headaches, or peripheral facial palsy) treatment-emerged adverse event (TEAE) grade 3 or worse occurring within seven days following vaccine injection. Escalating dose levels of 10 μg, 20 μg, and 30 μg were tested to determine the MTD. We used a Bayesian adaptive phase 1/2 design, stratified by age group, distinguishing between 2 and 5, 5–11, and 12–15 years [15]. In each age group, patients initially received the lowest dose. Then, for each age group, among the set of acceptable doses based on the updated dose-toxicity model, the 321 dose-finding algorithm allowed escalation above the current level in an age group only if three patients in that group or four patients overall had been treated at the current level [16] (**Fig. S1**). At the same time, patients or siblings aged over 12 years, received a dose of 30 μg according to French guidelines and following a European authorization extension in November 2021, siblings aged 5–11 could be included and received two doses of 10 μg.

The primary objective of the phase 2 trial was to assess the humoral immune response. The primary endpoint was the proportion of responders, defined as anti-Spike IgG level ≥ 260 BAU/ml, at month 2, i.e., one month after the second vaccine dose. The secondary objectives of the phase 2 trial were to evaluate the humoral response at months 3 (if a third dose was administered), and persistence of humoral response at month 6, and 12; others endpoints were as follow: neutralizing anti-Spike IgG response measured by lateral flow assay (Boditech®) at months 2, 3 (if a third dose was administered), 6, and 12; cellular immune response assessed by Elispot against S1, S2, N, and M proteins at months 2, 3 (if a third dose was administered), 6, and 12; and the incidence of symptomatic, laboratory-confirmed SARS-CoV-2 infection. Additional exploratory analyses compared immune responses between patients and their siblings and investigated predictive factors of humoral and cellular immune responses.

2.2. Participants

Eligible patients were aged 2 to 15 years. Patients with acute lymphoblastic leukaemia could be included during chemotherapy, at least two weeks after the last PEG-asparaginase injection, or within 12 months following treatment discontinuation. Patients with acute myeloid leukaemia could be included within 12 months of treatment discontinuation. Patients' siblings residing with the patients at least 50 % of the time served as the control group. Initially, only siblings aged 12 and above were eligible, but the protocol was amended in December 2021 to include siblings aged 5–11 to receive the 10-μg dose following French vaccine authorization. The main exclusion criteria were a history of COVID-19 within two months before inclusion, a positive Covid-19 PCR at inclusion and/or a fever or symptoms of COVID-19 in the previous 72 h, a history of severe post-vaccination adverse events or severe allergic manifestations, a known clinical allergy to polyethylene glycol (PEG), pregnancy, platelet count <50 G/L or absolute neutrophil count <0.5G/L at the time of vaccination, influenza vaccination within 14 days prior to the first injection, and other vaccinations in the four weeks prior to the first injection or scheduled to receive a licensed vaccine four weeks after the last injection. Patients with known HIV, HCV, or HBV infection were also excluded. Written informed consent was obtained from the parents/legal guardian of each participant before enrolment. The study was conducted in accordance with the Declaration of Helsinki and the national French legislation. This study was approved by the Institutional Review Board (CPP IDF1–2021-ND59-cat.1 NSI) and was registered under clinicaltrial.gov, number NCT0496960.

2.3. Procedures

All included children received two doses of BNT162b2 21 to 28 days apart. Dose escalation was then carried out for patients aged 2–12 years with three doses (10, 20, and 30 μg). All other patients and siblings aged above 12 years were administered 30-μg doses. Siblings between 5 and 12 received 10 μg. A third dose was offered to leukaemia patients with inadequate seroconversion two months post first vaccine dose (see “immunogenicity assessment” section). Patients were monitored for 30 min after each injection for any potential immediate reaction. Tolerance was monitored seven days after each injection by a medical visit and with the help of a patient logbook. Treatment-emergent adverse events (TEAEs) were defined as any adverse event that was not present before (or worsening with) therapy and graded according to the Common Terminology Criteria for Adverse Events (version 5.0)[17]. Serious adverse events, including those attributable to the vaccine, were immediately reported to the sponsor.

Medical visits were then scheduled at 2, 6, and 12 months after the first dose of vaccine. Serum samples were collected at inclusion, the time of each dose of vaccine, and then 2, 6, and 12 months after the first dose. Serum samples were also collected three months after the first dose for patients with AL who received a third dose of vaccine.

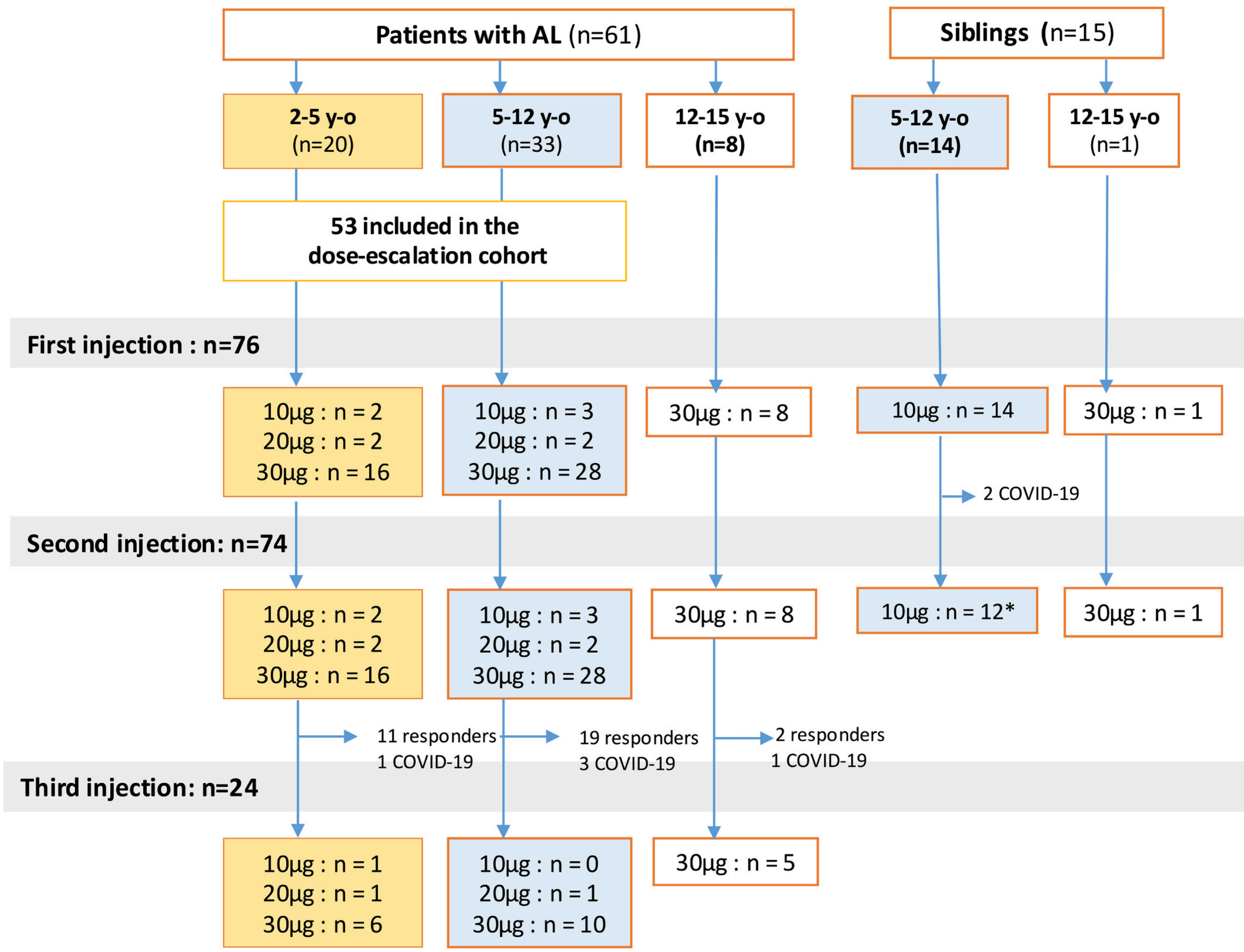

Fig. 1. Study flow chart.

IgG antibodies directed against the S1 domain of the SARS-CoV-2 Spike protein and the Nucleocapsid protein were assessed using the ALINITY chemiluminescence technique (Abbott). Antibody neutralizing activity was assessed using two techniques. First, a surrogate assay based on antibody-mediated blockage of the ACE-2-Spike protein interaction (ichroma™ Covid-19 nAb, Boditech, South-Korea) was used. Inhibition of fluorescence >30 % was considered positive. Second, neutralizing antibody titres were assessed on sera collected at the M2 visit using a whole virus replication assay (VNT) against the B.1, BA.2, and BQ.1.1 SARS CoV-2 variants, as described [18]. A titer above 10 was considered positive. Cellular responses were assessed by T-cell enzyme linked immunospot (EliSpot). Peripheral blood mononuclear cells were isolated using a density gradient (PBMCs) and lymphocytes enumerated by flow cytometry using the BD Tritest™ CD3FITC/ CD8PE/ CD45PerCP, and BD Trucount™ Tubes (BD Biosciences, Le Pont de Claix, France). The EliSpot assay was conducted as previously described [19]. Sterile PVDF strips (Millipore, Saint-Quentin-en-Yvelines, France) were coated overnight at 4 °C with an IFNγ antibody (U-CyTech, Utrecht, Netherlands) and then incubated for 1 h in culture medium (RPMI-1640, Sigma-Aldrich, Molsheim, France) supplemented with 10 % human AB serum) at 37 °C. PBMCs were then seeded at 0.2×10^6 $CD3^+$ T cells/well and stimulated for 18–20 h with pools of 15-mer peptides from each viral antigen at a final concentration of 10 µmol/L. On day 2, PBMCs were removed and IFNγ secretion revealed using a biotin-conjugated IFNγ antibody (U-CyTech), streptavidin-horseradish peroxidase (U-CyTech), and 3-amino-9-ethylcarbazole (AEC) (U-CyTech). Spots were enumerated using an automated EliSpot reader (Autoimmune Diagnostika (AID), Strassberg, Germany). Peptide pools for N-terminal (pool S1) and C-terminal (pool S2) SARS-CoV-2-Spike were purchased from (JPT Peptide Technologies GmbH, BioNTech AG, Berlin, Germany). M and N pools from the SARS-CoV-2 Nucleoprotein were used to detect responses to SARS-CoV-2 infection. Negative controls consisted of cells in culture medium. Positive controls consisted of cells stimulated with 1 mg/mL phytohemagglutinin PHA-P (Sigma-Aldrich) and 10 µmol/L CEFX Ultra SuperStim Pool (JPT Peptide Technologies GmbH, BioNTech AG, Berlin, Germany). Results are expressed as spot-forming units (SFU)/10^6 $CD3^+$ T lymphocytes subtracted from the background. Relevant clinical positivity thresholds for each peptide pool were determined used ROC curves as follows: S1 pool: 20 SFU/10^6 $CD3^+$, S2 pool: 60 SFU/10^6 $CD3^+$, M pool: 24 SFU/10^6 $CD3^+$, and N pool: 17 SFU/10^6 $CD3^+$.

2.4. Statistical analysis

In children aged <12 years with AL, an adaptive Bayesian phase 1 design was used. It consisted of carrying out dose finding in each age group, combining the data via a hierarchical model that allows estimation of a different maximum tolerated dose for each age group, and sharing the information between the groups to obtain a more accurate estimate of the maximum tolerated dose [20]. Bayesian estimation was performed for each enrolled patient using a skeleton for the power model of dose-limiting toxicity probabilities of (0.10, 0.20, and 0.50),

Table 1
Characteristics of patients at study entry according to age.

	2–5 $n = 20$	5–11 $n = 33$	12–15 $n = 8$	Total $n = 61$
Disease				
B-cell ALL	18 (90)	28 (85)	7 (88)	53 (87)
T-cell ALL	0 (0)	4 (12)	0 (0)	4 (7)
AML	2 (10)	1 (3)	1 (12)	4 (7)
Risk group for treatment				
B - SR	7 (35)	18 (55)	1 (12)	26 (43)
B - MR	7 (35)	7 (21)	5 (63)	19 (31)
B - HR	3 (15)	2 (6)	0 (0)	5 (8)
T - SR	0 (0)	3 (9)	0 (0)	3 (5)
T - HR	0 (0)	1 (3)	0 (0)	1 (2)
Other ALL	1 (5)	1 (3)	1 (12)	3 (5)
AML treatment	2 (10)	1 (3)	1 (12)	4 (7)
Complete remission	20 (100)	33 (100)	8 (100)	61 (100)
Time since complete remission, months	12.5 [8.3;19.3]	17.7 [10.1;25.4]	9.4 [7.5;14.6]	14.2 [8.5;21.9]
Time since last chemotherapy, months	4.8 [1.1;11.2]	9.5 [3.0;15.5]	2.0 [0.5;6.1]	6.4 [1.4;13.7]
Time since last PEG-Asparaginase, months	6.8 [5.5;16.5]	13.3 [5.2; 20.5]	3.8 [2.6;10.2]	11.0 [4.1;17.4]
Treatment stage				
Intensive	1 (5)	1 (3)	0 (0)	2 (3)
Maintenance	17 (85)	24 (73)	7 (88)	48 (79)
No chemotherapy	2 (10)	8 (24)	1 (12)	11 (18)
Chemotherapy < 7 days	18 (90)	25 (76)	7 (88)	50 (82)
Corticosteroids < 7 days	2 (10)	6 (18)	0 (0)	8 (13)
IV immunoglobulins < 1 month	3 (15)	2 (6)	0 (0)	5 (8)
SARS-CoV-2 humoral response				
Anti-S IgG titer ≥7 BAU/mL	11 (55)	10 (30)	4 (50)	25 (41)
Anti-S IgG titer ≥260 BAU/mL	4 (20)	2 (6)	0	6 (10)
Anti-N IgG titer ≥1.40	2 (10)	3 (9)	1 (2)	6 (10)
SARS-CoV-2 cellular response				
Positive Elispot against S1 or S2 proteins	4/14 (29)	7/29 (24)	2/7 (29)	13/51 (25)
Positive Elispot against M or N proteins	3 /14 (21)	8/29 (28)	1/7 (14)	12/51 (24)

* ALL, acute lymphoblastic leukaemia; AML, acute myeloblastic leukaemia; SR, standard risk; MR, medium risk; HR, high-risk; PEG, pegylated; IgG, immunoglobulin G; anti-S, anti-Spike protein; anti-N, anti-Nucleocapsid protein.
* Quantitative data are expressed as medians and inter-quartile ranges [IQRs] and qualitative data as numbers and percentages.

with a target toxicity rate of 0.30 and a threshold for determining whether a dose has an acceptable probability if toxicity of 0.20. Posterior inference used 10,000 MCMC samples following a period of 5000 iterations for burn-in. Approximately 60 AL patients (approximately 20 by age group) and 20 siblings were scheduled to be enrolled, with a maximum of 100. In September 2022, the sponsor and the DSMB recommended stopping the inclusions on December, 19, 2022, due to the decrease in the epidemic and difficulties in recruitment, although the scheduled sample size was not reached. The baseline characteristics were analysed using summary statistics. All enrolled patients were assessed by intention-to-treat analysis. Quantitative data are expressed as medians and inter-quartile ranges [IQRs] and qualitative data as numbers and percentages, unless otherwise stated. Comparisons across patients and siblings used exact Fisher tests or nonparametric Wilcoxon rank sum tests. All tests were two-sided, with *p*-values <0.05 denoting statistical significance. No adjustment for multiplicity was conducted.

Table 2
Treatment emergent adverse event that occurred following vaccine injection.

	Patients n = 61			Siblings $n = 15$	
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2
Any TEAE within 7 days	**34 (56)**	**20 (33)**	**0 (0)**	**8 (53)**	**2 (13)**
Pain at site of injection	26 (43)	11 (18)		7 (47)	1 (7)
Reaction at site of injection	7 (11)	1 (2)		0	0
Fever	4 (7)	12 (20)		2 (13)	1 (7)
Headache	5 (8)	1 (2)		3 (20)	1 (7)
Other AE within 7 days	**18 (30)**	**4 (7)**	**1 (2)**	**3 (20)**	**0**
Fatigue	5 (8)				
Nausea-Vomiting	4 (7)				
Diarrhea	1 (2)	3 (5)			
Aplasia			1 (2)		

* TEAE, Treatment emergent adverse event; AE, adverse event.

All statistical analyses were performed using R (version 4.1.1).

This study was registered with ClinicalTrials.gov, number NCT04969601, on the 19th July 2021.

3. Results

Between September, 29, 2021 and December 29, 2022, 76 children were enrolled (Fig. 1**)**, including 61 AL patients (median age 6 years [IQR, 4–9], 33 (54 %) males and 28 (46 %) females) and 15 siblings (median age 7 years [IQR, 6–9.5]; 5 (33 %) males and 15 (67 %) females). The accrual rate was close to that of the French number of cases [21]**.** Among the 61 patients enrolled, 57 (93 %) had acute lymphoblastic leukaemia (ALL) and 4 (7 %) acute myeloblastic leukaemia (AML). At study entry, 45 (74 %) patients were treated for B-lineage ALL (B-ALL) with a standard- or medium-risk protocol and were on maintenance therapy (Table 1). Patients were younger than their siblings and had lower frequencies of lymphocytes and gamma globulin, as expected. By contrast, both the SARS-CoV-2 humoral and cellular immune status were similar in the two groups **(Table S1)**. The two siblings who had positive Covid-19 serology at inclusion (anti-S IgG ≥ 7 BAU anti anti-N IgG ≥ 1.4) received only one dose of vaccine. All patients and the other 13 siblings received a second vaccine injection, which was delayed due to the occurrence of a documented SARS-CoV-2 infection for two siblings and three patients. Among the 29 patients who did not respond to the first two doses, 24 received a third dose, while the other five patients developed a SARS-CoV-2 infection between M2 and M3.

In part 1 of the study, five patients received a dose of 10 μg, four received 20 μg, and 44 received 30 μg (Fig. 1). No dose-limiting toxicity occurred for any dose. Afterwards, the recommended dose was 30 μg for each injection for the 53 enrolled patients, regardless of age group (2–5 and 5–12). No severe TAEs attributable to the vaccination were reported during the study. Principal side effects are reported in Table 2. Briefly, most adverse effects occurred in the days following vaccine administration and were local, such as pain or redness at the injection site.

A humoral immune response one month after the second injection (M2 visit) was observed in 47 participants (61.8 %, 95 %CI, 50.0–72.7), including 32 patients (52.4 %, 95 %CI, 39.3–65.4) and 15 siblings (100 %, 95 %CI, 78.2–100) ($p = 0.0003$). Anti-Spike IgG, with levels >260 BAU/mL, were all neutralizing (Table 3). Only 15 % of patients had antibody-neutralizing capacity against the more recent BQ.1.1 variant (8/52) vs 67 % of siblings (8/12) (Fig. 2**)**.

T-cell responses were assessed for 53 patients and 15 siblings (Table 3**,** Fig. 3**, Fig. S2**). Seventy-nine percent of patients (42/53) and 100 % of siblings (15/15) showed a positive T-cell response against S1 and/or S2 proteins ($p = 0.11$). Among them, 16 patients (14/42, 33 %) and seven siblings (7/15, 47 %) also showed a positive T-cell response

Table 3
Comparison of infections and immune responses over time across patients and siblings.

	Patients n = 61	Siblings n = 15	*p*
SARS-CoV-2 documented infections	32 (52)	6 (40)	0.56
Day 0-Day 21	3/32 (9)	2/6 (33)	
Day 21-Month 2	5/32 (16)	0 (0)	
Month 3-Month 6	14/32 (44)	4/6 (67)	
Month 7-Month 12	10/32 (31)	0 (0)	
Immune response at M2			
Anti-S IgG titer (median, range)	288.7 [40.75;1413]	2791 [1700;3542]	**0.0009**
Anti-S IgG titer ≥ 260 BAU	32 (52)	15 (100)	**0.0003**
Global anti-S neutralizing capacity (Boditech°)	32 (52)	15 (100)	
Anti-S neutralizing capacity by variant (VNT)			
B.1 Variant	35/52 (67)	12/12 (100)	**0.012**
BA.2 Variant	26/52 (50)	12/12 (100)	**0.0009**
BQ.1.1 Variant	8/52 (15)	8/12 (67)	**0.0008**
Anti-N IgG titer ≥ 1.4	9/60 (15)	4 (27)	0.28
Positive Elispot			
Against S1 or S2 proteins	42/53 (79)	15/15 (100)	0.11
Against M or N proteins	14/53 (26)	7/15 (47)	0.20
Immune response at M3 (in patients who received a third dose)			
Anti-S IgG titer (median, range)	140.3 [48.8;980.2]		
Anti-S IgG titer ≥ 260 BAU	10/23 (43)		
Global anti-S neutralizing capacity (Boditech°)	8/23 (35)		
Anti-N IgG titer ≥ 1.4	2/23 (9)		
Positive Elispot			
Against S proteins	13/19 (6)		
Against M or N proteins	4/19 (21)		
Immune response at M6			
Anti-S IgG titer (median, range)	394.5 [161.2;1851]	846.6 [681.9;1212]	0.17
Anti-S IgG titer ≥260 BAU	34/60 (57)	14 (93)	**0.007**
Global anti-S neutralizing capacity (Boditech°)	37/60 (62)	14 (93)	**0.027**
Anti-N IgG titer ≥ 1.4	9/60 (15)	7 (47)	**0.012**
Positive Elispot			
Against S1 or S2 proteins	42/55 (76)	13/13 (100)	0.06
Against M or N proteins	27/55 (49)	8/13 (62)	0.54
Immune response at M12			
Anti-S IgG titer (median, range)	728 [84.06;2199]	558.7 [463.6;659]	0.71
Anti-S IgG titer ≥260 BAU	38/58 (66)	13/15 (87)	0.20
Anti-S Neutralizing capacity (Boditech°)	38/58 (66)	14/15 (93)	0.05
Anti-N IgG titer ≥ 1.4	9/58 (16)	6/15 (40)	0.07
Positive Elispot			
Against S1 or S2 proteins	36/44 (82)	14/14 (100)	0.18
Against M or N proteins	29/44 (59)	11/14 (75)	0.51

* anti-S, anti-Spike protein; anti-N, anti-Nucleocapsid protein; IgG, immunoglobulin G;
* Quantitative data are expressed as medians and inter-quartile ranges [IQRs] and qualitative data as numbers and percentages.

against M or N proteins, indicating a post-infection response. Nine patients (56 %) and four siblings (57 %) already had a positive ELISpot at entry, with a post-infection profile. Eleven patients (20 %) had a negative ELISpot two months after the first injection. These patients are described in Table 4.

The factors associated with humoral immune responses of patients at M2 are summarized in Table 4**.** Briefly, the presence of B-cell lymphopenia and hypogammaglobulinemia (**Fig. S3**), or being in an intensive treatment phase were associated with a low humoral immune response.

Twenty-four patients received a third vaccine dose. The humoral response characteristics one month after the third injection are summarized in Table 3**.** Briefly, 10 patients (42 %) achieved significant humoral seroconversion one month after the third injection. T-cell responses were assessed in 19 patients. Among them, eight already had a positive ELISpot before the third injection. Among the 11 patients with a negative ELISpot before the third injection, six (55 %) obtained a positive T-cell response to SARS-CoV2-S proteins. The ELISpot transitioned from positive to negative for one patient.

The persistence of the vaccine response was evaluated by assessing the humoral and cellular responses at 6 and 12 months after the first vaccine dose. The results are summarized in Table 3**.** Briefly, anti-Spike antibodies and T-cell responses persisted in most patients and siblings. However, the percentage of patients and siblings with positive T-cell responses to M or N proteins increased with time.

A symptomatic and documented SARS-CoV2 infection was reported for 32 patients (52 %) and six siblings (40 %) during the study. Ten infections occurred within two months after the first vaccine injection in eight patients (13 %) and two siblings (13 %) **(**Table 3). None of the cases were severe, and no hospitalization was required. Chemotherapy was suspended for 19 patients for 5 to 14 days. The details of infections are summarized in **Table S2.**

4. Discussion

Preventing infections remains a major challenge for children with acute leukaemia. The PACIFIC trial was the first and only phase 1–2 study to assess the safety and immunogenicity of the BNT162b2 mRNA vaccine in children with AL and their siblings. This trial was set up very quickly (less than nine months between writing the protocol and the first inclusion) due to the Fast-track procedure put in place by France during the Covid-19 pandemic.

In this dose-ranging trial, we demonstrate that vaccination of children with AL with two 30 μg doses during maintenance treatment or after the end of chemotherapy is well tolerated and results in significant seroconversion with neutralizing antibodies in 52 % of patients one month after the second injection. Although this response rate was significantly lower than that of the siblings, it was still significant. The use of adult doses, which were three times the recommended pediatric dose, likely contributed to enhancing the immune response in our patients. Indeed, several studies, particularly involving the influenza vaccine, have demonstrated that increasing the vaccine dosage elicits a stronger immune response in immunocompromised patients [22,23].

The immunogenicity and safety of Covid-19 mRNA vaccines have been studied in adults with haematological malignancies (HMs). Systematic reviews and meta-analyses published in 2022 reported overall seroconversion rates ranging from 38 % to 99 % after two doses of vaccine in these patients. The lowest seroconversion rates were observed for patients with chronic lymphocytic leukaemia [24,25]. There were no studies specifically dedicated to patients with AL. However, in a subset of 166 AL patients reported in different studies, seroconversion rates in AML patients ranged from 43 % to 91 % and in ALL patients from 25 % to 100 % [24,25]. The heterogeneity of these results could be explained by the definition of "seroconversion", the type of treatment at the time of vaccination, and the timing of vaccination in relation to treatment [26]. The fact that we used adult doses of BNT162b2 probably helped us to obtain these results.

In August 2021, the US Food and Drug Administration (FDA) approved a third dose of mRNA vaccine of both BNT162b2 and mRNA-1273 for immunocompromised patients. Initial immunogenicity studies in patients with HMs showed good response rates, with one study reporting seroconversion in more than half of seronegative patients prior to the third dose [27]. In the absence of data on children at the time of our study, we decided to investigate the impact of a third dose in patients who did not achieve significant seroconversion after two doses. In accordance with the results observed in adult patients with HMs, this third injection resulted in significant seroconversion in 43 % of our children.

To study the overall vaccine response, we also assessed the T-cell

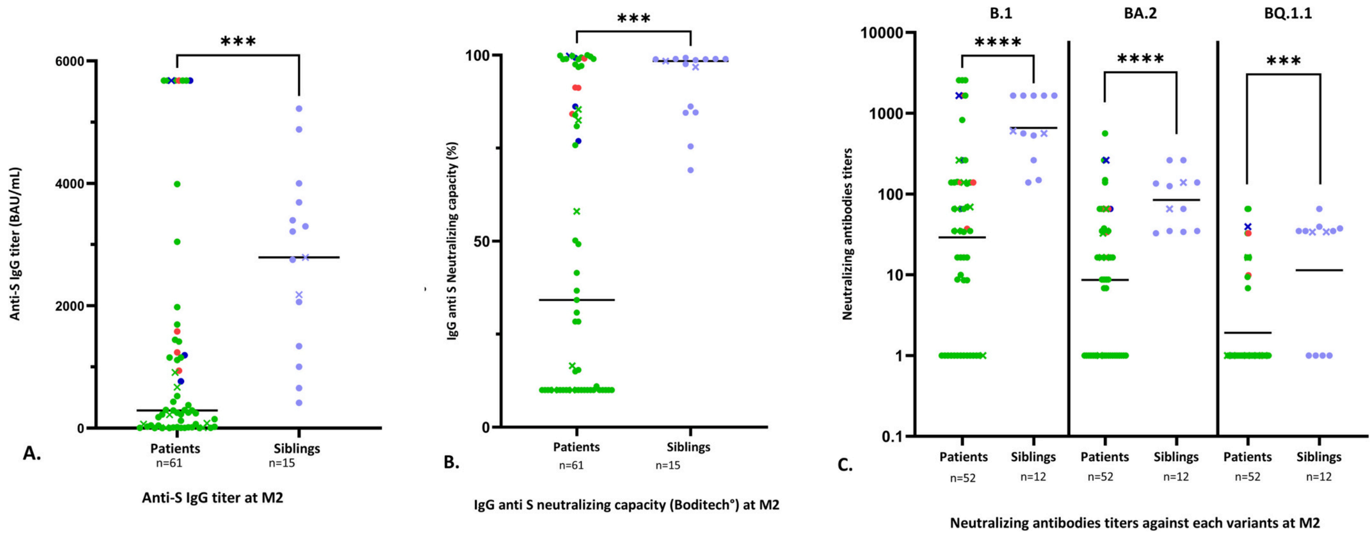

Fig. 2. Representation of humoral immune responses in patients and siblings one month after the second injection (M2 visit) based on anti-S IgG levels (BAU/ml) (A), anti-S IgG neutralizing capacity, (B) and neutralizing antibodies titers (C). Horizontal solid bars represent the median (A, B) or geometric mean (C). Each point corresponds to one participant. Patients with B-ALL are shown in green, those with T-ALL in violet, patients with AML in red, and siblings in blue. Crosses indicate children with a documented COVID-19 infection between M0 and M2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

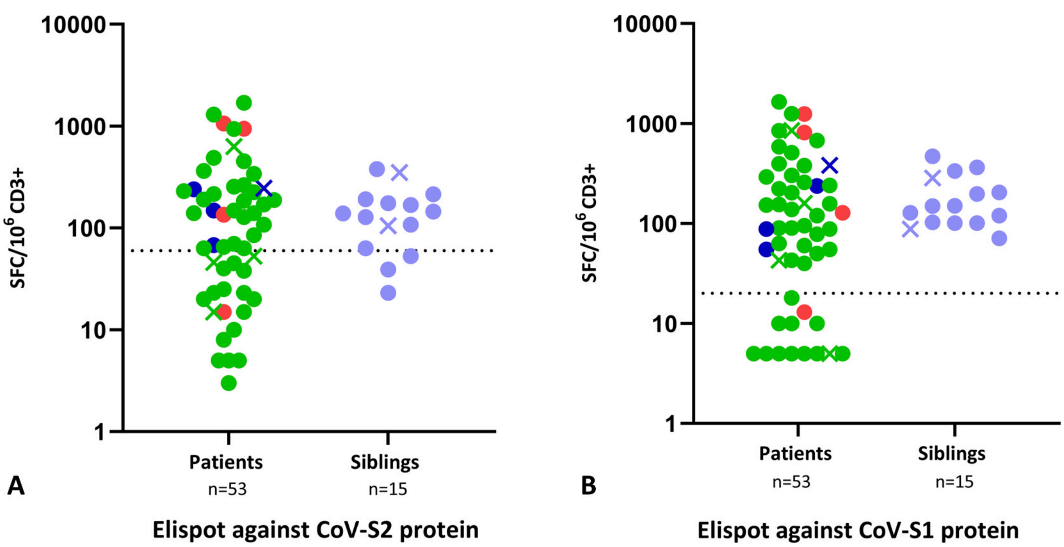

Fig. 3. Representation of cellular immune responses against SARS-CoV-S1 (A) and SARS-CoV-S2 (B) proteins measured with Elispot in patients and siblings one month after the second injection (M2 visit). Horizontal dotted bars represent the test's positivity threshold. Each point corresponds to one participant. Patients with B-ALL are shown in green, those with T-ALL in violet, patients with AML in red, and siblings in blue. Crosses indicate children with a documented COVID-19 infection between M0 and M2. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

response, which is crucial in these immunocompromised patients. Interestingly, we found that 80 % of patients achieved a T-cell response within one month after the second injection, a rate almost equivalent to that of the siblings. Robust T-cell responses, despite an impaired humoral response, have already been reported for adult patients with HMs in several studies that have assessed cellular immunogenicity [24], particularly in patients receiving CD20 antibody therapy [24]. Studies reporting discordant humoral and cellular immune responses showed that at least 20 % of patients have a cellular response in the absence of seroconversion [24]. This contrast between humoral and cellular response capacity is not specific to mRNA vaccines and has also been reported for other vaccines in immunocompromised patients. For example, in a study evaluating the response of adults with HMs to the subunit herpes zoster vaccine, a significant humoral response was observed in 65 % of patients, whereas a cellular response was observed in 84 % of patients one month after the second vaccine injection [28].

Our study had several limitations, including the small number of patients included and the difficulty in assessing long-term responses due to the frequent occurrence of infections between M2 and M12. Indeed, the number of SARS-CoV-2 infections increased significantly in the general population just after the start of our study. As in the general population, the children in our study were infected and developed diversified humoral and T-cell responses, making it impossible to draw any conclusions on the persistence of the vaccine response over long term.

5. Conclusions

In conclusion, we have demonstrated that vaccination against Covid-19 with 30 μg-doses of an mRNA vaccine is well tolerated and results in

Table 4
Factors measured at inclusion associated with patient immunogenicity at M2.

	Anti-S IgG titer < 260 BAU ($n = 29$)	Anti-S IgG titer ≥260 BAU ($n = 32$)	p-value	Negative Elispot ($n = 11$)	Positive Elispot ($n = 44$)	*p*
Sex M/F	14/15 (48)	19/13 (59)	0.45	7/4 (64)	22/22 (50)	0.51
Age group			0.28			0.23
2–5	9 (31)	11 (34)		4 (36)	13 (30)	
5–11	14 (48)	19 (60)		4 (36)	26 (59)	
12–15	6 (21)	2 (6)		3 (27)	5 (11)	
Dose group			0.25			0.34
10 μg	1 (3)	4 (13)		2 (18)	3 (7)	
20 μg	3 (10)	1 (3)		0	3 (7)	
30 μg	25 (86)	27 (84)		9 (82)	38 (86)	
Pathology			**0.02**			0.57
B-ALL	29 (100)	24 (76)		10 (91)	37 (84)	
T-ALL	0 (0)	4 (12)		0 (0)	4 (9)	
AML	0 (0)	4 (12)		1 (9)	3 (7)	
PEG-Asparaginase allergy	4 (14)	3 (9)	0.88	2 (18)	4 (9)	0.59
Phase of treatment			**0.0002**			0.39
Intensive	2 (7)	0 (0)		1 (9)	1 (2)	
Maintenance	27 (93)	21 (66)		9 (82)	33 (75)	
No treatment	0 (0)	11(34)		1 (9)	10 (23)	
Chemotherapy < 7 days	29 (100)	21(75)	**0.004**	10 (100)	34 (83)	0.32
Corticosteroids < 7 days	5 (17)	3 (9)	1.00	3 (30)	4 (12)	0.32
Biology						
ANC, G/L	2.2 [1.1; 3.3]	2 [1.4; 2.6]	0.63	2.5 [1.5;4.1]	2.1 [1.4;2.6]	0.31
IgG, G/L	4.8 [4.1;6.0]	6.6 [5.0;9.1]	**0.005**	4.7 [4.1;5.1]	6.3 [4.7;8.6]	**0.022**
Leucocytes, G/L	3.53 [2.6; 4.32]	3.72 [2.91;5.36]	0.63	3.93 [2.805;6.905]	3.615 [2.728;5.018]	0.39
Lymphocytes, G/L	0.67 [0.54;0.95]	1.35 [0.8;1.74]	**0.001**	0.93 [0.595;1.065]	0.95 [0.6325;1.605]	0.56
CD19+	0.02 [0.01;0.02]	0.06 [0.01;0.25]	**0.009**	0.02 [0.005;0.02]	0.03 [0.01;0.13]	0.085
CD3+	0.7 [0.5;1]	1 [0.63;1.43]	0.17	0.8 [0.54;0.9]	0.87 [0.57;1.4]	0.44
CD3 + CD4+	0.3 [0.24;0.54]	0.42 [0.29;0.85]	0.18	0.3 [0.25;0.455]	0.425 [0.255;0.8475]	0.31
CD3 + CD8+	0.31 [0.24;0.43]	0.41 [0.25;0.65]	0.28	0.31 [0.27;0.45]	0.38 [0.24;0.68]	0.87
CD16 + CD56+	0.04 [0.02;0.08]	0.09 [0.04;0.15]	0.095	0.05 [0.04;0.11]	0.06 [0.02;0.14]	0.42

* ALL, acute lymphoblastic leukaemia; AML, acute myeloblastic leukaemia; PEG, pegylated; ANC, absolut neutrophil count; IgG, immunoglobulin G
* Quantitative data are expressed as medians and inter-quartile ranges [IQRs] and qualitative data as numbers and percentages.

significant T-cell response children with AL, even during chemotherapy. As doses of 10 μg is currently recommended for children, these results support the value of increasing vaccine doses in immunocompromised patients. Furthermore, the possibility to elicit a strong T-cell response in children with AL through mRNA vaccination is of particular interest because this type of vaccine has the potential to be used against other infectious pathogens and could also have applications in anti-tumor strategies in these patients.

Availability Statement

Will individual participant data be available (including data dictionaries)?	Yes, according RGPD after contractualisation with promotor
What data in particular will be shared?	All of the individual participant data collected during the trial, after de-identification
What other documents will be available?	Study protocol, statistical analysis plan, informed consent form, clinical study report, analytic code
When will data be available (start and end dates)?	Immediately following publication; no end date
With whom?	Investigators whose proposed use of the data has been approved by an independent review committee (“learned intermediary”) identified for this purpose
For what types of analyses?	To achieve aims in the approved proposal
By what mechanism will data be made available?	Proposals should be directed to arnaud.petit@aphp.fr; to gain access, data requestors will need to sign a data access agreement

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CRediT authorship contribution statement

Fanny Alby-Laurent: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Amani Ouedrani:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Data curation. **Pierre Jateau:** Writing – review & editing, Writing – original draft, Resources, Investigation, Data curation. **Marion Strullu:** Writing – review & editing, Resources, Investigation. **Anouk Walter-Petrich:** Writing – review & editing, Resources, Formal analysis, Data curation. **Chaima Mrad:** Writing – review & editing, Investigation, Data curation. **Audrey Guilmatre:** Writing – review & editing, Investigation, Conceptualization. **Maxime Ferreboeuf:** Writing – review & editing, Investigation, Conceptualization. **Anne-France Ray-Lunven:** Writing – review & editing, Resources, Investigation. **Florentia Kaguelidou:** Writing – review & editing, Investigation. **Odile Launay:** Writing – review & editing, Writing – original draft. **Lucienne Chatenoud:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Data curation, Conceptualization. **Laurence Morand-Joubert:** Writing – review & editing, Writing – original draft, Resources, Methodology, Investigation, Data curation, Conceptualization. **Sylvie Chevret:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Methodology, Formal analysis, Data curation, Conceptualization. **Arnaud Petit:** Writing – review & editing, Writing – original draft, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2025.128130.

Data availability

Data will be made available on request.

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