

Pediatric posttransplant relapsed/refractory B-precursor acute lymphoblastic leukemia shows durable remission by therapy with the T-cell engaging bispecific antibody blinatumomab

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Supplementary Appendix

Online Supplementary Methods:

Clinical Monitoring

Clinical surveillance parameters were assessed in all patients throughout the whole period of the first cycle and at the start of each subsequent cycle. In patients who tolerated the treatment well, an outpatient basis for treatment was offered with clinical and laboratory monitoring every three days.

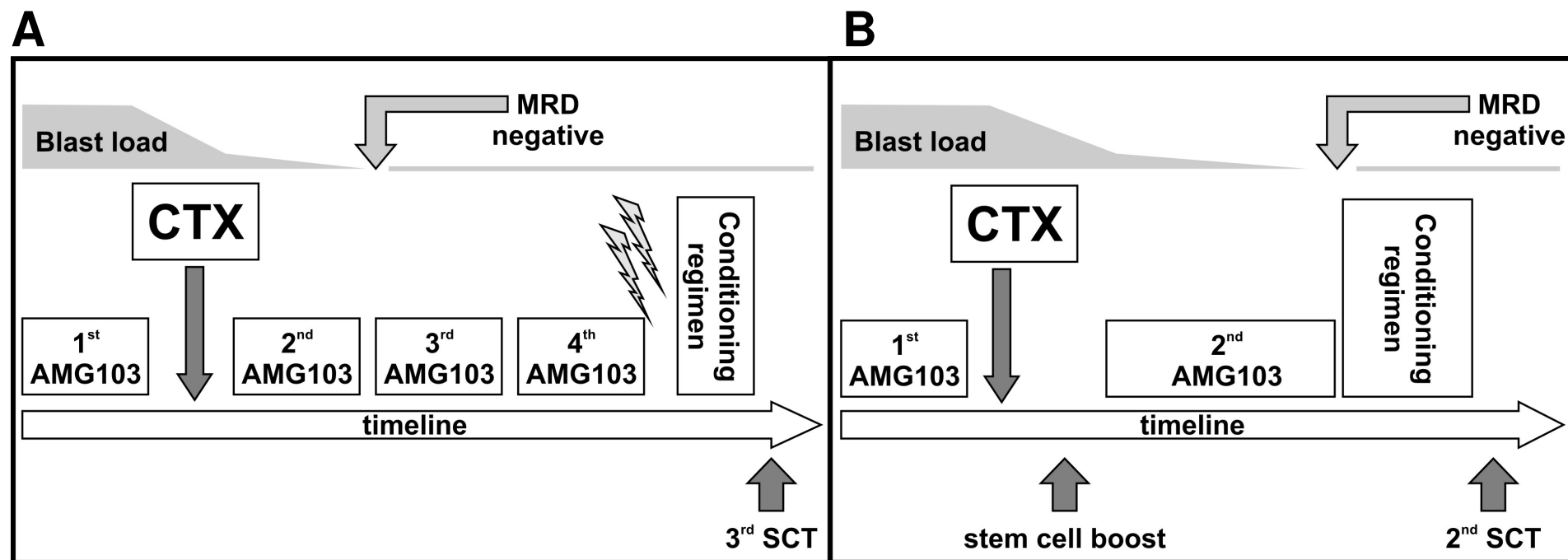
Acquisition of Laboratory Parameters

Standard laboratory parameters (differential blood count, liver enzymes, bilirubin, pancreatic enzymes, kidney retention values, albumin, immunoglobulins, coagulation parameters, C-reactive protein and electrolytes) were assessed daily at the start of each cycle.

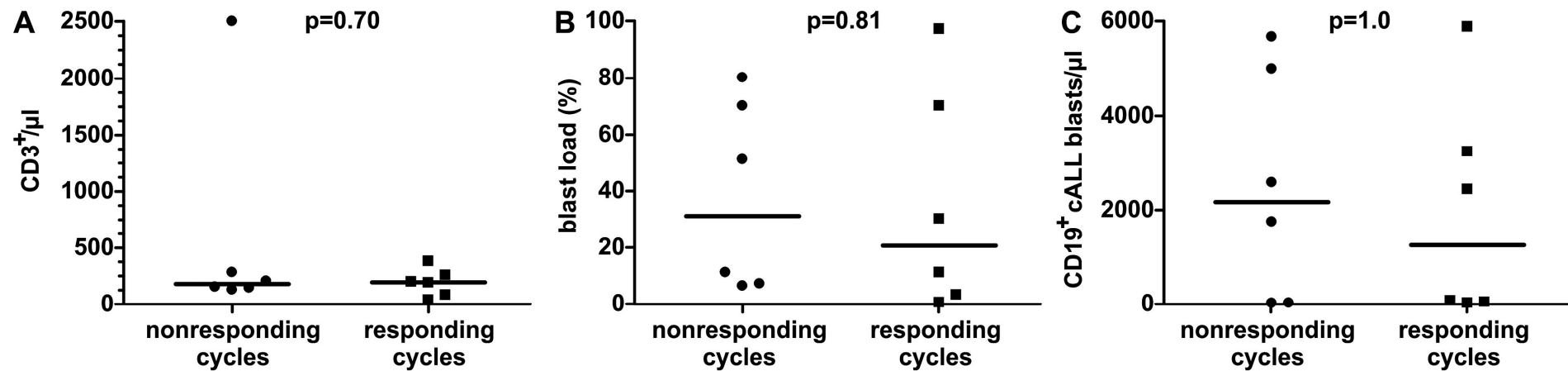
Statistical Analysis

Nonparametric two-tailed Mann-Whitney test was used to compare the data set of responding versus nonresponding cycles in terms of T-cell to blast ratio. Ratio was calculated as T-cell count per μl whole blood divided by blast count per μl bone marrow. The data was acquired prior to the start of each blinatumomab cycle (n=18). In the available data on T-cell and blast count, the comparison of time interval from HSCT to start of blinatumomab and time off immunosuppression in responding

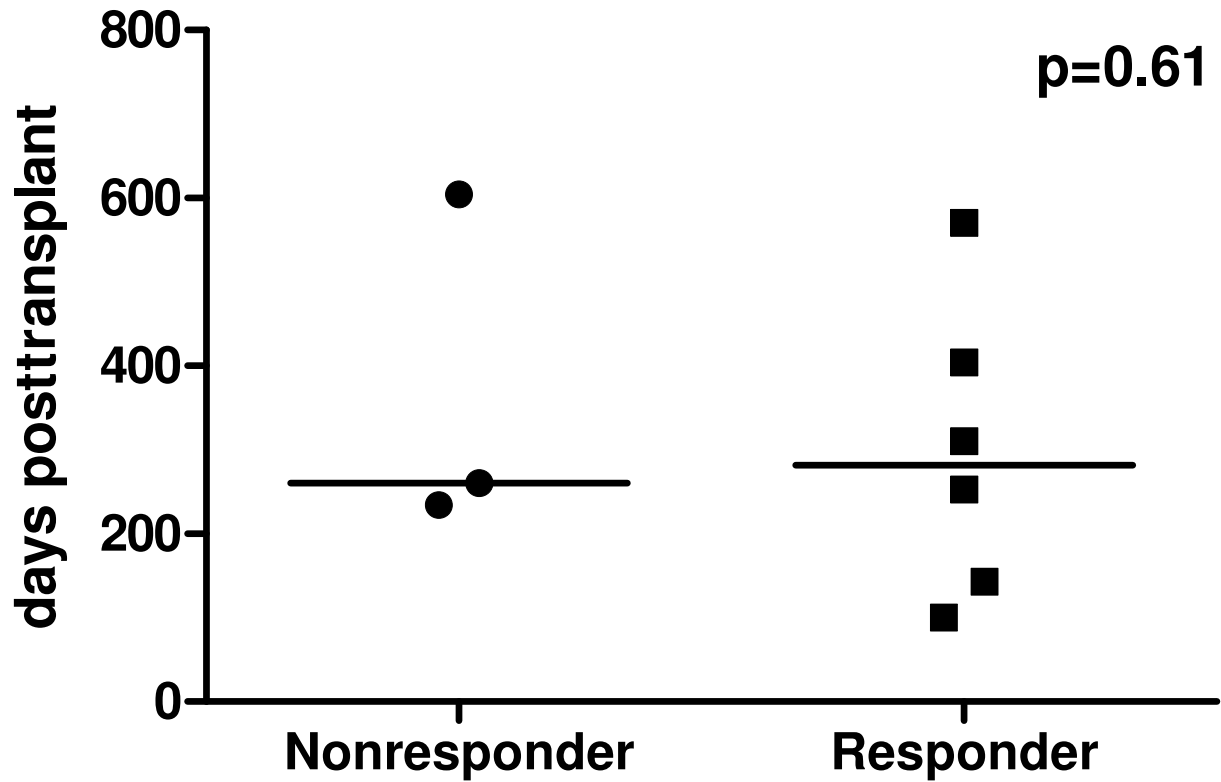
versus nonresponding cycles is descriptive and hypothesis generating but is not sufficient for drawing firm conclusions.



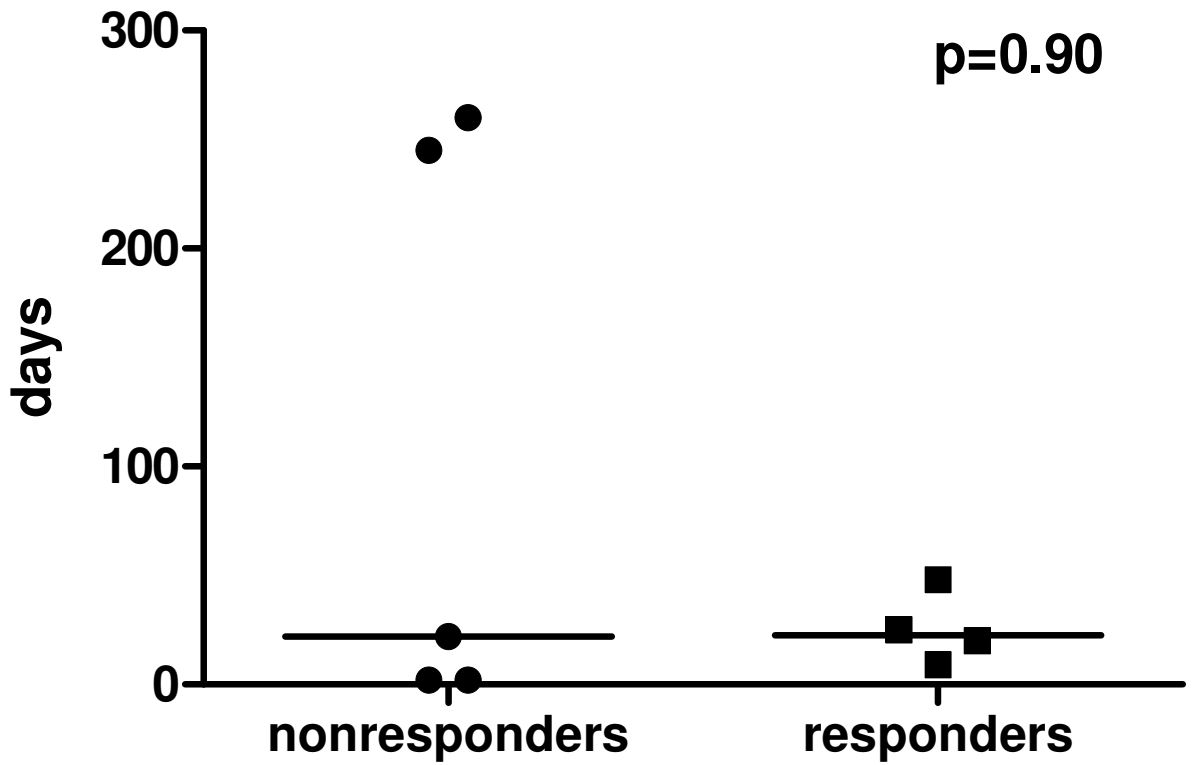
Online Supplementary Figure S1. Exemplary course of treatment (No. 5, 6), who showed secondary response. Blinatumomab (AMG 103), Chemotherapy (CTX), Irradiation (⚡). Patient **A** (No. 5) received one day of melphalan ($20\text{mg}/\text{m}^2$) and one day of cytarabine ($1000\text{mg}/\text{m}^2$) prior to the 2nd blinatumomab cycle at $15\text{ }\mu\text{g}/\text{m}^2/\text{day}$, two cycles to consolidate MRD and local irradiation 18 Gray (Gy) of the viscerocranium and 12 Gy of the neurocranium because of chloroma-like detected local leukemic infiltration in the bilateral mastoids and the jugulum. Patient **B** (No. 6) received one dose of vincristine ($1\text{ mg}/\text{m}^2$) and three days of clofarabine ($40\text{ mg}/\text{m}^2$), etoposide ($100\text{ mg}/\text{m}^2$) and cyclophosphamide ($400\text{ mg}/\text{m}^2$) with subsequent stem cell rescue ($5 \times 10^6/\text{kg}$ BW $\text{CD}34^+$ cells, T-cell depleted graft) from the previous parental haploidentical donor and then proceeded to the 2nd blinatumomab cycle of treatment at increased $15\text{ }\mu\text{g}/\text{m}^2/\text{day}$. Both patients then succeeded to receive another haploidentical HSCT in molecular remission.



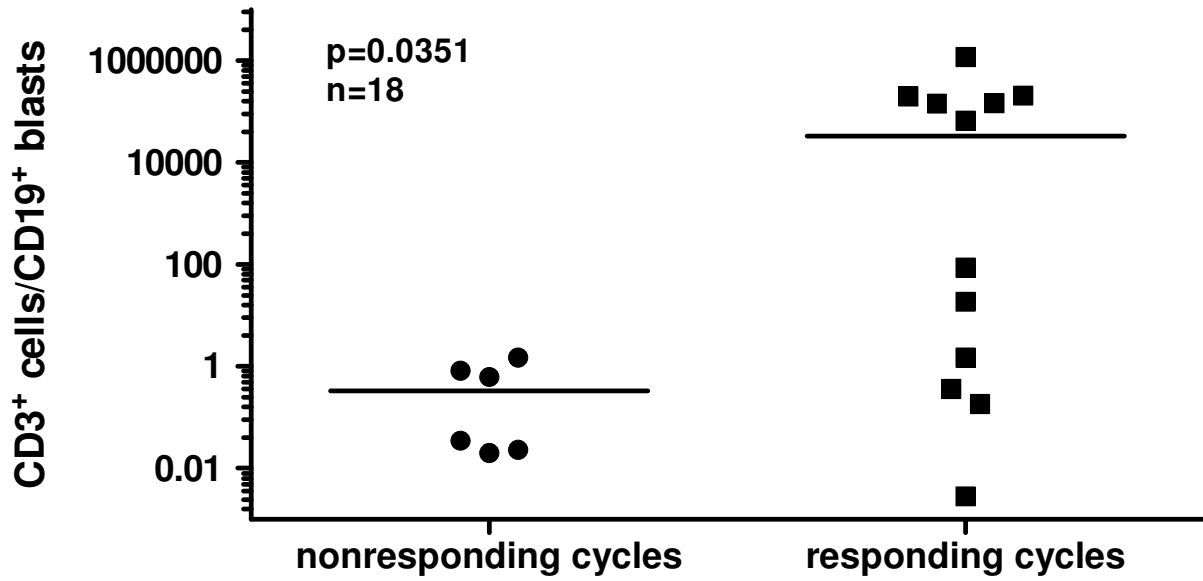
Online supplementary Figure S2. Comparison of nonresponding versus responding cycles (n=12) in respect to T-cell count in the peripheral blood, percental blast load as well as absolute blast count of CD19⁺ cALL blasts/μl in the bone marrow. The data was acquired prior to each corresponding blinatumomab cycle. Statistical comparison of nonresponding vs. responding cycles was performed using Mann-Whitney test. **A** T-cell count (CD3⁺/μl) in the peripheral blood. **B** Percental leukemic blast load in the bone marrow. **C** Absolute blast count in the bone marrow.



Online Supplementary Figure S3. Comparison of the time interval in days from HSCT (prior to the start of blinatumomab) treatment to the first day of blinatumomab treatment in all 9 patients. The line shows the median number of days, nonresponders 260 days, responders 282 days. Statistical comparison of nonresponders vs. responders was performed using Mann-Whitney test.



Online Supplementary Figure S4. Comparison of the time interval in days from last day of immunosuppression or last day of applied chemotherapy to the first day of blinatumomab treatment. The line shows the median number of days, nonresponders 22 days, responders 23 days. Statistical comparison of nonresponders vs. responders was performed using Mann-Whitney test.



Online supplementary Figure S5. T-cell to blast ratio acquired via flow cytometry. Absolute CD3⁺ cell count per μ l divided by absolute cell count per μ l blasts from bone marrow aspirates at the start of each blinatumomab cycle (n=18). Statistical comparison of nonresponding vs. responding cycles was performed using Mann-Whitney test.