BRIEF REPORT

Early relapse after rituximab chemoimmunotherapy. Report on a patient with common acute lymphoblastic leukemia in second relapse and review of the literature.

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ABSTRACT

In relapsed/refractory childhood acute lymphoblastic leukemia (ALL) of the B-cell lineage rituximab, a monoclonal anti-CD20 antibody, was used successfully in some cases. We report on a 15-year old girl with relapsed CD20-positive B-cell progenitor ALL treated with rituximab because of positive minimal residual disease signals after chemotherapy, as checked by flow cytometry and real time quantitative-PCR. Rituximab eliminated the CD20-positive subpopulation, but not the more immature leukemic cells. The patient died in fulminant aspergillosis before hematopoietic stem cell transplantation could be performed. A summarization of the literature and possible mechanisms behind treatment failure will be discussed.

INTRODUCTION

Rituximab, a chimeric monoclonal antibody directed against the B-cell lineage-restricted CD20 antigen was successfully applied in mature B-cell malignancies (1,2). It has recently been introduced in the treatment of CD20-positive B-cell progenitor (BCP) acute lymphoblastic leukemia (ALL) of adults (3) and children with relapsed/refractory disease (4-6).

Due to the lack of larger studies, the success/failure rate of rituximab treatment in childhood BCP ALL is unknown. Here we report that combined chemotherapy supplemented with rituximab treatment failed to induce a long-term remission in a young adult experiencing second relapse. The kinetics of minimal residual disease (MRD) status will be described in relation to the disease course. Based on the presented case and a review of the current literature**,** possible causes of rituximab treatment failure in childhood BCP ALL will be discussed.

MATERIALS AND METHODS

Case report

A 15-year old Caucasian girl with common-ALL, treated according to ALL-BFM 95 (7) experienced bone marrow (BM) relapse 48 months after diagnosis. Salvage therapy according to ALL R-87 (8) was accompanied by corticosteroid psychosis and avascular necrosis of the right femoral head. A second BM relapse occurred 67 months later. Due to the previous corticosteroid side-effects, FLAG-IDA (9,10) induction treatment was applied resulting in a third complete remission. Protocol M of ALL-BFM 95 was chosen as consolidation therapy. Since a further intensification of conventional chemotherapy was limited by severe myelosuppression and the

patient was MRD positive, two courses of rituximab (MabThera®, Roche, Hertfordshire, UK 375 mg/m^2 4 weeks apart) were applied as post-consolidation therapy without complications followed by maintenance therapy (Fig. 1). After identifying only one suitable, partially mismatched, unrelated donor the patient refused hematopoietic stem cell transplantation (HSCT). Ten months after second relapse MRD signals started to elevate followed by an overt, third BM relapse as checked on May-Grünwald-Giemsa-stained smear at 13 months**.** The patient, having consented to HSCT, was referred to the regional transplantation center. Due to the increasing ratio of BM blasts, she received a repeated course of FLAG-IDA and died of fulminant pulmonary aspergillosis in massive pancytopenia 14 months after the second relapse.

Immunophenotyping:

For BM flow cytometric analysis three-color panels using monoclonal antibodies (CD3-PerCP, CD4-FITC, CD7-FITC, CD8-PE, CD19-PerCP, CD20-FITC, CD22-PE, CD34-FITC, CD34-PE, CD34-PerCP, CD45-FITC, CD45-PerCP, CD56-PECy5, HLA-DR-PerCP, κ-FITC, λ-PE, Becton Dickinson: [BD], San Jose, CA) (CD10-PE, CD79α-PE, TdT-FITC, IgM-PE: DAKO, Glostrup, Denmark) were utilized. Surface and intracytoplasmic staining was executed according to our standard labeling procedures as published previously (11). For MRD detection 100 000 cells were analyzed using a FACS Scan flow cytometer with CellQuest 3.2 software (BD, San Jose, CA).

RQ-PCR:

Multiplex PCR reactions, heteroduplex analysis and sequencing were performed according to the standardized protocols of the BIOMED-1 Concerted Action (12). A single monoclonal T-cell

receptor δ (TCRδ) rearrangement was identified and sequenced, and a patient-specific forward primer, hybridizing to the junctional region, was designed:

5'-TACTACTGTGCCTGTGACTCCTGG-3'

RQ-PCR was performed according to standard protocols (12), using an ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). Reaction mixtures contained 500 ng gDNA template. Peripheral blood mononuclear cell (PBMC)-derived gDNA from 5 healthy donors was used as negative control.

RESULTS

Of the leukemic cell population CD10bright/CD19/TdT coexpression of lymphoblasts provided a useful aberrant immunophenotype for identification of MRD (13). Infiltrating the initial BM sample in 76% (3432/μL), these cells were present throughout the course of the disease, with a dramatic decrease exceeding 3 log ($<10^{-3}$) at Day 25, immediately after induction treatment (Fig.1). At this time point, both the ratio and the absolute number of CD10/CD19/TdT-positive lymphoblasts were below the detection limit of flow cytometry. The CD20 coexpressing subpopulation, as checked by the presence of CD10/CD/19/CD20-positive cells (59%, 1853/μL), made up over 50% of the initial leukemic population. This subpopulation decreased below detection limit by Day 25 but reappeared along with a small, increasing ratio of CD10/CD19/TdT-positive cells, present in 0.8% (68/μL) after consolidation treatment.

Application of 2 courses of rituximab resulted only in a delayed decrease in the CD10/CD19/TdT-positive subpopulation, although CD20-positive cells completely vanished and never reappeared. At Month 13, MRD level increased to 12% (879/μL). Interestingly, a more

immature CD19/TdT-positve/CD10-negative lymphoblast population was present in the sample. Relapse was obvious by checking MGG-stained smears.

Molecular analysis of gDNA isolated at second relapse identified a monoclonal, incomplete patient-specific TCRδV2-TCRδD3 gene rearrangement, which was used as a target sequence to monitor MRD by RQ-PCR. MRD level in follow-up samples was compared to the tumor load of the initial sample. Because of high PBMC background, a $4.75x10^{-2}$ quantization limit was set for the assay. MRD level decreased moderately, approximately to $5x10^{-2}$ in course of 4 months of therapy and fell below quantization limit before the first rituximab treatment and remained less than $4.75x10^{-2}$ until check-up at Month 10. At this data point MRD level by RQ-PCR started to rise and by Month 13 the tumor load was 20 times higher than at Day 1.

DISCUSSION

The limited number of reported pediatric patients having received rituximab for relapsed/refractory BCP ALL does not allow statistical evaluation of success/failure rate of anti-CD20 monoclonal antibody treatment. We have found 4 reported cases of rituximab treatment failure or severe side-effects in children with BCP ALL.

In the presented case rituximab was applied in a patient with relapsed childhood BCP ALL because of positive MRD status after FLAG-IDA chemotherapy. Treatment was well tolerated. Severe rituximab-related adverse effects have been registered in 0.04-0.07% of patients according to the manufacturer's post-marketing surveillance database (14). In 2 children, complications related to cytokine release syndrome have been reported (6,14). Another potentially severe side-effect of rituximab, elimination of normal B-cell clones, might have contributed to the fatal aspergillosis of our patient (15).

Therapy failure however, was determined by the third BM relapse of the patient. Two different biological mechanisms may interfere with eradication of the leukemic cell population. 1) Leukemic cells may escape in pharmacological sanctuaries: BM relapse has been reported in a boy treated with rituximab because of optic nerve relapse of common-ALL (16). 2) Stem cells**,** that are supposed to play a fundamental role in the maintenance of the leukemic population**,** represent a small pool of leukemic progenitors (17). Immunophenotype of these progenitors may be more immature than that of the bulky progeny. Hato et al reported on a patient with CD20-positive ALL acquiring resistance to rituximab. Leukemic cells at relapse exhibited the loss of CD20. Flow cytometry showed the preexistence of CD20-negative leukemic cells before rituximab therapy (18). Similarly, in the child treated with rituximab because of optic nerve relapse**,** the recurrent leukemic clone was CD20-negative (16). In our case, flow cytometry at second relapse identified a large CD20-negative subpopulation. Molecular-based MRD detected a different subpopulation than flow cytometry. Rituximab treatment resulted in a complete elimination of the CD20-positive subpopulation, but not the leukemic cells expressing a more immature phenotype. Indeed, a relapse was heralded by RQ-PCR at Month 10 followed by an overt relapse at Month 13. At this time leukemic blasts were CD20-negative**:** more immature**,** CD19/TdT-positive/CD10-negative cells were present. In conclusion rituximab may prove beneficial for the treatment of BCP ALL including pediatric cases. Large multicenter trials utilizing multiple MRD detection techniques are required to determine the success/failure rate and optimal application of this investigational drug.

Legend to figure 1 (Fig. 1):

Minimal residual disease (MRD) detected by flow cytometry and RQ-PCR in relation to treatment and the final stage of the clinical disease. Negative means below the detection limit. IMT: interim therapy.

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