Clonal stability of initial leukemia in a child with CNS relapse 7.4 years after bone marrow relapse of common ALL

Second central nervous system (CNS) relapses represent about 7.3% of subsequent recurrences of childhood acute lymphoblastic leukemia (ALL). In most children these subsequent CNS relapses occur during the first 18 months after diagnosis of the first relapse (mean 1.42±0.73 years). We present a patient who suffered a second ALL relapse in the CNS more than seven years after diagnosis of his first relapse. The leukemic clone was completely stable over more than ten years as shown by minimal residual disease techniques. Possible reasons for the recurrence of the leukemic clone after this very long period of dormancy (e.g. role of the disease site, immune system dysfunction) are discussed.

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Very late second central nervous system (CNS) relapses of childhood acute lymphoblastic leukemia (ALL) are rare, and there are no published case reports to date. Little is known about the pathogenesis of late first and second ALL relapses, but different theories regarding clonal evolution and immune system dysfunction have been discussed previously. Here, we present a case report of a very late subsequent CNS relapse of ALL without evidence of clonal evolution of the original clone.

Patient

In 1991, the now 20 year old male patient was diagnosed with common (c-) ALL at the age of 10 years. Due to his higher age and the presence of hyperleukocytosis, he was treated according to the high risk arm of protocol COALL-89 including cranial irradiation at a dose of 18 Gy. Two and a half years later, aged 13, the patient suffered a late isolated c-ALL bone marrow (BM) relapse and was treated according to protocol ALL-REZ BFM 90 (relapse trial of the Berlin-Frankfurt-Münster Group), group B, again including cranial irradiation (12 Gy).[1,2] The patient was included in a phase I/II-pilot study of active specific immunotherapy with the Newcastle-Disease-Virus. During maintenance therapy of first relapse treatment, cryo-conserved BM leukemic blasts from relapse diagnosis were injected subcutaneously every week after having been incubated with Newcastle-Disease-Virus and irradiated with 200 Gy, as previously described for renal cell carcinoma.[3] After cessation of relapse-chemotherapy (10/1996) and in continuous second remission, hypogammaglobulinemia was diagnosed resulting from a B-cell deficiency (< 0.5% of leukocytes), and consequently the boy received multiple courses of intravenous immunoglobulins during the following years until now.

During the whole period of salvage chemotherapy and also after cessation of therapy, he suffered from recurrent episodes of bacterial upper respiratory tract infections, and, in 02/1997, already in an immunodeficient state, he experienced a severe meningococcal meningoencephalitis.

In 07/2001, after prolonged headaches and partial 6th cranial nerve palsy, lumbar and BM puncture revealed an isolated CNS relapse of his c-ALL. Currently, he receives chemotherapy according to protocol ALL-REZ BFM 96 (strategic group S2) and is to date in third complete remission. The CNS-directed therapy consists of oral dexamethasone, intravenous methotrexate and triple intrathecal therapy (cytarabine, prednisone and methotrexate) every two months during the first year and every three months during the second year.

Material and Methods

The following materials were available for analysis: BM slides at initial disease; BM aspirates at diagnosis of first and second relapse; peripheral blood (PB) and cerebro-spinal fluid (CSF) at diagnosis of second relapse, and BM follow-up samples of first and second relapse treatment. Mononuclear cells were isolated by Ficoll density gradient centrifugation (Seromed; Biochrom KG, Berlin, Germany). The diagnosis of the immunophenotype was made according to French-American-British classification and standard immunophenotypic criteria.[4-6] Assessment of common chromosomal translocations in childhood ALL was performed with reverse-transcriptase polymerase chain reaction (PCR).[7] DNA was extracted using PuregeneTM DNA-Isolation kit (Biozym Diagnostic GmbH, Oldendorf, Germany). T-cell receptor delta and gamma (TCRD/TCRG) and immunoglobulin heavy and light chain (IgH/IgK-Kde) gene rearrangements were used as clone-specific MRD markers.[8,9] Clonality was assessed by homo-heteroduplex analysis.[10] PCR products for which monoclonality was confirmed were directly sequenced. Minimal residual disease (MRD) quantification of BM follow-up samples was performed using real-time quantitative (RQ) PCR with germline TaqMan-probes (TCRD-D3, and IgH-JH6) and clone-specific primers.[11]

Results

In the BM aspirate obtained at diagnosis of first relapse, two clonal markers were identified: an incomplete TCRD gene rearrangement Ddelta2-Ddelta3 and an IgH gene rearrangement VH4-VH3-DH3-JH6. Three primers, a Ddelta2-Ddelta3, a VH4-VH3 and a DH3-JH6 have been designed with a sensitivity of 10^{-5}, 10^{-4} and 10^{-4}, respectively. These primers were used to assess the stability of the clone at initial diagnosis and second relapse and to quantify MRD during relapse therapy. Screening for additional rearrangements was not done at presentation due to lack of available DNA and was negative in the CSF at second relapse diagnosis. In the BM, PCR screening for additional rearrangements was not performed at second relapse because of low leukemic blast counts.

The clone characterized by two monoclonal rearrangements detected with PCR at first relapse diagnosis was stable throughout the disease without evidence of emerging sub-clones or clonal evolution. This molecular clonal stability is reflected by the immunophenotype of the leukemic cells which did not change throughout the disease episodes. The mononuclear cells of the BM showed a high expression of CD10, CD19, TdT, CD24 and HLA-DR (c-ALL) at diagnosis of initial disease and first relapse. At second relapse diagnosis, the identical c-ALL was confirmed using the leukemic blasts in the CSF.

Neither at first nor at second relapse chromosomal translocations as *TEL-AML1* t(12;21), *BCR-ABL* t(9;22), *MLL-AF4* t(4;11) were detectable.

Figure 1 summarises the results of monitoring of minimal residual disease (MRD) from initial diagnosis to second relapse. During frontline treatment, BM follow-up samples were not available. MRD quantification of BM aspirates obtained during treatment of first relapse showed a rapid initial response to therapy. After the second therapy course, MRD was below the critical limit of 10-3.[12] However, during and at the end of maintenance

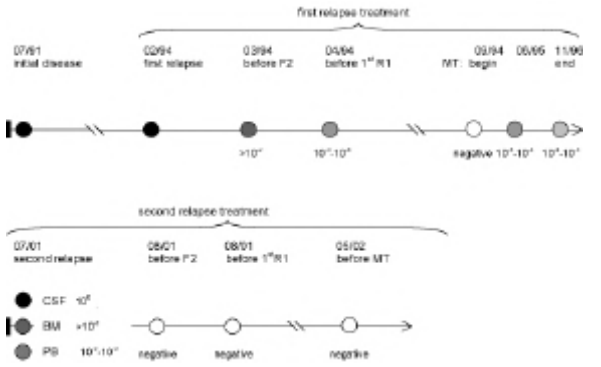

Figure 1. Results of monitoring of minimal residual disease (MRD) from initial diagnosis to second relapse. MT maintenance therapy, BM bone marrow, PB peripheral blood, CSF cerebro-spinal fluid, F2 second therapy course and 1st R1 third therapy course of ALL-REZ BFM protocols (strategic group B and S2).

therapy, MRD was again detectable at a level of $<10^{-3}$->10^{-4} and $<10^{-4}$->10^{-5}, respectively. At second CNS relapse diagnosis, residual leukemic cells were detected in the BM (>10^{-2}) and in the blood ($<10^{-2}$->10^{-3}), a phenomenon often observed in patients with isolated extramedullary relapse.[13,14] In subsequent BM samples during second relapse treatment, MRD was not detected.

Discussion

The vast majority of second isolated CNS relapses of children registered in the ALL-REZ BFM registry occurred during the first three years after first relapse diagnosis, between 0.16 and 2.98 years with a median of 1.4 years (n=61). Among these patients, the probability of experiencing a subsequent isolated CNS relapse beyond 4.2 years from diagnosis has been estimated to be less than 0.001. Our patient suffered a second ALL recurrence in the CNS 7.4 years after the first relapse in the BM, the latest second relapse reported in the literature to date.

There is a long and still ongoing discussion whether a late relapse of leukemia is clonally related to those present at diagnosis or a second leukemia. Late true relapses might still respond favourably to chemotherapy, whereas a secondary leukemia is associated with poor prognosis. The clonal identity of this very late second relapse with first relapse and to the initial disease was confirmed by the stability of two monoclonal gene rearrangements and the immunophenotype throughout the disease without evidence of emerging sub-clones or clonal evolution. How can leukemic cells persist over ten years?

A major factor contributing to the survival of the leukemic cells is thought to be the existence of extra-compartments, in particular the CNS, in which the cells are at least partially protected from chemotherapeutic agents by the blood brain barrier. In addition, there is evidence that leukemic cells in the CNS have a lower proliferation rate and another surface ultrastructure than in the BM because of a completely different microenvironment.[15-17] The CNS environment lacks several growth promoting factors present in the BM, such as high vascularisation and growth factor supply. It is of note that in our patient even a double cranial radiation therapy at a total cumulative dose of 30 Gy was not able to prevent the subsequent relapse.

Relapses originating from the CNS are more chemosensitive than those from the BM because leukemic cells derived from the CNS may be less exposed to previous chemotherapy than leukemic cells of the BM. Moreover, leukemic cells from the CNS had been exposed to prophylactic CNS-irradiation and may be already enervated. Furthermore, leukemic cells proliferating at relapse are more vulnerable by chemotherapy than dormant cells. Therefore, a repeated course of relapse chemotherapy rather than stem cell transplantation was chosen as second salvage therapy. Indeed, complete elimination of the leukemic cells could be seen after the first induction course in the CNS and, at a very sensitive MRD-level (reduction from >10^{-2} to <10^{-4}), in the BM.

In patients with an isolated or combined BM relapse, recent findings revealed that MRD response after the second induction course (cut off 10^{-3}) was the only independent and best factor predictive for subsequent relapses.[12] Although, the patient had a good molecular response (<10^{-3}), his subsequent CNS relapse after an exceptionally long remission period could not be predicted. The course of the patients disease demonstrates the limitations of MRD regarding the ability of leukemic cells to escape and to hide in extra-compartments.

Several theories exist as to the concept of cure in leukemia. It is believed that the remission induced by anti-leukemic therapy results in a marked reduction of the number of clonal blast cells under a certain threshold, resulting in regained control of the tumour cells by the host's immune system. During the interval between initial disease and first ALL-relapse, our patient developed symptomatic B-cell deficiency with multiple upper respiratory-tract infections. This defect resembles common variable immunodeficiency (CVI), a disease with the hallmarks pan-hypogammaglobulinemia and recurrent bacterial infection especially of the upper and lower respiratory tract.[18] Normally, patients with CVI have normal or near normal numbers of B-cells, which is not true in our patient having nearly no B-cells (CD19-positive cells < 0.5% of lymphocytes). Interestingly, only the B-cell compartment was affected whereas the number of T-cells was normal. According to the aforementioned theory, a defect of immune surveillance as described in our patient could lead to insufficient antineoplastic control and subsequent flare-up of the neoplasm. Interestingly, although the majority of patients with CVI suffering from a malignant disease registered in the Immunodeficiency Cancer Registry had B-cell non-Hodgkin lymphomas (46%), still eight per cent of them had leukemia, suggesting that the CVI-like immunodeficiency of our patient could at least have facilitated the ALL recurrences.[19]

The original pre-leukemic clone identified by two stable gene rearrangements, as demonstrated above by MRD and immunophenotype analysis, must have persisted for more than ten years. This could be explained by the "two step model" of leukemogenesis as proposed by Greaves et al. He assumes a pre-leukemic clone with the origin already in the prenatal period which is never completely eliminated during therapy.[20] One or more postnatal genetic alterations are then supposed to be necessary for the development of leukemia or recurrence, respectively. Selection pressure towards the "decisive-step" may be exerted by stress to the B-lymphoid progenitor compartment, for example after several episodes of infections as seen in our patient or even by aggressive treatment.[21,22]

In conclusion, we describe for the first time a very late subsequent isolated CNS relapse of a immunogenetically and -phenotypically stable clone. The leukemic cells of the dominant clone hidden in the growth inhibiting environment of the CNS could have achieved a growth advantage by accumulating genetic alterations and an either simultaneous or following lack of immune system control. Assuming that indeed the immune system is a

crucial factor for the control of leukemia, the question is whether the outgrowth of the dormant leukemic cells might be prevented. Recently, an association between a lower incidence of leukemia in children having received Haemophilus influenzae type b - Hib vaccination has been reported, and historically, a leukemia preventing effect of bacille Calmette-Guérin - BCG vaccination has been assumed.[23-25] Thus, the question may be raised whether long-term control of leukemia could be achieved or maintained by T-cell stimulating procedures such as repeated vaccinations?

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