Autologous Immune Enhancement Therapy in Philadelphia Chromosome Positive Acute Lymphoblastic Leukemia

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Abstract Advances in current treatment regimens in childhood acute lymphoblastic leukemia (ALL) have resulted in cure rates of 75–80 %. Some molecular genetic abnormalities confer a poor prognosis. Of these, the chromosomal translocation t (9;22)—Philadelphia chromosome is associated with the worst outcome in childhood ALL. Optimal therapy for this variant of ALL includes chemotherapy as per high risk schedule, imatinib and early stem cell transplantation. We report here the successful natural killer cell-based autologous immune enhancement therapy along with chemotherapy and imatinib in a patient with Philadelphia chromosome positive ALL.

Introduction

The translocation t (9;22), which generates the Philadelphia chromosome (Ph), occurs in 3–5 % of children with ALL. This results in a fusion protein of 210 kd (p210) when the

abl proto-oncogene moves from chromosome 9 to the major breakpoint cluster region (BCR) on chromosome 22. This is the usual finding in chronic myelogenous leukemia and occurs occasionally in Ph-positive ALL. The *abl* gene can also translocate to the minor BCR on chromosome 22, resulting in a 190-kd fusion protein (p190) that occurs exclusively in ALL. More than 90 % of children with Ph-positive ALL have this subtype of t (9;22). Overall, Ph-positive ALL has a poor prognosis with rates of event-free survival are 25–30 % in children [1]. Attempts to improve results for such subtypes of ALL include early stem cell transplantation and use of concomitant imatinib.

Immunotherapy has been used effectively to augment the effect of cancer chemotherapy. Improved prognosis has been reported when chemotherapy has been combined with immunotherapy than with chemotherapy alone [2]. Natural killer (NK) cells form a major part of our immune system that helps in mounting a specific response to viruses or malignant cells. They lock onto their targets and destroy them by inducing apoptosis and by releasing inflammatory cytokines [3]. Autologous immune enhancement therapy (AIET) involves isolation of the peripheral blood mononuclear cells (PBMCs) from a patient and expanding NK cells in vitro. The patient's own serum is used in the culture for expansion of the cells. After in vitro expansion and activation, the NK cells are re-introduced into the patient's system and allowed to attack the cancer cells [4–6]. We report here a case of Ph+ALL, treated with NK cell-based AIET along with chemotherapy and imatinib and followed up for a period of 5 years.

Case Presentation

A 15 year old girl presented to us in October 2004 with complaints of diffuse bone pain for 3 months, intermittent

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fever for 1 month and increasing pallor. On examination, she was found have pallor with hepatosplenomegaly. Her blood counts revealed haemoglobin 9.3 g/dl, platelet count of $21 \times 10^3/\mu\text{l}$ and a WBC count of $13.6 \times 10^3/\mu\text{l}$ with 83 % lymphoblasts. Bone marrow examination and flow cytometry were suggestive of acute lymphoblastic leukemia—CD 10+ CD 19+. Cytogenetics revealed Philadelphia chromosome and BCR–ABL was positive. Qualitative BCR–ABL was done serially to assess disease status.

She was started on chemotherapy as per UK MRC protocol—Regimen B along with Imatinib Mesylate (in view of her BCR–ABL positivity). She also received prophylactic cranial radiotherapy of 12 Gy at the end of delayed intensification. She had persistent disease with low positive BCR–ABL at 0.04 % at the end of delayed intensification. Thereafter, the patient was started on maintenance chemotherapy. Her family HLA typing revealed no matched family donors. As she had high risk disease we decided to treat her with AIET using autologous in vitro expanded and activated NK cells (CD3-CD56+ lymphocytes) in January 2007.

After informed consent was obtained, 60 ml of peripheral blood was collected from the patient in heparin tubes and transported to a GMP compliant cell processing facility lab under cold conditions. The peripheral blood was first centrifuged to separate the autologous plasma. The pellet containing white and red blood cells was mixed using phosphate buffered saline. LymphoPrep solution was used to isolate the PBMCs. The cell count was estimated using trypan blue dye-exclusion method and the NK cells were expanded for a period of 14 days using the methodology already reported [4–7]. Flow cytometry analysis was done to determine the percentage of NK cells employing markers like CD3, CD16 and CD56.

At the end of in vitro expansion and activation, 562×10^6 NK cells obtained were suspended in 70 ml of saline and infused to the patient intravenously, on an outpatient basis at the rate of 0.5 ml/min over a time period of 80 min. The premedication used before infusion was paracetamol and pheniramine maleate. Only one infusion of AIET was administered to the patient. The patient tolerated the procedure well. The patient was continued on maintenance chemotherapy. Four weeks later, BCR–ABL in peripheral blood turned negative. She completed her treatment (maintenance chemotherapy) in October 2007. She has been on regular follow up for the last 5 years and she continues to be in remission with negative BCR–ABL status.

Discussion

Cell-based immunotherapy has been in practice for the past 2 decades, with published randomized clinical trials

establishing its safety and efficacy in different cancers [8, 9]. Numerous studies have demonstrated efficacy of cell based immunotherapy in various solid tumours—ovarian, hepatic, pancreatic and breast carcinomas [4–6, 8–11]. However, there have been very few studies on the use of NK cells in pediatric leukemias [12]. Recurrence free interval of more than 24 months was reported in a 77 year old patient with ALL treated with immune cell therapy in combination with Imatinib Mesylate [13]. However, to our knowledge there has not been any study till date on the use of AIET in paediatric ALL and this is the first such report. The prognosis of children with very high risk ALL such as those with Philadelphia chromosome positivity is generally poor with chemotherapy alone. Early stem cell transplantation carries significant morbidity and mortality. Fregni et al. [14] suggest that even when conventional therapies may apparently result in complete remission, tumour relapse may occur due to the micrometastases of residual tumor cells and immunotherapy after conventional therapies can help in complete and durable eradication of tumors. The patient in our study after chemotherapy and Imatinib Mesylate followed by AIET using NK cells has been in remission for nearly 5 years and is still under follow-up, the duration of this remission being higher than what was reported in the earlier report on immunotherapy for ALL [13]. Thus AIET using NK cells can be explored as a novel approach in the treatment of very high risk ALL. We would recommend further studies to substantiate this mode of therapy in children with T ALL, Ph+ALL with persistent MRD at the end of 17 weeks.

Conclusion

AIET using NK cells in combination with other therapies has been found to be safe in our experience in this case of Ph+ALL and has yielded a BCR–ABL negative status, 4 weeks after the treatment with the patient continuing to be in remission for 5 years. We reiterate that this may be considered as an optional experimental therapy in similar cases and large clinical trials are needed to validate the efficacy.

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