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Infusion of *in-vivo* expanded cord blood lymphocytes: A new strategy to control residual disease?

Letter to the editor

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Donor lymphocyte infusion is a procedure for treatment or prevention of relapse after allogeneic stem cell transplant (ASCT), of hematological malignancies including multiple myeloma (MM)(1)(2). This procedure is effective alone or associated with chemotherapy or targeted therapy(3). The infusion of immune cells from the donor especially T cells leads to their activation against tumour cells, also known as graft versus disease effect. The inherent risk is its pathological counterpart, the graft versus host (GVH) disease. The main limitation of cord blood transplant is the ethical impossibility to collect lymphocytes directly from the donor. To overcome this issue, we considered that it might be possible to collect donor

lymphocytes from the transplanted patient after their *in vivo* expansion and maturation. Here we describe our first experience of such strategy.

In 2006, 56 years old man with a diagnosis of free light chain (FLC) kappa multiple myeloma was unsuccessfully treated with an association of conventional chemotherapy (vincristine, doxorubicin, dexamethasone). Finally, a first remission was achieved after bortezomib based treatment followed by high-dose melphalan and autologous stem cell transplantation consolidation. After 17 months, he experienced relapse and was rescued with lenalidomide and dexamethasone followed by a double cord blood unit (CBU) allogeneic transplantation in December 2009. Conditioning regimen included fludarabine, cyclophosphamide and total body irradiation (2 Grays). One year after allogeneic transplantation he achieved complete response (CR) with normal FLC ratio, undetectable medullary minimal residual disease (MRD) assessed by cytometry evaluation(4) and absence of active bone lesion in total body magnetic resonance imaging (MRI) and positron emission tomography (PET TDM). In terms of toxicities, the patient experienced stage 3 hepatic acute GVH disease followed by limited chronic GVH disease targeting mucosae with an oral lichen planus. Eighteen months after transplant, lymphocyte count was 2.8×10^6 elements per liter (G/L) and chimerism evaluation of circulating nucleated cells and circulating T lymphocytes showed more than 99% of donor cells indicating that an *in vivo* expansion of cord blood lymphocytes took place. Lymphocyte peripheral blood sub-populations included 51% of T cells CD3+ (including 7% of HLA DR+, 15% of CD4+, 35% of CD8+, 2% of CD8+/CD56+ cells), 5% of NK cells (CD3-/CD56+) and 42% of B cells (CD19+/CD20+). After providing oral and written information about the procedure and obtaining patient consent, we proceeded to leukapheresis and collected 4.25×10^7 CD3, viability was 99% as assessed by 7-AAD staining. Due to suboptimal venous access we stopped the collection that was divided into 2 samples and cryo-conserved.

The patient experienced extra-medullary relapse 32 months after the ASCT, with MRI and PET TDM showing active focal lesions on the right humerus and femur. Histological analysis of the humeral lesion showed a proliferation of tumoral kappa restricted plasma cells. Serum FLC ratio was increased (10.9), but no medullary involvement was found even by 8 colour multi-parametric cytometry evaluation. The lymphocyte count was 1,7 G/L. Blood lymphocytes sub-populations consisted of 52% of T cells CD3+ (HLA DR 5%, CD4+ 24%, CD8+ 27%, CD8+/CD56+ 3%), 8% of NK cells (CD3-/CD56+) and 40% of B cells (CD19+/CD20+). A 27 grays irradiation was delivered to the femoral lesion. The humeral lesion hadn't been irradiated, as it wasn't symptomatic. The FLC ratio was normalized after three courses of lenalidomide and dexamethasone. At this time, Pet-CT demonstrated no significant fluorodeoxyglucose (FDG) uptake and MRI showed stable bone lesions. Lenalidomide and dexamethasone were discontinued because of asthenia (ECOG 3) and diarrhoea. These symptoms resolved shortly after treatment interruption, and no chronic GVH disease had been observed. Nine months later, and 6 month after the last lenalidomide and dexamethasone intake, the lymphocyte count kept deteriorating to 0,30 G/L including 54% of T cells CD3+ (HLA DR 6%, CD4+ 24%, CD8+ 38%, CD8+/CD56+ 26%), 42% of NK (CD3-/CD56+) and 2% of B cells (CD19+/CD20+). No viral infection had been diagnosed, chimerism evaluation did not differ and patient remained in CR. An infusion of cryopreserved donor lymphocytes was carried out in May 2013. 0.5×10^7 CD3+ lymphocytes per kg of body weight were infused, viability was 62%. One month later the lymphocyte count increased to 0,54 G/L for the benefit of CD4+ and CD8+ lymphocytes (figure 1). One month after lymphocyte infusion, chronic GVH disease occurred with erosive oral lichen manifestation. MRI evaluation showed regression of the femoral lesion (that had been irradiated) and stability of the humeral lesion (figure 2). A second cryopreserved CD3+ lymphocyte infusion (2×10^7 CD3+ lymphocytes per kg of body weight with 61% of

viability) was carried out 6 months after the first infusion. The lymphocyte count continued to rise (1,3 G/L). T CD4+, T CD8+, B (CD19+/CD20+) and NK (CD3-/CD20+) subpopulations were those showing the greater absolute increase (figure 1). No toxicity was reported. One year after the first allogeneic lymphocyte infusion the patient was in complete remission with normal serum FLC ratio and disappearance of humeral bone lesion (not irradiated). Bone marrow aspiration demonstrated no plasma cell infiltration and undetectable MRD. Lymphocyte count and phenotype were normal with donor origin for more than 99% of total circulating nucleated cells and T lymphocytes. An incidental lung adenocarcinoma with spine metastasis was found on evaluation PET TDM. Subsequent follow-up showed biological relapse of myeloma after 18 months of CR, with slowly increasing FLC ratio of 2.6. The lymphocyte count at this new relapse remained stable.

We report here the first case, to our knowledge, of the collection of *in vivo* expanded lymphocytes and subsequent re-infusion for consolidation of myeloma relapse treatment after cord blood allogeneic transplantation.

ASCT is a possible strategy for treatment of refractory MM, especially for young patients relapsing after high dose chemotherapy followed by autologous stem cell transplantation, resistant to proteasome inhibitors and immune-modulatory drugs (IMiDs)(5)(6). In the European and Japanese experiences, this strategy provides a 24% rate of progression free survival at 3 years with 41 % grade II to IV acute GVH disease, 22% of chronic GVH disease and 29% of therapy related mortality (7)(8). An high proportion of extra-medullary relapses (24% of all ASCT in our experience) is observed after reduced intensity conditionings(9). The outcome of these extra-medullary relapses is similar to systemic relapses and combinations of proteasome inhibitors or immune-modulatory drugs with DLI or not are options(10), taking into account that DLI effectiveness requires a low residual disease. In the present case,

management of clinical relapse with lenalidomide allowed reducing tumour mass down to subclinical residual disease but this treatment could not be maintained.

CBU transplantation is not compatible with classical DLI because the limited number of cells contained in the cord blood requires complete and immediate re infusion of all cells at time of transplant to allow haematopoietic recovery. We report here the use of in-vivo expanded and cryopreserved cord blood lymphocytes(11)(12). The sequence “lenalidomide followed by infusion of these lymphocytes” allowed a 18 months disease free interval.

The collection of lymphocytes early after transplantation and *ex-vivo* expansion (directed against tumour antigens) was reported in a Hodgkin lymphoma case(13). This technique requires bio banking of personal tumour sample.

Ex-vivo lymphocyte expansion directed against Epstein Bar Virus antigens has been used for transplant related EBV proliferations but there is limited data on their anti-tumor effect. Another report describes classical lymphocyte collection in a case of sibling cord blood transplantation(14). However, this can only be performed in rare cases of directed donation, with ethical and medical issues about multiple collections in child.

In vivo expansion, maturation and collection of CBU-derived lymphocytes to control minimal residual disease is an original approach. The optimal term for collection is a point of discussion. Early lymphocyte collection after immune recovery appears to be a good option as most relapses occur between 2 and 5 years in hematological malignancies. Another option could be to collect lymphocytes during acute or chronic GVH disease to obtain allo-reactive lymphocytes. The last point was to collect after completion of vaccination program but timing was evaluated as too long. Due to poor venous access in the patient's arms, the number of cells collected was only $4,25 \times 10^7$ CD3 per kg. Cryoconservation alters viability of lymphocytes. Nevertheless, viable lymphocytes could be recovered and were sufficient for 2

DLI with 10E6 and 10E7 cells per kg. Authors have described cell amounts infused of up to 10⁸ cells per kg(15).

Our experience suggests that, in the situation of controlled post transplant relapse, prolonged remission can be obtained with consolidation by infusion of cord blood lymphocytes, collected after the allogeneic transplantation. However this is a single case report and there are many intrinsic biases. We postulate that infusions of *in-vivo* expanded donor lymphocytes could be a solution to be able to benefit from DLI in the CBU ASCT setting. This procedure is easy to set up in hospitals practicing blood and marrow transplantation with cell processing facilities for cryoconservation. A phase one clinical trial could determine the safety and effectiveness of the procedure, the ideal time to collect lymphocyte and the best approach in terms of indication: prophylaxis, pre-emptive strategy, curative in association with antineoplastic therapy or loss of chimerism.

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Conflict of Interest Statement

The authors declare that there are no conflicts of interest.

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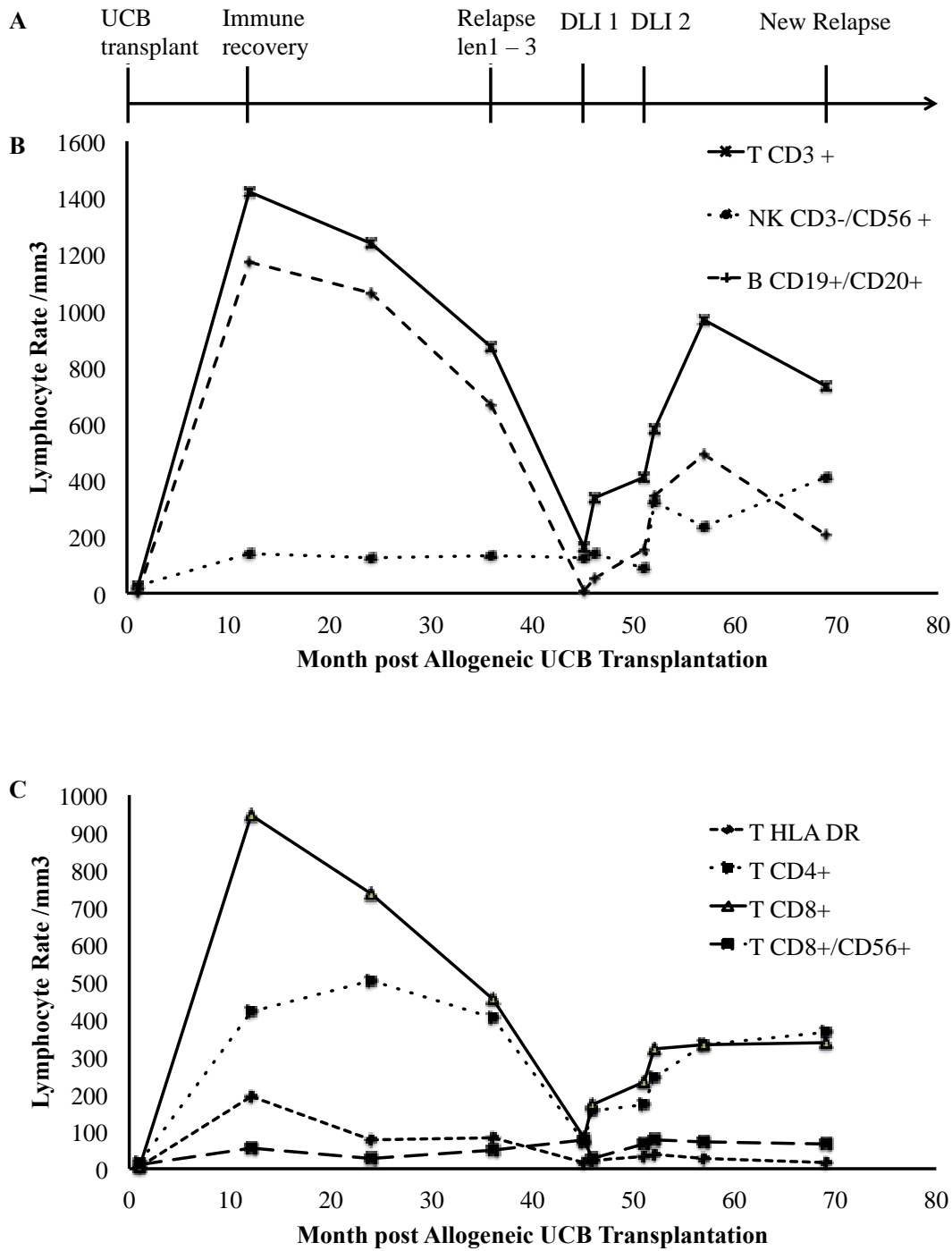


Figure 1: Evolution of lymphocyte rate and its subtype during post-transplant management of multiple myeloma. Panel A shows an overview of the procedure: 12 months after double cord blood unit transplant immune recovery had been shown and leukapheresis proceeded. The cryo-conserved lymphocytes were infused after extramedullary relapse and 3 courses of lenalidomide treatment. The CR lasted 18 months. Chart B and C shows rapid

immune recover during the first year post transplant. At relapse there was a trend to B (CD19+/CD20+), T CD4+ and T CD8+ lymphopenia, amplified by lenalidomide treatment. Shortly after donor lymphocyte infusion there is an increase in T CD4+, T CD8+ and a few month later an increase of NK (CD3-/CD56+) and B (CD19+/CD20+) lymphocytes. There is no change in T HLA DR and T CD8+ CD56+ population. Chart A shows T, B and NK population. Chart B shows T subpopulation.

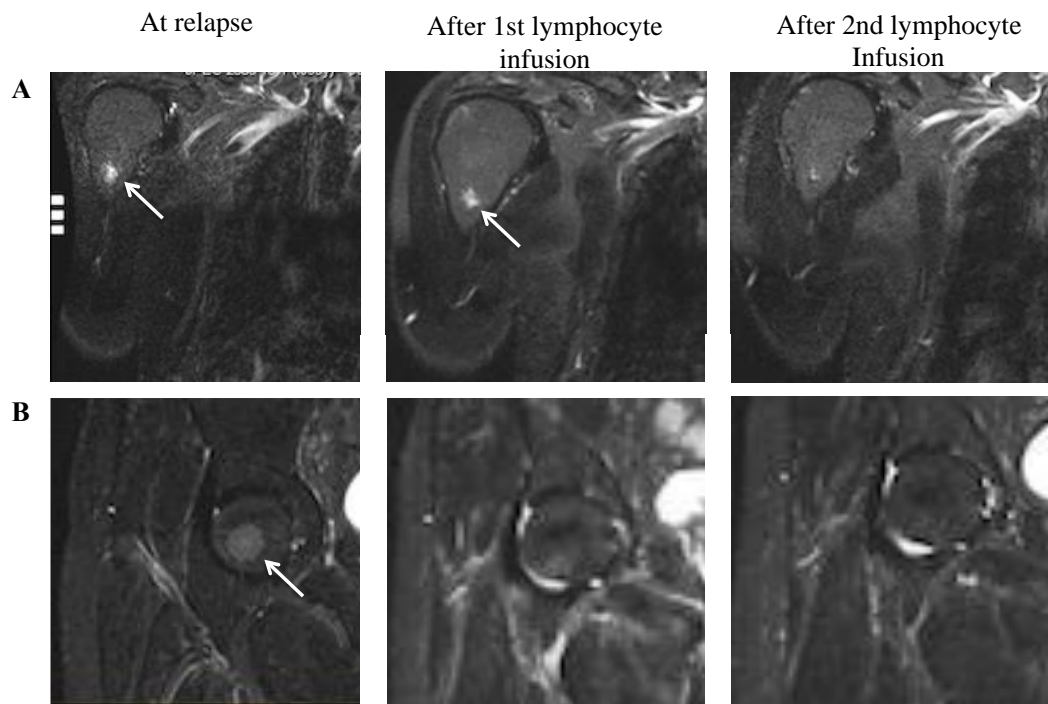


Figure 2: Tumour Control after *in-vivo* expanded infusion of allogeneic lymphocytes.

Panel A shows axial MRI (STAIR) of the right humerus head and panel B shows axial MRI (STAIR) of the right femoral head, sites of the myeloma relapse (proven by biopsy on humerus). The femoral lesion had been irradiated and disappeared after 1st allogeneic lymphocyte infusion; the humeral lesion disappeared after 2nd allogeneic lymphocyte infusion.