CLINICAL USE OF DONOR LYMPHOCYTE INFUSIONS (DLI)

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Donor lymphocyte infusions (DLI) have broadly been used after allogeneic hematopoietic stem cell transplantation (HSCT) primarily either to enhance donor chimerism after non-myeloblative/reduced-intensity conditionings or to treat disease relapse. In 1990, encouraging clinical data on the use of unmanipulated DLI were first reported by Kolb et al[1] in patients with chronic myeloid leukemia (CML) relapsed after an allograft. These findings were confirmed by other studies which reported up to >70% durable complete cytogenetic remissions in CML patients. The higher relapse rate reported after T depleted HSCT as compared with T replete HSCT is indirect evidence that donor CD3+ T cells play a crucial role in *graft-vs.-leukemia* (GvL) effects. More recently, natural killer (NK) cells and regulatory T cells (Tregs) have also been described as potential mediators of GvL. However, GvL may be offset by the association of DLI with *graft-vs.-host-disease* (GvHD). Acute and chronic GvHD following DLI occur in 40-60% and 33-61% of patients respectively with a mortality of approximately 6-11% [2,3]. Clinical manifestations of post-DLI GvHD are similar to those seen after HSCT. Skin is the most frequently involved organ, though a peculiar high rate of single organ liver GvHD is not uncommon [4,5]. Thus, DLI are not administered in patients with active GvHD. Among others, the DLI dose infused and timing after transplant are factors significantly associated with the development of severe GvHD. [6-8]. Response to DLI was less prominent in hematological malignancies such as acute myeloid leukemia (AML) and myelodisplastic syndromes where complete response rates were 15-29% and 33-40% respectively. In AML patients better outcome was observed with low tumor burden at the time of DLI and favorable cytogenetics[9]. Although the combination of chemotherapy with DLI may evoke a stronger GvL, treatment-related mortality is disappointingly high[10-12]. In acute lymphoblastic leukemia (ALL), response to DLI was observed in only in 5-18% of cases, whereas in Hodgkin lymphoma, non-Hodking Lymphomas and chronic lymphocitic leukemia the experience is rather limited. Stronger GVL effect was observed in indolent/low grade lymphomas and multiple myeloma where higher efficacy was seen with the combination of DLI and new drugs such as bortezomib and lenalidomide [13-16].

DLI have also been used for establishing a state of full donor chimerism from a state of mixed donor chimerism. This attempt has been rather successful in pediatric patients after reduced-intensity or non-myeloablative HSCT [17-19]. In adults a role for DLI in mixed chimeras has mostly been observed in T-depleted allografts [20,21].

DLI have also been explored in patients with post-transplant persistence or re-occurrence of minimal residual disease (MRD) [22]. A large prospective study on 814 patients with standard-risk acute leukemias who received T-repleted HSCT in first or second complete remission was reported by Yan et al. After HSCT, 709 patients were MRD- (group A) and 105 were MRD+, of whom 49 received low-dose IL2 (group B) and 56 modified-DLI obtained from G-CSF mobilized peripheral blood cells with/without IL2 (group C). Group C showed significantly higher overall survival (OS) and disease free survival (DFS) than group B (p=0.001 and p=0.002) whereas no difference were seen between group C and group A (p=0.695 and p=0.688). By multivariate analysis, MRD state and modified DLI correlated with better DFS suggesting that modified DLI may reduce relapse in MRD+ patients with standard risk AL after HSCT [23].

More recently, the use of unmanipulated DLI has also been investigated in haploidentical HSCT. High cumulative incidence of acute grade II-IV GvHD (53.2%) was initially described after T cell-repleted haploidentical HSCT by Yan et al [24]. Two other studies on DLI after haploidentical

HSCT with post-transplant cyclophosphamide (PT-CY) showed a safer profile, with lower rates of severe acute GvHD[25,26].

Several attempts to maximize GvL while reducing GvHD after DLI have been made in recent years. CD8+ T cells depleted DLI were shown to determine durable clinical responses with lower mortality than unmanipulated DLI, particularly in CML [27,28]. The underlying mechanism may be given to a GvL effect induced by donor CD4+ cells which reactivate *in situ* CD8+ T cells antitumor activity, previously exhausted by chronic exposure to tumor antigens [29,30]. Moreover, other forms of T cell therapies included CD4+ CD25+ Tregs depleted DLI, gamma/delta T cell subsets, and cytokine induced killer cells (CIK) [31,35]. Finally, bispecific T cells engaging molecules or chimeric antigen receptor (CAR) cells have been developed. Chimeric antigen receptors are synthetic proteins that comprise an antigen recognition portion and an intracellular activation domain. The incorporation of this receptor on T cells and, more recently, on NK cells allows to achieve a strong combination of antibody specificity and a direct cytotoxic power. This strategy of adoptive immunotherapy may potentially bypass the immune-escape ability of malignant cells. Overall, most encouraging clinical observations have been achieved in patients with $CD19^+$ B cell malignancies, especially in chronic and acute lymphoid leukemias [36].

In conclusion, although responses to DLI have been described, a clear and effective role of unmanipulated or minimally manipulated DLI remains to be defined. Novel T cell-based immunotherapies offer an encouraging scenario, but need to be validated in larger clinical trials.

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