




# Evidence for Graft versus Tumour Effect after Allogeneic Stem Cell Transplantation for Leukemia and Lymphoma

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ABSTRACT

Haematopoietic stem cell transplantation (HSCT) has been commonly used in the treatment of haematopoietic malignancy as a rescue regiment to prevent bone marrow toxicity subsequent to high dose chemotherapy. The harmful effects are leading to graft versus host disease (GVHD). However, studies have shown that GVHD, T-cell dependent mechanisms, and NK cells in allogeneic stem cell transplantation contributed to a lower risk of disease relapse by a mechanism known as graft versus tumour (GVT) effect. Evidence of graft versus tumour (GVT) effect has allowed development of cancer therapy with less toxic chemotherapy to allow tumour eradication. This essay aims to discuss evidences of GVT effect after allogeneic stem cell transplantation in both leukaemia and lymphoma and examine future prospects of maximizing GVT effect in the treatment of both diseases

**Keywords:** Graft rejection, graft versus host disease, graft versus tumour effect, leukemia, lymphoma, stem cell

ABSTRAK

Transplantasi dengan sel punca hematopoietik umumnya digunakan untuk pengobatan keganasan pada darah sebagai pencegahan toksisitas kemoterapi dosis tinggi terhadap sumsum tulang. Efek negatif dari pengobatan tersebut adalah *graft versus host disease* (GVHD). Namun, studi-studi menunjukkan efek *graft versus tumour* (GVT) dapat menurunkan risiko relaps melalui efek GVHD, mekanisme dependen sel T dan sel NK. Efek GVT ini memungkinkan pengembangan terapi kanker dengan toksisitas lebih sedikit untuk eradikasi tumor. Tulisan ini membahas bukti-bukti efek GVT setelah tranplantasi sel punca alogeneik pada leukemia dan limfoma serta prospek masa depan untuk memaksimalkan efek GVT pada terapi leukemia dan limfoma. **Meutia Ayuputeri Kumaheri. *Graft versus Tumour Effect* setelah Transplantasi *Allogeneic Stem Cell* untuk Leukemia dan Limfoma**

**Kata kunci:** *Graft rejection, graft versus host disease, graft versus tumour effect*, leukemia, limfoma, sel punca.

## INTRODUCTION

Haematopoietic Stem Cell Transplantation (HSCT) has been commonly used in the treatment of haematopoietic malignancy as a rescue regimen to prevent bone marrow toxicity subsequent to high dose chemotherapy. It has been demonstrated that both detrimental and beneficial effects to patients are produced by immune reactions between donor and recipient in allogeneic transplantation.[1,2] The harmful effects are mediated by host NK cells or T-cells that recognize HLA in donor cells, termed graft rejection or mediated by donor T-cells that recognize and react against recipient HLA-peptide complexes, leading to Graft versus Host Disease (GVHD). However, studies have shown that GVHD, T-cell dependent mechanisms, and NK cells in allogeneic stem cell transplantation contributed to a lower risk of disease relapse by a mechanism known as Graft versus Tumour (GVT) effect.[3] Evidence of Graft versus Tumour (GVT) effect has allowed development of cancer therapy with less toxic chemotherapy to allow tumour eradication.

### Mechanism of GVT

GVT effect of allogeneic HSCT is mainly mediated by T-cells. Several factors such as type and stage of the disease, HLA-matching between donor and recipient, and degree of chimerism as well as the presence of additional treatment to the disease may influenced the extent of GVT effect.[4,5]

GVT effect, like GVH effect is mediated by host's DCs that stimulate donor's T-cell (**Figure 1**). T-cell mediated effector pathway by and T-cell derived cytokine mediated pathway and direct cytolytic pathway. CD4+ T-cells produce cytokines such as IL-2, TNF-$\alpha$, and IFN-$\gamma$ that both recruit and potentiate the cytolytic activity of effector cells. Direct cytolytic pathway relies on Fas-FasL induced apoptosis or perforin/granzyme degranulation to eradicate tumour cells. Fas-dependent killing is predominantly used by CD4+ T-Cells while perforin degranulation is mainly performed by CD8+ T-cells.[6,7]

Both CD4+ T-cells and CD8+ T-cells are stimulated upon presented with peptide on the surface of HLA class I and HLA class II respectively. CD4+ T-cells will eventually exert

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its own effector mechanism or promote DCs to activate CD8+ T-cells by the presentation of HLA Class I restricted peptide. Furthermore, HLA Class II expression on DCs and stimulation of CD4+ T-cells are necessary for presentation of HLA Class I restricted peptide in DCs and other APCs to further activate graft's CD8+ cells.[4] The peptide bound to HLA Class I is supposed to be 'restricted' so that it is only expressed on haematopoietic cells, serving as potential tumor cells targets for donor T-cells in producing GVT without developing GVHD.[7] Minor Histocompatibility Antigens (mHAgs) are peptidesexclusively expressed in recipient capable to elicit T-Cell response in allogeneic HLA-matched transplantation.[7] A few known mHAgs selectively expressed on haematopoietic cells such as HA-1, HA-2, HB-1, and BCL2A1 are the potential restrictive peptides that serve as candidate target antigens to mediate GVT effect.[1,2]

Donor T-cells are found to be active against abberantly or overexpressed Tumour Associated Antigens (TAAs) such as proteinase 3 in myeloid leukaemia[3,5] and WT 1 in ALL (acute lymphoblastic leukemia).[7] The reactivity of donor T-cells to both mHAgs and TAAs formed to basic concept of DLI where isolation of donor lymphocyte followed by infusion to the recipient could optimize GVT effect in patients with relapsed malignancy.[5]

Recent evidence suggests that NK-cells are able to mediate GVT effect due to their interaction with HLA Class I molecules on target cell. The activation of NK-cells is regulated by the balance between inhibitory and activating Killer Cells Immunoglobulin Receptors (KIRs). NK-cells express KIR specific for self HLA Class I group that is absent on allogeneic NK-cells as the inhibitory receptor for self. Following allogeneic HSCT, donor NK-cells recognise the missing expression on the donor class I KIR ligand on the host HLA class I, are activated and mediate alloreaction.[7]

Welniak, *et al*, (2007) described the contribution of regulatory T-cells (Treg cells) to GVT effect. Depletion of CD25+ cells from graft or recipient produced both acute and chronic GVHD that correlate with maintenance of GVT against lymphoid malignancy in mice studies. However, recipient-derived CD4+ CD25+ Treg cells reduced acute and chronic GVHD, inhibited NK mediated graft rejection and improved GVT in murine models.[6]

Previous studies have revealed that humoral immunity may contribute to the emergence of GVHD by the discovery of both autoantibodies and alloantibodies in patients with chronic GVHD.[7,8] However, there are still limited studies addressing involvement of B-cells in mediating GVT effect, providing insufficient evidence to support this theory. Several studies claimed that antibodies respond to tumour associated antigen in association after administration of Donor Lymphocyte Infusion (DLI) in myeloma and CML.[9] Other studies demonstrated that development of antibodies H-Y mHAgs correlate with remission with an increased risk of developing GVHD.[10] Rituximab, a monoclonal antibody that mainly targets CD20 protein mainly expressed on B-cell surface is demonstrated to potentiate GVL (graft versus leukemia) effect in adult with CLL, but not with follicular lymphoma, disputing the role of either donor and recipient B-cells in mediating GVT effect.[11,12]

### Evidence of GVT in Leukaemia

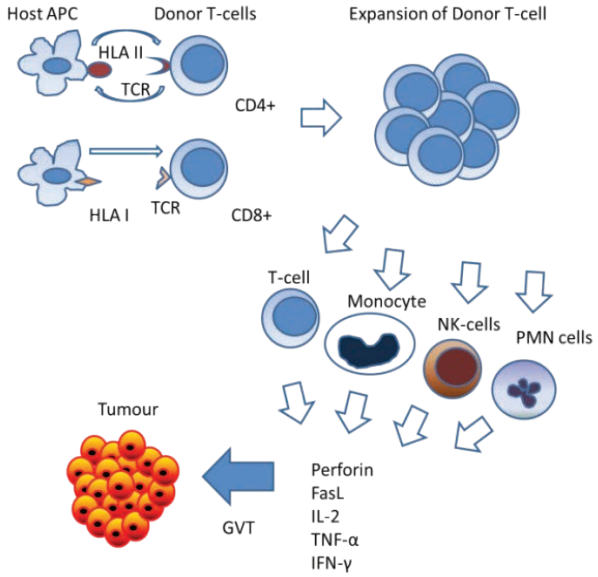
Acute GVHD is associated with stronger GVT effect in ALL compared to chronic GVHD. However, moderate and severe acute GVHD correlate with increased risk with non-leukaemiadeath. Thus, only mild acute and chronic GVHD produce sufficient GVT effect related with long term disease-free survival.[3]

Contrary to ALL, chronic as well as severe acute GVHD correlate with better GVT effect as opposed to mild moderate acute GVHD in AML and CML, but severe acute GVHD comes with higher transplant related mortality. Horowitz et al. (1990) indicated that the least risk of relapse is present in patients both acute and chronic GVHD compared to individual occurrences.[3] Moreover, mild acute and mild chronic GVHD is associated with the highest Leukaemia Free Survival (LFS).[7]

Following haploidentical HSCT in AML patients, a lower relapse probability and improved survival are associated with donor NK cells activity responding to the lack for inhibitory KIRs in recipients' HLA class I. However, several studies showed conflicting results on the significance of KIR-ligand mismatch between recipient and donor in mediating GVT effect. Some experts illustrated that KIR-ligand mismatch had no impact on patients' survival while other study observed that KIR-ligand mismatch unrelated donor gave rise to a higher infection-related mortality.[7]

Evidence of GVT effect in CLL has been demonstrated by Gribben, et al. Seven CLL with B-cell origin patients with clinical relapse following either HLA-matched T-cell depleted HSCT from related sibling and autologous HSCT were treated with DLI. Six out of seven patients showed response to DLI by decreasing level of CLL specific IgH rearrangement in bone marrow.[4,13]

**Figure 1**. Mechanism of GVT






Some of the Dendritic Cells (DC) in CML has been found to be of leukaemia origin and are able to present mHAgs and leukaemia specific peptide to donor lymphocytes. These leukaemia DCs are postulated to spontaneously differentiate from CML progenitor cells, and may explain decent response of DLI. Based on this finding, *in vitro* model had shown that combination of GM-CSF and TNF-α better induces DCs that are able to present restricted peptides on the surface of their HLA class I.[4]

Marijt et al. identified the role in mHAgs HA-1 and HA-2 T-cells in inducing remission of relapsed CML patients in two case studies. Patients were treated with T-cell depleted HLA-matched allogeneic HSCT and underwent relapse within the first two years following transplant. Both patients are mHAgs HA-1 and HA-2 positive patients and they both received DLI from mHAgs HA-1 and/or HA-2 negative donors. Emergence of HA-1 and HA-2 specific CD8+ T-cells in patients' blood are observed 5-7 weeks following DLI followed by a reduction in BCR/ABL fusion gene, which is the hallmark genetic abnormality of CML and conversion to 100% donor chimerism. *In vitro* inhibition of leukaemia precursor cell growth by HA-1 and HA-2 specific CD8+ T-cells suggests that these specific T-cells may be developed as adoptive immunotherapy to treat haematological malignancies.[14]

Paradoxical to the successful inhibition of leukaemia precursors growth in CML, mHAg-specific T-cells fail to provide decent frequency of complete remission Non-Hodgkin's lymphoma and AML (acute myeloid leukemia) (10-30%).[2] These poor responses are attributed to either the absence or low expression of costimulatory and adhesion molecules that give rise to incompetent antigen-presenting capacity of the malignant cells. Production of suppressive cytokines such as IL-10 or TGF-β is also hypothesised to be able to abrogate the immune response to malignant cells.[7]

Similarly, myeloid disease has better response to DLI than lymphoid disease. This phenomenon may be explained by the fact that lymphoid leukaemia derived from B-cell lineage such as Pre-B ALL lacks costimulatory molecules and therefore unable to function as alloantigen presenting cells and induces T-cell tolerance.[4,15]

T-cell depletion significantly alters antileukaemia effect in CML by decreasing the risk of developing GVHD. However, this effect is associated with a decrease in antileukaemia activity leading to a suggestion that GVHD may aid antileukaemia activity in T-cell depleted patients. This claim is proven to be incorrect as studies show that CML patients with GVHD had an extensive increased risk of relapse following T-cell depleted HSCT compared to their counterparts who had non-T-cell depleted transplant and did not develop GVHD. This leads to the proposition of additional antileukemic effect of T-cells independent of GVHD.[3]

**Table 1**. Response to donor lymphocyte transfusion (DLI) in common types of leukaemias

| Type of Disease | No. of Patients Responding | Total Patients Treated | %CCR |
|---|---|---|---|
| ALL^ | 0 | 12 | 0% |
| AML^ | 5 | 17 (128-855 days) | 29% |
| CLL* | 7 molecular relapse | 9 (>2 years) | 78% |
| CML^ | 54 | 75 (3 years) | 73% |

^ Data taken from centers participating in European Bone Marrow Transplantation (EBMT) in the experience treating relapsed leukaemias with DLI[16]

* Molecular relapse=absence of minimal residual disease (MRD), data taken from German Multicenter trial on molecular relapase an persistence[17]

**Table 2**. Non-myeloablative allogeneic HSCT results in non-hodgkin lymphoma[20]

| Patients' Status | Type of Disease | No. of Patients |
|---|---|---|
| Alive | | 13 |
| Complete Remission | Low grade | 4 |
| | Mantle cell | 2 |
| | Aggressive | 4 |
| Partial Remission | Low grade | 1 |
| Stable | Mantle cell | 1 |
| Relapse | Aggressive | 1 |
| Dead | | 13 |
| Complete | Low grade | 2 |
| Remission/GVHD | Aggressive | 2 |
| /Infection/hemorrhage | Low grade | 1 |
| Partial | Aggressive | 1 |
| Remission/GVHD/ | Low grade | 1 |
| Infection | Mantle cell | 1 |
| Progressive Disease | Aggressive | 5 |

### Evidence of GVT in Lymphoma

Several types of lymphoma such as chemotherapy sensitive Hodgkin Lymphoma, mantle cell lymphoma, and follicular lymphoma have been associated with lower relapse rate following allogeneic HSCT compared to autologous transplantation.[7] Low-grade lymphoma is reported to have a lower relapse rate following allogeneic HSCT accompanied by the absence of GVHD in some case.[4] However, there are differences in sensitivity to GVT effect among lymphoma subtypes.[18] The most obvious evidence of GVT effect is the patients' response to DLI after transplantation in persistent or relapsed lymphoma.[19]

Akpek, *et al*, illustrated that Hodgkin Lymphoma (HL) patients sensitive to conventional chemotherapy tended to have lower relapse rate, especially in patients with GVHD. This finding suggested that allogeneic HSCT produced a clinical GVT effect. However, the findings in this study failed to reach statistical significance due to the relatively small sample size. In addition, allogeneic HSCT in HL presented a lower risk of developing secondary AML/MDS than autologous HSCT.[21]

Response rate to DLI following allogeneic HSCT in HL is reported to vary between 32% and 52%, with a higher rate after *in vivo* T-cell depleted HSCT. However, this finding may reflect a reporting bias due to the fact that relapsed patients after T-cell-replete transplantation have T-cells affecting the growth of their tumour.

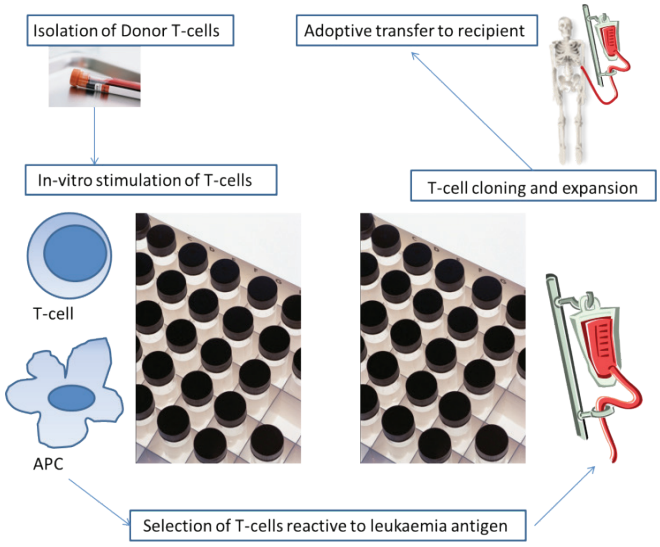
The GVT effect observed in patients with Mantle Cell Lymphoma (MLC) is considered dramatic. Five out of eight patients underwent

complete remission following HSCT from HLA-matched sibling donor or matched unrelated donor. Other evidence showed a MCL patient with disease progression after autologous HSCT that underwent second transplantation using matched unrelated donor. Regression of the disease with minimal GVHD was observed 60-days post-HSCT.[20]

Patients with Follicular Lymphoma[21] experienced a plateau in relapse risk in 2-5 years subsequent to allogeneic HSCT, suggesting clinical evidence of GVT effect.[22] Further evidence is shown with the success of a secondary transplantation from allogeneic donor after a failed autologous HSCT. The GVT effect in follicular lymphoma is associated with a lower recurrence rate in patients with chronic GVHD as opposed to those without GVHD.[23] DLI presents as an effective treatment for relapsed FL after HSCT with relatively mild GVHD.[24] In addition, withdrawal of immunosuppression followed by DLI in relapsed patients showed a marked response that suggest the capacity of GVL to maintain long term remission in FL.[22]

Interestingly, there is poor evidence of GVT effect in diffuse large B-cell lymphoma (DLBCL) patients receiving DLI upon the relapse following allogeneic HSCT.[25] The response from small minority of patients is hypothesised to be related to poor antigen presenting capability of DLBCL tumour cells.[19]

**Figure 2**. Principle of adoptive immunotherapy




## Outlook

Throughout the past decades, HSCT has been developed towards clinical immunotherapy to treat haematological malignancy by optimizing GVT effect in allogeneic transplant. The future will see a more individualized treatment, with risk assessment of GVHD and relapse, as well as monitoring of minimal residual diseases to detect disease relapse.[7] Researches to further exploit GVT effect while minimising GVHD are based on the development of adoptive immunotherapy with 1) identification of specific target antigen including mHAgs expressed only by leukaemia cells, 2) modification of T-cell by transferring specific TCR gene to appropriate T-cell population, 3) adoptive immunotherapy using organ-specific antigen, 4) adoptive immunotherapy using tumour-associated antigen (TAA) selectively expressed on malignant cells i.e WT1, proteinase-3 (3), MUC1, as well as development of WT1 vaccine, 5) adoptive transfer of NK-cells with KIR ligand mismatch, and development of combination immunosuppresive drugs that possess both antitumour and prevention of GVHD activity.

## REFERENCES


1. Bleakley M, Riddell SR. Molecules and mechanisms of the graft-versus-leukaemia effect. Nat Rev Cancer 2004;4(5):371-80.
2. Falkenburg JH, Van De Corput L, Marijt EW, Willemze R. Minor histocompatibility antigens in human stem cell transplantation. Exp Hematol. 2003;31(9):743-51.
3. Horowitz MM, Gale RP, Sondel PM, Goldman JM, Kersey J, Hans-Jochem K, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75(3):555-62.
4. Kolb HJ. Graft-versus-leukemia effects of transplantation and donor lymphocytes. Blood 2008;112:4371-83.
5. Roddie C, Peggs KS. Donor lymphocyte infusion following allogeneic hematopoietic stem cell transplantation. Expert Opin Biol Ther. 2011;11(4):473-87.
6. Welniak LA, Blazar BR, Murphy WJ. Immunobiology of allogeneic hematopoietic stem cell transplantation. Annu Rev Immunol. 2007;25:137-70.
7. Ringdén O, Karlsson H, Olsson R, Omazic B, Uhlin M. The allogeneic graft-versus-cancer effect. Br J Haematol. 2009;147(5):614-33.
8. Kapur R, Ebeling S, Hagenbeek A. B-cell involvement in chronic graft-versus-host disease. Haematologica 2008;93(11):1702-11.
9. Shimabukuro-Vornhagen A, Hallek MJ, Storb RF, Von Bergwelt-Baildon MS. The role of B cells in the pathogenesis of graft-versus-host disease. Blood 2009;114(24):4919-27.
10. Miklos DB, Kim HT, Miller KH, Guo L, Zorn E, Lee SJ, et al. Antibody responses to H-Y minor histocompatibility antigens correlate with chronic graft-versus-host disease and disease remission. Blood 2005;105(7):2973-8.
11. Khouri IF, Lee MS, Saliba RM, Andersson B, Anderlini P, Couriel D, et al. Nonablative allogeneic stem cell transplantation for chronic lymphocytic leukemia: Impact of rituximab on immunomodulation and survival. Experimental Hematol. 2004;32(1):28-35.
12. Khouri IF, McLaughlin P, Saliba RM, Hosing C, Korbling M, Lee MS, et al. Eight-year experience with allogeneic stem cell transplantation for relapsed follicular lymphoma after nonmyeloablative conditioning with fludarabine, cyclophosphamide, and rituximab. Blood 2008;111(12):5530-6.
13. Gribben JG, Zahrieh D, Stephans K, Bartlett-Pandite L, Alyea EP, Fisher DC, et al. Autologous and allogeneic stem cell transplantations for poor-risk chronic lymphocytic leukemia. Blood 2005;106(13):4389-96.
14. Marijt WAE, Heemskerk MHM, Kloosterboer FM, Goulmy E, Kester MGD, Van der Hoorn MAWG, et al. Hematopoiesis-restricted minor histocompatibility antigens HA-1- or HA-2-specific T cells can induce complete remissions of relapsed leukemia. Proc Natl Acad Sci USA. 2003;100(5):2742-7.





15. Cardoso AA, Schultze JL, Boussiotis VA, Freeman GJ, Seamon MJ, Laszlo S, et al. Pre-B acute lymphoblastic leukemia cells may induce T-cell anergy to alloantigen. Blood. 1996;88(1):41-8.
16. Kolb HJ, Schattenberg A, Goldman JM, Hertenstein B, Jacobsen N, Arcese W, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood 1995;86(5):2041-50.
17. Ritgen M, Stilgenbauer S, Von Neuhoff N, Humpe A, Brüggemann M, Pott C, et al. Graft-versus-leukemia activity may overcome therapeutic resistance of chronic lymphocytic leukemia with unmutated immunoglobulin variable heavy-chain gene status: Implications of minimal residual disease measurement with quantitative PCR. Blood 2004;104(8):2600-2.
18. Van Besien KW, De Lima M, Giralt SA, Moore DF Jr, Khouri IF, Rondón G, et al. Management of lymphoma recurrence after allogeneic transplantation: The relevance of graft-versus-lymphoma effect. Bone Marrow Transplant. 1997;19(10):977-82.
19. Chakraverty R, Mackinnon S. Allogeneic transplantation for lymphoma. J Clin Oncol. 2011;29(14):1855-63.
20. Maloney DG. Graft-vs.-Lymphoma effect in various histologies of non-Hodgkin's lymphoma. Leuk Lymphoma 2003;44(supp 3):99-105.
21. Akpek G, Ambinder RF, Piantadosi S, Abrams RA, Brodsky RA, Vogelsang GB, et al. Long-term results of blood and marrow transplantation for Hodgkin's lymphoma. J Clin Oncol. 2001;19(23):4314-21.
22. Khouri IF. Allogeneic stem cell transplantation in follicular lymphoma. Best Pract Res Clin Haematol. 2011;24:271-7.
23. Hunault-Berger M, Ifrah N, Solal-Celigny P. Intensive therapies in follicular non-Hodgkin lymphomas. Blood 2002;100(4):1141-52.
24. Mandigers CM, Verdonck LF, Meijerink JP, Dekker AW, Schattenberg AV, Raemaekers JM. Graft-versus-lymphoma effect of donor lymphocyte infusion in indolent lymphomas relapsed after allogeneic stem cell transplantation. Bone Marrow Transplantation 2003;32(12):1159-63.
25. Thomson KJ, Morris EC, Bloor A, Cook G, Milligan D, Parker A, et al. Favorable long-term survival after reduced-intensity allogeneic transplantation for multiple-relapse aggressive non-Hodgkin's lymphoma. J Clin Oncol. 2009;27(3):426-32.