AUTOLOGOUS STEM CELL-BASED GENE THERAPY OFFERS AN INNOVATIVE SOLUTION FOR TREATING INHERITED BLOOD CELLS DISORDERS

Nurul Faudhan [1*], Yayu Aisida [2]

[1]Faculty of Health Sciences, Universitas Muhammadiyah Sidoarjo, Indonesia

[2]Faculty of Pharmacy, University of Surabaya, Indonesia

[*] Corresponding author : fiknurulfaudhan@gmail.com

ABSTRACT

Recent advancements in medical treatments, particularly gene therapy using hematopoietic stem cells (HSCs), have significantly impacted the treatment of inherited blood disorders. HSCs can self-renew and differentiate into blood cells, making them essential for treating conditions like sickle cell anemia, thalassemia, and severe combined immunodeficiency (SCID). This study conducted a literature review on autologous stem cell therapy for genetic blood disorders, analyzing studies from databases such as PubMed and Scopus. Gene therapy corrects genetic defects in HSCs, offering an alternative to allogeneic transplantation by avoiding immune rejection. The therapy involves modifying stem cells in the lab, often through viral vectors or gene-editing tools, and reinfusing them into the patient to produce healthy blood cells long-term. Lentiviral vectors, considered safer than retroviruses, have been particularly effective in treating various conditions, including immunodeficiencies and hemoglobinopathies. The ex vivo gene transfer approach, commonly used for genetic disorders, has shown promise for one-time curative treatments, especially for pediatric diseases. However, early gene therapy efforts, such as the use of gamma-retroviral vectors for SCID, faced complications like leukemia, leading to a shift towards safer lentiviral vectors. Despite its complexity, the procedure has a low failure rate and provides a less risky alternative to traditional allogeneic stem cell transplants. Ultimately, HSC gene therapy holds significant potential for curing genetic blood disorders by permanently altering the stem cells, ensuring long-term benefits and improved treatment outcomes, with ongoing advancements in safety and efficacy.

Keywords : Hematopoietic stem cells; immunodeficiency; inherited blood disorders

INTRODUCTION

In medical science, the search for effective treatment methods continues to motivate researchers to develop new solutions that can enhance patient well-being. One significant advancement in this field is innovative gene therapy utilizing hematopoietic stem cells (HSCs) (Giommetti & Papanikolaou, 2024). The remarkable ability of HSCs to self-renew for long periods and differentiate into various blood cell types makes them highly valuable in the medical field. Hematopoietic stem cell transplantation (HSCT) has proven effective and is widely accepted as a treatment procedure for inherited metabolic diseases and blood-related disorders (Giommetti & Papanikolaou, 2024). Transplantasi sel punca hematopoietik (HSCT) alogenik pertama kali berhasil diterapkan dalam pengobatan defisiensi imun gabungan berat terkait-X (X-SCID) dan sindrom Wiskott-Aldrich (WAS) (Gatti et al., 1968; Bach et al., 1968). Autologous HSCT

represents a significant advancement in reducing the risk of graft rejection and complications often associated with allo-reactivity. Therefore, the combination of autologous HSCT with HSC gene therapy has been explored as an alternative treatment approach not only for addressing hematologic cancers but also for various genetic diseases, such as severe immunodeficiencies, hemoglobinopathies, and metabolic disorders (Hatzimichael & Tuthill, 2010).

Gene therapy involves the insertion of nucleic acids, either DNA or RNA, into target cells for therapeutic purposes. This approach aims to introduce new healthy genes (additive gene therapy) or to correct genetic mutations (gene editing). In this context, autologous gene therapy utilizing hematopoietic stem cells (HSCs) as targets offers an appealing alternative to allogeneic stem cell transplantation, as it avoids the risk of immune complications, including graft-versus-host disease (Dunbar et al., 2018).

Gene therapy using autologous HSCs opens new opportunities in treatment, expanding the scope of applications for allogeneic hematopoietic stem cell transplantation (allo-HSCT). In addition to addressing blood disorders such as primary immunodeficiencies (PID), hemoglobinopathies, or stem cell defects, this approach also offers potential in treating metabolic diseases. This is achieved through cross-correction, where circulating mature blood cells can produce missing or abnormal proteins, which are then directed to tissues in need. In some cases, this gene therapy may provide better outcomes than allo-HSCT, as therapeutic proteins expressed at higher-than-normal levels can reach affected non-hematopoietic cells and tissues, such as in mucopolysaccharidosis or Fabry disease. This approach is supported by over 30 years of experience in bone marrow manipulation, as well as a deep understanding of autologous and allo-HSCT, including the ease of isolating HSCs and HSPCs through CD34+ selection, and the ability of HSCs to return to their origin after intravenous infusion (Staal et al., 2019).

MATERIALS AND METHODS

This study employed a narrative literature review method to examine autologous stem cell therapy in the treatment of genetic blood disorders. Literature was sourced from PubMed, Scopus, and Google Scholar databases (2015–2024) using keywords related to the topic. Articles were selected based on relevance, qualitative or quantitative methodology, and availability in English, and were systematically evaluated using the PRISMA method. These criteria encompassed aspects such as relevance to the topic, the type of study, publication year, and the quality of the methodology. Data were analyzed descriptively and thematically to identify patterns, themes, and research gaps. The selected studies underwent a thorough screening process based on inclusion and exclusion criteria outlined in the protocol. Only studies that fulfilled these requirements were incorporated into the review.

RESULT AND DISCUSSION

Genetically inherited blood disorders became the first type of condition successfully addressed through gene therapy. These disorders, caused by single-gene mutations, affect the formation or function of blood cells and include: 1) hemoglobinopathies, which impact red blood cells, such as sickle cell anemia and thalassemia; 2) inborn errors of immunity (IEI), which affect immune cells like neutrophils, macrophages, or lymphocytes; 3) lysosomal storage diseases and certain types of leukodystrophies, involving tissue macrophages and microglial cells in the brain; and 4) conditions leading to

hematopoietic stem cell (HSC) dysfunction and genomic instability, such as Fanconi anemia. These diseases can be treated with transplantation of normal HSCs obtained from immunologically compatible healthy donors (allogeneic transplantation), enabling the regeneration and production of healthy blood cells (Kohn et al., 2023).

The most commonly explored gene transfer approach in hematopoietic stem cells to date is the ex vivo method. In this technique, HSCs are harvested from the patient and genetically modified through viral transduction or gene editing to correct abnormal phenotypes. After the patient undergoes preconditioning, the engineered cells are reintroduced into the body, where they can self-renew and differentiate into various types of blood cells, thereby establishing a long-term reserve of modified HSCs. By conducting the manipulation in a controlled laboratory environment, researchers can ensure the cells' characteristics and functionality before transplantation. This approach offers the potential for a one-time curative treatment, thanks to the ability of corrected HSCs to engraft. It presents a highly promising therapeutic option for addressing blood and immune system disorders (Giommetti & Papanikolaou, 2024).

Stem Cell Therapy Procedures for Patients with Inherited Blood Cell Diseases

The stem cell-based gene therapy approach for treating pediatric diseases involves collecting CD34+ cells, which are rich in HSCs, from the patient. These cells are typically sourced from bone marrow or, more recently, mobilized peripheral blood. In a GMP laboratory, the cells are cultured with cytokines and viral vectors, followed by a series of quality control steps before being re-infused into the patient. To ensure the process runs optimally, the cells are often cryopreserved temporarily to allow for quality testing and transportation to transplant facilities that may be located far from the production site. Once re-infused, the HSCs home back to their niche, differentiate into various types of mature blood cells, and effectively address the patient's clinical condition (Staal et al., 2019).

Various types of viral vectors have been employed in gene therapy; retroviruses are often chosen to transform hematopoietic stem cells (HSCs) due to their unique ability to stably integrate viral genes into the genome of the human host (Staal et al., 2008). In the context of gene therapy, retroviruses have been modified to be replication-incompetent, retaining only essential genes. These elements are integrated through co-transfection with different plasmids into trans-complementary cells, which encode the transgene construct as well as critical viral structural components such as gag, pol, and env, to produce infectious viral particles (Staal et al., 2019).

Therapy Success

Severe combined immunodeficiency (SCID) represents one of the earliest successes of gene therapy in the clinical field. This condition is the most severe form of congenital immunodeficiency in humans, characterized by the absence of T and B lymphocyte function, leaving infants highly susceptible to life-threatening infections and facing high mortality rates without treatment. SCID is the first genetic blood cell disease successfully cured through hematopoietic stem cell (HSC) transplantation from a donor (Gatti et al., 1968; Pai et al., 2014).

Early success in restoring immune function was achieved through the use of murine gamma-retroviral vectors to transfer the XSCID (IL2RG) and ADA SCID (ADA) genes into the patient's hematopoietic stem cells (HSCs) (Cavazzana-Calvo et al., 2000; Aiuti et al., 2002). This therapy resulted in significant clinical immune recovery and overall good health. However, two years or

more after these genetic procedures, serious complications in the form of leukemia were found in 6 out of 20 XSCID patients due to the vectors used. A similar issue was recently reported in ADA SCID patients (Hacein-Bey-Abina et al., 2003; Howe et al., 2008; Cavazzana et al., 2019).

In this field, there has been a shift towards the use of lentiviral vectors, which are considered safer due to their lower risk of genotoxicity compared to gamma-retroviral vectors, and are also more effective in transducing human HSCs. Various types of IEI have been successfully treated with HSCGT using lentiviral vectors, such as in cases of Wiskott-Aldrich syndrome, chronic granulomatous disease, and leukocyte adhesion deficiency type I (Aiuti et al., 2013; Kohn et al., 2020; Magnani et al., 2022; Kohn et al., 2022).

Complexity of Autologous HSC Production

The production process for genetically modified autologous hematopoietic stem cell (HSC)-based therapies involves complex steps. Each autologous product requires the collection of stem cells specifically sourced from the patient. This stage is clinically challenging as it involves administering G-CSF for 5–7 days for mobilization, inserting a central venous catheter for apheresis, and conducting 1–3 leukapheresis sessions (Kohn et al., 2023).

The isolation of CD34+ cells and genetic modification using lentiviral vectors or gene-editing technology via electroporation have established standards. However, these activities still require hours of cell processing in cleanroom-standard laboratory facilities. Although the production failure rate is relatively low, nearly all batches meet the requirements for purity, potency, identity, and safety. Furthermore, applying the modified stem cells in clinical transplantation with moderate to intensive conditioning chemotherapy necessitates intensive inpatient medical care for several weeks. Comparatively, the standard clinical procedure used for similar disorders, allogeneic stem cell transplantation (HSCT), has an equivalent or even higher level of complexity (Kohn et al., 2023).

Genetic Modification of HSCs

Gene therapy approaches for genetic blood cell disorders are based on the hypothesis that hematopoietic stem cell transplantation (HSCT) using genetically modified autologous stem cells (HSCs) can provide benefits comparable to allogeneic HSCT but with a lower risk of immunological complications. To achieve effectiveness, genetic modifications in HSCs must be permanent, ensuring that the corrections persist alongside the proliferation of HSCs and their derivatives. This process requires complex, patient-specific cell processing after HSC collection through various methods, such as bone marrow aspiration, PBSC mobilization via leukapheresis, or umbilical cord blood extraction. Processing follows Good Manufacturing Practice (GMP) standards, where HSCs are enriched using CD34 immunoaffinity selection and cultured in a cytokine mixture (e.g., c-kit ligand, flt-3 ligand, and thrombopoietin) on extracellular matrix protein layers (e.g., recombinant fibronectin). A gene delivery vector is then added after the pre-stimulation phase. Following this, the cells are formulated for intravenous infusion with a series of washing steps and suspension in specific media, such as Plasmalyte. Before use, the cell product must meet standards for viability and microbiological sterility. Further evaluations, such as colony formation assays, vector copy number quantification via quantitative PCR, and transgene expression, are conducted post-infusion on reserve samples from the final product (Kohn et al., 2013).

SUMMARY

Gene therapy has successfully addressed genetically inherited blood disorders caused by single-gene mutations. These disorders, such as sickle cell anemia, thalassemia, and SCID, impact blood cell formation or function. A key approach involves ex vivo modification of hematopoietic stem cells (HSCs), enabling long-term correction of genetic defects. HSCs are harvested, genetically modified using viral vectors or gene-editing tools, and reintroduced into the body after preconditioning, allowing the generation of healthy blood cells. Autologous HSC-based therapies, though complex, reduce immunological risks compared to allogeneic transplantation. Lentiviral vectors have largely replaced gamma-retroviral vectors due to their safety and efficacy. This process requires patient-specific cell collection, genetic modification in GMP labs, and rigorous quality control. Despite challenges, HSC gene therapy offers curative potential, particularly for severe immune deficiencies and blood disorders, with advancements continually improving safety and outcomes.

BIBLIOGRAPHY

Aiuti, A., Biasco, L., Scaramuzza, S., Ferrua, F., Cicalese, M. P., Baricordi, C., Dionisio, F., Calabria, A., Giannelli, S., Castiello, M. C., Bosticardo, M., Evangelio, C., Assanelli, A., Casiraghi, M., di Nunzio, S., Callegaro, L., Benati, C., Rizzardi, P., Pellin, D., di Serio, C., Schmidt, M., von Kalle, C., Gardner, J., Mehta, N., Meduva, V., Dow, D. J., Galy, A., Miniero, R., Finocchi, A., Metin, A., Banerjee, P. P., Orange, J. S., Galimberti, S., Valsecchi, M. G., Biffi, A., Montini, E., Villa, A., Ciceri, F., Roncarolo, M. G. and Naldini, L. (2013), "Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott-Aldrich syndrome", Science, Vol. 341 No. 6148.

Aiuti, A., Slavin, S., Aker, M., Ficara, F., Deola, S., Mortellaro, A., Morecki, S., Andolfi, G., Tabucchi, A., Carlucci, F., Marinello, E., Cattaneo, F., Vai, S., Servida, P., Miniero, R., Roncarolo, M. G. and Bordignon, C. (2002), "Correction of ADA-SCID by stem cell gene therapy combined with nonmyeloablative conditioning", Vol. 296 No. 5577, pp. 2410-3.

Bach, F. H., Albertini, R. J., Joo, P., Anderson, J. L. and Bortin, M. M. (1968), "Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome", Lancet, Vol. 2 No. 7583, pp. 1364-6.

Cavazzana, M., Bushman, F. D., Miccio, A., André-Schmutz, I. and Six, E. (2019), "Gene therapy targeting haematopoietic stem cells for inherited diseases: progress and challenges", Nature Reviews Drug Discovery, Vol. 18 No. 6, pp. 447-62.

Cavazzana-Calvo, M., Hacein-Bey, S., de Saint Basile, G., Gross, F., Yvon, E., Nusbaum, P., Selz, F., Hue, C., Certain, S., Casanova, J. L., Bousso, P., Deist, F. L. and Fischer, A. (2000), "Gene therapy of human severe combined immunodeficiency (SCID)-X1 disease", Science, Vol. 288 No. 5466, pp. 669-72.

Dunbar, C. E., High, K. A., Joung, J. K., Kohn, D. B., Ozawa, K. and Sadelain, M. (2018), "Gene therapy comes of age", Science, Vol. 359 No. 6372.

Gatti, R. A., Meuwissen, H. J., Allen, H. D., Hong, R. and Good, R. A. (1968), "Immunological reconstitution of sex-linked lymphopenic immunological

deficiency", Lancet, Vol. 2 No. 7583, pp. 1366-9.

Giommetti, A. and Papanikolaou, E. (2024), "Advancements in Hematopoietic Stem Cell Gene Therapy: A Journey of Progress for Viral Transduction", Cells, Vol. 13 No. 12, pp. 1039.

Hacein-Bey-Abina, S., Kalle, C. V., Schmidt, M., McCormack, M. P., Wulffraat, N., Leboulch, P., Lim, A., Osborne, C. S., Pawliuk, R., Morillon, E., Sorensen, R., Forster, A., Fraser, P., Cohen, J. I., de Saint Basile, G., Alexander, I., Wintergerst, U., Frebourg, T., Aurias, A., Stoppa-Lyonnet, D., Romana, S., Radford-Weiss, I., Gross, F., Valensi, F., Delabesse, E., Macintyre, E., Sigaux, F., Soulier, J., Leiva, L. E., Wissler, M., Prinz, C., Rabbitts, T. H., Deist, T. L., Fischer, A. and Cavazzana-Calvo, M. (2003), "LMO2-associated clonal T cell proliferation in two patients after gene therapy for SCID-X1", Science, Vol. 302 No. 5644, pp. 415-9.

Hatzimichael, E. and Tuthill, M. (2010), "Hematopoietic stem cell transplantation", Stem Cells and Cloning, Vol. 3, pp. 105-17.

Howe, S. J., Mansour, M. R., Schwarzwaelder, K., Bartholomae, C., Hubank, M., Kempski, H., Brugman, M. H., Pike-Overzet, K., Chatters, S. J., de Ridder, D., Gilmour, K. C., Adams, S., Thornhill, S. I., Parsley, K. L., Staal, F. J. T., Gale, R. E., Linch, D. C., Bayford, J., Brown, L., Quaye, M., Kinnon, C., Ancliff, P., Webb, D. K., Schmidt, M., von Kalle, C., Gaspar, H. B. and Thrasher, A. J. (2008), "Insertional mutagenesis combined with acquired somatic mutations causes leukemogenesis following gene therapy of SCID-X1 patients", Journal of Clinical Investigation, Vol. 118 No. 9, pp. 3143-50.

Kohn, D. B., Booth, C., Kang, E. M., Pai, S., Shaw, K. L., Santilli, G., Armant, M., Buckland, K. F., Choi, U., Ravin, S. S. D., Dorsey, M. J., Kuo, C. Y., Leon-Rico, D., Rivat, C., Izotova, N., Gilmour, K., Snell, K., Dip, J. X., Darwish, J., Morris, E. C., Terrazas, D., Wang, L. D., Bauser, C. A., Paprotka, T., Kuhns, D. B., Gregg, J., Raymond, H. E., Everett, J. K., Honnet, G., Biasco, L., Newburger, P. E., Bushman, F. D., Grez, M., Gaspar, H. B., Williams, D. A., Malech, H. L., Galy, A. and Thrasher, A. J. (2020), "Lentiviral gene therapy for X-linked chronic granulomatous disease", Vol. 26 No. 2, pp. 200-6.

Kohn, D. B., Chen, Y. Y. and Spencer, M. J. (2023), "Successes and challenges in clinical gene therapy", Gene Therapy, Vol. 30, pp. 738-46.

Kohn, D. B., Pai, S. and Sadelain, M. (2013), "Gene therapy through autologous transplantation of gene-modified hematopoietic stem cells", Biology of Blood and Marrow Transplantation, Vol. 19 No. 1, pp. S64-9.

Kohn, D. B., Sevilla, J., Rao, G., Chitty-Lopez, M., Almarza, D., Terrzas, D., Zubicaray, J., Gonzalez-Vicent, M., Chetty, K., O'Toole, G., Xu-Bayford, J., Nicoletti, E., Fernandes, A., Kuo, C., de Oliveira, S., Moore, T. B., Choi, G., Zeini, M., Mesa-Nunez, C., Thrasher, A. J., Bueren, J. and Schwartz, J. D. (2022), "Interim results from an ongoing phase 1/2 study of lentiviral-mediated ex-vivo gene therapy for pediatric patients with severe leukocyte adhesion deficiency-I (LAD-1)", Molecules Therapy, Vol. 30, pp. 550.

Magnani, A., Semeraro, M., Adam, F., Booth, C., Dupré, L., Morris, E. C., Gabrion, A., Roudaut, C., Borgel, D., Toubert, A., Clave, E., Abdo, C., Gorochov, G., Petermann, R., Guiot, M., Miyara, M., Moshous, D., Magrin, E., Denis, A., Suarez, F., Lagresle, C., Roche, A. M., Everett, J., Trinquand, A., Guisset, M.,

Bayford, J. X., Hacein-Bey-Abina, S., Kauskot, A., Elfeky, R., Rivat, C., Abbas, S., Gaspar, H. B., Macintyre, E., Picard, C., Bushman, F. D., Galy, A., Fischer, A., Six, E., Thrasher, A. J. and Cavazzana, M. (2022), "Long-term safety and efficacy of lentiviral hematopoietic stem/progenitor cell gene therapy for Wiskott-Aldrich syndrome", Nature Medicine, Vol. 28 No. 1, pp. 71-80.

Pai, S., Logan, B. R., Griffith, L. M., Buckley, R. H., Parrott, R. E., Dvorak, C. C., Kapoor, N., Hanson, I. C., Filipovich, A. H., Jyonouchi, S., Sullivan, K. E., Small, T. N., Burroughs, L., Skoda-Smith, S., Haight, A. E., Grizzle, A., Pulsipher, M. A., Chan, K. W., Fuleihan, R. L., Haddad, E., Loechelt, B., Aquino, V. M., Gillio, A., Davis, J., Knutsen, A., Smith, A. R., Moore, T. B., Schroeder, M. L., Goldman, F. D., Connelly, J. A., Porteus, M. H., Xiang, Q., Shearer, W. T., Fleisher, T. A., Kohn, D. B., Puck, J. M., Notarangelo, L. D., Cowan, M. J. and O'Reilly, R. J. (2014), "Transplantation outcomes for severe combined immunodeficiency, 2000-2009", The New England, Journal of Medicine, Vol. 371 No. 5, pp. 434-46.

Staal, F. J. T., Aiuti, A. and Cavazzana, M. (2019), "Autologous Stem-Cell-Based Gene Therapy for Inherited Disorders: State of the Art and Perspectives", Frontiers in Pediatrics, Vol. 7.