Short thesis for the degree of Doctor of Philosophy (PhD)

The role of autologous hematopoietic stem cell transplantation in oncohematological diseases

by Réka Ráhel Bicskó, MD

Supervisor: Lajos Gergely, MD, PhD

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By Reka Rahel Bicsko, MD

Supervisor: Lajos Gergely, MD, PhD

Doctoral School of Clinical Medicine, University of Debrecen

Head of the Defense Committee:	Gabriella Szűcs, MD, PhD, DSc
Reviewers:	Zsolt György Nagy, MD, PhD
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Introduction

In the treatment of malignant hematological diseases, there has been unprecedented progress in the last 10 years with the rise of immunotherapies, which primarily target the effector cells of the anti-tumor immune response: T cells. T-cell therapies expressing chimeric antigen receptors and bispecific antibodies that create immunological synapses have been able to achieve significant results even in previously untreatable patients in many cases. This highlights the crucial role of the immune system in the treatment of hematological diseases. Meanwhile, conventional chemotherapy treatments are still often effective today, and in some cases, the treatment can be supplemented with high-dose chemotherapy and autologous hematopoietic stem cell reinfusion (autologous stem cell transplantation). During the intervention, a fully myelo- and lymphoablative conditioning regimen is applied, which also modulates the immune response by restoring the adaptive immune system to its baseline state. This effect can be observed in certain autoimmune diseases (such as multiple sclerosis and systemic sclerosis), where autologous stem cell transplantation performed after lymphoablative treatment can temporarily or permanently eliminate autoimmunity. In cases of hematological disorders, while chemotherapy alone may not suffice, combining it with autologous stem cell transplantation can lead to survival outcomes comparable to modern therapies. Given that autologous bone marrow transplantation is now a well-known, widely accessible, and safe procedure, with significantly lower costs than newer immunotherapies, its application will remain justified for a long time. The author examines the role of autologous bone marrow transplantation in the era of modern therapies in two very common malignant hematological diseases.

AUTOLOGOUS STEM CELL TRANSPLANTATION

During stem cell transplantation, we destroy the patient’s bone marrow hematopoiesis with appropriate chemotherapy and radiation therapy, and subsequently reinfuse healthy mononuclear cells, including pluripotent CD34+ stem cells, which possess the ability to rebuild the hematopoietic system. Today, we primarily distinguish three main types of transplantation based on the origin of the graft: autologous, allogeneic, and syngeneic hematopoietic stem cell transplantation.

In autologous stem cell transplantation, reinfusing the patient's own stem cells prevents any alloimmune reactions, so the essence of autologous transplantation is not the stem cell infusion itself, but rather the high-dose conditioning treatment that precedes it. This treatment aims to achieve complete eradication of residual tumor cells, causing lethal bone marrow depression. By eradicating the bone marrow, the restoration of the adaptive immune system to its baseline state also occurs, which can have an anti-lymphoma effect through the immune response generated against the abnormal cells.

The process of autologous stem cell transplantation consists of three main steps: stem cell collection, conditioning treatment, and reinfusion of the stem cells. The mobilization of stem cells from the bone marrow to the peripheral blood can occur after chemotherapy or, in certain cases, with the standalone use of granulocyte colony-stimulating factor (GCSF). If mobilization is insufficient, the use of the chemokine receptor-4 blocker plerixafor can be helpful. We aim to collect an optimal number of viable stem cells, specifically 4-6 x 10^6 cells per kilogram of body weight, using a cell separator through a peripheral or central catheter. The collected stem cell product undergoes quality assurance testing, and then it is cryopreserved in liquid nitrogen at -180°C using 5-10% dimethyl sulfoxide until reinfusion.

After stem cell collection, the patient may receive additional treatment, but transplantation generally occurs only when the patient is confirmed to be free of tumors. Achieving a good therapeutic response is crucial for a successful transplantation. The intensity and type of chemotherapy protocol used for conditioning primarily depend on the diagnosis, as well as the patient's age and comorbidities. In lymphomas, the BEAM (BCNU, etoposide, cytarabine, melphalan) regimen is most commonly used, often supplemented with rituximab if necessary, while in the case of plasma cell myeloma, high-dose melphalan is typically used as monotherapy.

Next comes the infusion of stem cells delivered through a central venous catheter. The role of the administered progenitor stem cells is to navigate to the appropriate microenvironment through cytokines and adhesion molecules released by the bone marrow stromal cells, where they can engraft, followed by division and multi-lineage differentiation. After autologous stem cell transplantation, this process typically takes about 10-14 days. Granulocyte colony-stimulating factor is necessary to facilitate the production of white blood cells. The success of the engraftment process is first indicated by an increase in the number of platelets and white blood cells, with the threshold for platelets being 20 G/L. For white blood cells, reaching an absolute neutrophil count of >0.5 G/L is considered a clear sign of successful engraftment.

During the transplantation, supportive care is extremely important, primarily aimed at preventing infections. Nevertheless, infectious events are among the most common early complications, where prolonged neutropenia, reduced phagocytic function, and damage to the mucosal barrier play significant roles. Thanks to advancements in supportive therapy, the peritransplantation mortality rate has significantly decreased over the past decades, currently varying between approximately 3-4% per conditioning treatment. As a late complication, secondary malignancies, solid tumors, and hematological disorders may occur. The immunoreconstitution after transplantation typically becomes complete within about six months to a year.

Autologous stem cell transplantation has become a widely used procedure that can potentially lead to cures for various malignant and autoimmune diseases. The main indications are malignant hematological disorders. The complications and side effects are well known, making this a safe therapeutic tool in diseases where recovery is based on high-dose chemotherapy conditioning treatment. However, in recent years, with the emergence of immunotherapies, small molecules, and cell therapies, the hope for chemotherapy-free salvage treatments and cures has sparked questions about the role of autologous stem cell transplantation.

DIFFUSE LARGE B-CELL LYMPHOMA

Diffuse large B-cell lymphoma (DLBCL) is the most common non-Hodgkin lymphoma, with its incidence increasing with age. Its prevalence has significantly risen over the past decade due to advancements in therapy and improved survival outcomes. Genetic alterations, gene

mutations, chromosomal translocations, and other cytogenetic abnormalities play a role in its pathogenesis, resulting in extraordinary heterogeneity. The introduction of rituximab was a milestone in its treatment; today, with first-line immunochemotherapy (R-CHOP – rituximab, cyclophosphamide, vincristine, doxorubicin, prednisone), approximately 60% of patients achieve remission. However, due to this genetic diversity, the remaining 40% of patients have relapsed or refractory disease with poor prognosis, and their treatment continues to pose significant challenges.

Treatment of Relapsed/Refractory DLBCL

The second-line treatment for relapsed patients is relatively well-defined as immunochemotherapy. For patients eligible for transplantation, the R-DHAP protocol (rituximab, dexamethasone, high-dose cytarabine, cisplatin) is recommended for the CG subtype, while the R-ICE protocol (rituximab, ifosfamide, carboplatin, etoposide) is suggested for the ABC group, followed by autologous stem cell transplantation in cases of complete metabolic remission. In elderly, frail patients where transplantation is not feasible, the combination of polatuzumab vedotin + rituximab + bendamustine is recommended as second-line therapy.

For primary chemoresistant patients, immunotherapy is suggested instead of immunochemotherapy in the second line. Chimeric antigen receptor T-cell (CAR-T) therapy has revolutionized salvage therapy. With second-generation, CD19-targeting axicabtagene ciloleucel (axi-cel) and lisocabtagene maraleucel (liso-cel), significantly higher complete remission rates can be achieved compared to conventional treatments (32% vs. 65% and 33% vs. 66%, respectively). However, the success of CAR-T therapy is limited by the number of prior chemotherapy treatments, the duration between leukapheresis and reinfusion of the trained cells, and specific side effects. The introduction of bispecific antibodies was also a milestone in treatment. In the relapsed/refractory group, the complete remission rates were 37% with CD3xCD20 glofitamab and 39% with epcoritamab in third-line treatment. Regarding side effects, bispecific antibodies are primarily associated with neurological issues and cytokine release syndrome (CRS).

The aforementioned therapies are only available in Hungary as part of clinical trials from the second line onward. In relapsed/refractory patients who are not candidates for transplantation, the CD19-targeting tafasitamab combined with lenalidomide has resulted in a

33% remission rate, and the antibody-drug conjugate loncastuximab tesirin has also proven effective as a monotherapy. Polatuzumab vedotin can also be used as a second- and third-line salvage treatment with high cure rates, provided the first-line treatment did not include it. Successful treatment with polatuzumab followed by autologous transplantation offers additional survival benefits.

Autologous Transplantation in the treatment of diffuse large B-cell lymphoma

In diffuse large B-cell lymphoma, autologous stem cell transplantation can be performed in first-line treatment for high-risk patients and as part of salvage therapy in relapsed and refractory cases. However, the clear indications for the procedure are only partially clarified alongside new therapeutic options. First-line transplantation is recommended for primary CNS lymphoma and for high-risk patients with a high initial IPI, as well as in double-hit and triple-hit cases. In the case of true relapse, suitable patients ideally receive salvage chemotherapy followed by autologous stem cell transplantation.

For the success of transplantation, it is optimal to have a tumor-negative status confirmed by PET/CT prior to the procedure, which can only be achieved in about a quarter of patients with conventional treatments. In primary chemoresistant cases, immunotherapy is now clearly recommended as salvage treatment instead of chemotherapy, raising questions about the necessity of transplantation afterward; however, such studies are lacking. Currently, the literature is open regarding whether autologous stem cell transplantation is necessary after successful CAR-T therapy. At the same time, CAR-T therapy is only available to a limited extent and is not a realistic second-line therapeutic option in Hungary.

Thus, despite the expanding array of new therapeutic options, most patients with relapsed/refractory DLBCL in Hungary still receive traditional chemo-immunotherapy as salvage treatment, followed by autologous stem cell transplantation. Even in the era of new therapies, autologous stem cell transplantation remains an important modality for managing relapsed/refractory cases. However, the selection of suitable patients, as well as the timing and factors influencing the success of transplantation, are critical.

PLASMA CELL MYELOMA

Plasma cell myeloma is the most common malignant disease of the bone marrow. It primarily affects older patients, with incidence increasing in parallel with age; prevalence is continuously rising due to improved survival outcomes. Clinical manifestations include plasma cell myeloma, plasmacytoma, and plasma cell leukemia. Cytogenetic abnormalities, gene mutations, and epigenetic modifications all play a role in its pathogenesis. Among these, two foundational abnormalities can be distinguished: hyperdiploidy and translocations involving the immunoglobulin heavy chain (IgH) coding gene segment, along with numerous other secondary events that occur later.

First-Line Treatment of Plasma Cell Myeloma

Plasma cell myeloma is currently an incurable disease, characterized by alternating periods of remission and relapse. The main goal of first-line treatment is to achieve the deepest possible therapeutic response and to delay relapse. High-dose melphalan combined with autologous transplantation is the cornerstone of treatment. Therefore, for patients eligible for transplantation, our first-line therapeutic plan consists of induction, stem cell collection, conditioning, transplantation, and maintenance therapy. We use a triplet regimen as induction therapy, combining immunomodulators, proteasome inhibitors, and steroids.

Although survival data have significantly improved with the current first-line treatment, relapse must always be anticipated. Definitive cure could potentially be achieved through allogeneic stem cell transplantation, but this option is limited due to the average age of patients and their comorbidities.

Treatment of Refractory/Relapsing Myeloma

There is no standard protocol for second-line treatment of myeloma. The selection of salvage therapy requires careful consideration, as the early use of modern agents can quickly lead to multidrug resistance, while poorly chosen therapeutic combinations may yield suboptimal responses despite high costs.

In the treatment of patients who are primarily refractory to the VRd triplet, we can utilize second-generation proteasome inhibitors and alternative immunomodulatory agents. Effective options include carfilzomib-based doublets and triplets, as well as combinations containing pomalidomide. Generally, if the t(4;14) translocation is present, the salvage therapy is

recommended to include a proteasome inhibitor. In cases of del17p and/or TP53 mutations, combinations containing cyclophosphamide are not recommended; instead, pomalidomide may provide a solution. If the t(11;14) translocation is confirmed, adding venetoclax may be an alternative.

For the treatment of relapses occurring after VRd therapy, monoclonal antibodies against CD38 have shown high efficacy, and the SLAMF7 inhibitor elotuzumab can also be used effectively. In lenalidomide-refractory patients, alternative immunomodulatory agents should be considered. In third-line treatment, we may attempt new-generation proteasome inhibitors and/or immunomodulators combined with other monoclonal antibodies, such as carfilzomib-lenalidomide-dexamethasone (KRd), isatuximab-carfilzomib-dexamethasone, or elotuzumab-pomalidomide-dexamethasone triplets.

The expanding therapeutic options have resulted in longer overall survival, but simultaneously, the number of relapses and lines of therapy used has increased. The concept of so-called pentarefractory disease, which does not respond to traditional treatments, has emerged. In this patient group, a new therapeutic option is belantamab mafodotin, a B-cell maturation antigen (BCMA) inhibitor conjugated to a cytotoxic microtubule inhibitor, which demonstrated a one-year progression-free survival (PFS) in the DREAMM-2 trial. CAR T-cell therapies and bispecific antibodies have shown remarkable efficacy in the treatment of multidrug-resistant diseases. In CAR T-cell therapy, the most common surface target is BCMA. The efficacy of ciltacabtagene-autoleucel was evaluated in the CARTITUDE-1 study, where, applied in the fourth line of therapy, a PFS of 54.9% was achieved with a median follow-up of 27.7 months. In the KarMMA study, idecabtagene-vicleucel achieved one-year progression-free survival in pentarefractory patients.

Of course, the limiting factors discussed in the previous chapter regarding CAR T-cell treatment also apply to myeloma. Despite the encouraging results, it is evident that some patients still relapse after CAR T-cell therapy. In such cases, bispecific antibodies are very promising. The CD3-synchronized BCMA-targeting teclistamab and elranatamab have shown approximately 12 months of PFS, while the GPRC5D-targeting talquetamab has reported a 70% overall response rate (ORR). However, cytokine release syndrome occurred in 67-70% of patients. The availability of bispecific antibodies in myeloma is still quite limited.

The Role of Second Autologous Stem Cell Transplantation in Plasma Cell Myeloma

Second stem cell transplantation remains an important option for relapsing/refractory patients. In the first large retrospective study conducted in 2006, a median progression-free survival (PFS) of 6.9 months and overall survival (OS) of 29 months were reported after the second autologous stem cell transplant (ASCT). Another study found a median PFS of 8.5 months and an OS of 20.7 months. Later, it was confirmed that a second autologous transplantation is recommended if at least 24 months of remission is achieved after the first transplant. In patients where relapse occurred within one year, the intervention yielded only 9.8 months of PFS, whereas in those with relapse occurring after 24 months, a PFS of 17.3 months was documented.

Results from a further study indicated that the therapeutic response achieved prior to the second transplantation significantly influences outcomes; with at least partial remission, the two-year overall survival is 85.9%, while in cases of poorer therapeutic response, it drops to only 51.3%. At Karolinska University, the outcomes of second AHSCT were compared with those treated with proteasome inhibitors and immunomodulatory agents in relapsing patients. In the case of second autologous transplantation, a four-year OS was achieved, compared to 3.3 years in the other group. It was also established that the second stem cell transplant should ideally be performed after the first relapse following the initial transplantation, as this most effectively improves PFS and OS.

These results highlight the importance of patient selection and optimal timing. A second transplantation is advisable when the first high-dose melphalan treatment has resulted in sustained remission, and the deeper the therapeutic response achieved before transplantation, the better the expected survival. With the advent of immunotherapy and CAR T-cell therapy, the therapeutic arsenal has expanded even in relapsing cases, leading to a relative overshadowing of second autologous transplantation. The extraordinary costs, side effect profiles, and availability of new therapies limit their application; in contrast, second autologous stem cell transplantation remains a well-known, safe, and funded treatment option for relapsing plasma cell myeloma.

OBJECTIVES

In our work, we set the following goals for patients with diffuse large B-cell lymphoma:

1. To investigate the safety of autologous stem cell transplantation, including the occurrence rates of complications and second malignancies.
2. To assess the impact of the number of prior salvage therapies on the outcome of autologous stem cell transplantation.
3. To examine the laboratory factors influencing the success of transplantation.
4. To determine the prognostic value of pre-transplant PET/CT imaging.
5. To investigate the healing outcomes achievable with autologous transplantation and assess its role among modern therapies.

Additionally, we aim to investigate in patients with plasma cell myeloma:

6. The safety of second autologous transplantation, including the occurrence rates of complications and second malignancies.
7. The effect of the number of prior salvage therapies on the outcome of second autologous stem cell transplantation.
8. The role of the timing of stem cell collection in post-transplant survival.
9. The correlation between the conditioning melphalan dose and transplantation outcomes.
10. The place of salvage autologous transplantation in the era of modern therapeutic options.

MATERIALS AND METHODS

Diffuse large B-cell lymphoma patients

We retrospectively analyzed the demographic data and clinical caracteristics of patients with DLBCL treated and transplanted at the University of Debrecen, Department of Hematology. All patients were included who underwent autologous transplantation with the diagnosis of diffuse large B-cell lymphoma between January 1, 2010, and June 30, 2021. The patient's clinical data were retrospectively collected from patient record databases. The data was also cross-checked with the data reported towards the European Bone Marrow Transplantation Society (EBMT). The diagnosis of DLBCL was based on histological sampling in all cases, Hans algorithm was used to define the histological subgroup. Treatment modalities chosen were in accordance with the therapeutic guidelines in force at the time of diagnosis. Lugano classification was used to define response assessment on PET/CT. Primary refractory cases were defined as not responding after four cycles of first-line chemotherapy determined by interim PET/CT, or not achieving CR after first-line therapy based on end-of-treatment PET/CT, or relapsing within 12 months of first-line rituximab-containing therapy. Relapse was defined as recurrence of DLBCL more than one year from the last cycle of first-line treatment. Rebiopsy was not routinely performed, only in case of late relapse (>5 years) or unusual localization. Administration of salvage therapy continued until PET-negative status was reached. PET positivity was considered only an acceptable result before transplantation in those cases, where no other salvage therapeutic option was available, but at least a partial remission developed. Transplantation was not performed in case of stable or progressive disease. A PET/CT scan was performed before and after the transplantation to assess the stage of the disease. During the peritransplantation period all patients received antimicrobal, antifungal and antiviral prophylaxis. Filtered and irradiated blood suspensions were given if needed. Transplant-related mortality was defined as death occurring from any cause other than disease progression within 100 days from the date of transplantation.

Data were presented as means±standard deviation or medians (upper and lower quartiles). The relationship between normally distributed variables was performed with Pearson tests. The Kaplan-Meier method analyzed and compared unadjusted survival distributions using the log-rank (Mantel-Cox) test. Hazard ratios were calculated using the Log-rank test. The effect of variables on outcome was investigated using the ROC analysis to define cutoff points where needed. Cutoff values were always rounded to the nearest non-decimal number, except noted. $P \leq 0.05$ probability values were considered statistically significant. Statistical analyses

were performed using GraphPad Prism v9.5 and SPSS v 28.0 software. Survival graphs were created using GraphPad Prism v9.5 software.

The study was carried out in accordance with the Declaration of Helsinki and was approved by the local and regional ethical committees (DE RKEB/IKEB H.0290-2021, date of approval: 30 April 2021).

Multiple myeloma patients

We retrospectively analyzed the demographic and clinical characteristics of patients with multiple myeloma, who underwent a second autologous stem cell transplantation between 1 January 2008 and 31 December 2023. Data were obtained from patient record databases and were also cross-checked with the data reported towards the European Bone Marrow Transplantation Society (EBMT). No patients underwent planned tandem transplantation were included in the study. Performing a second autologous transplantation was considered if the relapse occurred after a remission of at least 24 months after the first transplant. A relapse was defined as a reappearance of serum or urine M-protein by immunofixation or electrophoresis, development of >5% plasma cells in the bone marrow or any other sign disease progression based on the IMWG criteria. The upper age limit was 75 years with appropriate Karnofsky and ECOG status, with an expected survival of at least 6 months. Severe heart failure, chronic obstructive pulmonary disease and liver failure were exclusion criteria. Renal failure due to cast-nephropathy, even hemodialysis dependency, was not an exclusion factor. Conditioning regimen was high dose melphalan in all cases. Most patients received reduced dose (140mg/m2) according to age and previous exposure. Stem cell suspension was infused 48 hours after a single dose melphalan. During the peritransplantation period all patients received antimicrobal, antifungal and antiviral prophylaxis. Filtered and irradiated blood suspensions were given if needed. The therapeutic response to transplantation was assigned by bone marrow examination 100 days after the transplant. Complete remission was stated in case of negative immunofixation and <5% abnormal plasma cells in the bone marrow. Very good partial response was defined as more than 90% reduction of serum M-protein and <100mg/day urinary M-component level. In the case of partial remission, there was >50% decrease in the serum monoclonal protein level, plus at least a 90% reduction of the M-component in the urine. Transplant-related mortality was defined as death occurring from any cause other than disease progression within 100 days from the date of transplantation.

Statistical analysis was performed using GraphPad Prism 10.1 computer software. Survival times were compared using Kaplan-Meier analysis and log-rank (Mantel-Cox) test. Two-sided p-values of <0.05 (5%) were considered statistically significant.

The study was managed according to the Declaration of Helsinki. The protocol was approved by the local and regional ethical committees (DE RKEB/IKEB 6739/2024, date of approval: 30 April 2021).

Patients' characteristics – diffuse large B-cell lymphoma patients

116 patients were enrolled in the study. The median age of patients at the diagnosis was 55 years (range 17-73), 63 (54.3%) were male. Centrum germinativum histological subtype was found in 19 patients (16.3%), non-GC in 36 cases (31%), while the largest proportion (52.5%) could be classified into the NOS subtype. Most patients (80.2%) were diagnosed in advanced Ann Arbor stage, but in good general condition (87.9% ECOG 0-1). Low initial IPI was found in a quarter of the patients (25.8%), while 40.5% of them belonged to the high-risk group. In 24 cases, autologous stem cell transplantation was performed as first-line consolidation, and another 39 primary refractory and 53 relapsing patients underwent the intervention as part of salvage treatment. Most patients received R-CHOP immunochemotherapy as first line treatment, in case of simultaneous systemic and central nervous system involvement, this was supplemented with high-dose methotrexate, while in primary central nervous system lymphoma patients received R-MPV treatment. The most frequently used salvage protocol was R-DHAP (62%), followed by RICE with 13.7%. Patients received an average of 2.22 cycles of salvage treatment, and 42 patients required more than 1 salvage protocol. Most patients (59.5%) achieved complete remission before transplantation, but the others failed to do so, they underwent the transplant in partial remission status. The median age of patients at the time of transplantation was 58 years (range 18-74). Conditioning regimen was TBC (thiotepa, busulfan, cyclophosphamide) in case of central nervous system involvement, the other patients received RBEAM (rituximab, carmustine, etoposide, cytarabine, melphalan). We aimed to infuse a minimum of $5x10^6$/tskg CD34+ viable stem cells during the transplantation, but this was not achieved in all cases.

Patients' characteristics – multiple myeloma patients

30 patients with multiple myeloma underwent two autogolous stem cell transplantation in the defined time interval in our institution. The median age of the patients at diagnosis was 53.5 years (range 37-68). IgG subtype was confirmed in most cases (50%), followed by IgA (23.3%) and light chain diseases (kappa and lambda 13.3-13.3%). All patients received bortezomib-based induction treatment. The conditioning treatment was predominantly (97%) full-dose (200mg/m^2) intravenous melphalan. Two-thirds of patients (66.6%) achieved complete remission after the first stem cell transplant. The median follow-up time was 86 months from diagnosis, while the median overall survival was 118 months. After the first transplant, the median progression-free survival was 39 months. The median time between the diagnosis and the second stem cell transplant was 94 months (range 27-305), while the time between the two transplants was 59.1 months (range 19-138). Thus, the average age of patients at the time of the second transplant was 59 years (range 43-73). Between the two stem cell transplants, 2-6 (median 2) lines of salvage therapy were used in one patient. The most common protocols were VRd (11 cases), KRd (7 cases), bortezomib-thalidomide-dexamethasone-cisplatin-doxorubicin-cyclophosphamide-etoposide and bortezomib-dexamethasone (6 cases each), while 4 patients received daratumumab-based treatment (2 DRd, 2 DPd). For the second transplantation, stored frozen stem cells from the collection before the first transplantation were used in 17 cases, while in 13 cases a re-mobilization and apheresis was performed. Most patients (22) received a reduced-dose (140mg/m^2) conditioning, while in the other cases full dose (200mg/m^2) was used. 14 patients received maintenance treatment, which was lenalidomide monotherapy in most cases.

RESULTS

Results of consolidative autologous transplantation in DLBCL

In a study of 116 patients, the median follow-up was 46 months (range 8-133). The median overall survival (OS) for the entire population was 105 months, while the median event-free survival (EFS) was 75 months.

Impact of Initial Prognostic Factors on Transplant Outcomes

The Ann Arbor stage at diagnosis was not a significant predictor of post-transplant survival, and extensive initial disease did not correlate with shorter survival (EFS p=0.1, OS p=0.24). No significant differences were found among different IPI risk groups. Higher initial

LDH levels did not impact survival (ROC cut-off 220 U/L, p=0.38). Patients without B symptoms had a median EFS of 93 months, compared to 25 months with B symptoms (p=0.12). The initially better-prognosed CG subtype did not correlate with longer survival (EFS p=0.52, OS p=0.74). The impact of primary vs. secondary central nervous system involvement was also not significant (p=0.12).

Impact of Prognostic Factors Before Transplant on Survival

No significant differences were observed in EFS or OS between primary refractory and relapsed cases. The number of chemotherapy treatments before autologous stem cell transplantation did not affect survival. However, PET/CT results prior to transplantation significantly influenced outcomes. Patients with complete metabolic remission had a significantly longer 5-year OS compared to PET/CT positive cases (62.5% vs. 30%, p=0.0009). The median OS for the latter group was 36 months, while the PET-negative group did not reach the median (p=0.001). For partial remission, median EFS was 15 months, while the complete remission group also did not reach the median (p=0.0015).

Objective measures of bone marrow function, such as white blood cell count, lymphocyte/monocyte ratio, and hemoglobin levels, did not significantly affect survival outcomes. Notably, renal function impacted survival; higher serum creatinine (ROC cut-off 90 umol/L) and urea levels (ROC cut-off 4.5 mmol/L) were associated with significantly poorer outcomes. In patients with normal creatinine levels, median EFS was 93 months, compared to 13 months with elevated levels (p=0.028). This difference was also evident in OS (p=0.04).

In cases of ongoing infection, transplantation was postponed. However, slightly elevated CRP without clear infectious symptoms allowed for the procedure. Elevated CRP levels (ROC cut-off 6 mg/L) were linked to significantly shorter EFS and OS. For EFS, this was 105 vs. 35 months (p=0.04), and OS was not reached with normal CRP levels, but was 36 months with elevated levels (p=0.038). The quantity of stem cells reinfused did not significantly affect survival. Faster engraftment (7-18 days, median 9) was associated with better outcomes for both EFS (36 months vs. not measurable, p=0.025) and OS (41 months vs. not measurable, p=0.01; ROC cut-off 9 days). The examined variables and their associated survival outcomes with significance levels are presented in Table 1.

In the first 100 days post-transplant, five patients were lost due to treatment-related toxicity and infection, resulting in a transplant-associated mortality rate of 4.3% (16.7% for TBC conditioning, 1% for RBEAM conditioning). Ten early relapses were recorded (4 with

TBC conditioning, 6 with RBEAM), with 7 patients lost within 100 days. No second solid tumors developed in the study group, though one case of myelodysplastic syndrome was diagnosed.

	Univariate analysis			
	EFS		**OS**	
	Hazard Ratio	p value	Hazard Ratio	p value
Factors at diagnosis				
AnnArbor Stage III-IV	2.254	0.1053	1.728	0.2435
IPI 3-5	1.425	0.1934	1.411	0.2412
Bulky disease	1.385	0.4724	1.042	0.9140
B symptoms	1.922	0.0760	1.606	0.1006
CG histology	0.765	0.5259	0.887	0.7456
Normal LDH	0.810	0.5468	0.828	0.6033
Factors at transplant				
PET negative	***0.422***	***0.0015***	***0.382***	***0.0010***
More than 1 salvage lines	1.527	0.133	1.599	0.1158
Primary refractory disease	1.014	0.9624	1.024	0.9566
Engraftment before 9 days	***0.531***	***0.0253***	***0.452***	***0.0101***
More than $4x10^6$/kg CD34+ graft	0.684	0.2195	1.031	0.9402
Hgb over 100 g/L	0.890	0.7276	0.779	0.4666
Absolute lymphocyte over 1 G/L	1.078	0.8176	1.212	0.5321
Thrombocyte over 100 G/L	0.704	0.2103	***0.491***	***0.0241***
CRP less than 6 mg/L	***0.558***	***0.0453***	***0.530***	***0.0382***
Creatinin below 90 umol/L	***0.504***	***0.0288***	***0.550***	***0.0408***
Blood urea nitrogen less than 4.5 mmol/L	***0.437***	***0.0184***	***0.375***	***0.0121***

Table 1. *Factors affecting the outcome of autologous transplantation in diffuse large B-cell lymphoma patients. Overall survival (OS) and event free survival (EFS) are calculated from the date of transplant. Significant difference was stated where the p value was less than 0.05. All significant values are highlighted in bold text. CRP - C reactive protein, PET - positronemission tomography, Hgb - hemoglobin, CG - germinal center type by Hans algorythm, IPI - international prognostic index, LDH - lactate dehydrogenase enzyme*

Results of Second Autologous Transplantation in Patients with Plasma Cell Myeloma

In a study involving 30 patients who underwent a second stem cell transplantation, the median follow-up was 34 months (range 11-110). The median progression-free survival (PFS) for the entire group was 24 months, while the median overall survival (OS) was 48 months.

The number of prior salvage treatments did not significantly affect survival. Patients receiving two types of salvage treatment had a median PFS of 32 months, compared to 24 months for those with multiple types (p=0.47). The use of previously collected stem cells versus fresh mobilization for the graft did not impact survival either; the median PFS was 28 months for previously collected cells and 21 months for fresh grafts (p=0.94). Median engraftment time was 9 days for prior mobilization and 10 days for new collection (p=0.22). There were no differences in survival based on the conditioning dose; full-dose (200 mg/m^2) melphalan resulted in a median PFS of 34 months, while reduced dose (140 mg/m^2) showed 24 months (p=0.47).

Median neutrophil engraftment time was 9 days (range 8-13), and slower engraftment was not associated with shorter PFS (32 vs. 22 months, p=0.27) or OS (48 vs. 39 months, p=0.82).

Transplant-associated mortality was 3%, with one patient lost to nosocomial pneumonia 32 days post-transplant. At 100 days post-procedure, 60% of patients achieved complete remission (CR). For those with CR or very good partial remission (VGPR) status at the time of second transplantation, the median PFS was 32 months, compared to 14 months for those with partial remission (PR) (p=0.18). Among the 10 patients who underwent the second autologous stem cell transplant with PR, three achieved VGPR, three achieved CR, while four remained in PR.

CR and VGPR detected during the 100-day bone marrow check were associated with significantly longer PFS and OS compared to those achieving only PR (PFS 32 months vs. 8.5 months, p=0.0006; OS 58 months vs. 18 months, p=0.002).

The use of maintenance therapy did not provide benefits in either PFS or OS. Patients receiving maintenance had a median PFS of 32 months, compared to 24 months without it (p=0.83). Median OS was 52 months with maintenance versus 39 months without (p=0.77). During the follow-up period, one case of second malignancy (lung tumor) was reported (3%). No cases of myelodysplastic syndrome or acute leukemia were noted. The parameters studied, their associated survival data, and significance levels are presented in Table 2.

	Median PFS (months)	**p-value**	**Median OS (months)**	**p-value**
2 lines of salvage therapy	32	0.47	48	0.82
>2 lines of salvage	24		58	
Stored stem cells	28	0.94	48	0.76
Fresh collection	21		39	
Melphalan 200mg/m^2	34	0.47	34	0.56
140mg/m^2	24		52	
Engraftment <9 days	32	0.27	48	0.82
>9 days	22		39	
Pre-TX CR+VGPR	32	0.18	52	0.19
Pre-TX PR	14		15	
Post-TX CR+VGPR	**32**	**0.0006**	**58**	**0.002**
Post-TX PR	**8.5**		**18**	
Maintenance therapy	32	0.83	52	0.77
No maintenance therapy	24		39	

Table 2. *Factors affecting the outcome of second autologous transplantation in myeloma patients. Overall survival (OS) and event free survival (EFS) are calculated from the date of transplant. Significant difference was stated where the p value was less than 0.05. All significant*

values are highlighted in bold text. CR = complete remission, PR = partial remission, TX = autologous stem cell transplantation, VGPR = very good partial remission,

DISCUSSION

In our study, we examined the effects of autologous hematopoietic stem cell transplantation in patients with diffuse large B-cell lymphoma (DLBCL) and plasma cell myeloma, comparing the results with those of new therapies.

In the treatment of relapsing/refractory DLBCL, autologous peripheral stem cell transplantation remains an effective therapeutic option, provided the disease is chemosensitive and responds to salvage therapy. Based on this, our study sought clinical parameters whose presence or absence could help identify patients who would benefit from transplantation and improve outcomes.

In the entire population, we confirmed a long-term survival rate exceeding 50%, which is comparable to previously reported results in the literature. The RBEAM conditioning regimen proved to be well-tolerated, with a low complication rate. In the subgroup receiving RBEAM, we recorded a 1% transplantation-associated mortality rate, which is somewhat lower than the previously reported 2-3%. In the subgroup conditioned with TBC, due to higher toxicity, this rate was higher at 16.7%, consistent with literature data. However, it should be noted that the lower number of cases in this latter category (n=24) may distort the results in either direction.

Our study confirmed that the IPI score recorded at diagnosis does not affect transplantation outcomes. This observation contrasts with previous studies indicating that a lower baseline IPI is associated with better post-transplant survival. There was no significant correlation between cell origin and survival outcomes after AHSCT, nor regarding the presence of B symptoms or elevated LDH values at the time of disease diagnosis. Thus, we can state that initial prognostic factors lose their significance by the time of transplantation.

Transplantation was performed as primary consolidation in primary chemoresistant and relapsing cases. We found no differences in survival between these subgroups, contrary to observations in the literature suggesting that refractory patients have poorer survival compared to relapsing ones. The results of PET/CT performed prior to transplantation emerged as a clear determinant of survival. Here, it is not the number of types of salvage therapies the patient

underwent prior to transplantation that matters, but the quality of the therapeutic response achieved with those therapies. These observations are consistent with international publications.

We observed that the number of infused stem cells did not affect outcomes; however, the time required for neutrophil engraftment was a determining factor, with faster engraftment significantly associated with longer event-free and overall survival. We confirmed that an elevated CRP value detected without clear infectious symptoms, along with higher urea and creatinine levels, are important determinants of survival.

In summary, consolidative AHSCT is an effective and rational treatment option for chemosensitive DLBCL patients. With careful patient selection, the incidence of toxicity and side effects can be reduced, and survival outcomes can be comparable to those of immunotherapies.

Analyzing data from patients with plasma cell myeloma who underwent second autologous stem cell transplantation, the procedure proved to be a safe and effective therapeutic option, even for patients previously treated multiple times. We found no significant survival differences based on the dose of the conditioning regimen. The transplantation-associated mortality rate was 3%. The occurrence of secondary malignancies was also low, at only 3%, compared to the 5-7% frequency reported in the international literature.

Consistent with existing literature, we established that a very good partial remission or complete remission status prior to transplantation is optimal for better progression-free and overall survival; however, some patients in partial remission can also benefit from the procedure. Patients who achieve a partial remission post-transplant tend to have significantly shorter survival, indicating a need for further active myeloma treatment in such cases.

Shorter progression-free survival is expected after the second transplantation; in our study, this was 24 months, which is somewhat better than previously reported data indicating a median PFS of 20.4 months.

In our study, the overall 5-year progression-free survival rate for the entire patient group was 17%, and there was no significant difference between subgroups receiving and not receiving maintenance therapy. However, this may be influenced by the small patient numbers associated with specific maintenance therapies. We could not assess the relationship between

maintenance therapy and secondary malignancies, as only one case of a second solid tumor developed.

Based on these findings, the authors believe that second autologous stem cell transplantation is an effective and safe therapeutic option for appropriately selected patients with plasma cell myeloma. A key consideration is timing; transplantation is not recommended for remissions shorter than 24 months following the first transplant. We should strive for the deepest possible therapeutic response before conditioning, and if a partial remission is confirmed after the second transplantation, further active treatment will be necessary. The mortality associated with transplantation is minimal, as is the rate of secondary malignancies occurring during follow-up.

NEW FINDINGS

Diffuse large B-cell lymphoma consolidative autologous transplantation:

1. We have confirmed that autologous stem cell transplantation in diffuse large B-cell lymphoma is a safe procedure, associated with low toxicity and a 1% transplantation-associated mortality rate when using RBEAM conditioning. The occurrence of secondary malignancies is minimal.
2. We demonstrated that the number of salvage treatments prior to transplantation has no significant impact on the outcome of autologous stem cell transplantation; however, the quality of the therapeutic response achieved with these treatments greatly determines survival.
3. We established that patients with higher pre-transplant serum CRP, urea, and creatinine levels have poorer survival compared to those without these laboratory abnormalities.
4. We observed that the prognostic factors recorded at diagnosis lose their significance by the time of transplantation. Furthermore, the results of PET/CT performed before autologous stem cell transplantation have strong prognostic significance. A partial remission status is an independent risk factor for shorter event-free and overall survival.

5. We confirmed that the survival outcomes achievable with autologous transplantation in a well-selected patient group are comparable to those obtained with chimeric antigen receptor (CAR) T-cell therapy, with tolerable side effects and lower costs.

Second Autologous Transplantation in Plasma Cell Myeloma:

6. We established that second autologous transplantation in patients with plasma cell myeloma is a safe procedure, associated with low toxicity and transplantation-associated mortality. The frequency of secondary malignancies is also minimal.

7. We observed that there is no correlation between the number of salvage treatments prior to the second transplantation and its outcome; however, a deeper therapeutic response achieved with these treatments is associated with better survival.

8. We demonstrated that the timing of stem cell collection for transplantation does not affect either the engraftment time or survival.

9. We confirmed that the use of reduced-dose melphalan is not associated with shorter survival and is equivalent to the full dose.

10. We established that the progression-free and overall survival achievable with salvage autologous transplantation is comparable to data reported for CAR T-cell therapy, with tolerable side effects and lower costs.

Summary

Diffuse large B-cell lymphoma and plasma cell myeloma are two very common hematologic malignancies. The pathogenesis is a multistep process entailing the accumulation of multiple genetic lesions due to genetic predisposition and environmental factors. The prevalence is constantly increasing, which is due to the improved survival results due to the development of diagnostics and therapeutic options. Nowadays, DLBCL have become a highly curable, and multiple myeloma have become a well-treatable disease, but the management of relapsed and refractory cases still remains a challenge. In these cases, autologous hematopoietic stem cell transplantation has been a fundamental therapeutic modality in recent decades, but its role has been questioned with the introduction of novel immunotherapies.

Our study aimed to define the efficacy and safety of autologous transplantation among DLBCL patients and of the second autologous transplantation in the MM patient group,

comparing them with the results achieved with immunotherapies. Furthermore, we aimed to identify clinical parameters whose presence or absence affects the outcome after the transplant.

Autologous hematopoietic stem cell transplantation proved to be a well-tolerated and effective treatment option with low toxicity even in a heavily pretreated cohort. The frequency of second malignant diseases was insignificant. The number of salvage treatments before transplantation was not, but the depth of therapeutic response achieved with them, was a significant determinant of survival. The survival of patients less responsive to salvage treatment was inferior compared to the subgroups that achieved complete remission. In both diseases, both the progression-free and overall survival results are nearly equivalent to the data reported with CART treatments.

In summary, despite all efforts and expanding therapeutic options, the treatment of relapsed and refractory hematology patients is still challenging. Among the available therapeutic modalities, our goal is to achieve the best therapeutic response, reduce toxicity and improve the quality of life, in which autologous stem cell transplantation appears to be a safe and effective therapeutic tool.

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List of publications related to the dissertation

1. **Bicskó, R. R.**, Nyilas, R., Szász, R., Váróczy, L., Kiss, A., Udvardy, M., Illés, Á., Gergely, L.: The efficacy and safety of second salvage autologous transplantation in myeloma patients.
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