REVIEW

NON–MYELOABLATIVE ALLOGENEIC STEM CELL TRANSPLANT: ACHIEVEMENTS AND PERSPECTIVES

*T. Saito, S. Mineishi**
Hematopoietic Stem Cell Transplantation Unit,
National Cancer Center Hospital, Tokyo 104-0045, Japan

НЕМИЕЛОАБЛАТИВНАЯ АЛЛОГЕННАЯ ТРАНСПЛАНТАЦИЯ СТВОЛОВЫХ КЛЕТОК: ДОСТИЖЕНИЯ И ПЕРСПЕКТИВЫ

*Т. Сайто, С. Минейши**
Отделение трансплантации стволовых клеток крови клиники
Национального онкологического центра, Токио, Япония

In the past concept, conditioning regimens for allogeneic hematopoietic stem cell transplantation (HSCT) were assumed to have two roles: myeloablation and immunosuppression. Recent clinical and experimental data have shown that intense immunosuppression, rather than myeloablation, is the primary requirement for stable engraftment and that the main therapeutic component of allogeneic HSCT is attributed to graft-versus-leukemia/tumor (GVL/T) effect mediated by donor-derived T cells. Based on these findings, a new strategy of non-myeloablative stem cell transplantation (NST) has been developed for patients who are ineligible for conventional stem cell transplantation (CST) because of the old age or organ dysfunction. Recent reports from many transplant centers have shown that application of NST decreased regimen-related toxicities (RRT), while associated with consistent stable engraftment. In this review we overview the clinical development (background, clinical experience), remaining problems and future directions of NST.
Key Words: **non-myeloablative stem cell transplantation, graft-versus-leukemia/tumor effect, leukemia.**

Согласно существующим до настоящего времени концепциям, режимы кондиционирования, используемые при аллогенной трансплантации стволовых клеток, направлены на достижение миелоаблации и иммуносупрессии. Данные недавно проведенных клинических и экспериментальных исследований свидетельствуют о том, что для достижения устойчивой трансплантации стволовых клеток в первую очередь необходима интенсивная иммуносупрессия, а не миелоаблация. Эффекты, достигаемые при аллогенной трансплантации стволовых клеток, обусловлены главным образом реакцией "трансплантат против лейкоза/опухоли", которая, в свою очередь, опосредуется Т-клетками донора. На основе этих данных разработана новая стратегия немиелоаблативной трансплантации стволовых клеток, приемлемая для больных, которым по причине возраста или сопутствующих заболеваний противопоказано проведение обычной трансплантации стволовых клеток. По данным, полученным в ряде центров по трансплантации стволовых клеток, применение немиелоаблативной схемы приводит к достижению более устойчивой трансплантации одновременно со снижением токсичности. В обзоре рассмотрены история вопроса, опыт клинического применения немиелоаблативной трансплантации и дальнейшие перспективы этого метода.
Ключевые слова: **немиелоаблативная трансплантация стволовых клеток, реакция "трансплантат против лейкоза/опухоли", лейкозы.**

BACKGROUND OF NON–MYELOABLATIVE TRANSPLANT

Allogeneic HSCT has been shown to provide potentially curative therapy for many patients with hematological malignancies, aplastic anemia, and immunodeficiency disorders [1–3]. The use of myeloablative conditioning regimens has been mandatory for allogeneic HSCT. However, high–dose chemo–radiotherapy can not avoid substantial RRT and TRM. Therefore, HSCT is usually offered to patients under 60 without underlying organ dysfunctions.

Previous theory suggested that conditioning regimens for allogeneic HSCT consisting of high–dose chemo–radiotherapy facilitate engraftment by creating space (niche) and by providing host immunosuppression for grafts to be accepted. In other words, conditioning regimens have been assumed to have two roles, myeloablation and immunosuppression. However, in 1990s pioneering works have shown that engraftment of allogeneic cells can take place in non–myeloablated animal models [4–6]. F. Stewart *et al.* [4] have demonstrated that allogeneic cells engraft into normal mice without conditioning and induce stable engraftment for more than 25 months. Furthermore, with a BALB/c male/female

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*Correspondence. Fax: (81–3) 3542–3815;
E–mail: smineish@ncc.go.jp

Abbreviations used: ALL — acute lymphoid leukemia; AML — acute myeloid leukemia; ATG — anti–thymocyte globulin; CLL — chronic lymphoid leukemia; CML — chronic myeloid leukemia; CMV — cytomegalovirus; CR — complete remission; CsA — cyclosporine A; CST — conventional stem cell transplantation; DLI — donor lymphocyte infusion; GVL/T — graft–versus–leukemia/tumor; HSCT — hematopoietic stem cell transplantation; MDS — myelodysplastic syndrome; MM — multiple myeloma; NHL — non–Hodgkin's lymphoma; NST — non–myeloablative stem cell transplantation; RCC — renal cell carcinoma; RRT — regimen–related toxicities; SCID — severe combined immunodeficiency; TBI — total body irradiation; TRM — transplant related mortality.

murine marrow transplant model the same group showed that exposure of host mice to doses of irradiation that induce minimal myeloablation (50 to 100 cGy) had led to very high levels of donor chimerism and resultant stable engraftment [5]. Using a DLA–matched dog model, R. Storb *et al*. [6] also showed that mixed lympho–hematopoietic chimerism can be established following low–dose TBI (200 cGy) as a sole preparative regimen, when followed by post–transplant immunosuppression with mycophenolate mofetil (MMF) and CsA. In this experimental model, they have shown that post–transplant immunosuppression is critical for facilitating engraftment. Using this combination of minimal TBI followed by post–grafting immunosuppression, 11 of 12 dogs became stable mixed chimeras for up to 30 weeks.

On the other hand, in human solid organ transplantation, attempts to induce donor–specific transplantation tolerance via formation of micro–chimerism through donor stem cell infusion have been reported in a few clinical trials [7, 8]. C. Ricordi *et al*. [7] have demonstrated improved patient and graft survival in the setting of combined cadaveric donor liver and bone marrow transplantation as compared with control those who did not receive (donor bone) marrow cells.

Additionally, A. Tsaroucha *et al*. [8] have demonstrated induction of prolonged micro–chimerism after simultaneous peripheral blood stem cell transplantation from the same donor in the setting of living–related liver transplantation. These findings prompted the investigation of efficient immunosuppressive regimen to facilitate engraftment of allogeneic stem cells by overcoming the host–versus–graft reaction without cytotoxic conditioning regimens. Thus, severe RRT can be avoided.

ALLOGENEIC CELL THERAPY

Since 1950s, anti–tumor effect mediated through immune system involving lymphocytes as an effector has been explored and existing GVL/T effect has been studied. D. Barnes *et al*. [9] first revealed the presence of GVL/T effect in a murine transplantation model in 1956. Other investigators were able to induce an anti–leukemic effect without associated GVHD by immunizing MHC compatible donors with allogeneic lymphocytes from the host [10]. More recently, human GVL responses have been studied in SCID mice model transplanted with human leukemia cells [11]. Besides, some clinical data support the presence of GVL effect. M. Horowitz *et al*. [12] retrospectively analyzed the IBMTR data and disclosed that the survival became better in patients who developed grade I acute GVHD as compared to those who did not develop acute GVHD at all, and that the risk of leukemia relapse became higher after syngeneic HSCT [13] or T–cell depleted allogeneic HSCT compared to CST [12, 14]. Altogether, these data confirmed that alloreactive T cells play an important role in preventing leukemia relapse.

Based on these observations, H. Kolb *et al*. [15] showed that many patients who relapsed after allogeneic HSCT could be reinduced into long–lasting CR by DLI and this has been confirmed by series of subsequent studies [16, 17]. DLI nowadays has become a standard therapy for CML which relapsed after allogeneic HSCT. This has given a rationale that CML might be cured by cell–mediated immune therapy without intensive chemo–radiotherapy. Thereafter, this strategy has been applied to a variety of tumors including relapsed AML, MDS, NHL, MM, and solid tumors. [16–22]. The results of these trials revealed that marked disease–to–disease difference exists in the generation of GVL response. Notably in CML, approximately 70% of relapsed patients can achieve CR after DLI, while only the minority of patients with ALL does [16, 17]. Although, 30% of relapsed patients with AML and MDS respond to DLI, remission duration is generally short and most of them eventually relapse within one year [16, 17] (Table 1). Through these clinical experiences, there has been a speculation that major part of anti–leukemic effect carried by allogeneic HSCT resides in an intimate interaction between the quantity and quality of leukemia–specific target molecules, antigen presenting pathway and types of immune effector cells.

Table 1. GLV effect for hematological malignancies

Disease	Remission probability of DLI after allo–HSCT, %	Median duration of remission, months	References
Myeloid malignances			
CML			
chronic phase	60–82	NA	15,16
blastic phase	17–33		
AML	15–29	6–18	15,16
MDS	25–40	7–13	15,16
Lymphoid malignances			
ALL	0–18	0–10	15,16
NHL	0–22	0–20	16,18
MM	9–22	2–30	16,19,20

NA — not achieved.

Previously it used to be believed that the eradication of leukemia/tumor cells depends on the intensity of conditioning chemo–radiotherapy. However, in a number of clinical trials, attempts to prevent the leukemia relapse by intensifying conditioning regimens have been offset by increasing toxicities [23, 24]. Thus, it has become recognized that the main therapeutic component of allogeneic HSCT is attributed to T–cell mediated GVL effect [9–12, 15–22, 25–29] and consequently, the concept that improvement of transplant results can not be achieved by means of intensification of conditioning regimens has been established.

EARLY CLINICAL EXPERIENCES OF NON–MYELOABLATIVE STEM CELL TRANSPLANT

Based on the findings described above, the concept of non–myeloablative conditioning regimens has recently been clinically applied to allogeneic HSCT by more than a few groups [30–35]. Their reports have shown that RRT associated with NST were mild compared to that with CST, while engraftment capability was retained. Thus, NST has been accepted by many transplant physicians as an alternative to CST for patients who cannot tolerate CST.

The basic strategy of developing NST is to utilize less intensive conditioning regimens designed not to eradicate the host cells but to provide sufficient immunosuppression to achieve engraftment of allogeneic stem cells, thus allowing the development of GVH reaction and GVL effect. Several investigators have evaluated this strategy by using sublethal doses of chemotherapy and/or minimal TBI as the conditioning regimen and have demonstrated the feasibility of achieving allogeneic engraft–

ment [30–35]. By definition, non–myeloablative conditioning should allow prompt hematopoietic recovery within 4 weeks even if rescue transplant was not done. As a result, host cells may recover promptly, and thus mixed chimerism may be seen after engraftment [36]. Therefore, DLI may be required only in case when mixed chimerism is detected, to convert it to complete donor chimerism. The results of several clinical trials are shown in Table 2 and discussed below.

1. M. D. Anderson Cancer Center: Purine analog based regimen without ATG. S. Giralt *et al.* [30] first reported a strategy to use purine analog containing non–myeloablative conditioning regimens for myeloid malignancies. Their preliminary experience involved 13 patients with AML and 2 patients with MDS. These patients were ineligible for conventional myeloablative transplantation because of older age or organ dysfunction. Eight patients received fludarabine at 30 mg/m^2/day for 4 days with idarubicin at 12 mg/m^2/day for 3 days and Ara–C at 2 g/m^2/day for 4 days (n = 7) (Flag/Ida regimen) or melphalan at 140 mg/m^2/day (n = 1) (FM regimen). Seven patients received cladribine (2–CdA) at 12 mg/m^2/day for 5 days and Ara–C at 1 g/m^2/day for 5 days. Thirteen patients received peripheral blood stem cell transplants from HLA–identical donors and two from one–antigen mismatched sibling donors. Conditioning regimens were generally well tolerated with only 1 death of multiorgan failure. Thirteen (87%) of 15 patients achieved neutrophil engraftment. Acute GVHD grade $\geq$ II was observed in 3 (20%) patients. Six of 15 patients remain alive between 34 and 175 days post–transplant and 2 of 15 patients remain in remission. In a follow up of this study, 36 patients with AML, MDS, and CML were treated with Flag/Ida regimen followed by HSCT [36]. More than 90% of patients had engraftment with donor cells. Treatment–related mortality was 20%, grade II–IV acute GVHD developed in 32% of patients, and 52% of patients developed chronic GVHD. One–year and 2–year survival of patients who were in CR at the time of transplant was 71% and 59%, respectively. However, only 10% of patients who had refractory disease at the time of transplant remained in CR for 1 year after transplant.

I. Khouri *et al*. [32] treated 15 patients with lymphoid malignancies, such as CLL and NHL, using non–myeloablative conditioning regimens. These regimens consisted of fludarabine at 30 mg/m^2 for 3–5 days and cyclophosphamide at 300–1,000 mg/m^2 for 2–3 days (FC regimen) or fludarabine at 30 mg/m^2 for 2 days, Ara C at 500 mg/m^2 for 2 days, and cisplatin at 25 mg/m^2 for 4 days (PFA regimen) [32]. Eleven (73%) patients had engraftment of donor stem cell, and remaining four (three in FC regimen and one in PFA regimen) rejected the graft. Although five (33%) patients developed acute GVHD, only 1 (7%) patient had grade II disease. Furthermore, they extended this study to low–grade lymphoma and reported a superior result in NST arm compared to CST arm [37].

2. Hadassah University Hospital (Israel) and National Cancer Center Hospital (Japan): Purine analog based regimen with ATG. S. Slavin *et al.* [31] reported non–myeloablative conditioning regimen consisting of fludarabine 30 mg/m^2/day for 6 days, busulfan 4 mg/kg/day for 2 days, and horse ATG 10 mg/kg/day for 4 days (Flu/BU/ATG regimen). They treated 26 patients who may be able to tolerate standard allogeneic HSCT, including acute leukemia (n = 10), chronic leukemia (n = 8), NHL (n = 2), MDS (n = 1), MM (n = 1), and genetic diseases (n = 4). Engraftment was documented in all patients. In addition, in 9 patients absolute neutrophil counts were always above 0.1 · 10^9/L, and platelet counts were always above 20 · 10^9/L in 4 patients. Grade $\geq$ I acute GVHD was observed in 12 (46%) of 26 patients. Grade III to IV acute GVHD was diagnosed in 6 (25%) patients and was the cause of mortality in four patients. Relapse was observed in 2 patients with AML and 1 with NHL. Cytogenetic relapse was diagnosed in 1 patient with CML who did not develop acute GVHD. However, 22 (85%) of 26 patients are alive, 21 (81%) maintain disease–free with excellent quality of life. Later, the same group treated 21 patients with CML using the same Flu/BU/ATG regimen. Acute and chronic GVHD occurred in 12 and 9 patients, respectively, and two died of GVHD. However, all surviving patients stayed in molecular remission [38].

In National Cancer Center Hospital in Tokyo, Japan, we performed a phase I/II study to establish a novel 2–CdA–based regimen for NST [39, 40]. Patients with hematological malignancies who were older than 50 years old or had organ dysfunction were eligible to this study. The regimen consisted of 2–CdA at 0.11 mg/kg/day for 6 days, busulfan at 4 mg/kg/day for 2 days, and rabbit ATG at 2.5 mg/kg/day for 4, 2, or 0 days (2–CdA/BU/±ATG regimen). Sixteen patients were enrolled in this study. The underlying diseases included AML (n = 6), CML (n = 2), MDS (n = 6), and NHL (n = 2).

Table 2. Clinical results of non–myeloablative transplantation

	Regimen	Disease	Stem cell source	Engraftment, %	acute GVHD II–IV, %	Outcome
Purine analog based regimen						
Giralt *et al*. [30] (n = 15)	Flag/Ida FM 2–CdA/Ara C	AML, MDS	PB, BM	87	20	6 patients are alive. 2 patients remain in CR.
Khouri *et al*. [32] (n = 15)	FC PFA	NHL, CLL	PB, BM	73	7	7 patients are alive. 5 patients remain in CR.
Purine analog and ATG based regimen						
Slavin *et al*. [31] (n = 26)	Flu/BU/ATG	AML, ALL, CML, NHL, MM, GD	PB	100	38	22 patients are alive. 21 patients remain in CR.
Low dose TBI based regimen						
MacSweeney *et al*. [34] (n = 50)	TBI (200 cGy)	AML, ALL, MDS, CML, NHL, CLL	PB	83 (#50)	36	NA

Flag/Ida — fludarabine/idarubicin/Ara C; FM — fludarabine/melphalan; 2–CdA/Ara C — cladribine/Ara C; FC — fludarabine/cyclophosphamide; PFA — fludarabine/Ara C/cisplatin; Flu/BU/ATG=fuludarabine/busulfan/ATG; GD — genetic diseases; PB — peripheral blood; BM — bone marrow; (#50) — 50% patients with CML have experienced graft rejection; NA — not available.

This non–myeloablative conditioning regimen was generally well tolerated except for one who developed congestive heart failure. Engraftment was achieved in 14 of 16 patients, including one–antigen mismatched patients. Overall and disease–free survival in this group of patients was comparable to that in standard risk patients who underwent standard transplant. Comparing our results to the recent report of S. Giralt *et al*, NCCH regimen used much less 2–CdA compared to M.D. Anderson regimen, which used 12 mg/m^2 for 5 days of 2–CdA and reported severe toxicity with this regimen [41].

3. Fred Hutchinson Cancer Research Center: Low dose TBI based regimen. P. McSweeney *et al*. [34] reported conditioning regimen consisting of TBI 200 cGy as a single fraction, based on their dog model showing that mixed chimerism is reliably achieved in this method [6]. They demonstrated the feasibility and safety of this non–myeloablative regimen, which has been confirmed in more than 50 patients [42]. Although myelosuppression was very mild and no blood or platelet transfusions were required in many cases, eight (17%) patients have experienced graft rejection between 60 and 90 days post–transplant. The risk of rejection was especially high in patients with CML and MM. Therefore, they added fludarabine at 30 mg/m^2/day for 3 days before TBI 200 cGy, and they achieved 100% engraftment afterwards [43].

REMAINING PROBLEMS AND FUTURE DIRECTIONS

Is DLI prerequisite for NST? Several studies have shown that DLI may not be necessary as often as originally thought. It has gradually become apparent whether DLI is necessary or not largely depends on the conditioning regimens. Some regimens achieve complete donor chimera within 30 days and other regimens take about 100 days or so [32, 35, 40, 44]. In regimens which are more myeloablative, complete chimera may be achieved sooner, and the possibility to produce mixed chimera is smaller, and DLI is required less often. This is typically seen in regimens containing melphalan or busulfan in addition to fludarabine, such as fludarabine/melphalan or fludarabine/busulfan. On the other hand, fludarabine/CY regimen generally achieves donor–type complete chimera slower [35]. It has also been shown that TBI 200cGy only regimen requires DLI more often than fludarabine /TBI 200cGy regimen [43].

Incidence of GVHD. It has been controversial whether non–myeloablative regimens reduce the incidence of acute GVHD [30–35, 45]. The hypothesis that the cytokine release caused by RRT enhances acute GVHD [46] may favor the decreased incidence of acute GVHD after NST as reported by I. Khouri *et al.* [32]. However, the majority of reports so far have shown that the incidence of acute GVHD after NST is equivalent to that after CST. The incidence of GVHD may be related to the GVHD prophylaxis, DLI, or whether other potent immunosuppressive drugs such as ATG or Campath–1H (anti–CD52 antibody) are given or not [45]. Also, in NST, in order to induce GVL, CsA may be tapered down sooner than in CST. Thus, the incidence of GVHD in NST cannot be compared directly to that in CST.

Immune reconstitution and incidence of infections. The impact of NST on infectious complications or the immune reconstitution has not been addressed very well. M. Mohty *et al*. [47] reported high incidence of viral and bacterial infections in patients who underwent NST. In their report, early viral infections before day 45, especially CMV, occurred at a high rate (65%) and 33% of patients presented with late bacterial infections. In other report, S. Chakrabarti *et al.* [48] demonstrated that high incidence of early and late CMV infections after NST using Campath–1H. However, these are the only reports which indicated increased infections in NST. Other investigators do not report increased incidence of infections.

The main purpose of non–myeloablative conditioning regimen is immunosuppression to achieve engraftment of donor cells rather than myeloablation. Therefore, the immune reconstitution after NST might be different from that after CST. Although the immune reconstitution after CST has been extensively studied, only a few published studies have analyzed that after NST [40, 45–49]. We showed that immune reconstitution after NST using 2–CdA/BU/ATG was slower than that after CST using BU/CY or CY/TBI regimens [40]. However, the incidences of bacterial, fungal, and viral infections were not different between the two groups. A British group also reported the delayed immune reconstitution after NST using Campath–1H [45]. On the other hand, other group reported faster immune reconstitution using fludarabine/cyclophosphamide conditioning for NST. Therefore, the incidence of infections and the rate of immune reconstitution may largely depend on the conditioning regimens, particularly whether they are with or without ATG, and depend on stem cell sources, bone marrow or peripheral blood stem cells.

Indication and optimal regimen. The therapeutic effect of NST depends on GVL/T effect. Therefore, this strategy is only suitable for diseases susceptible to GVL/T effect. The best candidates for this strategy may be patients with myeloid malignancies, particularly CML in chronic phase, MDS, or low–grade lymphoid malignancies. However, it could be indicated for other diseases, particularly benign diseases [50]. It has been controversial whether NST is useful to treat aggressive malignancies, such as leukemias not in remission. Also, it is not known whether lymphoid leukemias can be cured by NST. Recently EBMT reported that 1–year survival of ALL patients after NST was only 15% and significantly worse than other diseases [51].

It has not been decided yet whether the optimal regimens should be decided disease by disease, or one universal regimen is good for most diseases, just like CY/TBI for CST. Although the optimal conditioning regimen is still to be determined, at least the results of Flu/BU/±ATG regimen for myeloid malignancies and Flu/CY regimens for low–grade lymphoma are promising [37, 38].

Non–myeloablative regimens in mismatch transplant. HSCT from an HLA–mismatched, or haploidentical donor has been investigated for patients who lack HLA–matched donors [52–54]. The major obstacles for such technique are GVHD and rejection. To prevent GVHD, techniques to deplete T cells from the graft have been developed. It has been known that GVHD can be prevented almost completely if more than 4–log T cell depletion is done. However, such manipulations further

increase the risk of rejection [55]. To overcome this problem, very intensive conditioning regimens to suppress residual host immune system have been used [52]. More recently, so called mega–dose stem cell transplant has been developed using large dose peripheral stem cell harvest and it further improved the results [53, 54]. However, RRT in addition to infections and relapse are still problematic in this kind of transplant. Recently less intensive and more immunosuppressive conditioning regimens, although still quite myeloablative, are being sought even in haploidentical transplant [53, 54]. The Perugia group, which pioneered in haploidentical transplant, is trying to apply non–myeloablative conditioning regimens for haploidentical transplant using alloreactive NK cells in conditioning [55].

Other groups also try to establish a method to perform haploidentical non–myeloablative transplant. Based on their animal experiments, M. Sykes *et al.* [33] reported a non–myeloablative conditioning regimen for haploidentical transplant consisting of cyclophosphamide at 50 mg/kg for 4 days and thymic irradiation 700 cGy before transplant, with ATG before and after transplant. Five patients with refractory NHL underwent bone marrow transplantation from a haploidentical related donor using this regimen. All evaluable patients showed engraftment in the state of sustained mixed chimerism. This study suggested that non–myeloablative conditioning regimens can induce mixed chimerism even in haploidentical transplant. However, in their study, all five patients developed grade II to III acute GVHD, and 2 died within 7 weeks post–transplant. Thus, haploidentical non–myeloablative transplant is still in its infancy, and at this point it is quite difficult, whether T cell depleted or not. Nevertheless, less–toxic mismatch transplant is ideal for patients who do not have HLA–matched donors and more studies in this direction are warranted.

NST for Solid Tumors. Allogeneic cell effect has been noticed in some solid tumor patients since before [21, 22, 56]. However, because of high TRM in allogeneic HSCT, solid tumors have not been considered as a target for allogeneic cell therapy. Now that allogeneic HSCT can be performed safely using non–myeloablative regimen, it can be used for solid tumors as a form of immunotherapy. R. Childs *et al.* applied this method to solid tumors first [35, 57, 58]. They have published their result of NST for solid tumors, particularly for RCC. They have shown that durable CR can be induced in metastatic RCC. Their results are being reproduced by many groups across the world. So far, RCC is the only tumor which showed unequivocal response to NST, but other tumors are also being tested very actively in many transplant centers.

Future Directions. NST is a powerful tool which may change the whole idea of allogeneic HSCT. Until recently allogeneic transplant has been a very dangerous treatment option which can serve only for life–saving purposes. Now, allogeneic HSCT can be performed safer, and its application extended to solid tumors. In the near future it may be utilized for more benign conditions such as autoimmune diseases. In this case, allogeneic HSCT may be used not only as a life–saving procedure, but also as a tool to improve the quality of life. Although NST has unlimited possibilities to change the therapeutic options in many diseases, it can be dangerous if performed inappropriately. Thus, it is very important to establish a large, high–quality multicenter study soon to determine the role of NST in the treatment scheme of many diseases.

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