

REVIEW

Going straight to the point: intra-BM injection of hematopoietic progenitors

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Intra-BM injection (IBMI) has been used clinically as a technique to deliver medications, blood products and fluids to critically ill children and war-wounded soldiers. Interest in IBMI has now been renewed in the setting of hematopoietic cell transplantation, in particular when umbilical cord blood is the graft source. Clinical studies have not yet unequivocally shown improvement in hematopoietic recovery. However, most intriguing is the observation, both in the clinical setting and in murine models, that the IBMI delivery of hematopoietic grafts and lymphocytes may reduce in the risk of acute GVHD. The underlying mechanism of the reduced risk of GVHD requires further investigation. In this study, we review the rationale as well as the clinical and pre-clinical data that support the study of IBMI as a method to deliver hematopoietic cells.

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Introduction

As a technique, intra-osseous injection was initially developed in humans for the purposes of drug infusion and resuscitation, and has been safely performed in thousands of patients.^{1,2} Intra-BM injection (IBMI) into the sternum was first reported by Josefson³ in 1934 as a method to treat pernicious anemia. As IBMI provides for rapid absorption and easy access, it was subsequently used to deliver blood and other fluids, specially in critically ill children and war-wounded soldiers.⁴ More recently, there has been renewed interest in IBMI as a technique to minimize nonspecific cell loss when transplanting a limited number of hematopoietic progenitor cells (HPCs) and hematopoietic stem cells (HSCs), such as with umbilical cord blood (UCB) grafts.^{5,6}

Allogeneic hematopoietic cell transplantation (HCT) has become an accepted treatment for hematologic malignancies, hereditary immunodeficiencies and storage diseases.^{7–11} The conventional transplant procedure involves conditioning with chemotherapy, with or without radiation therapy to ablate the recipient's BM and immune system, followed by i.v. infusion of hematopoietic cells to repopulate the BM and reconstitute the peripheral blood.^{12–15} However, it has been shown that only a small number of i.v.-infused HPC/HSC will ultimately home to the BM, with most being nonspecifically lost by trapping in organs that do not support hematopoiesis.^{16–18}

UCB has been increasingly used as a source of HPC/HSC for transplantation of children and adults.^{19–21} However, the utilization of UCB for transplantation of larger patients has been impeded by the limited nucleated cell dose available from individual UCB units.^{22–24} To overcome the cell dose limitation, investigators have been studying strategies such as *ex vivo* expansion,^{25,26} administration of multiple unit UCB units^{27–30} and treating the graft with agents that potentially enhance homing to the marrow niche.^{31,32} In view of the limited cell availability in UCB grafts, nonspecific loss of progenitors is of particular importance and has prompted investigators to study the injection of UCB grafts directly into the BM space to improve engraftment. In this review, we summarize the clinical and pre-clinical data on IBMI in the setting of HPC/HSC transplantation in particular with UCB grafts.

Clinical application of IBMI in HSC transplantation

Prospective clinical trials studying IBMI in the setting of allogeneic HPC/HSC transplantation have used different approaches depending on the source of stem cells, volume of the product and supportive measures used.^{5,6,33}

Clinical outcomes after HCT by IBMI

The clinical experience with IBMI is still limited by the relatively low numbers of patients and transplant centers, as compared with the number of HCT procedures performed worldwide and the outcomes of those reported in peer-reviewed journals (Table 1).

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Table 1 Clinical outcomes after hematopoietic cell transplantation by IBMI

Variable	Hägglund <i>et al.</i> ³³			Frassoni <i>et al.</i> ⁵	Brunstein <i>et al.</i> ⁶
Route of injection	i.v. + IBMI	IBMI	i.v.	IBMI	IBMI + i.v.
No. of patients	10	8	20	32	10
Diagnosis (<i>n</i>)		AML (<i>n</i> = 17), CML (<i>n</i> = 14), lymphoma, (<i>n</i> = 3), myeloma (<i>n</i> = 1), CLL (<i>n</i> = 1), MLD (<i>n</i> = 1)		AML (<i>n</i> = 20), ALL (<i>n</i> = 12)	ALL (<i>n</i> = 4), AML (<i>n</i> = 3), NHL (<i>N</i> = 3)
Source stem cells		BM		Single umbilical cord blood	Double umbilical cord blood
Conditioning regimen		Myeloablative		Myeloablative	Myeloablative
TNC dose × 10 ⁷ /kg (range)	23 (11–32)	20 (14–23)	23 (13–29)	2.6 (1.4–4.2)	i.v. 2.0 (1.2–3.0) IBMI 1.8 (1.4–3.4)
Median time ANC recovery, days (range)	19 (16–26)	20 (17–32)	18.5 (15–23)	23 (14–44)	21 (17–49)
Cumulative incidence of ANC recovery (%)	NR	NR	NR	85%, day 44	NR
Median time platelet recovery, days (range)	17 (12–33)	21 (15–30)	18 (12–40)	36 (16–64)	69 (30–272)
Cumulative incidence of platelet recovery (%)	NR	NR	NR	81% day 64	NR
Grade II–IV acute GVHD (<i>n</i>)	0	1	2	Grade II (<i>n</i> = 4) grade III–IV (<i>n</i> = 0)	Grade II (<i>n</i> = 5) grade III (<i>n</i> = 2)
Chronic GVHD (<i>n</i>)	5	5	6	Limited = 4 extensive <i>n</i> = 1	2 out of 7
Graft failure (<i>n</i>)		NR		1/32	1/10
TRM	10% 2 years	25% 2 years	21% 2 years	37%	50%
Survival	60%, LFS 2 years	75%, LFS 2 years	55%, LFS 2 years	45%, OS 1 year	47%, OS 1 year

Abbreviations: HL = Hodgkin lymphoma; IBMI = intra-BM injection; MDS = myelodysplastic syndrome; MLD = metachromatic leukodystrophy; NHL = non-Hodgkin lymphoma; NR = not reported; TNC = total nucleated cell.

In 1998, the first prospective clinical trial studying IBMI in HCT was reported by Hägglund *et al.*³³ In their study, 37 adult patients with hematologic malignancies who received matched or mismatched related BM grafts after myeloablative conditioning were randomized to either i.v. or IBMI delivery of the graft. All patients received GVHD prophylaxis with cyclosporine and MTX. To ensure safety, the first nine patients randomized to IBMI injection received half of the volume by IBMI and the other half by i.v. A subsequent 8 patients received their grafts by IBMI, and another 20 patients received it by i.v. injection. Engraftment was similar in all groups of patients with a median of neutrophil engraftment of 19 days for the IBMI + i.v. route, 20 days for the IBMI route and 18.5 days for the i.v. route. In addition, time to plt recovery, transfusion requirement, GVHD, TRM, disease-free survival and time from transplant to hospital discharge were similar. Only days of total parenteral nutrition and number of patients with bacteremia the first month after transplant were significantly lower in IBMI injections. A remarkable feature of this study was the investigator's attempt to track BM homing by labeling grafts with Technetium (Tc-99m). Curiously, scintigraphy studies showed no differences in the distribution between patients infused by i.v. or IBMI. However, this finding needs to be considered with caution as only five patients were studied (one control, two i.v. and two IBMI). Overall, clinical outcomes were similar among the treatment cohorts (Table 1). This group continues to study the clinical utilization of IBMI and updated results will soon be available (H. Hägglund, personal communication).

Frassoni *et al.*⁵ evaluated the role of IBMI in the setting of single unit unrelated UCB transplantation in 32 patients with acute leukemias. UCB grafts were required to be 4–6/6 HLA-matched to the recipient, and have a nucleated cell dose of at least 1.0×10^7 /kg of recipient body weight. A majority of the patients received myeloablative conditioning and all patients received immunosuppression with cyclosporine, mycophenolate mophetil and anti-thymocyte globulin. The median number of total nucleated cells infused was 2.6×10^7 /kg recipient. Median time to neutrophil and plt recovery was 23 days (range 14–44) and 27 days (range 16–64), respectively. It is noteworthy that there were no differences in hematopoietic recovery between patients receiving bilateral or unilateral IBMI. One patient had graft failure (3%). Chimerism at day 60 was 100% as measured in peripheral blood and in injected and not injected hemi-pelvis. Remarkably, the incidence and severity of acute GVHD was low with 6 of 26 patients at risk developing grade I–II acute GVHD. More recently, the European Group for Blood and Marrow Transplantation and Eurocord presented at the 7th Annual International Umbilical Cord Blood Transplantation Symposium (Los Angeles, CA, USA, June 2009) preliminary results of a matched-pair analysis comparing single UCB unit IBMI vs i.v. Although hematopoietic recovery was similar between the two routes, there was still a reduced risk of acute GVHD.

We recently published our experience with IBMI injection of double UCB.⁶ In this study, 10 patients received one of two UCB units by IBMI. All patients

received the same myeloablative conditioning and immunosuppression regimens³⁴ followed by a double UCB transplant. Units were randomized to IBMI or i.v. injection stratifying by cell dose (larger vs smaller) with the IBMI unit always infused first. Interestingly, in our study neither time to hematopoietic recovery was shorter nor chimerism skewed toward the IBMI unit (four patients from IBMI unit and five patients from i.v. unit). Five patients had grade II acute GVHD and two patients grade III, which was also not different from historical controls receiving both units by i.v. Statistical modeling determined that the chance of observing a significantly shorter time to neutrophil recovery in IBMI patients as compared with historical controls was only 2% with the enrollment of an additional 20 patients; thus, the study was terminated early. In spite of the IBMI being safe in the setting of double UCB grafts, the procedure provided no clinical benefit over conventional i.v. transplantation (Table 1).

Many factors including, but not limited to, diagnosis, conditioning, immunosuppression, source of stem cells and volume of infusion affect clinical outcomes and likely influence the results of each one of these trials. When the UCB studies were planned, the main focus was to improve neutrophil engraftment. However, neither of the UCB studies showed a significant advantage in terms of neutrophil recovery. Several factors can potentially explain the lack of engraftment advantage observed in the UCB studies. One has to consider that after myeloablation the damage to the BM microenvironment may reduce the area that can support HPC/HSC homing and proliferation. It may also be possible that IBMI does not improve seeding efficiency and that the graft simply falls into the systemic circulation. Finally, there is yet limited experience with the IBMI technique, and the current technique may lead to inadequate homing of HPC/HSC either because the infusion is too fast or the volume infused is too large, both of which could result in further damage to the BM microenvironment.

By far the most intriguing observation with respect to IBMI is the reduced risk and severity of acute GVHD observed by Frassoni *et al.*⁵ Although this finding needs to be confirmed in properly designed clinical trials and the mechanism remains to be elucidated, murine models of IBMI have also shown reduced GVHD as discussed below.

As GVHD remains an important cause of morbidity and mortality in HCT, this finding provides a rationale for conducting further studies with an IBMI platform.

The IBMI technique

The technical aspects of the IBMI procedure in each clinical trial are summarized in Table 2.

Hägglund *et al.*³³ first reported on IBMI allogeneic HPC/HSC from related donors. In their study, IBMI was performed using two BM aspiration needles (Adolfo 1.8 × 50 mm, Unimed, Lausanne, Switzerland) that were inserted into each side of the posterior iliac crests under sterile conditions in the patient's room. Before the procedure, patients received vancomycin or cloxacillin 1 g i.v. and sedation with 5 mg morphine hydrochloride and local anesthesia. Cells were resuspended in dextran before infusion in a total volume of 1–1.5 L. In spite of the volume the procedure was generally well tolerated, and there were no procedure-related complications.

More recently, Frassoni *et al.*⁵ reported on IBMI in the setting of single UCB transplantation. In this report, patients were positioned in flank posture. No antibiotic prophylaxis was given prior to the procedure. IBMI was performed under sedation with propofol. After conventional processing,³⁵ UCB units were resuspended in 20 mL of saline solution plus dextran and albumin and aliquoted in four 5-mL syringes for IBMI. Initially, patients received IBMI in a single site in both iliac bones (*n* = 11). Subsequently, IBMI was performed through 4–5 injections limited to one iliac crest at a distance of 2–3 cm from the previous site (Figure 1).

Our group studied IBMI in the setting of double UCB transplantation in which the graft is composed of two partially matched UCB units.⁶ Units were randomized to either IBMI or i.v. administration, which was given as usual. Both UCB were thawed according to standard methods.³⁵ However, the UCB grafts randomized for IBMI were resuspended in a reduced volume (40 mL) to be injected in equal aliquots to both posterior iliac crests. Vancomycin prophylaxis was administered before the procedure and subsequently patients were given bedside sedation with i.v. lorazepam (1–2 mg) and morphine sulfate (1–5 mg). Then under local anesthesia with 1% lidocaine, the UCB unit was injected into a single site on each hemipelvis over approximately 10 min.

Table 2 Graft processing and clinical IBMI technique

	Hägglund <i>et al.</i> ³³	Frassoni <i>et al.</i> ⁵	Brunstein <i>et al.</i> ⁶
Stem cell source	BM	Single UCB	Double UCB
Graft processing	NR	Thaw per Rubinstein <i>et al.</i> ³⁵ Resuspension in saline, dextran and human albumin	Thaw per Rubinstein <i>et al.</i> ³⁵ Resuspension in saline, dextran and human albumin
Sites of injection	Bilateral posterior superior iliac crests one injection per side	Bilateral posterior superior iliac crests (two injections per side) unilateral posterior superior iliac cresta (four injections in one side)	Bilateral posterior superior iliac crests (one injection per side)
Volume injected	1000–1500 mL	20 mL	40 mL
Sedation	Local anesthesia morphine	Propofol	Local anesthesia lorazepam + morphine
Antibiotic prophylaxis	NR	Cloxacillin or vancomycin	Vancomycin
Monitoring	NR	NR	HR, BP, O ₂ sat

Abbreviations: BP = blood pressure; HR = heart rate; IBMI = intra-BM injection; NR = not reported; O₂ sat = oxygen saturation; UCB = umbilical cord blood.

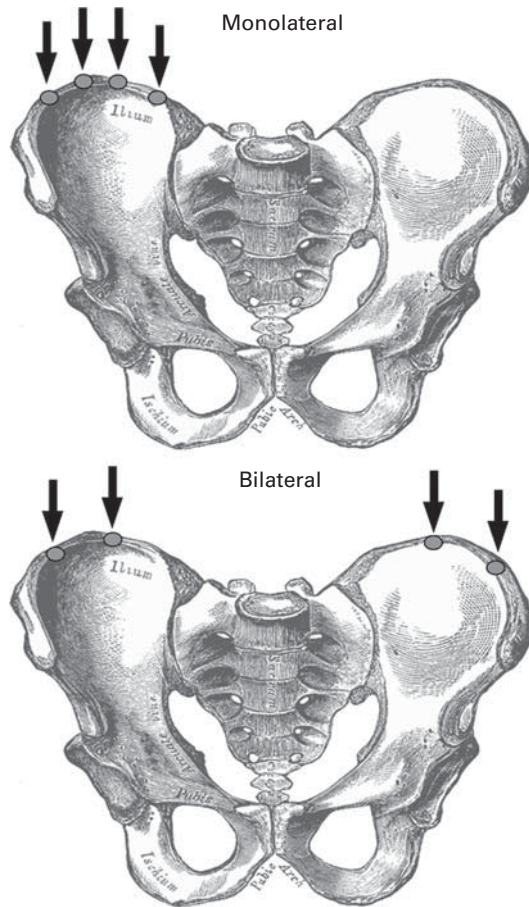


Figure 1 Posterior iliac crest IBMI technique (from Frassoni *et al.*⁵).

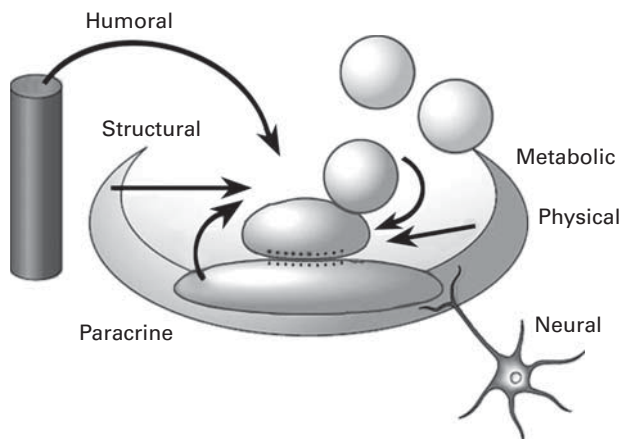


Figure 2 Schematic depiction of factors influencing hematopoietic cell fate in the niche (from Scaden.⁴³).

Although there are variations in the IBMI technique among studies, it seems that the procedure can be performed at the bedside with mild sedation and local anesthesia. Potentially, serious complications such as infections and air or fat embolism could result from IBMI administration. However, in the small number of patients reported, no embolism as manifested by respiratory symptoms or drop in oxygen saturation was observed. So

far the slow infusion of a low volume graft suspension seems to be adequate in preventing embolic complications. Infections, either local or systemic could result from IBMI. Careful handling of the cell products, sterile technique and possibly prophylactic antibiotics before the procedure seems to be effective in preventing serious infections. Fortunately, no serious procedure-related adverse events other than local discomfort/pain were reported in the studies. Currently, IBMI clinical trials have been using conventional marrow aspirate needles. As new clinical trials are planned, new techniques such as the 'marrow-miner'³⁶ may be considered as a method to potentially improve the delivery of the graft into the BM space.

Biology of homing and engraftment

Direct IBMI of hematopoietic grafts has been studied as a strategy to reduce nonspecific loss of progenitors in organs that do not support hematopoiesis and therefore improve hematopoietic engraftment. In murine models, IBMI was shown to facilitate engraftment and possibly reduce the risk of GVHD.³⁷⁻⁴² As most i.v.-injected HPC/HSC are 'trapped' elsewhere and never reach the BM niches, the rationale for the IBMI approach is that it will allow HPC/HSC to reach supportive 'niches' in the BM, wherein they will proliferate.

Interactions between hematopoietic graft and the niche

The concept that hematopoietic progenitors in the BM were localized in tridimensional structures (Figure 2) was first proposed by Schofield in 1978.^{44,45} He called these structures niches. The specific place where the interactions between HPC/HSC and the niche components takes place as well as the real location and number of niches remains to be elucidated.⁴⁷⁻⁴⁹ The interactions between HPC/HSC and the niche microenvironment are very complex and involve a number of adhesion molecules and cell surface receptors.⁵⁰ The best-known axes and receptors involved in HPC/HSC trafficking include CXCR4/SDF-1,^{51,52} VLA4/VCAM-1,^{53,54} CXCR2/GRO β ⁵⁵ and CD26 peptidase⁵⁶ although current data suggest the CXCR4/SDF-1 and VLA4/VCAM-1 axes have the most relevant role in adult donor graft homing to and engraftment in their niches.^{54,57-61} Less is known regarding trafficking and homing of UCB HPC/HSC within the adult niche and the role of different adhesion molecules in UCB homing and engraftment after transplantation. However, there is no evidence to suggest that these mechanisms after UCB transplantation are very different from those of adult donor grafts.⁶²⁻⁶⁴

Murine models of IBMI

I.v. hematopoietic graft injection has low seeding efficiency
Seeding efficiency, defined as the proportion of injected hematopoietic graft cells that ultimately home to the BM space after i.v. hematopoietic graft injection, has been studied in murine models. van Hennik *et al.*¹⁶ showed that the BM seeding efficiency for PBSCs was 5.3%, UCB cells

4.4% and BM 0.8%. Cui *et al.*¹⁷ labeled cells with indium-111, PKH26 or a detectable transgene reporter to track donor BM cells in irradiated and non-irradiated mice; they detected cells not only in the BM but also in the kidneys, liver, lungs, heart and muscle. Transgene detection also was shown in the BM, peripheral blood, spleen, liver, lungs and kidneys. They estimated that 17% of the donor BM cells localize to the BM of non-irradiated recipients, whereas only 3% of cells in irradiated mice. Taken together, these results suggest that there is a relatively low seeding efficiency after i.v. injection of hematopoietic grafts.

Improving seeding efficiency by IBMI injection

Preclinical studies have shown that UCB CD34 + 38- cells administered by IBMI to severe combined immunodeficient mice results in human-derived multi-lineage differentiation of UCB progenitors in the BM of the injected tibia, non-injected tibia^{40,42} and spleen.⁴⁰ Hematopoietic recovery and restoration of T-cell function may be rapidly achieved even across the MHC barriers after IBMI.³⁷ Moreover, the frequency of severe combined immunodeficient mice repopulating cells may be as high as 15-fold as compared with tail vein injection.^{38,40,65} Finally, IBMI was associated with a 15-fold greater seeding efficiency as compared with i.v. transplantation.⁴² This may be particularly important when there is a limited number of HPC/HSC available for transplantation, as in UCB grafts. These murine data show a BM repopulating advantage for IBMI and has now become a standard laboratory technique in the study of HPC/HSC biology. These findings set the basis for clinical trials that studied IBMI in the setting of HCT.

Lower risk of GVHD and IBMI

GVHD is one of the main sources of morbidity and mortality after allogeneic HCT. More recently, there have been reports that in murine models of allogeneic HCT by IBMI there is a decreased incidence of GVHD.^{37,66–68} In a study of the effects of donor lymphocyte infusions, mice that received i.v. DLI rapidly died of severe GVHD, whereas no mice died of GVHD after receiving IBMI-DLI regardless of having previously been transplanted with HPC/HSC by i.v. or IBMI routes. Interestingly, in spite of a similar number of circulating lymphocytes, mice that received IBMI DLI had a higher frequency of splenic T_{regs}, greater numbers of IL-10-producing cells and higher levels of transforming growth factor- β and hepatocyte growth factor in BM stromal cells.^{37,66–68} It has been shown that after IBMI, donor's stromal cells replace host BM stromal cells.³⁷ The ultimate reasons for the decreased incidence of GVHD are not fully understood. It appears that in case this is effective, multiple interactions are involved in this phenomenon including the presence of allogeneic mesenchymal stromal cells in the graft that have the ability to regulate immune response, direct interaction between T_{regs} and BM cells, regulation of immune cells in the BM microenvironment, migration of infused immune cells to different niches depending on the administration route of the cells.

In summary, in murine models, IBMI of allogeneic lymphocytes is associated with lower risk of GVHD. However, the mechanism of GVHD control after IBMI is

still poorly understood and may be related to modifications of T-cell regulation through cytokine production and stromal cell interaction.

The way forward

'Going straight to the point' and injecting hematopoietic progenitors directly into the BM space to improve the chance of cells homing to supportive niches and proliferate there is an attractive idea. However, none of the available clinical studies shows a clear advantage in hematopoietic recovery after IBMI. Little is known regarding the expression and function of adhesion and migration proteins after i.v. injection of a hematopoietic graft, in particular an UCB graft that had been frozen. Preliminary data from our group shows 100% expression of CXCR4 and VLA-4 on thawed UCB 34+ cells. Priming of the graft with reagents that activate adhesion molecule pathways may facilitate homing after IBMI. It is possible that being exposed to plasma cytokines, chemokines and/or other components after i.v. infusion is critical for proper homing of previously frozen UCB. Moreover, future studies will have to address technical questions such as the maximum volume and rate of IBMI to prevent immediate escape from the BM microenvironment into the vascular system. Currently, the most intriguing clinical data are from the study by Frassoni *et al.*⁵ on IBMI in single UCB transplantation showing a reduced risk of acute GVHD. Although clinical studies support the safety of the IBMI technique, a clinical trial randomizing single UCB grafts to be given by the i.v. or IBMI route is required to obtain a definitive answer in terms of GVHD risk and engraftment. Clinical trial design would need to consider: (1) the intensity of the conditioning regimen, (2) the standardization of the conditioning regimen, (3) the use of anti-thymocyte globulin and (4) the use of bedside sedation vs anesthesia support. The answer to each one of these questions may affect transplant center participation and accrual, how applicable the results will be to a broader transplant population and the cost of the clinical trial. On the basis of our experience, we would favor a randomized study using a single myeloablative conditioning regimen, without anti-thymocyte globulin but with bedside sedation. The clinical data available are encouraging, but at this point IBMI can only be recommended in the context of a prospective clinical trial.

Conflict of interest

The authors declare no conflict of interest.

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