Autologous Pancreatic Islet Transplantation in Human Bone Marrow

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The liver is the current site of choice for autologous pancreatic islet transplantation, even though it is far from being ideal. We recently have shown in mice that the bone marrow (BM) may be a valid alternative to the liver, and here we report a pilot study to test feasibility and safety of BM as a site for islet transplantation in humans. Four patients who developed diabetes after total pancreatectomy were candidates for the autologous transplantation of pancreatic islet. Because the patients had contraindications for intraportal infusion, islets were infused in the BM. In all recipients, islets engrafted successfully as shown by measurable posttransplantation C-peptide levels and histopathological evidence of insulin-producing cells or molecular markers of endocrine tissue in BM biopsy samples analyzed during follow-up. Thus far, we have recorded no adverse events related to the infusion procedure or the presence of islets in the BM. Islet function was sustained for the maximum follow-up of 944 days. The encouraging results of this pilot study provide new perspectives in identifying alternative sites for islet infusion in patients with type 1 diabetes. Moreover, this is the first unequivocal example of successful engraftment of endocrine tissue in the BM in humans. Diabetes 62:3523–3531, 2013

Islet transplantation represents an important therapeutic option for adults with unstable type 1 diabetes (TID) who, despite their best efforts, have wide and unpredictable fluctuations of glucose levels or who are no longer able to sense hypoglycemia with an increased risk of acute and chronic complications of diabetes and a significant worsening of quality of life (1). The liver is the current site of choice for autologous islet transplantation, even though it is far from being ideal because of immunologic (2–4), anatomic (5), and metabolic (6–8) factors leading to significant early graft loss. Along with preexisting and transplant-induced autospecific and allospecific immune responses (9), a nonspecific response, predominantly mediated by innate inflammatory processes related to mechanics and site, plays a major role in the loss of islets and islet function after transplantation in the liver (4,10–13).

As reported by many studies, an estimated 60–80% of the transplanted islet mass is lost within hours or days after intrahepatic islet infusion (12,14,15), mainly because of immediate blood-mediated inflammatory reaction (16), thrombosis (11,17), and hepatic tissue ischemia (18,19) with release of liver enzymes (20,21). Furthermore, from a clinical point of view, the process of islet infusion in the liver is associated with an increase of portal pressure proportional to the islet mass (22), thus limiting the total islet mass to be transplanted (23). Recognizing these problems has increased the interest in the search for alternative sites for islet transplantation to avoid liver-specific problems (24). Despite the success of experimental islet transplantation in mouse models using different sites, the results of only a few of those studies were applied in large animal models and none was applied in human models.

Bone marrow (BM) may be an alternative site for autologous islet transplantation because it offers a protected and extravascular, although well-vascularized, microenvironment (25). Because of BM broad distribution and easy access, islet infusion in the BM may overcome technical limitations and reduce complications of islet infusion in the liver through the portal vein (24). In a recent preclinical study, we tested whether syngeneic pancreatic islets could engraft in the BM of diabetic mice by comparing survival, function, and morphology of syngeneic islets infused in the BM or in the liver (26). Islets engrafted efficiently in the BM of diabetic mice and for >1 year posttransplantation, the glucose metabolism of those animals was similar to that of nondiabetic mice. Furthermore, mice with islets infused in the BM were more likely to reach euglycemia than mice with islets infused in liver. Islets in the BM showed a compact morphology with a preserved ratio between α-cells and β-cells, with only marginal effects on bone structure. Moreover, the presence of islets in the BM did not affect hematopoietic activity, even when this function was strongly upregulated in response to virus-induced BM aplasia. Based on these results, we were granted approval to use this approach in humans, and we performed a pilot study in which patients with diabetes and hepatic contraindications for liver islet...
In-hospital AEs

Islet isolation and transplantation

Reimplantation procedures already approved for intraportal infusion and islet isolation, and to proceed with the second choice (16°C plus 1% penicillin–streptomycin and 1% glutamine (Lonza, Basel, Switzerland), Ficoll (Biochrom, Berlin, Germany) gradient. Puriﬁcation was performed under general anesthesia. Surgeons included total pancreatectomy or complete pancreatectomy. If a tumor was resected, then 1 cm of the pancreatic remnant in the proximity of the pancreatic margin was resected and sent to the pathologist to conﬁrm that margins were not inﬁltrated. Pancreas remnants were immediately ﬂushed with cold preservation solution (University of Wisconsin) and brought to the islet isolation facility. Islets were isolated and puriﬁed according to the automated method described by Ricordi (27), with local modiﬁcations. Brieﬂy, the pancreatic duct was catheterized and distended by intraductal injection of a cold collagenase solution. After digestion at 37°C in a modiﬁed Ricordi chamber, islets were puriﬁed and washed on a Cobe 2991 using continuous Hanks’ balanced salt solution–Ficoll (Biochrom, Berlin, Germany) gradient. Puriﬁed islet fractions were pooled in an 150-mm diameter (islet equivalents [IEQ]).

Intraoperative collection of the pancreas for islet isolation and puriﬁcation. Open surgery was performed under general anesthesia. Surgery included total pancreatectomy or complete pancreatectomy. If a tumor was the reason for pancreatic resection, then 1 cm of the pancreatic remnant in the proximity of the pancreatic margin was resected and sent to the pathologist to conﬁrm that margins were not inﬁltrated. Pancreas remnants were immediately ﬂushed with cold preservation solution (University of Wisconsin) and brought to the islet isolation facility. Islets were isolated and puriﬁed according to the automated method described by Ricordi (27), with local modiﬁcations. Brieﬂy, the pancreatic duct was catheterized and distended by intraductal injection of a cold collagenase solution. After digestion at 37°C in a modiﬁed Ricordi chamber, islets were puriﬁed on a Cobe 2991 using continuous Hanks’ balanced salt solution–Ficoll (Biochrom, Berlin, Germany) gradient. Puriﬁed islet fractions were pooled in an 150-mm diameter (islet equivalents [IEQ]).

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Islet transplantation. Islets were transferred back to the operating room without time in culture (n = 2) or were infused after being in culture for a maximum of 48 h (n = 2). The intra-BM infusion was performed after the same procedures used for the BM administration of cord blood cells in patients with acute leukemia (28). Brieﬂy, a needle for BM aspiration (14 gauge) was inserted into the left superior-posterior iliac crest under local anesthesia and the islet suspension (1:2.5 ratio of tissue to Ringer’s lactate solution) was infused. The entire injection procedure lasted 9–15 min. All islet

autotransplantation (IAT) received a single intra-BM islet infusion in the iliac crest.

RESEARCH DESIGN AND METHODS

Pilot study. A pilot study to test feasibility and safety of BM as a site for IAT in humans was approved by the Italian Transplant Regulatory Agency (Centro Nazionale Trapianti) and by the Institutional Review Board of the Ospedale San Raffaele in August 2009 (NCT01346098). We were granted permission to perform islet infusion in the BM of the iliac crest in patients with contra-indications for intraportal infusion ("second choice"). The Institutional Review Board asked us to follow-up for indications for intra-BM infusion in the same patients. In four patients after total pancreatectomy. All patients signed informed consent before enrollment in this study.

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antibodies were used: cytokeratin 8, eosin, Giemsa, and silver impregnation. For immunohistochemistry, the following antisera were employed: glucagon (polyclonal Rb [1:50]; Novocastra); somatostatin (polyclonal Rb [1:50]; Novocastra); CD34 (QBEND/10 [1:100]; Novocastra); insulin (2D11-H5 [1:100]; Novocastra); Tyne, U.K.); chromogranin A (LK2H10 [1:600]; Biogenex, San Ramon, CA); and pancreatic polypeptide (polyclonal Rb [1:50]; Novocastra); and pancreatic polypeptide (polyclonal Rb [1:50]; Novocastra). Sections were counterstained with hematoxylin. Also, 1:600  

Preparations used had negative Gram stain results immediately pretransplantation and negative microbial culture results at the time of infusion. Perioperative monitoring and follow-up. A target glucose level of ~100 mg/dL using intensive capillary glucose monitoring and continuous regular intravenous infusion of insulin plus intermittent insulin injections was maintained in all patients for at least 5 days. Adverse events (AEs) related to the procedure were recorded and classified according to the terminology criteria for AEs in Trials of Adult Pancreatic Islet Transplantation version 4.1 (16 July 2008) (http://www.isletstudy.org/CITDocs/CIT-TC/CAE/4204.pdf). Serious AEs were reviewed by an Independent Data Safety Monitoring Committee. Follow-up outpatient visits were scheduled at 1, 3, 6, and 12 months and every year after hospital discharge. We updated medical history and performed a physical examination during each visit. In case of malignancy, adjuvant chemotherapy or radiotherapy was administered when indicated, and computed tomography scan was performed and blood neoplastic markers were measured every 3 or 6 months, according to the risk of recurrence. 

β-Cell function. β-Cell function was assessed by measuring fasting C-peptide, HbA1c, glycemia, average daily insulin requirement, glucagon, C-peptide, and insulin levels during (basal fasting and 10–120 min) an arginine test or a mixed-meal tolerance test. Laboratory and stimulation tests were performed as previously described (29). 

BM aspiration and biopsy. We planned to perform BM aspiration and biopsy of both iliac crests (the one infused with islets and the contralateral one) at 1, 3, and 12 months after islet infusion. Histology and fluorescence-activated cell sorter analyses of BM leukocyte populations were performed as previously reported (30). Histopathological analysis was performed on Bouin solution-fixed, paraffin-embedded BM biopsy specimens. Basic stains included hematoxylin-eosin, Giemsa, and silver impregnation. For immunohistochemistry, the following antibodies were used: cytokeratin 8–18 (5D3 [1:200]; Novocastra, Newcastle-upon-Tyne, U.K.); chromogranin A (LK2H10 [1:600]; Biogenex, San Ramon, CA); CD34 (QBEND/10 [1:100]; Novocastra); insulin (2D11-H5 [1:100]; Novocastra); glucagon (polyclonal Rb [1:50]; Novocastra); somatostatin (polyclonal Rb [1:600]; Novocastra); and pancreatic polypeptide (polyclonal Rb [1:500]; Novocastra). For all antigens the retrieval procedure was performed with ER2 solution (pH 9). Reactions were developed using an automated immunostainer (i6000; Biogenex, San Ramon, CA). Sections were counterstained with hematoxylin. Also, 1–2 mm of the biopsy sample was cut before fixation and preserved in RNAlater (Qiagen, Hilden, Germany). Tissue was then disrupted with a homogenizer (Tissue Ruptor; Qiagen) and total RNA was extracted using mirVana Isolation Kit (Applied Biosystem, Foster City, CA). We obtained good-quality RNA from all the samples, with a mean yield of 16.2 ± 11.3 μg of total RNA. Reverse-transcription was performed using 5 μg total RNA with SuperScriptIII (Roche, Basel, Switzerland). Real-time quantitative RT-PCR was performed to study the expression of selected genes with TaqMan Gene Expression Assays (Applied Biosystems). Results were analyzed with RT2 Profiler PCR Array software (Qiagen). 

RESULTS 

Patients and surgery. Four patients with contra-indications for liver IAT received an intra-BM islet infusion in the iliac crest. Patient characteristics are summarized in Table 1. Patient 1 underwent a complete pancreatectomy 34 days after pancreaticoduodenectomy because of uncontrolled bleeding from the gastroduodenal artery caused by a grade C pancreatic fistula (31). Liver IAT was contraindicated because of portal vein thrombosis. Patients 2, 3, and 4 were initially scheduled for pancreaticoduodenectomy, but the procedure was changed to total pancreatectomy at the time of surgery because the pancreatic anastomosis was deemed to be at high risk for leakage. Liver IAT was contraindicated because of the high risk of complications.
during percutaneous cannulation of the portal vein in patients 2 and 3, whereas patient 4 had diffuse small liver metastases.

**Follow-up: AEs related to BM islet infusion and patient survival.** AEs that occurred during hospitalization are reported in Table 1. A total of 21 AEs were recorded; 18 were mild to moderate grade 1–2 AEs, 2 were serious grade 3 AEs, and 1 was grade 5 (death). None of the AEs was related to the islet infusion procedure. A detailed description of the grade 5 AE is provided. Patient 1 was on hemodialysis and underwent pancreaticoduodenectomy because of a mass of the pancreatic head. Massive bleeding from the gastroduodenal artery occurred on day 12 after surgery because of leakage of the pancreatic anastomosis. The bleeding was treated with selective endovascular embolization. The complete pancreatectomy and subsequent IAT were performed 18 days after embolization because of massive bleeding (second instance of bleeding). The patient died of bleeding (third instance of bleeding) on day 4 after IAT. The postmortem examination documented the rupture of the gastroduodenal artery and the event was considered unrelated to the intra-BM islet infusion by the Independent Data Safety Monitoring Committee. Patient 2 (pancreatic ductal carcinoma) received adjuvant chemotherapy and had sustained insulin production, although all required exogenous insulin injections during follow-up. At the last metabolic follow-up (mean follow-up, 545 ± 369 days), they had stable HbA1c (7.0 ± 0.7% [53 ± 8 nmol/mol]) while using exogenous insulin treatment (0.37 ± 0.2 IU/kg/day) and showed sustained endogenous insulin secretion (fasting C-peptide, 0.127 ± 0.097 nmol/L; stimulated C-peptide, 0.196 ± 0.057 nmol/L). Moreover, after intravenous arginine stimulation we observed an insulin secretory response, which documented that regulated insulin secretion was restored with islet transplantation in the BM (Supplementary Fig. 1). Because all patients had undergone total pancreatectomy, C-peptide detection unequivocally proves the successful engraftment of pancreatic islets in the BM.

**Follow-up: primary graft function, glycemic control, and graft survival.** In all recipients, islets engrafted successfully as shown by circulating C-peptide levels after islet transplantation (Fig. 1). Patient 3 gained insulin independence for 1 week after islet infusion but it was subsequently lost. Patients 2, 3, and 4 maintained good metabolic control and had sustained insulin production, although all required exogenous insulin injections during follow-up. White blood cell, erythrocyte (red blood cells), and platelet counts were not affected by the presence of islet in the BM. Recovery of peripheral blood cells after adjuvant cytotoxic chemotherapy was efficient and did not differ from what was expected in these conditions. At the last follow-up, white blood cell counts (7.4 ± 1.99 x 10⁹/L), leucocyte formula (neutrophils, 60 ± 14%; lymphocytes, 30 ± 8%; monocytes, 8.3 ± 0.5%), and platelet counts (230 ± 12 x 10⁹/L) were within the normal range.
TABLE 2
BM biopsy morphology

|                  | Patient 2 | Patient 3 | Patient 4 |
|------------------|-----------|-----------|-----------|
|                  | 1 month   | 3 months  | 12 months | 1 month   | 3 months  | 12 months |
| Site             | L 2:3     | 1:3       | 1:3       | 1:1       | 1:3       | 1:5       |
|                  | R 2:3     | 1:3       | 1:3       | 1:1       | 1:3       | 1:4       |
| B cells (CD20+)  | Nor       | Nor       | Nor       | 2:3       | 1:6       |           |
| Myeloid/erythroid ratio | L 3:1     | 1:1       | 3:1       | 3:1       | 4:1       | 3:1       |
|                  | R 3:1     | 1:1       | 3:1       | 3:1       | 3:1       | 3:1       |
| Myeloid component | L Red     | Red       | Red       | Inc       | Inc       | Red       |
|                  | R Red     | Red       | Red       | Inc       | Inc       | Nor       |
| Erythroid series maturation | L Nor    | Mild right shift | Mild left shift | Nor       | Nor       | Mild left shift |
|                  | R Nor     | Mild right shift | Mild left shift | Nor       | Nor       | Mild left shift |
| Myeloid series maturation | L Red     | Red       | Red       | Inc       | Nor       | Red       |
|                  | R Red     | Red       | Red       | Inc       | Nor       | Red       |
| Erythroid component | L Nor    | Mild right shift | Mild left shift | Nor       | Nor       | Mild left shift |
|                  | R Nor     | Mild right shift | Mild left shift | Nor       | Nor       | Mild left shift |
| Lymphoid component | L Nor    | Nor       | Nor       | Reactive lymphoid aggregates with a predominance of | Nor       | Nor       |
|                  | R Nor     | Nor       | Nor       | B cells (CD20+) | Nor       | Nor       |
| Lymphoid series maturation | L Nor    | Mild right shift | Mild left shift | Nor       | Nor       | Mild left shift |
|                  | R Nor     | Mild right shift | Mild left shift | Nor       | Nor       | Mild left shift |
| Megakaryocyte numbers | L Nor    | Red       | Nor       | Nor       | Nor       | Red       |
|                  | R Nor     | Red       | Nor       | Nor       | Nor       | Red       |
| Megakaryocyte morphology | L Nor    | Hypolobate | Nor       | Hypolobate | Nor       | Naked nucleus |
|                  | R Nor     | Hypolobate | Nor       | Hypolobate | Nor       | Naked nucleus |
| Bone              | L Nor     | Nor       | Nor       | Nor       | Nor       | Nor       |
|                  | R Nor     | Nor       | Nor       | Nor       | Nor       | Nor       |

Inc, increased; L, graft-bearing iliac crest; Nor, normal; R, not graft-bearing iliac crest; Red, reduced.

normal range in all patients. Expected mild anemia associated with exocrine pancreatic insufficiency was present (red blood cells, 4.1 ± 0.2 10¹²/L).

BM biopsies (both graft-bearing and contralateral iliac crests) and aspirates (contralateral iliac crest only) were planned at 1, 3, and 12 months after IAT. A total of eight biopsies were performed in patients 2, 3, and 4. Moreover, postmortem BM tissue at day 4 after IAT was obtained from patient 1 (Fig. 2). Myeloid-erythroid ratios, hematopoietic series maturation, lamellar bone, and reticulin fiber content (with the exception of the areas in which pancreatic islet were detected) were within physiological range in all patients at all time points. Overall cellularity showed changes during follow-up (Table 2), with a trend toward slightly hypocellular marrow. This was expected considering that patients 2 and 4 displayed slight maturation defects involving both erythroid and myeloid lineages. Reactive lymphoid aggregates were observed in patient 3. None of these findings was specific of the graft-bearing BM. The morphologic and flow cytometry analyses of the available BM aspirates did not show specific unexpected changes during follow-up (Supplementary Table 1).

The presence of cytokeratin or chromogranin A–positive cell aggregates within the graft-bearing BM was detected by immunohistochemistry in four out of eight biopsies performed (Table 3). Cytokeratin-positive and chromogranin A–positive components were generally surrounded by a stromal reaction that was focal and close to the bone lamellae (Figs. 2 and 3). All four islet cell types, e.g., insulin, glucagon, somatostatin, and pancreatic polypeptide cells, were present in the BM 1 year after islet infusion (Fig. 3). Moreover, the presence of CD34-positive endothelial cells inside and around the islets was suggestive of islet neovascularization.

Molecular analysis revealed increased expression of genes related to endocrine pancreatic cells in six out of eight biopsy samples (Table 3). In these six cases, the graft-bearing BM showed 8,077-fold, 21,318-fold, and 41-fold increases in insulin, glucagon, and chromogranin A mRNA levels compared with the contralateral BM in the same patient. Moreover, in addition to these markers of endocrine fully differentiated pancreatic cells, we analyzed the expression of transcription factors involved in normal pancreatic development and differentiation. As shown in Fig. 4, the expression of transcription factors of endocrine-committed progenitor cells and mature β-cells, such as Pdx1, Nkx2.2, Nkx6.1, NeuroD/beta2, and Pax6, was higher in the graft-bearing iliac crest BM than in contralateral BM, whereas the expression of transcription factors expressed in early pancreatic precursors and late-stage pancreatic bud precursor cells, such as Ptf1alpha, Onecut, and Ngn3, was not different.

Follow-up: BM imaging. On MRI T2-weighted images, the site of islet infusion at the posterior-superior iliac spine appeared as a small hypointense area inside the normal hyperintense signal of the iliac BM (Fig. 5). This hypointense area did not significantly change over time. One year after islet infusion, bone structure was unaffected by the presence of the infused islets. Moreover, a gadolinium-enhanced MRI perfusion study did not reveal areas of anomalous enhancement surrounding the site of islet infusion. Computed tomography scans showed the presence of small calcified spots at the site of islet infusion.

DISCUSSION

To the best of our knowledge, our article represents the first unequivocal example of successful engraftment of endocrine tissue in BM. Our recent preclinical studies in mice (26) showed that the amount of success and the timing of reverse hyperglycemia were superior after islet infusion in the BM than in the liver. Therefore, we
translated our preclinical findings to a proof-of-concept pilot phase 1 study in which four patients with pancreateogenic diabetes and hepatic contraindications for receiving islet transplant in the liver received a single intra-BM islet infusion at the iliac crest. This study has limitations, intrinsic to all phase 1 studies, such as the limited number of patients enrolled, the nonrandomized design, and the absence of a control group. Furthermore, because of the heterogeneity of the pancreatic disease of the patients enrolled in the study, the results cannot be directly compared with those observed with autologous islets transplanted to the liver.

Although conducted with a small number of patients, this pilot experience has generated some important data. First, we were able to document the feasibility and the safety of this approach for islet infusion. The direct islet infusion in the BM was performed according to the same procedure used in our institution for the administration of cord blood cells in patients with acute leukemia (28). The procedure was easy and reproducible and, thus far, we have recorded no AEs related to the islet infusion in the iliac crest. Islets in the BM did not affect hematopoietic activity, even when it was strongly upregulated in response to adjuvant chemotherapy. Moreover, bone structure and trabecular compartments were not significantly affected by the presence of the infused islets.

Second, and equally important, we demonstrated the presence of insulin-producing cells in BM biopsy specimens, and this presence was associated with detectable levels of fasting and stimulated circulating C-peptide. This implies that the BM microenvironment is able to support islet revascularization and function, providing an appropriate oxygen tension, a suitable pH, clearance of toxic metabolites, and access to nutrients. Our study unequivocally proves that islets can successfully engraft in the BM, and it provides the rationale for testing the BM as a site for islet infusion in patients with T1D selected to receive allotransplantation of pancreatic islets. A phase 2 trial in which patients with T1D will be randomized to receive islets either in the liver or in the BM is currently ongoing at our institution (NCT01722682). This trial will allow us to assess whether islet infusion in the BM may improve the outcome of an islet transplant infused in the liver, as measured by glycemic control.

Third, we have shown that islet sampling in the BM is highly feasible. Because BM is an easily accessed and well-confined site ideal for serial multiple biopsies, we have the unique opportunity to monitor, over time, different markers of engraftment or survival of islets directly at the site of islet infusion. Although sequential biopsies are often used to monitor acute or chronic events in solid organ transplants, no study in humans has ever attempted to harvest liver biopsy samples and monitor the fate of islets infused via the portal vein. This is because islets are rapidly and randomly scattered throughout the liver after intraportal infusion, and subsequent liver needle biopsies would have limited value because of the low yield of islets in biopsy samples (32). In contrast, this study has shown that islet sampling in BM is feasible and allows the histological and immunohistochemical analyses of the transplanted tissue and surrounding BM and the real-time quantitative PCR analyses of messenger RNAs. Molecular analysis allowed us to detect the presence of endocrine-specific proteins with higher sensitivity than with immunohistochemical analysis, and to search for the expression of transcription factors of pancreas development (33) that may be markers
of the proliferation of pancreatic precursor cells. Notably, this monitoring strategy has the potential not only to help our mechanistic understanding of the various harmful events affecting islet graft survival but also to allow the identification of biomarkers for the prompt treatment of such events, hopefully leading to improved islet survival and prolonged insulin-independence of transplant recipients.

Advances in islet transplantation research have led to remarkable improvements in clinical outcomes. During the 2007–2010 period, the reported insulin independence rates were 66% at 1 year, 55% at 2 years, and 44% at 3 years (1). To achieve these results we still need to infuse a large number of islets because almost half of the islets infused in the liver die during or soon after transplantation (12). Since the first report of successful pancreatic islet transplantation to reverse hyperglycemia in diabetic rodents, there has been great interest in identifying the optimal site for implantation. The liver was suggested by Lacy and colleagues (34) based on their experience with a rat model of diabetes, and the first case of insulin independence in a patient with T1D after infusion of islets through the portal vein consecrated the liver as the site of choice for islet transplantation in humans (35). Although the liver remains the most frequently used site, several alternative sites for islet transplantation (pancreas, gastric submucosa, genitourinary tract, muscle, omentum, kidney capsule, anterior eye chamber, testis, and thymus) have been explored in experimental animal models with the goal of improving engraftment and minimizing surgical complications. The results of a few of these studies were translated in large animal models and only rarely in human models (36). However, at this time the ideal site for islet implantation has not yet been identified. The BM has the potential for being an alternative site for islet transplantation because of its protected and extravascular, although well-vascularized, microenvironment. If the results of this pilot study are confirmed by randomized clinical trials, then islet

**FIG. 3. BM morphology at 1 year posttransplantation.** Photomicrographs of BM biopsy samples from patient 3. A: Histological appearance (left panel, magnification 200×; right panel, inset of left panel, magnification 400×) of transplanted tissue. Hematoxylin and eosin staining. B: Representative immunohistochemical stainings (magnification 200×) with anti-insulin, antiglucagon, antichromogranin A, antisomatostatin, antipancreatic polypeptide, and anti-CD34 antibodies.

**FIG. 4. BM biopsy molecular assay.** Scatter plot comparing the normalized expression (2^(-ΔΔCt)) of every gene with graft-bearing and contralateral BM. The central line indicates unchanged gene expression and the dashed line indicates the boundary (fold regulation cutoff set = 3). Expression changes greater than the selected boundary (filled circles) and expression changes smaller than the selected boundary (gray circles) are shown.
infusion in BM may become an ambulatory procedure of limited invasiveness, well-suited for repeated infusions, with the possibility of performing repeated graft biopsies with a low-risk and simple procedure. Moreover, BM also may be an appropriate site to test, in future trials, the impact of coinjecting islets with cells of putative immunomodulatory capacity, such as T-regulatory cells (37) or mesenchymal stem cells (38), that could help prevent or minimize detrimental autoimmune and alloimmune responses. T-regulatory cells or mesenchymal stem cells would benefit from the close proximity of islet antigens, the target of their tolerogenic function, and from the favorable microenvironment of the BM. The demonstration that pancreatic islets can efficiently engraft in BM holds the potential to revolutionize the field of islet transplantation, thus allowing new lines of research with significant clinical impact on the treatment of diabetes and, more generally, on cell therapy.

ACKNOWLEDGMENTS

This study was supported by the Italian Minister of Health (Ricerca Finalizzata RF-2009-1409891) and by the European Union (HEALTH-F5-2009-241883-BetaCellTherapy).

No potential conflicts of interest relevant to this article were reported.

P.M. and G.B. managed patients. M.P. performed the histopathological analysis of the bone marrow. R.N. performed islet isolations. V.S. performed the molecular analysis of the bone marrow biopsy samples. R.M. and A.M. performed islet isolations. M.S. reviewed and edited the manuscript and contributed to discussion. A.E. performed magnetic resonance imaging studies. J.P. developed the intra-bone marrow islet infusion method and performed the transplantsations. E.C. conceived the intra-bone marrow strategy. C.M. and M.B. developed the intra-bone marrow islet infusion method and performed the transplantsations. A.D.M. performed magnetic resonance imaging studies. C.S. reviewed and edited the manuscript and contributed to the discussion. C.D. performed the histopathological analysis of the bone marrow. F.C. developed the intra-bone marrow islet infusion method and performed the transplantsations. A.S. reviewed and edited the manuscript and researched data. L.P. conceived the intra-bone marrow strategy, developed the concept, designed the experiments, wrote the manuscript, promoted the study, and researched data. L.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

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