



[haematologica reports]
2006;2(10):119-121

In utero transplantation for genetical diseases

F. PORTA*
F. BOLDA
E. BAGGIO
S. BERTA
R. BAFFELLI
M. RIDELLA
S. GUARISCO
V. GRASSI
D. DE MARTIS
K. TETTONI
L.D. NOTARANGELO
A. LANFRANCHI

Centro di Terapia Cellulare e Genica
delle Oncoemopatie Infantili
"A.Nocivelli", Spedali Civili, Brescia,
Italy *

Bone marrow transplantation (BMT) is the therapy of choice for several genetic diseases of blood. Since 1968 there is evidence that stem cell transplantation can cure diseases characterized by lack or miss-function of blood cells. Infact the first correction of a genetic disease by stem cells infusion has been realized in a child affected by a form of immunodeficiency, severe combined immunodeficiency (SCID), in a child who lacked T cells.

The Pediatric Department of the Spedali Civili di Brescia is the Italian referent center for the treatment by bone marrow transplantation of children affected by primary immunodeficiencies. More than 340 transplants have been realized since 1990 when the centre started the activity. BMT is conventionally realized from matched related family donors, nevertheless, in genetical diseases, the chance that an affected child could have a matched family donor has been calculated in 1/20. Therefore in recent years the transplants are performed from alternative donors. When a matched family donor was unavailable, a transplant from one of parents have been realized. In a consecutive series of 100 transplants from a partially matched family donor the survival didn't exceed 60%. Therefore a great effort has been made to increase the number of the volunteer donors enrolled within the International Registry of Bone Marrow Donors. In 1991 the first transplant from a matched unrelated donor (MUD) within our country in a child affected by Chediak-Higashi syndrome has been realized in our institution. The success of the procedure allowed enrollment of several other patients, so that now MUD transplants is the therapy of choice in children who lack a matched family donor. Beside the efforts to ameliorate the chance of survival of the affected children, the studies conducted on the families allowed the fine characterization of the molecular defects that prone to the diseases. Therefore prenatal diagnosis is now an option, since most genetic defect under-

lying the most common immunodeficiencies are known.

Prenatal diagnosis opens the option, whenever the child is affected, to interrupt the pregnancy, but, as well, whenever a cure is available, to face the opportunity to cure the child after birth. In primary immunodeficiencies, however, once the child is born the chance to contract an infection is very high. Therefore in 1996, both in USA within the State of California and in Italy in Brescia, two mothers asked the doctors, once they had the information the foetus was affected by a severe form of immunodeficiency, why it was not possible to treat the disease by stem cells during pregnancy.

As a consequence, in our institution since 1996 a clinical project on prenatal treatment of genetic diseases by injection of stem cells has been started.

The clinical experience of Department of Pediatrics, Brescia, Italy

The first case of in utero transplant (IUT) has been realized in a foetus affected by a form of SCID characterized by lack of both T and NK cells (Figure 1). Namely this is the form that is easily transplantable since the recipient cannot reject the graft. At the fifth month of gestation, purified CD34 marrow stem cells from the father have been injected intraperitoneally in the foetus. At birth the child had normal numbers of both T cells and NK cells reconstituted from the marrow of the father. The child is now 11 years old is alive and well (Figure 2).

Up to now we have treated 5 children affected by different forms of severe combined immunodeficiency (SCID). Among this group of patients three were the different forms of SCID: SCID T-B+NK-; SCID T-B-NK+; Omenn syndrome (with presence of non functional T cells). If the clinical result of prenatal graft in the SCID T-B+ fetuses is satisfactory with good engraftment and immunological reconstitution, more difficult is engraft and reconstitute fetuses who are affected by forms of SCID with presence of NK cells. The clinical protocol that

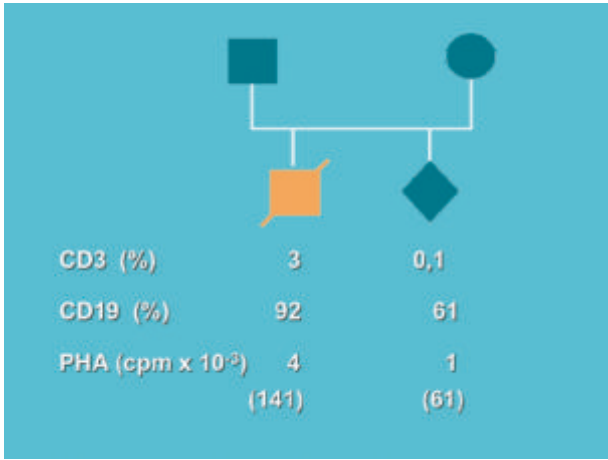


Figure 1.

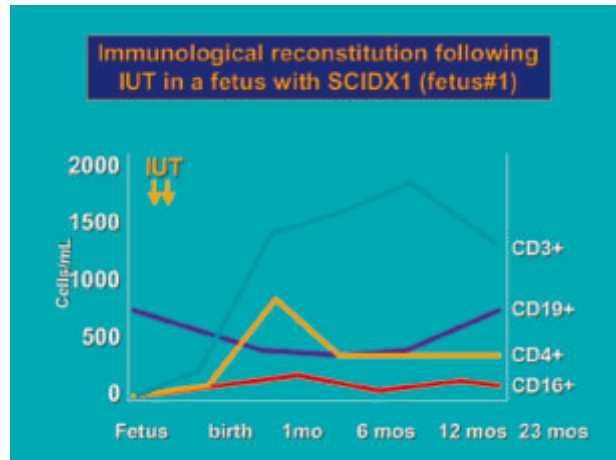


Figure 2.

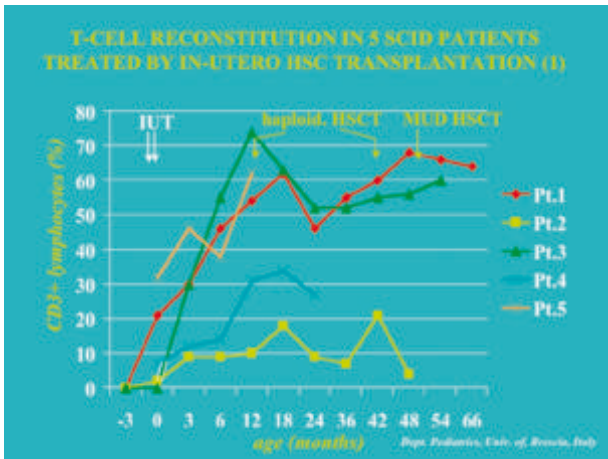


Figure 3 [left column].

we have applied in 4 cases consisted of positive selection of CD34 of an adult sibling haploidentical donor and a subsequent double intraperitoneal injection in the fetus at the fifth month of gestation, has been modified in one SCID with NK cells. In fact in this case after the first injection that consisted exclusively of CD34 positive cells, the second injection has been realized by a coinfusion of donor CD34 and expanded stromal cells.

In one case of SCID with non-functional T cells and presence of activated NK cells (Omenn syndrome),

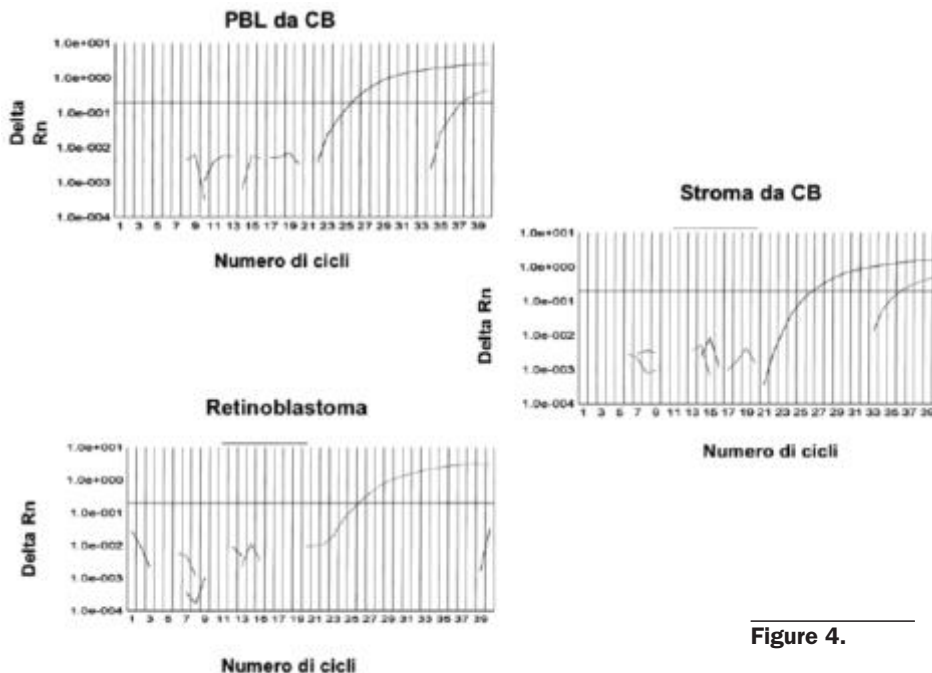


Figure 4.

since engraftment is particularly difficult even in the post-natal setting, the mother has been chosen as a donor. Engraftment occurred, despite stem cells derived from peripheral blood (Figure 3).

The overall experience in SCID, demonstrated that the procedure didn't expose the foetuses neither to interruption of pregnancy nor to immunological reactions after stem cell transplant, graft versus host reaction (GVHD).

Recently it has been demonstrated that stem cell transplantation can correct genetical diseases other than SCID such as metabolic diseases, bone diseases (osteopetrosis, osteogenesis imperfecta). Bone marrow transplantation has been realized in 10 children affected by osteogenesis imperfecta (OI) both from matched family and volunteer donors. The procedure demonstrated to ameliorate the quality of life of the affected children with increase of the height rate, reduced number of fractures.

In utero protocol has been therefore applied in 2 cases of prenatal OI using the mother as a stem cell donor with the aim to correct the diseases prenatally. The diagnosis came after ultrasound investigation at the 20th week of gestation (femur fracture 1 one case, multiple in the second). In both cases maternal CD 34 cells were injected together with a suspension of enriched non-expanded non-haematopoietic marrow stem cells.

In the first case the child is 15 months old, has a mild form of OI, the bone trophine do not demonstrate

osteoblasts engraftment with conventional methods to detect chimerism (>1% sensitivity). On expanded fibroblasts 4% of the cells are of donor origin. Few months later the child presented bilateral retinoblastoma with no signs of mixed chimerism (Figure 4), that after chemotherapy, is currently in complete remission. The second child was born by caesarian cut at the 32nd week because of the costal fractures that impaired pulmonary growth. He died on a ventilator due to the severity of the clinical picture and no information on chimerism are available.

Grant FIRB n.RBNEO1R4MJ 006

References

1. Chandross KJ, Mezery E. Plasticity of adult bone marrow stem cell. Mattson, M.P. and Van Zant, G. eds (Greenwich, CT: JAI Press) 2001.
2. Slack JM. Stem cells in epithelial tissues. *Science* 2000;287:1431-3.
3. Odorico JS, Kaufman DS, Thomson JA. Multilineage differentiation from human embryonic stem cell lines. *Stem Cell* 2001;19:193-204.
4. Porta F, Mazzolari E, Lanfranchi A, Ugazio AG. Il trapianto di midollo nelle immunodeficienze primitive. *Riv Ital Ped* 1999;25:35-38.
5. K Le Blanc, C Götherström, O Ringdén, M Hassan, R McMahon, E Horwitz et al. Fetal Mesenchymal Stem Cell Engraftment in Bone after in Utero Transplantation in a Patient with Severe Osteogenesis Imperfecta. *Transplantation* vol.6, June, 2005.
6. Porta F, Bolda F, Baggio E, Baffelli R, Mazzolari E, Guendalini F, et al. Prenatal therapy of severe combined immunodeficiencies and of osteogenesis imperfecta by non-hematopoietic stem cell. VII International Meeting on in Utero Stem Cell Transplantation: new frontiers in regenerative therapy. Roma 2004.