
# Nuovi orientamenti nel "cord blood banking"

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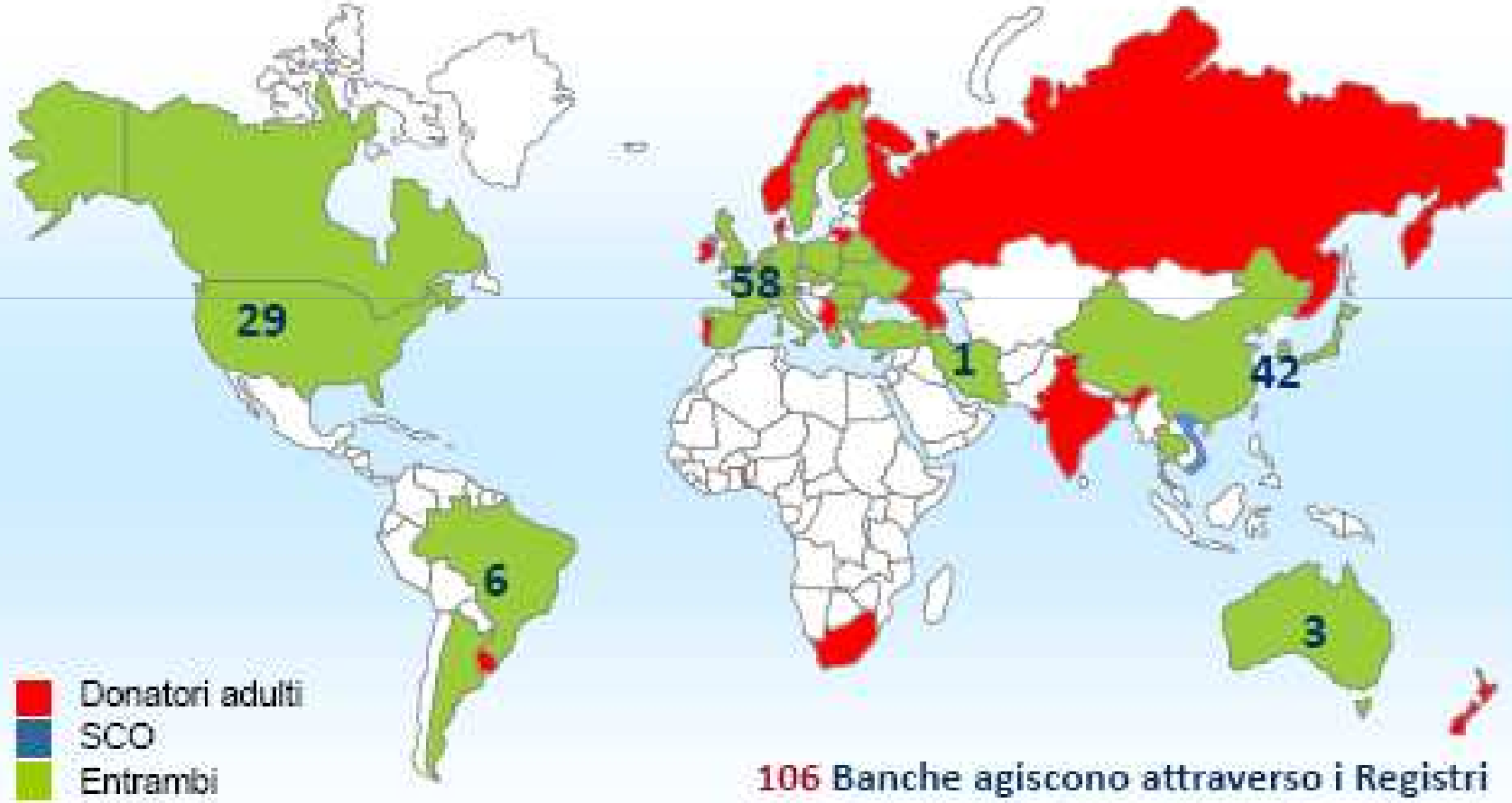
# Il network internazionale delle Banche di CSE da SCO

**139 Banche/reti di Banche SCO**




**106 Banche agiscono attraverso i Registri**

**11 Banche indipendenti**

# Unità SCO in ciascun continente

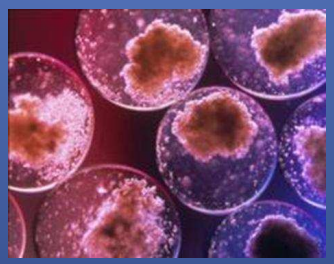

**Totale 537.276**

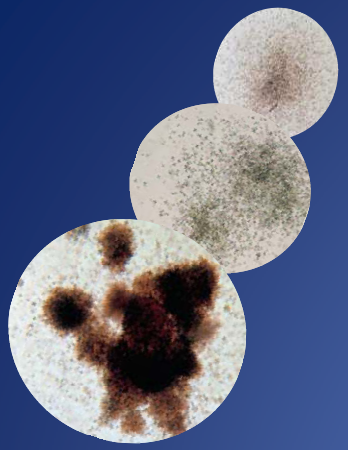
# New directions in Cord Blood Banking

- Cord blood (CB) transplantation is successfully used to treat malignant and non-malignant disorders
- The national and international inventories need to be expanded in order to provide CB units with high cellular dose and an increased chance of matching for ethnic minorities

# Current research is focused on

- increasing the limited number of CB stem cells, as a low cell dose may cause:
  - granulocyte and platelet recovery delay (increased infection risk)
  - graft failure
  - disease relapse
- finding new therapeutic uses of CB stem cells





# The Network of CB Banks

Quality control shall be planned and regularly performed to ensure the quality of products for clinical use

Written policies and procedures (SOPs) covering all relevant aspects of CB Banking (from collection to transplantation) in compliance with international and national standards guidelines and laws shall be in place to achieve FACT-Netcord accreditation (after on-site inspection)

**CELL DOSE** = number of total nucleated cells

**OK**

pediatric patient

**1. Double CB transplantation (Minneapolis)**

**2. Single CB transplantation with reduced intensity conditioning (Minneapolis)**

***3. ex vivo cellular expansion* (Huston)**

**4. Intra-bone CB transplantation (Genova)**

adult patient

# Immunological privileges

Reduced risk for immunomediated complications (naiveté of CB immune system)

- transplantation is possible between non relatives
- transplantation is possible between mismatched donor and recipient (up to 2 mismatches)

# Advantages of Cord Blood

- LOW incidence and severity of graft versus host disease (GvHD)
- LOW risk of viral contamination (CMV, EBV)
- AVAILABILITY for unrelated patients (reduced search times)

# Minority matters

Compared to the register of bone marrow donors, the CB inventory has a low number of units, but a greater genetic variability (partially due to the higher number of donors from non-Caucasian ethnic groups)

- **EASY TO COLLECT**

- **SAFE FOR THE DONOR**

# How to overcome the disadvantages of CB transplantation

Disadvantages:

- limited number of cells obtained from a single CB collection
- slower time for neutrophil and platelet engraftment

**Improvement in CB transplantation**:

- collecting more haematopoietic stem and progenitor cells from placental and CB
- ex vivo expansion of haematopoietic stem and progenitor cells
- enhancing homing and engrafting of CB stem cells

Broxmeyer HE, 2011

# Collecting more cells from placental and CB

- Collect as much blood as possible from the umbilical cord and placental blood vessels
- Perform the perfusion of the placenta after collecting the greatest volume of blood from the umbilical cord
- The placenta is also rich of progenitor cells, and represents a new source of stem cells

Critical:

  - The perfusion of the placenta may cause the collection of maternal cells. This contamination must be carefully monitored as an excess maternal cell has the potential to elicit higher levels of graft-versus-host disease

# Perfusion of the placenta

- Using solutions able to mobilize stem/progenitor cells (AMD3100 is used to enhance mobilization of stem cells from adult peripheral blood)

Critical:

  - Need to check and set the method
  - Compare the cell yield obtained by perfusion with the yield obtained by traditional collections to demonstrate that the mobilizing agent really increases the cell number of collection
  - Because of great variability in numbers of cells collected between CB units from different donors, we must use the same placenta and cord as internal control to show that after perfusion the placenta has exhausted further release of cells

# Ex vivo expansion of haematopoietic stem cells

- The ex vivo expansion of long-term engrafting human haematopoietic stem cells has not been yet adequately demonstrated
- Research efforts are focused on enhancing the environment in vitro to improve survival, proliferation and self-renewal of the stem cells, including use of potent combinations of cytokines and growth factors
- Identifying receptors and intracellular signals that are selectively involved in enhancing hematopoietic stem cell expansion will allow us to bypass the need for stromal cell interactions of the niche and specific cytokines

# Pharmacological-induced expansion

- In mice, the over-expression of Rheb2 enhances the haematopoietic progenitor cell growth in vitro and a transient short-term repopulation in vivo
- In mammalian cells, Rheb1 and Rheb2 are able to activate the signal of the target of rapamycin (mTOR)
- The means to modulate the signalling pathway that incorporates rapamycin-sensitive mTOR pathway may, in the presence of cytokines, enhance functional capacity of and/or expand, human haematopoietic stem cells in vitro

# Enhancing homing and engraftment capabilities of haematopoietic stem cells

Ligand–receptor interaction is strongly implicated in homing and engraftment of haematopoietic stem and progenitor cells:

stromal cell-derived factor-1 (SDF-1 /CXCL12) acts as a chemotactic (directed cell migration) agent, but also enhances survival of early haematopoietic cells

# Other uses of cord blood

There are numbers of papers reporting presence and/or generation of non-haematopoietic cell types from cord blood for **regenerative medicine**.

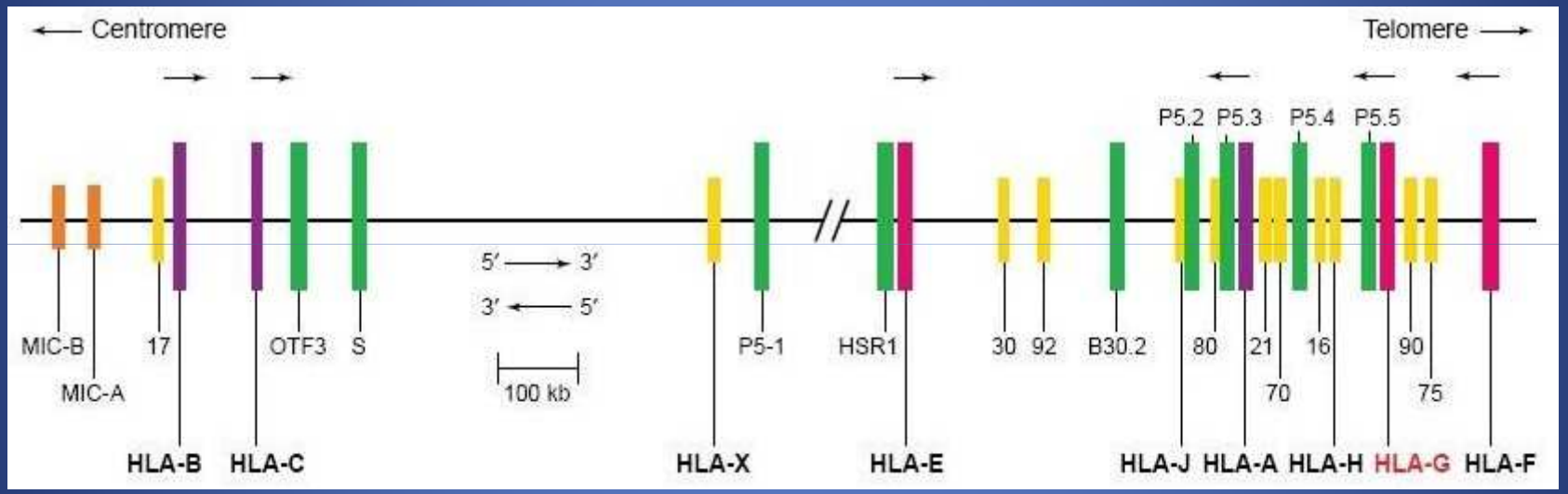
Included in such reports are references to mesenchymal stem/stromal cells, endothelial progenitor cells and induced pluripotent stem cells.
Such laboratory and pre-clinical animal studies are of interest, and can provide us with insights into biological processes.

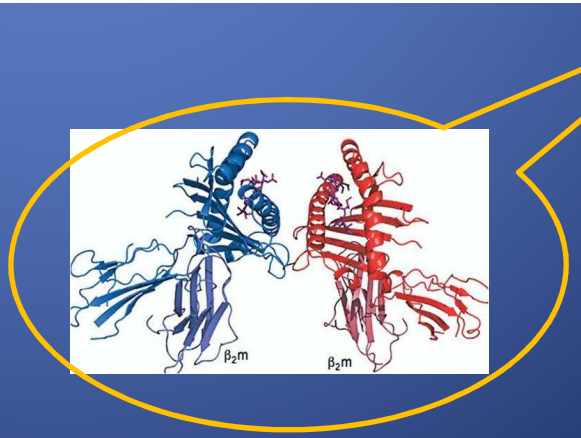
However, there is little or no rigorous evidence yet that such studies can or will translate into relevant clinical treatments. These clinical studies will need to be well controlled before any conclusions regarding clinical efficacy are put forth for non-haematopoietic uses of cord blood.

# Hot topics in cord blood banking

- Provide high quality certified products for clinical use
- Provide units with high cellular dose for clinical use
- Give a prompt genomic analysis of each CB unit typing HLA-A, -B, -C, and -DRB1 at high resolution
- Investigate the HLA-G immunosuppressive potential on transplantation outcomes
- Study the NIMA and NImMA immunotolerant effects on transplantation outcomes

# Immunosuppressive effect of HLA non classical class I molecules in Cord Blood

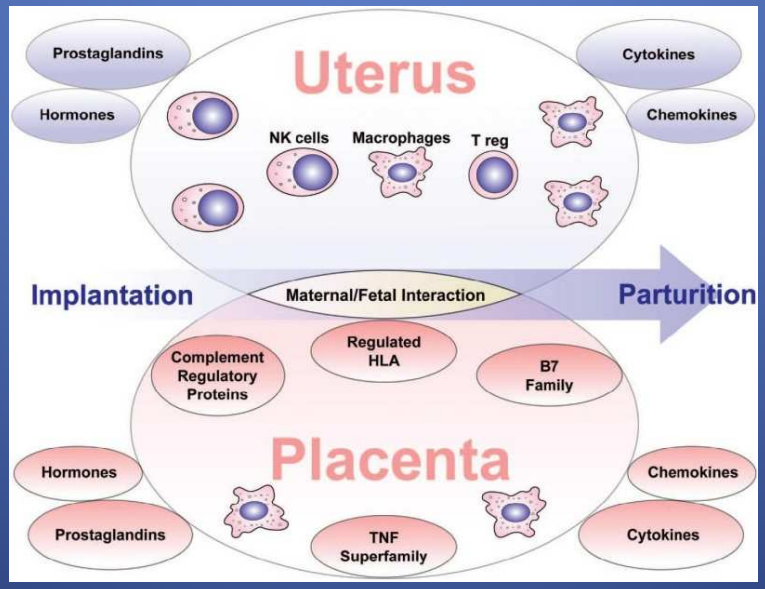



**HLA-G**

# The feto-maternal interplay

Pregnancy is an immunological balancing act in which the mother's immune system has to remain tolerant of paternal antigens and yet maintain normal immune competence for defense against microorganisms

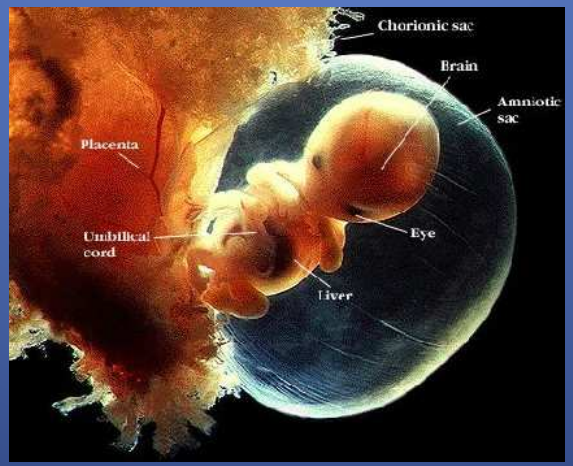


# Changes at the feto-maternal interface

- Uterine decidual and placental cells produce a huge array of cytokines which, in part, contribute to the deviation of the immune response from Th1 to Th2
- The trophoblast expresses Fas ligand conferring immune privilege: maternal immune cells expressing Fas will undergo apoptosis at the placenta/decidua interface
- During pregnancy there is a low peripheral NK activity, and early pregnancy the majority of uterine lymphocytes are CD56(bright) granulated NK cells, which do not express CD16 or CD3

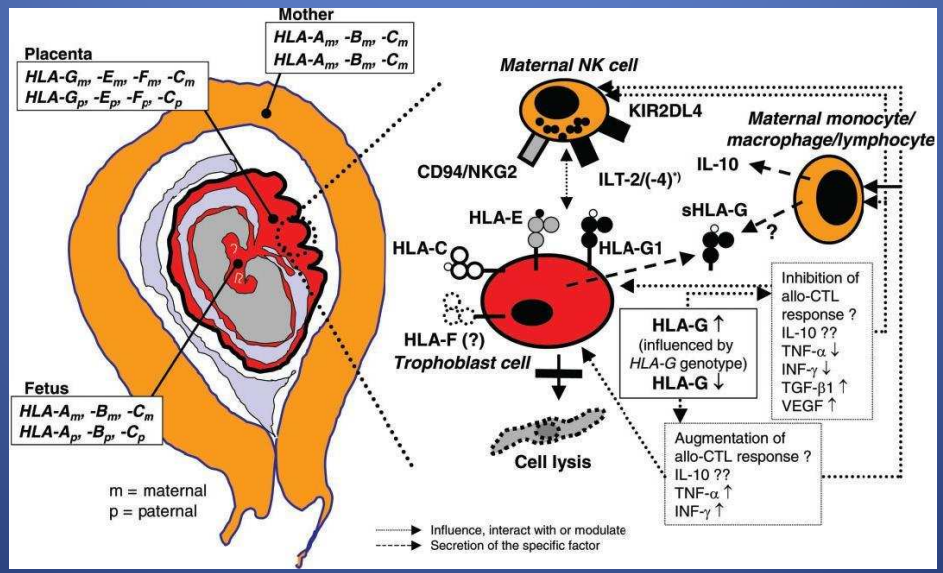
# Fetal protection against maternal rejection

- Trophoblast cells fail to express HLA class I or class II molecules to prevent normal T cell responses
- Cells lacking HLA molecules undergo cytolisis by Natural Killer cells, thus the trophoblast cells strongly express the non classic class I **HLA-G**, -E, -F molecules, which may downregulate Natural Killer cell function



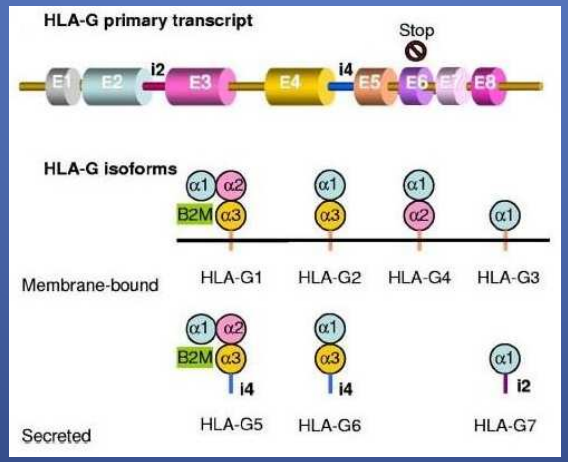
# HLA-G functions against maternal rejection

- inhibits the cytolytic function of uterine and peripheral blood NK cells
- stimulate the production of cytokines and angiogenic growth factors that allow implantation and placental vascularization



# HLA-G differs from classical class I molecules

- lower polymorphism
- restricted tissue distribution (trophoblast, cornea, timus)
- particular expression pattern as seven protein isoforms generated from alternative splicing of a single mRNA transcript (transmembrane isoforms: G1, G2, G3, G4; soluble forms: G5, G6, G7)
- Further soluble molecule, namely G1 shed, that is proteolitically clived from the G1 transmambrane isoform



# HLA-G inhibitory functions

## HLA-G binds to:

- **KIR2DL4** ⇨ inhibiting cytotoxic function of Natural Killer cells, stimulating the production of pro-angiogenic factors after internalization
- **ILT2** ⇨ inhibiting the proliferation of natural killer cells, CD8+ and CD4+ lymphocytes, inhibiting the alloreactivity of CD4+ lymphocytes
- **ILT4** ⇨ inhibiting the alloreactivity of CD4+ lymphocytes and growth of dendritic cells (DC)
- **CD8** ⇨ stimulating the apoptosis of natural killer cells and CD8+ lymphocytes
- **CD160** ⇨ stimulating the apoptosis of endotelial cells

# HLA-G enhances tolerance in transplantation setting

- **Soluble HLA-G levels in sera and bioptic tissues from heart transplanted patients correlate with lower incidence of rejection**
- **High soluble HLA-G levels correlate with lower incidence of rejection in kidney and kidney/liver transplanted patients**
- **Pre-transplantation high soluble HLA-G levels correlate with lower incidence of acute GvHD after hematopoietic stem cell transplantation**

# HLA-G and xeno-transplantation

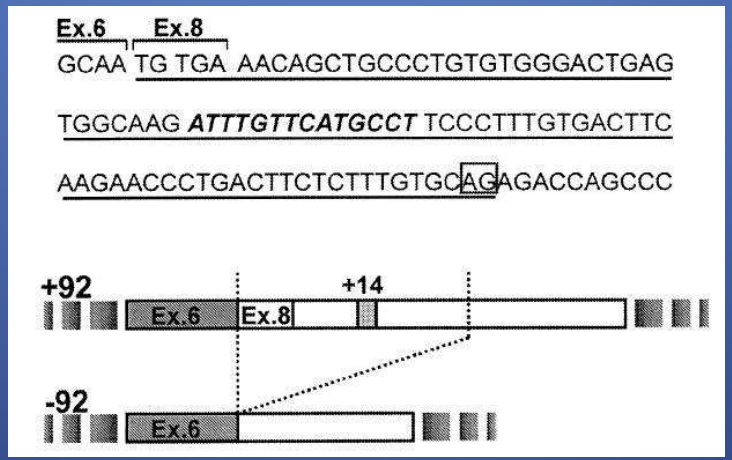
- Low genomic polymorphism
- Soluble proteins isoforms
- Immunosuppressive function as ligand for inhibitory receptors of T cells, Natural Killer cells, and APC

**HLA-G tollerogenic potential in xeno-transplantation**

# Current research

- Measurement (immunoassay) of soluble HLA-G molecules in CB plasma before and after cryopreservation, to verify if these molecules are stable also after a long time storage

- Analysis of the HLA-G 14 bp insertion/deletion polymorphism (rs16375, 3'UTR), affecting mRNA stability, to verify a possible correlation between soluble HLA-G expression and genotype in CB donors



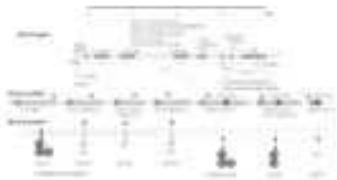

**manuscript in preparation**

## GENDER AND HLA-G GENE POLYMORPHISM SEEM TO INFLUENCE THE LEVELS OF HLA-G MOLECULES IN CORD BLOOD

Bergamaschi F[1], Capittini C[2], Sachetto S[3], Tinelli C[3], Pasi A[3], Viola M[1], Truglio M[1], Guarene M[4], Giordano V[1], Romano B[1], Marchesi A[1], Poma R[1], Salvaneschi L[1,2], Martinetti M[1]

Soluble HLA-G (sHLA-G) is an immunosuppressive molecule involved in establishing immunotolerance at the maternal-fetal interface during pregnancy and in transplantation. sHLA-G is also advocated in preventing graft versus-host disease (GvHD), a major complication of allogeneic stem cell (SC) transplantation.

Cord blood (CB) is obtained at infant donor's birth then is cryopreserved to be available for donor search.
We previously demonstrated the presence of detectable levels of sHLA-G in CB plasma even after long-term storage (4-8 years at -196°C).



HLA-G antigens are encoded by a non-classical HLA class I gene, whose 14bp polymorphism, in exon 8, has been associated with stability of HLA-G mRNA. Besides HLA-G genotype, other factors could affect sHLA-G levels in CB.

For this purpose, we retrospectively reviewed the data of 80 CBs cryopreserved from 2003 to 2005. sHLA-G concentration was determined on thawed aliquots of CB plasma by sHLA-G ELISA kit (Exbio, Praha, Czech Republic). 14bp HLA-G genotyping was performed by PCR.

### RESULTS

sHLA-G concentration was 38.8 ng/ml on average (range: 0.58-93.0) and was correlated according to sex (21 males, 29 females), fetal weight (2810-4210 g), gestational age (38-42 weeks), 14bp genotype (+14bp/+14bp in 14 CB, -14bp/+14bp in 20 CB, -14bp/-14bp in 18 CB) by multivariate analysis.
The highest sHLA-G levels (mean value 50.73 ng/ml), although not statistically significant, were observed in males with homozygous 14bp insertion (p=0.346).

### CONCLUSIONS

This trend should be confirmed by increasing the sample size of CBs for which sHLA-G levels have been ascertained. Our findings open a new scenario in pregnancy where fetus and delivery may play a role in enhancing sHLA-G levels in cord blood.

**Special Mention by tutor**
**ISBT Congress 2010**

## THE VALUE OF SOLUBLE HLA-G MOLECULES CONTENT IN CORD BLOOD. PERSPECTIVES FOR STEM CELL TRANSPLANTATION

Sachetto S[1], Capittini C[2], Viola M[1], Truglio M[1], Tinelli C[1], Bergamaschi F[1], Bodoli C[1], Pasi A[1], Poma R[1], Martinetti M[1], Salvaneschi L[1]

Soluble HLA-G (sHLA-G) is an immunosuppressive molecule able to inhibit both T and NK cell activity and to induce anergic T cells by interacting with receptors on dendritic cells.

HLA-G antigens are encoded by a non-classical HLA class I gene with limited polymorphism. The insertion of 14bp in the 3'UTR of the HLA-G gene is known to associate with low production of more stable transcription of HLA-G mRNA. sHLA-G is involved in establishing immunotolerance at the maternal-fetal interface during pregnancy and in transplantation.

In solid organ transplantation, low risk of serious rejection and good prognosis have been described in presence of high levels of sHLA-G. In haematopoietic patients receiving unrelated allogeneic stem cell transplantation, the homozygous deletion of 14bp has been recently associated to higher risk of acute graft-versus-host disease (aGvHD) and worse outcome. Cord blood (CB) is obtained at the end of pregnancy and some evidence are coming out that it may be rich of sHLA-G, thus accounting (at least in part) for the lower incidence of GvHD associated to CB as respect to other stem cell sources.

We previously investigated the correlation between sHLA-G concentration and 14bp HLA-G genotype in 29 CB units of the Pavia CB Bank cryopreserved from 2003 to 2005. Here we show the results of the retrospective data review extended to 80 CBs. sHLA-G concentration was determined on thawed aliquots of CB plasma (stored in the same conditions of the cells) by sHLA-G ELISA kit (Exbio, Praha, Czech Republic). 14bp HLA-G genotyping was performed by PCR.

### RESULTS

14 samples showed the +14bp/+14bp (mo), 20 the -14bp/+14bp (ho/e) and 18 the -14bp/-14bp (ho0) genotype, respectively. The mean values of sHLA-G concentration are depicted in the table.
Higher sHLA-G levels were observed in presence of the homozygous 14bp insertion, although with no statistical significance.

### CONCLUSIONS

It confirmed our observation might indicate the idea that in nature the 14bp polymorphism of CB could be utilized by the physician as a predictive factor of GvHD to be evaluated for pre-transplantation risk-assessment.
In our hands, the quantification of sHLA-G by ELISA demonstrated to be reliable despite the time (ranging from 4 to 8 years) and conditions of storage (-196°C, in liquid nitrogen).

In particular, the analysis of sHLA-G levels according to the storage time showed no differences in HLA-G concentration over the years (see the table), suggesting that sHLA-G molecules could remain stable and detectable levels can be demonstrated in CB plasma also after long-term storage.

It confirmed that testing could suggest a different destination for plasma which is usually discarded after volume reduction of CB units. In order to possibly find a role of its sHLA-G content. Future investigations will help us to further increase the sample size, to cover a longer storage time (most CB Banks are keeping CB units stored for 15 years and more) and to definitively assess the relevance of HLA-G for adopted strategies of CB banking.

**Best Poster Session Award**
**EFI Congress 2010**

# From implantation to transplantation

REVIEW

Arch. Immunol. Ther. Exp., 2006, 54, 165–172
PL ISSN 0004-069X

DOI 10.1007/s00005-006-0018-y

## Impact of fetal-maternal tolerance in hematopoietic stem cell transplantation

**Takanori Teshima[1], Ken-ichi Matsuoka[2] and Tatsuo Ichinohe[3]**

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[2] Biopathological Science, Okayama University Graduate School of Medicine and Dentistry, 2-5-1 Shikata-cho, Okayama 700-8558, Japan
[3] Department of Hematology/Oncology, Graduate School of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

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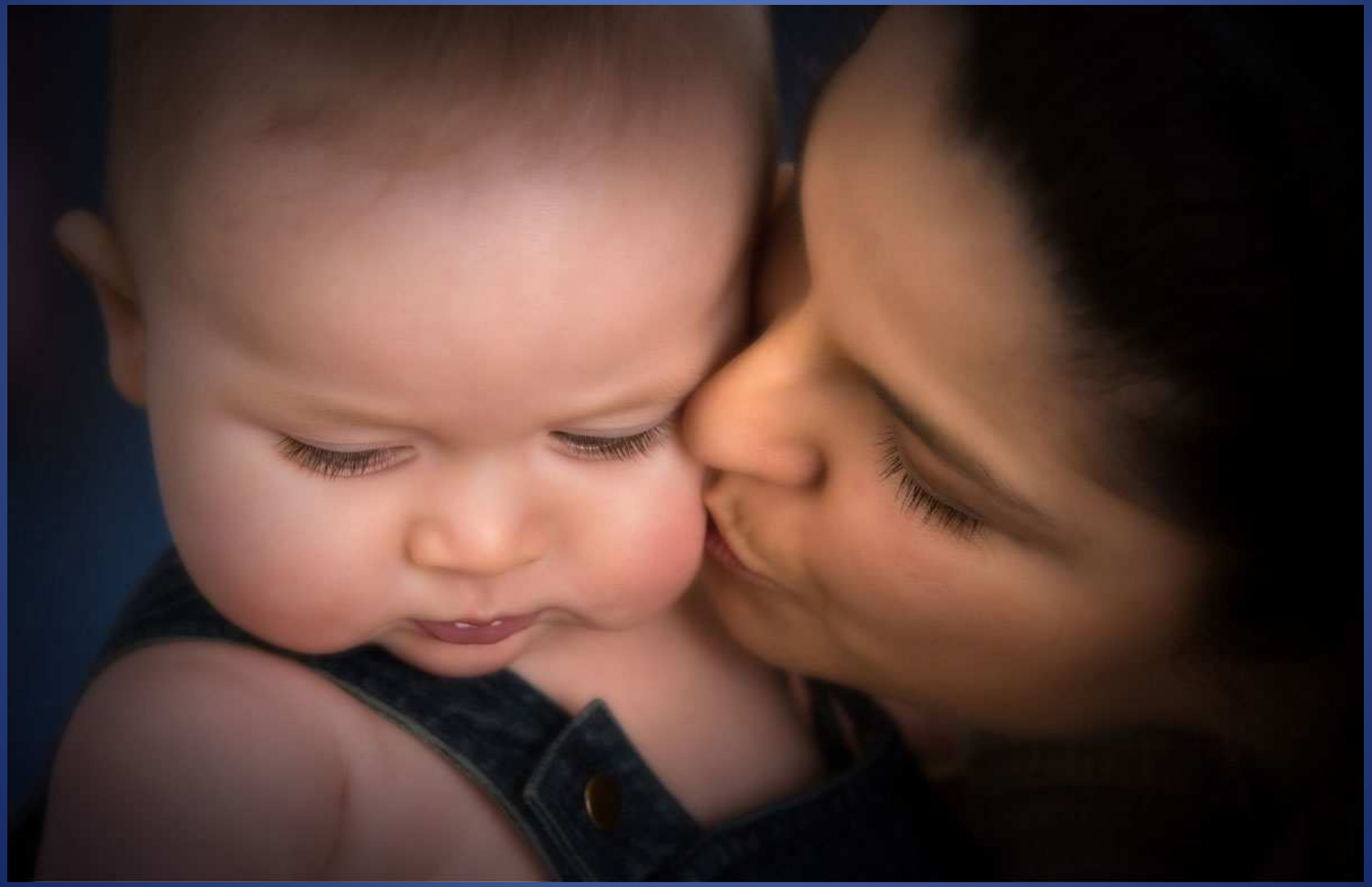
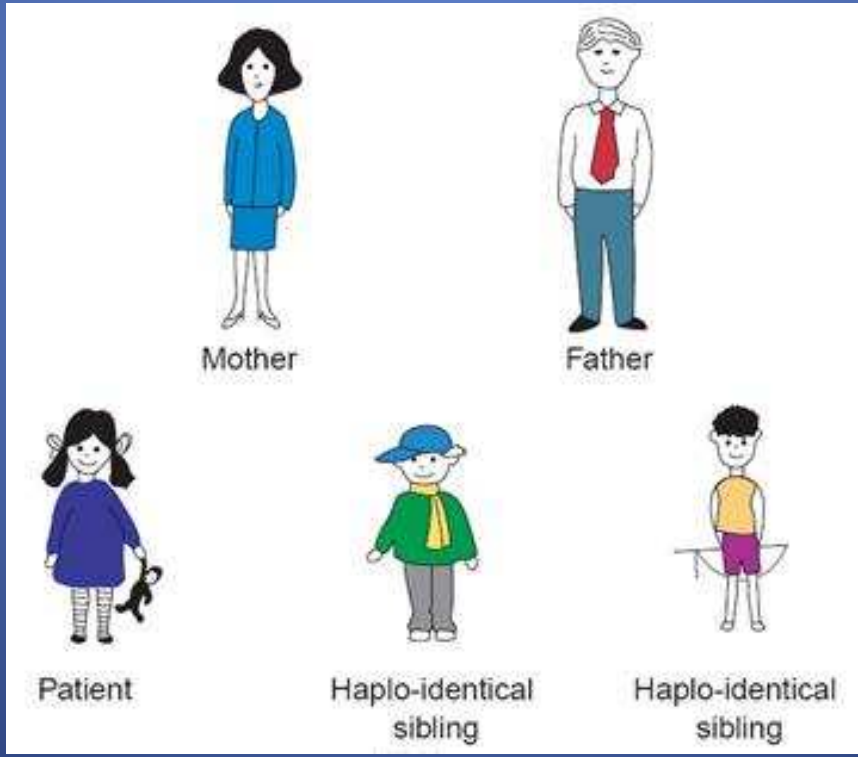
# Non inherited maternal antigens
# NIMA

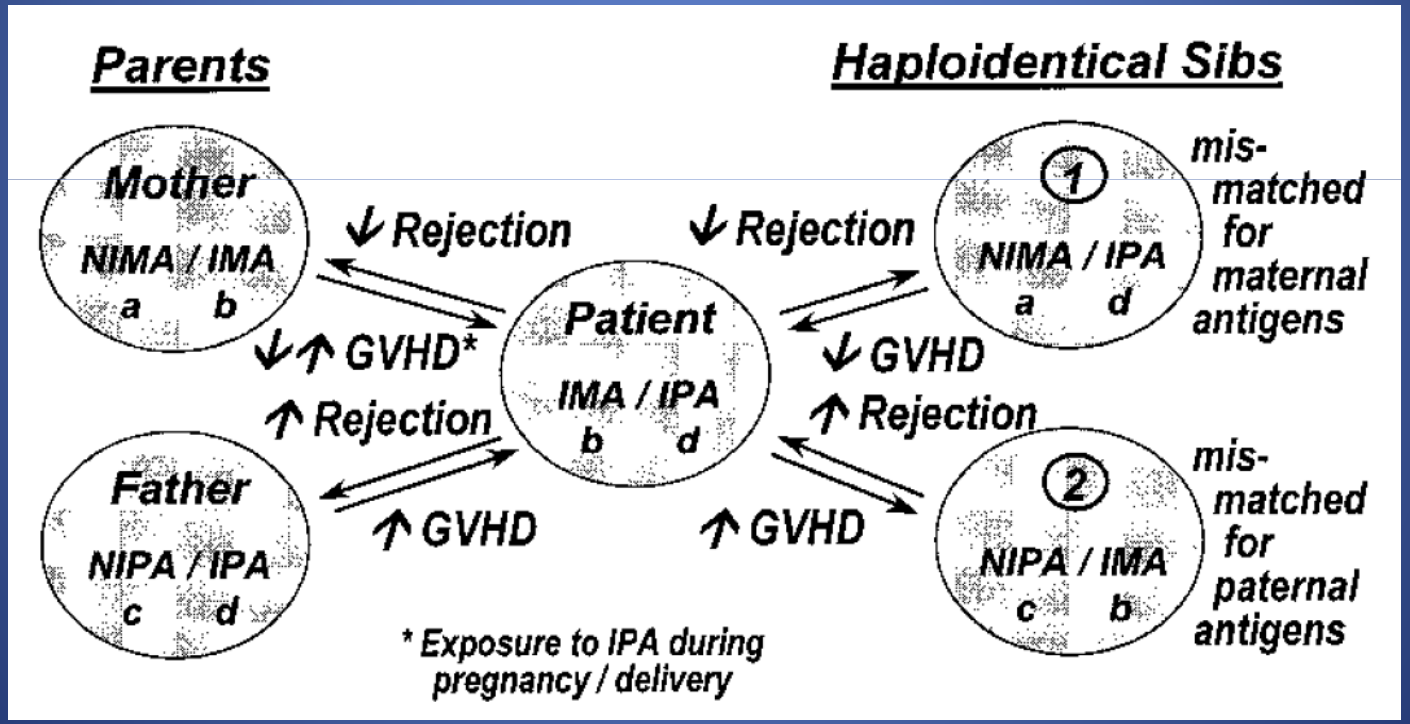


van Rood et al., 2002

Haploidentical siblings may provide an alternative transplant option when HLA-matched related donors are not available, the disadvantages being essentially the increased risk for GvHD and graft rejection

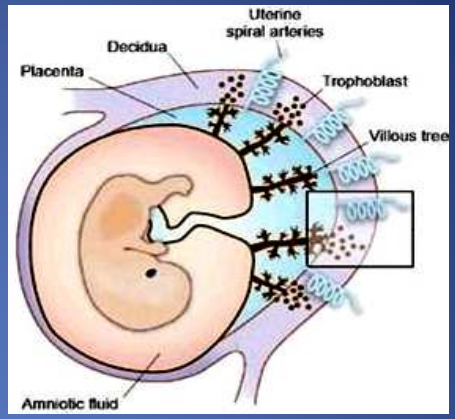


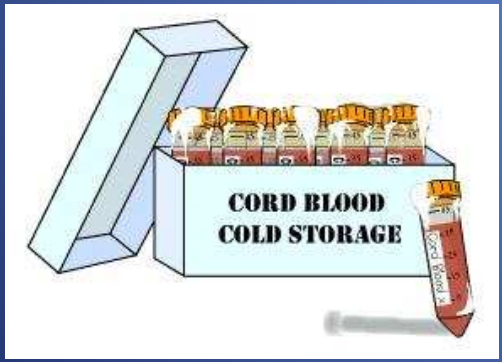
ASCT from an inherited paternal HLA antigen (IPA)/ non inherited maternal antigen (NIMA) – mismatched donor has been previously demonstrated to be feasible, decreasing GvHD and preserving graft-versus-leukaemia (GvL) effect




van Rood et al., 2002

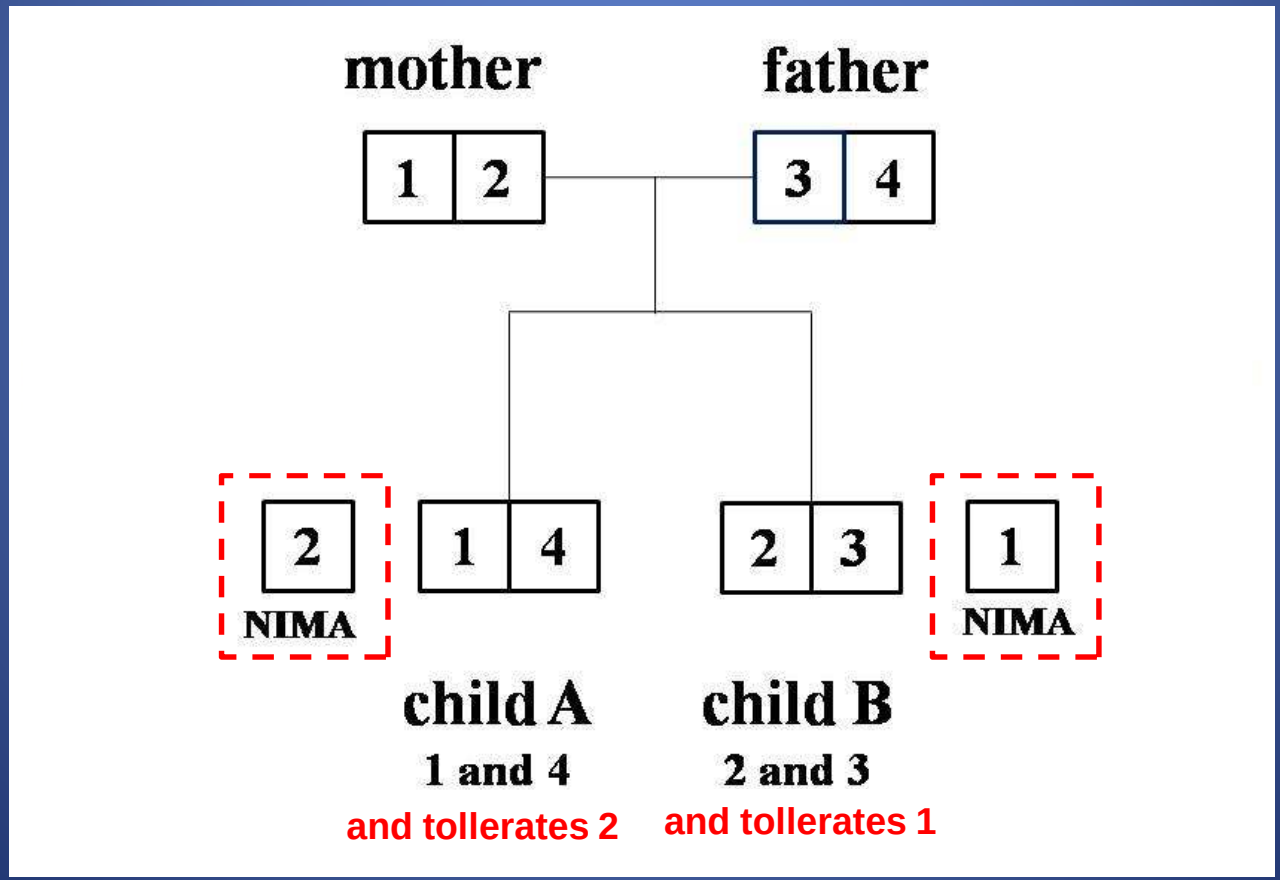
Maternal cells passing
the placenta
during pregnancy
express HLA molecules
which are acknowledged
by the foetus,
who develops a tolerance
to NIMAs maintained in adulthood




The maternal haplotypes
are easily available
for cord blood (CB) units
while this information
is not provided for adult donors

As NIMAs should be tolerated by the CB immune system, the knowledge of the third donor's NIMA haplotype could further augment the chance for finding a compatible CB donor and reduce immune complications of ASCT



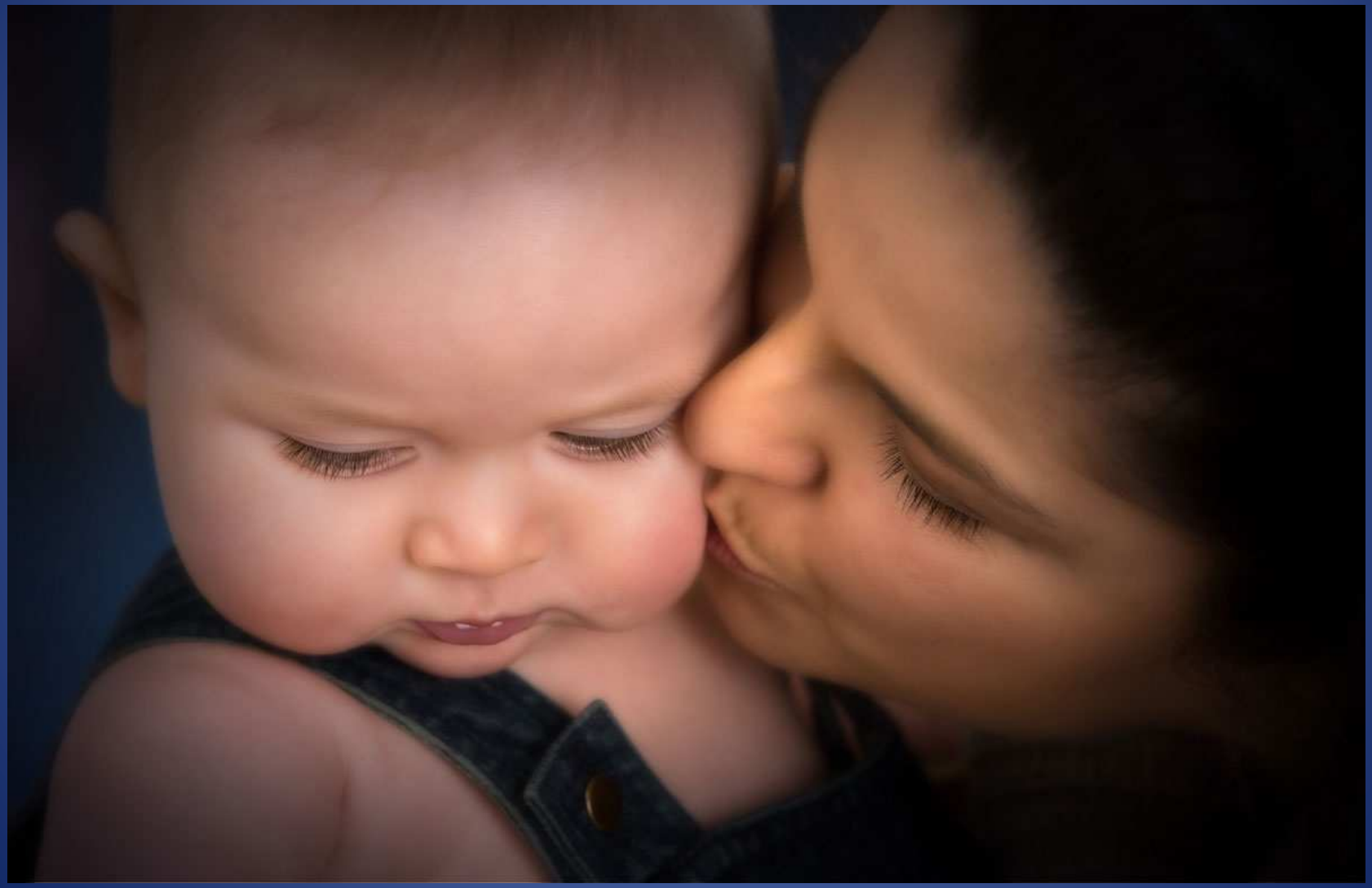
# *Current research on NIMA Pavia Cord Blood Bank*

EUROCORD multicenter study:
**"IMMUNOGENETIC DETERMINANTS OF OUTCOMES OF UNRELATED CORD BLOOD TRANSPLANTATION: KIR AND NIMA EFFECT"**

**EUROCORD** records and updates data regarding patients transplanted with CB stem cells
**EUROCORD** sets procedures and guidelines to improve outcomes after CB transplantation

**manuscript submitted**

# Non inherited maternal MINOR antigens NIMmA



# Minor histocompatibility antigens mHAg

- minor histocompatibility antigens (mHAg) are polymorphic peptides derived from the proteolitic cleavage of intracellular proteins encoded by genes mapping on autosomes and Y chromosome

- in transplantation setting, all intracellular peptides may be considered virtually mHAg if these peptides are able to bind HLA class I and II molecules and be recognized by alloreactive T cells

- as mHAg are expressed by genes mapping on crhomosomes following the mendelian segregation, HLA-identical brothers may have a different mHAg repertoire. This genetic diversity, due to the natural genetic variability, influences the production of polymorphic mHAg

Besides major histocompatibility complex antigens (HLA), minor histocompatibility antigens (mHAgs) may be considered to play a role in transplantation setting.

In HLA identical sibling transplantation, mHAgs mismatches can lead to T-cell activation with specific alloreactivity against recipient's or donor's antigens, thus supporting the GvHD or rejection response, respectively.

Naturally acquired tolerance and sensitization to minor histocompatibility antigens in healthy family members
Blood 2009;114(11):2263-72

Cord blood comprises antigen-experienced T cells specific for maternal minor histocompatibility antigen HA-1
Blood 2005;105(4):1823-7.

# *Current research on NImMA Pavia Cord Blood Bank*

- **Evaluate the NImMA effect in CB sibling transplantation considering the post transplantation complications, such as acute and chronic GvHD, relapse and total-related mortality in patients affected by non malignant diseases**

- **Analyze the influence of NIMmA on anti-tumoral GvT effect in malignant patients transplanted the CB of their siblings**

# Aknowledgments

Paola Bergamaschi
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Marco Guarene

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Università degli Studi di Pavia
IRCCS Policlinico San Matteo Pavia