European Frontiers in Ocular Pharmacology

Catania January 15 ,2015

« IMMUNOREGULATION & PERSONALIZED IMMUNOGENETICS »
FROM HLA TO REGENERATIVE THERAPY

Dominique CHARRON MD,PhD

dominique.charron@sls.aphp.fr
XX th Century

HLA, MHC, Cytokines, Receptors, …

… TRANSPLANTATION, AUTOIMMUNITY, INFECTIONS

XXI st Century

HLA & MEDICINE (Schizophrenia/Parkinson … )
IMMUNO PHARMACOGENETICS (Abacavir/Carbamazepin, Allopurinol…)
REGENERATIVE MEDICINE/CELL & IMMUNO THERAPIES

TOWARDS «SYSTEM BIOLOGY/PERSONALIZED SYSTEM MEDICINE »
Pre DNA area

- Serology
  - Leucoagglutination
  - Microlymphocytotoxicity
- Genetics
  - Familial segregation
  - Population study (frequency)
HLA DIVERSITY = BIOLOGICAL SELF = PERSONALIZED MEDICINE

1958  MAC  first allele HLA-A2
1970’s  20 to 50 alleles (serology)

2014  :  >10 000  ALLELES
A,B,C,DR,DQ,DP (dna typing)

NGS

WE ARE THE LIMIT
Population Genetics Worldwide
XXI Century
HLA, MHC AND MUCH MORE
TRANSPLANTATION, AID AND MUCH MORE

IMMUNOPHARMACOGENETICS
REGENERATIVE MEDICINE
SYSTEMS BIOLOGY
XXth century

T CELL MEDIATED : REJECTION (ORGANS) & GVH/GVL (HSCT)

HLA MATCHING - HLA TYPING
HLA-A+B+DR Mismatches
Deceased Donor, First Kidney Transplants 1985-2006

% Graft Survival (log)

20-Year Estimate | Half-Life (Years)
--- | ---
0 MM | 41 % | 16.4
1 MM | 35 % | 14.4
2 MM | 33 % | 13.7
3 MM | 32 % | 13.4
4 MM | 30 % | 12.7
5 MM | 26 % | 11.2
6 MM | 24 % | 10.9

0 MM n= 8,170
1 MM n=11,652
2 MM n=26,175
3 MM n=36,175
4 MM n=27,201
5 MM n=12,946
6 MM n= 3,903
Kaplan-Meier probability of survival of the 14th IHWG HCT recipients according to 0, 1, 2 or 3 or more HLA disparities at HLA-A, B, C, DRB1 and DQB1.
HLA HISTO-INCOMPATIBILITY/ALLOGENICITY IN TRANSPLANTATION

ANTI HLA ANTIBODY MEDIATED:

VASCULAR REJECTION (ORGANS) & NO ENGRAFTMENT (HSCT)

Anti HLA AB Detection & Characterisation (DSA)
Antibody-mediated vascular rejection of kidney allografts: a population-based study


LANCET Nov 23, 2012
Population based study

2079 patients (nck/sls) + 602 validation samples (foch)

302 biopsy proven rejection (1998-2008)

CINICAL, HISTO PATHOLOGICAL (including C4d) & IMMUNOLOGICAL (DSA) DATA

Hierarchical cluster analysis/ unsupervised principal component

4 patterns of rejection

TCMR/V+: T cell mediated rejection (26 = 9° /° )

ABMR/V+: Antibody mediated rejection (64 = 21° /° )

TCMR/V-: T cell mediated rejection without vasculitis (139 = 46° /° )

ABMR/V-: Antibody mediated rejection without vasculitis (73 = 24° /° )
Figure 2: Comparison of morphological and immunological variables in the four rejection patterns

Bars represent SD. NS=not significant. TCMR/V−=T cell-mediated rejection without vasculitis.
TCMR/V+ = T cell-mediated vascular rejection. ABMR/V+ = antibody-mediated vascular rejection.
ABMR/V− = antibody-mediated rejection without vasculitis.
Figure 2: Identification of four distinct rejection patterns according to clinical, histological, and immunological variables. The unsupervised principal component analysis examined kidney recipients with acute biopsy-proven rejection with seven variables: glomerulitis, peritubular capillaritis, donor-specific anti-HLA antibodies, C4d deposition, interstitial inflammation, tubulitis, and endarteritis. The horizontal axis opposes cellular rejection (interstitial inflammation and tubulitis) and antibody-mediated rejection (donor-specific anti-HLA antibodies, glomerulitis, peritubular capillaritis and C4d), as recognized by the international Banff classification. The vertical axis defines the presence or absence of lesions of endarteritis (appendix).
Figure 3: Kaplan-Meier curves for kidney graft survival by acute rejection phenotype
Initial diagnoses as per (A) Banff classifications and (B) our new approach. Graft survival in patients without rejection is purely illustrative; graft survival in these
Complement-Binding Anti-HLA Antibodies and Kidney-Allograft Survival

Alexandre Loupy, M.D., Ph.D., Carmen Lefaucheur, M.D., Ph.D., Dewi Vernerey, M.P.H., Christof Prugger, M.D., Jean-Paul Duong van Huyen, M.D., Ph.D., Nuala Mooney, Ph.D., Caroline Suberbielle, M.D., Ph.D., Véronique Frémeaux-Bacchi, M.D., Ph.D., Arnaud Méjean, M.D., François Desgrandchamps, M.D., Ph.D., Dany Anglicheau, M.D., Ph.D., Dominique Nochy, M.D., Dominique Charron, M.D., Ph.D., Jean-Philippe Empana, M.D., Ph.D., Michel Delahousse, M.D., Christophe Legendre, M.D., Denis Glotz, M.D., Ph.D., Gary S. Hill, M.D.,* Adriana Zeevi, Ph.D., and Xavier Jouven, M.D., Ph.D.

NEJM 2013 september 26
KYDNEY TRANSPLANTS

5 Year Graft Survival

- 1016 patients from 01/2005 to 01/2011 – All cross match – (CDC IGg T& B cells)
  - C1q + DSA + (77) °/°
  - C1q - DSA + (239) °/°
  - C1q - DSA - (700) °/° (p<0.001)

C1q + correlates with AMVR, microvascular inflammation & C4d deposition
Immunogenetics today: HLA, MHC and much more

Editorial overview
Dominique Charron

Current Opinion in Immunology 2005, 17:493–497
This review comes from a themed issue on
Immunogenetics
Edited by Dominique Charron
Available online 8th August 2005
0952-7915/$ – see front matter
© 2005 Elsevier Ltd. All rights reserved.
DOI 10.1016/j.coi.2005.07.007

HLA, MHC AND MUCH MORE....

...TRANSPLANTATION, AUTOIMMUNITY AND MUCH MORE

HLA in MEDICINE
IMMUNOPHARMACOGENETICS
REGENERATIVE MEDICINE
SYSTEMS BIOLOGY
The human MHC: epicenter of disease association as determined by GWAS

Autoimmune
Cancer
Viral
Bacterial
Others

* Top Hit
Overview of the Primary GWA Scan Involving 931 Family Trios

Type I diabetes
Association analysis across the MHC
HLA GWAS IN EYE DISEASES

• Acute Anterior Uveitis: + HLA B27 IL23R, ERAP1, IL10, IL6R, ILR1 …
• Birdshot Retinopathy: + HLA A29 ERAP2…
• Behcet Disease: + HLA B51, B57 – B35 ERAP1, IL23R, IL10 …

Towards System Biology - System Medicine
Association between Parkinson's disease and the HLA-DRB1 locus

Ismail Ahmed1,2, Ryad Tamouza3, Marc Delord4, Rajagopal Krishnamoorthy5, Christophe Tzourio1,2, Claire Mulot6,7, Magali Nacfer6,7, Jean-Charles Lambert8, Philippe Beaune6,9, Pierre Laurent-Puig6,9, Marie-Anne Loriot6,9, Dominique Charron3, Alexis Elbaz1,2

Previous GWAS: association with DRA (non polymorphic) & DRB5 (gene present in only 20° /°)

THIS STUDY

2 population based case control (499/1123) studies of ethnically homogeneous PD vs 51 HLA-DR region SNPs (logistic regression-permutation method)

Imputation HLA* Imp software)

Rs 660895 DR B1 (OR 0.70 cP 0.01)

META ANALYSIS confirmation 7996 cases 36455 controls (OR: 0.85 P 0.0001)

HLA typing (23 cases Rs 660895) = DRB1*O4
HLA AND MEDICINE

MAJOR PSYCHOSIS: SCHIZOPHRENIA(S)/BIPOLAR DISORDER (BD)

INFLAMMATORY STATUS
ALTERED CYTOKINE PATHWAYS
AUTOIMMUNITY – VIRAL IMMUNITY

1970-2001
S: HLA A9/A 28/ A 10 (Wright et al 2001)
BD: DISCREPANT DATA

2009
GWAS – 6p21.3. 221 (Stephansson 2009, Jianxin 2009)
S – SUBGROUP DRB1*03 /B*08 AUTOIMMUNE HAPLOTYPE ?
BD – SIMILAR DATA (MHC REGION) (Purcell et al, 2009)

2013 « Fondamental » data (unpublished)

HLA ASSOCIATIONS WILL SPECIFY THE IMMUNE ORIGIN/STATUS OF DISEASES NOT EXPECTED TO BE ORIGINALLY IMMUNE
Evolutionary forces → fight against pathogenicity

Genetic systems → Adaptative Immunity → HLA-ABC / -DR/DQ/DP... → > 2000 alleles

Xenobiotic metabolizing enzyme → CYP + GST, UGT... → e.g. CytP450 > 50

Haplotypic organization - Population variability

Environment
External milieu
Foreign microbial pathogens
Foreign chemicals

Environment
Foreign microbial pathogens
Foreign chemicals

Evolutionary forces

Genetic systems
Adaptative Immunity
Xenobiotic metabolizing enzyme

Diversity
> 2000 alleles
e.g. CytP450 > 50

Haplotypic organization - Population variability
A single CYP can Metabolize Chemicals
Numerous Peptides

A single Chemical can be Metabolized by several CYP

Chemical Peptide Presented

HLA redundancy
can be
Presented

CYP degeneracy

HLA
HLA and HIV

Progression to AIDS

Rapid  →  HLA-B35 (Homozygosity class I)
          HLA-A1, B8, DR3

Slow  →  HLA-B27, -B57 (Heterozygote advantage)

Drug reaction

ABACAVIR  →  HSR (4-5%)  HLA-B5701 (B5701, DR7, DQ3 haplotype) AUS + USA # But not in American Blacks
+HSP70-Hom M493T variant (57.1 ancestral haplotype)  
  (p<0.0001)  A.M Martin et al. PNAS 2004

NEVIRAPINE  →  HSR (4.9%)  HLA-DRB1*0101(pc 0.001)

Antiviral treatment  →  HLA-DRB1*13, -DQB1*06

Viral suppression and cellular immunity  
  A.M Martin et al.  AIDS 2005
# Recent Associations -- SCAR and HLA Variants

<table>
<thead>
<tr>
<th>Patients</th>
<th>Drugs</th>
<th>Diseases</th>
<th>SNPs</th>
<th>Odds etc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>56 White</td>
<td>Carbamazepine</td>
<td>DIHS</td>
<td>HLA-B*1502</td>
<td>All neg.</td>
</tr>
<tr>
<td>8 White</td>
<td>Carbamazepine</td>
<td>SJS/TEN</td>
<td>HLA-B*1502</td>
<td>All neg.</td>
</tr>
<tr>
<td>4 Asian</td>
<td>Carbamazepine</td>
<td>SJS/TEN</td>
<td>HLA-B*1502</td>
<td>All pos.</td>
</tr>
<tr>
<td>60 Han</td>
<td>Carbamazepine</td>
<td>SJS</td>
<td>HLA-B*1502</td>
<td>1357</td>
</tr>
<tr>
<td>51 Han</td>
<td>Allopurinol</td>
<td>SCAR</td>
<td>HLA-B*5801</td>
<td>580</td>
</tr>
<tr>
<td>31 White</td>
<td>Allopurinol</td>
<td>SJS/TEN</td>
<td>HLA-B*5801</td>
<td>80 (61%)</td>
</tr>
<tr>
<td>3 Japanese</td>
<td>Allopurinol</td>
<td>SJS/TEN/DIHS</td>
<td>HLA-B*5801</td>
<td>All pos.</td>
</tr>
<tr>
<td>40 Japanese</td>
<td>Multiple</td>
<td>SJS/TEN</td>
<td>HLA-A*0206</td>
<td>5.5</td>
</tr>
</tbody>
</table>

Genetic [HLA] marker variance across ethnicity & drug
HLA

Immunogenetics Impact

Biological Self

Therapeutic Self

Diagnosis
(Susceptibility)

Response to treatment
HLA, MHC AND MUCH MORE…. 
...TRANSPLANTATION, AUTOIMMUNITY AND MUCH MORE

HLA in MEDICINE
IMMUNOPHARMACOGENETICS
REGENERATIVE MEDICINE
SYSTEMS BIOLOGY
STEM CELL THERAPIES FOR REGENERATIVE MEDICINE

BENEFITS

- PLURIPOTENCY / MULTIPOTENCY
- SELF RENEWAL
- IN VITRO SPECIFIC DIFFERENTIATION
- IMMUNE PRIVILEGE?

LIMITS OF IN VIVO ENGRAFTMENT AND FUNCTIONALITY

- IMMUNOGENICITY/ALLOGENICITY/REJECTION/AUTOIMMUNITY?
- DISPONIBILITY – TIMELINE
- AGING
- SAFETY
- ETHICAL – REGULATORY ISSUES
THE IMMUNITY FACTORS IN REGENERATIVE CELL THERAPIES

- THE IMMUNOGENETIC FACTOR: ALLOGENICITY
  HLA, MHC and Much More…

- THE IMMUNE EFFECTORS: DIRECT vs INDIRECT PATHWAYS
  OF ALLO RECOGNITION
  Cells, Mediators and Allo Antibodies...

- THE AGING FACTOR: IMMUNO SENESCENCE

Toward an IMMUNOLOGICALLY EDUCATED CHOICE OF SCs
ALLOGENEIC STEM CELLS ARE NOT IMMUNO PRIVILEGED

- MHC EXPRESSION
- IMMUNOGENICITY INCREASES UPON DIFFERENCIATION
- IN VIVO REJECTION

3 SUPPORTING PAPERS
CHARACTERIZATION OF THE EXPRESSION OF MHC PROTEINS IN HUMAN EMBRYONIC STEM CELLS

M. DRUKKER, G. KATZ, A. URBACH, M. SCHULDINER, G. MARKEL, J. ITSKOVITZ-ELDOR, B. REUBINOFF, O. MANDELBOIM, N. BENVENISTY

PNAS, 2002, 99:9864
Embryonic Stem Cell Immunogenicity Increases Upon Differentiation After Transplantation Into Ischemic Myocardium


_Circulation. 2005;112:I-166-I-172_

Graft infiltration of immune cells after transplantation of _in vivo_ differentiated ESCs

![Images showing infiltration of immune cells](image-url)

### Cellular Composition of Graft Infiltrates Over Time After Intramyocardial ESC Injection

<table>
<thead>
<tr>
<th></th>
<th>1 Week*</th>
<th>2 Weeks*</th>
<th>4 Weeks*</th>
<th>8 Weeks*</th>
<th>2 Weeks After HTX†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sham</td>
<td>Syn</td>
<td>Allo</td>
<td>Sham</td>
<td>Syn</td>
</tr>
<tr>
<td>CD3</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>CD4</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>CD8</td>
<td>+/-</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
<td>+/−</td>
</tr>
<tr>
<td>B220</td>
<td>+/-</td>
<td>+</td>
<td>+</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>CD11c</td>
<td>+/-</td>
<td>+/−</td>
<td>+/−</td>
<td>−</td>
<td>+/−</td>
</tr>
<tr>
<td>Mac-1</td>
<td>++</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Gr-1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Immunosuppressive Therapy Mitigates Immunological Rejection of Human Embryonic Stem Cell Xenografts


IN VIVO VISUALIZATION OF HESC SURVIVAL

PNAS, 2008, 105:12991
THE 2014 IMMUNOLOGICAL CHALLENGE

2002 - 2010

- Allogeneic ESCs are Immunogenic : alloimmunity

2010 - 2012

- Reprogrammed iPSCs are immunogenic : autoimmunity
- Gene Transduced cells are immunogenic: autoimmunity
- MSCs are immunogenic & Immunoregulatory

Toward an IMMUNOLOGICALLY EDUCATED CHOICE OF SC

Endomyocardiac stem cells ?
STEM CELLS THERAPEUTICS

Compelling choice
treating, repairing, restoring, maintaining or enhancing organ function

STEM CELLS THERAPEUTICS

Stem Cells

Embryonic  Cord Blood  Adult  Induced-Pluripotent

FAST TRACK
CARDIAC THERAPEUTICS

Terminal Heart Failure
11 million patients (USA & EU)
5000 new case/year
18% mortality rate

Heart Transplantation
unique treatment (4000/year)* donor
scarcity * high cost
* heavy immunosuppression

IMMUNE PRIVILEGED vs IMMUNOGENICITY

Autologous
* unavailable
* limited disponibility
Not over the shelf

Allogenic
* more available
* less limited
could be over the shelf
Human Cardiac-derived Stem/Progenitor Cells

Pluripotency (Oct4, Sox2, Nanog) / Stem (SSEA 1/4, CD 73/90 105/166) + Cardiac Lineage Markers (Mef2c, Nkx2.5, Islet-1, GATA-4)

Promote cardiac repair
Restore cardiac function

PBS
hCPC

Cardiomyocytes
Endothelial cells
Smooth muscle cells

Allogeneic Immunity: Cellular Responses

**INDUCTION/TRIGGERING/EFFECTOR PHASE (REJECTION)**

**Allogeneic T cells (CD8/CD4)**
- Immediate Response
  - 1-5% of Circulating T cells vs <0.5% for Ag
  - 2 Pathways
    - Direct
    - Indirect

**Natural Killer cells**
- Immediate Response
  - 2 activities
    - Cytotoxicity
    - Cytokine secretion
    - activating/inhibitory receptors
    - ligand
    - Perforins
    - Granzymes
    - Lysis
    - IFN\(_\gamma\) & TNF\(_{\alpha}\)
T cell response to allo-hCPC: hCSC are Immunomodulators

1) IL-10 producing CD4+ cells

2) Prevent CD4 and CD8 T cells activation (IFNγ, IL-2) but promote IL-10

3) Regulatory T cells activation & expansion (CD4+/CD25high/CD127low/FoxP3high)
Co-stimulatory/Co-regulatory molecules on hCPC

HLA-class I
hCPC 99.7%
IFN-γ-hCPC 99.8%

HLA-class II
hCPC 1.8%
IFN-γ-hCPC 78.5%

IOW IMMUNOGENIC PROFILE

Programmed Cell Death Ligand 1 (PD-L1)

- Expressed on leukocytes and non-hematopoietic cells in lymphoid and non-lymphoid tissues
- Binding partner for PD-1 and B7-1 (CD80)
- Exert vital and diverse range of immunoregulatory roles in T cell activation, tolerance, and immune-mediated tissue damage
- Co-stimulate T cell proliferation and IL-10 secretion in response to polyclonal and allogenic stimuli
- PD-L1/PD1 and PD-L1/B7-1 control engraftment of solid organs, including heart, and GVHD
- Control regulatory T cell induction and expansion
- Expression on non-hematopoietic donor cells is essential in acquired tolerance to fully allogenic vascularized cardiac grafts

From Butte MJ et al, Immunity, 2007
PD-L1 orchestrates interactions of allogenic CPC with T cells

1) Allo-Treg generation
2) Allo-Treg expansion blockage (anti PD-L1)

3) Immune-modulation (si RNA)
4) IL-10 production inhibition (siRNA)

Main findings:

- Do not trigger a conventional allogeneic Th1 and Th2 response
- Trigger a PD-L1-dependent regulatory T cell response
- Have immunomodulatory capacities

- Low immune risk even within inflammatory environment
- Reparatory by promoting Treg and by controlling immune-mediated injury
- PD-L1 immune-biomarker (identify & select low/risk allogenic cardiac repair cells)

PD-L1 expressing hCSC are attractive Low risk/high benefit cells for cardiac repair clinical translation
Susceptibility of cardiac progenitor cells to allogeneic NK cell lysis
Mechanisms of NK-mediated killing

Natural cytotoxicity
- Activating and inhibitory receptors
- NK
- Target cell
- Ligand
- Granules
- Lysis
- Perforins
- Granzymes

Antibody-dependent cell-mediated cytotoxicity (ADCC)
- Fc-RIII (CD16)
- NK
- Target cell
- Granules
- Perforins
- Granzymes
- Lysis
- Allo-antibodies
  - anti-HLA I
  - anti-HLA II
  - anti-non-HLA

Death receptor pathways
- Death Receptor (FAS, TRAIL-R)
- NK
- Target cell
- Ligand
- Caspases
- Apoptosis

NK optimum activity occurs upon priming by cytokines including IL2, IL15, IL12-18
Expression of NK receptors Ligands by hCPC and IFNγ-hCPC

- Both hCPC and IFNγ-hCPC might be susceptible to NK cell lysis
- Inflammatory conditions increase expression of ligands for inhibitory receptors
NK cell degranulation and cytotoxicity towards hCPC

Allogeneic co-cultures:
- CD107a expression by NK: marker of NK degranulation
- 7-AAD staining of hCPC: marker of cell death

- hCPC and IFNγ-hCPC are only susceptible to cytokine-activated NK lysis
- Inflammatory conditions protect hCPC against NK lysis
Implication of death receptors

hCPC express death receptors

IFNγ-hCPC are sensitized to induced cell death

Use of blocking antibodies

- IFNγ treatment sensitizes hCPC to TRAIL-induced cell death
- hCPC are killed by NK cell through natural cytotoxicity
Engagement of NK cells in immune synapses with hCPC

- NK form less conjugates and less polarized synapses with IFNγ-hCPC
Conclusions and Perspectives

1) hCPC are susceptible to NK cells killing but are not a preferred target

2) Inflammatory conditions sensitize to TRAIL-induced cell death but generally protect hCPC from NK-mediated lysis
   - Less conjugates and less polarization
   - Higher expression of ligands for NK-inhibitory receptors

3) Nkp46 is the main NK activating receptor responsible for hCPC lysis

ALLOGENEIC hCPC ENGAGE T & NK CELL PATHWAYS

Are allogeneic hCPC immunologically safe? Beneficial?
Allogenic Cellular Responses

No CD8 activation

Protection from moderate NK lysis

Lauden et al, Cardiovascular, in press 2014

“Allogenic-driven benefit”
“First-in human” European Clinical Trial
Acknowledgement

Luis Borlado
Pilar Sepulveda
Itziar Palacios

Coretherapix (Madrid, Spain)

Bernardo Nadal-Ginard
Liverpool John Moores University (Liverpool, UK)

Dominique Charron
Reem Al Daccak

Laura Lauden
Wahid Boukouaci
Noemie Dam
Thank You
NOW THIS IS NOT THE END
IT IS NOT EVEN THE BEGINNING OF THE END
BUT IT IS PERHAPS, THE END OF THE BEGINNING
Impact of donor specific anti-HLA antibodies on graft failure & survival after reduced intensity conditioning regimen unrelated Cord Blood Transplantation.

A Eurocord, SFGM-TC and SFHI study

Dominique CHARRON  laboratoire «Jean Dausset »
Hopital Saint-Louis ; Paris

On behalf of


Hematologica 2014
Patients Selection Criteria

- UCBT from 2000 to 2010
- Single and double UCBT, performed in France
- Reduced Intensity Conditioning regimen
- Availability of pre-transplant serum samples to evaluate DSA

294 patients were evaluable

Median Follow-up, months: 36 (3-98)

<table>
<thead>
<tr>
<th>Category</th>
<th>Count</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Children, n</td>
<td>60</td>
<td>20%</td>
</tr>
<tr>
<td>Female gender, n</td>
<td>136</td>
<td>46%</td>
</tr>
<tr>
<td>Non malignant disease, n</td>
<td>50</td>
<td>17%</td>
</tr>
<tr>
<td>Previous Auto-HSCT, n</td>
<td>112</td>
<td>38%</td>
</tr>
</tbody>
</table>
RESULTS

- Neutrophil engraftment
  - 78% (median time: 20 days [13 – 60])
  - 73 graft failure
    - 8 DSA (5 single, 3 double)
  - Engraftment depending of Ab status:

- Multivariate analysis:
  - DSA before engraftment: only factor independently associated with engraftment (p=0.002, HR:1.69)
  - Graft failure was associated with increased TRM and lower OS

No DSA: 77%
DSA: 44%  

p=0.003
Transplant-Related Mortality at 1-year

Cumulative Incidence of TRM

- No DSA, n=280
- DSA, n=14

No DSA: $32 \pm 3\%$

DSA: $46 \pm 9\%$

$p=0.06$
The presence of DSA(HLA) is associated with delayed engraftment and graft failure.

Trend towards increased TRM and lower OS.

Role of Ab intensity:
- Higher Ab Titer associated with lower engraftment.
- Further studies (larger groups) to establish a threshold for CB selection.

CB selection:
- HLA compatibility, TNC, anti-HLA Ab.
Figure 1. Summary of the Association Between the HLA-DRA, DRB, and DQ Loci and PD. P-values for the 102 SNPs, derived from univariate logistic regression models (additive model), are presented on the left Y-axis on the logarithmic scale according to the position of the SNPs on chromosome 6 (X-axis). Each SNP is depicted by a dot whose colour reflects linkage disequilibrium estimates ($r^2$) with the top SNP (rs660895 in purple); linkage disequilibrium estimates were calculated based on 1622 subjects included in the analysis. The correlation between rs660895 and other SNPs was low to moderate. The blue line represents the recombination rate (right Y-axis). The plot was produced with the LocusZoom software (29).
IFN-\(\gamma\) induction of MHC-I in human ES cells is dose and time dependent
Differenciation of allogeneic mesenchymal stem cells induces immunogenicity & limits their long-term benefits for myocardial repair

Xi-Ping Huang & coll  Circulation .2010 ;122:2419-242

- Wistar and Lewis rats
- MSCs untreated vs MSCs cultured with 5-azacytidine (to induce myogenic differentiation)

Flow cytometric & mRNA evaluation of MHC Ia, Ii and CD86 is increased by >30% upon differentiation while MHC Ib is decreased

------ GFP+ MSCs Implanted into the infarcted myocardium 3 weeks after MI express low level of MHC Ia when undifferenciated (alpha-SMA-) at day seven & high level of MHC Ia when differenciated (alpha-SMA+) at Day 14 (differenciated)

------ Implanted Allogeneic MSCs induce a local immune reaction after 7 days and are not detected in situ after 5 weeks

------ Allogeneic MSCs restore cardiac function as effectively as Syngeneic MSCs for 3 months but not 6 months after implantation

While immunoprivileged in their undifferenciated state MSCs become immunogenic in vitro & in vivo when differenciated (biphasic immune response)
Cardiac Stem Cells

hCSC purified & expanded from cardiac samples

C-kit-positive cells
Upon injection in experimental MI
Regeneration (Brdu+ cells)
Differentiation (3 lineages)
Restoration (cardiac function)
Beltrami, AP et al, Cell, 2003

Cardiac Stem Cells Therapy

Clinical trials using AUTOLOGOUS cells
(Feasibility/efficiency)

SCIPIO Bolli et al. Lancet.2011

ALLOGENIC cells are more REALISTIC
Immediate availability (off the shelf) Manufacturing Quality/Safety

IS ALLOGENICITY A BARRIER TO SUCCESS?

Experimental Interrogation

Allo-immune Response To Cardiac Stem/Progenitor Cells
Mechanisms involved in natural cytotoxicity

Use of blocking antibodies in cytotoxicity assays at 10:1 E:T ratio

Activating NK receptors

- **Nkp46** is the major NK activating receptor responsible for hCPC and IFNγ-hCPC lysis

<table>
<thead>
<tr>
<th>Ligands for inhibitory NK receptors</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG2a</td>
</tr>
<tr>
<td><img src="chart1.png" alt="Graph" /></td>
</tr>
</tbody>
</table>

- Blocking of HLA I on IFNγ-hCPC increases their susceptibility to NK cell lysis
- Increase of HLA I on IFNγ-hCPC could explain their resistance to NK killing
Modulation of NK cell proliferation by hCPC

Allogeneic cocultures: CFSE labeled NK cells + IL15 for 6 days

- In allogenic settings hCPC modulate NK cytokine-induced proliferation
- This modulation is more pronounced under inflammatory conditions
Modulation of NK cell cytotoxicity by hCPC

IL15-activated NK and hCPC or IFNγ-hCPC

NK - K562 conjugates

NK + K562

NK degranulation towards K562

K562 specific lysis

- hCPC modulate capacity of allogeneic NK to form conjugates with, to degranulate towards and to lyse a well-known target
- This modulation is cell-contact dependent and seems higher with IFNγ-hCPC.