Hôpitaux Universitaires
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European Frontiers in Ocular Pharmacology

Catania January 15 ,2015

« IMMUNOREGULATION & PERSONALIZED IMMUNOGENETICS »
FROM HLA TO REGENERATIVE THERAPY

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HISTOCOMPATIBILITY – IMMUNOGENETICS
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LabEx TRANSPLANTEX

HLA , IMMUNOGENETICS & MEDICINE

XX th Century

HLA, MHC ,Cytokines,Receptors….

…TRANSPLANTATION, AUTOIMMUNITY,INFECTIONS

XXI st Century

HLA & MEDICINE (Schizophrenia/Parkinson …)

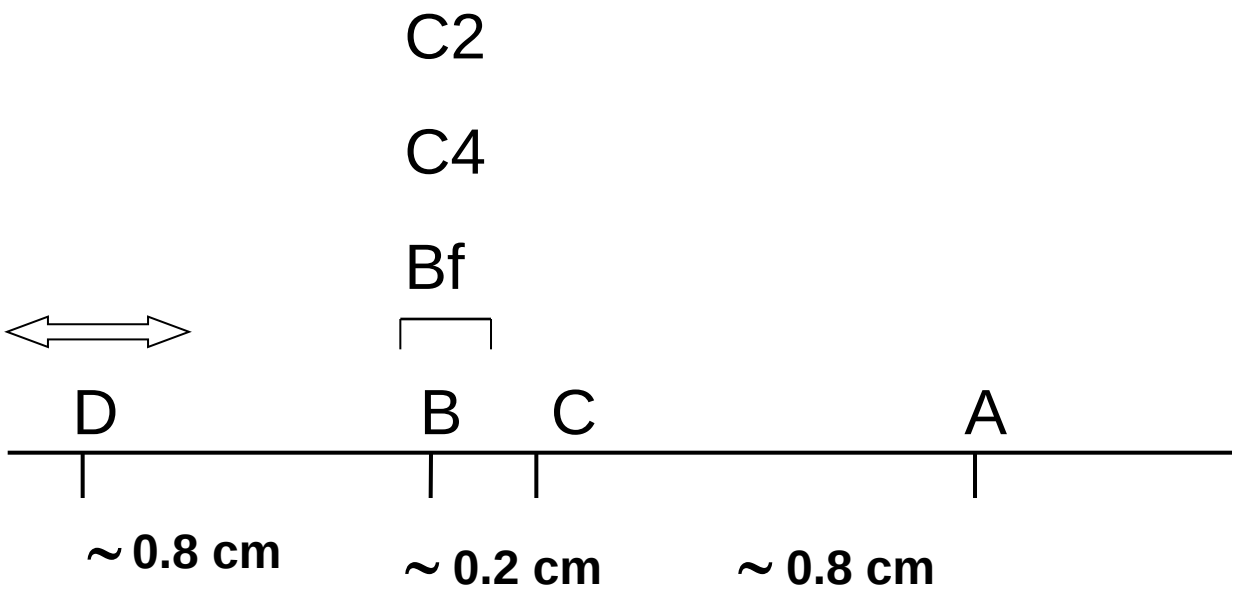
IMMUNO PHARMACOGENETICS (Abacavir/Carbamazepin ,Allopurinol…)

REGENERATIVE MEDICINE/CELL & IMMUNO THERAPIES

TOWARDS

«SYSTEM BIOLOGY/ PERSONALIZED SYSTEM MEDICINE »

HLA MAP 70’S



Pre DNA area

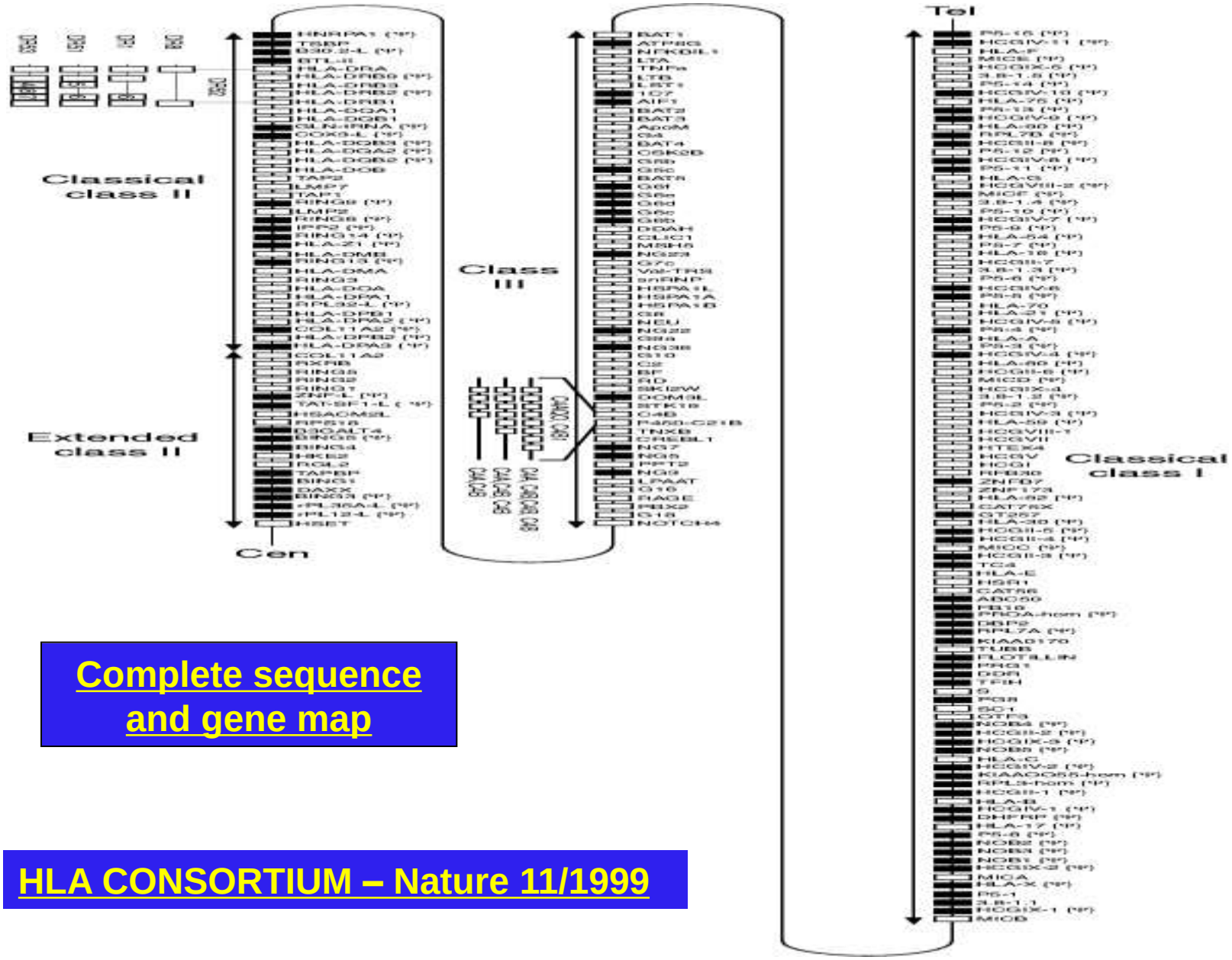
- Serology

- Leucoagglutination
- Microlymphocytotoxicity

- Genetics

- Familial segregation
- Population study (frequency)

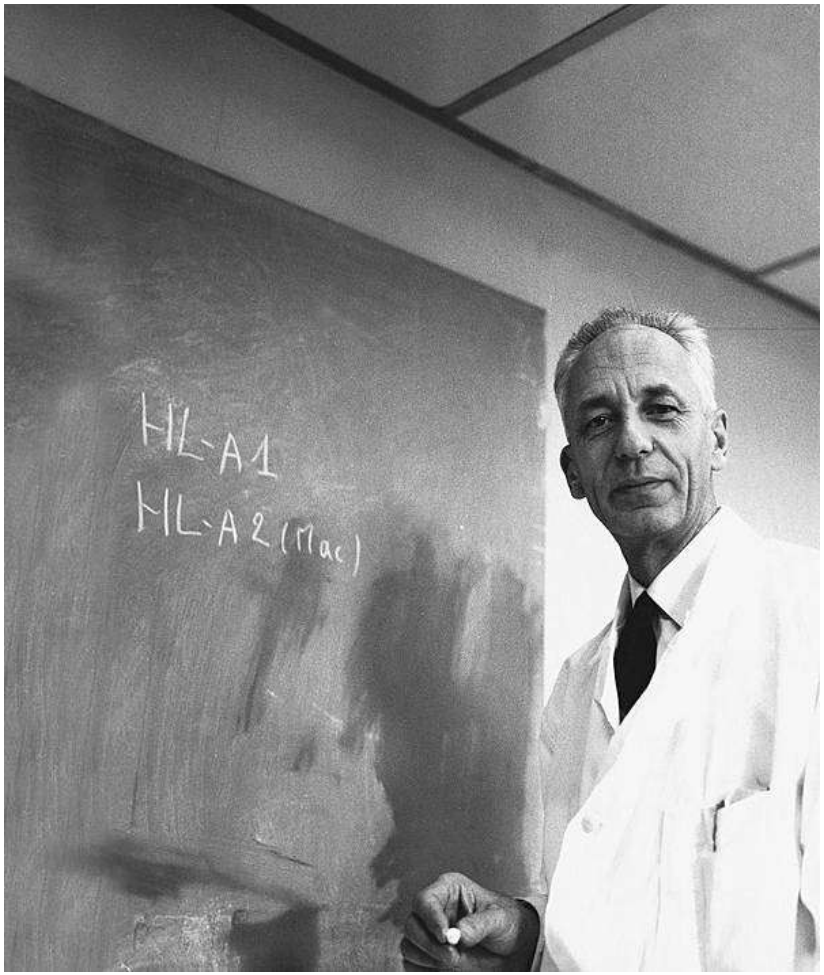
MHC / HLA



Complete sequence and gene map

HLA CONSORTIUM – Nature 11/1999

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

HLA HISTO-INCOMPATIBILITY/ALLOGENICITY IN TRANSPLANTATION

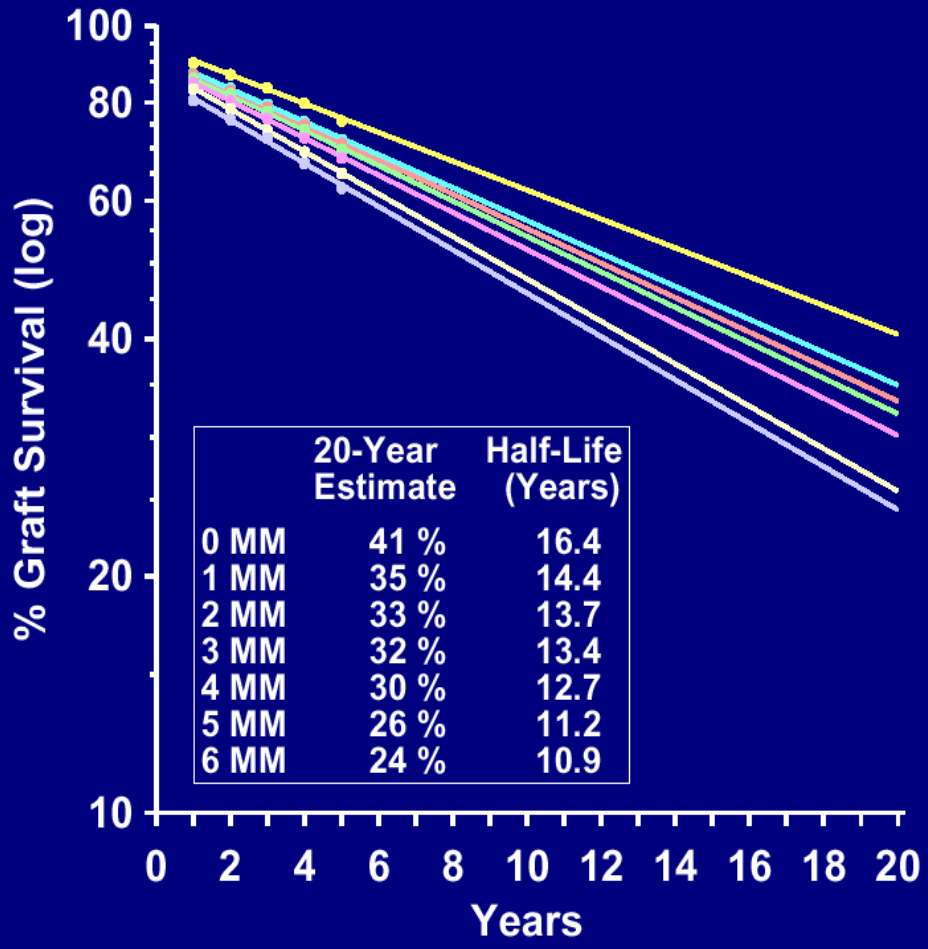
XXth century

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

HLA MATCHING - HLA TYPING

HLA-A+B+DR Mismatches

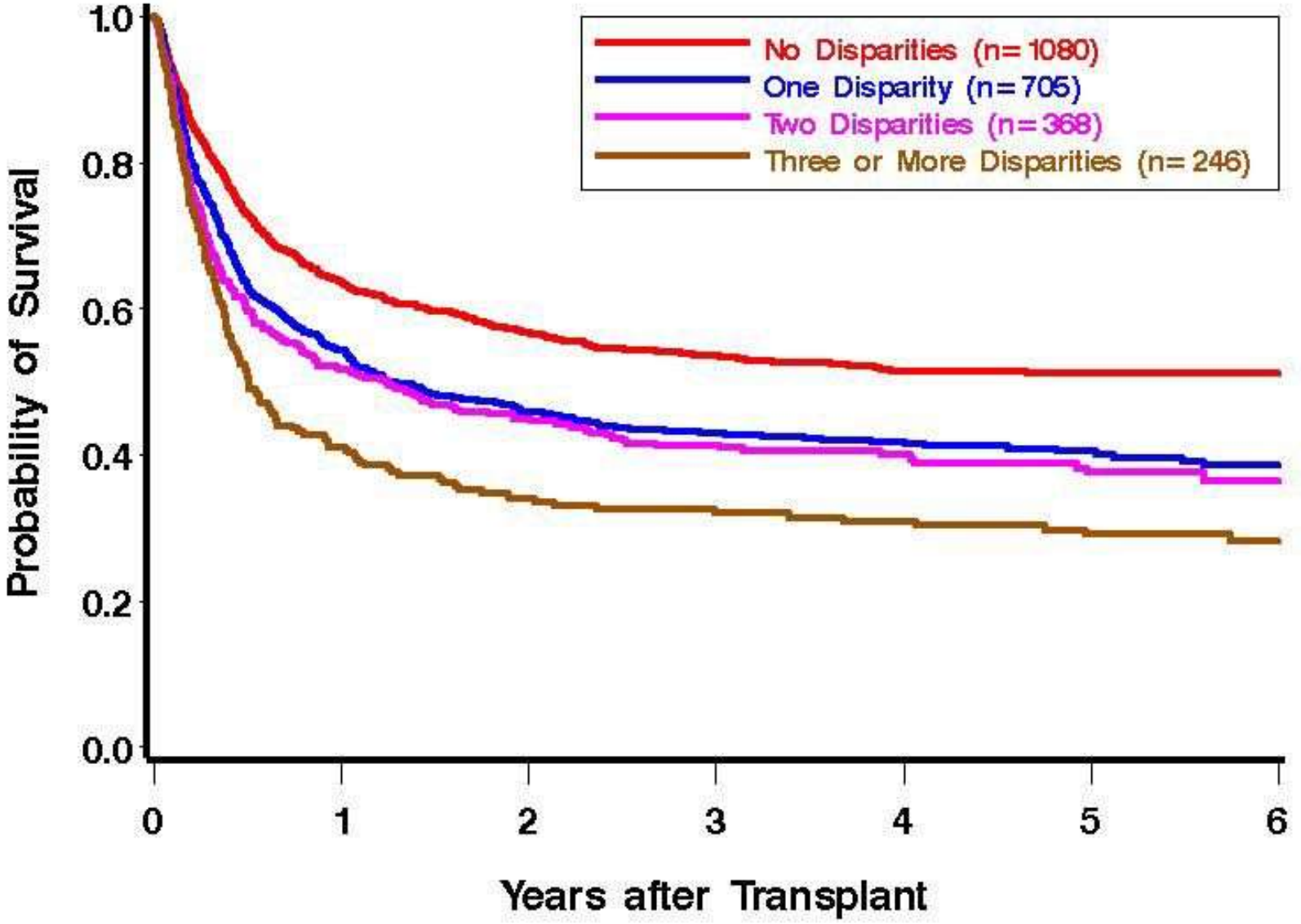
Deceased Donor, First Kidney Transplants 1985-2006



	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

BMT SURVIVAL according to the Number of HLA DISPARITIES



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

XXIth CHANGE OF PARADIGM

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

Carmen Lefaucheur, Alexandre Loupy*, Dewi Vernerey, Jean-Paul Duong-Van-Huyen, Caroline Suberbielle, Dany Anglicheau, Jérôme Vérine, Thibaut Beuscart
, Dominique Nochy, Patrick Bruneval, Dominique. J. Charron
, Michel Delahousse, Jean-Philippe Empana, Gary S Hill, Denis Glotz, Christophe Legendre, Xavier Jouven*

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° /°)

PATHOLOGICAL & IMMUNOLOGICAL PHENOTYPES
DISTRIBUTION OF THE 4 REJECTION PATTERNS

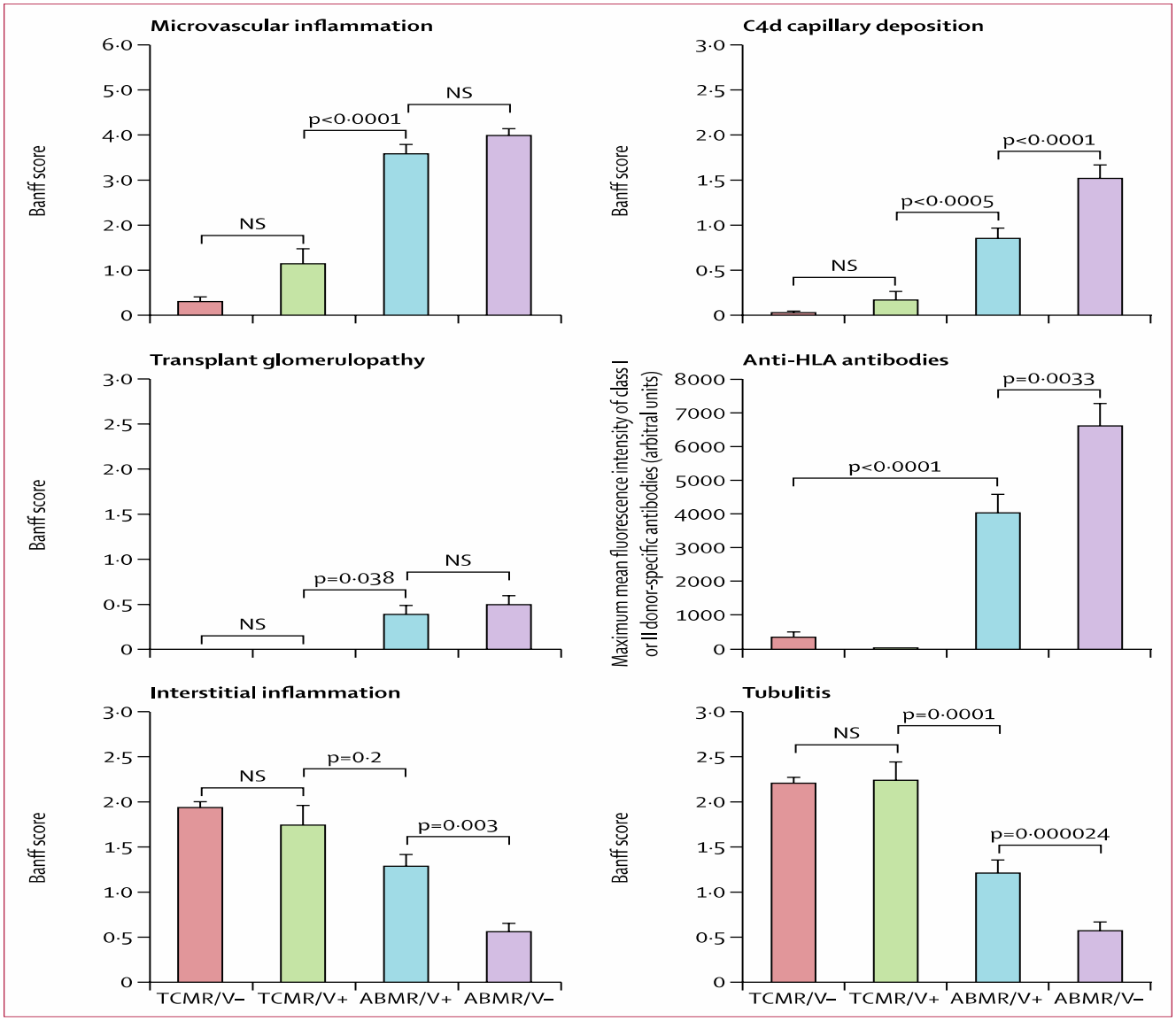


Figure 2: **Comparison of morphological and immunological variables in the four rejection patterns**
Bars represent SD. NS=not significant. TCMR/V–=T cell-mediated rejection without vasculitis. TCMR/V+=T cell-mediated vascular rejection. ABMR/V+=antibody-mediated vascular rejection. ABMR/V–=antibody-mediated rejection without vasculitis.

Cellular (Tcell) Rejection **Antibody Mediated Rejection**

Endarteritis --

Endarteritis +

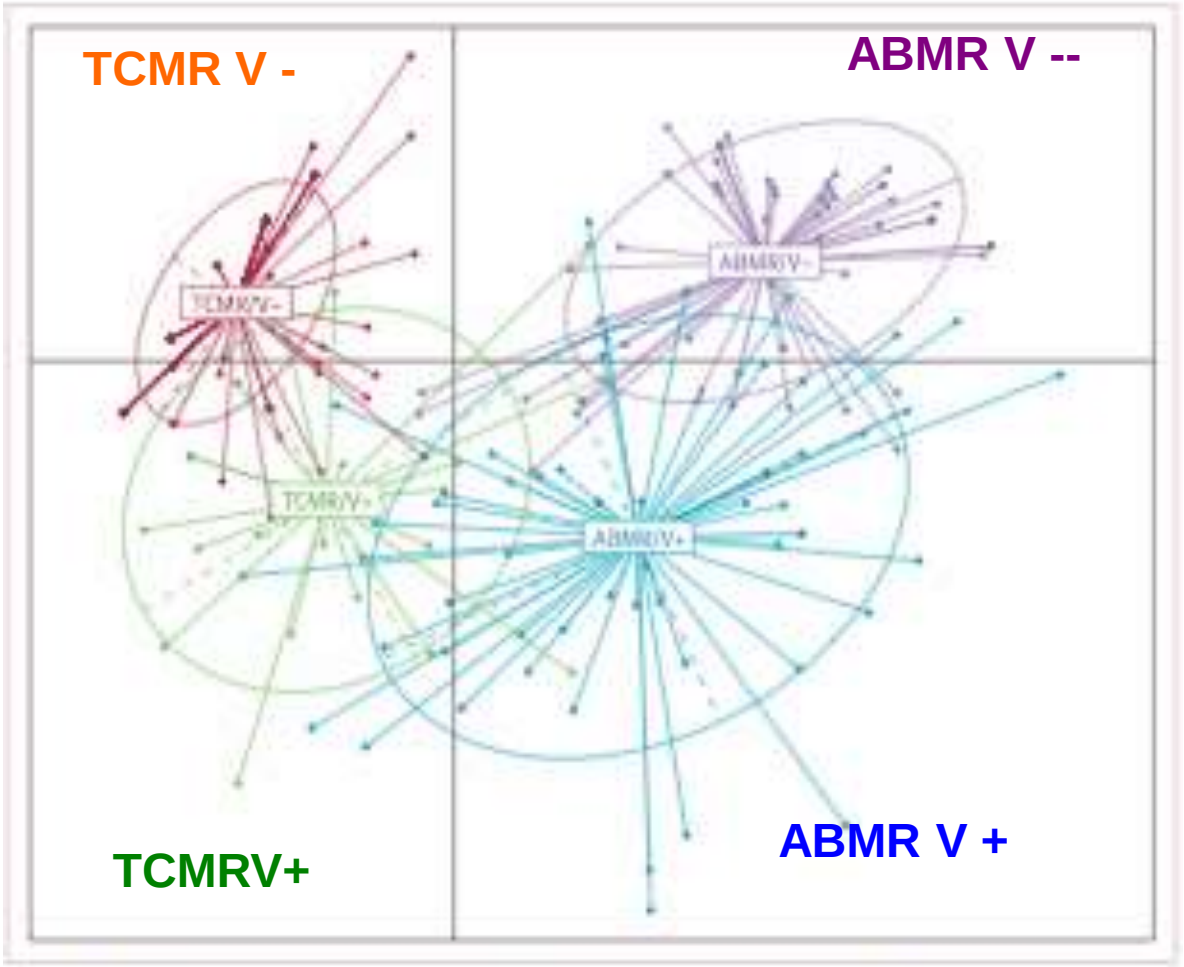
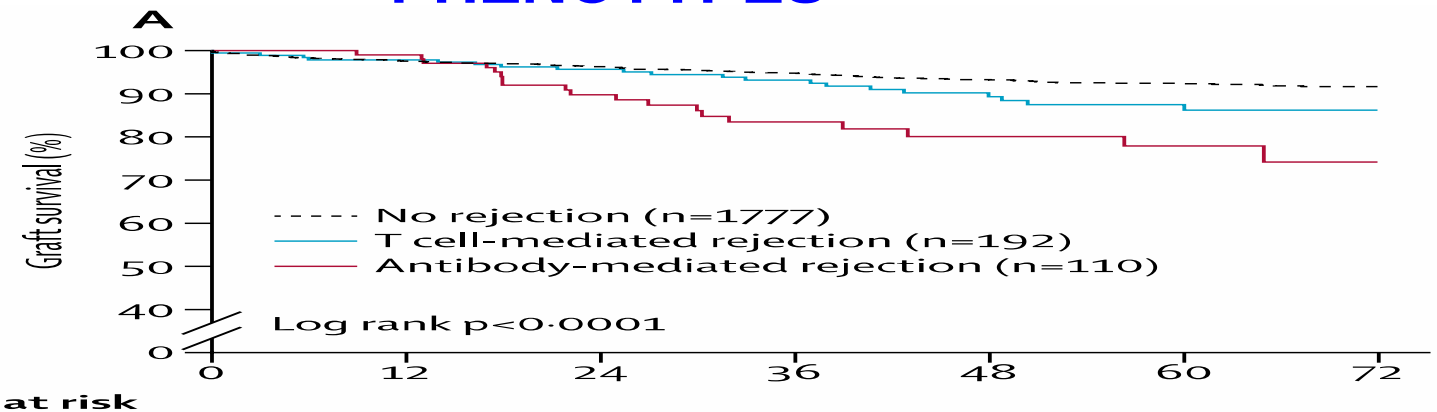


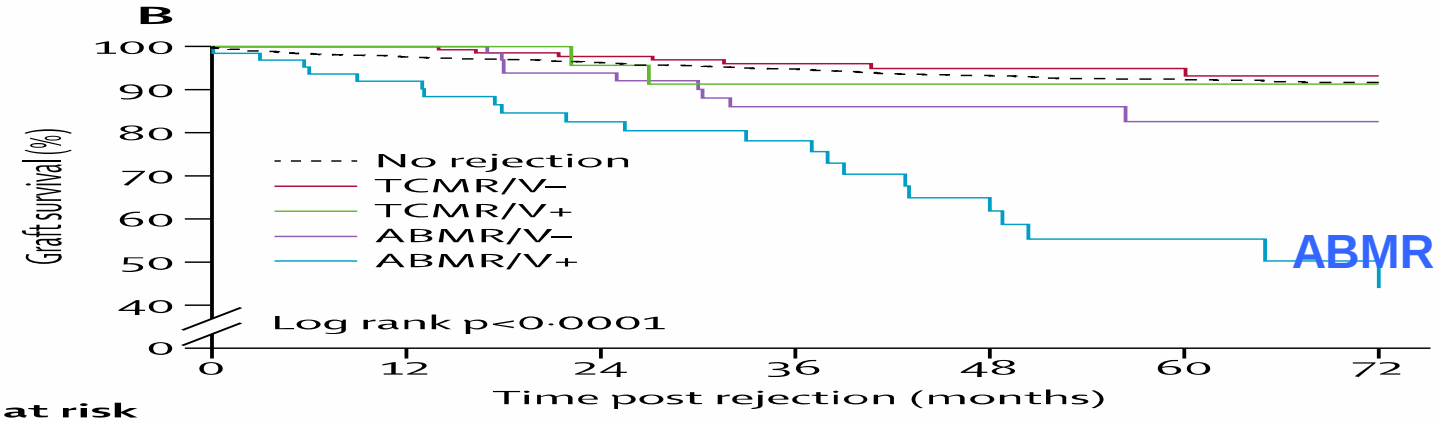
Figure 2: Identification of four distinct rejection patterns according to clinical, histological, and immunological variables

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 recognised by the international Banff classification. The vertical axis defines the presence or absence of lesions of endarteritis (appendix).

GRAFT SURVIVAL IN THE 4 REJECTION PHENOTYPES



Number at risk							
No rejection	1777	1600	1408	1152	933	673	473
T cell-mediated rejection	192	182	163	134	101	71	37
Antibody-mediated rejection	110	100	78	61	40	26	12



ABMR V+

Number at risk							
No rejection	1777	1600	1408	1152	933	673	473
TCMR/V–	139	136	121	101	77	54	29
TCMR/V+	26	26	23	20	15	12	4
ABMR/V–	73	68	56	42	28	19	7
ABMR/V+	64	52	41	32	21	12	9

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.,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) 54
  ° /°
- C1q - DSA + (239) 93
  ° /°
- C1q - DSA - (700) 94
  ° /° (p<0.OO1)

C1q + correlates with AMVR, microvascular inflammation & C4d deposition

ELSEVIER

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

DOI 10.1016/j.coi.2005.07.007

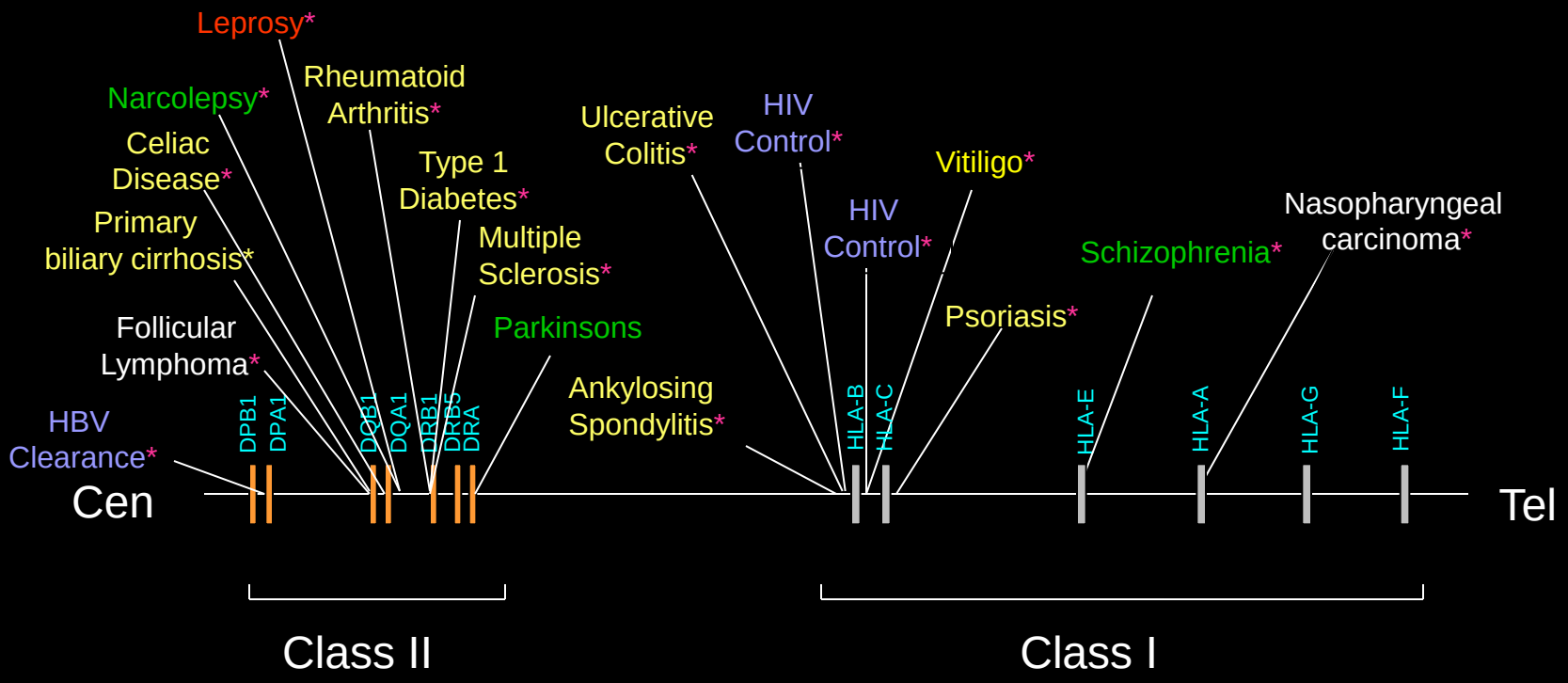
HLA, MHC AND MUCH MORE….

…TRANSPLANTATION, AUTOIMMUNITY AND MUCH MORE

HLA in MEDICINE

IMMUNOPHARMACOGENETICS
REGENERATIVE MEDICINE
SYSTEMS BIOLOGY

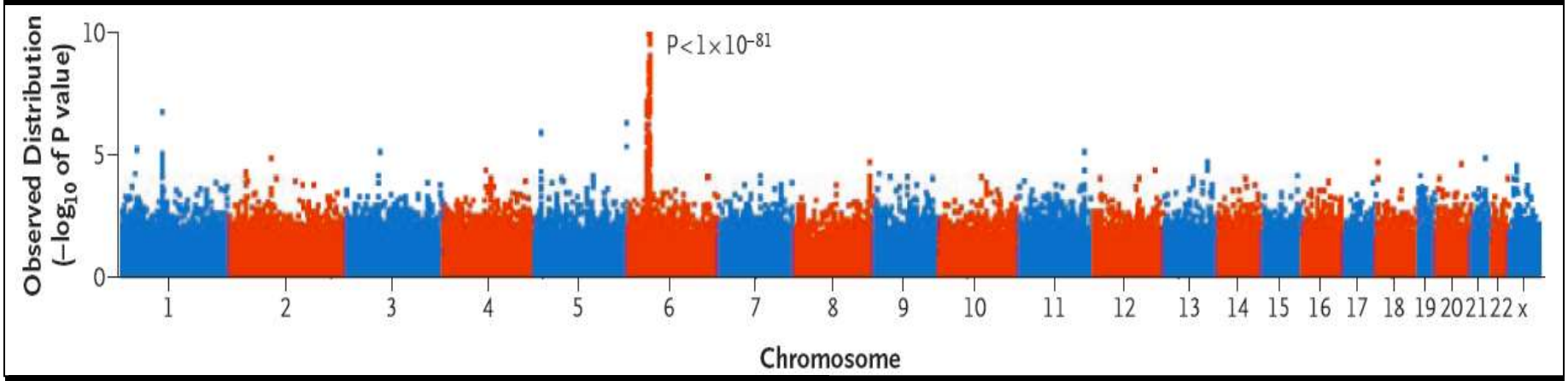
The human MHC: epicenter of disease association as determined by GWAS



Autoimmune
Cancer
Viral
Bacterial
Others

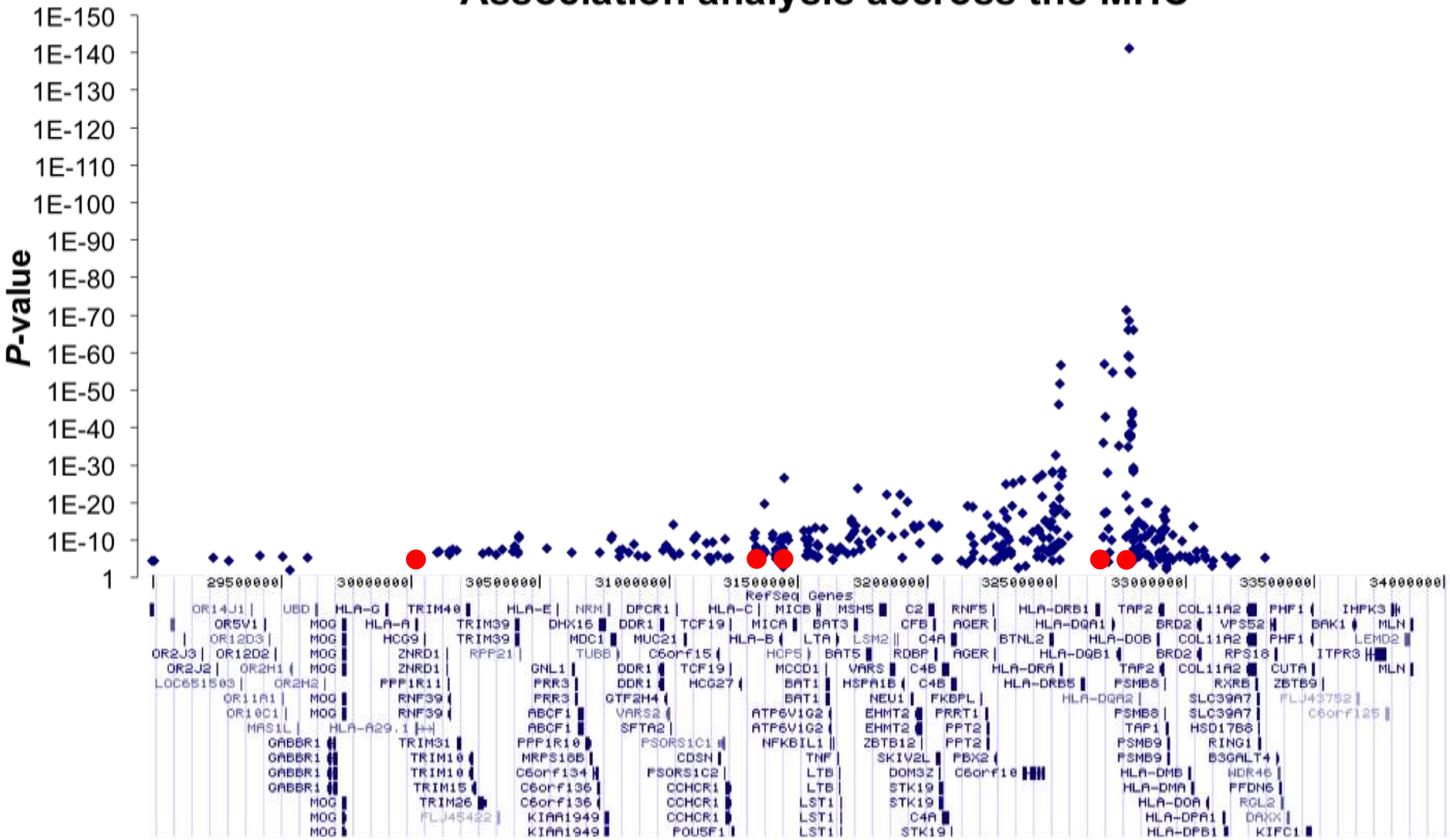
* Top Hit

Overview of the Primary GWA Scan Involving 931 Family Trios



International Multiple Sclerosis Genetics Consortium, N Engl J Med 2007

Type I diabetes
Association analysis accross 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

Ismaïl Ahmed[1,2], Ryad Tamouza[3], Marc Delord[4], Rajagopal Krishnamoorthy[5], Christophe Tzourio[1,2], Claire Mulot[6,7], Magali Nacfer[6,7], Jean-Charles Lambert[8], Philippe Beaune[6,9], Pierre Laurent-Puig[6,9], Marie-Anne Loriot[6,9], Dominique Charron[3], Alexis Elbaz[1,2]

Mov Disord 2012 ,9 1104-10

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: O.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

HLA ?

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


IMMUNO
PHARMACO
GENETICS

genetics

Immuno Pharmaco

Environment
External milieu

Foreign microbial pathogens

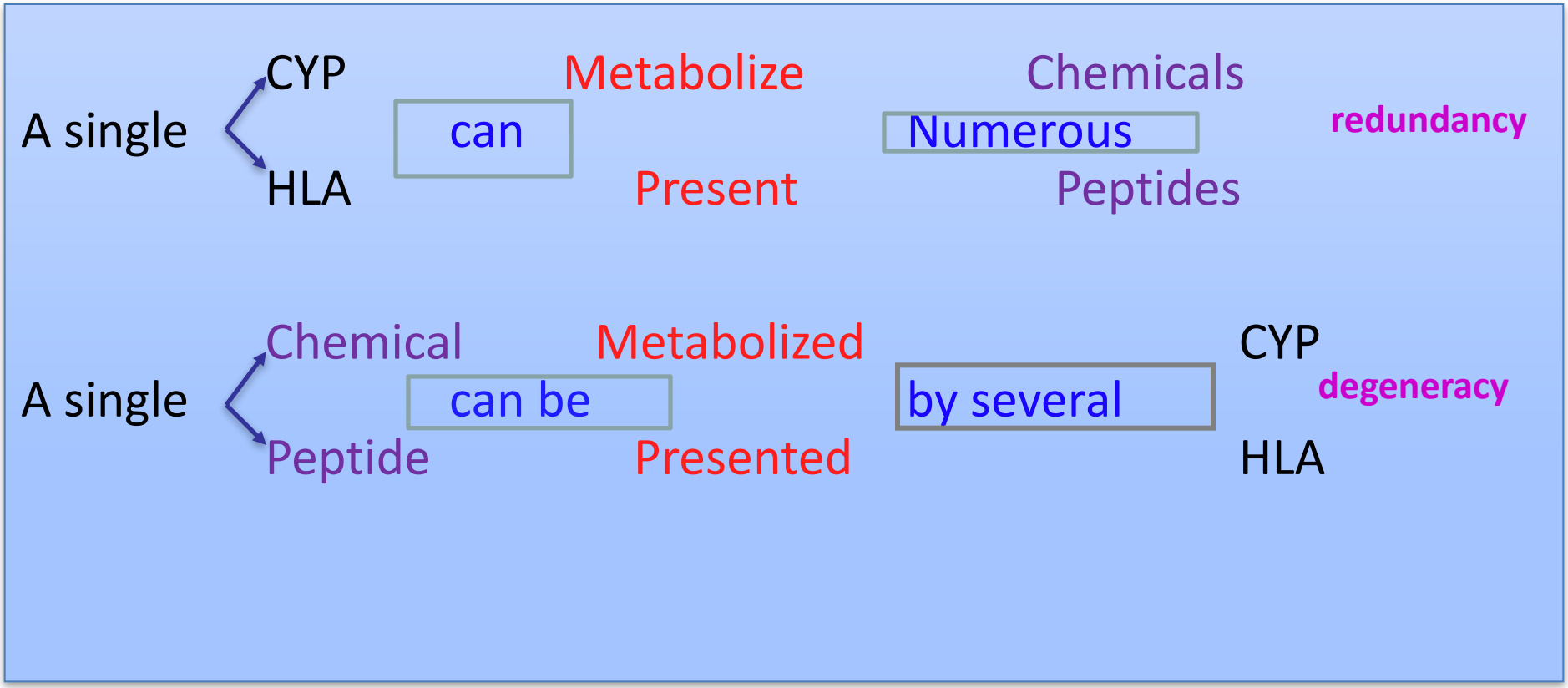
Foreign chemicals

Evolutionnary forces → fight against pathogenicity

Genetic systems	Adaptative Immunity	Xenobiotic metabolizing enzyme
	HLA-ABC / -DR/DQ/DP…	CYP + GST, UGT…
Diversity	> 2000 alleles	e.g. CytP450 > 50

Haplotypic organization - Population variability

HLA CYP


A single
CYP
HLA
can
Metabolize
Present
Chemicals
Numerous
Peptides
redundancy
A single
Chemical
Peptide
can be
Metabolized
Presented
by several
CYP
HLA
degeneracy

HLA and HIV

Susceptibility

Progression to AIDS

Rapid →	HLA-B35	(Homozygozity 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**

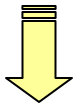
NEVIRAPINE → HSR (4,9%) HLA-DRB1*0101(pc 0.001)

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

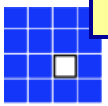
Viral suppression and cellular immunity **A.M Martin et al.** **AIDS 2005**

Recent Associations -- SCAR and HLA Variants

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



Genetic [HLA] marker variance across ethnicity & drug

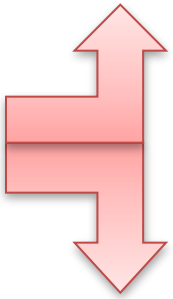


HLA

Immunogenetics
Impact

Diagnosis

(Susceptibillity)



Response to treatment

Biological Self

Therapeutic Self

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

- ❑ PLURI / MULTIPOTENCY
- ❑ SELF RENEWAL
- ❑ IN VITRO SPECIFIC DIFFERENCIATION
- ❑ **IMMUNE PRIVILEGE ?**

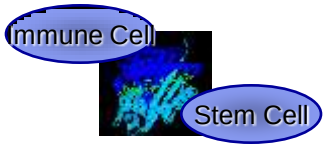
LIMITS OF IN VIVO ENGRAFTMENT AND FUNCTIONALITY

- ❑ **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**

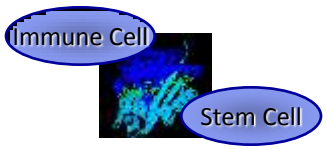
Toward an IMMUNOLOGICALLY EDUCATED CHOICE OF SCs



2002 -2008

ALLOGENEIC STEM CELLS ARE NOT IMMUNO PRIVILEGED

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

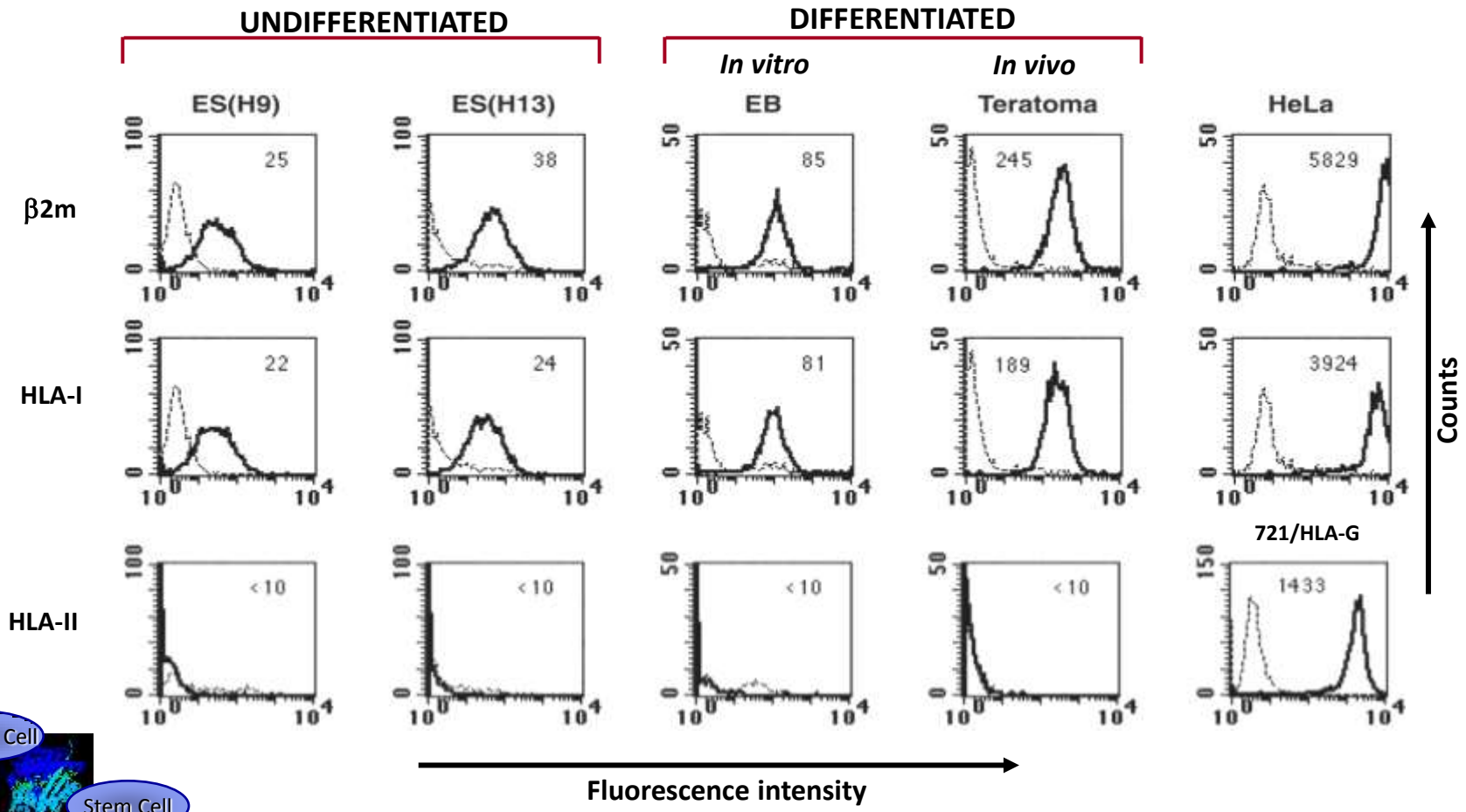


3 SUPPORTING PAPERS

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



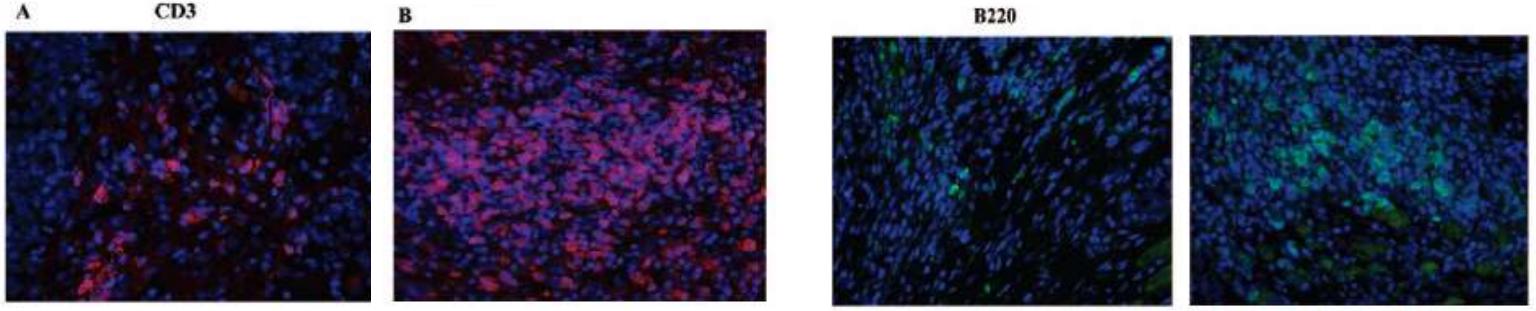
Embryonic Stem Cell Immunogenicity Increases Upon Differentiation After Transplantation Into Ischemic Myocardium

R-J Swijnenburg, M. Tanaka, H. Vogel, J. Baker,T. Kofidis, F. Gunawan, D.R. Lebl, A.D. Caffarelli, J.L. de Bruin, E.V. Fedoseyeva, R.C. Robbins

Circulation. 2005;112:I-166-I-172

Graft infiltration of immune cells after transplantation of *in vivo* differentiated ESCs

T cells



B cells

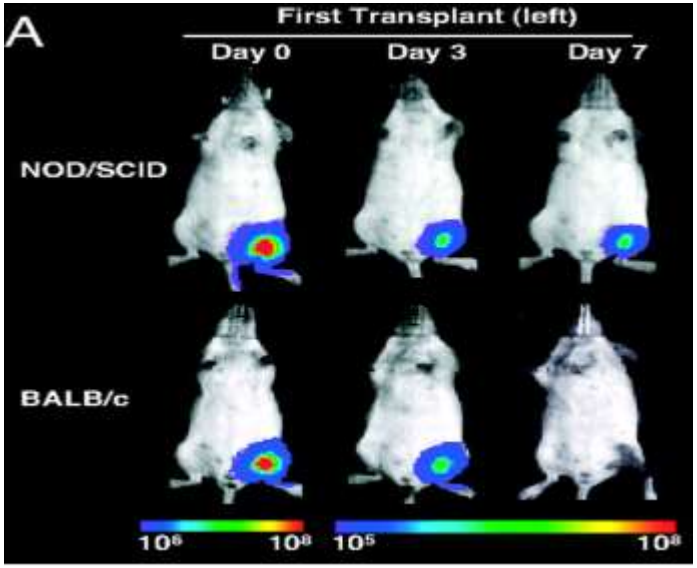
Cellular Composition of Graft Infiltrates Over Time After Intramyocardial ESC Injection

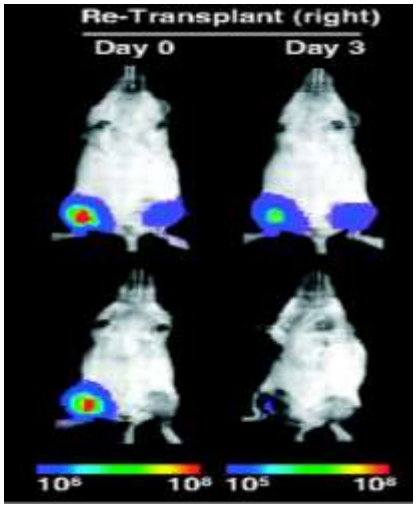
	1 Week*			2 Weeks*			4 Weeks*			8 Weeks*			2 Weeks After HTX†	
	Sham	Syn	Allo	Sham	Syn	Allo	Sham	Syn	Allo	Sham	Syn	Allo	Sham	Allo
CD3	+/−	+	+	+/−	+	++	+/−	+	+++	+/−	+	+++	+/−	+++
CD4	+/−	+	+	+/−	+	++	+/−	+	+++	+/−	+/−	+++	+/−	+++
CD8	+/−	+/−	+/−	+/−	+/−	++	+/−	+/−	+++	+/−	+/−	+++	+/−	++
B220	+/−	+	+	+/−	+	++	+/−	+	++	−	+/−	+	+/−	+++
CD11c	+/−	+/−	+/−	−	+/−	+	−	+	++	−	+/−	++	−	+
Mac-1	++	++	++	++	+++	+++	+	++	+++	+	++	+++	++	+++
Gr-1	+	+	+	+	+	++	+	+	+++	+	+	++	+	+++

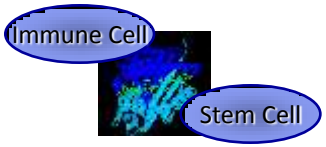
Immunosuppressive Therapy Mitigates Immunological Rejection of Human Embryonic Stem Cell Xenografts

R.J SWIJNENBURG, S. SCHREPFER, J.A. GOVAERT, F. CAO, K. RANSOHOFF, A.Y SHEIKH, M. HADDAD, A.J CONNOLLY, M.M DAVIS, R.C ROBBINS, J.C WU

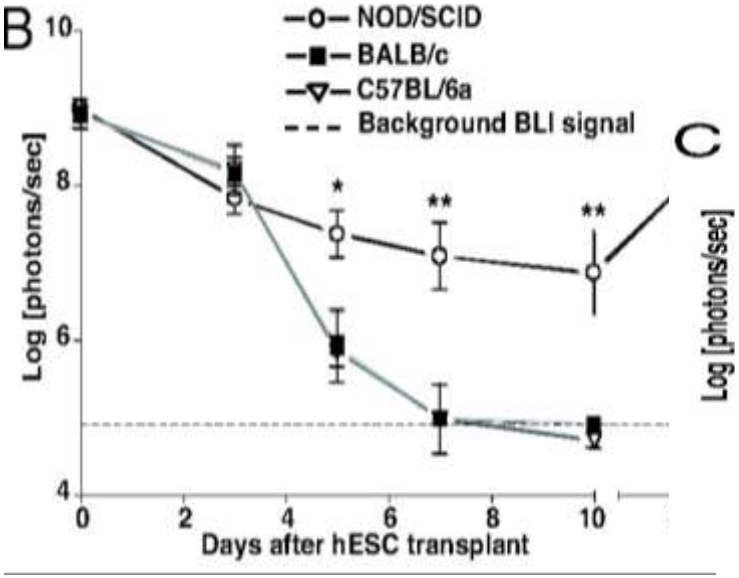
PNAS, 2008,105:12991

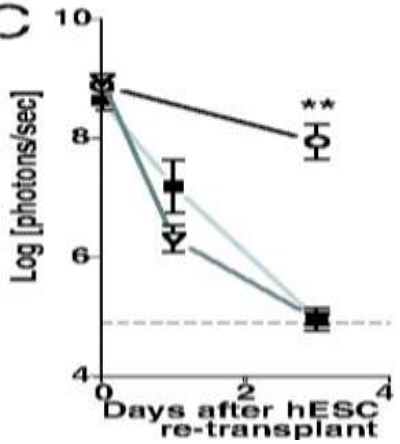






IN VIVO VISUALIZATION OF HESC SURVIVAL





THE 2014 IMMUNOLOGICAL CHALLENGE

2002 - 2010

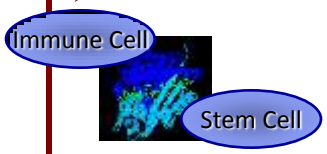
- **Allogeneic ESCs are Immunogenic : alloimmunity**

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

Stem Cells

- Embryonic
- Cord Blood
- Adult
- Induced-Pluripotent

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

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

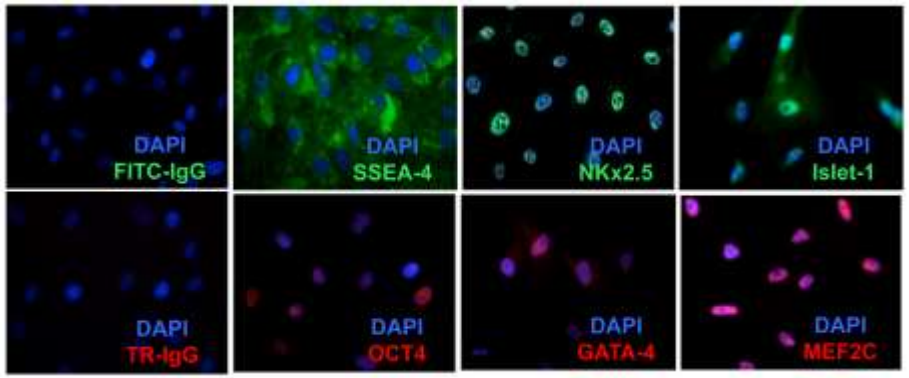
Autologous

*unavailable
*limited disponibility
Not over the shelf

Allogenic

*more available
*less limited
could be over the shelf

Human Cardiac-derived Stem/Progenitor Cells

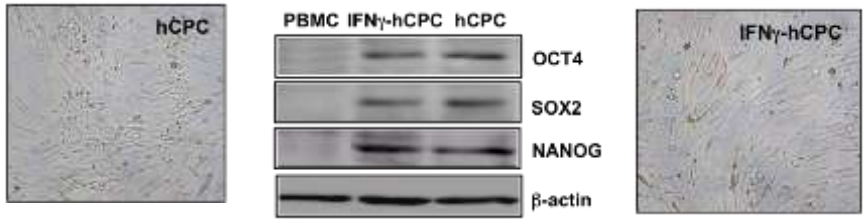


Pluripotency (Oct4,Sox2,Nanog) /Stem (SSEA 1/4,CD 73/90 105/166) + Cardiac Lineage Markers (Mef2c, Nkx2.5,iIslet-1, GATA-4)

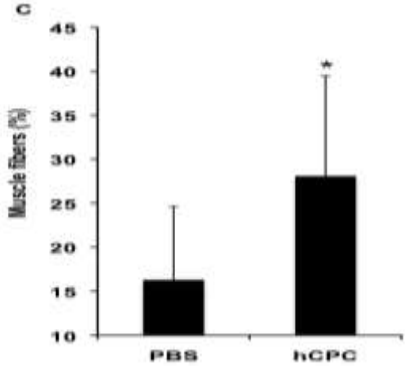
3% O_2

Non-manipulated

IFNγ-activated



hCPC Cardiac differenciation potency(in vitro)



Cardiomyocytes
Endothelial cells
Smooth muscle cells

Promote cardiac repair
Restore cardiac function

Lauden, L et al, Circ Res, 2013

Allogeneic Immunity : Cellular Responses

INDUCTION/TRIGGERING/EFFECTOR PHASE (REJECTION)

Allogenic T cells (CD8/CD4)

↓

Immediate Response

↓

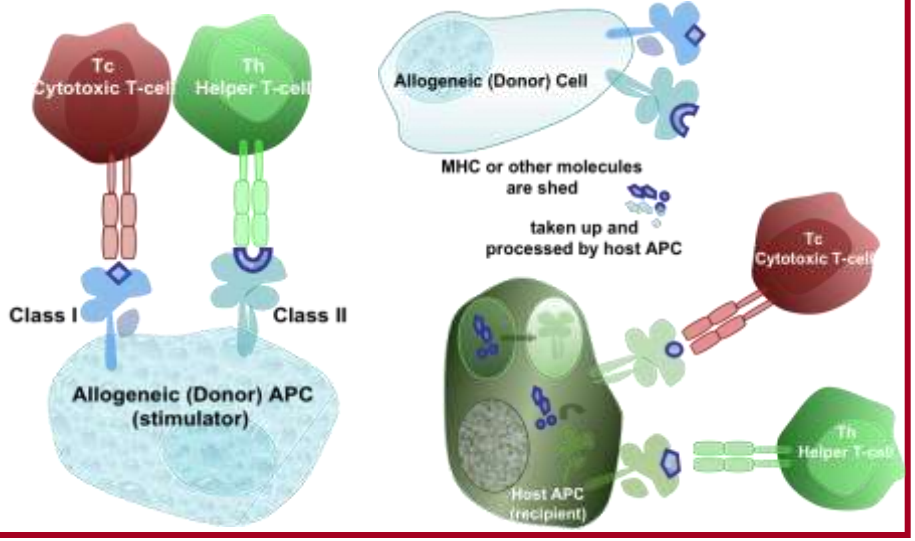
1-5% of Circulating T cells *vs* <0.5% for Ag

↓

2 Pathways

↙ ↘

Direct **Indirect**



Natural Killer cells

↓

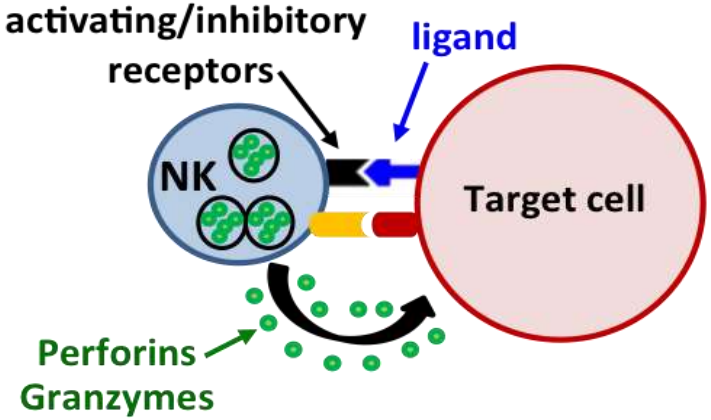
Immediate Response

↓

2 activities

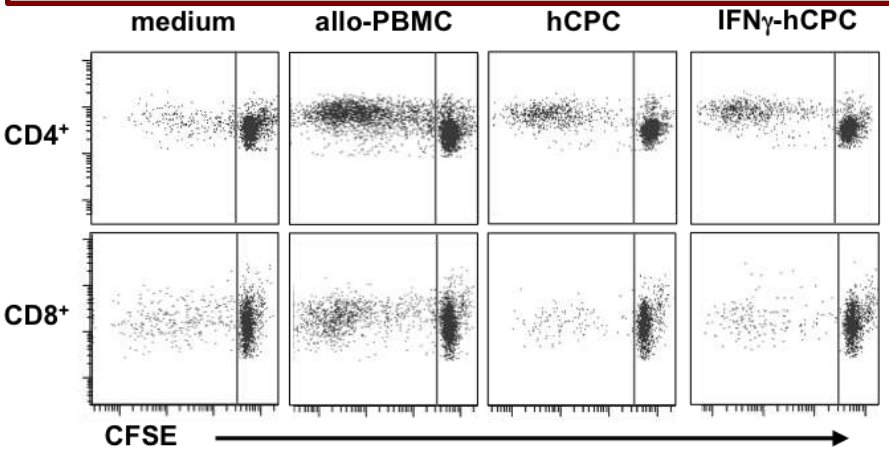
↙ ↘

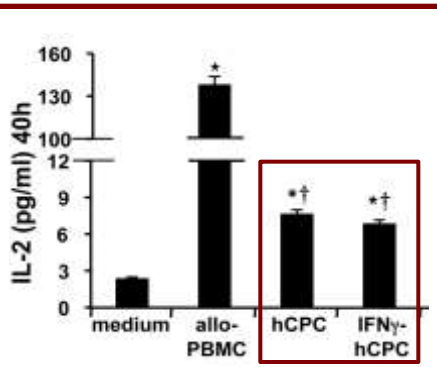
Cytotoxicity **Cytokine secretion**

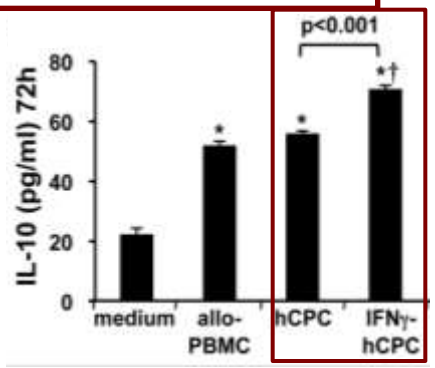


Lysis **IFNγ & TNFα**

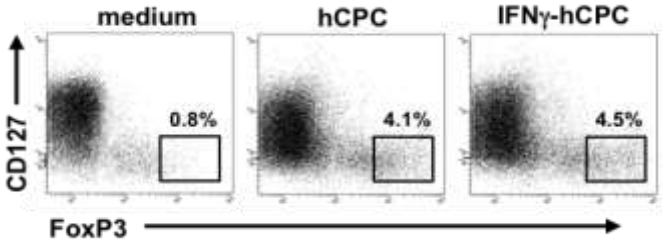
T cell response to allo-hCPC :hCSC are Immunomodulators

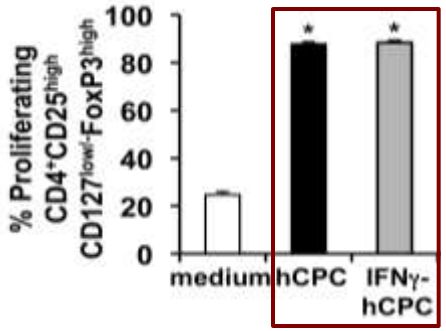




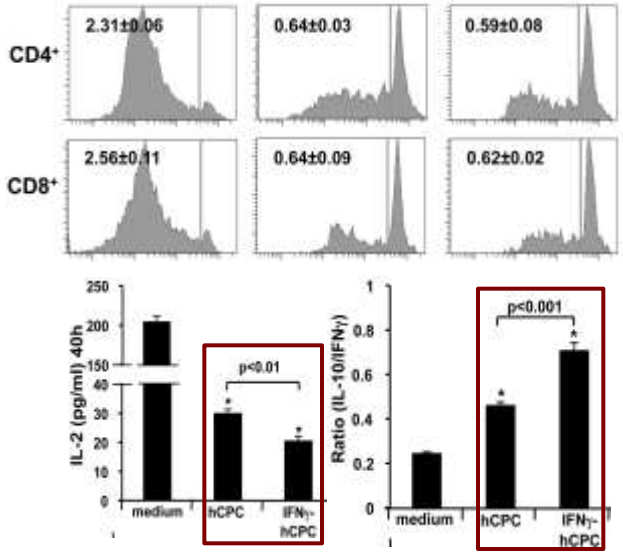


1) IL-10 producing CD4+ cells



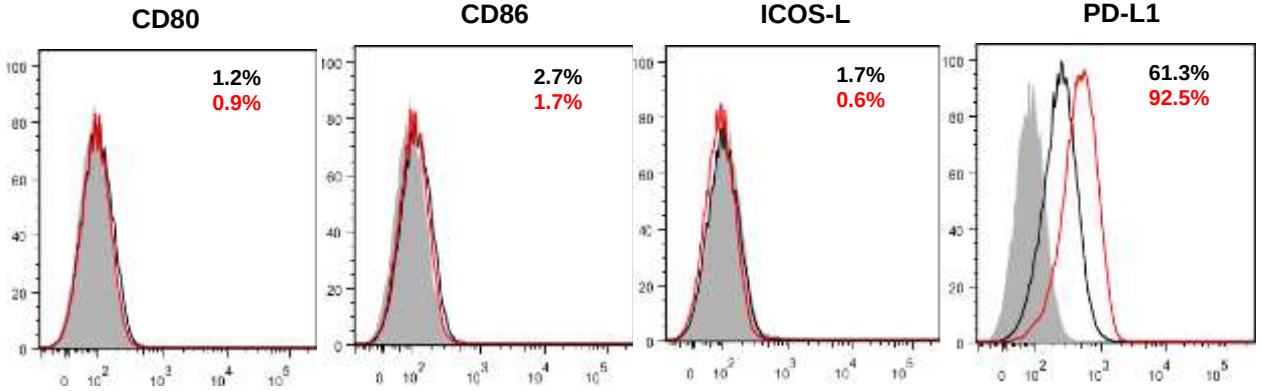


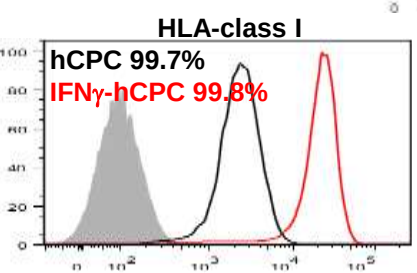
2) Prevent CD4 and CD8 T cells activation (IFNγ, IL-2) but promote IL-10

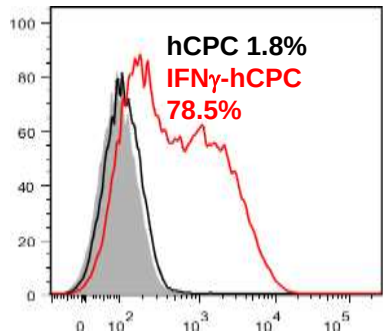


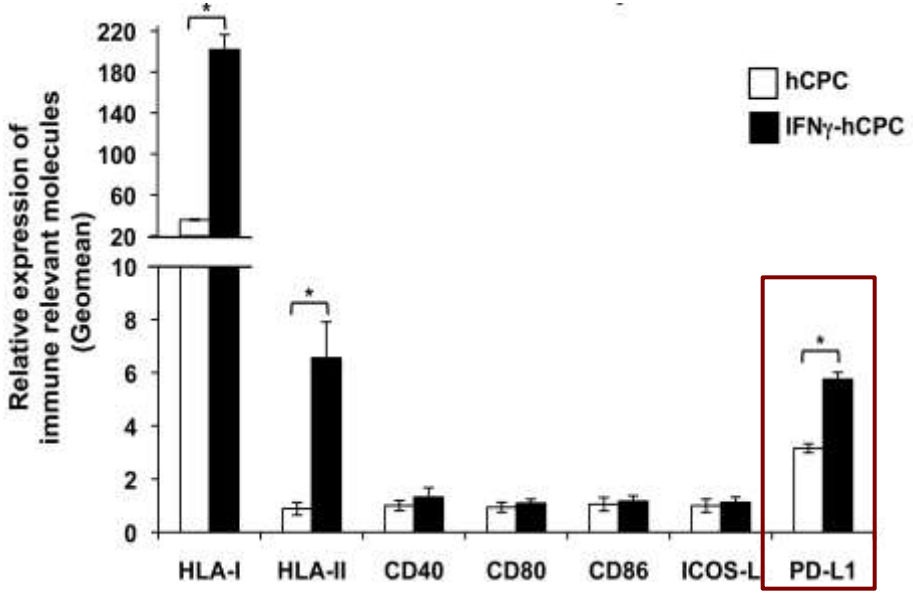
3) Regulatory T cells activation & expansion (CD4+/CD25high/CD127low/FoxP3high)

Co-stimulatory/Co-regulatory molecules on hCPC





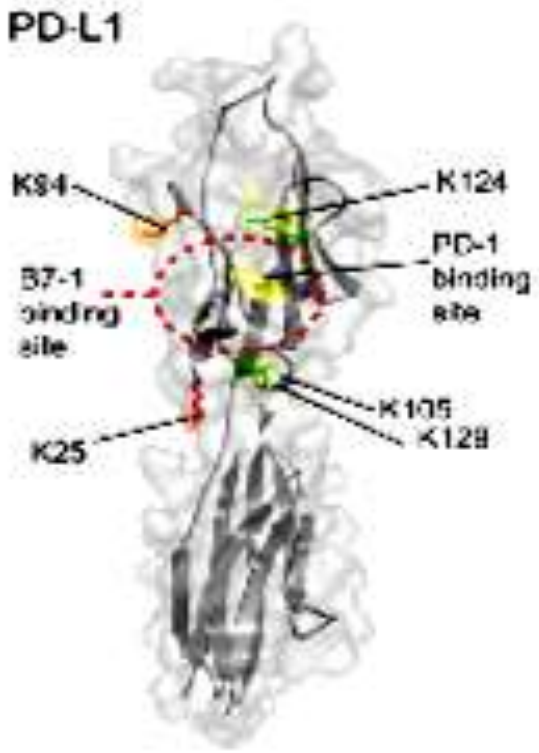




IOW IMMUNOGENIC PROFILE

Lauden, L et al, Circ Res, 2013

Programmed Cell Death Ligand 1 (PD-L1)

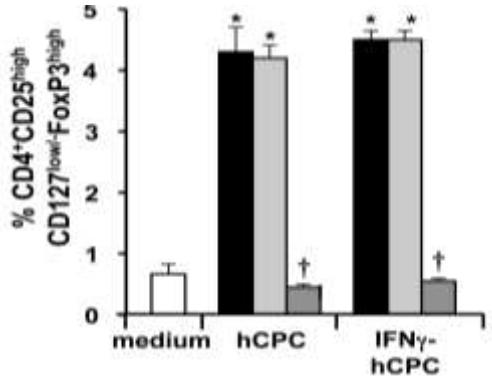


From Butte MJ et al, Immunity, 2007

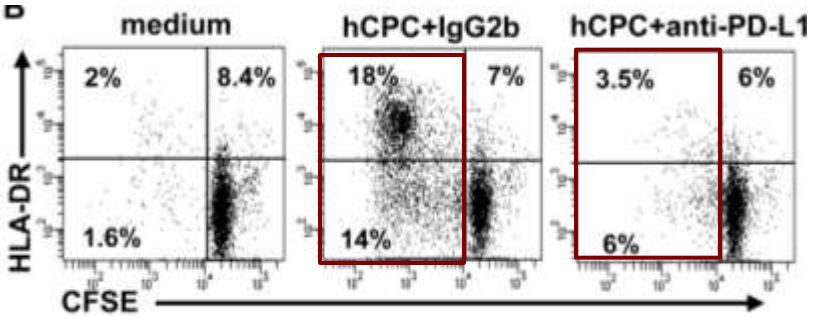
- Expressed on leukocytes and non-hematopoietic cells in lymphoid and non-lymphoid tissues
- Binding partner for PD-1 and B7-1 (CD80)
- Exert vital and diverse range of immunoregulatory roles in T cell activation, tolerance, and immune-mediated tissue damage
- **Co-stimulate T cell proliferation and 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

PD-L1 orchestrates interactions of allogenic CPC with T cells

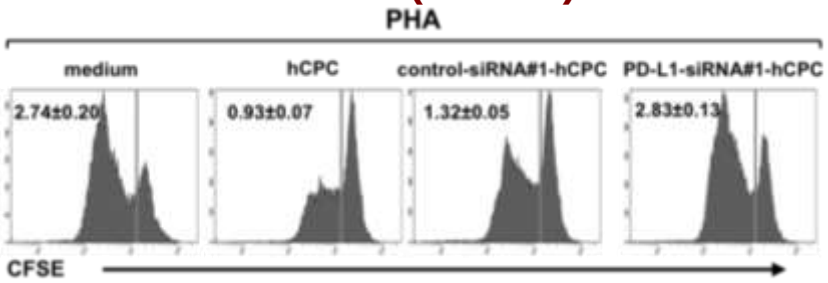
1) Allo-Treg generation

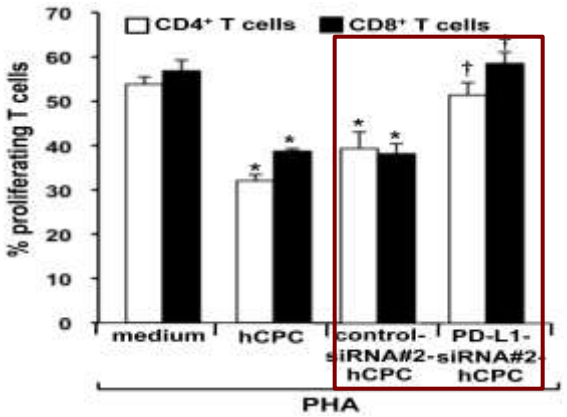


2) Allo-Treg expansion blockage(anti PD-L1)

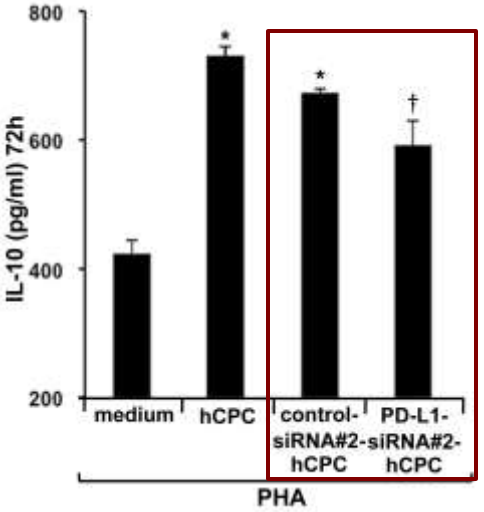


3) Immune-modulation(si RNA)





4) IL-10 production inhibition(siRNA)



Lauden, L et al, Circ Res, 2013

Adaptative T cells Response against Allogeneic hCPC

Main findings: **hCSC**

- 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)**

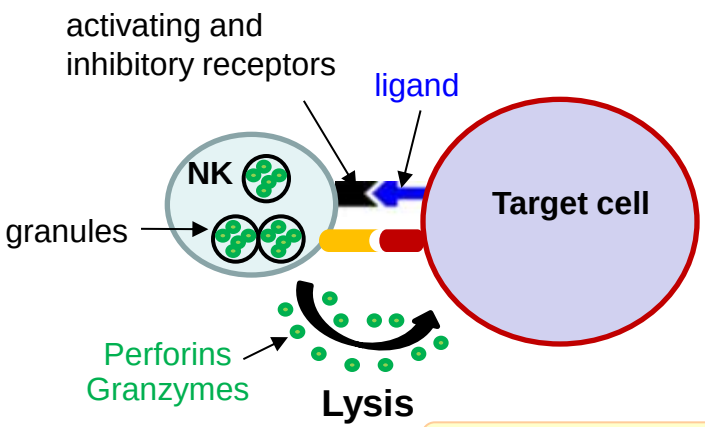
PD-L1 expressing hCSC are attractive Low risk/high benefit cells for cardiac repair clinical translation

Allogeniciy of Human Cardiac Stem/Progenitor Cells Orchestrated by Programmed Death Ligand 1L Lauden, W Boukouaci, L R. Borlado, Itziar P. Lopez, Pilar Sepulveda, Ryad Tamouza, D Charron, R Al-Daccak, **Cir Res 2013**

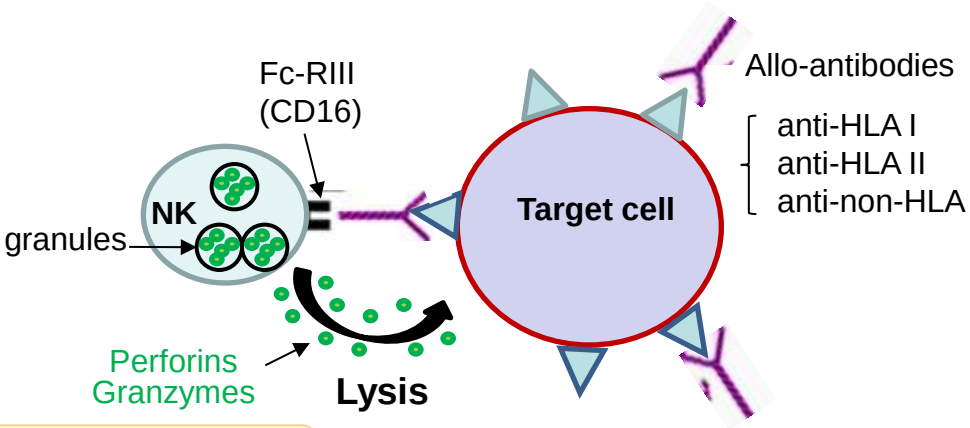
Susceptibility of cardiac progenitor cells to allogeneic NK cell lysis

Mechanisms of NK-mediated killing

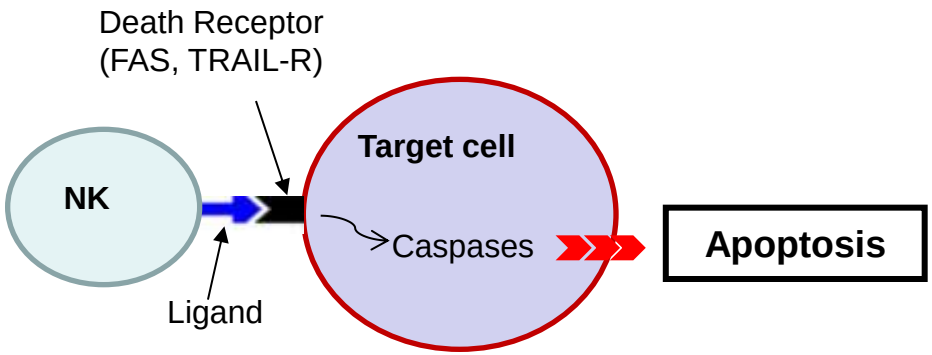
Natural cytotoxicity



Antibody-dependent cell-mediated cytotoxicity (ADCC)

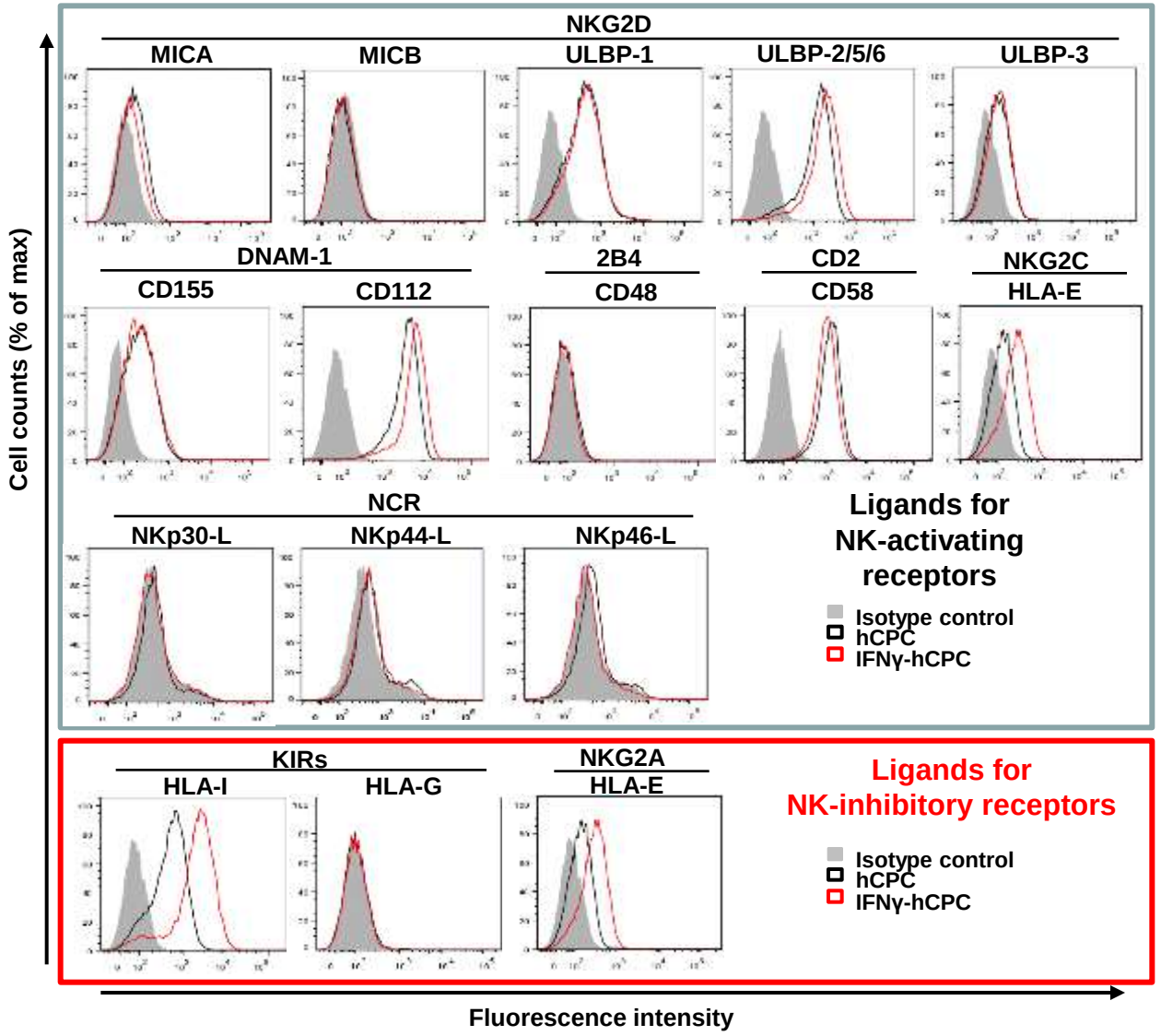


Death receptor pathways



NK optimum activity occurs upon priming by cytokines includingIL2, IL15, IL12-18

Expression of NK receptors Ligands by hCPC and IFNγ-hCPC



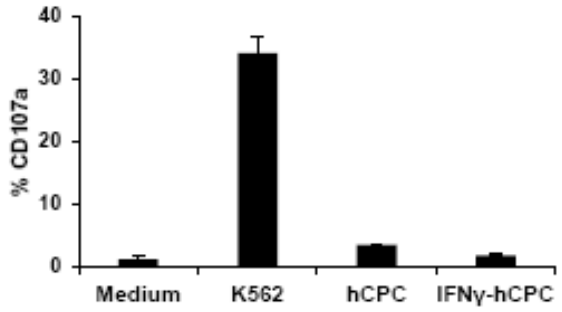
- Both hCPC and IFNγ-hCPC might be susceptible to NK cell lysis
- Inflammatory conditions increase expression of ligands for inhibitory receptors

NK cell degranulation and cytotoxicity towards hCPC

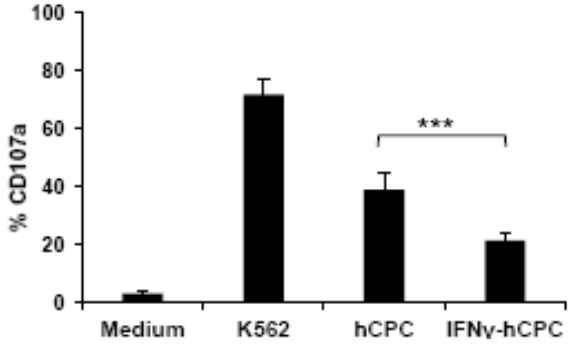
Allogeneic co-cultures:

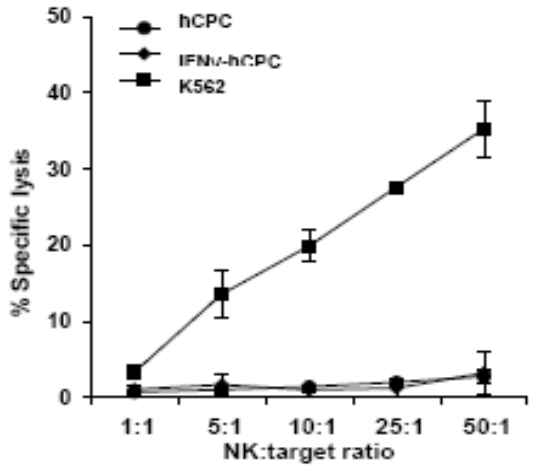
- CD107a expression by NK: marker of NK degranulation
- 7-AAD staining of hCPC: marker of cell death

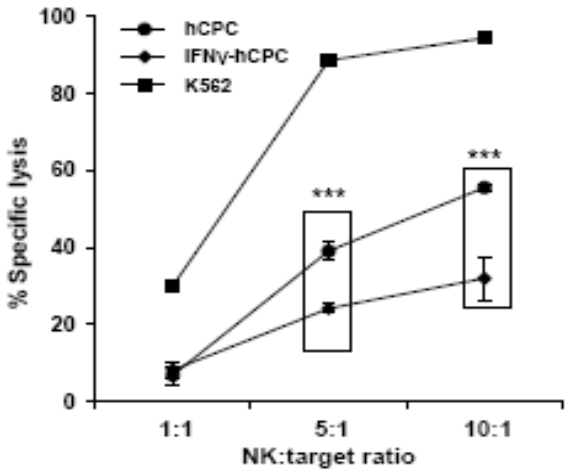
Resting NK



IL15-activated NK



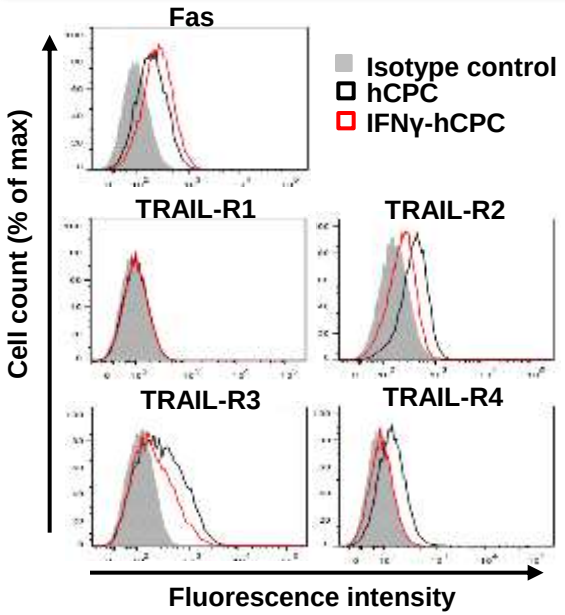




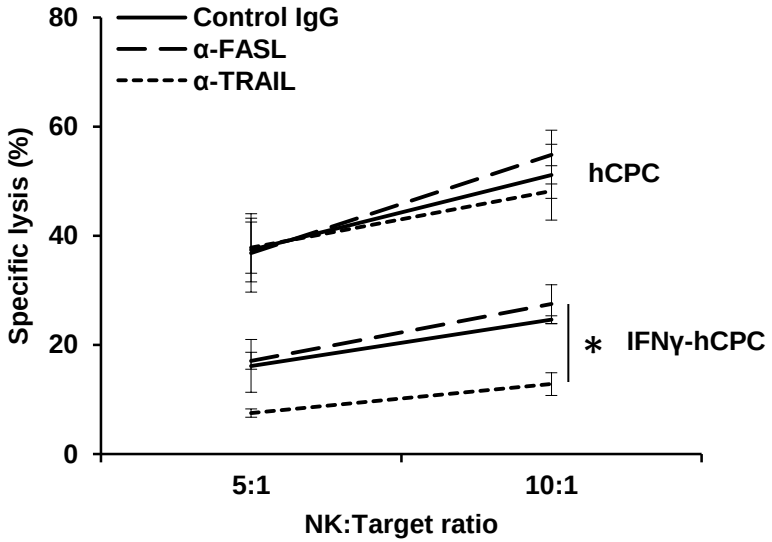
- hCPC and IFNγ-hCPC are only susceptible to cytokine-activated NK lysis
- Inflammatory conditions protect hCPC against NK lysis

Implication of death receptors

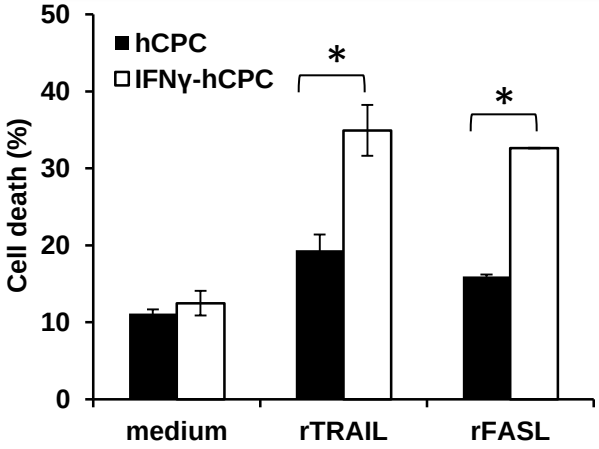
hCPC express death receptors



Use of blocking antibodies



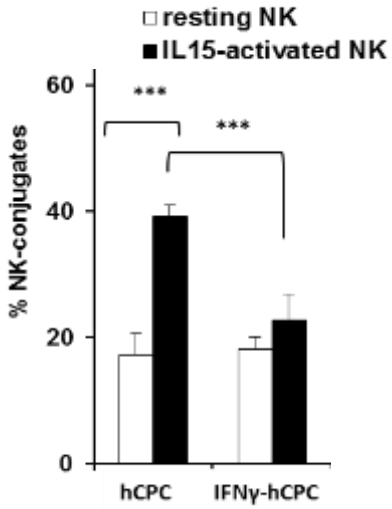
IFNγ-hCPC are sensitized to induced cell death



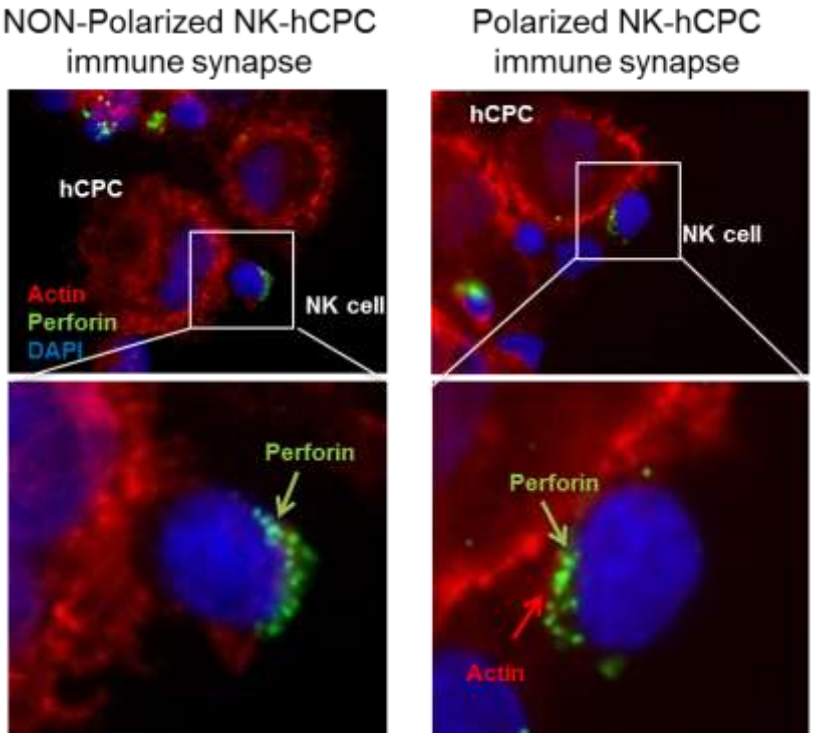
- IFNγ treatment sensitizes hCPC to TRAIL-induced cell death
- hCPC are killed by NK cell through natural cytotoxicity

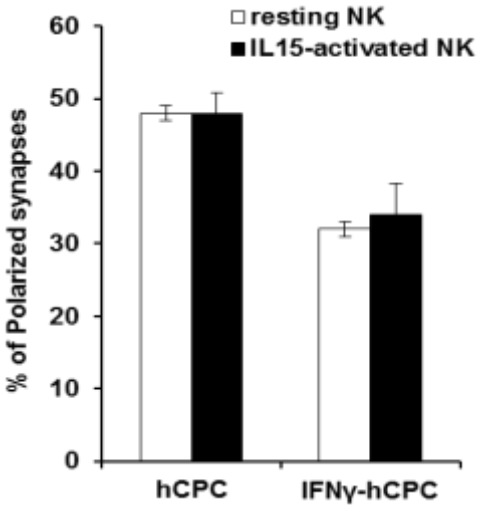
Engagement of NK cells in immune synapses with hCPC

Determination of conjugates by FACS



Analysis of conjugates polarization by microscopy





- 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 prefered 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 lysi

ALLOGENEIC hCPC ENGAGE T & NK CELL PATHWAYS

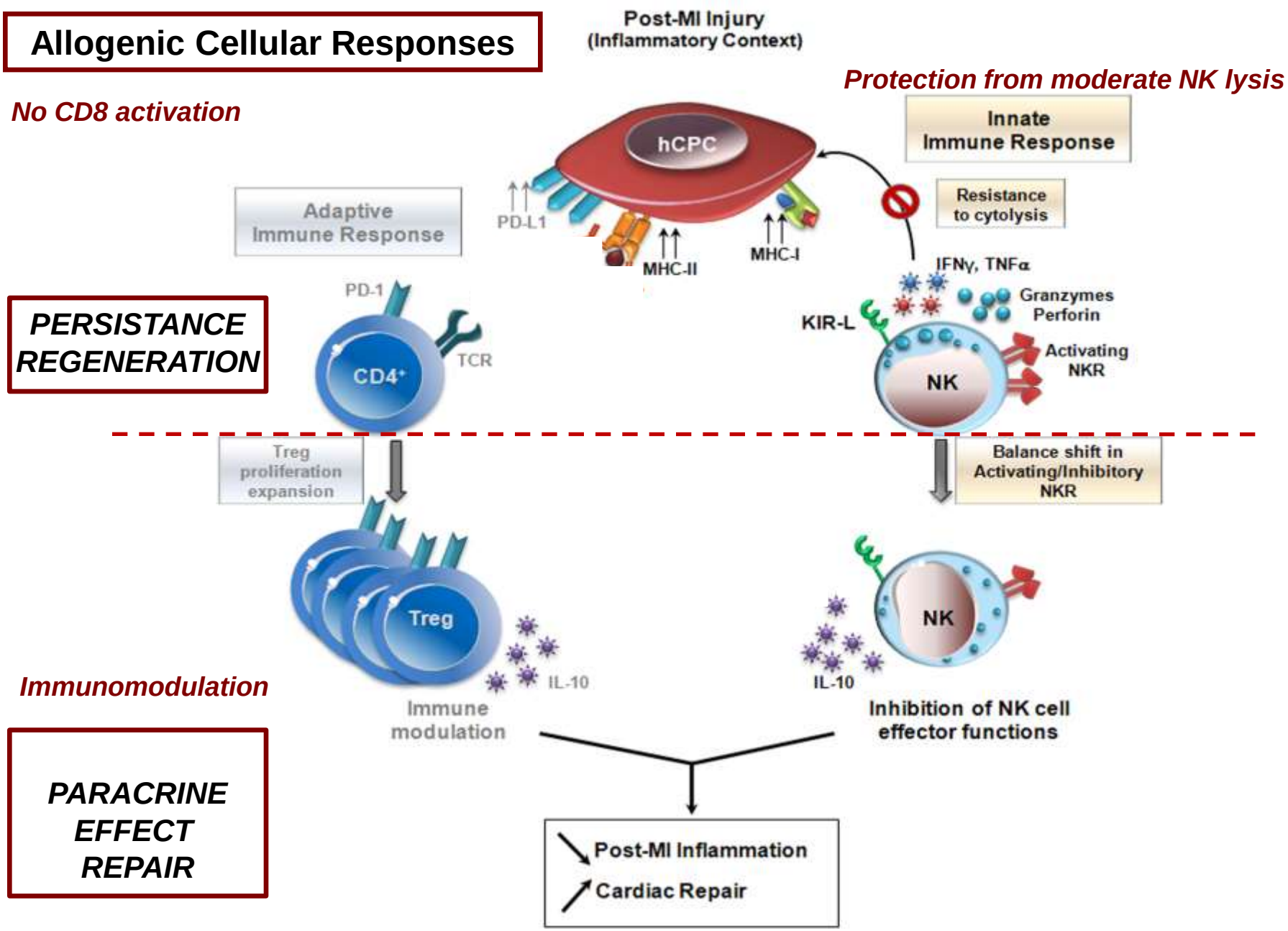
Are allogeneic hCPC immunologically safe? Beneficial ?

Cell response T and NK

Allo-antibodies reactivity

Monocytes/ Macrophages

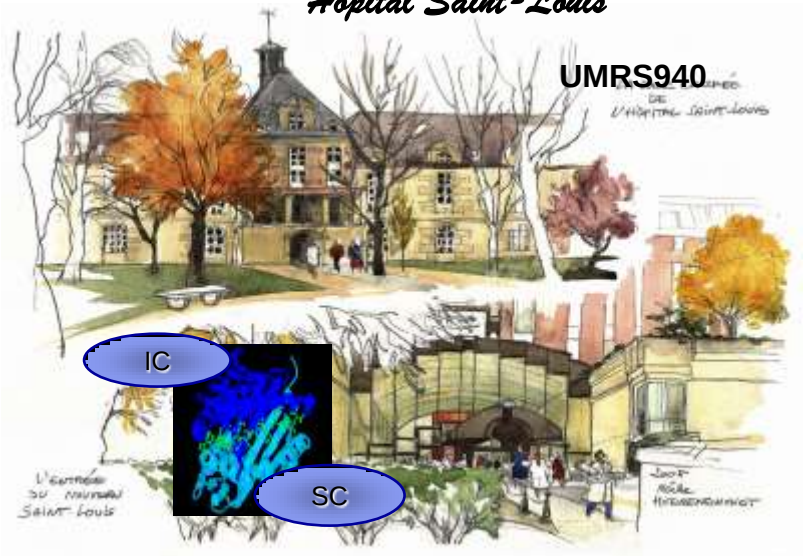
Allogenic Cellular Responses



"Allogenic-driven benefit"
"First-in human" European Clinical Trial

Lauden, L et al, Circ Res, 2013
Lauden et et, Cadiovascular, in press2014

Acknowledgement



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CARE-MI Cardio Repair European Multidisciplinary Initiative


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

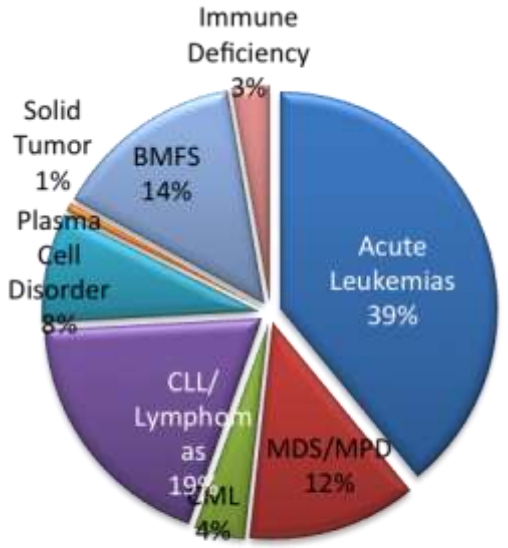
Annalisa Ruggeri, Vanderson Rocha, Emelyne Masson, Renato Cunha,
Lena Absi, Ali Boudifa, Brigitte Coeffic,, Anne Devys, Muriel De Mattei,
Valerie Dubois, Daniel Hanau, Francoise Hau, Isabelle Jollet,
Dominique Masson, Beatrice Pedron, Pascale Perrier,
Dominique Charron, Eliane Gluckman, Pascale Loiseau

Hematologica 2014

Patients Selection Criteria

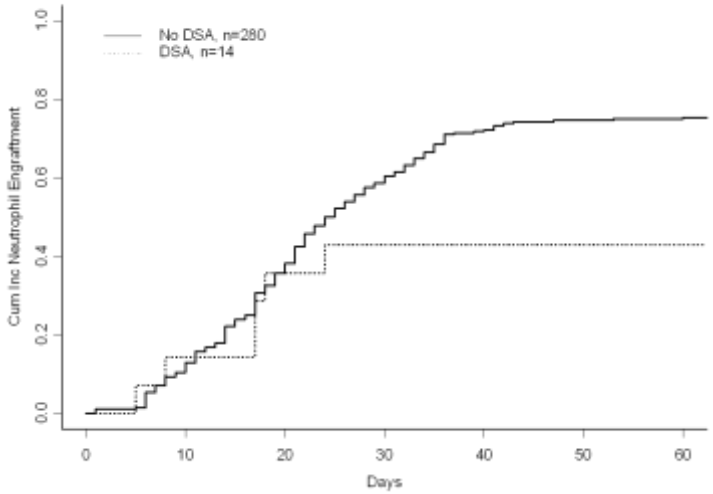
- UCBT from 2000 to 2010
- Single and double UCBT, performed in France
- Reduced Intensity Conditioning regimen
- Availability of pre-transplant serum samples to evaluate DSA

Median Follow-up, months	36 (3- 98)
Children, n	60, 20%
Female gender, n	136, 46%
Non malignant disease, n	50, 17%
Previous Auto-HSCT, n	112, 38%



RESULTS

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



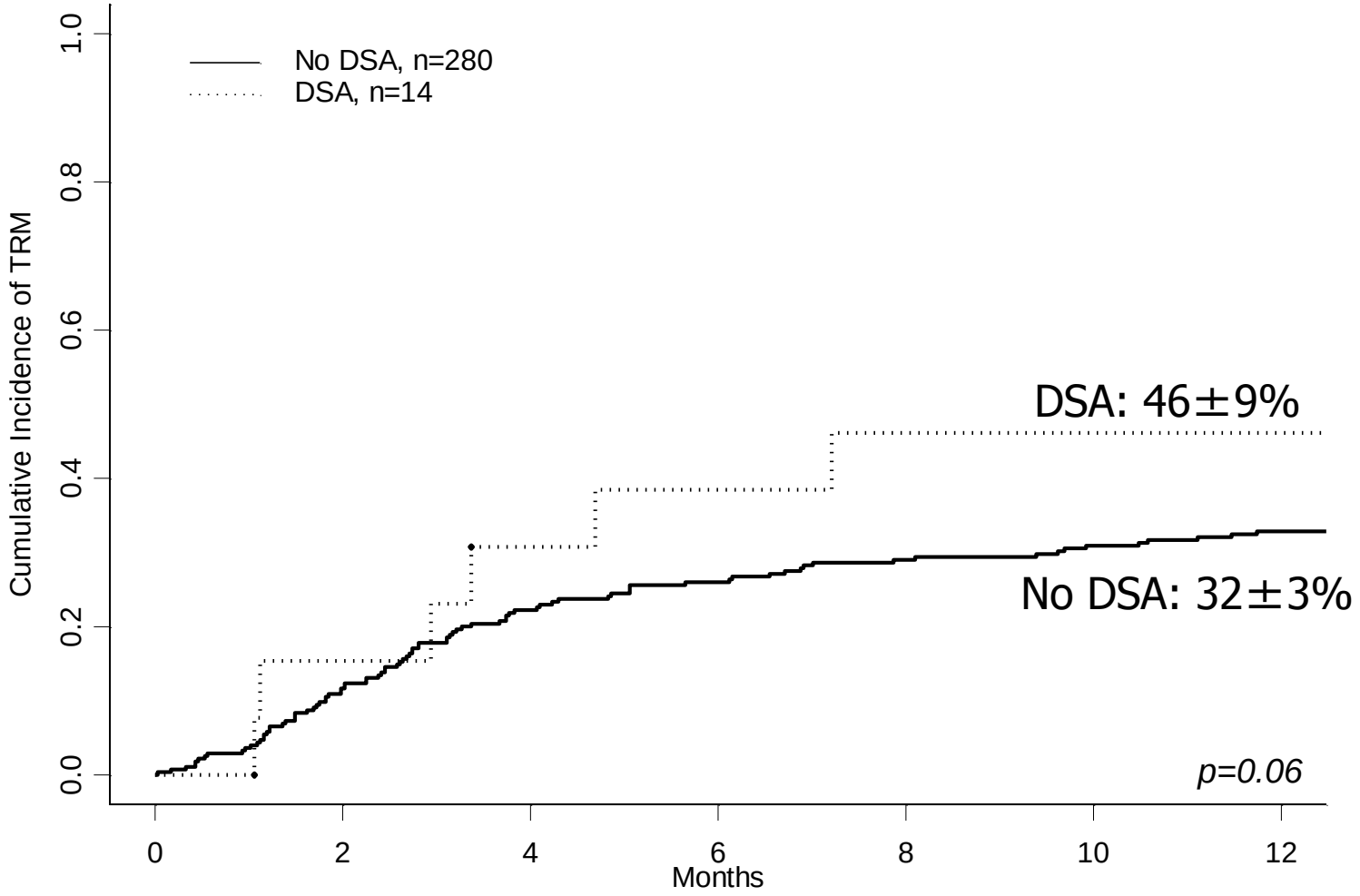
No DSA : 77 %

DSA : 44 %

p=0,003

  - **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**

Transplant-Related Mortality at 1-year



DISCUSSION – CONCLUSION CBT

- 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

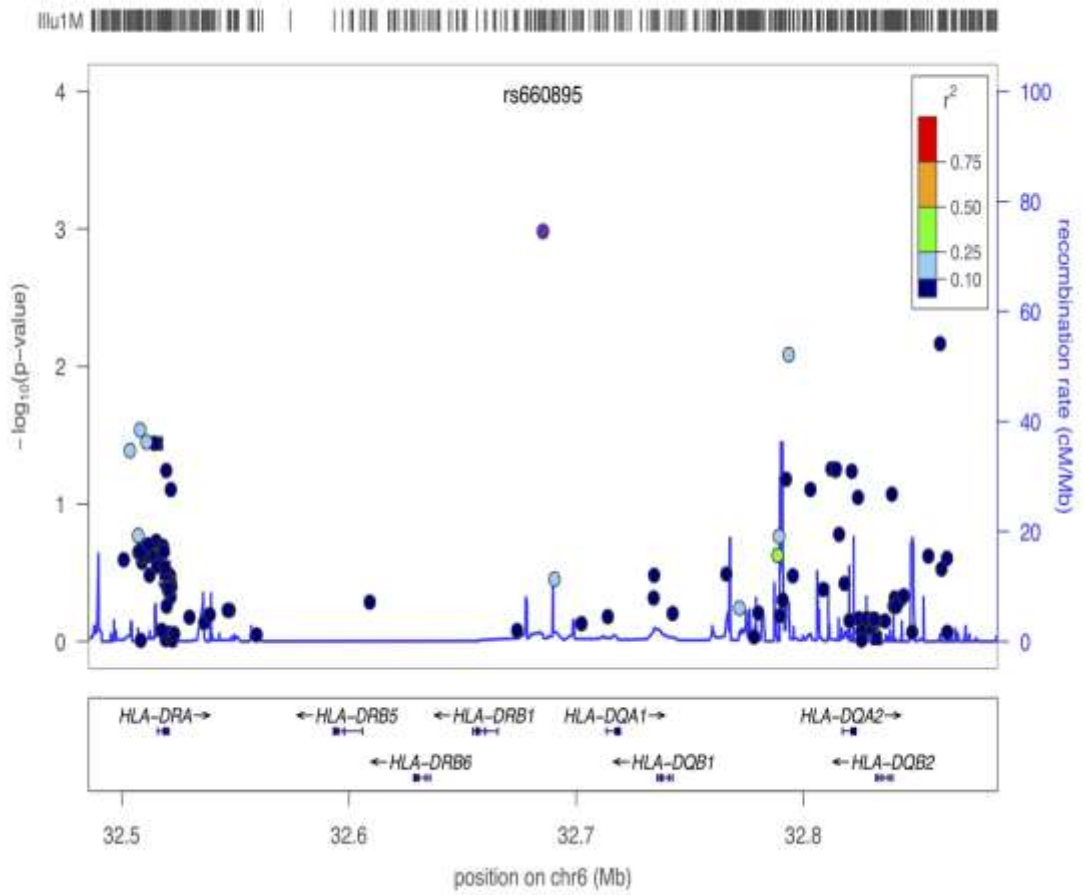
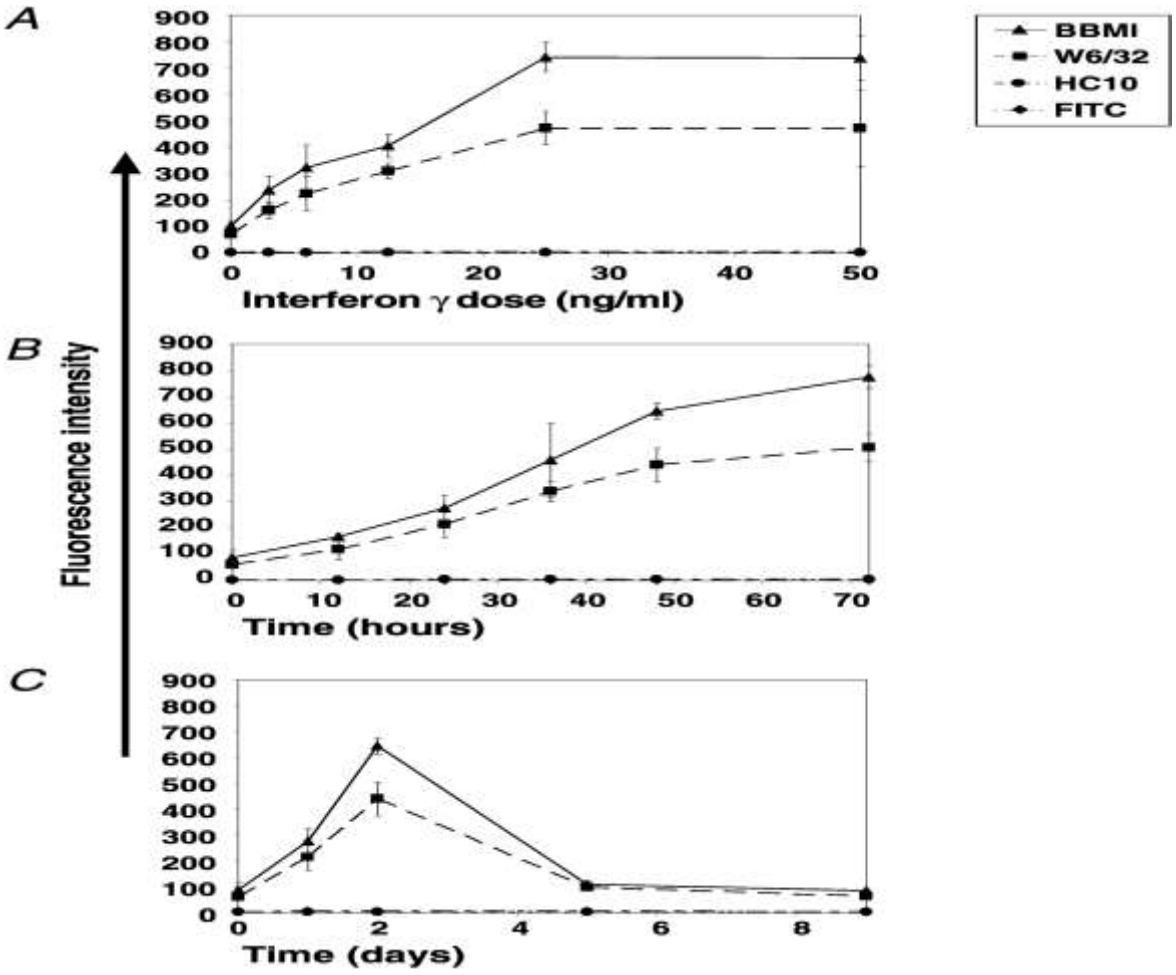


Figure 1. Summary of the Association Between the HLA-DRA, DRB, and DQ Loci and PD. P-values for the 102 SNPs, derived from univariate logistic regression models (additive model), are presented on the left Y-axis on the logarithmic scale according to the position of the SNPs on chromosome 6 (X-axis). 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-γ induction of MHC-I in human ES cells is dose and time dependent



2O10

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)

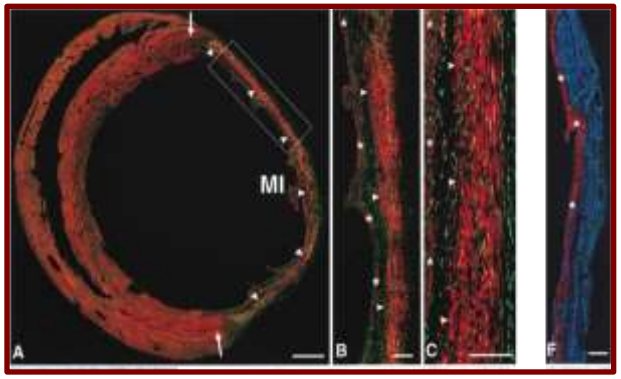
------ Implanted Allogeneic MSCs induce a local immune reaction after 7 days and are not detected in situ after 5 weeks

-------Allogeneic MSCs restore cardiac function as effectively as Syngeneic MSCs for 3 months but not 6 monts 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
(Feasability/efficiency)

SCIPIO ***Bolli et al.Lancet.2011***
CADUCEUS ***Makkar et al. Lancet.2012***

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

IS ALLOGENICITY A BARRIER TO SUCESS ?

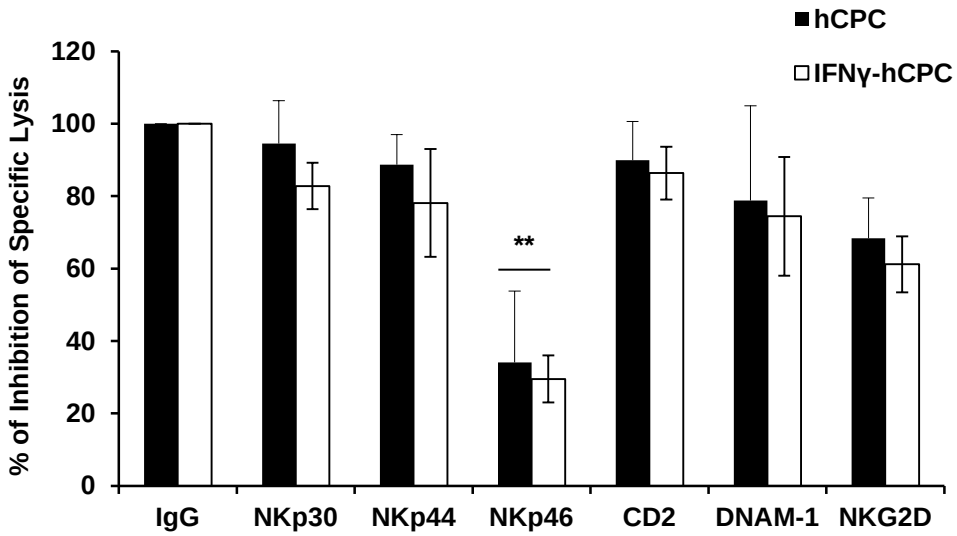
Experimental Interrogation

Allo-immune Response To Cardiac Stem/Progenitor Cells

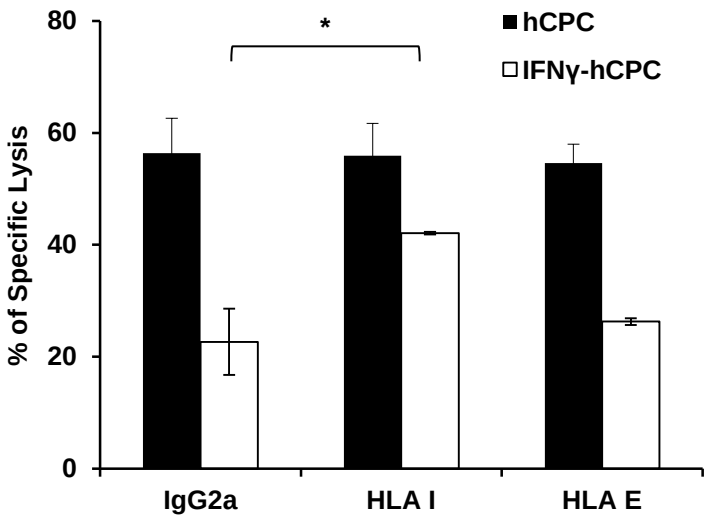
Mechanisms involved in natural cytotoxicity

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

Activating NK receptors



Ligands for inhibitory NK receptors

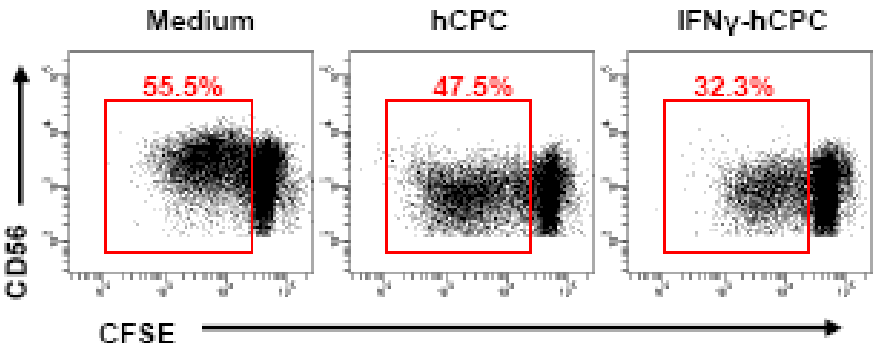


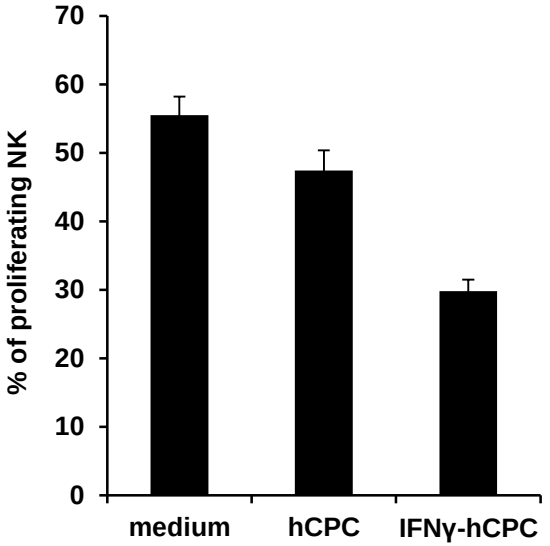
- Nkp46 is the major NK activating receptor responsible for hCPC and IFNγ-hCPC lysis

- 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

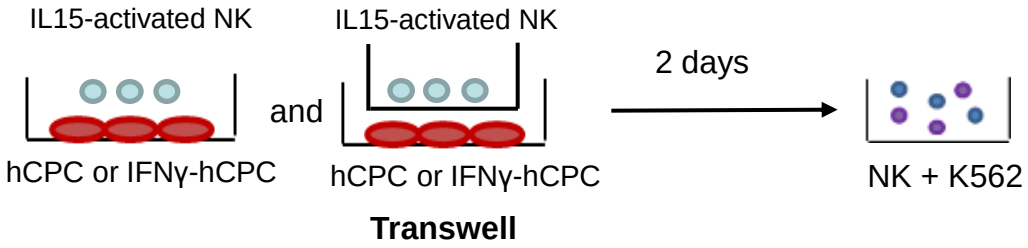
Allogeneic cocultures: CFSE labeled NK cells + IL15 for 6 days



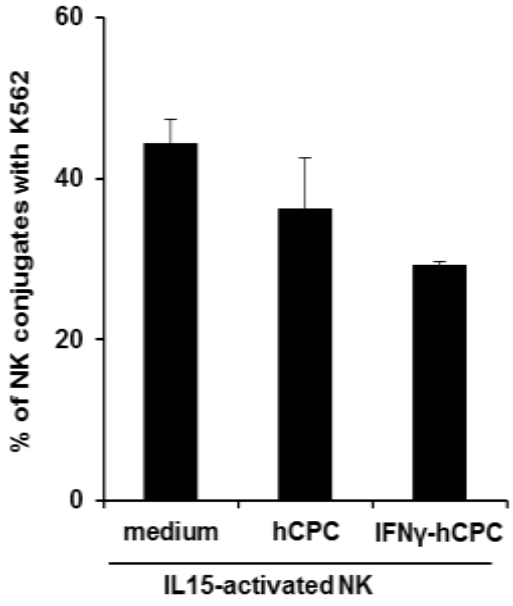


- In allogenic settings hCPC modulate NK cytokine-induced proliferation
- This modulation is more pronounced under inflammatory conditions

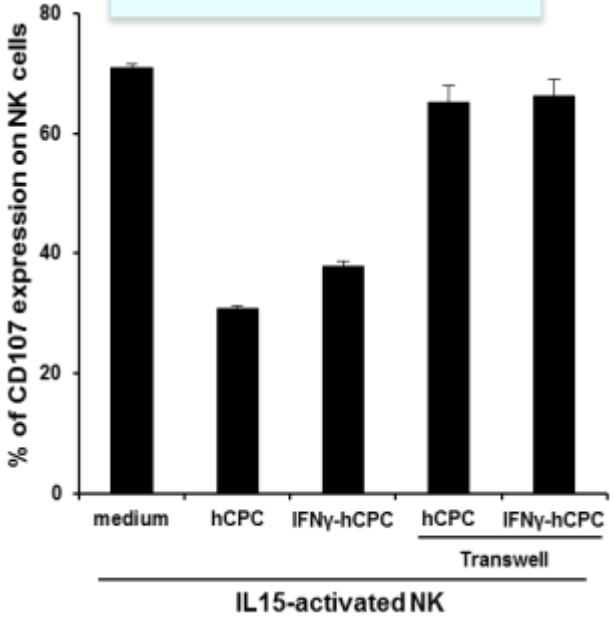
Modulation of NK cell cytotoxicity by hCPC



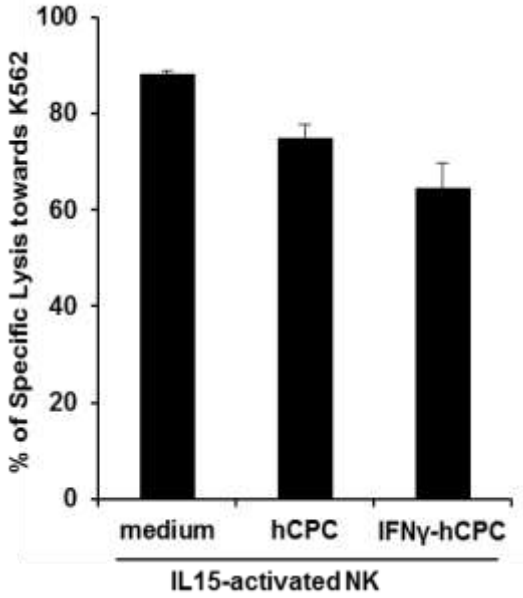
NK-K562 conjugates



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.

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