Belgian Red Cross Flanders

Immunogenicity of Stem Cells in Therapeutic Applications

MDPB-Registry meeting nov 25th 2016

Stem cell immunogenicity in therapeutic applications

Has it been underestimated?

Transplantation of any type of cell
- possibility of triggering an immune response
- unless: ABSOLUTELY identical in every respect, including epigenetics

Predicting immunogenicity of a stem cell therapy is essential to enable safe and effective strategies to prevent immune attack

Immunogenicity of Stem Cells

Differences in cell types

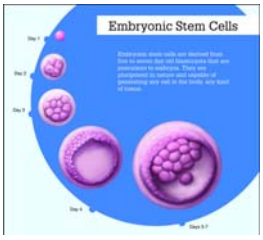
Mechanisms of attack

How to avoid immune attack (incl. MHC measures)

Belgian Red Cross Flanders

Embryonic stem cells

- Most likely from an unrelated donor;
  - may express mismatched MHC
  - and/or minor (miH)
  - they can trigger immune response

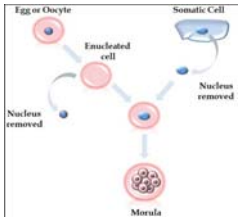


MHC (miH) expression of ESC

- Low expression
  - Risk for attack by NK cells
  - Absence of NK cells (rodent model) -> teratoma
- MHC Expression is expected to increase
  - during differentiation
  - Following cytokine release in case of immune response

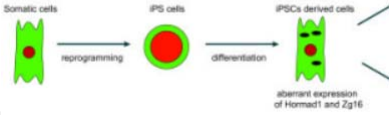
Nuclear transfer-derived embryonic stem cells (NT-ESC)

- Transplanting the nucleus of an autologous somatic cell in an enucleated oocyte
  - Contains allogeneic mitochondria that are immunogenic
  - miH



Induced Pluripotent stem cells (iPSC's)

- Are adult differentiated somatic cells that underwent nuclear reprogramming immunogenic?
  - Can be autologous
  - Undifferentiated iPSC's may express molecules from embryonic origin that may trigger immune response;
  - fully differentiated autologous iPSC's are less immunogenic



iPSC from autologous somatic cells should lack immunogenicity (auto immunity)

- The more differentiated, the less immunogenic
- Cave remaining embryonic molecules (incomplete differentiation of all cells in culture)
- The length of time cells remain in culture can
  - Result in epigenetic changes
  - Induce new molecules
- Immune senescence
- Should iPSC's be purified after culture to eliminate undifferentiated ESC's?

Type of cell, differentiated from autologous may affect potential immunogenicity in vivo

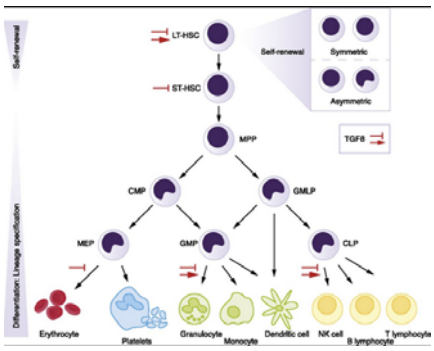
- Smooth muscle -> rejection
- Retinal pigmented epithelial cells -> not immunogenic?

- Dependent on many factors, including epigenetic abnormalities
- Intrinsic immune characteristics of different cell types
- Site implementation

(Zhao T et al. Cell Stem cells (2015) 17, 353-359

Hematopoietic stem cells (HSC's)

- Are multipotent cells
- Chimerism
- Can be used to induce tolerance



Other aspects

- Blood group antigens are expressed

  Mölne et al (2008) Transplantation 86, 1407-1413

- Absence of self tolerance to wild type functional gene

  Wang J et al (2008) Nat. Biotech. 26, 901-908

- MSC's

  Najar M. et al. (2016) Cytotherapy. 18: 160-171
  Jacobs S. et al. (2013)Immunology and Cell Biology 91, 32–39

Allo Immunity

- **Direct pathway**: recognition of intact mismatched MHC molecules by host T cells usually triggered by donor-derived APCs that express costimulatory molecules.

Given that cells and tissues differentiated from stem cells are unlikely to have a significant APC content, this pathway is unlikely to play a major role in triggering an immune response to stem cell-derived tissues *in vivo*.

- **Indirect pathway**: recognition of allopeptides derived from MHC or miH antigens, including those induced by epigenetic changes by host APCs to T cells (i.e., the physiological pathway of T cell recognition).

This is likely to be the dominant pathway for triggering a T cell response to stem cell therapy.

- **Semi-direct pathway**: the acquisition of MHC molecules through membrane exchange by host APC.

This pathway could play a role in triggering a response to stem cells, but there is no evidence that this is the case as yet.

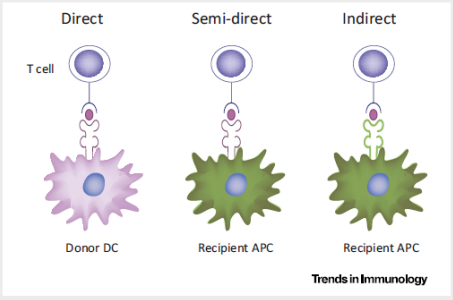


Figure I. Pathways of Alloantigen Recognition. Immune recognition of implanted cells can occur through varied mechanisms as discussed above. Abb

Trends in immunology, jan 2016, Vol. 37, No.1

Mechanisms of attack – are T cells involved?

- Indirect route of antigen presentation
  - Dominant pathway
    - miH antigens
    - Abnormally expressed molecules
- MHC mismatched stem cells
  - *Direct pathway if costimulatory molecules are expressed*
  - APC function molecules

  -> generally low expression both in ES & iPSC lines

  -> of marginal importance?
  - Memory T cells!! –require low levels of co stimulation only

Mechanisms of attack – Tissue Damage

- Tissue damage at moment of implementation
  - Toll like receptors -> conflicting literature data
  - Danger signals: DAMPS & PAMPS

Impact?

- Site of implementation
- Amount of tissue disruption created by the procedure
- Whether antigens that can be recognised by the immune system are present

Mechanisms of attack – Insulin producing cell clusters (IPCC's)

- Less immunogenic than allogeneic allografts
  - Less DAMP expression (not tested - hypothesised)
  - Less infiltration of immune cells (passenger lymphocytes, macrophages,…)
- Less to absence of direct presentation

  -> Even if innate reactivity triggered after transplantation, they were compromised in their ability to activate the adaptive immune system

  absence of co stimulatory factors

Boyd, A.S. and Wood, K.J. (2010) PLoS ONE 5, e10501

Mechanisms of attack – autologous derived dopamine neurons

- Disruption of the blood-brain barriers

  -> although good activity; insufficient data on the number of cells that survived the procedure

  -> early inflammatory response?

  -> injecting more cells than theoretically required?

Hallet P. et al (2015) Cell Stem Cell 16, 269-274

Mechanisms of attack – use of scaffolds

- Polymer & chemical scaffolds
- Decellularised organ scaffolds (e.g. Trachea)

-> used for replacement therapy with structural purpose

-> pose both advantage & create new targets for immune response

Clinical impact currently not known.

Controlling Stem Cell immunogenicity

- Immunomodulation
- Immunomonitoring
- Induction of Tolerance
- Immunogenetic selection / Stem cell banks / HLA matching

Controlling Stem cell immunogenicity

- Production of immunomodulatory molecules (role for MSC's?)
- Costimulation and accessory molecule blockade
- Combination of immunosuppressive drugs
  - risk benefit analysis
  - duration of required treatment
- Regulatory T cells
- Inducing immunological unresponsiveness
  - Mixed chimerism? Conditioning?
  - Tolerogenic DC's?
  - Encapsulation
- Clinical grade GMP iPS cell lines ("haplo" banks)

Mechanisms to overcome immunogenicity

- HLA matching
  - Requirements to be evaluated in view of immunogenicity
  - What level of HLA match is required?
  - Somatic cells don't express HLA-class II – contrary to tissues and organs
- MHC –expression knock out?
  - NK cell attack

-> iPSC – haplobanks (e.g. homozygous for HLA-A B DR)

Mechanisms to overcome immunogenicity

What is a reasonable HLA match?

Mechanisms to overcome immunogenicity

- 150 cell lines to provide 20% of population with HLA match at 4 loci (HLA-A B C and DR) Taylor CJ et al. 2002

Prof Sir Ian Wilmut said: "Calculations suggest that within the UK cells from approximately 150 selected people would provide a useful immunological match for the majority of people. Similar numbers are likely to be required elsewhere."

Mechanisms to overcome immunogenicity

- HLA matching – ESC banks : "reasonable" HLA match
  - 50 most frequent haplotypes could lead to a "Zero Mismatch" for HLA-A B DR for 60% of potential recipients.

    Bradley JA et al. Nat.Rev. Immunol (2002) 2(11): 589-871
  - As few as 10 cell lines homozygous for the most frequent haplotypes could provide a zero HLA mismatch for 38% of UK population.

    Taylor CJ et al. Lancet (2005) 366(9502): 2019-2025

Mechanisms to overcome immunogenicity

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Is the situation of UK comparable to e.g. Belgium?

B*0801

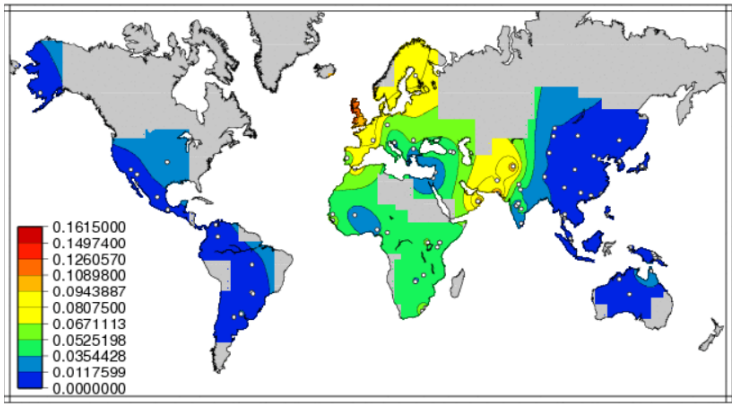
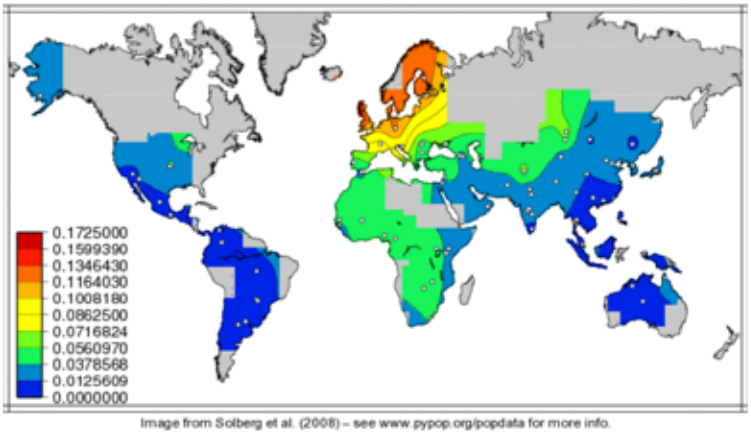


Image from Solberg et al. (2008) – see www.pypop.org/popdata for more info.

1	**A*01**:01-**B*08**:01-**DRB1*03:01**	Ireland Northern	9.00	1,000	

B*0702



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A*03:01-**B*07**:02-**DRB1*15**:01	Ireland Northern	4.90	1,000	

	A	B	C	DR	DQ	Flanders 6933 haplotypes in 26644 donors
1	A*01	B*08	C*07	DRB1*03	DQB1*02	5,51%
2	A*03	B*07	C*07	DRB1*15	DQB1*06	3,31%
3	A*03	B*35	C*04	DRB1*01	DQB1*05	2,25%
4	A*02	B*07	C*07	DRB1*15	DQB1*06	1,99%
5	A*02	B62	C*03	DRB1*04	DQ8	1,52%
6	A*29	B*44	C*16	DRB1*07	DQB1*02	1,51%
7	A*02	B60	C*03	DRB1*13	DQB1*06	1,35%

	A*03:01-**B*07**:02-**DRB1*15**:01 — Ireland Northern	4.90	1,000	
1	**A*01**:01-**B*08**:01-**DRB1*03:01** — Ireland Northern	9.00	1,000	

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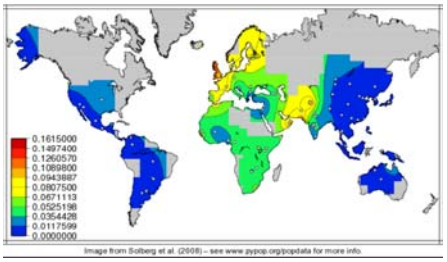

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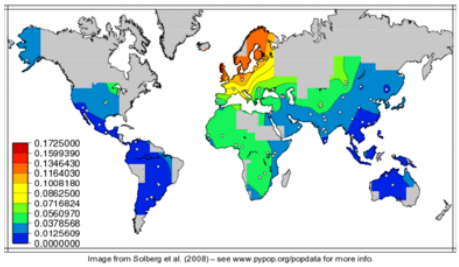

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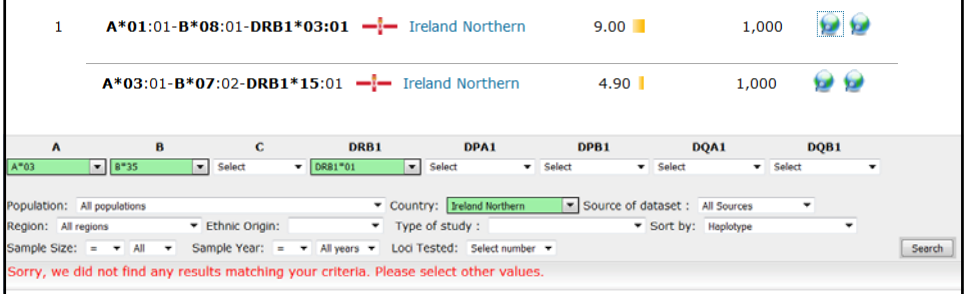


Donor cell line selection

HLA matching – Reducing HLA mismatching

- Pre transplant: screen for HLA antibodies & Identify specificity if present

- Post transplant: continue monitoring (cfr organ transplantation)

Diabetes patients waiting for or transplanted with a (kidney) pancreas

HLA antibodies & HLA mismatches

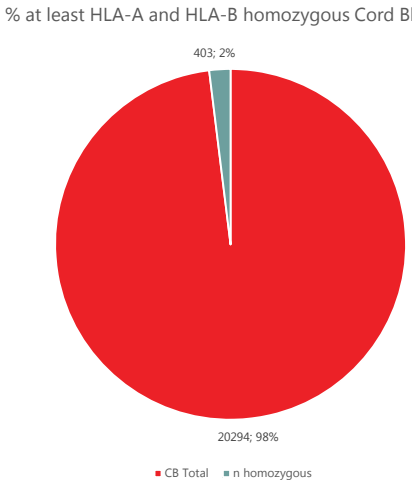
9/64 patients have HLA antibodies
All are female

88% is HLA-DR3 and/or DR4 positive

Create or use:
Stemcell (haplo) banks

Belgian CB Registries : nov 2016 - homozygotes

Total: 20697



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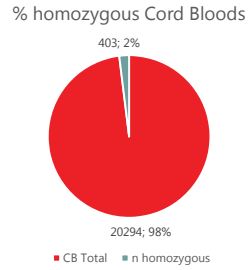
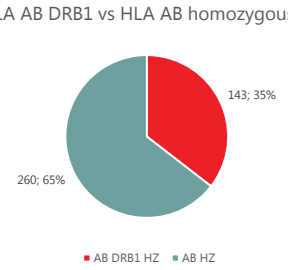


Total: 403



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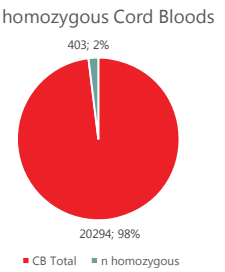

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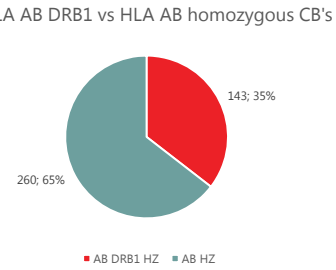
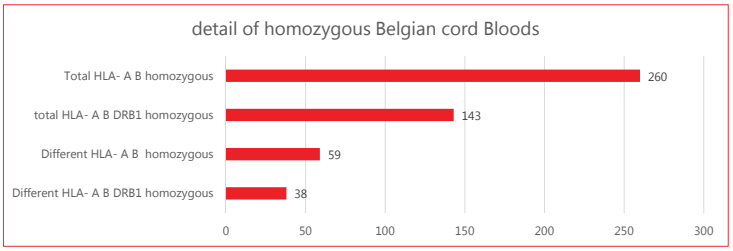




Belgian CB Registries : 38 different HLA-A,B DRB1 homozygotes

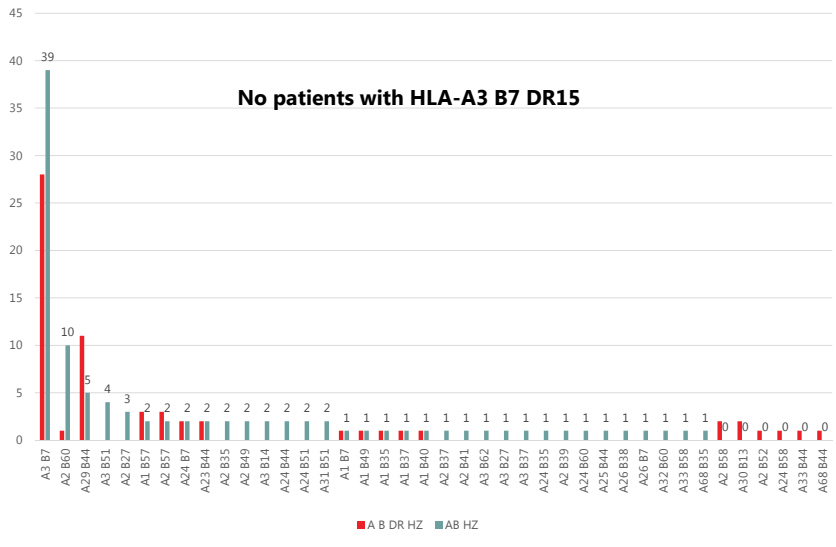
A1	B49	DR13
A1	B57	DR7
A1	B8	DR13
A1	**B8**	**DR3(DR17)**
A2	B18	DR11
A2	B44	DR1
A2	B44	DR11
A2	B44	DR13
A2	B44	DR4
A2	B51	DR11
A2	B52	DR15
A2	B57	DR13
A2	B57	DR7
A2	B58	DR11
A2	B58	DR16
A2	B60	DR13
A2	B62	DR13
A2	B62	DR4
A2	B7	DR15
A2	B8	DR11
A2	B8	DR3(DR17)
A23	B44	DR7
A23	B49	DR11
A24	B58	DR3(DR17)
A24	B7	DR15
A24	B7	DR4
A29	B44	DR7
A3	**B35**	**DR1**
A3	B35	DR11
A3	B35	DR7
A3	B7	DR11
A3	**B7**	**DR15**
A3	B8	DR3(DR17)
A30	B13	DR7
A30	B18	DR3(DR17)
A31	B18	DR3(DR17)
A33	B44	DR7
A68	B44	DR11

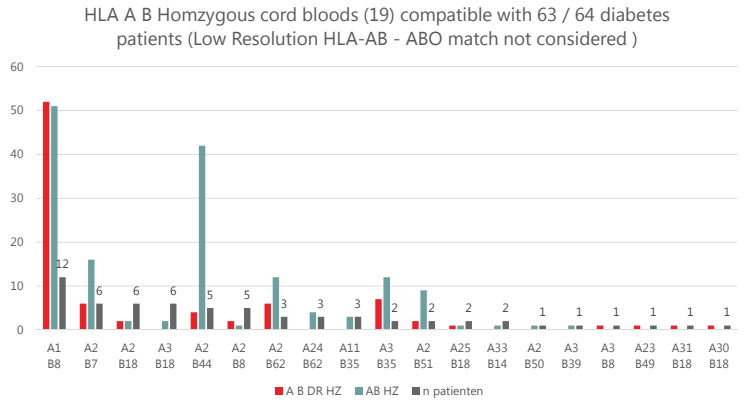
Belgian CB Registries : 38 different HLA-A,B DRB1 homozygotes

A1	B49	DR13	
A1	B57	DR7	
A1	B8	DR13	2
A1	**B8**	**DR3(DR17)**	**9**
A2	B18	DR11	1
A2	B44	DR1	1
A2	B44	DR11	
A2	B44	DR13	
A2	B44	DR4	3
A2	B51	DR11	
A2	B52	DR15	
A2	B57	DR13	
A2	B57	DR7	
A2	B58	DR11	
A2	B58	DR16	
A2	B60	DR13	
A2	B62	DR13	
A2	B62	DR4	5
A2	B7	DR15	
A2	B8	DR11	
A2	B8	DR3(DR17)	5
A23	B44	DR7	
A23	B49	DR11	
A24	B58	DR3(DR17)	
A24	B7	DR15	
A24	B7	DR4	
A29	B44	DR7	
A3	B35	DR1	
A3	B35	DR11	1
A3	B35	DR7	
A3	B7	DR11	
~~**A3**~~	~~**B7**~~	~~**DR15**~~	
A3	B8	DR3(DR17)	1
A30	B13	DR7	
A30	B18	DR3(DR17)	1
A31	B18	DR3(DR17)	1
A33	B44	DR7	
A68	B44	DR11	

47% of patients HLA-A B DRB1 match with 11 cell lines

HLA A B homozygous cords not required for this Diabetes cohorte (64 patients)





30/64 (47%) with 11 cell lines HLA-AB-DRB1 matched
18/64 (28%) with 6 cell lines HLA-AB –DRB1 matched
40/64 (62%) with 6 cell lines HLA-AB matched
63/64 (98%) with 19 cell lines HLA-AB matched

Same % as UK – But cell lines with different HLA types

ABO, HR-HLA not yet taken into account

Final remarks

+ **Minimize immunogenic differences**
  - It is unlikely that autologous cells will be used
  - HLA matching should be a primary strategy

    Bolton E., Bradley J, (2015) Regen. Med. 10(3), 287-304
  - Cfr strategies in organ and tissue transplantation
    - Evaluate the immunization status of the patient prior to iPSC implementation
    - Monitor allogenicity post implementation
    - Immunosuppressive treatment? – increased risk for infection/malignancy

+ **Follow up on tolerance induction**
  - Targeted genome modifications (also for minimizing immunogenicity)
    - Hurdle: Lack of biomarkers for immunological Tolerance characterisation

Final remarks

Be pragmatic

e.g. consider short course of immunosuppression/ HLA matching

Final remarks

Be pragmatic

(e.g. consider short course of immunosuppression/ HLA matching)

Be Utopic

-> hope for tolerance one day

2016-nov-25 - Rode Kruis-Vlaanderen - Immunogenicity of stem cells


Questions?