HaploIPS Cost Action

WG1 Sample selection: Deliverable D1.1.

Report on the common understanding of the haplo-selected CB samples selection procedure

Summary written by: S. Querol, Chair WG1 on behalf WG1 on Donor Selection (12th/Oct/2024)

1. Introduction

HAPLO-iPS aims to create a collaborative network to provide a framework for human induced pluripotent stem cells (hiPSC) generation of hiPSC homozygous for frequent human leukocyte antigen (HLA) haplotypes, compatible with a significant percentage of the population to be used for cell therapy clinical trials and to create a data collection system (REGISTRY) for such lines. This network should include all the relevant stakeholders: hiPSC generation/banking centers, Cord Blood (CB) banks that will supply cord blood units manufacturing centers (GMP complying), immunology experts, chemistry and manufacturing controls, regulatory bodies, National Agencies, and ethics experts.

The objective of working group 1 (WG1) is to identify the source material and define screening, selection, collection and tracing of the proposed samples. For these, two task forces are planned:

- Task force 1 for developing a common understanding of the HLA / CB sample selection procedure by:

- Revision of initiatives and literature on hiPSC generation from haplo-selected CB samples (including coordination and communication opportunities to understand the diversity and commonalities of approaches in different centres/countries).
- Establishment of a methodology to define the most suitable haplotypes to cover most of the European population. For this, an expert team on immunogenetics is created to further develop this topic.

- Task force 2 to coordinate information seeking traceability, collection and data curation of the selected samples by:

- Proposing a CB bank expert meeting to establish minimal HAPLO-iPS criteria for sample quality (identifying appropriate standards for starting materials), data and traceability. A preliminary contact with interested CB banks was made through the Cord Blood Working Group of the World Marrow Donor Association.
- Discussing the procedure to obtain the samples including:

a) Definition of the way to access raw material: planning to target qualified units listed in WMDA database, including non-EU CB banks, if necessary, willing to participate in this initiative. In addition, adult registries could be a second option if key haplotypes are not covered in the CB repertoire.
b) Description of eligibility criteria: Proposing the minimum donor characteristics, proposing a procedure for re-consenting, donor follow-up and donor counselling, if required. Finally, definition of verification test to confirm HLA typing and other cell characteristics and discussing which additional genetic testing should be performed for safety reasons.
c) Discussion of the most appropriate starting biological material: Including selected CD34+ cells or other progenitors and the amount of sample required for developing the pluripotent cell lines.

This will be further developed in a second deliverable (D1.2.).

Overall, this proposal will pioneer new approaches that will foster the progress of a haplo-selected hiPSC generation for therapeutics by the development, implementation and exploitation of a registry with all the information for the benefit of patients.

2. Literature review and initiatives:

Three papers of reference were found to guide in this task all local initiatives that aim to cover an important fraction of their population using few cell lines. Below a summary of these works is presented:

- Japan (Ref. 1):

  Through cooperation with several organizations, the CiRA Foundation recruited donors whose HLA involved in immunorejection were homozygous. The peripheral or umbilical cord blood collected from the donors was used for hiPSC production by electroporation of episomal vectors. These hiPSC lines were then subjected to testing, including genome analyses and sterility, to maximize safety. This initiative has already constructed a clinical-grade haplobank of 27 hiPSC lines from 7 donors according to good manufacturing practice regulations. However, some reasons decided to avoid using hiPSC lines include the presence of residual episomal vectors or genetic mutations in cancer-related genes. This haplobank provides HLA-matched hiPSC lines for approximately 40% of the Japanese population. Since the haplobank's release in 2015, these hiPSC lines have been used in more than 10 clinical trials. The establishment of this haplobank is an important step toward the clinical application of hiPSCs in cell therapies.

- Australia (Ref. 2):

  Many clinical trials are in progress using cells derived from hiPSC for immunotherapies and regenerative medicine. The success of these new therapies is underpinned by the quality of the cell population used to create the hiPSC lines, along with the creation of hiPSCs in a fully Good Manufacturing Practice (GMP)-compliant environment such that they can be used safely and effectively in the clinical setting. Umbilical cord blood (CB) from public cord blood banks is an excellent source of starting material for creation of hiPSCs. All CB units are manufactured under GMP-conditions, and have been screened for infectious diseases, with known family and medical history of the donor. Furthermore, the HLA tissue typing is known, thereby allowing identification of CB units with homozygous HLA haplotypes. CB cells are naïve with less exposure to environmental insults and hiPSC can be generated with high efficiency. Paper describes a protocol that can be adopted by those seeking to create clinical-grade hiPSC from banked CB. This protocol uses a small volume of thawed CB buffy to first undergo ex-vivo expansion towards erythroid progenitor cells, which are then used for reprogramming using the CytoTune™-iPS 2.0 Sendai Reprogramming Kit. Resultant hiPSC lines are tested to confirm pluripotency, genomic integrity, and stability. Cells are maintained in a feeder-

free, xeno-free environment, using fully defined, commercially available reagents. Adoption of this protocol, with heed given to tips provided, allows efficient and robust creation of clinical-grade hiPSC cell lines from small volumes of cryopreserved CB.

- Spain (Ref. 3).

The Spanish Stem Cell Transplantation Registry was screened for cord blood units (CBUs) homozygous for the most common HLA-A, HLA-B and HLA-DRB1 haplotypes. Seven donors were selected with haplotypes covering 21.37% of the haplotypes of the Spanish population. CD34-positive hematopoietic progenitors were isolated from the mononuclear cell fraction of frozen cord blood units from each donor by density gradient centrifugation and further by immune magnetic labeling and separation using purification columns. Purified CD34 + cells were reprogrammed to hiPSCs by transduction with the CTS CytoTune-iPS 2.1 Sendai Reprogramming Kit. The hiPSCs generated from the 7 donors were expanded, characterized, banked and registered. Master cell banks (MCBs) and working cell banks (WCBs) from the hiPSCs of each donor were produced under GMP conditions in qualified clean rooms. Paper presents characteristics of the first clinical-grade, hiPSC haplobank in Spain made from CD34 + cells from seven cord blood units homozygous for the most common HLA-A, HLA-B and HLA-DRB1 haplotypes within the Spanish population. A description of their generation by transduction with Sendai viral vectors and their GMP-compliant expansion and banking is presented and conclude these haplolines will constitute starting materials for advanced therapy medicinal product development (ATMP).

Finally, a working subgropup within WG1 wrote a revision paper to discuss initiatives on creation of HLA-Homozygous hiPSC Lines as a Source of Hypoimmunogenic Cell Therapies (Ref. 4).

Authors discussed the use of allogeneic hiPSC-derived cell therapies for regenerative medicine offers an affordable and realistic alternative to producing individual hiPSC lines for each patient in need. HLA- homozygous hiPSCs matched in hemi-similarity could provide cell therapies with reduced immune rejection covering a wide range of the population with a few hiPSC lines. Several banks of HLA-homozygous hiPSCs (haplobanks) have been established worldwide or are underway, to provide clinical grade starting material for cell therapies covering the most frequent HLA haplotypes for certain populations (see attached table).

Table 1 Summary of the main features of currently manufactured iPSC haplobanks from cord blood units and peripheral blood

	Country	Manufacturer	N. haplolines	N. haplotypes	Cell of origin	Availability research units	Availability GMP units	Ref
PUBLIC	Japan	CiRA	27	5	PB / CB CD34+	Yes	Yes	(31,32)
	S. Korea	CiSTEM (CUK)	13	7	PB / CB CD34+	Not yet	Not yet	(33)
	S. Korea	CHA SCI	10	10	CB CD34+	Not yet	Not yet	(34)
	Spain	IPS-PANIA (BST-IDIBELL)	7	7	CB CD34+	Yes	Not yet	(35)
	Germany	Univ. Hosp. Düsseldorf		10	CB CD34+	UD		(37)
	Germany	iPStemRNA			Skin fibroblasts	UD		(38)
	France	CiTHERA			CB CD34+	UD		(39)
	Norway	NIBCA			Skin fibroblasts	UD		(40)
	Australia	AusCord			CB CD34+	UD		(41)
	Saudi Arabia	KAIMRC			PB	UD		(44)
PRIVATE		Fujifilm CDI	2	2	CB	No	Yes	(47)
		CATALENT	4	4	PB CD34+	No	Yes	(48)

PB, peripheral blood; *CB*, cord blood; *UD*, under development

Harmonizing quality standards among haplobanks and creating a global registry could minimize the collective effort and provide a much wider access to HLA-compatible cell therapies for patients with less frequent haplotypes.

3. Defining the most suitable HLA-haplotypes in Europe:

3.1. WMDA donor database

Established in 1994, the World Marrow Donor Association (WMDA) is a global society of registries, cord blood banks, donor centres, collection centres, HLA-experts, regulators, researchers, technologists, and industry partners with a shared vision to strive for a world where access to life-saving cellular therapies for all patients is assured and donor's rights and safety are protected.

WMDA is the global leader operating a search and request platform to facilitate that more patients can be transplanted, thereby advancing digitalisation in healthcare. WMDA offers a unique collaborative environment that addresses three key areas of translation: selection of the most suitable stem cell source, donor care and quality. Through strong relationships with global regulatory agencies, registries, and industry partners, WMDA drives the advancement of harmonisation globally.

Comprised of over 1,700 experts across five geographic regions and representation from over 57 countries, WMDA members are part of a global community of peers, thought leaders, and organisations invested in donor care and graft selection.

Currently, in the WMDA donor database, there are 41,723,048 total adult donors and 768,546 Total Cord Blood Units reported in the WMDA database from 57 different countries. Of them, for the interest of this project, there are 20 countries that report 282924 CB units for transplantation, as shown in table below:

Country	Total		
ES	60963	SE	4428
IT	39728	HR	3729
FR	38526	CY	3245
		PL	3239
DE	38100	FI	3123
GB	28307	TR	1666
BE	21518	SK	1645
IL	16645	AT	1421
CH	5570	BG	591
GR	5410	SI	341
NL	4729	20	282924

In addition, almost 20.000.000 of European adult donors are also listed in the database.

3.2. Establishing a collaboration with GAIT to develop a join project:

GAiT formed in 2013 to investigate the possibility of building a library of haplotyped stem cells for use globally (Ref. 5).

GAiT is an international resource to facilitate the therapeutic use of immunogenetically matched, clinical-grade new types of stem cells (hiPSCs) for the benefit of patients globally. GAiT's vision is that patients globally will have equity of access to this new generation of hiPSC-derived cell therapy products.

GAiT's mission is to enable the global human community the opportunity to benefit from the new generation of cell therapies by facilitating the development of, and access to, clinical-grade and haplotyped hiPSC for the manufacture of cell therapy products (Ref. 6).

As their objectives are aligned, HAPLOIPS and GAiT agreed to study the European HLA haplotypes and population coverage for the purpose of designing an efficient hiPSC haplobank for cell therapies.

GAiT has previously explored which is the best ranking of HLA haplotypes in order to cover as much patients as possible. Under previous projects, they have already developed IT tools for ranking and clustering of HLA-haplotypes to make the most precise recommendation in this connection. Given the goal of COST Action Haplo-iPS to map HLA haplotypes for Europe per countries, geographical region and on a European level, meeting participants discussed the best approaches and the adaptation of tools for implementing these tasks.

Agreement between both initiatives comprises some members of GAiT joining the project as COST Action Haplo-iPS members facilitating a common work with the goal of writing a scientific publication. This paper is necessary to guide in which bank will be donor sources for developing the European Haplobank.

3.3. Immunogenetic methodology for sample selection

GAiT and NMDP joined to develop a coverage model for hiPSC banks of HLA A, B, DRB1 homozygous cells that cover patients in target population(s). For that the need of an algorithm to prioritize haplotypes that optimally treat national populations and assess coverage of the 'global' population was foreseen.
To develop such an algorithm, it is necessary first to answer two questions:
1. How many patients are 'covered' (matched) by a particular HLA haplotype homozygous donor?
2. How to build a set of cell lines that provide optimal coverage of patients in local and 'global' populations?

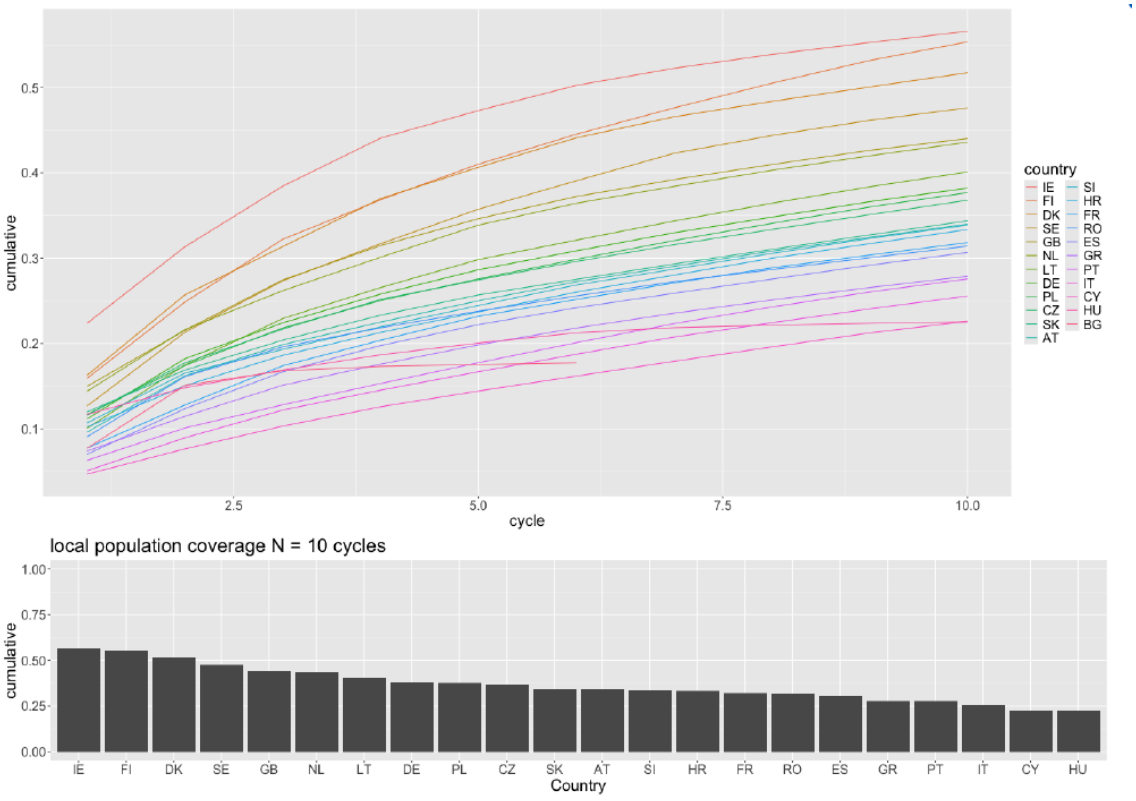
A proposal for this was initially published (Ref. 7). Following, an optimal population coverage based on iterative greedy algorithm was proposed:
- Start with a patient set (sampled from donor pool)
- Determine a candidate set of genotypes from the source population
- Select the genotype that covers the most patients
- Remove those patients
- Repeat N times
- Resulting set of N genotypes are the "optimal" coverage set

3.4. Preliminary results of the Haploips-GAiT coverage study

a) Local coverage models:

Frequency of population covered by top 10 haplolines, based in:
- 23 countries
- Top 10 haplotype coverage
- With overlap

Table below shows that coverage per country after including their top 10 local haplotypes. This is an indirect measure of their HLA diversities. Coverage ranged between 25 to 50%.



In more detail, we show haplotype examples of countries with low (Ireland) and high (Hungary) HLA diversity: First haplotype in Ireland (IE) has 392 potential CB haplodonors in the registry but the 10th only 2. On the contrary, Hungary (HU) has the same first haplotype but falling soon the number of donors for the next, and 6 of the most frequent ones have only 1 or 0 CB donors represented.

IE

	donors	cord
A*01:01+A*01:01^B*08:01+B*08:01^DRB1*03:01+DRB1*03:01	65503	392
A*03:01+A*03:01^B*07:02+B*07:02^DRB1*15:01+DRB1*15:01	16617	110
A*02:01+A*02:01^B*44:02+B*44:02^DRB1*04:01+DRB1*04:01	4607	22
A*02:01+A*02:01^B*07:02+B*07:02^DRB1*15:01+DRB1*15:01	6131	68
A*29:02+A*29:02^B*44:03+B*44:03^DRB1*07:01+DRB1*07:01	3389	109
A*01:01+A*01:01^B*57:01+B*57:01^DRB1*07:01+DRB1*07:01	1511	13
A*02:01+A*02:01^B*08:01+B*08:01^DRB1*03:01+DRB1*03:01	877	10
A*02:01+A*02:01^B*44:02+B*44:02^DRB1*07:01+DRB1*07:01	42	0
A*32:01+A*32:01^B*14:01+B*14:01^DRB1*07:01+DRB1*07:01	88	1
A*02:01+A*02:01^B*44:02+B*44:02^DRB1*15:01+DRB1*15:01	252	2

HU

	donors	cord
A*01:01+A*01:01^B*08:01+B*08:01^DRB1*03:01+DRB1*03:01	65503	392
A*02:01+A*02:01^B*13:02+B*13:02^DRB1*07:01+DRB1*07:01	1650	10
A*03:01+A*03:01^B*35:01+B*35:01^DRB1*01:01+DRB1*01:01	4059	29
A*02:01+A*02:01^B*27:02+B*27:02^DRB1*16:01+DRB1*16:01	307	1
A*02:01+A*02:01^B*15:01+B*15:01^DRB1*14:01+DRB1*14:01	12	1
A*30:01+A*30:01^B*13:02+B*13:02^DRB1*07:01+DRB1*07:01	1162	17
A*03:01+A*03:01^B*44:02+B*44:02^DRB1*16:01+DRB1*16:01	33	0
A*24:02+A*24:02^B*15:01+B*15:01^DRB1*16:01+DRB1*16:01	3	0
A*01:01+A*01:01^B*40:01+B*40:01^DRB1*14:01+DRB1*14:01	0	0
A*01:01+A*01:01^B*40:06+B*40:06^DRB1*14:01+DRB1*14:01	0	1

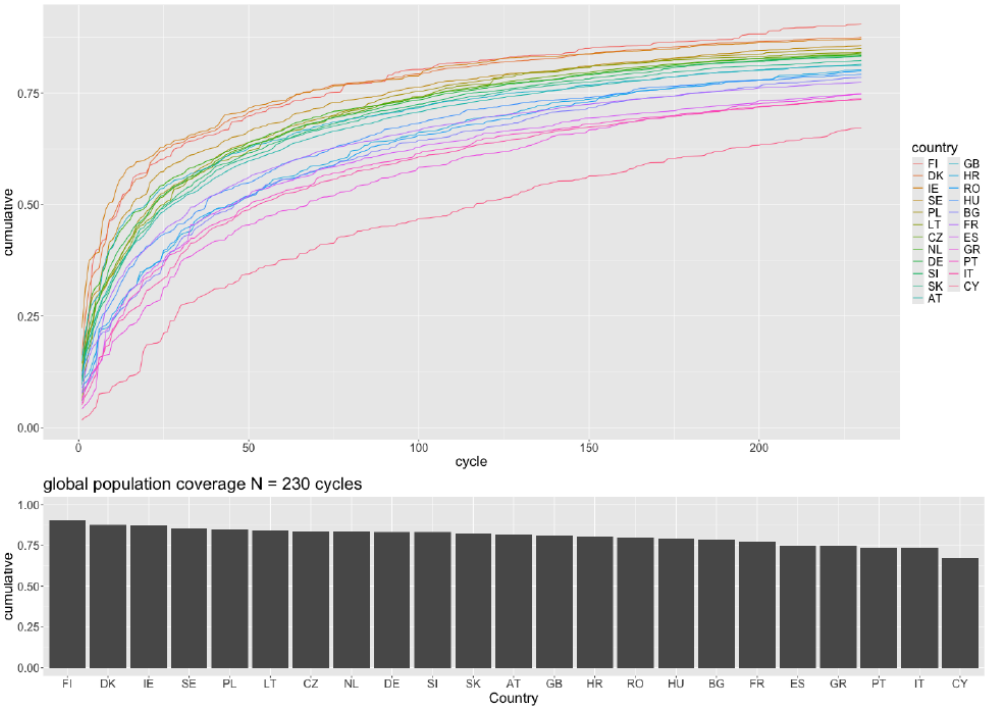
To gain a vision of the cross-linked distribution of local haplotypes we ranked them between 23 participants. This table shows in how many countries is that particular haplotype present and in which position. This table suggests that a global approach will facilitate the creation of the HaploIPS bank.

Homozygous Genotype	AT	BG	CY	CZ	DE	DK	ES	FI	FR	GB	GR	HR	HU	IE	IT	LT	NL	PL	PT	RO	SE	SI	SK	banks
A*01:01+A*01:01^B*08:01+B*08:01^DRB1*03:01+DRB1*03:01	1	1	9	1	1	1	2	2	1	1	2	1	1	1	1	3	1	1	1	1	1	1	1	23
A*03:01+A*03:01^B*35:01+B*35:01^DRB1*01:01+DRB1*01:01	6			6	5	7		1	7	9		8	3		9	7	4	7	8	6	7	3	8	18
A*03:01+A*03:01^B*07:02+B*07:02^DRB1*15:01+DRB1*15:01	2			2	2	4	4	5	2	2				2	5	2	3	2	6		4	2	2	17
A*02:01+A*02:01^B*07:02+B*07:02^DRB1*15:01+DRB1*15:01	3			4	3	2	6	3	5	4				4		4	2	4			2	10	4	15
A*02:01+A*02:01^B*13:02+B*13:02^DRB1*07:01+DRB1*07:01	4			3	7			7			10	5	2			1		3		4			3	11
A*02:01+A*02:01^B*44:02+B*44:02^DRB1*04:01+DRB1*04:01	5			10	6	5			4	3				3			7				5		7	10
A*02:01+A*02:01^B*15:01+B*15:01^DRB1*04:01+DRB1*04:01	10			7	4	3		4		7							5	9			3	7		10
A*29:02+A*29:02^B*44:03+B*44:03^DRB1*07:01+DRB1*07:01					9	10	1		3	5				5	6		8		2					9
A*02:01+A*02:01^B*18:01+B*18:01^DRB1*11:04+DRB1*11:04		2	2								1	2			2					2		6	6	8
A*02:01+A*02:01^B*51:01+B*51:01^DRB1*11:01+DRB1*11:01	9						8		8		4				3				10	9		4		8

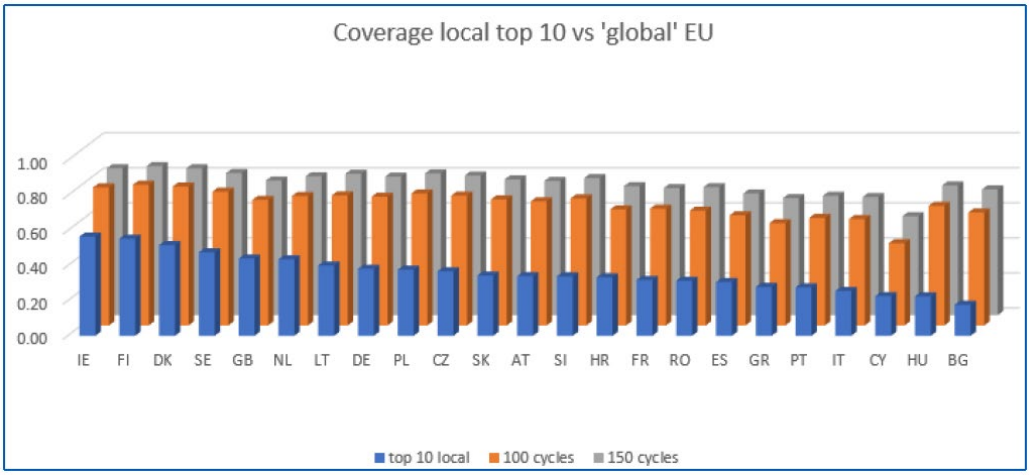
b) Global coverage model:

Calculation of frequency covered using the top 230 haplotypes, based in:
- 23 countries
- Top 230 haplotype coverage
- With no overlap

Table below shows that coverage per country after including top 230 global haplotypes. This is an indirect measure of the benefit of a common initiative. Now coverage increase more than 2-times.



Following chart exemplifies the benefit of using local lists versus a coordinated pan-European initiative. A comparison between local 10 vs global 100 and 150 selected haplotypes is shown.



c) Availability of CB units for the top ranked haplotypes:

Now, an exercise to explore the capacity of the already banked CB units to fulfil the need of source material for developing the European haplobank is described.

Table shows availability of CB units in the WMDA database with the 20 top ranked homozygous haplotypes included in European CB banks.

genotype	rank	donors	cord
A*01:01+A*01:01^B*08:01+B*08:01^DRB1*03:01+DRB1*03:01	1	65503	392
A*03:01+A*03:01^B*07:02+B*07:02^DRB1*15:01+DRB1*15:01	2	16617	110
A*02:01+A*02:01^B*07:02+B*07:02^DRB1*15:01+DRB1*15:01	3	6131	68
A*03:01+A*03:01^B*35:01+B*35:01^DRB1*01:01+DRB1*01:01	4	4069	29
A*02:01+A*02:01^B*13:02+B*13:02^DRB1*07:01+DRB1*07:01	5	1650	10
A*02:01+A*02:01^B*18:01+B*18:01^DRB1*11:04+DRB1*11:04	6	964	37
A*02:01+A*02:01^B*44:02+B*44:02^DRB1*04:01+DRB1*04:01	7	4607	22
A*29:02+A*29:02^B*44:03+B*44:03^DRB1*07:01+DRB1*07:01	8	3389	109
A*02:01+A*02:01^B*15:01+B*15:01^DRB1*04:01+DRB1*04:01	9	3968	17
A*02:01+A*02:01^B*51:01+B*51:01^DRB1*11:01+DRB1*11:01	10	537	15
A*01:01+A*01:01^B*57:01+B*57:01^DRB1*07:01+DRB1*07:01	11	1511	13
A*02:01+A*02:01^B*08:01+B*08:01^DRB1*03:01+DRB1*03:01	12	877	10
A*02:01+A*02:01^B*27:05+B*27:05^DRB1*01:01+DRB1*01:01	13	630	0
A*02:01+A*02:01^B*44:03+B*44:03^DRB1*07:01+DRB1*07:01	14	376	8
A*11:01+A*11:01^B*35:01+B*35:01^DRB1*01:01+DRB1*01:01	15	729	6
A*02:01+A*02:01^B*15:01+B*15:01^DRB1*13:01+DRB1*13:01	16	822	5
A*25:01+A*25:01^B*18:01+B*18:01^DRB1*15:01+DRB1*15:01	17	799	5
A*02:01+A*02:01^B*44:02+B*44:02^DRB1*16:01+DRB1*16:01	18	200	0
A*33:01+A*33:01^B*14:02+B*14:02^DRB1*01:02+DRB1*01:02	19	631	9
A*30:01+A*30:01^B*13:02+B*13:02^DRB1*07:01+DRB1*07:01	20	1162	17

As shown, even in top 20 of 'global' European haplotypes, some are not available in cord banks. This increase if we consider top 100 of 'global' European haplotypes: 16% have 10 or more cords, but 59% have only 1-9 cords and up to 25% have no

cords. Also need to consider attrition from need for blood group O donors, if required.

Interestingly, if we extend the search for homozygous CB units to international CB banks, the following is observed:

- For e.g. 13th ranked haplotype in Europe has 0 cords in European banks, but 32 examples in Brazil/Canada/US cord banks
- But 18th ranked haplotype in Europe has 0 cords in European banks and 0 in international banks

So likely there will be a need for other donor sources for many haplotypes

d) Remarks:

A bank of 230 HLA-A, -B, -DRB1 homozygous genotypes optimized for European population provided patient coverage that ranges from 67.2% to 90.3% with an average patient coverage of 80.8% (100 haplotypes = 69% and 150 = 75%).

By contrast, 23 countries developing their own bank of 10 cell lines (local top 10) would provide patient coverage that ranges from 22.5% to 56.6% with an average patient coverage of 36.7%.

An European-wide coordinated strategy will reduce redundancy and allow more patients to be treated with a much smaller investment.

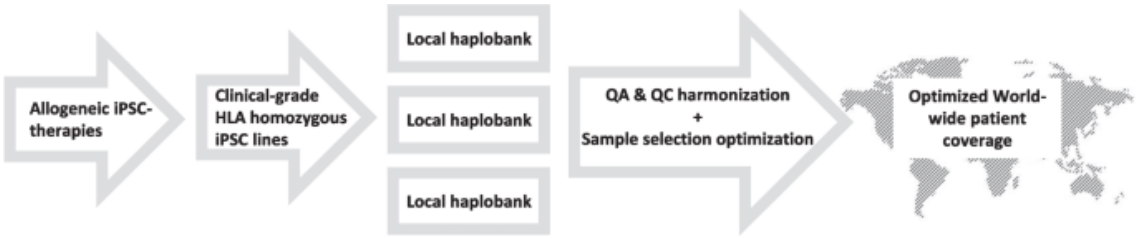
Optimal coverage genotypes (based on donor + CBU) may have 0 CBU in European banks. May be able to source some from non-European banks, but use of other sources may be necessary

4. Conclusion:

The first deliverable of HaploIPS WG1 (D1.1.) aims to report on the common understanding of the haplo-selected CB samples selection procedure.

Task force 1 worked on developing a common understanding of the HLA / CB sample selection procedure and presented a revision of initiatives and literature on hiPSC generation from haplo-selected CB samples and the preliminary data of which are the best donor samples available for clinical applications in public cord blood banks. Here, a methodology to define the most suitable HLA-haplotypes to cover most of the European population is shown.

Main conclusions are summed up in this picture:



First, allogeneic hiPSC therapies are paramount as future ways of treating many conditions under the fields of regenerative medicine and immunotherapy.

Clinical-grade HLA homozygous hiPSC lines could be the most cost-benefit way to facilitate access to such therapies to a substantial amount of people worldwide.

Development of local banks achieves a certain degree of coverage depending on the HLA diversity of the population. For instance, top 7 local lines already created and published cover 40% of Japanese population but only 21% of Spain. Here, we present preliminary data studying 23 European countries, showing the benefit of a coordinated initiative of proposing a global haplobank for Europe that could increase coverage. A bank of top 230 best ranked HLA-haplotypes covers an average of 80% of their population but the same number of lines developed by each of these countries (top 10 within 23 countries considered) only covers an average of 37%. This means HaploIPS cost-action will result in an optimization of resources, saving up to two times the investment required, and covering much of the European population.

A worldwide vision of this initiative maybe can result in the development of “local banks” but covering regional areas that could be grouped by studies on genetic ancestry. This is a common goal with GAiT (http://www.gait.global).

Such banks must harmonize quality assurance and quality control procedures and optimize sample selection strategies. These areas are also developed within HaploIPS initiative and are part of other working groups (https://haplo-ips.eu).

5. References

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