PIRCHE

How to integrate PIRCHE in today's routine

Introduction

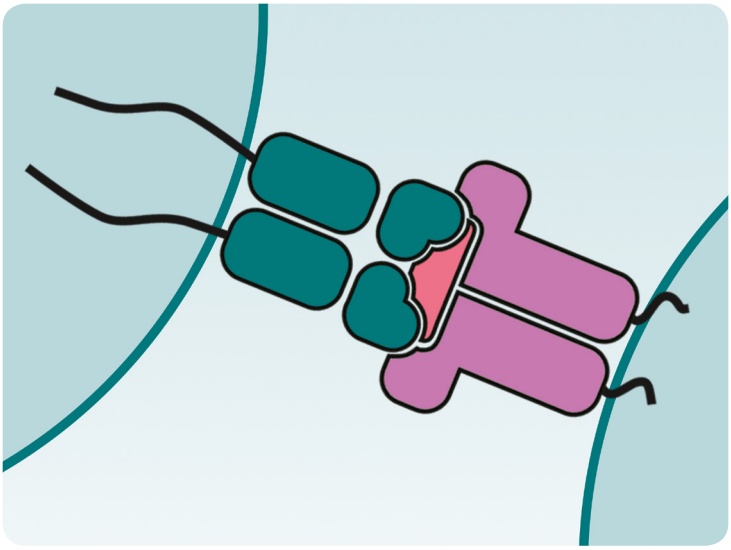
Previous studies showed the beneficial effect of low PIRCHE® epitope matching scores in both kidney-transplanted patients and patients who received hematopoietic stem cell transplantation. In kidney transplantations with high PIRCHE-II scores, the incidence of de novo donor specific antibodies was significantly increased compared to cases with a low PIRCHE-II score. This indicates, selecting kidney donors with a lower PIRCHE-II score reduces immunological risk after transplantation. In stem cell transplantations PIRCHE may play a role in development of GvHD, which suggests selecting mismatch donors with low PIRCHE scores. Here we want to describe how the PIRCHE® matching technology can be used in the work-up of a patient for kidney or stem cell transplant and demonstrate the different tools to stratify the risk to your patients.

The PIRCHE algorithm was invented by Eric Spierings at the University Medical Center Utrecht in the Netherlands. In 2013, his group published the proof of concept, which showed that an increased PIRCHE-II score in renal transplant patients correlates with the immunogenicity of donors' HLA mismatches.[1] Recently, the group of Nils Lachmann validated the technology in a larger single center cohort at the Charité University Hospital in Berlin, Germany.[2] In parallel, the PROCARE team – a

consortium of all Dutch kidney transplant centers – independently demonstrated, that the PIRCHE-II score furthermore correlates with graft survival.[3] In the stem cell transplantation setting two smaller cohort studies focused on the impact of PIRCHE on GvHD in HLA-C- and HLA-DPB1-mismatched hematopoietic stem cell transplantations (HSCT).[4,5] In both studies it was observed, that in patient groups having lower numbers of PIRCHE mismatches there was a lower incidence of GvHD.

PIRCHE-II are correlated to post-kidney-transplant donor-specific HLA antibodies

These findings are supported by a Dutch multicenter study for 9/10 matched HSCTs. Patient-



donor-constellations with low PIRCHE scores had a comparable transplant outcome to fully HLA matched transplantations.[6] A German research group reproduced these findings independently.[7]

The PIRCHE scores are calculated between a patient and donor at the time of donor selection and does not change over time. As it is a pure bioinformatics approach relying on the HLA typing, no additional work at the lab is required. Thus, there are no additional wet test costs involved.

1) H.G. Otten, J.J. Calis, C. Keşmir, A.D. van Zuilen, and E. Spierings, "Predicted indirectly recognizable HLA epitopes presented by HLA-DR correlate with the de novo development of donor-specific HLA IgG antibodies after kidney transplantation." Human Immunology. 2013; 74, no. 3, 290-296.
2) N. Lachmann, M. Niemann, P. Reinke, K. Budde, D. Schmidt, F. Halleck, A. Pruß, C. Schönemann, E. Spierings, and O. Staeck, „Donor Recipient Matching Based on Predicted Indirectly Recognizable HLA Epitopes Indipendently Predicts the Incidence of De Novo Donor-Specific HLA Antibodies Following Renal Transplantation." Am J Transplant. 2017; Jun 14.
3) K. Geneugelijk, M. Niemann, J. Drylewicz, A. D. van Zuilen, I. Joosten, W. A. Allebes, A. van der Meer, L. B. Hilbrands, M. C. Baas, C. E. Hack, F. E. van Reekum, M. Verhaar, E. G. Kamburova, M. L. Bots, M. A. J. Seelen, J.S.- F. Sanders, B. G. Hepkema, A. J. Lambeck, L. B. Bungener, C. Roozendaal, M. G. J. Tilanus, J. Vanderlocht, C. E. M. Voorter, L. Wieten, E. M. van Duijnhoven, M. Gelens, M. H.- L. Christiaans, F. J. van Ittersum, A. Nurmohamed, N. M. Lardy , W. Swelsen , K. A. van der Pant, N. C. van der Weerd, I. J. M. ten Berge, F. J. Bemelman, A. Hoitsma, P. J. M. van der Boog, J. W. de Fijter, M. G. H. Betjes, S. Heidt, D. L. Roelen, F. H. J. Claas, H. G. Otten, and E. Spierings, „PIRCHE-II is related to graft failure after kidney transplantation". Front. Immunol. 2018.
4) K.A.Thus, L.Te Boome, J. Kuball, and E. Spierings, "Indirectly recognized HLA-C mismatches and their potential role in transplant outcome." Frontiers in Immunology. 12th May 2014.
5) K.A. Thus, M.T. Ruizendaal, T.A. de Hoop, E. Borst, H.W. van Deutekom, L. Te Boome, J. Kuball , and E. Spierings, „Refinement of the definition of permissible HLA-DPB1 mismatches with predicted indirectly recognizable HLA-DPB1 epitopes." BBMT. November 2014, Volume 20, Issue 11, Pages 1705–1710.
6) K.A. Thus, K. Geneugelijk, H.W. van Deutekom, J. Calis, E. Borst, C. Kesmir, M. Oudshoorn, B. van der Holt, E. Meijer, S. Zeerleder, M. R. de Groot, P. von dem Borne, N. Schaap, J. Cornelissen, J. Kuball, and E. Spierings, "Identifying Permissible HLA-Mismatches in Unrelated-Donor Hematopoietic Stem-Cell Transplantation Using Predicted Indirectly Recognizable HLA Epitopes." Abstract at BMT Tandem 2017, unpublished data.
7) F. Ayuk, M. Bornhäuser, M. Stelljes, T. Zabelina, E.M. Wagner, C. Schmid, M. Christopeit, N. Kröger, W. Bethge, "Predicted Indirectly ReCognizable HLA Epitopes (PIRCHE) are associated with poorer outcome after single mismatch unrelated donor stem cell transplantation: a study of the German Cooperative Transplant Study Group (GCTSG) within the German working group for bone marrow and blood stem cell transplantation (DAG-KBT)." Abstract at EBMT 2017, unpublished data.

The PIRCHE Technology

The novel PIRCHE® technology predicts T cell related immune responses against HLA derived peptides after transplantation. In contrast to existing technologies, the indirect pathway of allo-recognition is in focus. PIRCHE uses the important functional peptide binding properties of HLA molecules. In environmental immune responses these peptides are derived from foreign proteins on, for example, viruses and bacteria. These bound peptides are presented to specific T cell receptors to evoke an immune response.

The same pathway leads to detection of exogenous proteins in the solid organ transplantation setting, which may be followed by an immune response against the transplant. Since hematopoietic stem cell transplantation is the transfusion of active, immunocompetent cells, unlike solid organ trans-plantation, these cells can recognize non-self peptides in the HLA mismatches of the patient, which may invoke an immune response targeted against the patient.

The genes in the HLA region are the most poly-morphic in the whole human genome. Transplanting tissue introduces foreign HLA proteins, which will inevitably be processed into smaller peptides by the lysosome. The active immune system may have T cells, which bind some of these peptides

and will cause an immune response. This means, the lower the number of such presented peptides, the lower the likelihood of an immune response. PIRCHE effectively simulates that concept and provides you the number of donor-mismatched peptides, which may be detected by the patients' T cells.[9]

There are over 17.000 HLA alleles known[8] - every day new alleles are found

To enable incorporating PIRCHE into today's laboratory workflow, we have developed a web-based platform to determine the PIRCHE scores. The service only requires entering the typing data of your patient and donors. The HLA typings can be entered as a serological type, as a molecular low or high resolution typing, or using multi allele codes. In case high-resolution HLA typings are unavailable, a multiple imputation method estimates the most probable genotypes of your patient and donors from given serologic or molecular low resolution HLA typings.

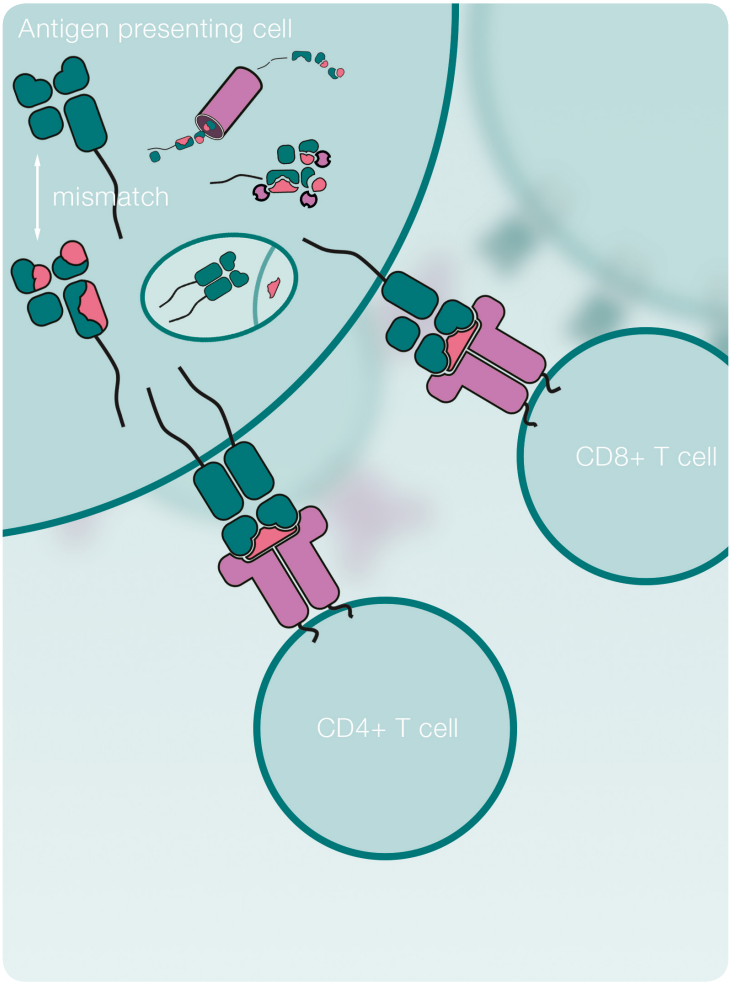
Epitope matching despite low resolution HLA data

We apply complex calculations dealing with hundreds of proteins, gigabytes of peptide data, predicting thousands of genotype constellations – just for a single match between one patient and

8) J. Robinson, J.A. Halliwell, J.H. Hayhurst, P. Flicek, P. Parham, S.G.E. Marsh, „The IPD and IPD-IMGT/HLA Database: allele variant databases", Nucleic Acids Research (2015) 43:D423-431.
9) K. Geneugelijk and E. Spierings, „Matching donor and recipient based on predicted indirectly recognizable human leucocyte antigen epitopes", Int J Immunogenet, 2018.

one donor. Our easy-to-use web service generates your results within fractions of a second.

The PIRCHE platform provides several modules using PIRCHE® epitope matching which are described in the following.



Solid Organ Transplant Risk Profile

It is well known, that for some patients it is more difficult to find a suitable donor than for others as some combinations of HLA molecules are rare in the population. These patients will typically get a poor HLA match leading to increased immunological risk.

With the PIRCHE SOT Risk Profile, this issue is addressed from an epitope perspective by calculating a PIRCHE-II histogram for a cohort of random donors from the selected ethnic back-

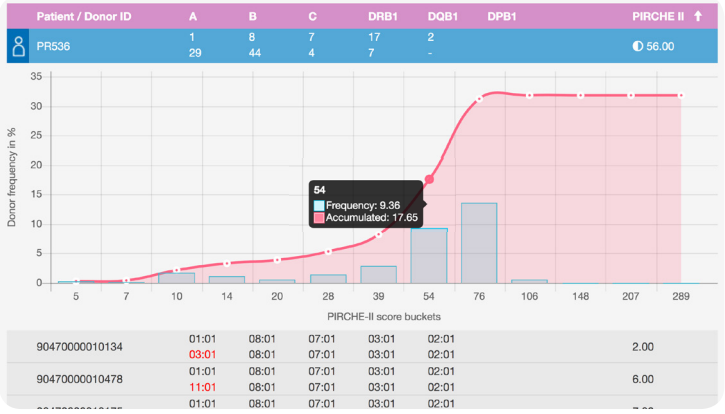


Figure 1: The PIRCHE SOT Risk Profile shows the PIRCHE-II scores to be expected with random organ donors in the local population, which in this example is of European origin. The allocation mismatch schema was 1 A-, 1 B-, 2 C-, 1 DRB1- and 2-DQB1-mismatches. The histogram shows the donor frequency (y-axis) in increasing PIRCHE intervals (x-axis). The red curve indicates the cumulated donor frequency. The median PIRCHE-II score for this patient is 56, indicating half of the donors having a lower score and half of them having a higher PIRCHE-II score. The list at the bottom of the display shows some donor genotypes with the lowest number of PIRCHE mismatches.

ground and using the allocation schema. After copying the HLA data from the laboratory system into the PIRCHE portal, a detailed analysis is provided of the likelihood, that the patient will be offered an organ with low PIRCHE-II scores. The calculated median PIRCHE-II score is where 50% of the donors will have a lower (i.e. better) PIRCHE-II score and 50% of the donors will have a higher (i.e. worse) PIRCHE-II score (see figures 1 and 2).

Calculate the epitope background risk without any donor

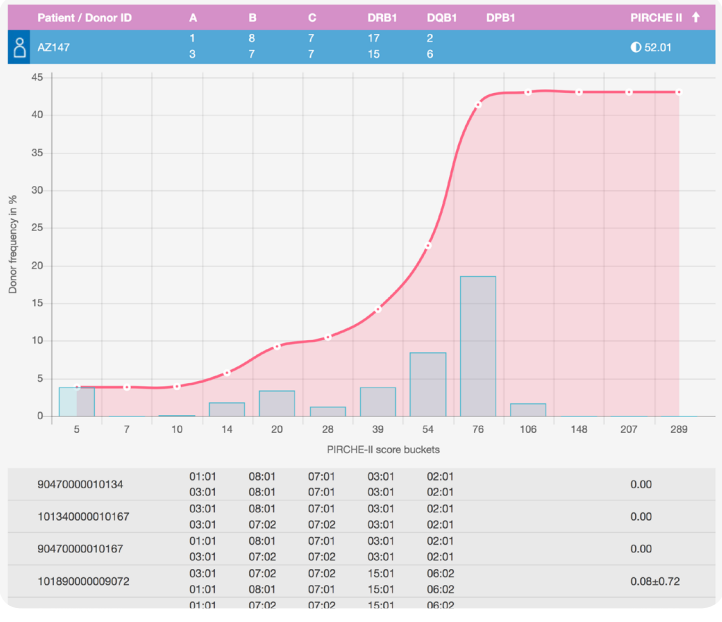
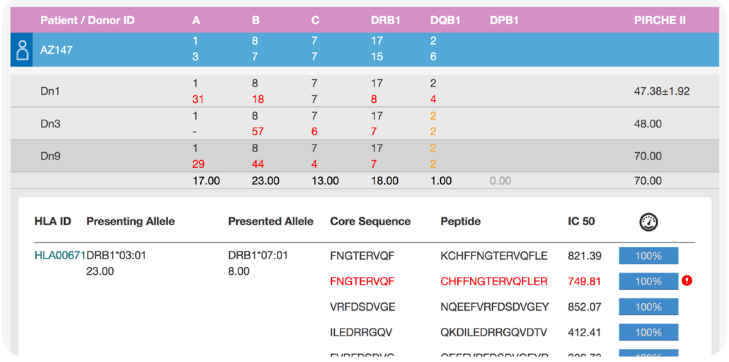


Figure 2: Compared to the patient in figure 1, this example's patient has only a mildly decreased PIRCHE-II median score of 52. The histogram however indicates, the likelihood of finding a donor with a very low PIRCHE-II is increased, as the frequency of donors with 5 or less PIRCHE is higher.

The Single Patient modules allows the user to determine – depending on the setting – the PIRCHE-I and -II scores for particular donor HLA types. For kidney transplantation this is useful since a particular deceased donor offer or a certain living donor might not be the best choice for the patient, if the PIRCHE-II score is above the median calculated in the SOT Risk Profile. Figure 3 shows the PIRCHE-II score of three donors for a patient that has a Risk Profile median PIRCHE-II score of 52. Donor Dn9 has a much higher PIRCHE-II score whereas Dn1 has a lower score of ~47. However, if only Dn9 is available, it may be worthwhile considering waiting for a better donor with a PIRCHE-II score below the median PIRCHE-II score obtained in the Risk Profile analysis for this patient (see figure 2).



Patient / Donor ID	A	B	C	DRB1	DQB1	DPB1	PIRCHE II
AZ147	1 3	8 7	7 7	17 15	2 6		
Dn1	1 31	8 18	7 7	17 8	2 4		47.38±1.92
Dn3	1 -	8 57	7 6	17 7	2 2		48.00
Dn9	1 29	8 44	7 4	17 7	2 2		70.00
	17.00	23.00	13.00	18.00	1.00	0.00	70.00

HLA ID	Presenting Allele	Presented Allele	Core Sequence	Peptide	IC 50	
HLA00671	DRB1*03:01 23.00	DRB1*07:01 8.00	FNGTERVQF	KCHFFNGTERVQFLE	821.39	100%
			FNGTERVQF	CHFFNGTERVQFLER	749.81	100%
			VRFDSDVGE	NQEEFVRFDSDVGEY	852.07	100%
			ILEDRRGQV	QKDILEDRRGQVDTV	412.41	100%

Figure 3: Comparing the donors' match result with the median PIRCHE of 52 as calculated in the Risk Profile (see figure 2) suggests selecting donor Dn1 or Dn3.

In stem cell transplantations, the impact of a single mismatch of available 9/10 donors on the PIRCHE-I and -II scores can be determined by this module as well. In figure 4 donor Dn1 (DQB1 mismatch) has the lowest combined PIRCHE-I and -II score of 1, with donor Dn2 (A mismatch) and Dn3 (C mismatch) having cumulated scores of 21 and 34 respectively.

Choose between manual input with validation, semi-automatic data upload or API access

In contrast to the Single Patient modules, the Multi Patient modules allow the user to calculate PIRCHE scores of multiple cases at once e.g. for retrospective analyses. Given a csv-formatted input, the web service will return a machine-readable output file.

Patient / Donor ID	A*	B*	C*	DRB1*	DQB1*	DPB1*	PIRCHE I ↑	PIRCHE II
KL824	01:01 29:02	08:01 44:03	07:01 16:01	03:01 07:01	02:01 02:02			
Dn1	01:01 29:02	08:01 44:03	07:01 16:01	03:01 07:01	02:01 03:02		0.00	1.00
Dn2	02:01 29:02	08:01 44:03	07:01 16:01	03:01 07:01	02:01 02:02		7.00	14.00
Dn3	01:01 29:02	08:01 44:03	04:01 16:01	03:01 07:01	02:01 02:02		16.00	18.00

HLA ID	Presenting Allele	Core Sequence	Peptide	IC 50
HLA00001	A*01:01 1.00	YWDRETQNY	YWDRETQNY	360.56
HLA00475	C*16:01 11.00	AVLVVLAVL	AVLVVLAVL	303.21
		LLLSGGLAL	LLLSGGLAL	27
		RVMAPRALL	RVMAPRALL	23.75
		SSQPTIPIM	SSQPTIPIM	375.58
		VAGLAVLVV	VAGLAVLVV	280.63
		KTHVTHHPL	KTHVTHHPL	73.21
		VMAPRALLL	VMAPRALLL	21.59

Figure 4: All three donors have one mismatch with the patient's typing. However, PIRCHE allows to further stratify donor suitability by calculating T cell epitope mismatches. Donor Dn1 has 0 PIRCHE-I and 1 PIRCHE-II and therefore represents a low risk donor for the patient.

Definition of Acceptable Mismatches

The SOT Risk Profile calculates the patient's median PIRCHE-II score against a cohort of random donors from different population groups. However, the PIRCHE portal can also determine which mismatches should be avoided and which mismatches are more acceptable. Avoiding high-risk mismatches and reducing the number of HLA antibodies is beneficial for subsequent transplants, making more donor options available in the future.

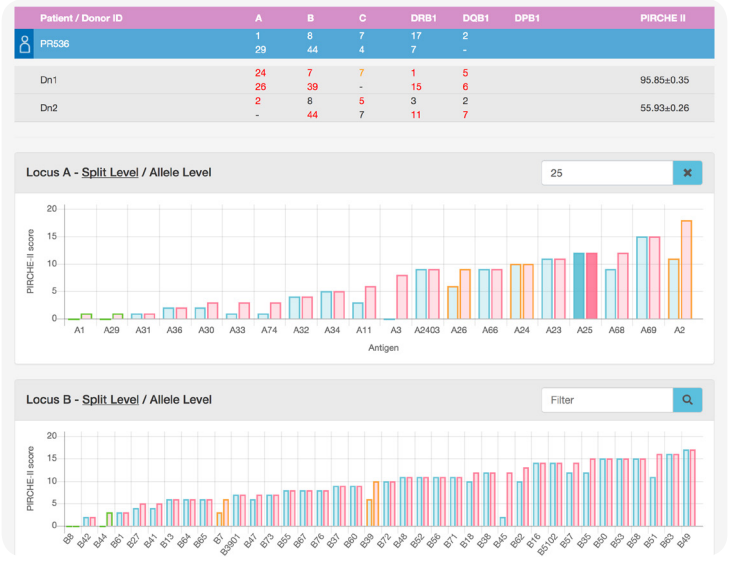


Figure 5: The Acceptable Mismatch Profile shows the impact of individual mismatches on the PIRCHE-II score. Where classical HLA matching does not differentiate between antigen mismatches, PIRCHE clearly can discriminate between the different types of mismatches. In this example, a B42 mismatch contributes to the overall score with 2 PIRCHEs, whereas a B49 mismatch yields in 17 PIRCHEs. The blue bar indicates the lowest allelic PIRCHE-II score within the serologic split, the red bar the highest allelic PIRCHE-II score.

The PIRCHE Acceptable Mismatch Profile allows the individual evaluation of each antigen and allele (see figures 5 and 6). Mismatches with a high PIRCHE-II score or repeated mismatches from previous transplantations may be considered unacceptable for future donor offers. On the other hand, mismatches with a low score may be considered as acceptable mismatches. This enables the user to determine low- versus high-risk mismatches for their patients, both at the serological split and the allelic levels. Especially when defining unacceptable mismatches

Figure 6: Browsing through the immunogenicity of mismatched alleles reveals there can be PIRCHE-II score variability within the same antigen group. In this example, a mismatch with DRB1*14:07 has a PIRCHE score of 5 compared to DRB1*14:06, 14:04 and 14:01 with PIRCHE scores of 6, 7 and 8 respectively.

for a kidney transplant recipient it is interesting to correlate epitope matching scores with the current HLA antibody status. Therefore, the PIRCHE Acceptable Mismatch Profile supports loading MFI values of HLA single antigen assay runs and enriches these values with the antigen's individual PIRCHE scores (figure 7).

Connect HLA antibodies and PIRCHE scores

Figure 7: The Antibody Matrix plots MFI values of HLA single antigen assays (y-axis) and PIRCHE scores (x-axis). Circles show HLA antigen beads and their respective PIRCHE score, whereas plus signs stand for antigens that aren't represented in the antigen assay. Triangles facing up highlight self-antigen, whereas triangles facing down symbolize donor antigen.

HSCT Search Profile

For stem cell donor search centers with no direct access to stem cell donor registry databases, it might be useful to create a search profile for the patient to guide the local search strategy.

The PIRCHE HSCT Search Profile supports this process by matching virtual donor populations with the patient. Currently, there are 4 populations available that are based on haplotype frequency tables.[10] The result is a list of 9/10 matched genotypes, with their phenotype frequency and PIRCHE scores. This allows the user to determine the mismatches with low PIRCHE values as well as the frequency they

Patient / Donor ID	A*	B*	C*	DRB1*	DQB1*	DPB1*	PIRCHE I	PIRCHE II ↑	Frequency
BG899	01:01 29:02	08:01 44:03	07:01 16:01	03:01 07:01	02:01 02:02				
90480000011449	01:01 29:02	08:01 44:03	07:01 16:01	03:01 07:01	06:02 02:02		0.00	0.00	1.2888
90560000011443	01:01 29:02	08:01 44:03	07:01 16:01	07:01 03:01	03:03 02:01		0.00	1.00	0.1165
92020000011405	01:01 29:02	44:03 08:01	16:02 07:01	07:01 03:01	02:02 02:01		0.00	1.00	1.5412
90470000011356	01:01 29:01	08:01 44:03	07:01 16:01	03:01 07:01	02:01 02:02		0.00	1.00	18.6432
91380000011449	01:01 29:02	08:01 44:03	07:07 16:01	03:01 07:01	02:01 02:02		0.00	2.00	1.1047
91980000011664	01:01 31:01	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		1.00	3.00	11.8056
90470000011460	01:01 29:02	08:01 44:04	07:01 16:01	03:01 07:01	02:01 02:02		3.00	5.00	4.4744
91980000011856	01:01 32:01	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		3.00	7.00	15.1691
10329000011405	03:02 29:02	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		2.00	8.00	9.2403
10286000011405	03:01 29:02	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		2.00	8.00	77.1434

Figure 8: The HSCT Search Profile allows to find mismatches with low PIRCHE values, sorted on PIRCHE-II score. The frequency is shown "per million donors" and allows estimating how often a donor may be present in a registry.

10) M. Maiers, L. Gragert, W. Klitz, "High resolution HLA alleles and haplotypes in the US population", Human Immunology (2007) 68, 779-788.

will be found in the population. The example in figure 8 shows the expected 9/10 donors ordered according to PIRCHE-II score. The donor with the lowest PIRCHE score (zero) is a DQB1 mismatch with a frequency of ~1 per million donors. The fourth donor listed is an A mismatch with a total PIRCHE score of 1 but with a much higher frequency of ~18 per million donors.

There is more than one needle in the haystack

Figure 9 orders the 9/10 donors according to their frequency. The donor genotype with the highest frequency (~304 per million donors) is an A mismatch (A*02:01 versus A*29:02) and the donor with the second highest frequency (~182 per million donors) is also an A mismatch (A*02:01 versus A*01:01).

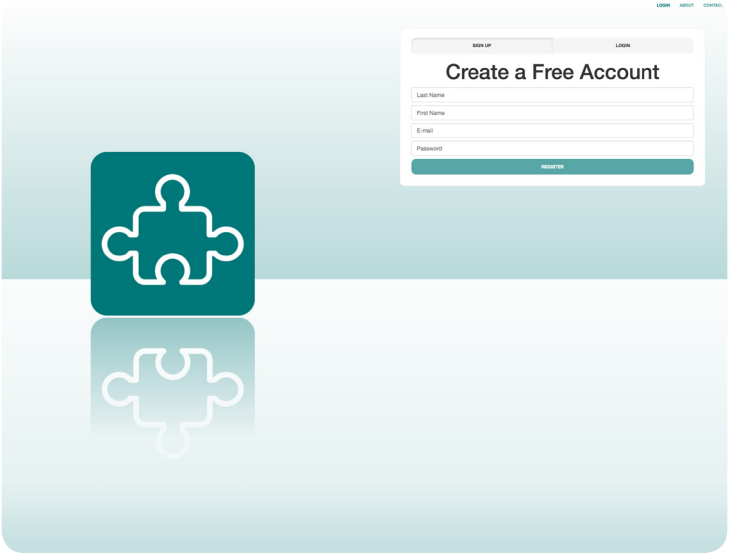
Patient / Donor ID	A*	B*	C*	DRB1*	DQB1*	DPB1*	PIRCHE I	PIRCHE II	Frequency ↓
BG899	01:01 29:02	08:01 44:03	07:01 16:01	03:01 07:01	02:01 02:02				
91980000009588	01:01 02:01	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		8.00	13.00	304.4497
98190000011405	02:01 29:02	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		7.00	14.00	182.3270
91980000009047	01:01 01:01	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		13.00	18.00	161.0774
102860000011405	03:01 29:02	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		2.00	8.00	77.1434
91980000010134	01:01 03:01	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		13.00	16.00	76.1585
110520000011405	24:02 29:02	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		7.00	18.00	72.8942
91980000010478	01:01 11:01	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		13.00	18.00	55.5689
91980000010900	01:01 24:02	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		13.00	17.00	49.0272
111260000011449	25:01 29:02	08:01 44:03	07:01 16:01	03:01 07:01	02:01 02:02		10.00	18.00	21.9099
114490000011405	29:02 29:02	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		10.00	23.00	20.9893
105710000011405	11:01 29:02	44:03 08:01	16:01 07:01	07:01 03:01	02:02 02:01		4.00	11.00	19.5670
	68:01	44:03	16:01	07:01	02:02				

Figure 9: Sorting the HSCT Search Profile by frequency per million donors allows to evaluate, which mismatches will be found more often in the selected population and which PIRCHE scores these will result in.

Start Today

The PIRCHE web service is available 24/7, worldwide from any computer with Internet access. There is no need to install any software locally. Simply create a free test account and try it out.

We are always keen to hear your ideas on how we may collaborate and further improve our service.



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