PIRCHE II Risk And Acceptable Mismatch Profile Analysis in Solid Organ Transplantation

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Summary/Abstract

To optimize outcomes in solid organ transplantation, the HLA genes are regularly compared and matched between the donor and recipient. However, in many cases a transplant cannot be fully matched, due to widespread variation across populations and the hyperpolymorphism of HLA alleles. Mismatches of the HLA molecules in transplanted tissue can be recognized by immune cells of the recipient, leading to immune response and possibly organ rejection. These adverse outcomes are reduced by analysis using epitope-focused models that consider the immune relevance of the mismatched HLA.

PIRCHE, an acronym for Predicted Indirectly ReCognizable Epitopes, aims to categorize and quantify HLA mismatches in a patient-donor pair by predicting HLA-derived T-cell epitopes. Specifically, the algorithm predicts and counts the HLA-derived peptides that can be presented by the host HLA, known as indirectly-presented T-cell epitopes. Looking at the immune-relevant epitopes within HLA allows a more biologically-relevant understanding of immune response, and provides an expanded donor pool for a more refined matching strategy compared with allele-level matching. This PIRCHE algorithm is available for analysis of single transplantations, as well as bulk analysis for population studies and statistical analysis for comparison of probability of organ availability and risk profiles.

Materials

PIRCHE Web Portal

The fully integrated PIRCHE prediction pipeline is provided as a web service (https://pirche.com/), that is accessible through any modern web browser. The default "clinical mode" of the application provides a basic feature set as used by previously published studies. The "lab mode" unlocks experimental features that are in development and under investigation. These modes can be switched within the application.

HLA Typing Data

Several input formats are supported; genotypes can be provided using standard HLA nomenclature,***(42)*** as serological types, or antigens with multi-allele codes. Internally, the PIRCHE-II method processes HLA protein-level typing data of both patient and donor to identify corresponding full-length amino acid sequences. A multiple imputation approach using NMDP Haplotype frequencies ***(43, 44)*** was implemented to estimate PIRCHE-II scores in cases of low resolution HLA typing data or missing HLA loci. This approach extracts potential haplotype pairs from given inputs and extracts the haplotypes' frequencies from respective tables. This may yield multiple potential protein-level genotypes for the provided typing information, which are further considered in PIRCHE-II analyses and finally aggregated based on the corresponding genotypes' frequencies in the specified population. ***(45)***

Generally, three ways to load typing data into the PIRCHE web service are available: (1) entering typing data manually, supported by auto-completion and nomenclature validation, (2) extraction of HLA data from common standardized data formats (CSV, HML ***(46)*** and GLString ***(47)***), and (3) using the service's REST API for machine-to-machine (M2M) communication. Example input files are provided both within the web service and on Github (https://github.com/PIRCHE/pipeline for CSV, https://github.com/nmdp-bioinformatics/hml for HML).

Access, Analysis and Extraction Scripts

Optional scripts that support M2M access to the PIRCHE service and data transformation of output files are provided on the PIRCHE Github Page for Python and R (https://github.com/PIRCHE/pipeline). For integration in 3rd party applications, PIRCHE provides an OAuth2-secured API, that can be obtained upon request. Various HLA diagnostic software suites have already been connected through this API to allow PIRCHE analysis without manual steps in exchanging data (Immucor® MatchIt!® Integration Utility; OneLambda® Fusion™ 4.4+ specialty report "LABScreen - PIRCHE"; GenDX® NGSengine®; Omixon® HLA Twin™). For submitting HLA typing data from these software suites to the PIRCHE portal we refer to the respective vendor's documentation.

Risk and Acceptable Mismatch Profile (RAMP)

As explained, the PIRCHE-II score is dependent on the patient's HLA typing in both 1.) the characteristic HLA self-peptidome and 2.) the presenting HLA proteins. Consequently, the distribution of PIRCHE-II scores for a fixed donor panel (i.e. the PIRCHE-II Risk Profile) between patients can vary significantly (Fig 4). Likewise, the PIRCHE-II Risk Profile for a

single patient may be different depending on distinct donor populations. Consequently, it can be difficult to define reasonable static thresholds for the PIRCHE-II score that can be applied to a majority of patients. The absolute thresholds suggested by Lachmann *et al.**(28)* simplify visualization in retrospective studies, but may lack applicability for every patient considering the patient specific score range.

The RAMP module creates a PIRCHE-II Risk Profile for an input patient typing considering a predefined virtual donor population or custom uploaded donor panels, which may be based on e.g. the last 100 organ offers. RAMP also converts specific donor typings' PIRCHE-II scores into a rank considering the applied donor population. In contrast to the reported absolute ranges, this approach allows identifying relative thresholds for each patient. A rank of e.g. 10% can be interpreted as "10% of my donor pool yield a lower (preferable) PIRCHE-II score" but also means "90% of my donor pool yield a higher (inferior) PIRCHE-II score". The example of Patient A provided in Fig 4 indicates that a donor with a PIRCHE-II of 100, which is categorized as "high risk" by Lachmann *et al*. (i.e. > 90; ***(28)***) may still have a low rank, suggesting that there are only low chances of finding donors with a lower PIRCHE-II score.

The Acceptable Mismatch Profile of the RAMP module provides PIRCHE-II scores of individual alleles, which helps identify the strongest contributors to the overall PIRCHE-II sum. Selecting alleles as unacceptable mismatches within the Acceptable Mismatch Profile excludes virtual donors carrying the respective allele from the Risk Profile, which also has an impact on the PIRCHE-II distribution amongst the remaining donors.

Alternatively, the Acceptable Mismatch Profile also allows selecting alleles as Previous Immunizers according to the previously published concept of repeated T cell epitope mismatches (Tmem). ***(40, 41)***

Methods

The PIRCHE-II methodology is flexible, and can be applied in several analysis scenarios (Fig 5). These scenarios vary based on the source and format of the HLA genotypes, as well as the specific use case, whether it is used in comparing risk of patients or applying to population studies. Therefore, techniques in analysis do not follow a single path, and depend on the user's needs. These paths are visually illustrated in figure 5, and some general techniques are described below for the cases of Living Donor analysis, Population studies, and RAMP analysis without a donor.

Prepare Genotype Data

PIRCHE-II is flexible in regards to the source of input HLA genotyping data for patients and donors. Standard data formats are supported, and HLA genotypes can be entered in the PIRCHE interface manually.

Note: By using supported 3rd party software suites, typing data is transmitted automatically into the PIRCHE application, allowing to skip manual steps in input preparation and entry.

2.1 Extract patient or donor HLA typing data manually

Located HLA typing report within any HLA genotyping software. A minimum requirement is serological typing for HLA-A, HLA-B, and HLA-DR, however more accuracy is obtained by providing 2-field allele-level genotypes for HLA-A, HLA-B, HLA-C, HLA-DRB1,2,3; and HLA-DQB1. Be sure to select the Molecular or Serological toggle depending on how the typing should be interpreted and extrapolated.

These genotypes can be entered by copy-pasting or manually entering into the corresponding patient and donor fields in the PIRCHE interface.

2.2 Extract patient or donor HLA typing data as a GL-String:

Genotypes can also be prepared as a GL-String, a standardized text format. GLStrings can be prepared using intermediate tools, may be present in Lab Management Systems, or exported from a genotyping software. This format is convenient for reducing some error from manually inputting several genotypes.

```
A*24:02+A*26:02^B*15:01+B*40:01^DRB1*14:06^DQB1*03:01
```

2.3 Extract one or more donors' HLA typing data and prepare a csv input

In a PIRCHE .csv format, each line represents an individual patient or donor. The comma-separated line contains first a unique ID, and then HLA typing. Generating the input data can be performed using e.g. Excel (although care should be taken when creating this input file, see Notes). The data input files can also be created to include context-specific data using custom-built scripts or programs. Data from a laboratory management system, or even simulated populations, can be converted into the PIRCHE CSV input format.

The PIRCHE csv format can be used to prepare an individual patient genotype which

is entered separately from the paired donors. This applies to the Living Donor or RAMP scenarios. A single line can be prepared containing a patient's genotypes:

```
patientB,A*01,A*02,B*51,B*08,C*06,C*07,DRB1*11,DRB1*09,DQB1*03
```

In the case of Living donor analysis, each potential donor is separated on a different row. When the csv is inputted using the input wizard, the donor genotypes automatically populate their corresponding fields:

```
d1,A*33:03,B*44:03,DRB1*13:02,DQB1*06:04
d2,A*33:03,A*24:02,B*15:01,B*44:03,DRB1*14:06,DRB1*13:02,DQB1*03:01,DQB1*06:04
d3,A*24:02,A*01:01,B*15:01,B*48:01,DRB1*14:06,DRB1*03:01,DQB1*03:01
```

For CSV population analysis, the same input format is used to pair patients with donors. In this format, patient and donor HLA genotypes are provided on separate lines. Patient and donor pairs are separated into analysis groups, separated by a line containing a single comma (,), to specify which pairings should be analyzed.

The module provides an example CSV input by pushing the Example button (Fig 5 step 5.2, Fig 6). In the given example, a PIRCHE-II score will be calculated for a transplantation from “donorA” in the context of recipient “patientA”. Similarly, for the recipient “patientB”, two separate donors (donorB1 and donorB2) are analyzed and compared as potential donors.

2.4 HML

An HML (Histoimmunogenetics Markup Language) export is supported in many of the modern genotyping software packages. This XML-based file is exported directly from a genotyping software or laboratory management system, it can contain HLA genotyping data, as well as some related metadata.

The HML file can be prepared for a patient as well as donor, and (Fig 5 step 5.1) can be uploaded using the upload button next to the input wizard.

PIRCHE-II in Living Donor Scenarios

When a clinician encounters a patient who requires a transplant, they can use PIRCHE to compare potential donors for their PIRCHE compatibility. A focus on epitope compatibility gives a more refined perspective than allele-level matching, and can give indications on the relative risk between multiple potential donors.

For this example, we're considering the scenario of a patient with three potential living donors.

1.1 Locate typing data. This is likely contained in a HLA genotyping software or the Laboratory Management system.

Depending on the format of the data, or the HLA typing vendor's PIRCHE integration, step 2.X applies:

2. Extract and prepare the HLA genotyping data for the patient(s) and donor(s) (See "Prepare Genotype Data" section above.

3.1. Login to pirche.com using your credentials, or create a new account.

4.1. Select "Single Patient" from the "SOT" section on the top menu bar

5.1.a Paste patient HLA typing (GL-String, CSV) into Input Wizard's "Paste data here", or import HML file via the upload button next to the Input Wizard

5.1.b Paste multiple donor HLA typings (GL-String, CSV) into Input Wizard's "Paste data here", or import HML file via the upload button next to the Input Wizard

See Notes: Data Input

5.1.c (Optionally) Add or remove the Patient or Donor HLA Genotypes manually

5.1.d (Optionally) Select population for imputation for patient and donors separately. Will be ignored if two-field data is provided.

6.1 Press "Match" to perform PIRCHE calculations.

7.1 After matching, the result page is loaded automatically (Fig 7).
The typing result can also be exported and downloaded in a CSV or PDF format.
PIRCHE-II scores of donors provided in a table with lower scores indicating higher compatibility.
Pressing the folder button at the end of a donor row shows additional results of a specific match result, including a heatmap of PIRCHE-II scores and a table listing the identified T cell epitopes (Fig 8).

Risk and Acceptable Mismatch Profile (RAMP)

The first steps for performing a PIRCHE RAMP analysis are similar to matching for a single solid organ transplant. The patient genotype, as well as any previous transplantations, can be provided using the same set of input formats. The major difference in RAMP is in evaluation of the results, as this module provides an informative set of results for the patient across a virtual donor population.

1.1-5.1.d (see above)

5.1.e Select the virtual donor population in the top Settings panel. You may either select a predefined population, or upload your own local donor pool via Settings -> Populations for your own PIRCHE account

6.2 Press “RAMP”' to perform the PIRCHE RAMP analysis. The analysis may take up to a few minutes.

7.2 The RAMP analysis is divided into several sections (Fig 9).

7.2.a Genotype Bar

Near the top of the RAMP results you can find the genotypes of the patient and previous donors. If needed, these have been extrapolated based on population frequencies provided during data input. A median PIRCHE value for the patient with respect to the virtual donor population is shown, as well as a PIRCHE score for each previous donor. Previous donors are ranked according to the virtual donor population, suggesting how this donor compares to a random donor of the virtual donor population. By left-clicking alleles in the bar charts, they can be marked as unacceptable mismatches. A calculated PRA is then provided based on the selection. This shows a percentage of the population that the patient’s antibody serum is likely to react against.

7.2.b Population Histogram

The simulated virtual population is compared as potential donors for the provided patient information. The potential donors are sorted by their PIRCHE-II scores and assigned into “buckets'' in the histogram. Each “bucket” shows a range of PIRCHE scores, and can quickly give a visual indication if you can expect relatively high or low PIRCHE scores for the patient in the population.

The histogram is overlaid with a curve which shows the cumulative population frequency, moving left to right. Donors are accumulated, from low, to high PIRCHE score, and contribute to the cumulative curve. This gives another indication of how the population profile relates to the patient in terms of PIRCHE-II score. The curve approaches 100%, in the case where the whole population is considered. If unacceptable mismatches are selected, a portion of the donor pool is excluded from this histogram, and the curve approaches a lower proportion of the population according to the remaining acceptable donors.

See Notes: RAMP

7.2.c Acceptable Mismatch Barcharts

These barcharts are divided by HLA loci, and list specific alleles which are present in the simulated virtual donor population. Aside from allele frequency, the bar charts indicate the PIRCHE-II score derived from this specific allele. If MFI values from single antigen bead assays are uploaded via Immucor® MatchIt!® or OneLambda® Fusion™ export files, the corresponding beads MFI values are also shown.

The plot can be re-ordered by a few select parameters, the MFI or the PIRCHE-II score from these alleles. When questionable alleles are selected, they can be assigned as unacceptable alleles by left clicking the corresponding bar. As unacceptable alleles are selected, the PIRCHE-II Risk Profile and the donor ranks are automatically updated to reflect the reduced pool of donors. The calculated PRA is also updated.

See Notes: RAMP

7.2.d Repeated T cell epitope mismatches (Tmem)
Within the Acceptable Mismatch Barcharts, alleles can be selected as Previous Immunizers by right clicking the respective bar. Based on the selection, overlapping T cell epitopes between all other alleles and the selected Previous Immunizers are indicated with orange bars.

PIRCHE-II in Research & Donor Cohorts

In addition to analysis of individual transplantations, and exploring the population risk for individual patients, PIRCHE is also commonly used in cohort analysis. This can be for retrospective studies that consider the differences in using PIRCHE modeling compared with traditional HLA matching. For large-scale PIRCHE analysis of patients against an arbitrary number of donors, a researcher can use the csv input module. The HLA genotypes can be encoded in a comma-separated format, which is generated using Excel or other computational tools.

An advantage of csv-based PIRCHE analysis is that data can be created using specially designed scripts. Genotypes can be exported, converted, or generated from many software tools, databases, or frequency tables. This allows flexibility in analyzing e.g. retrospective transplant cohorts, real world patient data against specially designed donor pools, or HLA genotypes representing cells to be used in a cellular therapy.

1.1 Locate typing data. This is likely contained in a Laboratory Management system, from a HLA genotyping software, or from a database or tool with potential donors.

2. Extract and prepare the HLA genotyping data for the patient(s) and donor(s) (See “Prepare Genotype Data” section above. The .csv format is the most suitable for entering data for cohort analysis.

3.1 Login to pirche.com using your credentials, or create a new account.

4.3 To access the CSV module, select the “SOT” in the top menu, then “Multi Patient (CSV)”

5.2.a Paste your generated csv input data into the PIRCHE analysis field. (Figure 6)

5.2.b Adjust settings if deviating from defaults. With large datasets, it may be required by the application to store the results on the server to avoid session timeouts.

6.3 Press “Match” to perform PIRCHE calculations. After matching, the result page is loaded automatically

7.3.a Interpret Results - CSV Result Format

The analysis results are returned as a CSV file, which can be imported and read by a variety of applications. The analyzed donors are separated to separate lines, and the separated results are stored in columns within the spreadsheet. It is discouraged to use Excel for following analysis given automatic data transformations alter produced output data. To create an Excel-friendly result file, the data transformation scripts provided on https://github.com/PIRCHE/pipeline to compress output files are recommended.

The combined PIRCHE-II score is in the column labeled “PIRCHE II”. This score represents the estimated number of peptides likely to be presented by the patient’s HLA Class II. The sequences of the presented peptides are specified in separate columns, e.g. DRB1_1_Presents_Epitopes and DRB1_2_Presents_Epitopes. DRB1_1 and DRB1_2 represent the two DRB1 alleles by the patient. Alternatively, the columns such as “DRB1_Presents_B_Epitopes”, specify epitopes that are presented by HLA-DRB1 and derived from donor HLA-B.

7.3.b Interpret Results - Individual Epitope

The csv results can be analyzed by comparing the general PIRCHE scores, and this analysis can be even further refined by analyzing the specific predicted epitopes.

The epitopes are listed in a specific format, for example

FQKWASVVV GTFQKWASVVVPSGQ (603.57) [0.6094]

In this format, 4 pieces of information are separated by spaces.

- “FQKWASVVV” represents the 9-mer peptide “core,” which is the section of a peptide that is aligned to the HLA binding groove. This sequence is a subset of the full 15-mer peptide.
- “GTFQKWASVVVPSGQ” is the 15-mer peptide, derived from an HLA sequence.
- (603.57) is an IC50 concentration, as predicted by the PIRCHE model. An IC50 is the half-maximal inhibitory concentration. Peptide binding assays may report an IC50 as a result, and this measure is often thought of as a rough estimation of binding strength, although this is not completely accurate. Lower IC50s indicate a peptide may be more likely to bind the HLA, and in some scenarios IC50 cutoffs are used to categorize Strong Binders, Weak Binders, or Nonbinders. By default, PIRCHE-II considers an IC50 threshold of 1000 nM.
- [0.6094] represents a “weight” of a given PIRCHE peptide. The weight can be considered as a probability of a given PIRCHE actually being present, as

genotype ambiguities may be introduced by multiple imputations based on serological, molecular low or intermediate resolution typing.

Notes

Data Input

1. Patient and donor IDs must be unique within the transmitted dataset (both in Single Patient and CSV cases).

2. When Generating CSV files for analysis in the CSV module, care should be taken to ensure that the format matches the expected input of the PIRCHE files.
   This includes:
   a. The input uses a comma (,) delimiter, as opposed to semicolon (;), tab, or other delimiters.
   b. Analysis blocks (patient-donor pairings) should be separated by a line with a single comma (,)
   c. Gene names for HLA are specified as e.g. DRB1*01:01, instead of HLA-DRB1*01:01

3. When saving files in Excel or other Spreadsheet software, take care in selecting the exported format (CSV), and inspect the output files using a standard text editor (*e.g.* Notepad). Spreadsheet editor tools may often perform unexpected or inappropriate data conversions without warning the user.

4. By using supported 3rd party software suites, typing data is transmitted automatically into the PIRCHE application, allowing to skip manual steps in input preparation and entry.

5. The Input Wizard overwrites previous inputs. It processes multiple donor rows at once.

6. Identify and fix any input errors, indicated by red boxes (e.g. typos, unknown alleles, missing loci, unknown multi-allele codes, toggle molecular or serology input validation).

7. By using the “X” or “-” buttons, typing data can be cleared or donors removed.

Heatmap

8. In case of ambiguous input typings, the heatmap will only provide locus-specific scores. If unambiguous typing is provided, a toggle allows switching between locus-specific and allele-specific PIRCHE-II scores, providing a fine-grained visualization of results.

9. DQ and/or DP presentation is enabled, if in Lab mode and DQA1 or DPA1/DPB1 HLA typings are provided.

10. Currently, DQ and DP presentation considers both cis- and trans-configurations of HLA heterodimers and will show them individually in the heatmap.

RAMP

11. Alternative to the Risk Profile histogram, boxplot visualizations of PIRCHE-II distributions are available in the Risk Profile panel.

12. Single Antigen Bead assay data may be loaded from Immucor® MatchIt!® via Immucor's PIRCHE integration utility.

13. Single Antigen Bead assay data may be loaded from OneLambda® Fusion™ by using the "Load MFI" button, loading the respective report file and selecting one of the runs.

14. Since patients are matched against very large donor pools, RAMP Analysis can take some extra time, depending on how much extrapolation is needed for the input patient genotypes. This can be reduced by providing unambiguous allelic typing.

15. Donor populations for RAMP can be uploaded to fit, if the provided simulated populations are not expected to match the local donor pool. More information can be found at (Settings -> Populations)

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