PIRCHE®

TxPredictor

The Handbook

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List of Acronyms

AMR	antibody-mediated rejection
APC	antigen-presenting cell
API	application programming interface
ATG	antithymocyte globulin
BCR	B-cell receptor
CNI	calcineurin inhibitors
dd-cfDNA	donor-derived cell-free DNA
dnDSA	de novo donor-specific HLA antibody
DSA	donor-specific HLA antibody
ELISPOT	Enzyme-LInked immunoSPOT
EMS	Electrostatic Mismatch Score
FAQ	Frequently Asked Questions
GvH	graft vs host
GvHD	graft vs host disease
GvL	graft vs leukemia
HLA	human leukocyte antigen
HLA-EMMA	HLA-Epitope Mismatch Algorithm
HML	Histocompatibility Markup Language
HSCT	hematopoietic stem cell transplantation
HvG	host versus graft
IEDB	Immune Epitope DataBase
MAC	multi allele codes
MFI	mean fluorescence intensity
MMUD	mismatched unrelated donor
NGS	next-generation sequencing
NMDP	National Marrow Donor Program
nonDSA	non-donor-specific anti-HLA antibody
OPM	Outcomes Prediction Module
OPTN	Organ Procurement and Transplantation Network
PDB	Protein Data Bank
pHLA	peptide:HLA complex
PIRCHE	Predicted Indirectly Recognizable HLA Epitopes
ptCy	post-transplant cyclophosphamide
QA	Quality Assurance
RAMP	Risk and Acceptable Mismatch Profile
SAB	single-antigen bead
SOT	solid organ transplantation
SRTR	Scientific Registry of Transplant Recipients
T_{FH}	T follicular helper
TCMR	T cell-mediated rejection
TCR	T-cell receptor
UNOS	United Network for Organ Sharing

1 Introduction

In the evolving landscape of transplant medicine, the shift from traditional antigen-level matching to molecular matching represents a definitive leap forward in our ability to predict and prevent alloimmune responses. This booklet is dedicated to the science and application of two complementary computational technologies delivered by the TxPredictor™: Predicted Indirectly Recognizable HLA Epitopes (PIRCHE) and Snow molecular matching. Together, these tools redefine how we view immunological compatibility by addressing both arms of the adaptive immune response.

Invented by Eric Spierings at the University Medical Center Utrecht in the Netherlands, the PIRCHE algorithm is designed to quantify the risk of indirect alloresponse in transplantation. PIRCHE predicts the specific mismatched peptides likely to be presented by human leukocyte antigen (HLA) to T-cells (*i.e.* T-cell epitopes). The later developed PIRCHE® Snow algorithm focuses on antibody recognition, utilizing advanced structural modeling to predict which mismatched amino acids on the donor HLA surface are accessible to antibodies (*i.e.* B-cell epitopes). By combining the T-cell perspective of PIRCHE with the antibody-accessibility insights of Snow, TxPredictor™ enables a ”dual-pathway” view of immunological risk that classic antigen matching misses.

Within TxPredictor - The Handbook, you will find a comprehensive overview of the biological mechanisms driving allorecognition, a summary of the robust clinical evidence linking these scores – both individually and in conjunction – to de novo donor-specific HLA antibody (dnDSA) formation, rejection and graft survival, as well as a practical user guide for integrating TxPredictor™ into your workflow. Our goal is to empower you with the tools to move towards truly personalized, comprehensive risk stratification for improved patient management.

1.1 Intended Audience

TxPredictor - The Handbook is tailored for the multidisciplinary professionals driving the future of transplantation. It is designed for clinicians eager to understand the predictive logic behind molecular matching algorithms to refine risk stratification, as well as HLA laboratory scientists and immunologists preparing to validate and integrate these digital diagnostics into their operational workflows.

1.2 Symbols and Conventions

The following symbols and conventions are used throughout this document to alert you to important information:

Provides supplemental information, tips, or clarification.

Indicates a potentially hazardous situation which, if not avoided, could result in data corruption or software malfunction.

2 Molecular Matching in Transplant Immunology

2.1 The Role of the HLA System

The HLA system constitutes the most polymorphic region in the human genome and plays a pivotal role in the adaptive immune system to distinguish "self" from "non-self" [1]. Consequently, HLA compatibility between donor and recipient is a fundamental determinant of clinical outcomes in both solid organ transplantation (SOT) and hematopoietic stem cell transplantation (HSCT). In SOT, particularly kidney transplantation, HLA mismatches can trigger alloimmune responses via different pathways, leading to downstream effects that significantly shorten graft survival [2]. In the context of HSCT, HLA incompatibility is a major risk factor for graft vs host disease (GvHD), transplant-related mortality, and overall survival. The "gold standard" for unrelated donor selection typically requires high-resolution allelic matching at HLA-A, -B, -C, -DRB1, and -DQB1 (10/10 match) to mitigate these risks while balancing the beneficial graft vs leukemia (GvL) effect. However, due to the extreme polymorphism of the HLA system, finding fully matched donors is often challenging, necessitating the use of mismatched or haplo-identical donors [3].

While traditional serological HLA matching – which typically assesses whole-antigen mismatches at the HLA-A, -B, and -DR loci – has historically been vital for reducing hyperacute rejection and improving overall graft survival, it has significant limitations due to its stepwise scale of measurement, inherently failing to capture the underlying structural and functional determinants of HLA proteins and scoring each individual mismatch with an equal risk. Because the immune system targets specific molecular subunits rather than whole antigens, it has become increasingly clear that not all HLA mismatches elicit the same degree of alloreactivity. For instance, many amino acid differences between a donor and recipient are simply not accessible for binding by a B-cell receptor, or they may naturally possess a lower immunogenic potential. Consequently, the field of organ transplantation is shifting toward advanced "molecular matching". To introduce these concepts, the underlying determinants of allorecognition are summarized in section 2.2.

2.2 The Immune Response Pathways

While advances in pre-transplant diagnostics have significantly reduced hyperacute rejection, long-term graft survival remains a challenge due to antibody and T cell-mediated rejection. To improve histocompatibility, it is essential to understand the distinct pathways through which the immune system distinguishes "self" from "non-self," a process known as allorecognition (fig. 1) [1].

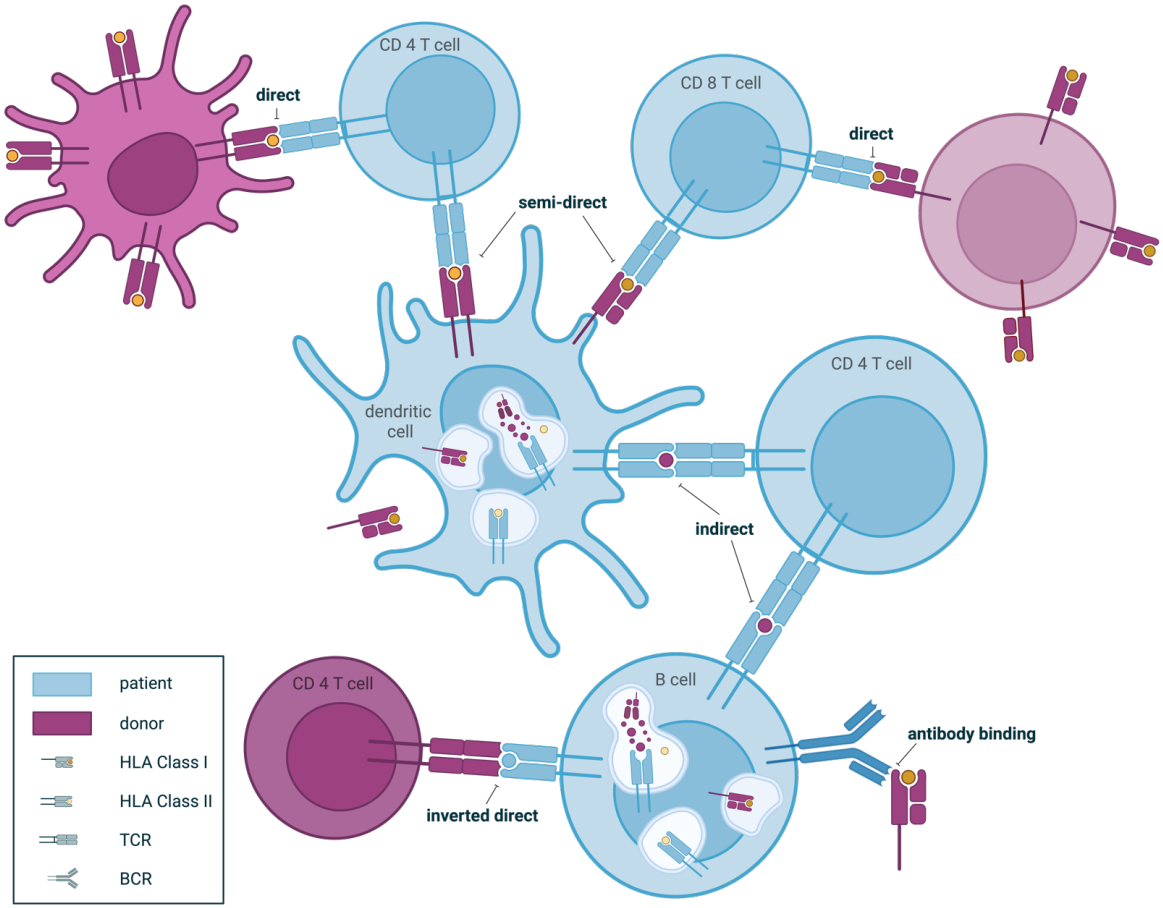


Figure 1: Different pathways of allorecognition are described by Murphy et al. [1] and Charmetant et al. [4]. (Created in BioRender. Niemann, M. (2026) `https://BioRender.com/lzzbvfw`)

2.2.1 The Direct Pathway

Direct allorecognition involves the recipient's immune system recognizing intact, three-dimensional donor HLA molecules directly on the surface of the donor's cells. Importantly, the recognition of these intact HLA targets by recipient antibodies and B-cell receptors occurs in a direct or semi-direct fashion. Alongside this

humoral recognition, naive T cells of the recipient also directly recognize these intact HLA complexes when presented by ”passenger” leukocytes (such as donor dendritic cells) that are transferred along with the donor organ. This pathway generates an immediate and robust immune response, typically leading to acute T cell-mediated rejection (TCMR). While this pathway poses an early threat to graft survival, its impact can be mitigated by depleting passenger leukocytes from the donor organ prior to transplantation [1, 4].

2.2.2 The Semi-Direct Pathway

The semi-direct pathway acts as a biological hybrid between direct and indirect recognition. In this mechanism, the recipient's own antigen-presenting cells (APCs) acquire intact, functional donor HLA molecules through direct cell-to-cell contact or extracellular vesicles, and display them on their own cell surfaces. Because the recipient's APCs can continue to present these intact donor molecules long after the original donor passenger leukocytes have been cleared from the graft, the semi-direct pathway can sustain an ongoing immune response, contributing to late occurrences of TCMR or chronic graft rejection [1, 4].

2.2.3 The Indirect Pathway

The indirect pathway is fundamentally different from the direct and semi-direct pathways, as it relies on the processing of donor antigens by the recipient's immune system. It is considered the dominant contributor to chronic rejection. The indirect pathway unfolds in the following sequence:

1. **Antigen Acquisition:** Cells from the donor graft undergo natural turnover or damage (such as apoptosis or necrosis), shedding donor proteins, including HLA, into the surrounding environment.
2. **Processing:** The recipient's dendritic cells internalize this donor antigen *e.g.* via macropinocytosis and phagocytosis. Inside the endosomes of the dendritic cells, the donor proteins are degraded and cleaved into smaller, linear peptides.
3. **Presentation:** These donor-derived peptides are loaded into the binding groove of the recipient's own HLA molecules. The dendritic cell then transports this peptide:HLA complex (pHLA) to the cell surface, presenting it to the extracellular space.
4. **T Cell Activation:** HLA-restricted, naive recipient CD4+ T cells encounter this presented pHLA. If the T-cell receptor (TCR) is properly stimulated by the pHLA complex – supported by co-stimulatory signals like the CD28 interaction with B7 on the dendritic cell – the T cell becomes activated. The activated T cell then survives, proliferates, and differentiates into specific effector cells, such as T follicular helper (T_{FH}) cells.

The activation of CD4+ T cells via the indirect pathway is highly consequential, as these helper cells are mandatory to co-stimulate downstream immune responses, including the development of CD8+ cytotoxic T cells and the activation of B cells [1, 4].

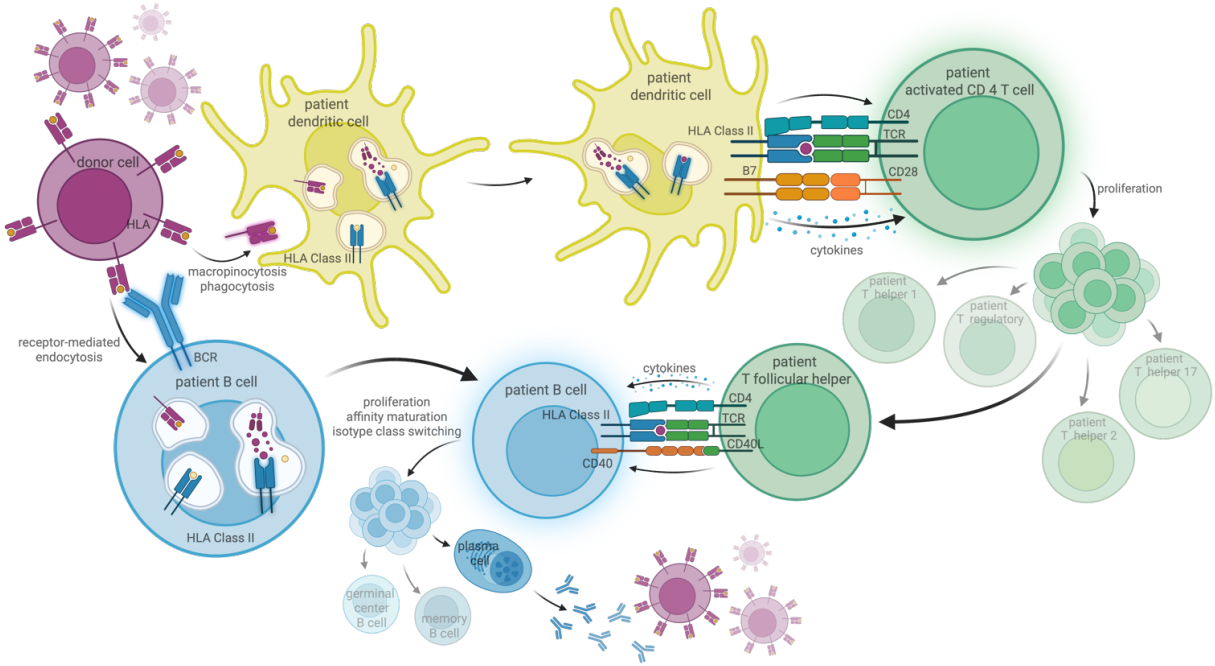


Figure 2: Linked allorecognition describes the cross-talk between activated T_{FH} cells and B cells to produce antibodies. (Created in BioRender. Niemann, M. (2026) `https://BioRender.com/px6rzp3`)

2.2.4 Linked Allorecognition

The production of destructive dnDSA is a primary cause of late kidney allograft loss. This humoral immune response is not driven by B cells acting alone; rather, it is the result of a highly orchestrated, T cell-dependent process known as ”linked recognition”. Linked allorecognition describes the essential collaboration between the indirect T cell pathway and the B cell pathway (fig. 2). Importantly, the activation of T_{FH} cells is not merely a promoter of the immune response but is mandatory to drive antibody responses [5].

1. **B Cell Antigen Engagement:** Recipient B cells possess B-cell receptors (BCRs) that recognize and bind to the intact, conformational (three-dimensional) structures of the donor HLA proteins on the surface of the allograft or on shed vesicles. Upon binding, the B cell endocytoses the donor HLA molecule.

2. **B Cell as an Antigen-Presenting Cell:** Once inside the B cell, the donor HLA antigen is degraded into linear peptides, much like in a dendritic cell. The B cell then loads these donor-derived peptides onto its own HLA Class II molecules and presents them on its surface.

3. **The T Cell Handshake:** For the B cell to fully activate and mature, it requires stimulation from a T_{FH} cell that was previously activated by a dendritic cell via the indirect pathway. The T_{FH} cell must recognize the exact same donor-derived pHLA complex presented by the B cell.

4. **Maturation and Antibody Production:** When the T_{FH} cell recognizes the target on the B cell, it triggers the CD40 receptor on the B cell via CD40L and releases necessary cytokines, such as IL-21. These signals stimulate the B cell to proliferate, undergo affinity maturation (mutating to bind the antigen more tightly), and perform isotype class switching (altering its effector function). Finally, the B cell differentiates into a plasma cell, which secretes large quantities of highly specific donor-specific antibodies (DSA) that target the original conformational epitope on the donor HLA.

Understanding the distinct roles of B cells and T cells in allorecognition has paved the way for advanced computational models that are discussed in section 3. Unlike traditional serological matching, these algorithms evaluate the biological structures driving both the B cell and T cell components of the immune response.

3 Molecular Matching Algorithms

A new family of advanced histocompatibility models known as ”epitope matching”, or more recently ”molecular matching” algorithms have been suggested, which evaluate the structural and functional drivers of the immune response through two primary approaches: B-cell epitope matching models (*e.g.* HLAMatchmaker, HLA-EMMA, and PIRCHE® Snow) that predict antibody recognition of intact donor proteins, and T-cell epitope matching models (*e.g.* PIRCHE) that predict the indirect presentation of donor-derived peptides to recipient T cells. Central to the function of all these computational approaches is their ability to shift the scale of compatibility assessment down to the highly granular biological unit known as an epitope.

3.1 What is an Epitope?

An epitope is the specific molecular target or structural subunit of a foreign antigen that is recognized and bound by the adaptive immune system. In the context of organ transplantation, histocompatibility assessment focuses on two distinct classes of epitopes derived from mismatched HLA proteins:

- B-cell (or antibody) epitopes are three-dimensional, conformational structures located on the surface of intact donor HLA proteins that can be engaged by recipient B-cell receptors or circulating antibodies. To analyze these structurally, computational models often translate them into smaller components called "Eplets" which are specific configurations of polymorphic, surface-accessible amino acids situated in close spatial proximity (typically within a 3 Angstrom radius) to one another.

- Indirect T-cell epitopes, conversely, are small, linear peptide fragments created when donor HLA proteins are degraded and processed by the recipient's antigen-presenting cells. These linear epitopes – specifically a "core peptide" of roughly nine consecutive amino acids – sit within the binding groove of the recipient's own HLA molecules, where the combined pHLA complex is recognized by T-cell receptors to drive cellular immune responses and essential T-cell help.

3.2 Antibody Epitopes

Antibody epitopes (or B-cell epitopes) are specific conformational structural subunits of intact donor HLA proteins. When a recipient's B-cell receptor engages these conformational regions, it can trigger the endocytosis of the HLA molecule, eventually leading to B-cell maturation and the production of dnDSA. Because not all mismatched amino acids between a donor and recipient are accessible or immunogenic enough to bind a B-cell receptor, identifying and quantifying these surface targets provides a much more refined picture of immunological risk. To evaluate this, several advanced computational molecular matching algorithms have been developed to model the structural and physiochemical distinguishability of intact donor HLA proteins.

3.2.1 HLAMatchmaker and Eplet Matching

Introduced by Rene Duquesnoy, HLAMatchmaker was the pioneering computational model for antibody epitope matching. Early iterations of the tool translated HLA sequences into linear three-amino-acid sequences called "Triplets" [6]. This concept was later refined to assess "Eplets," which are defined as structural configurations of polymorphic, surface-accessible amino acids situated in close spatial proximity to one another, typically within a 3 Angstrom radius [7]. The Eplet load is the number of donor-originating Eplets

that are absent from the recipient's own self-HLA molecules [8]. A curated repository of these structural motifs and their experimental verification status is maintained in the online HLA Eplet Registry (`https://www.epregistry.com.br`).

3.2.2 TerEp Model

Running parallel to computational approaches, the TerEp model was developed by Paul Terasaki's group using a purely experimental methodology. This approach identifies epitopes by performing absorption and elution experiments with patient sera, tracing antibody reactivity patterns back to shared amino acid configurations across various HLA proteins [9].

3.2.3 Electrostatic Mismatch Score

Beyond physical structure, the physiochemical properties of an antigen significantly influence antibody binding. The Electrostatic Mismatch Score (EMS) model evaluates donor HLA amino acid mismatches by calculating the minimum difference between the isoelectric points (the pH value at which the amino acid is neutrally charged) of the mismatched donor amino acids and the recipient's corresponding self-amino acids [10]. This approach was subsequently expanded into EMS-3D, which utilizes experimental and predicted HLA structures to model the electrostatic potential of the proteins in a three-dimensional space, calculating the minimum electrostatic similarity distance between the donor and recipient HLAs [11].

3.2.4 HLA-Epitope Mismatch Algorithm

The HLA-EMMA model introduced the use of predictors of solvent-accessible surface area [12]. The algorithm generates locus-specific maps of exposed amino acid positions. These maps function as a spatial filter for amino acid mismatching, attempting to restrict the model to evaluate only those mismatches predicted to be exposed on the accessible surface of the HLA molecule.

3.2.5 Snow

The recently suggested PIRCHE® Snow algorithm is specifically designed to model the B-cell/antibody antigenicity of mismatched HLA amino acids. To accurately predict which mismatched donor amino acids can act as targets for recipient B-cell receptors, the algorithm evaluates the three-dimensional conformational structure of HLA proteins and predicts surface-accessibility per protein [13, 14]. The algorithm will be discussed in detail in section 3.3.

3.3 The Snow Algorithm

A central challenge in predicting HLA surface-accessible targets is that generalizing structural information from HLA proteins fails to capture the structural nuances of individual representatives. Furthermore, relying exclusively on absolute solvent-accessible surface area can introduce a size-based bias; it inherently underestimates the accessibility of smaller amino acids (like glycine) and overestimates that of larger ones (like tryptophan). To overcome these limitations, the Snow algorithm integrates two distinct deep learning-based modules – Snowflake and Snowball – to evaluate both protein-specific surface area and local ellipsoid protrusion [14]. The integration of amino acid physicochemical properties into the matching algorithm (internally designated as the "Snowfall" module) did not demonstrate superior predictive performance compared to unweighted amino acid mismatching; consequently, this module was excluded from the final model configuration. Figure 3 provides a visual outline how the matching algorithm calculates PIRCHE-B scores.

3.3.1 Snowflake: Protein-Specific Surface Accessibility

The Snowflake module is a computational deep learning pipeline designed to calculate the allele-specific solvent-accessible surface area of HLA proteins. Because experimental crystal structures are only available for a fraction of known HLA variants in the Protein Data Bank (PDB), Snowflake utilizes the AlphaFold 2 protein folding predictor to supplement structural data. Using a "rolling probe" method, the surface area for each amino acid is calculated. This massive dataset of experimental and predicted structures is then used to train a bidirectional recurrent neural network. The trained neural network can subsequently infer protein-specific solvent-accessible surface area for the amino acid sequence of any known – or not yet discovered – HLA allele [13].

3.3.2 Snowball: Repeated Local Ellipsoid Protrusion

To complement surface area calculations and compensate for amino acid size biases, the Snowball module calculates the local protrusion rank of amino acids. While previous methods (such as ElliPro) approximated protrusion by fitting a single, global ellipsoid to the entire protein, Snowball recognizes that the characteristic, asymmetric shape of HLA molecules – particularly the helical structures forming the antigen-binding groove – is poorly described by a single ellipsoid. Instead, Snowball utilizes a repeated local ellipsoid fitting technique. The algorithm iterates over the atoms of an HLA structure, selects neighboring atoms within a 15 Angstrom radius (an area corresponding to the typical footprint of an antibody binding site), and fits an ellipsoid specifically to that localized substructure. Within each fitted ellipsoid, atoms are ranked based on

their distance from the ellipsoid's center, factoring in the lengths of the ellipsoid's axes. By aggregating the median protrusion ranks from all the locally fitted ellipsoids, Snowball generates a highly sensitive, consensus protrusion rank for each amino acid [14].

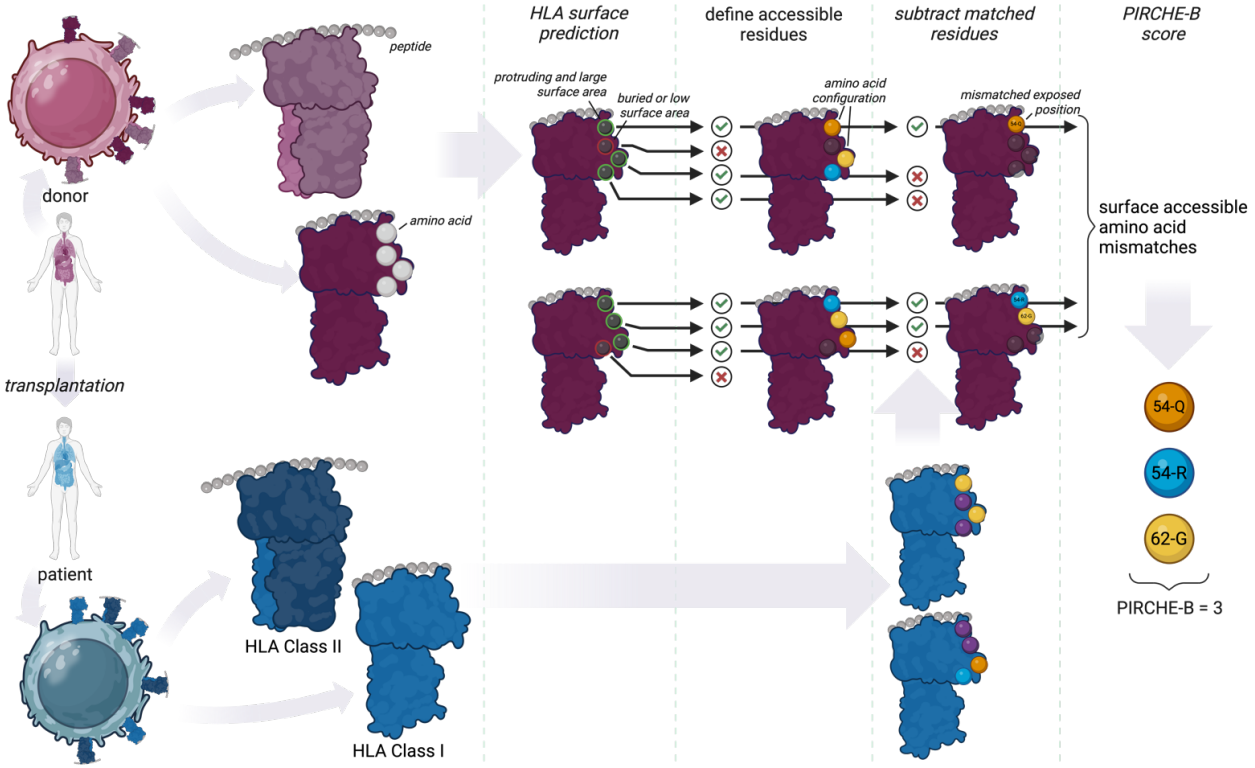


Figure 3: The Snow algorithm predicts the number of distinct, surface-accessible interlocus Class I /intralocus Class II HLA amino acid mismatches. (Created in BioRender. Niemann, M. (2026) https://BioRender.com/0m8ishu)

3.3.3 Snow Molecular Mismatch Score

In clinical and computational application, the Snow algorithm evaluates histocompatibility by combining the outputs of both modules. When comparing a donor's HLA sequence to a recipient's, the algorithm first identifies all donor-specific amino acid mismatches with respect to the recipient's self-HLA (interlocus Class I,

intra-locus Class II). For a mismatched amino acid to be considered a true, exposed B-cell epitope mismatch, it must exceed predefined thresholds for both predicted surface area (via Snowflake) and local protrusion rank (via Snowball). Mismatches that exceed these dual thresholds – empirically defined by Niemann et al. [15] as 0.26 and 0.68, respectively (figure 5b) – are summed to calculate the final PIRCHE-B score (also referred to as "Snow score", fig. 3) [15]. Higher numbers indicate a greater number of potential antibody targets on donor HLA.

3.4 Indirect T Cell Epitopes

As introduced in section 2.2.4, the activation of CD4+ T cells is paramount for the generation of a profound humoral immune response. Unlike the three-dimensional, conformational structures of B-cell epitopes, an indirect T-cell epitope is a small, linear peptide fragment generated when donor proteins are internalized and degraded by the recipient's antigen-presenting cells. While the open binding groove of the recipient's HLA Class II molecule can accommodate peptides of varying lengths (often modeled computationally as 15-mers), the essential functional unit is the core peptide. This core consists of typically nine consecutive amino acids that anchor directly into the binding groove. Crucially, T cells are HLA-restricted, meaning they cannot recognize an isolated, foreign peptide; rather, the presenting cell and the T cell must "speak the same language" by utilizing the recipient's own self-HLA molecules for presentation. When the TCR engages this target, it establishes contact with both the exposed residues of the bound core peptide and the surrounding domains of the presenting HLA molecule, evaluating this combined pHLA complex as a singular, highly specific unit. To computationally model this indirect pathway of allorecognition and quantify the immunological risk, the PIRCHE algorithm was developed.

3.5 The PIRCHE Algorithm

The PIRCHE algorithm operates through a robust, multi-step bioinformatics pipeline to assess these interactions (fig. 4).

3.5.1 Sequence Completion and Peptide Cleavage

The first step in the pipeline involves fetching all known HLA protein amino acid sequences from the IPD-IMGT/HLA database[16] and completing any missing structural data. Because many historically submitted HLA sequences only cover the highly polymorphic regions (such as exons 2 and 3 for HLA Class I and exon 2 for HLA Class II), a normalizing sequence completion for the remaining unsequenced exons is performed. This is achieved using an automated homology-based nearest-neighbor extrapolation [17]. Within this process,

each incomplete HLA sequence is aligned against more completely sequenced alleles from the exact same locus. The algorithm identifies the best-aligned "nearest neighbor" sequence and replaces the missing amino acid positions in the incomplete sequence with the known amino acids from that matched reference sequence.

Because the open binding groove of HLA Class II molecules typically accommodates peptides of varying lengths and aligns them, the algorithm computationally generates a set of candidate peptides by applying a 15-amino-acid sliding window across the donor's and patient's HLA protein sequences.

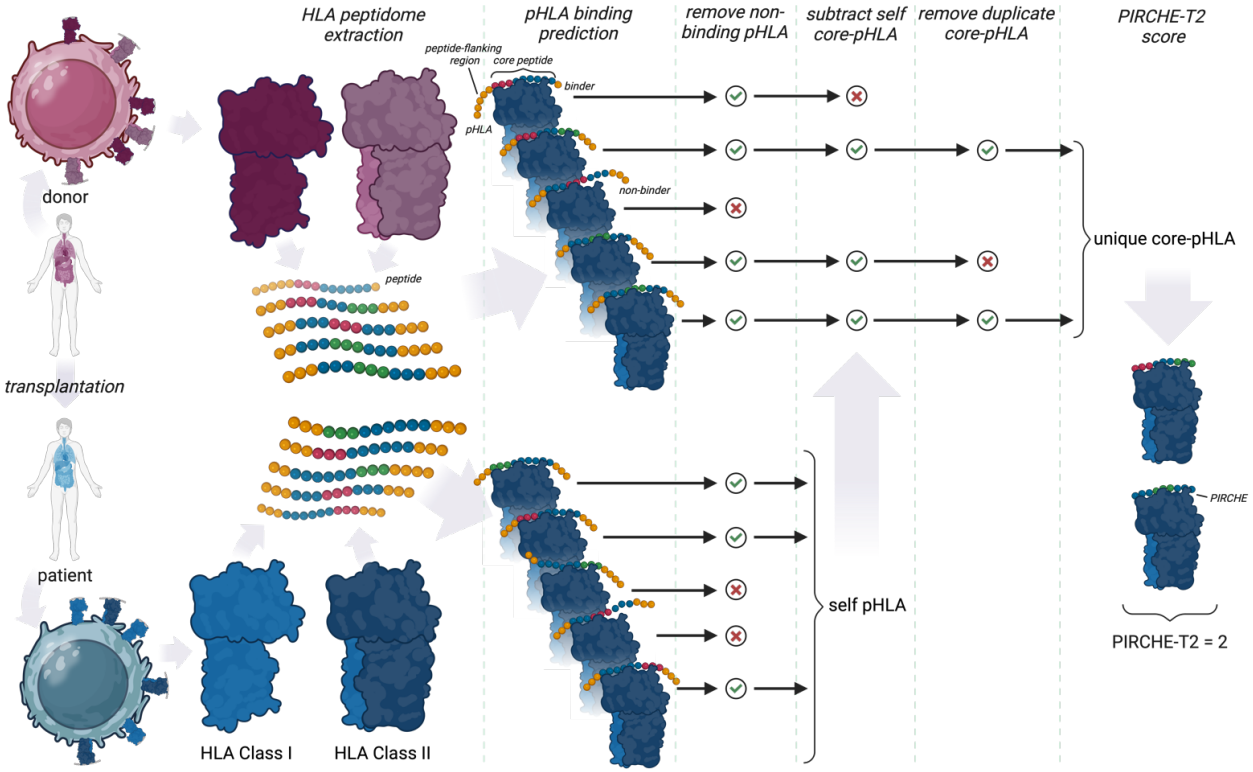


Figure 4: The PIRCHE algorithm predicts the number of distinct allopeptides bound by HLA. (Created in BioRender. Niemann, M. (2026) https://BioRender.com/qf6xrns)

3.5.2 Peptide-HLA Binding Prediction

Not all cleaved peptides will successfully bind to the recipient's HLA Class II molecules. While earlier versions of the algorithm (PIRCHE v3, [18]) utilized the netMHCpan/netMHCIIpan for pHLA binding prediction, the current iteration (PIRCHE v4) by default utilizes an advanced proprietary predictor known as PIRCHE® Frost [15]. Frost evaluates peptide binding using an optimal ensemble of 128 Artificial Neural Networks that were trained on experimental binding data from the Immune Epitope DataBase (IEDB). During binding prediction, the optimal alignment of the peptide to the anchor positions is estimated, effectively predicting a binding core, which is expected to be interacting with the TCR. To accommodate for allele-specific binding promiscuity or training data imbalance, the predicted binding affinity is then normalized by ranking it against 10,000 human control peptides, effectively eliminating the need for previously suggested post-hoc corrections [19]. The optimal threshold for identifying relevant T cell epitopes using Frost has been empirically defined at a binding rank of 300‰ (the top 30%, figure 5a), which purposely includes relatively weak binders that may still drive alloresponses during continuous antigen exposure [15].

3.5.3 Negative Selection

To accurately model the biological reality of thymic education – where T cells recognizing "self" peptides are deleted –, the algorithm applies a paradigm of negative selection. The recipient's own self-peptidome is computationally constructed and subtracted from the pool of predicted donor candidate pHLA. The final PIRCHE-T1 and PIRCHE-T2 scores are calculated by counting the number of remaining, unique donor-derived 9-mer core pHLAs that are successfully bound by the recipient's HLA Class I and Class II molecules, respectively (fig. 4). Higher numbers indicate a greater number of potential T cell targets introduced by the donor HLA.

3.5.4 Presenting HLAs

In the context of SOT, the algorithm models an immunocompetent host, operating under the premise that all expressed HLA molecules serve as peptide-presenting molecules capable of eliciting a T-cell response. Conversely, HSCT entails the reconstitution of a donor-derived immune system, necessitating immunological cross-talk between host and donor cells. Following the HLA-restriction of TCRs, the PIRCHE model restricts its evaluation of presenting molecules exclusively to HLA alleles shared (*i.e.* P Group-identity) by both the donor and recipient.

Historically, studies utilizing the PIRCHE algorithm have primarily focused on peptide presentation by the HLA-DRB1 locus simply because typing data for other loci was often unavailable. Niemann et al. [19] evaluated the inclusion of additional presenting loci like HLA-DQ and HLA-DP in the context of child-specific HLA antibodies developed during pregnancy. They revealed that T-cell epitope load scores are highly correlated across the different presenting HLA Class II molecules. Because of this strong inter-locus correlation, combining multiple presenting loci into a single predictive model of molecular mismatch load only marginally improves its overall predictive performance. Therefore, assessing presentation solely via HLA-DR is considered sufficient for risk stratification based on load scores, and thus the current Frost prediction pipeline explicitly restricts its analysis to HLA-DR presentation since incorporating HLA-DQ provides limited added predictive value.

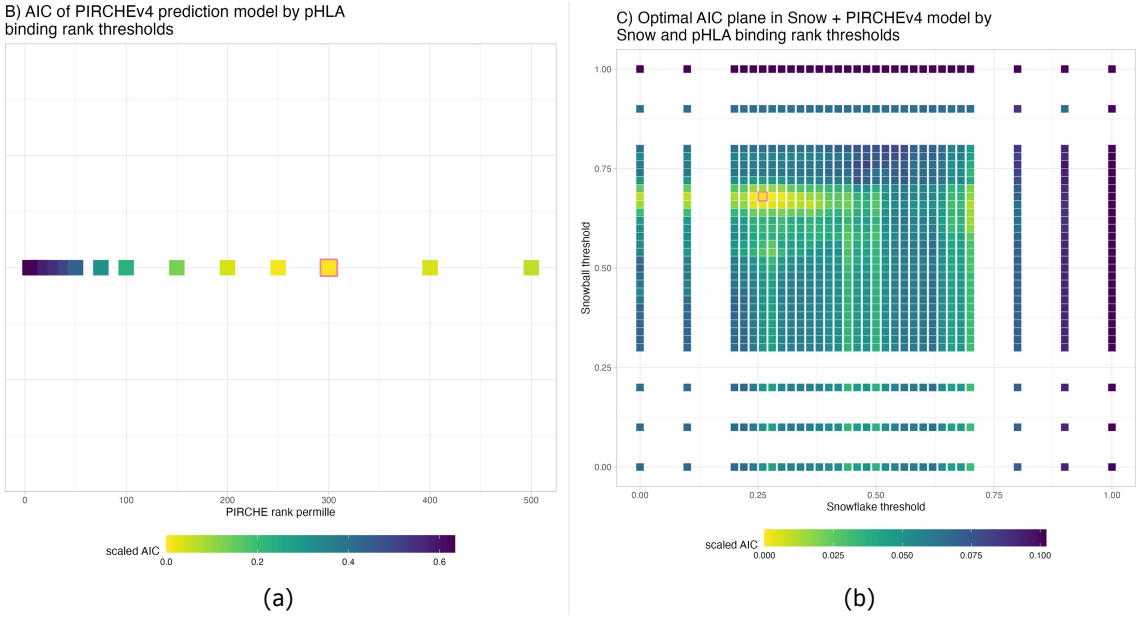


Figure 5: Optimal binding rank (a) and protrusion/surface area values (b) were evaluated empirically by Niemann et al. [15]. Image credit: Figures 5a and 5b: Niemann et al. [15] (derivative: cropped), licensed under CC-BY-4.0.

3.6 Shared T Cell Epitopes

Detecting pre-transplant donor-reactive memory CD4+ T cells *in vitro* is notoriously challenging. While tools like the Enzyme-LInked immunoSPOT (ELISPOT) assay exist, they predominantly detect T cells activated via the direct allorecognition pathway, leaving the indirect pathway poorly evaluated. An in silico alternative based on the PIRCHE® technology has been suggested to quantify the risk of a memory recall response [20]. To that extent the PIRCHE peptides derived from a patient's initial immunizing HLA are compared with the peptides derived from a new donor's HLA. The resulting overlap is quantified as Tmem score: the number of distinct shared T cell epitopes. Evidence in the literature is discussed in section 4.1.5 and how to evaluate Tmem scores within the application is presented in section 7.3.5.

3.7 HLA Genotype Imputation

Molecular matching algorithms require high-resolution HLA genotype data – specifically protein-level, *i.e.* two-field resolution – to accurately model the molecular surface and peptide-binding capabilities of the HLA proteins. Although the field of solid organ transplantation is increasingly moving towards modern HLA genotyping techniques, it is economically infeasible to re-analyze large datasets retrospectively. Furthermore, obtaining high-resolution genotyping is not always feasible in clinical practice, particularly for deceased donors where time constraints often limit typing to serological split level or low-resolution DNA methods.

To bridge the gap between available low-resolution clinical data and the high-resolution requirements of molecular matching algorithms, multiple imputation methods have been suggested. Based on linkage-disequilibrium, it is recommended to avoid imputation per-allele and preferably impute entire HLA haplotypes utilizing population-specific haplotype frequency tables to infer the most likely high-resolution genotypes and quantify the molecular mismatch load probabilistically.

Different imputation strategies have been implemented: Many articles in the literature employed a single imputation strategy, where only the most likely high-resolution explanation of the provided HLA typing data is selected (*i.e.* the winner takes it all). This strategy has the drawback, in that analysis is biased towards common genotypes. Furthermore, depending on probability distribution, the second-likeliest genotype may be nearly as likely, yet is ignored. These drawbacks are overcome by multiple imputation strategies, as has been implemented by TxPredictor™.

The core of the multiple imputation approach relies on the existence of large-scale HLA haplotype frequency tables, such as those provided by the National Marrow Donor Program (NMDP). The imputation process follows a stepwise algorithm to transform a low-resolution phenotype into a set of weighted high-resolution genotype probabilities.

3.7.1 Multiple Imputation Algorithm

In **step 1**, for a given serological split level or molecular low resolution-level HLA typing, the algorithm identifies all potential pairs of haplotypes (spanning loci HLA-A, -B, -C, -DRB1, and -DQB1, *i.e.* "strict" imputation) that, when combined, yield the observed phenotype. In **step 2**, for these haplotypes, matching high-resolution haplotypes are looked up in the haplotype frequency table (*i.e.* "natural" haplotypes). A critical feature of this approach is its ability to handle phenotypes that do not correspond to known haplotype pairs in the frequency tables. In these cases, an optional **step 3** is executed, that relaxes the linkage disequilibrium constraints between loci and repeats the search for haplotypes in the hapotype frequency tables. This "fallback" approach removes links between loci in a specific order:

1. Full haplotype (A-B-C-DRB1-DQB1), *i.e.* strict
2. Unlinking Class I and Class II (A-B-C / DRB1-DQB1)
3. Unlinking A from B-C (A / B-C / DRB1-DQB1)
4. Further unlinking (A / B-C / DRB1 / DQB1)
5. Allele frequency level (A / B / C / DRB1 / DQB1).

While strict imputation would abort if no natural haplotype is found, this fallback mechanism ensures a result is generated, albeit with lower frequency weights to penalize the unnatural combinations. In **step 4**, a list of potential high-resolution genotypes is compiled by joining two constituent haplotypes and calculating a genotype frequency by multiplying the corresponding haplotype frequencies. These absolute frequencies are normalized in **step 5** within the set of identified candidates. In **step 6**, molecular matching algorithms are performed for all high-resolution pairs of recipients and donors, generated in step 5 respectively. Finally in **step 7**, molecular mismatch loads of the individual analyses are aggregated into a single result by a weighted sum according to their normalized frequencies. This approach accounts for less frequent HLA genotypes and provides a risk assessment that reflects the uncertainty inherent in the low-resolution data.

Notably, this algorithm also supports mixed and intermediate typing resolution or even missing loci. Given the reduced certainty in the absence of loci, TxPredictor enforces input of at least A-B-DRB1 typing data.

3.7.2 Haplotype Frequencies in the Absence of Ancestry Information

Genotype imputation relies heavily on selecting a corresponding ancestry table (*e.g.* Caucasian, African American, Asian) to ensure accuracy. However, self-identified ancestry may be unavailable, ethically restricted, or inaccurate (mislabeled). To address this, using an ancestry-combined haplotype frequency approach has been described to facilitate imputation when specific ancestry is unknown [21]. The combined population is an artificial frequency table generated by aggregating all available haplotypes from the various distinct sub-populations found in the NMDP database. The frequencies of these haplotypes are accumulated and normalized based on their relative weights across all groups. This creates a "generic" frequency table that covers the breadth of human HLA diversity contained in the registry. A validation study comparing ground-truth high-resolution genotypes against those imputed using the combined population demonstrates that this aggregated approach serves as a valuable "middle ground": Imputation using the combined population introduces more error than using a correctly matched specific ancestry table. However, it introduces less error than using a specific but incorrect ancestry table (*e.g.* imputing an African American donor using Hispanic donors' frequencies). While the combined population yields a higher number of allele-level mismatches compared to ancestry-matched imputation (due to the flattening of population-specific linkage patterns), it achieves a high success rate in finding valid haplotype pairs because of its vast diversity. In scenarios where ancestry data cannot be practically or ethically collected, the combined population provides a viable, low-risk strategy for multiple imputation, serving as a safety net against the high error rates associated with guessing a specific, incorrect ancestry.

3.7.3 Validation and Factors Affecting Reliability

Reliability of molecular matching based on imputed genotypes has been controversial. It must be acknowledged, that despite all efforts (*e.g.* multiple over single imputation, consideration of standard deviation, best- or worst-case aggregation, larger haplotype frequency datasets, ancestry-driven imputation), imputation adds a level of uncertainty. Depending on its application the deviation may exceed acceptance criteria. Matern et al. [21] validated the reliability of ancestry-guided multiple imputation in the context of molecular mismatch load analysis (*Note: not* predicting one or more immunodominant epitopes) by comparing imputed scores against reference scores, which were based on the appropriate high-resolution typing. For the majority of donor-recipient pairs, the deviation between the imputed and actual molecular mismatch scores is minimal. In validation studies, over 70% of imputed PIRCHE-T2 values did not or only marginally deviate from the reference with practically no clinical impact (fig. 6)[22, 21].

The accuracy of imputation is sensitive to the breadth of the input data. The omission of HLA-C or HLA-DQ from the input typing significantly increases the deviation between imputed and actual scores. This suggests that even at low resolution, typing for HLA-C and HLA-DQ is a valuable addition to standard A-B-DR typing to maintain the reliability of molecular mismatch predictions. Moreover, observations indicated a directionality of imputation error, *i.e.* whether the recipient or the donor is being imputed:

- **Recipient Imputation:** Errors in imputing the recipient's genotype have a greater impact on T-cell epitope predictions (PIRCHE-T2). This is because PIRCHE-T2 models peptides presented by the recipient's HLA-DR; an incorrectly imputed recipient allele may alter the entire set of presented peptides.
- **Donor Imputation:** Errors in imputing the donor's genotype appear to have a greater impact on antibody epitope predictions (*e.g.* Eplets, PIRCHE-B). This can be attributed to candidate epitopes being derived from the donor HLA structure, whilst self epitopes may be redundantly covered through interlocus analysis.

Because high-resolution typing is still time-prohibitive for deceased donors but feasible for recipients on waiting lists, a hybrid strategy is highly effective. Using high-resolution data for the recipient and imputed low resolution data for the donor results in molecular mismatch load estimations nearly as accurate as fully high-resolution matching would have resulted. This "two-field recipient / split-level donor" approach is considered a feasible and reliable standard for clinical implementation [23, 21].

In conclusion, multiple HLA genotype imputation is a powerful computational tool that unlocks the benefits of molecular matching even when high-resolution data is unavailable. By using weighted genotype averages and aggregated frequency tables for unknown ancestries, immunological risk can be estimated reliably, though some inherent margin of error remains. While high-resolution genotyping remains the gold standard, these computational strategies make advanced molecular matching accessible for the majority of transplant pairs and a viable approach to analyze large datasets retrospectively.

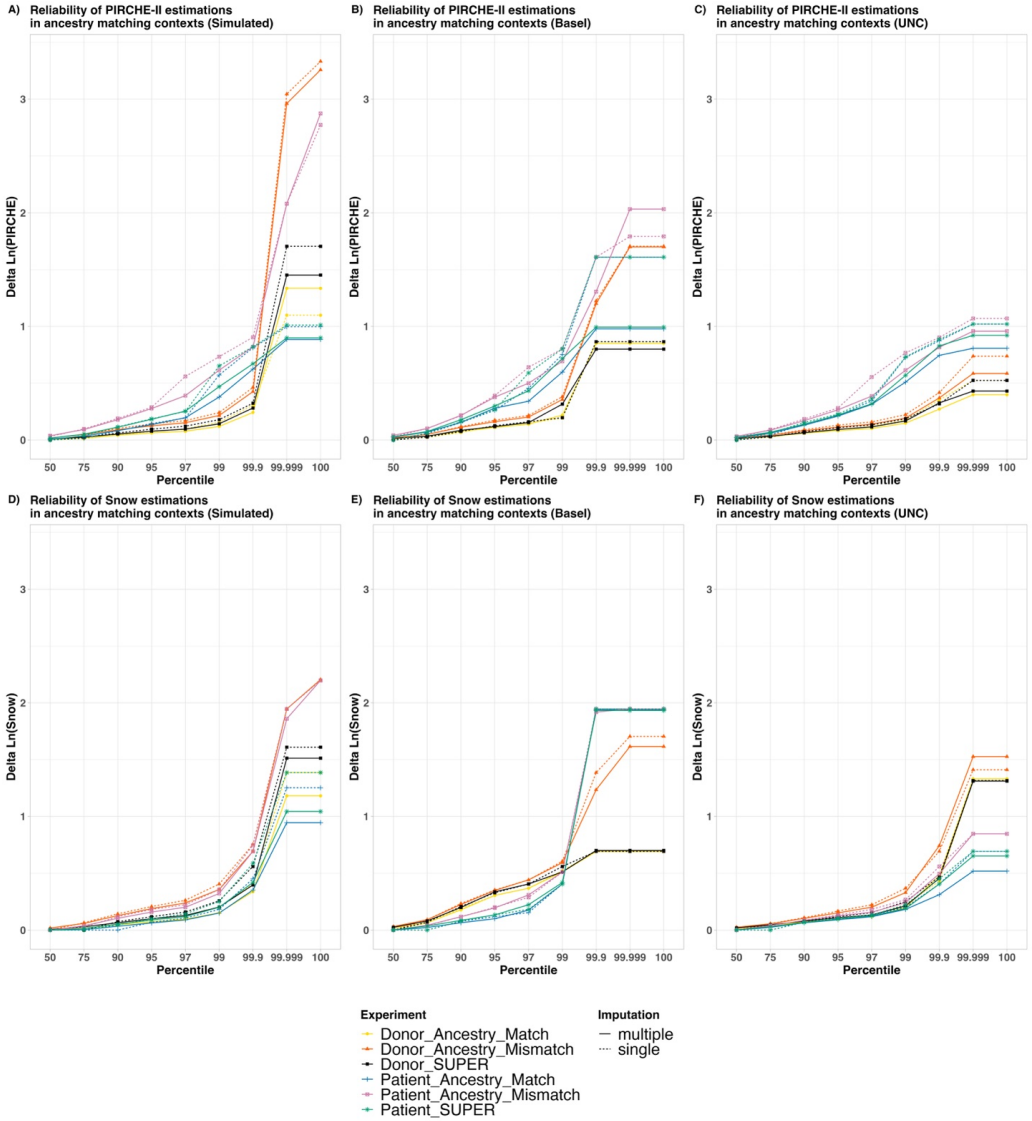


Figure 6: Performance of TxPredictor™'s ancestry-guided multiple imputation approach. Image credit: Matern et al. [21], licensed under CC-BY-4.0.

4 Clinical Evidence

There is growing evidence of PIRCHE-T2 and PIRCHE-B scores being correlated with various outcomes in different transplant domains. In the following sections, a brief summary will be given on different focus areas. An overview of publications in SOT and their corresponding key parameters are furthermore provided in table 1.

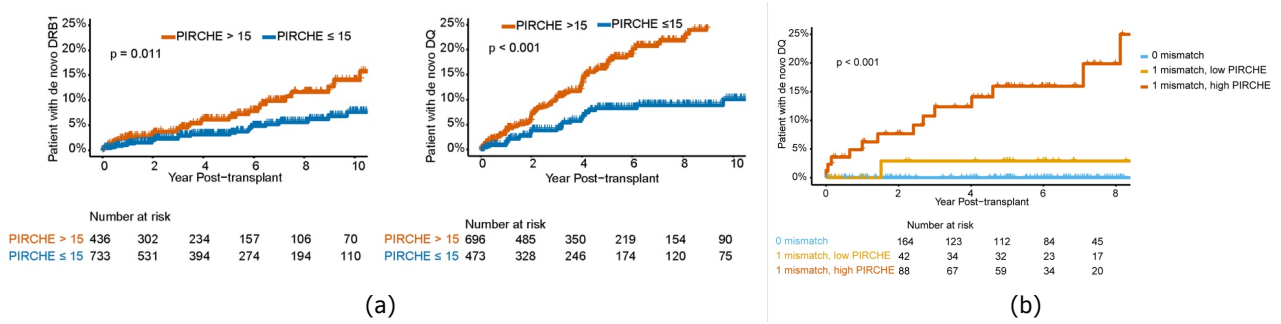
4.1 Kidney Transplantation

4.1.1 Donor-specific Antibodies

Multiple studies consistently establish the PIRCHE-T2 (formerly PIRCHE-II) as a robust, independent predictor of dnDSA formation following kidney transplantation. Building upon early proof-of-concept observations [24], a seminal large-scale study by Lachmann et al. [25] demonstrated in 2,787 transplantations that elevated PIRCHE-T2 scores, especially at the HLA-DRB1 and -DQB1 loci, strongly correlate with dnDSA development and serve as a better predictor than classical HLA antigen matching. These results were confirmed by a recent study of Tian et al. [26], who conducted a rigorous analysis of 1,296 kidney transplant recipients (figures 7a and 7b), noting that this risk is significantly exacerbated in younger recipients. This association has since been widely corroborated across diverse international cohorts – including living donor transplants [27, 28] and simultaneous pancreas-kidney transplants [29, 30] – confirming that higher T-cell epitope mismatch loads are a primary driver of humoral alloimmunity. Furthermore, PIRCHE-T2 successfully predicts dnDSA risk in complex clinical scenarios, such as when maintenance immunosuppression must be minimized during BK virus or SARS-CoV-2 infections [31, 32]. Recent research has further refined its clinical utility by demonstrating correlations between PIRCHE-T2 and early anti-donor T-cell responses [33, 28].

4.1.2 Graft Survival

Large-scale, multi-center, and registry-based studies have consistently demonstrated that elevated PIRCHE-T2 scores correlate significantly with an increased risk of kidney graft failure and reduced long-term graft survival. The foundational single-center study by Lachmann et al. [25] first established that higher PIRCHE-T2 scores predict impaired 10-year death-censored allograft survival, suggesting thresholds for the formerly used netMHCIIpan-based peptide binding database versions. This correlation was robustly validated by Geneugelijk et al. [22] in the Dutch PROCARE cohort of nearly 2,918 patients, which identified PIRCHE-T2 as a strong independent risk factor for graft failure featuring both early and late detrimental effects (figure 8a), particularly in first-time transplant recipients. Delving into specific mechanisms, Senev et al. [34]



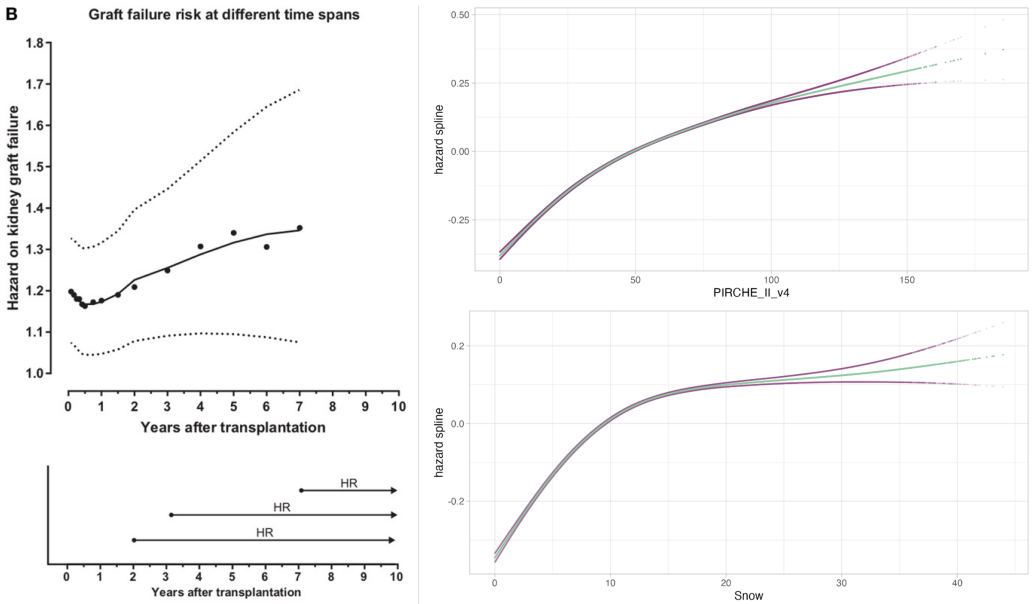
(a) (b)

Figure 7: Patients with elevated PIRCHE-T2 scores showed increased incidence of dnDSA, which is more pronounced for HLA Class II (figure 7a). This effect is independent of HLA antigen matching (figure 7b). Image credit: Figures 7a and 7b: Tian et al. [26] (derivative: cropped), licensed under CC-BY-4.0.

found that PIRCHE-T2 scores derived specifically from HLA-DRB1 and HLA-DQB1 mismatches independently predict both death-censored and all-cause graft failure, underscoring the dominant role of Class II molecular mismatches. Massive international registry analyses have further cemented this correlation: Unterrainer et al. [35] analyzed over 68,000 kidney transplants from the Collaborative Transplant Study and confirmed PIRCHE-T2 as a highly significant predictor of 5-year death-censored graft loss, while subsequent analyses of the US Scientific Registry of Transplant Recipients (SRTR) dataset by [36] and [15] demonstrated its strong predictive power for long-term death-censored graft survival (fig. 9). Furthermore, Niemann et al. [15] confirmed previous observations of non-linearity of molecular mismatch metrics (figure 8b), which can be compensated by log-transformation, and suggested a new set of thresholds for the newly introduced PIRCHE® Frost-based pHLA binding prediction.

4.1.3 Rejection

Elevated PIRCHE-T2 scores strongly correlate with both TCMR and antibody-mediated rejection (AMR) in kidney transplant recipients, underscoring the vital role of indirect CD4+ T-cell allorecognition in driving both cellular and humoral alloimmunity. For TCMR, Senev et al. [34] established that PIRCHE-T2 scores derived specifically from HLA-DRB1 and HLA-DQB1 molecular mismatches are independent predictors of early acute TCMR, notably even in the complete absence of dnDSA. Reinforcing this direct link to cellular rejection, Zhao et al. [38] showed that both the total PIRCHE-T2 score and Class I-derived PIRCHE-T2 scores are significantly elevated in patients who experience TCMR (fig. 10), demonstrating that the PIRCHE algorithm successfully



(a) (b)

Figure 8: Elevated PIRCHE-T2 scores increase hazard not only early after transplantation, but are at continuously elevated risk (a). Notably, molecular matching scores appear to increase risk supra-linear (b). Image credit: Figure 8a: Geneugelijk et al. [37] (derivative: cropped), licensed under CC-BY-4.0; figure 8b: Niemann et al. [15] (derivative: cropped), licensed under CC-BY-4.0.

captures T-cell presentation risks that antibody epitope matching algorithms miss. Furthermore, Lezoeva et al. [39] and Spitznagel et al. [40] found that in patients presenting with subclinical or borderline rejection, high total PIRCHE scores – particularly at the HLA-A locus – can accurately predict progression to clinically significant TCMR or AMR in subsequent follow-up biopsies. Similarly, Meneghini et al. [33] linked elevated PIRCHE-T2 scores to biopsy-proven acute rejection and the primary activation of donor-specific T-cells, while Naef et al. [31] associated high PIRCHE-DR and total scores with both TCMR and AMR occurrences when maintenance immunosuppression is minimized due to BK virus infections. A large multicenter analysis by Demir et al. [41] identified HLA-DQB1 and HLA-DRB1-derived PIRCHE-T2 scores as robust, independent determinants of AMR, regardless of whether patients had preformed donor-specific HLA antibody (DSA) at the time of transplantation.

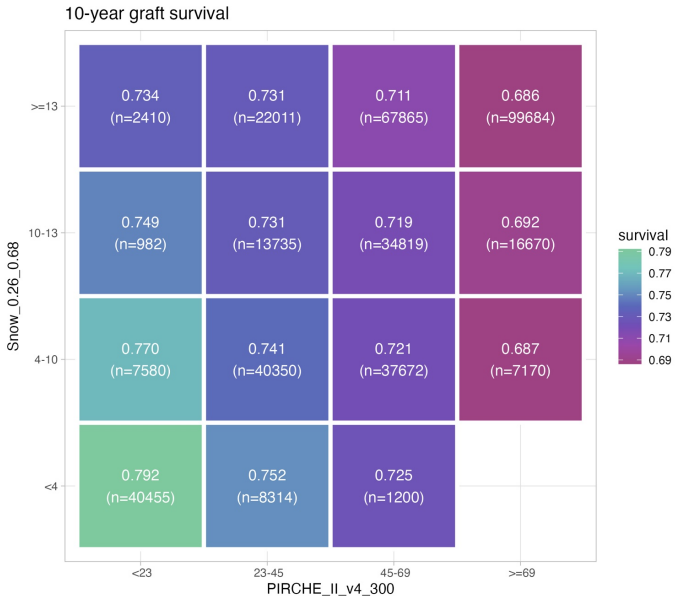


Figure 9: Death-censored graft survival at 10 years is reduced with higher molecular mismatch loads. Notably, within a risk bucket of one predictor (*e.g.* PIRCHE-T2), the secondary predictor provides further discrimination (*e.g.* PIRCHE-B). Image credit: Niemann et al. [15] (derivative: cropped), licensed under CC-BY-4.0.

4.1.4 Combining Predictors

Because the development of dnDSA relies on a coordinated interplay between B cells and T cells – a concept known as linked recognition (section 2.2.4) – researchers have increasingly combined multiple molecular matching algorithms, simultaneously capturing both humoral and cellular allorecognition pathways. Early large-scale efforts by Lachmann et al. [25] have shown that both HLAMatchmaker (*i.e.* antibody epitopes) and PIRCHE-T2 (*i.e.* indirect T cell epitopes) are independent predictors in multivariable models, evaluating their predictive power for dnDSA formation. Building on this, Sakamoto et al. [27] demonstrated the clinical synergy of these tools, finding that kidney transplant recipients with low scores in both Class II eplet mismatches and PIRCHE-T2 had a remarkably low (2.2%) incidence of Class II dnDSA (figure 11a). In pediatric heart transplantation, Mangiola et al. [42] showed that a similar combination effectively reclassifies patients; specifically, those with a high eplet mismatch load but a low PIRCHE-T2 score are actually at a lower risk for

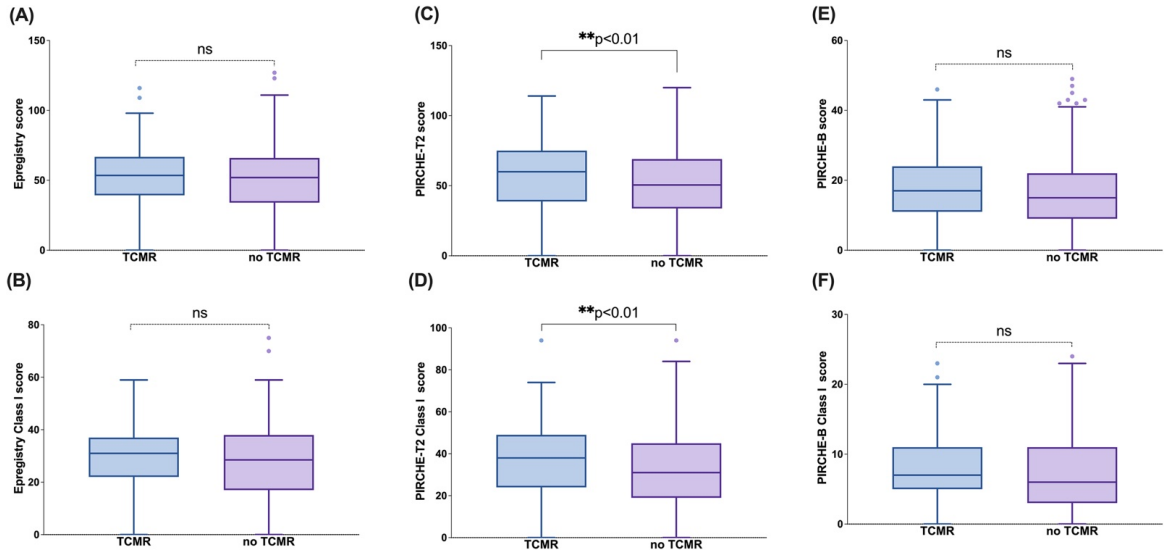


Figure 10: While PIRCHE-T2 was significantly higher in cases of TCMR, no significant correlation was found for Eplet matching or PIRCHE-B. Image credit: Zhao et al. [38], licensed under CC-BY-4.0.

dnDSA and AMR than those high in both, which was later on confirmed in a combination of another antibody epitope algorithm (HLA-EMMA) with PIRCHE-T2 [43]. Jäger et al. [44] demonstrated that integrating three molecular mismatch approaches – the eplet mismatch load, the number of highly immunogenic eplets, and the PIRCHE-T2 score – can successfully stratify standard-risk renal transplant recipients into distinct low- and high-risk groups for predicting clinical and subclinical rejection within the first year post-transplant. More recently, Niemann et al. [15] have shown in over 400,000 kidney transplantations of the SRTR registry, that integrating specialized T-cell and B-cell molecular mismatch metrics outperforms isolated predictors or classical amino acid matching for estimating death-censored graft survival (fig. 9). The optimal configurations of the PIRCHE-II and Snow algorithms established in that study were subsequently validated for dnDSA risk stratification in an independent kidney transplant cohort (figure 11b) [45]. Finally, pushing this integrative approach even further, Zhao et al. [38] proposed a comprehensive algorithm merging eplet matching (*i.e.* HLA Eplet Registry), PIRCHE-T2, and PIRCHE-B scores, demonstrating that this multi-algorithm approach offers superior predictive accuracy and robust risk discrimination for dnDSA formation compared to evaluating either immune pathway in isolation.

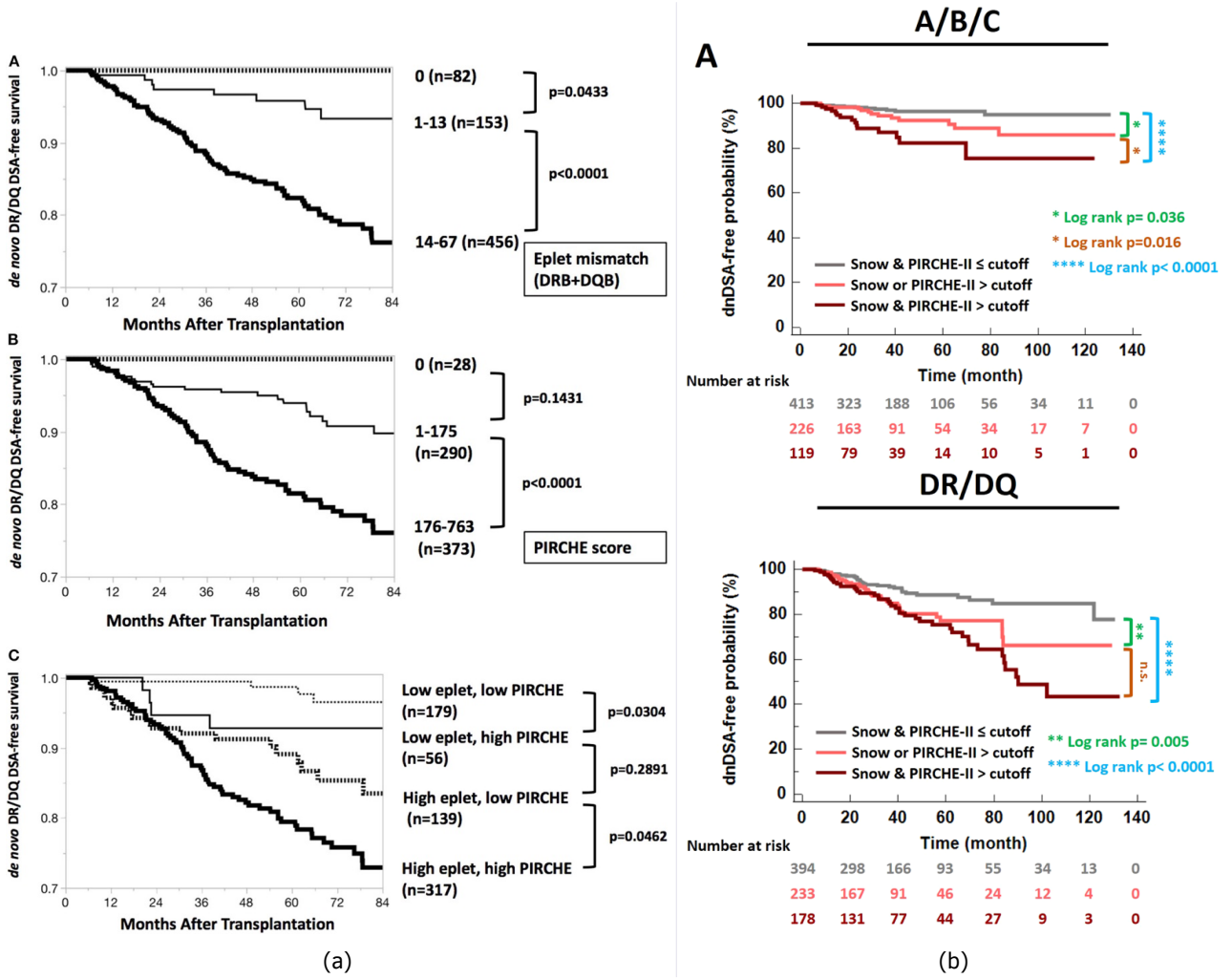
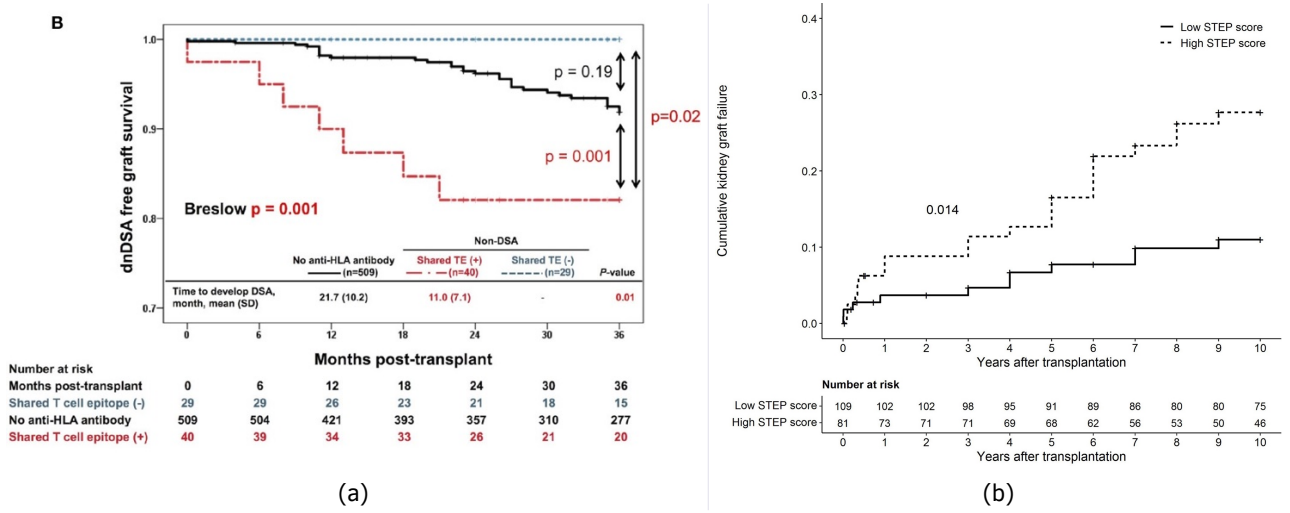


Figure 11: Combining B-cell epitope (*e.g.* Eplets, or PIRCHE-B *i.e.* PIRCHE® Snow) and T-cell epitope (PIRCHE-T2 *i.e.* PIRCHE-II) load appears to improve the ability to stratify patients into low and high risk groups, with both metrics indicating low values being optimal. Image credit: Figure 11a: Sakamoto et al. [27] (derivative: cropped), licensed under CC-BY-4.0; figure 11b: Chou-Wu et al. [45] (derivative: cropped), licensed under CC-BY-4.0.

4.1.5 Sensitized Patients

A study of 578 living donor kidney transplantations by Tomosugi et al. [20] analyzed patients who had pre-transplant non-donor-specific anti-HLA antibodys (nonDSAs). Among these pre-sensitized patients, those who possessed Tmem (shared PIRCHE) between their "dominant immunizer" – the HLA specificity yielding the highest mean fluorescence intensity (MFI) – and the donor HLA exhibited a significantly higher incidence of early dnDSA formation compared to both Tmem-negative patients and patients with no pre-transplant anti-HLA antibodies (figure 12a). Interestingly, this analysis outperformed traditional antibody epitope analysis.



(a) (b)

Figure 12: Patients with more PIRCHE Tmem had a higher incidence of dnDSA (a) and graft failure (b) than Tmem-negative patients. Image credit: Figure 12a: Tomosugi et al. [20] (derivative: cropped), licensed under CC-BY-4.0; figure 12b: Peereboom et al. [46], licensed under CC-BY-4.0.

In a confirmatory study, Peereboom et al. [46] showed this effect is not only relevant to early antibody formation, but the presence of Tmem is strongly associated with long-term graft survival. In multivariable Cox proportional hazards analyses, the log-transformed Tmem score (*i.e.* STEP) was found to be the only significant variable associated with death-censored graft failure. Specifically, a one-unit increase in the STEP score corresponds to a 48% increased risk of 10-year kidney graft failure. In conclusion, the shared T cell epitope concept represents a paradigm shift in understanding pre-sensitization and immunological risk in organ transplantation. By recognizing that memory CD4+ T-helper cells are specific to pHLA rather than

whole HLA molecules, clinicians can better understand why recipients with pre-transplant nonDSA frequently experience diminished graft function and decreased graft survival. Utilizing the TxPredictor™ in silico model to calculate Tmem scores allows for the objective estimation of the indirect allorecognition pathway. This provides a precise, epitope-based risk stratification method to predict early dnDSA production and long-term graft failure, paving the way for highly personalized immunosuppression and improved organ allocation strategies.

4.2 Liver Transplantation

Evidence in liver transplantation indicates PIRCHE-T2 may be a valuable tool for stratifying the immunological risk of humoral and cellular alloreactivity, though its impact on graft survival is more nuanced compared to other organ transplants. Studies by Meszaros et al. [47] and Hamada et al. [48] have demonstrated that elevated PIRCHE-T2 scores – particularly at the HLA-DRB1 and HLA-DQB1 loci – are significant, independent predictors of dnDSA formation in both adult and pediatric liver transplant recipients. Regarding cellular alloimmunity, Ono et al. [49] found that elevated PIRCHE-T2 scores specifically at the HLA-DQB1 locus strongly predict early TCMR and enhanced anti-donor T-cell responses in living-donor liver transplantation, while Vionnet et al. [50] associated higher PIRCHE-T2 scores with clinically silent fibro-inflammatory damage and higher TCMR-related transcript levels in long-term stable recipients. PIRCHE-T2 also shows promise in guiding immunosuppression protocols; for instance, in liver transplantation patients undergoing calcineurin inhibitors (CNI)-free maintenance immunosuppression, high PIRCHE-T2 scores were an independent risk factor for reduced liver graft survival [51]. However, the universal application of PIRCHE-T2 matching in LT remains debated; a large retrospective study by Kok et al. [52] involving 736 patients found no overall associations between PIRCHE-T2 scores and mortality, general graft loss, or clinical rejection across the broader cohort. This suggests that routine HLA-epitope matching may not be strictly necessary for standard non-autoimmune liver transplant indications.

4.3 Cardio-thoracic Transplantation

In adult heart transplantation, Zhang et al. [53] found that higher HLA-DQB1-derived PIRCHE-T2 scores are significantly associated with lower freedom from HLA-DQ dnDSA and an increased incidence of AMR. In the pediatric heart transplant setting, specific immunologic risk cut-offs for both dnDSA and AMR were established [42, 43]. They demonstrated that combining indirect T cell epitope mismatch loads with antibody epitope mismatch loads (*e.g.* HLAMatchmaker or HLA-EMMA) successfully refines risk stratification, revealing

that patients with high eplet mismatches but low PIRCHE-T2 scores actually have a low risk for developing an immune response. In lung transplantation, Lobashevsky et al. [54] identified the PIRCHE-T2 score as a superior, independent predictor of dnDSA formation when scores exceeded specific thresholds. Similarly, Kleid et al. [55] confirmed that elevated PIRCHE-T2 scores – particularly when utilizing comprehensive 11-loci high-resolution typing – strongly predict Class II dnDSA development, advocating for the combined use of PIRCHE-T2 and highly immunogenic eplet mismatches for optimal risk assessment. Reinforcing these organ-specific findings, Bedford et al. [56] evaluated a combined cardiothoracic (heart and lung) cohort and found that HLA-EMMA and Class II PIRCHE-T2 scores are statistically significant for predicting dnDSA production, mostly related to HLA-DQ loci.

Table 1: Retrospective cohort studies on molecular matching in SOT that included PIRCHE.
Abbreviations: 11 = A-B-C-DRB1-DRB345-DQB1-DQA1-DPB1-DPA1; 4 = A-B-C-DRB1; 5 = A-B-C-DRB1-DQB1; 5+A = A-B-DRB1-DQB1-DQA1; 8 = A-B-DRB1-DRB345-DQB1-DQA1; 8+ = A-B-C-DRB1-DRB345-DQB1; 9 = A-B-C-DRB1-DRB345-DQB1-DQA1; AA = amino acid mismatches; AMR = antibody-mediated rejection; BKV = BKV-DNAemia; CE = composite endpoint; CL = number of chronic lesions; CSA = child-specific HLA antibodies; DSA = de novo donor-specific HLA antibody; EM = HLA-EMMA; EMS = EMS / EMS3D; EP = Eplets; GS = graft survival; He = Heart; IS = Ischemic Cholangiopathy; Is = Islet; Ki = Kidney; KLC = KIR Ligand Calculator; Li = Liver; liv = living; Lu = Lung; OS = Overall Survival; p = pediatric; Pa = Pancreas; PIR = PIRCHE; Pr = Pregnancy; RAS = restrictive allograft syndrome; Rej = Rejection; RPL = Recurrent Pregnancy Loss; SPI = Severe Portal Inflammation; TCMR = T cell-mediated rejection; TG = Transplant Glomerulopathy.

Reference	Organ	Algorithms	Era	N	Loci	Endpoints
Zhang et al. (*2020*) [53]	He	EP, PIR	2011-2015	548	8+	AMR, DSA, OS
Bedford et al. (*2022*) [56]	He, Lu	EM, EP, PIR	2015-2020	79	11	DSA
Lobashevsky et al. (*2022*) [54]	Lu	EP, PIR	2014-2018	220	5+A	CLAD, DSA
Mangiola et al. (*2022*) [42]	He, p	EP, PIR	2014-2016	131	11	AMR, DSA
Ellison et al. (*2023*) [43]	He, p	EM, PIR	2014-2016	274	11	AMR, DSA
Kleid et al. (*2023*) [55]	Lu	EP, PIR	2012-2020	183	11	DSA
Hiho et al. (*2024*) [57]	Lu	AA, EM, ES, EP, PIR	2008-2015	277	11	CLAD, DSA, OS, RAS
Hiho et al. (*2024*) [58]	Lu	PIR	2008-2015	310	11	CLAD
Daniëls et al. (*2025*) [59]	Lu	EM, EP, KLC, PIR	2015-2021	128	5	CLAD, DSA, GS, Rej
Otten et al. (*2013*) [24]	Ki	PIR	1990-2008	21	4	DSA
Lachmann et al. (*2017*) [25]	Ki	EP, PIR	1995-2015	2787	5	DSA
Geneugelijk et al. (*2018*) [37]	Ki	EP, PIR	1995-2005	2918	5	GS
Daniëls et al. (*2018*) [60]	Ki	EP, PIR	1985-2016	36	5	DSA
Sakamoto et al. (*2020*) [27]	Ki, liv	EP, PIR	2008-2015	691	8	DSA

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Table 1: (continued)

Reference	Organ	Algorithms	Era	N	Loci	Endpoints
Senev et al. (*2021*) [61]	Ki	EP, PIR	2004-2013	954	11	TG
Naef et al. (*2021*) [31]	Ki	PIR	2009-2019	860	5	BKV, GS, TCMR
Peereboom et al. (*2021*) [46]	Ki	EP, Tmem	1995-2005	190	5	GS
Tomosugi et al. (*2021*) [20]	Ki	EP, Tmem	2012-2018	578	8	DSA
Unterrainer et al. (*2021*) [35]	Ki	PIR	1990-2016	68606	5	GS
Meneghini et al. (*2021*) [33]	Ki	EP, PIR	2014-2016	169	5	dnDST
Senev et al. (*2022*) [62]	Ki	EM, EMS, EP, PIR	2004-2013	893	11	AMR, TCMR
Betjes et al. (*2022*) [63]	Ki	PIR	1995-2005	688	5	Rej, TCMR
Senev et al. (*2022*) [34]	Ki	EP, PIR	2004-2013	893	11	GS, TCMR
Lezoeva et al. (*2022*) [39]	Ki	PIR	2009-2019	60	5	AMR, DSA, GS, TCMR
Lemieux et al. (*2022*) [36]	Ki	PIR	2000-2015	118309	5	GS
Kim et al. (*2023*) [64]	Ki, p	AA, EMS, PIR	N.A.	177	8+	AMR, DSA, TCMR
Castrezana-Lopez et al. (*2023*) [32]	Ki	PIR	2020-2021	47	5	DSA
Jäger et al. (*2024*) [44]	Ki	EP, PIR	2014-2022	439	11	DSA, Rej
Yamane et al. (*2024*) [28]	Ki, liv	PIR	2011-2023	105	9	dnDST, DSA
Ashimine et al. (*2024*) [65]	Ki, liv	EP, PIR	2008-2015	691	8	DSA
Strehler et al. (*2024*) [66]	Ki	EP, PIR	2000-2019	273	5	AMR, DSA, GS, TCMR
Gramkow et al. (*2024*) [67]	Ki, p	EM, PIR	2009-2020	49	11	DSA, GS, Rej
Jabbour et al. (*2024*) [68]	Ki	EM, EP, PIR	2016-2020	117	9	CL
Santos et al. (*2024*) [69]	Ki	EM, EP, PIR	2020-2022	419	9	DSA
Crane et al. (*2024*) [23]	Ki	PIR	2017-2023	419	5	DSA, Rej
Kamoun et al. (*2025*) [70]	Ki	EP, PIR	2012-2018	112	9	DSA, GS
Zhanzak et al. (*2025*) [71]	Ki	PIR	2016-2019	594	11	DSA, Rej
Niemann et al. (*2025*) [15]	Ki	AA, EP, PIR, Snow	1991-2022	400935	5	GS
Chou-Wu et al. (*2025*) [45]	Ki	EP, PIR, Snow	2010-2020	843	5	DSA
Tian et al. (*2025*) [26]	Ki	PIR	2008-2024	1296	5	DSA
Demir et al. (*2025*) [41]	Ki	EP, HED, PIR	2004-2017	5159	5	AMR
Zhao et al. (*2026*) [38]	Ki	EP, PIR, Snow	1981-2021	594	5	AMR, DSA, TCMR
Meszaros et al. (*2020*) [47]	Li	PIR	2015-2018	41	5	AMR, GS, SPI, TCMR
Hamada et al. (*2020*) [48]	Li	EP, PIR	1991-2019	540	5	DSA

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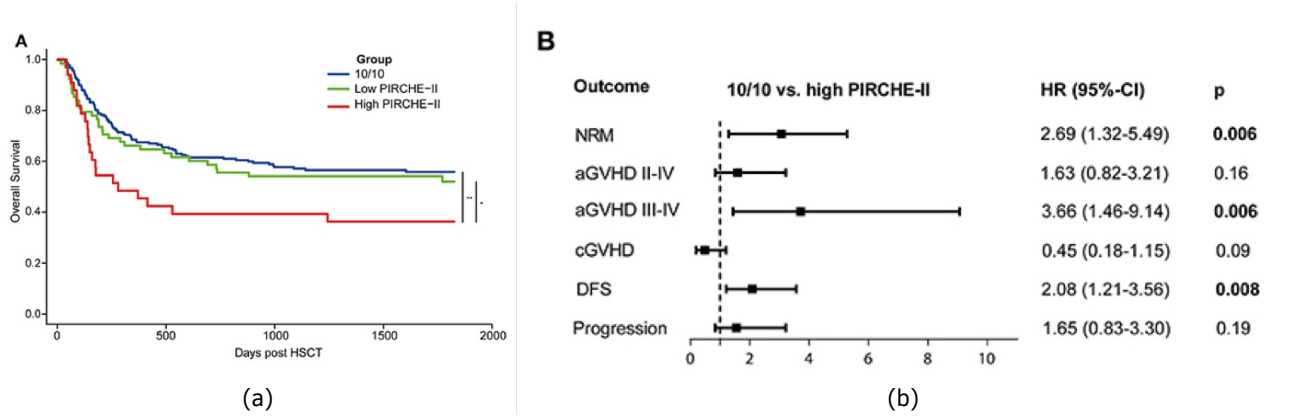
Table 1: (continued)

Reference	Organ	Algorithms	Era	N	Loci	Endpoints
Ono et al. (*2021*) [49]	Li, liv	EP, PIR	2010-2019	45	5	DSA, TCMR
Vionnet et al. (*2021*) [50]	Li	AA, PIR	2015-2019	166	5	DSA, TCMR
Kok et al. (*2022*) [52]	Li	EP, PIR	2000-2019	736	5	GS, IS, OS, Rej
Meszaros et al. (*2022*) [51]	Li	EP, PIR	2000-2018	385	5	DSA
Geneugelijk et al. (*2015*) [72]	Pr	EP, PIR	2009-2011	229	4	CSA
Niemann et al. (*2021*) [19]	Pr	PIR	2009-2011	231	11	CSA
Krog et al. (*2024*) [73]	Pr	PIR, Tmem	2008-2014	54	5	CSA, RPL
Chaigne et al. (*2016*) [29]	Is, Pa	EP, PIR	2008-2014	43	5	DSA, GS
Ladowski et al. (*2021*) [30]	Ki, Pa	EM, EP, PIR	2002-2017	113	9	AMR, DSA, GS
Raineri et al. (*2024*) [74]	Ki, Pa	PIR	2009-2019	455	5	DSA
Parajuli et al. (*2025*) [75]	Ki, Pa	EP, PIR, Snow	2012-2021	238	5	CE

4.4 Stem Cell Transplantation

4.4.1 Unrelated Donor Transplants with 9/10 HLA Matches

In the setting of mismatched unrelated donor HSCT, several studies confirm the adverse effects of high PIRCHE-T scores. Thus et al. [76] initially investigated 48 patients and found that higher numbers of PIRCHE derived specifically from antigenic HLA-C mismatches correlated with an increased probability of acute GvHD. Expanding on this in a large multicenter cohort of 424 adult recipients, Ayuk et al. [77] demonstrated that the presence of PIRCHE-T (PIRCHE-T1 + PIRCHE-T2 $>$ 0) was significantly associated with lower overall survival, higher non-relapse mortality, and a higher incidence of chronic GvHD. Similarly, Geneugelijk et al. [78] studied a Dutch multicenter cohort of 103 patients with early-stage disease and reported that a high PIRCHE-T2 score significantly impaired overall survival due to an increased risk of severe acute GvHD (figures 13a and 13b). Stenger et al. [79] investigated a pediatric cohort of 105 patients and determined that a high PIRCHE-T1 score was associated with a significantly higher risk for developing acute GvHD in patients with 9/10 matched donors. More recently, Grubic et al. analyzed 108 adult patients with 9/10 matched donors and noted that a PIRCHE-T2 score above 10 in the graft vs host (GvH) direction resulted in a notably higher incidence of GvHD.



(a) (b)

Figure 13: Early-disease stage patients with elevated PIRCHE-T2 scores showed impaired overall survival, whilst low PIRCHE-T2 patients had an overall survival similar to a 10/10 matched reference group. Image credit: Figures 13a and 13b: Geneugelijk et al. [78] (derivative: cropped), licensed under CC-BY-4.0.

4.4.2 HLA-DPB1 Mismatches in 10/10 Matched Donors

A specialized area of research focuses on isolated HLA-DPB1 disparities in otherwise fully matched (10/10) donor transplants. Thus et al. [76] analyzed 80 such patients and concluded that the presence of PIRCHE-T1 or -T2 significantly increased the hazard of acute GvHD. Buhler et al. [80] evaluated 909 recipient-donor pairs and demonstrated that the presence of PIRCHE-T2 significantly increased the risk of acute GvHD, particularly when used to complement the nonpermissive T-cell epitope (TCE) matching model. In a massive cohort of 1,514 patients, Zou et al. [81] revealed that high PIRCHE-T scores in the GvH direction were strongly associated with increased acute GvHD and a beneficial reduction in disease relapse. In a subsequent study of the same patient cohort, Zou et al. [82] also demonstrated that higher PIRCHE-T1 and PIRCHE-T2 scores in the GvH direction were linked to an increased risk of developing subsequent solid cancers post-transplant.

4.4.3 Haploidentical Stem Cell Transplantation

The impact of PIRCHE in haploidentical SCT appears to depend on the type of GvHD prophylaxis utilized. Huo et al. [83] investigated 577 patients receiving haploidentical transplants with antithymocyte globulin (ATG) prophylaxis and found no significant associations between PIRCHE-T scores and clinical outcomes. Conversely, Rimando et al. [84] evaluated 148 patients undergoing haploidentical transplantation with post-transplant cyclophosphamide (ptCy) and established that a higher PIRCHE-T1 score in the GvH direction was

an independent risk factor for increased acute GvHD. Gil-Etayo et al. [85] studied 145 haploidentical transplant patients using ptCy and concluded that a high overall PIRCHE-T score accelerated acute GvHD, a high HLA-B PIRCHE-T1 score increased chronic GvHD, and a high PIRCHE-T2 score in the host versus graft (HvG) direction increased the incidence of disease relapse. Iwasaki et al. [86] analyzed a Japanese registry of 1,037 haploidentical transplant patients, identifying a distinct interaction between the algorithm and prophylaxis type: higher PIRCHE-T1 scores reduced relapse risk and improved survival in patients receiving ptCy, whereas higher PIRCHE-T2 scores lowered non-relapse mortality in standard-risk patients receiving ATG.

4.4.4 Umbilical Cord Blood Transplantation

In cord blood transplantation, Thus et al. [87] evaluated 134 pediatric recipients and found a beneficial GvL effect, where high PIRCHE-T1, scores were associated with a significantly reduced risk of disease relapse and improved disease-free survival without exacerbating GvHD risks. Sugio et al. [88] evaluated the immunogenicity of mismatched HLA in cord blood transplantation by exploring how epitopes derived from recipient-mismatched HLA presented on matched – but not mismatched – donor HLA are associated with transplant outcomes, supporting the ”shared” HLA concept applied by PIRCHE in HSCT(section 3.5.4).

4.4.5 Minor Histocompatibility

Saliba et al. [89] expanded the PIRCHE in silico model beyond HLA to evaluate Y chromosome minor histocompatibility antigens (PIRCHyE) in 194 female-to-male stem cell transplants, discovering that higher PIRCHyE-T1 scores were associated with disease progression, while PIRCHyE-T2 scores correlated with the development of chronic GvHD.

4.5 Published Thresholds

Although molecular mismatch loads are technically fine-grained ordinal variables, they are typically treated as continuous in clinical analyses. This simplification allows for weighted aggregation following multiple imputation (section 3.7) and enables the use of more versatile statistical models. For practical application, many studies suggested cohort-specific optimal thresholds or validated previously suggested thresholds. Notably, optimal thresholds depend on the provided loci and the configuration of the applied predictors. Given molecular matching algorithms provide raw values instead of normalizing to the number of input alleles/loci, with increasing HLA typing breadth molecular mismatch loads typically increase. Based on the recently introduced PIRCHE® Frost and Snow predictors, thresholds reported by articles listed in table 2 may be considered a starting point for local implementation.

Table 2: PIRCHE thresholds as published for kidney transplantation considering 5-loci HLA typings (HLA-A, -B, -C, -DRB1 and -DQB1), Frost 1.1 peptide binding prediction (30% binding rank) and Snow 1.1 surface prediction (0.26 surface area threshold, 0.68 protrusion rank threshold).

Reference	**Endpoints**	**PIRCHE-T2**	**PIRCHE-B**
Niemann et al. (*2025*) [15]	graft failure	[0, 23), [23, 45), [45, 69), [69, -)	[0, 4), [4, 10), [10, 13), [13, -)
Chou-Wu et al. (*2025*) [45]	dnDSA	Class I: [0, 43], (43, -) Class II: [0, 30], (30, -)	Class I: [0, 9], (9, -) Class II [0, 7], (7, -)
Zhao et al. (*2026*) [38]	dnDSA, AMR, TCMR	Class I: [0, 30.5], (30.5, -) Class II: [0, 17], (17.5, -)	Class I: [0, 5.5], (5.5, -) Class II: [0, 5.5], (5.5, -)

In stem cell transplantation, the Frost predictor family is currently under evaluation. Literature-reported thresholds (table 3) are consequently still based on the previous generation of binding predictors.

Table 3: PIRCHE-T thresholds as published for stem cell transplantation considering 5-loci HLA typings (HLA-A, -B, -C, -DRB1 and -DQB1) and a netChop/netMHCpan/netMHCIIpan-based database (0.5/500nM/1000nM configuration).

Reference	**Mode**	**PIRCHE-T**
Geneugelijk et al. (*2019*) [78]	9/10	PIRCHE-T2: [0, 13], (13, -)
Ayuk et al. (*2019*) [77]	9/10	PIRCHE-T1+T2: [0, 0], (0, -)
Rimando et al. (*2020*) [84]	haplo	PIRCHE-T1: [0, 7], [8, 15], [16, 56]
Gil-Etayo et al. (*2024*) [85]	haplo	aGvHD PIRCHE-T1 + PIRCHE-T2: [0, 38), [38, -) cGvHD PIRCHE-T1 B: [0, 1], (1, -) relapse: HvG PIRCHE-T2: [0, 28], (28, -)

5 From Selection to Survival – Why to Use Molecular Matching?

Molecular matching algorithms offer a refined approach to histocompatibility assessment. Their practical utility may span the entire transplant timeline, from optimizing pre-operative allocation to precision post-transplant management.

5.1 The Smart Match – Optimize Allocation & Access

The pre-transplant phase relies heavily on assessing molecular compatibility to accurately stratify immunological risk and ensure equitable access to organs.

- **Donor Selection:** When a recipient has multiple compatible donors – such as in living donation workups or paired kidney exchanges – molecular mismatch loads may be utilized to select the candidate with the lowest long-term immunological risk. Computational simulations of allocation systems have demonstrated that incorporating T-cell epitope matching is both feasible [90] and effective in kidney allocation [91].
- **Defining Unacceptable Mismatches**: For highly sensitized patients, identifying alleles with shared incompatibilities is critical because these can trigger memory responses. Research has demonstrated that sensitized patients who receive a transplant with a repeatedly mismatched predicted T cell epitope have a higher likelihood of developing early dnDSA, which models a memory T cell response [20, 46]. Using the Tmem analysis module to flag previous immunizers may help clinical teams identify overlapping T cell epitopes between a patient's historical immunizers and new potential donor candidates.
- **Risk Prediction:** Identifying high-risk, "high-velocity" responder pairs prior to surgery may allow transplant teams to prepare more aggressive induction protocols [92]. Understanding a patient's molecular mismatch load may predict the risk of immune responses, underscoring the dependency of immunologic high-risk patients on sufficient, robust immunosuppression.

5.2 The Precision Management – Maximize Longevity & Quality of Life

Following transplantation, molecular mismatch metrics may provide a framework for individualized patient care, balancing the prevention of rejection against the toxicities of over-immunosuppression.

- **Personalized Immunosuppression:** Molecular matching is currently evaluated for facilitating personalized immunosuppression protocols by differentiating low-risk patients, who may be eligible for safe drug minimization, from high-risk patients requiring robust blockade. Evidence suggests a strong potential for molecular mismatch-guided maintenance strategies; for instance, patients identified as having low immunologic risk have achieved improved graft survival without an increased risk of immune-related events when placed on specific maintenance immunosuppression therapies [93, 51, 94].
- **Adaptive Surveillance:** Surveillance strategies can potentially be tailored based on the patient's specific immune risk, adjusting the frequency of invasive monitoring (such as protocol biopsies) and non-invasive biomarker testing (such as donor-derived cell-free DNA)[95]. Elevated molecular mismatch successfully predict clinical events, including elevated donor-derived cell-free DNA (dd-cfDNA) levels, dnDSA formation, and rejection. Adjusting post-transplant immune monitoring schedules according to this risk stratification may present valuable health economical benefits.
- **Diagnostic Clarity:** In cases where clinical presentation is ambiguous, molecular mismatch risk scores may support diagnostic interpretation, helping clinicians decide whether to actively treat or carefully watch. To that extent, high PIRCHE-T2 scores have been shown to allow for the effective risk stratification of borderline rejection results in kidney transplant recipients [39].

6 User Guide for TxPredictor™

6.1 Getting Started

6.1.1 System Requirements

General	Requires an internet-enabled environment allowing standard HTTPS access (via appropriate firewall configuration) to the TxPredictor™ platform using a supported web browser
Minimum supported browsers	Edge v80+, Firefox v75+, Chrome v80+, Safari v14+
Recommendation on browsers	Only use browsers still supported by their vendor and with latest/recommended security patches applied
Minimum supported operating systems	Depends on the availability of supported browsers and their minimum supported versions
Recommendation on operating systems	Only use operating systems still supported by their vendor and with latest/recommended security patches applied
Minimum screen resolution	1280 x 1024
Recommended screen resolution	2560 x 1440

6.1.2 Account and Access

To **activate** your account after its creation by your Organization Manager (section 6.1.5) or PIRCHE administrator, you will receive an email containing a link for password initialization. Once your password is set, you can **log into TxPredictor™** (fig. 14). Navigate to `https://pirche.com/pirche/` in your web browser and follow these steps to log in:

1. Enter registered email address.
2. Enter password.
3. Click LOGIN.

If you need to **reset your password**, click the Forgot Password link (fig. 14, #4). On the next screen:

1. Enter registered email address.
2. Click SEND RESET PASSWORD E-MAIL. *Note:* The Cancel link will take you back to the Login page.

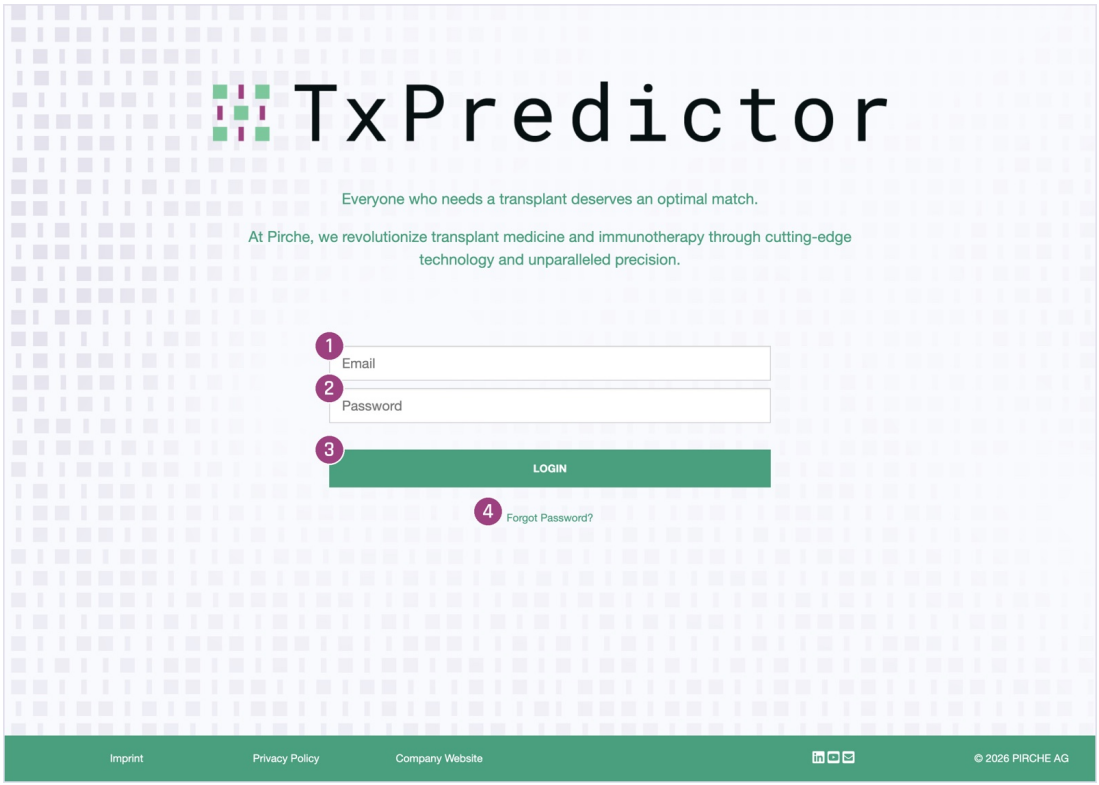


Figure 14: The login screen of the TxPredictor™ platform

6.1.3 Navigation

The primary method of navigation is the Top Navigation Bar, located at the top of every screen (fig. 15). Use this menu to easily select any of the core components:

Figure 15: The Top Navigation Bar serves as the main menu for the TxPredictor™.

Home A direct link to the main dashboard, which features a blog that is used to communicate software updates and key educational material (*e.g.* scientific publications and educational webinars).

HSCT Reveals a drop-down menu containing matching modules for hematopoietic stem cell transplantation.

SOT Reveals a drop-down menu containing matching modules for solid organ transplantation.

Academy Access educational resources, featuring a literature compass, publication list, and several webinars.

Help Find general support resources, such as Frequently Asked Questions (FAQ).

Settings Configure account and organization settings.

A Breadcrumb Trail is displayed at the top left of the screen to indicate your current location and allow for quick backward navigation.

6.1.4 Organization Settings

Users with administrative privileges, termed "Organization Manager", can select certain settings for all users within their organization.

- **Default Prediction Pipeline:** The default prediction pipeline is automatically selected in the drop-down menu on the HLA input screens. If needed, a different selection may be made before running a match. *Note:* Unless specified by the organization, the default is set to System Setting, which is PIRCHE's global default. As new prediction pipelines are released continuously (*e.g.* based on IPD-IMGT/HLA database [16] upgrades), it is highly recommended to update this default in accordance with your institution's policies and procedures.
- **Default Donor Panel:** In SOT Risk and Acceptable Mismatch Profile (RAMP) and HSCT Search Profile, the default donor panel is automatically selected in the drop-down menu. This setting may be manually overridden before running a match. Unless specified, the default is set to NMDP EUR haplotypes (2007) for both SOT RAMP and HSCT Search Profile. If needed, select a different default panel to maintain a consistent workflow.

To **Customize Organization Settings** (fig. 16):

1. Under *Settings*, select *Organization Settings*. *Note:* This menu selection is only available to the Organization Manager.
2. Use the drop-down menu to select the desired default setting.
   (a) *Prediction Pipeline*: A separate drop-down menu is available for SOT and HSCT
   (b) *Donor Panel*: A separate drop-down menu is available for SOT RAMP and HSCT Search Profile.
3. The **X** button reverts the selection to System Setting.
4. The **Cancel** button clears unsaved changes. This will preserve a previously saved custom default.
5. Click **Save** to apply changes across the organization.

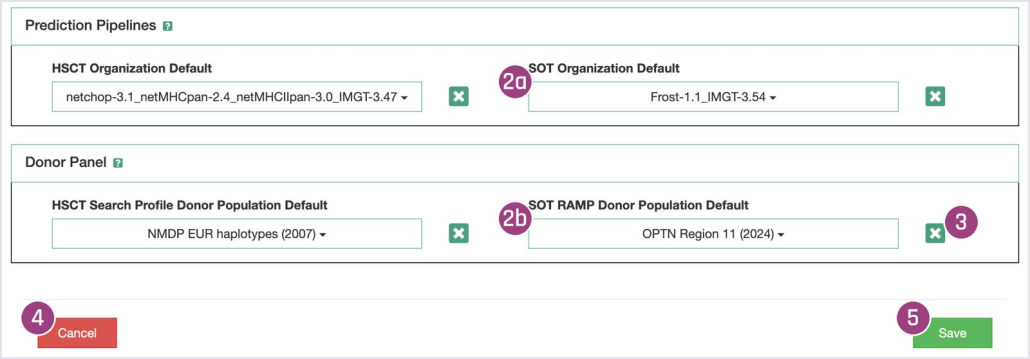


Figure 16: The configuration panel defines global default settings for your institution.

6.1.5 User Management

The system provides two levels of user profile management:

My Organization

The My Organization interface is restricted to users with elevated administrative permissions (*i.e.* Organization Managers). This dashboard serves as the central utility for scaling institutional access and auditing user privileges (fig. 17).

Manage Existing Users:

1. The **Administrator** column displays the current permission status of each member. **Organization Managers** are denoted by a user icon wearing a Crown. Click the icon to toggle administrative status. A confirmation window will open to prevent accidental permission changes.
2. **Deactivate** an account by clicking the **- Remove User** button. A window will open to **Confirm** or **Cancel** user inactivation.

Invite New Users:

3. Click the **+ Invite New User** button to start the invitation process.
4. Enter the user's Last Name, First Name, and Email Address into the designated text fields.
5. Click the **+ Invite New User** button again. A window will open to **Confirm** or **Cancel** the invitation. *Note:* Upon confirmation, the system sends an automated email containing a secure, time-sensitive link to set a password. The invitation recipient remains in the *Pending Invitations* panel until activating their account.

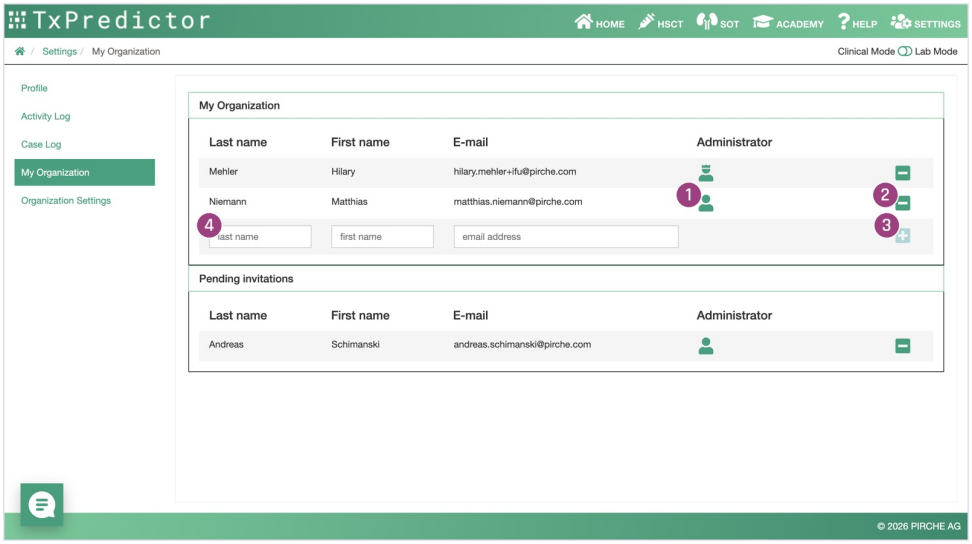


Figure 17: Manage user accounts for your organization.

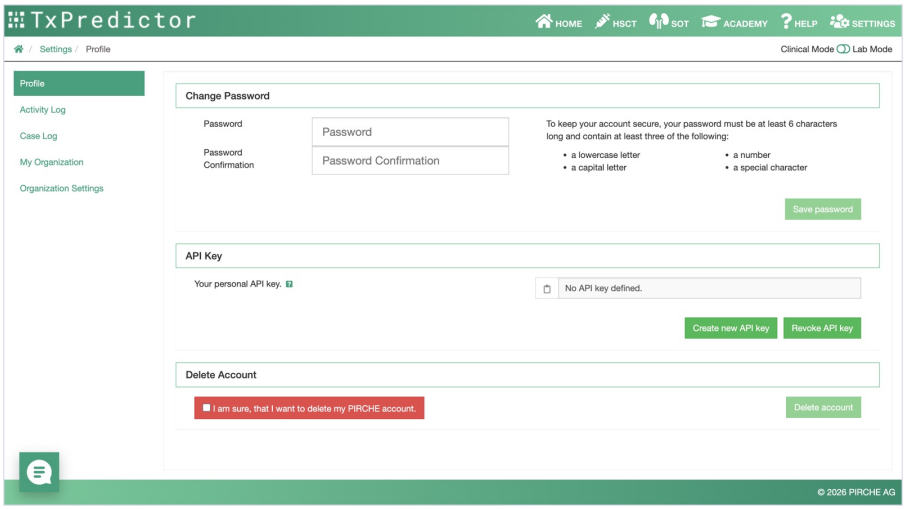


Figure 18: Manage your login credentials.

Edit Profile

Registered users can manage their personal credentials and integration keys through the Edit Profile interface (fig. 18):

- **Change Password**: Users may update their account password at any time. Follow the onscreen password requirements to ensure the new credentials meet system security standards and click the **Save Password** button to confirm.
- **API Key**: Click the **Create New API Key** button to generate a unique integration token for programmatic access without sharing your personal password. Use the **Clipboard** icon to easily copy the key to save locally. Existing keys can be revoked using the **Revoke API Key** button or by creating a new key, which will instantly terminate external integrations.
- **Delete Account**: To permanently delete your account, select the confirmation checkbox at the bottom left and click the **Delete Account** button to the bottom right.

API Key: For security reasons, the API key is only displayed once. It must be saved in a secure location immediately; it cannot be retrieved once the page is refreshed or closed.

6.2 Data Input for Core Analysis Modules

The platform offers two distinct modes for analysis, which can be selected through a toggle underneath the Top Navigation Bar:

Clinical Mode Capabilities are well-established and thoroughly validated in major journal articles (*e.g.* 5-loci input).

Lab Mode Includes extended features that may not yet be as widely published or are experimental (*e.g.* 13-loci input).

For each patient and donor entered into TxPredictor™, you must provide unique identifiers (*e.g.* Patient ID, Donor ID), which supports distinguishing cases unambiguously. The PIRCHE (section 3.5) and Snow (section 3.3) molecular matching algorithms are performed based on the provided HLA data. All HLA typing input is validated against the selected version of the IPD-IMGT/HLA database [16]. Furthermore, a dynamic drop-down menu assists with allele selection (*i.e.* auto-completion). For example, typing "02:" will display all valid alleles in that group, helping to "fill in the blanks" and reduce transcription errors.

Data Privacy: To ensure patient and donor confidentiality, it is prohibited to enter personal information such as names or dates of birth. Always use de-identified codes that can only be mapped back to the patient record internally by the laboratory users.

Requirements for solid organ transplantation: For SOT analyses, TxPredictor™ performs matching on at least 5 loci (HLA-A, -B, -C, -DRB1, and -DQB1), allowing for variable typing resolution. The system performs a multiple imputation algorithm to derive protein level typings from low-resolution input (see section 3.7). However, the following minimum criteria are required to ensure valid imputation:

- **HLA-A, -B, and -DRB1** must be provided at a minimum.
- HLA-C and -DQB1 are optional; if omitted, these fields will be imputed.
- The second field of HLA-A, -B, -C, -DRB1 and -DQB1 will be imputed if non protein-level typing data is provided.

- High-resolution typing is **strictly required** for optionally provided HLA loci (HLA-DRB3/4/5, -DQA1, -DPA1, or -DPB1).

- Imputation relies on NMDP haplotype frequency tables. If an individual's ancestry is unknown, the **NMDP Combined (2007)** population may be used (section 3.7.2) [21].

Managing Ambiguities: Although multiple imputation is performed, protein-level typing remains optimal. When dealing with allelic ambiguities, acknowledge:

- **P and G groups** are not supported. TxPredictor™ flags P and G groups as invalid. *Note:* Consideration of P or G groups would force the pipeline to consider every possible allele in that group, potentially introducing unnecessary ambiguity.

- **NMDP multi allele codes (MAC)** are supported, with the imputation algorithm filtering haplotypes for specific allele strings defined by the MAC.

Requirements for hematopoietic stem cell transplantation: Due to the critical nature of allele-level matching in stem cell transplantation, imputation is not used for HSCT. Thus the minimum requirements for HLA input data are:

- Protein-level typing (2-field) is strictly required.

- Third- and fourth-field HLA typing input is accepted, but does not impact matching calculation.

- **HLA-A, -B, -C, -DRB1, and -DQB1** must be provided at minimum.

Maintain Workflow Consistency: PIRCHE scores are not normalized, which typically yields higher molecular mismatch loads when more loci are provided. Valid risk stratification requires a consistent workflow of loci to be provided. A 5-loci analysis is not comparable to 6- or 11-loci analyses.

Recipient/Donor Consistency: To avoid inconsistencies in the algorithms, TxPredictor™ enforces providing the same HLA loci for all recipients and donors within a case.

Homozygosity: If you enter only a single allele for a locus, TxPredictor™ assumes the locus is homozygous. Consequently, a second allele will **not** be imputed.

6.3 Assisted Methods for HLA Data Entry

TxPredictor™ supports numerous methods for inputting HLA typing data, ranging from manual entry to automated system integration. It is recommended to use automated workflows to eliminate the risk of manual transcription errors.

6.3.1 Manual Entry

Users can type HLA data directly into the web interface field-by-field, assisted by real-time validation and auto-completion.

6.3.2 Input Wizard

The Input Wizard allows users to paste data or import Histocompatibility Markup Language (HML) files (fig. 19).
Note: The Patient and Donor sections have distinct Wizards in order to minimize the risk of data transposition.

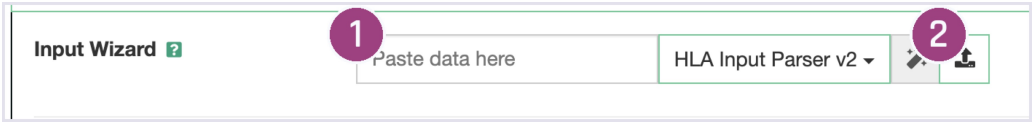


Figure 19: The Input Wizard allows semi-automated entry of HLA data.

Supported Formats for Copy & Paste: Paste text directly into the Input Wizard field (fig. 19, #1), and the system parses the information into the corresponding HLA input fields. Follow the examples below to convert your HLA typings to a supported format.

CSV format:

High-Resolution `Patient163,A*02:01,A*80:01,B*07:02,B*57:03,C*07:02,C*08:02,DRB1*12:01,DRB1*09:01,DRB3*02:02,DRB4*01:01,DQA1*01:05,DQA1*03:03,DQB1*05:01,DQB1*02:02,DPA1*01:03,DPA1*02:01,DPB1*104:01,DPB1*131:01`

Low-Resolution `Donor927,A*29,A*24,B*07,B*51,C*15,C*07,DRB1*04,DRB1*10,DQB1*04,DQB1*05`

Serology `Patient365,A26,A11,B46,B35,C1,C3,DR4,DR11,DQ4,DQ7`

Mixed-Resolution `Donor741,A2,B*15:13,B*46:01,C*08,C*01,DR12,DRB3*03:01,DQB1*03:01,DQB1*05:02`

GL String format:

High-Resolution `Donor484,A*11:01+A*29:01^B*40:02+B*07:05^C*15:02+C*15:05^DRB1*11:12+DRB1*10:01^DRB3*02:02^DQA1*05:05+DQA1*01:05^DQB1*03:01+DQB1*05:01^DPA1*01:03^DPB1*02:01`

Low-Resolution `Donor937,A*29+A*68^B*14+B*57^C*06+C*08^DRB1*07+DRB1*13^DQB1*03+DQB1*03`

Serology `Patient561,A2+A33^B44+B58^C5+C3^DR13+DR4^DQ6+DQ8`

Mixed-Resolution `Patient038,A*11+A*29^B18+B44^C*07+C*16^DRB1*11+DRB1*07^DRB3*02:02^DRB4*01:01^DQA1*05:05+DQA1*02:01^DQB1*03:01+DQB1*02:02^DPA1*02:01+DPA1*01:03^DPB1*11:01+DPB1*04:01`

HML File format: Click the Upload button to the right of the Input Wizard (fig. 19, #2) to upload an HML file. This standardized file format can be exported directly from many HLA next-generation sequencing (NGS) analysis software programs.

Why use HML? HML is a standardized data format developed specifically to facilitate HLA data exchange between software systems [96]. It is significantly more reliable than parsing unstructured reports.

6.3.3 Case Import via File Upload

The **Import Button** located at the bottom-left of the interface is designed for uploading complete case information. Both patient and donor information are parsed into the appropriate input fields. Different file formats are supported, *e.g.* *PIRCHE CSV*, which is a comma-separated file with one transplant per row, having the

columns `TXID`, `Patient ID`, `P-A1`, `P-A2`, `P-B1`, `P-B2`, `P-C1`, `P-C2`, `P-DRB11`, `P-DRB12`, `P-DRB31`, `P-DRB32`, `P-DRB41`, `P-DRB42`, `P-DRB51`, `P-DRB52`, `P-DQA11`, `P-DQA12`, `P-DQB11`, `P-DQB12` , `P-DPA11`, `P-DPA12`, `P-DPB11`, `P-DPB12`, `Donor ID`, `D-A1`, `D-A2`, `D-B1`, `D-B2`, `D-C1`, `D-C2`, `D-DRB11`, `D-DRB12`, `D-DRB31`, `D-DRB32`, `D-DRB41`, `D-DRB42`, `D-DRB51`, `D-DRB52`, `D-DQA11`, `D-DQA12`, `D-DQB11`, `D-DQB12`, `D-DPA11`, `D-DPA12`, `D-DPB11`, and `D-DPB12`.

6.3.4 API Integration

The application programming interface (API) acts as a secure digital bridge that connects your local laboratory software (such as a LIMS or NGS software) directly to the TxPredictor™. The API allows you to create a seamless, customized workflow. A number of 3rd party vendors and clinics already directly integrated the PIRCHE® API to communicate HLA typing data between the systems without manual intervention. If you are interested in connecting your own software to the PIRCHE® API, the following options are available

WebLink Browser By creating a rich HTML link, you can launch TxPredictor™ in your browser with patient and donor data already pre-loaded. This streamlines the workflow by removing manual data entry steps while still giving users full access to TxPredictor™'s visual tools and epitope maps.

WebLink REST Alternatively, your 3rd party software can send HLA typings to TxPredictor™ and receive risk scores back instantly. This happens in the background, allowing you to retrieve PIRCHE scores without ever opening a web browser.

Integration Support: For assistance developing a custom workflow, please contact support@pirche.com. We will provide your development team with the full API documentation and technical assistance to ensure a seamless integration.

7 Running Analyses: Solid Organ Transplant Core Modules

7.1 Donor Selection

This module is designed to evaluate the immunological risk of specific donor candidates for a given recipient (*i.e. many to one*), for example when comparing multiple living donor options for one patient.

To start this module, select **SOT** → **Donor Selection** from the Top Navigation Bar.

7.1.1 Input Screen

1. **Enter Patient Data:** In the top panel, input the recipient's unique ID and HLA typing (fig. 23).See section 6.2 for input requirements and methods.

2. **Enter Donor Data:** In the bottom panel, enter the unique ID and HLA typings for one or more donors. See section 6.2 for input requirements and methods.

   - To add more donors for manual entry, click the **+ Add a New Donor** button (fig. 20).
   - To add multiple donors simultaneously while extracting their typing data, paste a list of typings into the **Input Wizard** (figs. 19 and 23). For all supported formats, each donor should be separated with a newline. The system will automatically parse the data and create an entry for each donor.

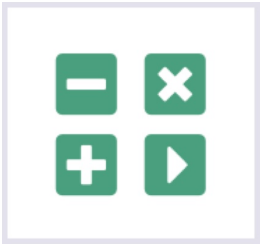


Figure 20: This button group allows you to remove donor or patient rows (-), clear input fields (x), add donor or patient rows (+) and copy over the recipient typing (→). Buttons are not visible if they do not apply in the specific context or module.

3. **Specify Typing Method:** *(Optional)* Use the DNA/SER toggles to define whether molecular (DNA) or serological (SER) nomenclature is provided (fig. 23). *Note:* This step is obsolete if protein-level typings are provided.

   (a) Select DNA or SER under the ID field to change all locus toggles for the patient/donor.

   (b) Adjust individual loci' toggles to handle mixed typing data.

4. **Verify Population:** *(Optional)* If using low-resolution data, verify the target population for imputation in the drop-down menu (figs. 21 and 23). *Note:* The population drop-down is automatically disabled if high-resolution typing is provided for all loci.

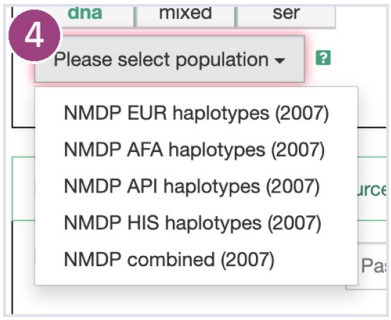


Figure 21: Select the population to define the reference haplotypes to be used for imputation. For more details, see section 3.7.

5. **Verify Prediction Pipeline:** Select the prediction pipeline to use for analysis (fig. 22). *Note:* The default selection for your institution may be adjusted under **Settings** → **Organization Settings** (section 6.1.4).
6. **Run Analysis:** Click the **Match** button at the bottom right. The results screen will load automatically.

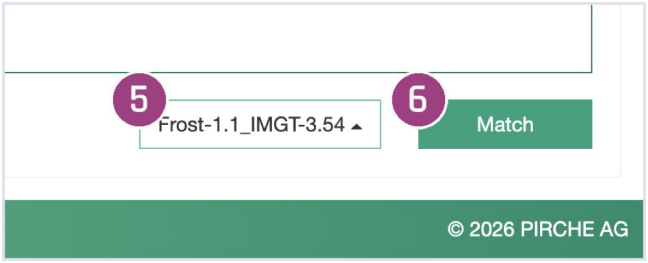


Figure 22: Select the prediction pipeline and click Match to trigger the PIRCHE® analysis.

Expected Processing Time: With only a few individuals provided, the SOT Donor Selection module will respond within seconds. With highly increased numbers of provided donors (*e.g.* entire paired donation pools) or highly ambiguous input HLA typings, the processing time may increase.

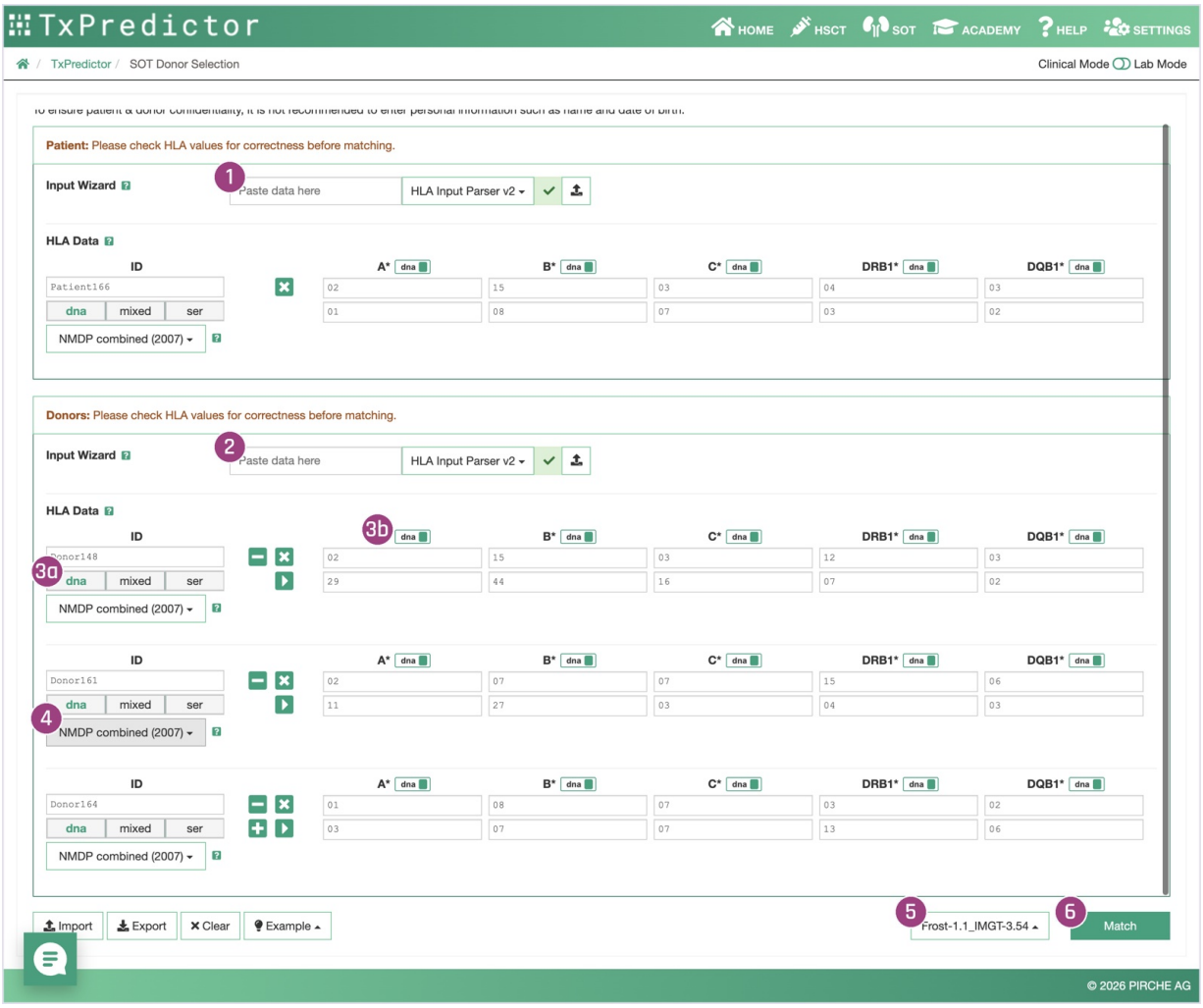


Figure 23: Verify patient and donor information, select the prediction pipeline, and click Match to submit the SOT Donor Selection analysis.

7.1.2 Results Screen

The Results screen provides a comprehensive overview of the immunological risk for each patient-donor pair. The following steps walk through the primary components of the Donor Selection Results screen (fig. 24):

1. **Results Summary Table:** At the very top of the screen, the summary table anchors the patient in the first row, with all evaluated donors listed below. Review the displayed input HLA typings and IDs for both the patient and the donors to verify that the initial data entry was accurate. *Note:* Mismatched HLAs are highlighted.

2. **PIRCHE® Scores:** To the right of each donor row, you will find the PIRCHE-T2 and PIRCHE-B scores for the patient-donor pair. These values represent the overall molecular mismatch load.

    - **PIRCHE-T2:** The number of unique core peptides presented by HLA Class II molecules (section 3.5).
    - **PIRCHE-B:** The number of interlocus donor-specific amino acid mismatches with high protein-specific surface area and high protein-specific local protrusion rank (section 3.3).

Score Fractions: Although defined as an integer (sections 3.3, 3.5 and 4.5), TxPredictor™ may output the molecular mismatch load as a decimal value accompanied by a standard deviation. This occurs when imputation is applied and the results are weighted and aggregated by haplotype frequency.

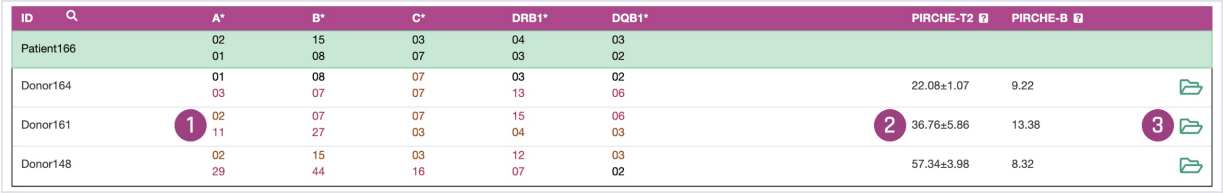
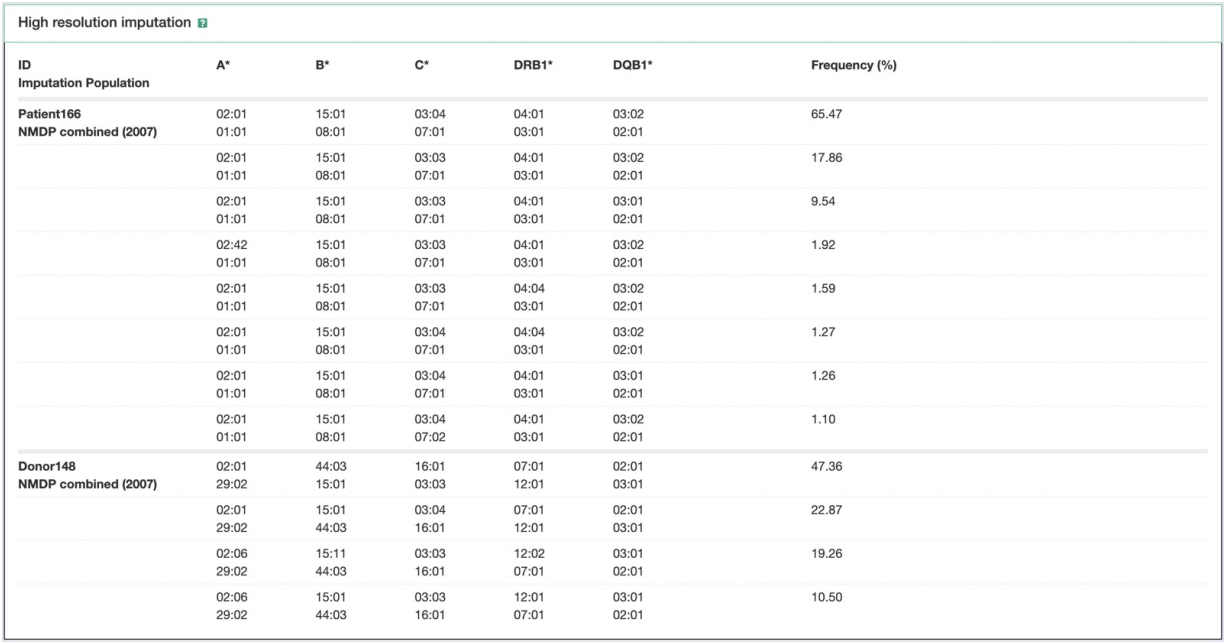


Figure 24: View patient and donor HLA typings, total PIRCHE® scores, and expand details for a patient-donor pair.

3. **Expand Details:** *(Optional)* To visualize the specific mismatched epitopes driving the molecular mismatch scores, click the folder icon next to any donor row in the summary table. This expands the row to reveal a detailed data panel for the patient-donor pair, including:

- **Imputation Details:** *(Optional)* When imputation is required, all considered genotype pairs are displayed alongside their frequencies (fig. 25). The list is sorted descending by likelihood, with the most probable combinations appearing at the top. Section 3.7 describes the imputation algorithm TxPredictor™ applies.



High resolution imputation

ID Imputation Population	A*	B*	C*	DRB1*	DQB1*	Frequency (%)
Patient166 NMDP combined (2007)	02:01 01:01	15:01 08:01	03:04 07:01	04:01 03:01	03:02 02:01	65.47
	02:01 01:01	15:01 08:01	03:03 07:01	04:01 03:01	03:02 02:01	17.86
	02:01 01:01	15:01 08:01	03:03 07:01	04:01 03:01	03:01 02:01	9.54
	02:42 01:01	15:01 08:01	03:03 07:01	04:01 03:01	03:02 02:01	1.92
	02:01 01:01	15:01 08:01	03:03 07:01	04:04 03:01	03:02 02:01	1.59
	02:01 01:01	15:01 08:01	03:04 07:01	04:04 03:01	03:02 02:01	1.27
	02:01 01:01	15:01 08:01	03:04 07:01	04:01 03:01	03:01 02:01	1.26
	02:01 01:01	15:01 08:01	03:04 07:02	04:01 03:01	03:02 02:01	1.10
Donor148 NMDP combined (2007)	02:01 29:02	44:03 15:01	16:01 03:03	07:01 12:01	02:01 03:01	47.36
	02:01 29:02	15:01 44:03	03:04 16:01	07:01 12:01	02:01 03:01	22.87
	02:06 29:02	15:11 44:03	03:03 16:01	12:02 07:01	03:01 02:01	19.26
	02:06 29:02	15:01 44:03	03:03 16:01	12:01 07:01	03:01 02:01	10.50

Figure 25: Imputed high-resolution genotypes and their frequencies are shown in a table within the matching details section.

- **Presentation Details:** This panel provides a table with a detailed breakdown of the T-cell epitopes driving the PIRCHE-T2 score (fig. 26).
  - *Presenter Allele/Dimer:* The patient's HLA molecule responsible for presenting peptides to T-cells.
  - *Presented Allele:* The mismatched donor HLA allele from which the presented peptides are derived. The count below the allele indicates the number of distinct core peptides derived from it (weighted by imputation likelihood, if applicable).

- *Core Peptide:* The peptide core sequence (typically a 9-mer) that is predicted to bind within the HLA presenter's binding groove.
- *Peptide:* The full-length peptide predicted to bind. If multiple peptides share the exact same core peptide, TxPredictor™ counts and highlights only one: the peptide with the highest probability of binding (lowest binding rank) is shown in black text with a star icon on the far right.
- *Imputation:* The likelihood of the pHLA being present in the donor HLA and absent in the patient HLA, considering multiple genotype imputation.

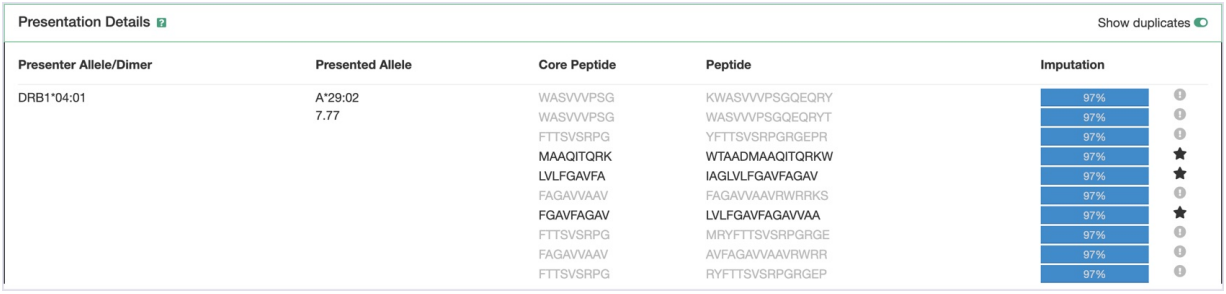


Figure 26: T-cell epitope presentation details are provided in a table, showing presented pHLA grouped by the patient's presenting HLA molecules and the mismatched donor alleles from which they originate.

4. **Matching Heatmap:** Directly below the summary table (fig. 24) are the Matching Heatmaps (fig. 27). Locus-specific scores are quantified and visualized here, where color intensity corresponds to higher values. This allows for a quick visual breakdown of the total score — highlighting, for example, if the cumulative scores are skewed towards specific HLA loci.

5. **Metadata:** Located at the bottom left of the screen, the metadata displays the exact calculation parameters applied to the current case (fig. 28). This information supports standard laboratory Quality Assurance (QA) procedures, compliance audits, and troubleshooting by documenting:

    - **Calculation Parameters:** The specific prediction pipeline (algorithm and database versions) alongside the underlying algorithmic thresholds applied.
    - **System Information:** The environment (*e.g.* RUO), server version, and a precise server timestamp.
    - **Evaluated Loci:** A summary of the specific presenter and presented HLA loci used for the calculation.

Figure 27: Matching Heatmaps visualize molecular mismatch load per HLA locus using a color gradient (purple for PIRCHE-T2, green for PIRCHE-B). Higher scores are represented by more intense colors.

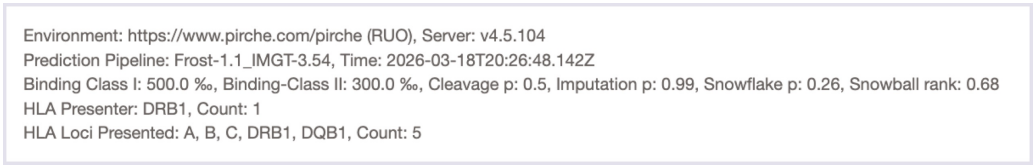


Figure 28: Example of the Metadata provided on the SOT result screens, providing a comprehensive summary of calculation parameters, server versions, and evaluation criteria.

Version numbers: TxPredictor™ server versions use a three-part format (*e.g. 4.5.104*). The first two fields (major platform and matching algorithm versions) can impact match result calculations, while the third introduces non-impacting features or fixes. Similarly, predictor versions (*e.g. Frost 1.1*) use two fields: the first indicates major updates affecting scores, and the second covers minor, non-impacting fixes and extensions. The IPD/IMGT HLA version denotes the source protein sequence and nomenclature. We will notify users of any major, score-impacting changes, compatibility details, and suggested validation procedures.

6. **Export Results:** By default, matching cases are **not** permanently stored on the TxPredictor™ server. If you want to keep the results of your match run, click the **Get PDF** button in the bottom right corner to generate and download a printer-friendly summary of the analysis. The PDF mirrors the layout of the results screen for easy cross-reference (fig. 29). You may save this PDF locally for internal laboratory documentation and to support your standard reporting procedures.

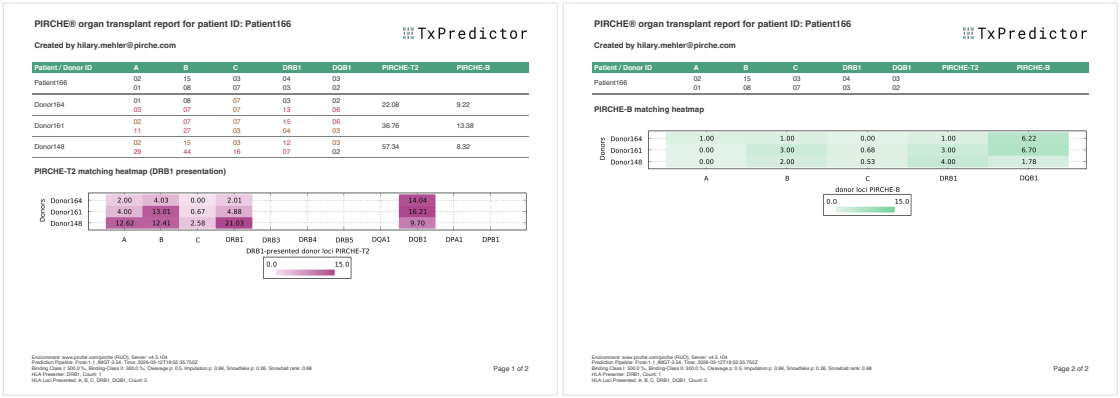


Figure 29: To permanently save your SOT match run's data, you can export it as a PDF.

7.2 Donor Allocation

The Donor Allocation module is designed to evaluate the immunological risk of several patient candidates simultaneously against a single donor (*i.e. one to many*), such as in kidney exchange or deceased donor allocation. This interface allows for the direct comparison of predicted indirect T cell epitope load across the selected group, providing a centralized view of the immunological compatibility for each patient-donor pair.

To start this module, select **SOT** → **Donor Allocation** from the Top Navigation Bar.

7.2.1 Input Screen

1. **Enter Patient Data:** In the top panel, enter the unique ID and HLA typing for one or more patients (fig. 30). See section 6.2 for input requirements and methods.

    - To add patients for manual entry, click the **+ Add a New Patient** button.

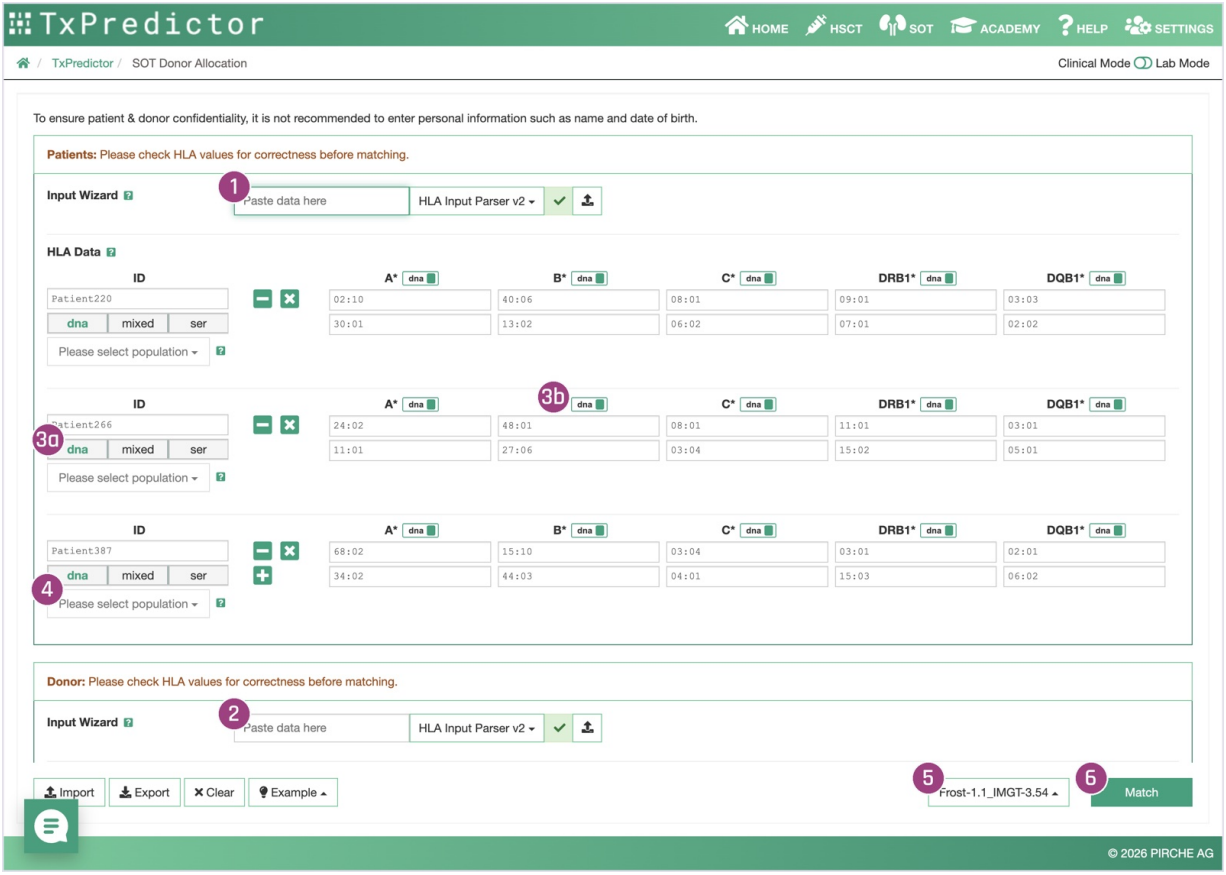


Figure 30: Verify patient and donor information, select the prediction pipeline, and click Match to submit the SOT Donor Allocation analysis.

- To add multiple patients simultaneously, paste a list of them into the **Input Wizard**. For all supported formats, each patient should be separated with a newline (figs. 19 and 30). The system will automatically parse the data and create an entry for each patient.

2. **Enter Donor Data:** In the bottom panel, input the donor's unique ID and HLA typing. See section 6.2 for input requirements and methods.

3. **Specify Typing Method:** *(Optional)* Use the DNA/SER toggles to define whether molecular (DNA) or serological (SER) nomenclature is provided (fig. 30). *Note:* This step is obsolete if protein-level typings are provided.
   (a) Select DNA or SER under the ID field to change all locus toggles for the patient/donor.
   (b) Adjust individual loci' toggles to handle mixed typing data.
4. **Verify Population:** *(Optional)* If using low-resolution data, verify the target population for imputation in the drop-down menu (figs. 21 and 30). *Note:* The corresponding population drop-down is automatically disabled if high-resolution typing is provided for all loci.
5. **Verify Prediction Pipeline:** Select the prediction pipeline to use for analysis. *Note:* The default selection for your institution may be adjusted under **Settings** → **Organization Settings** (section 6.1.4).
6. **Run Analysis:** Click the **Match** button at the bottom right. The results screen will load automatically.

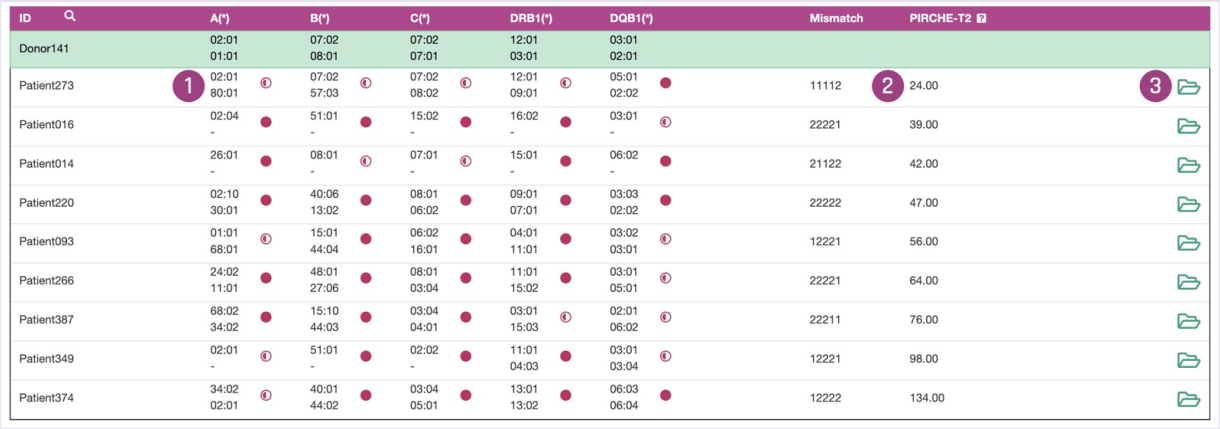
Expected Processing Time: With only a few individuals provided, the Donor Allocation module will respond within seconds. With highly increased numbers of provided patients (*e.g.* entire waiting lists) or highly ambiguous input HLA typings, the processing may increase.

7.2.2 Results Screen

The Results screen displays the molecular mismatch loads for each patient-donor pair. This view allows for the simultaneous review of multiple recipient candidates against a single donor profile. This section describes the components of the Donor Allocation Results screen (fig. 31):

1. **Results Summary Table:** At the top of the screen, the Results Summary Table provides a comparative overview of all patient-donor pairs (fig. 31). In this module, the donor is anchored in the top row, while recipient candidates are listed in the subsequent rows for direct comparison. Review the displayed input HLA typings for both the patients and the donor to verify that the initial data entry was accurate. *Note:* Mismatched HLAs are shown both visually and numerically:
   - **Circular Indicators:** Visualize the degree of mismatch at each locus. A *full circle* indicates two mismatches, a *half-full circle* one mismatch, and an *empty circle* a complete match.
   - **Numerical Summary:** A shorthand string (*e.g. 22211*) representing the number of mismatches per locus in the fixed order HLA-A, -B, -C, -DRB1, and -DQB1.

2. **PIRCHE Scores:** To the right of the Mismatch column (fig. 31), you will find the PIRCHE-T2 score for the patient-donor pair. This value represents the overall T-cell molecular mismatch load, specifically the number of unique donor-derived core peptides predicted to be presented by the recipient's HLA Class II molecules (section 3.5).



ID	A(*)	B(*)	C(*)	DRB1(*)	DQB1(*)	Mismatch	PIRCHE-T2	
Donor141	02:01 01:01	07:02 08:01	07:02 07:01	12:01 03:01	03:01 02:01			
Patient273	02:01 80:01	07:02 57:03	07:02 08:02	12:01 09:01	05:01 02:02	11112	24.00	
Patient016	02:04 -	51:01 -	15:02 -	16:02 -	03:01 -	22221	39.00	
Patient014	26:01 -	08:01 -	07:01 -	15:01 -	06:02 -	21122	42.00	
Patient220	02:10 30:01	40:06 13:02	08:01 06:02	09:01 07:01	03:03 02:02	22222	47.00	
Patient093	01:01 68:01	15:01 44:04	06:02 16:01	04:01 11:01	03:02 03:01	12221	56.00	
Patient266	24:02 11:01	48:01 27:06	08:01 03:04	11:01 15:02	03:01 05:01	22221	64.00	
Patient387	68:02 34:02	15:10 44:03	03:04 04:01	03:01 15:03	02:01 06:02	22211	76.00	
Patient349	02:01 -	51:01 -	02:02 -	11:01 04:03	03:01 03:04	12221	98.00	
Patient374	34:02 02:01	40:01 44:02	03:04 05:01	13:01 13:02	06:03 06:04	12222	134.00	

Figure 31: View patient and donor HLA typings, total PIRCHE scores, and expand details for a patient-donor pair.

3. **Expand Details:** *(Optional)* Click the folder icon at the end of a patient row to view the specific peptides driving the results. This section provides granular data for the specific patient-donor pair as described in section 7.1.2.

4. **Metadata:** Located at the bottom left of the screen, the metadata displays the exact calculation parameters applied to the current case. This information supports standard laboratory QA procedures, compliance audits, and troubleshooting by documenting:

    - **Calculation Parameters:** The specific prediction pipeline (algorithm and database versions) alongside the underlying algorithmic thresholds applied.
    - **System Information:** The environment (*e.g.* RUO), server version, and a precise timestamp.
    - **Evaluated Loci:** A summary of the specific presenter and presented HLA loci used for the calculation.

7.3 RAMP

Evaluate a candidate's *immunological profile* to determine the likelihood of finding a well-matched donor in a specific deceased donor population. RAMP features three main components:

- **Risk Profile:** Matches the patient's HLA typing against a reference donor population or an uploaded custom donor panel (*e.g.* the last 100 organ offers). This provides a statistical distribution of PIRCHE-T2 and PIRCHE-B scores for that candidate with respect to the donor pool.
- **Acceptable Mismatch Profile:** Analyzes individual alleles to identify the primary contributors to the overall PIRCHE-T2 and PIRCHE-B mismatch loads. Selecting alleles as *Unacceptable Mismatches* excludes the donors carrying the respective alleles from the Risk Profile, illustrating the resulting impact on the molecular mismatch load distribution among the remaining donor pool.
- **Predicted Memory T-Cell Epitopes:** Compares T-cell epitopes of selected *Immunizers* with HLA alleles to identify overlap in T-cell peptidomes, indicating potential risk of memory responses.

To start this module, select **SOT** → **RAMP** from the Top Navigation Bar.

7.3.1 Input Screen

1. **Select Donor Panel:** Under Risk Profile Settings, select the reference population or donor panel to be used for generating the patient's Risk Profile (figs. 32 and 33). *Note:* The default selection for your institution may be configured under **Settings** → **Organization Settings** (section 6.1.4).
2. **Enter Patient Data:** In the Patient section, enter the unique ID and HLA typing for the patient (fig. 33). See section 6.2 for input requirements and methods.
3. **Enter Donor Data:** *(Optional)* The Risk Profile may be generated with or without specific donors. To observe where specific individuals fall within the Risk Profile, click the **+ Add Donors** button to the far right of the Donor panel. This expands the Donor panel for HLA input (fig. 33, #3a). See section 6.2 for input requirements and methods. *Note:* When the Donor section is expanded a **- Remove Donors** button replaces the **+ Add Donors** button in the Donor heading.
    - To add additional donors for manual entry, click the **+ Add a New Donor** button located in the last donor row, positioned to the left of the HLA input fields.

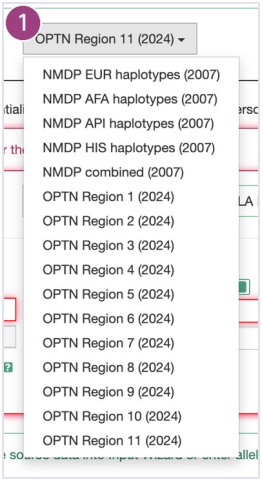


Figure 32: Select the Donor Panel to match the Patient with.

- To add multiple donors simultaneously while extracting their typing data, paste a list of typings into the **Input Wizard** (fig. 33, #3b). For all supported formats, each donor should be separated with a newline. The system will automatically parse the data and create an entry for each donor.

4. **Specify Typing Method:** *(Optional)* Use the DNA/SER toggles to define whether molecular (DNA) or serological (SER) nomenclature is reported.

   (a) Select DNA or SER under the ID field to change all locus toggles for the patient/donor.

   (b) Adjust individual locus toggles to handle mixed typing data.

5. **Verify Population:** *(Optional)* If using low-resolution data, verify the target population for imputation in the drop-down menu. *Note:* The population drop-down is automatically disabled if high-resolution typing is provided for all loci.

6. **Verify Prediction Pipeline:** Select the prediction pipeline to use for analysis (fig. 33). *Note:* The default selection for your institution may be adjusted under **Settings** → **Organization Settings** (section 6.1.4).

7. **Run Analysis:** Click the **RAMP** button at the bottom right (fig. 33). The results screen will load automatically.

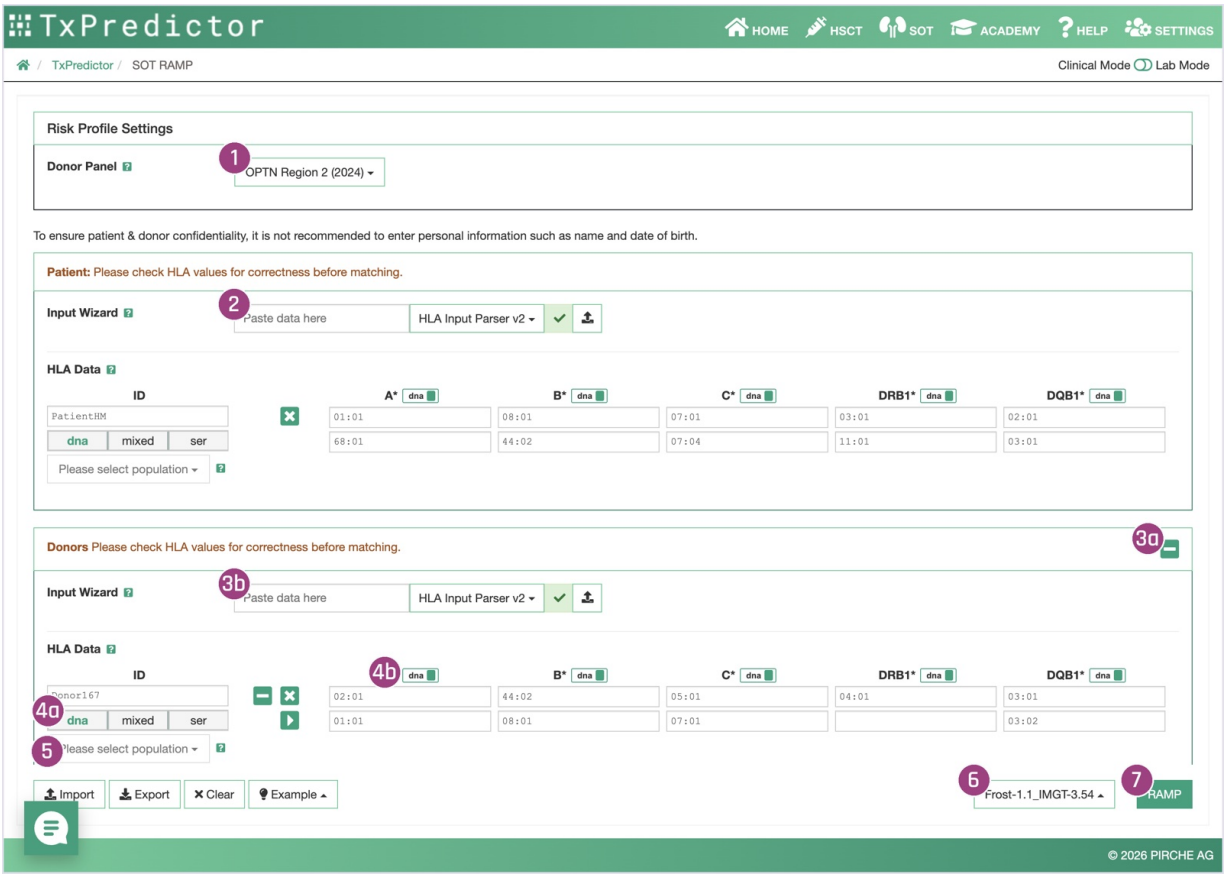


Figure 33: Verify Donor Panel, patient and donor HLA typings, and the prediction pipeline before clicking RAMP to submit the RAMP analysis.

Expected Processing Time: The RAMP module matches entire donor pools with the provided patient, which is computationally very complex. Particularly with ambiguous patient HLA typings, the processing may exceed one minute.

7.3.2 Results Summary and Export

The RAMP module utilizes the **Results Summary Table**, **Matching Heatmaps**, and **Export** functionality to provide a familiar workflow for analyzing and documenting *immunological risk*. These components, common with the *Donor Selection* module, are described in the following steps (fig. 34):

1. **Results Summary Table:** At the very top of the screen, the summary table anchors the patient in the first row, with all evaluated donors listed below (fig. 34). Review the displayed input HLA typings for both the patient and the donors to verify that the initial data entry was accurate. *Note:* Mismatched HLAs are highlighted.

2. **Results Summary Table:** To the right of the patient row are a few metrics derived from the Risk Profile:
   (a) **Median PIRCHE Scores**: The median scores of matching the patient genotype with all donors in the virtual donor pool indicate the scores at which half of the available donors have lower scores and the other half have higher scores. *Note:* This median is updated whenever the donor pool changes due to the selection of Unacceptable Antigens.
   (b) **cPRA**: The cPRA is calculated using the selected Unacceptable Antigens relative to the selected Donor Panel.

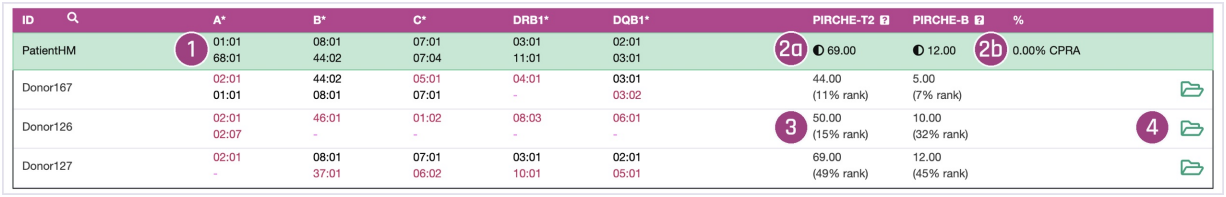


Figure 34: View patient and donor HLA typings, total PIRCHE scores, and expand details for a patient-donor pair.

3. **Total PIRCHE Scores:** As in Donor Selection, the PIRCHE-T2 and PIRCHE-B scores for the patient-donor pair are located to the right of the respective donor row. Below each score in parentheses is a percent rank, which indicates the percentage of virtual donors that have a lower score (fig. 34). This rank denotes the proportion of better matched donors.
   - **PIRCHE-T2:** The number of unique core peptides presented by HLA Class II molecules (section 3.5).
   - **PIRCHE-B:** The number of interlocus donor-specific amino acid mismatches with high protein-specific surface area and high protein-specific local protrusion rank (section 3.3).

4. **Expand Details:** *(Optional)* To visualize the specific mismatched epitopes driving the molecular mismatch scores, click the folder icon next to any donor row in the summary table. This expands the row to reveal a detailed data panel for the patient-donor pair as described in section 7.1.2.

5. **Matching Heatmap:** Directly below the summary table (fig. 34) are the Matching Heatmaps (fig. 35). Locus-specific scores are quantified and visualized here, where color intensity corresponds to higher values. This allows for a quick visual breakdown of the total score — highlighting, for example, if the cumulative scores are skewed towards specific HLA loci.

   A percent rank is displayed directly below each locus-specific score. This percentage indicates the proportion of donors in the selected donor pool that have a lower PIRCHE score for that specific locus. This allows for a quick assessment of whether a high score is unique to the current donor or typical for the patient across the entire population.

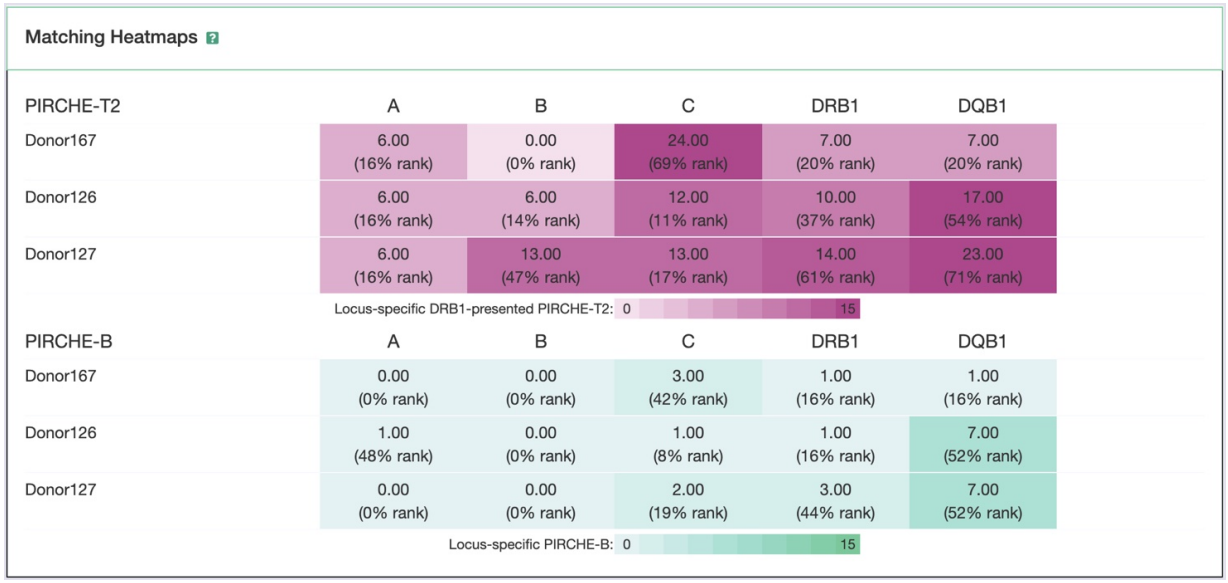


Figure 35: Matching Heatmaps visualize molecular mismatch load per HLA locus using a color gradient (purple for PIRCHE-T2, green for PIRCHE-B). Higher scores are represented by more intense colors.

6. **Metadata:** Located at the bottom left of the screen, the metadata displays the exact calculation parameters applied to the current case. This information supports standard laboratory QA procedures, compliance audits, and troubleshooting by documenting:

    - **Calculation Parameters:** The specific prediction pipeline (algorithm and database versions) alongside the underlying algorithmic thresholds applied.
    - **System Information:** The environment (*e.g.* RUO), server version, and a precise server timestamp.
    - **Evaluated Loci:** A summary of the specific presenter and presented HLA loci used for the calculation.

7. **Export Results:** By default, matching cases are **not** permanently stored on the TxPredictor™ server. If you want to keep the results of your RAMP match run – including the major charts –, click the **Get PDF** button in the bottom right corner to generate and download a printer-friendly summary of the analysis. The PDF mirrors the layout of the results screen for easy cross-referencing (fig. 36). You may save this PDF locally for internal laboratory documentation and to support your standard reporting procedures. *Note:* The RAMP PDF reflects the entire donor pool; it reflects neither selection of Unacceptable Mismatches nor Previous Immunizers.

7.3.3 Risk Profile

TxPredictor™ RAMP's Risk Profile provides a dynamic visualization of PIRCHE-T2 and PIRCHE-B scores within a donor pool. These graphs allow you to see the distribution of scores across the donor panel, providing essential context for how frequently a patient is expected to be well-matched and guiding how a specific patient-donor pair compares to *e.g.* a deceased donor pool. All visualizations update in real-time based on the currently selected Unacceptable Mismatches. Users can toggle between three chart types using the drop-down menu to the right.

1. **Scatter Chart** The scatter chart illustrates the relationship between PIRCHE-T2 and PIRCHE-B scores for the entire donor pool, including the provided donor candidates (figure 37a).

    - **PIRCHE-T2 (X-Axis):** Represents the T-cell mismatch load (section 3.5), increasing from left to right.
    - **PIRCHE-B (Y-Axis):** Represents the B-cell mismatch load (section 3.3), increasing from bottom to top.

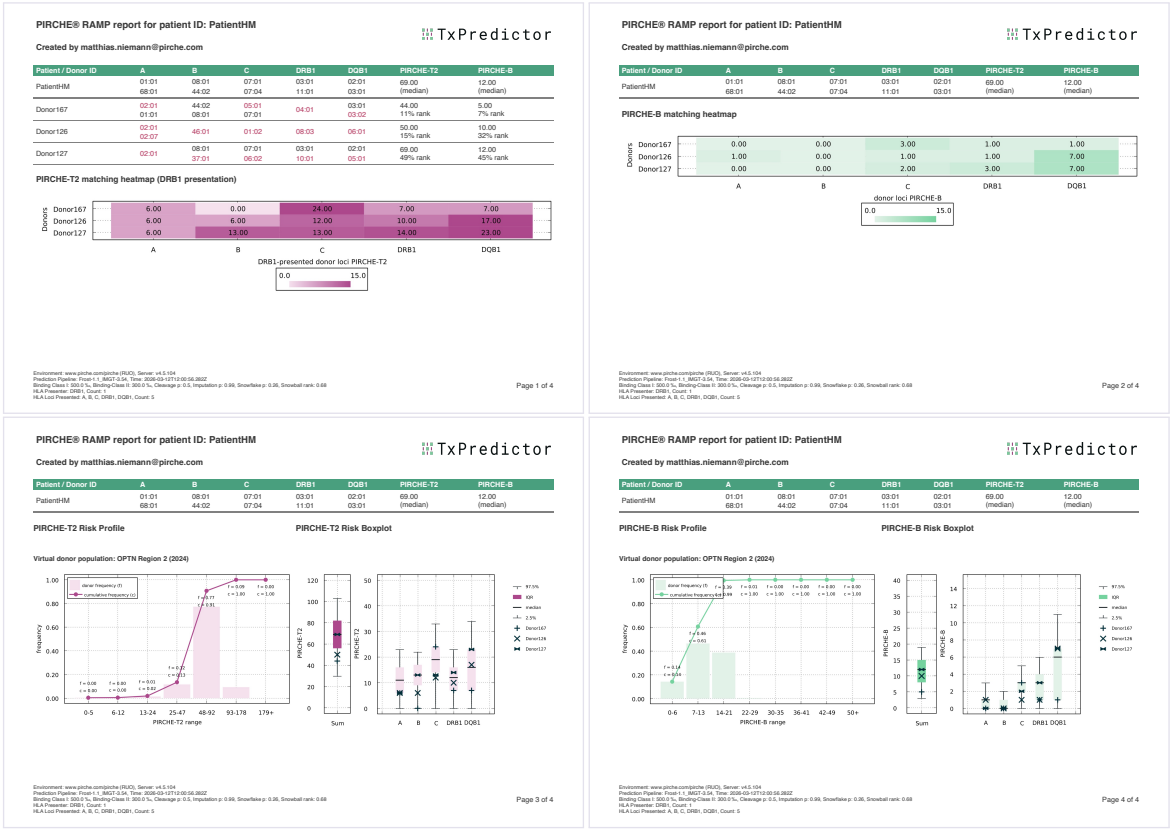
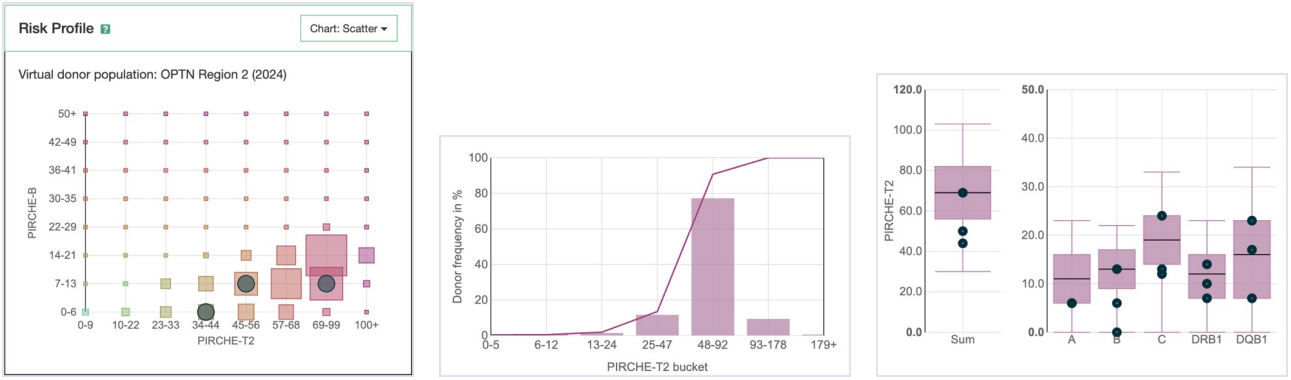


Figure 36: To permanently save your SOT RAMP match run's data, you can export it as a PDF.

- **Risk Buckets:** Data is grouped into boxes representing specific score ranges denoted by the respective coordinate. The size of the box is proportional to the frequency of donors that fall within the respective score ranges. *Note:* You can hover over a risk bucket to see exact numerical ranges and donor frequency.

- **Donor Mapping:** If specific donors are included in the analysis, each donor is depicted as a circle within its corresponding risk bucket. *Note:* Overlapping donors may not be individually depicted. You can hover over a donor circle to view the specific scores for that patient-donor pair.



(a) Scatter Plot (b) Histogram (c) Boxplot

Figure 37: RAMP's population analysis allows comparison of score correlation (a), frequency distribution (b), and locus variance (c). Examples show only PIRCHE-T2 visuals, yet TxPredictor™ includes charts for both PIRCHE-T2 and PIRCHE-B.

2. **Histograms** The histograms provide the donor frequency per risk bucket and a cumulative distribution function indicating the summed frequency of donors with the current or lower risk bucket's molecular mismatch load (figure 37b).

    - **Risk Buckets:** Each bar represents a "bin" of donors. The height of the bar is proportional to the bin's cumulative donor frequency. *Note:* You can hover over a bar to see the exact range of scores and the specific donor frequency.
    - **Accumulated Frequency:** The trend line represents the sum of the frequencies of the current bin and all preceding bins (*i.e.* cumulative distribution function). This allows you to see what percentage of the population falls at or below a certain score. *Note:* You can hover over the accumulated frequency line to view the cumulative numeric details.
    - **Configuration Options:** Drop-down menus located above the histograms allow for data refinement:
        - *Scale*: Adjust the ranges for molecular mismatch load risk buckets.
        - *Locus*: Select a locus to display score distributions exclusively for that particular locus.

3. **Boxplots:** The boxplots of score distributions are shown for total scores and per locus simultaneously (figure 37c).

- **Donor Mapping**: If specific donors are included in the analysis, their scores are represented by dots overlaid on the distribution. *Note:* Hover over a dot to see the specific value for that patient-donor pair.
- **Statistical Markers**: The boxplot provides a visual summary of the score distribution across the donor panel.
  - *Median:* The bold line separating the box represents the 50th percentile. Half of the donors fall below this score, and half of them have greater scores.
  - *Quartiles (The Box):* The lower edge represents the 25th percentile (Q1) and the upper edge represents the 75th percentile (Q3). This "box" contains the middle 50% of the donor population.
  - *Whiskers:* The vertical lines extend to the 2.5th percentile (bottom) and the 97.5th percentile (top).
  - *Outliers:* Any donors outside the score range of 2.5% to 97.5% of the population are considered statistical outliers and omitted.
  - *Note:* Hover over a box to see exact values for the median, quartiles, and whiskers.

7.3.4 Acceptable Mismatch Profile

The TxPredictor™ Acceptable Mismatch Bar Charts provide PIRCHE scores and allele frequencies of individual alleles. The alleles included are based on presence within the selected donor panel and common single-antigen bead (SAB) panels. This component allows a deep dive into molecular mismatch load induced by individual alleles, allele frequencies, and antibody data through imported data of SAB panels. By interacting with individual bars, users can assign *Unacceptable Mismatches* or evaluate T-cell epitope overlap (*i.e.* Tmem) by designating *Previous Immunizers*.

1. **General Chart Features**

   - **Dynamic Updates to Risk Profile:** Any change to the *Unacceptable Mismatch* list triggers an immediate recalculation of the patient's cPRA, median PIRCHE scores, ranks, and all associated population charts.
   - **Incorporate Antibody Data:** MFI values from SAB assays can be loaded into the chart using the Load MFI button at the bottom of the screen. In the case of heterodimer antibodies, the chart displays the maximum MFI of all bead combinations for that chain (*i.e.* pessimistic aggregation).

- **Axes and Limits:** The y-axis is standardized to a maximum MFI of 5000, a PIRCHE-T2 value of 30, a PIRCHE-B value of 60, and an allele frequency of 50% to ensure visual consistency across different patients. While PIRCHE-T2 and MFI will appear above the center line, PIRCHE-B, Tmem and allele frequency will be stacked underneath the center line. *Note:* Hover over any bar to view a detailed tooltip containing: PIRCHE scores, allele frequency in the donor pool, Tmem, and normalized MFI values.

- **Configuration Options:**

  - *Sorting:* Use the drop-down above the chart to sort alleles by specific metrics (*e.g.* allele name, MFI, or Tmem).

  - *Automatic Unacceptable Selection:* The left-hand drop-down allows for quick automatic exclusion of antigens based on predefined MFI or PIRCHE-T2 cutoffs.

  - *Bar Mode*: At the top left of each chart, switch between two visualization modes:

    * *Stacked: (Default)* View metrics stacked on top of each other.

    * *Grouped:* View individual bars positioned side by side.

2. **Unacceptable Antigens** Evaluate the impact of a patient's Unacceptable Mismatches on the overall Risk Profile. This feature allows you to visualize the changing molecular mismatch load distribution depending on selected unacceptable antigens, as this may increase or decrease average mismatch load. By **Left Clicking** a bar, its corresponding allele is assigned as an Unacceptable Mismatch (fig. 38).

   - **De-selecting:** The allele is added to the *Assigned Unacceptable Mismatches* list on the right. Clicking the bar a second time removes it from the list (fig. 38).

   - **Donor Panel Filtering:** All virtual donors carrying the selected allele are excluded from the analysis.

   - **Instant Updates:** The cPRA, median PIRCHE scores, ranks, and all Risk Profile charts update to reflect the immunological landscape of the remaining donor population.

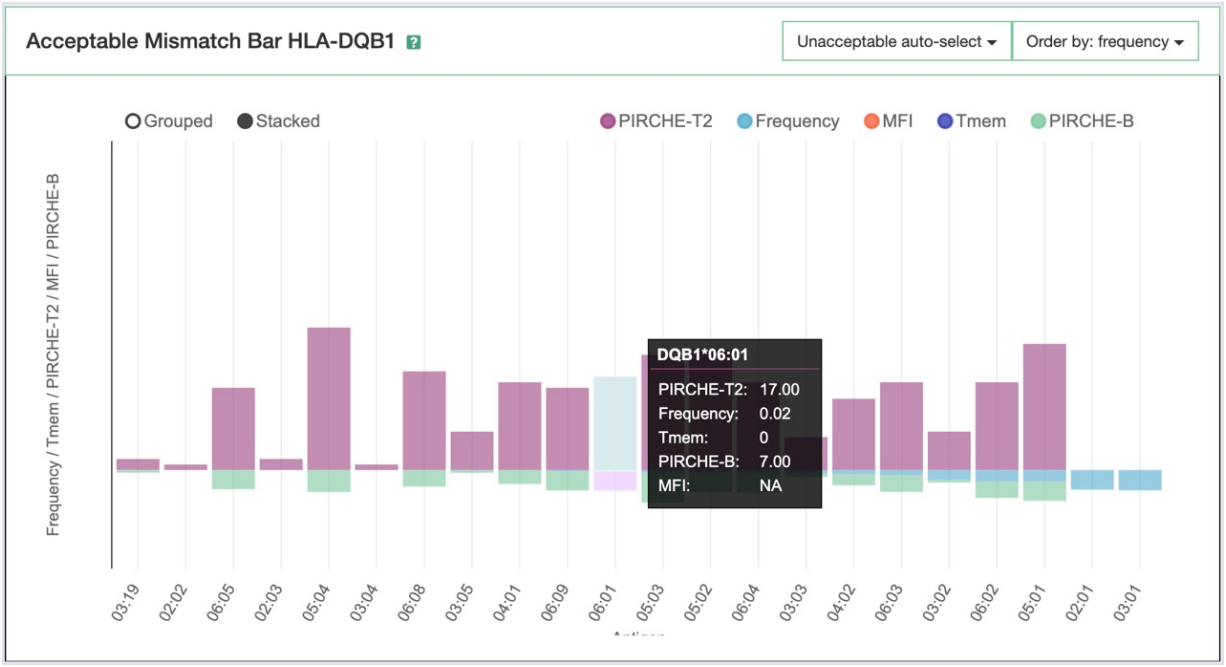


Figure 38: Presents allele-specific contribution to molecular mismatch load.

7.3.5 Tmem

The Tmem component calculates the number of overlapping peptides shared between selected previous immunizers and all other alleles. Elevated Tmem scores were shown to correlate with early immune response (section 4.1.5).

Right Click a bar in the Acceptable Mismatch Bar Charts to designate the corresponding allele as a *Previous Immunizer*. To visually distinguish this bar, it will obtain a striped overlay (fig. 39).

- **De-selecting:** Right click the bar a second time to remove it from the list of Previous Immunizers.
- **Tmem Analysis:** Purple bars will extend downward from the baseline, indicating the number of overlapping T-cell epitopes. *Note:* The bar will be capped at a Tmem load of 5.0.

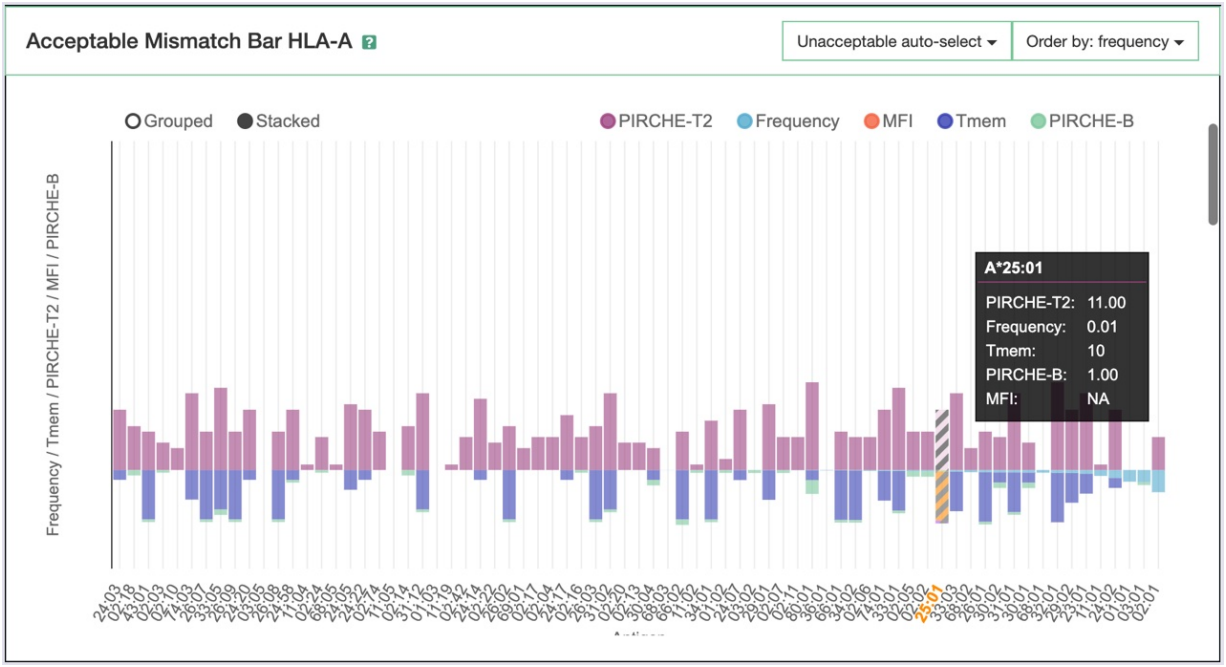


Figure 39: Avoid re-exposure to T-cell epitopes based on previous sensitization.

Interlocus Overlap: Overlapping peptides are not limited to the same locus; an HLA-A immunizer can reveal shared T-cell epitopes with alleles at *e.g.* HLA-B.

7.3.6 Custom Populations

Risk profiles are dependent on the considered donor pool. To reflect the diversity of real-world datasets, we have recently introduced Organ Procurement and Transplantation Network (OPTN) region-specific donor populations based on the 2024 United Network for Organ Sharing (UNOS) dataset. To further refine this, TxPredictor™ also supports upload of a center's own donor population data (fig. 40), which can be selected for PIRCHE® RAMP analyses.

To upload your own population, select **Settings** → **Populations** from the Top Navigation Bar.

1. Enter a name for the population. This name appears in the population selection menu in RAMP.
2. Click the **Add...** button located in the last row under **Content File**. A file selection dialog will open, prompting you to select the input file.
3. Resolution settings:
    - Toggle the arrow to switch between high-resolution and low-resolution.
    - When low-resolution is selected, a drop-down menu appears to the left of the **Add...** button. This menu is used to specify the reference population for HLA imputation.
4. Click the **+ Add Population** button to finalize the upload. Upon successful upload, allele frequency details are shown for verification.

Input File Structure: Custom populations must be provided as a standard Comma-Separated Values (CSV) file. Each row represents an individual donor genotype, with specific columns designated for each HLA locus. Alleles must be encoded using standard nomenclature, including the locus prefix and an asterisk separator (*e.g. A*01:01* or *B*44*).

Note: TxPredictor™ supports both high-resolution and low-resolution data strings. If low-resolution HLA typings are provided, the integrated multiple imputation algorithm (section 3.7) estimates high-resolution genotype frequencies for the custom population.

Example:

```
po98,A*03:01,A*01:01,B*51:01,B*37:01,C*15:02,C*06:02,DRB1*11:01,DRB1*01:01,DQB1*03:01,DQB1*05:01
fd24,A*11:01,A*68:02,B*53:01,B*14:02,C*04:01,C*08:02,DRB1*13:02,DRB1*01:02,DQB1*06:04,DQB1*05:01
aa44,A*29:02,A*68:01,B*44:03,B*18:01,C*16:01,C*05:01,DRB1*07:01,DRB1*03:01,DQB1*02:01,DQB1*05:01
```

Organization Access: Uploaded Custom Populations are available to all users of the same organization.

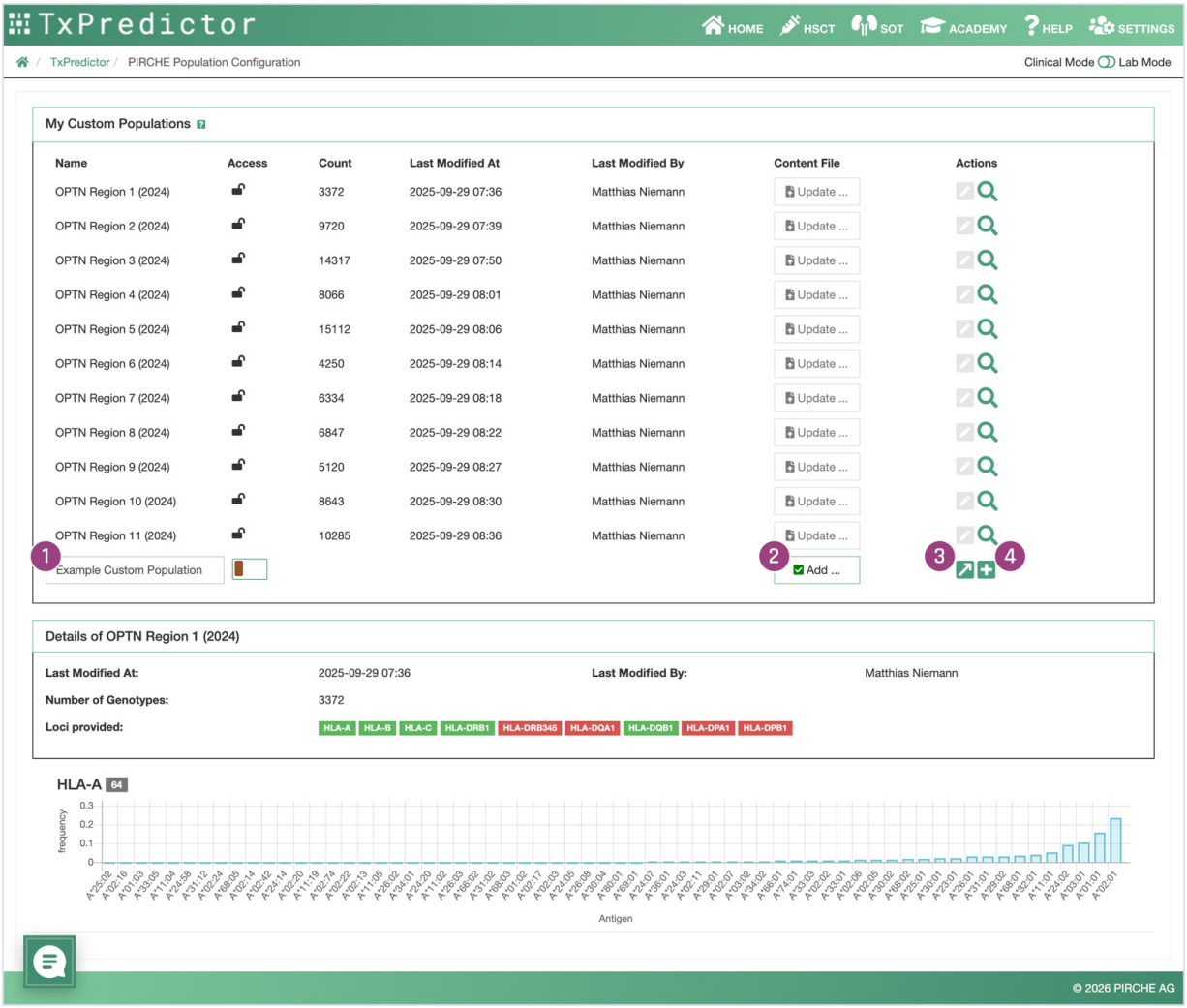


Figure 40: Upload a Custom Population to tailor Risk Profiles to reflect local demographics or institutional cohorts.

8 Running Analyses: Stem Cell Transplant Core Modules

8.1 Donor Selection

This module is designed to evaluate the *immunological risk* of specific hematopoietic stem cell transplantation (HSCT) donor candidates for a given recipient (*i.e.* *many to one*). This analysis may support prioritizing between multiple candidates during a registry search, such as comparing several 10/10 or 9/10 mismatched unrelated donors (MMUDs).

To start this module, select **HSCT** → **Donor Selection** from the Top Navigation Bar.

8.1.1 Input Screen

1. **Enter Patient Data:** In the top panel, input the recipient's unique ID and high-resolution HLA typing (fig. 42). See section 6.2 for input requirements and methods.

2. **Enter Donor Data:** In the bottom panel, enter the unique IDs and high-resolution HLA typings for one or more donors (fig. 42). See section 6.2 for input requirements and methods.

    - To add more donors for manual entry, click the **+ Add a New Donor** button (fig. 41).
    - To add multiple donors simultaneously while extracting their HLA typing data, paste a list of typings into the **Input Wizard** (figs. 23 and 42). For all supported formats, each donor should be separated with a newline. The system will automatically parse the data and create an entry for each donor.
    - For donors with very similar HLA typings to the patient (*e.g.* 9/10, 11/12), the **Copy Patient Typing** button can quickly copy over the patient typing into a donor row. This minimizes manual entry, requiring only minor adjustments to specific HLA alleles (fig. 41).

3. **Global DPB1 Removal:** *(Optional)* Imported donor HLA typing datasets frequently exhibit inconsistencies regarding the availability of the DPB1 locus. In contexts such as 9/10 match analyses or when managing incomplete DPB1 profiles (*e.g.* an unavailable patient DPB1 typing), batch-adjusting all typings simultaneously ensures analytical consistency. Clicking the Unlink button adjacent to the patient's DPB1 label clears the DPB1 data fields for all individuals in the case.

4. **Verify Prediction Pipeline:** Select the prediction pipeline to use for analysis (fig. 42). *Note:* The default selection for your institution may be adjusted under **Settings** → **Organization Settings** (section 6.1.4).

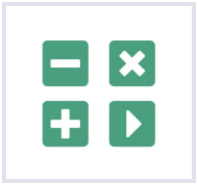


Figure 41: This button group allows you to remove donor rows (-), clear input fields of an individual (x), add donor or patient rows (+) and copy over the recipient typing (→). Buttons disappear if they do not apply in the specific context or module.

5. **Run Analysis:** Click the **Match** button at the bottom right (fig. 42). The results screen will load automatically.

Maintain Workflow Consistency: PIRCHE scores are not normalized, which typically yields higher molecular mismatch loads when more loci are provided. Valid risk stratification requires a consistent workflow of loci to be provided. A 5-loci analysis is not comparable to 6- or 11-loci analyses.

Recipient/Donor Consistency: To avoid inconsistencies in the algorithms, TxPredictor™ enforces providing the same HLA loci for all recipients and donors within a case.

Expected Processing Time: With only a few Donors provided, the HSCT Donor Selection module will respond within seconds. With highly increased numbers of provided donors (*e.g.* partially matched registry pools), the processing time may increase.

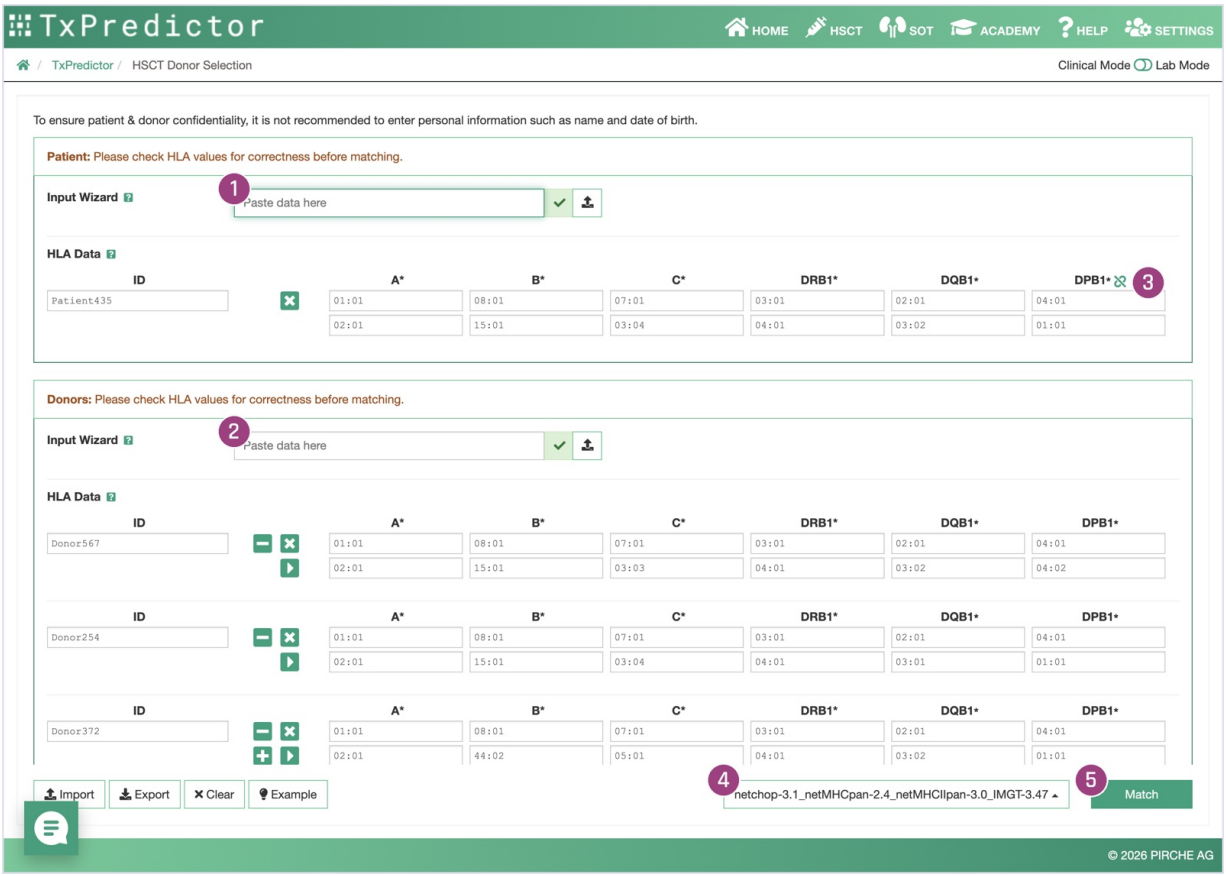


Figure 42: Verify patient and donor information, select the prediction pipeline, and click Match to submit the HSCT Donor Selection analysis.

8.1.2 Results Screen

The Results screen provides a comprehensive overview of the molecular mismatch loads for each patient-donor pair. The following steps walk through the primary components of the HSCT Donor Selection Results screen (fig. 43):

1. **Results Summary Table:** At the very top of the screen, the summary table anchors the patient in the first row, with all evaluated donors listed below. Review the displayed input HLA typings and IDs for both the patient and the donors to verify that the initial data entry was accurate. *Note:* Mismatched HLAs are highlighted in donor typings.

2. **PIRCHE Scores:** To the right of each donor row, you will find the PIRCHE-T1 and PIRCHE-T2 scores for the patient-donor pair (section 3.5). These values represent the overall molecular mismatch load.

    - **PIRCHE-T1:** The number of unique core pHLA presented by shared HLA Class I molecules.
    - **PIRCHE-T2:** The number of unique core pHLA presented by shared HLA Class II molecules.

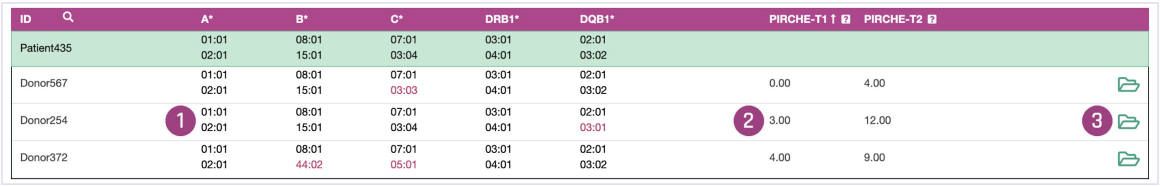


Figure 43: View patient and donor HLA typings, total PIRCHE scores, and expand details for a patient-donor pair.

3. **Expand Details:** *(Optional)* To show the specific mismatched epitopes driving the molecular mismatch scores, click the folder icon next to any donor row in the summary table. This expands the row to reveal a detailed data panel for the patient-donor pair, including:

    - **Presentation Details:** This panel provides a table with a detailed breakdown of the peptides (T-cell epitopes) driving the PIRCHE-T1 and PIRCHE-T2 scores. (fig. 44).
        - *Presenter Allele/Dimer:* The HLA molecule responsible for presenting peptides to T-cells. Class I presenters contribute to the total PIRCHE-T1 score while Class II presenters contribute to the total PIRCHE-T2 score.
        - *Presented Allele:* The mismatched recipient HLA allele from which the presented peptides are derived. The count below the allele indicates the number of derived distinct core peptides.
        - *Core Peptide:* The peptide core sequence (typically a 9-mer) that is predicted to bind within the HLA presenter's binding groove.

– *Peptide:* The full-length peptide predicted to bind. If multiple peptides share the exact same core peptide, TxPredictor™ counts and highlights only one: the peptide with the highest probability of binding (lowest binding rank) is shown in black text with a star icon on the far right, whilst duplicates are grayed out.

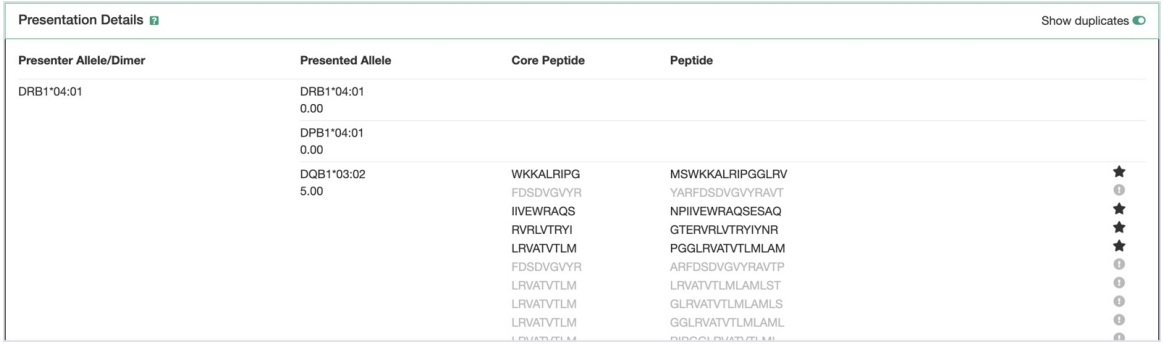


Figure 44: T-cell epitope presentation details are provided in a table, showing presented pHLA grouped by the presenting HLA molecules and the mismatched patient alleles from which they originate.

4. **Metadata:** Located at the bottom left of the screen, the metadata displays the exact calculation parameters applied to the current case (fig. 45). This information supports standard laboratory QA procedures, compliance audits, and troubleshooting by documenting:
   - **Calculation Parameters:** The specific prediction pipeline (algorithm and database versions) alongside the underlying algorithmic thresholds applied.
   - **System Information:** The environment (*e.g.* RUO), server version, and a precise server timestamp.
   - **Evaluated Loci:** A summary of the specific presenter and presented HLA loci used for the calculation.

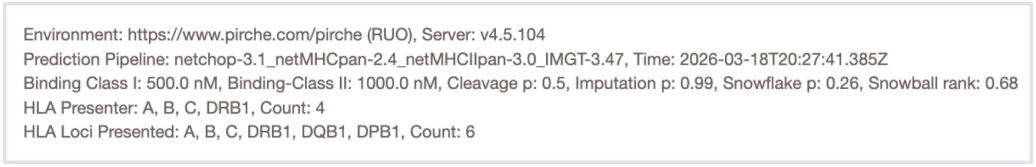
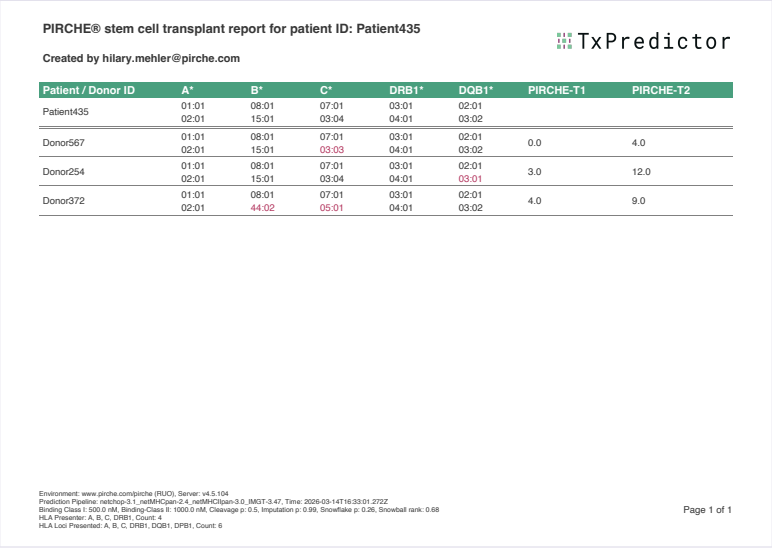


Figure 45: Example of the Metadata provided on the HSCT result screens, providing a comprehensive summary of calculation parameters, server versions, and evaluation criteria.

Version numbers: TxPredictor™ server versions use a three-part format (*e.g. 4.5.104*). The first two fields (major platform and matching algorithm versions) can impact match result calculations, while the third introduces non-impacting features or fixes. Similarly, predictor versions (*e.g. Frost 1.1*) use two fields: the first indicates major updates affecting scores, and the second covers minor, non-impacting fixes and extensions. The IPD/IMGT HLA version denotes the source protein sequence and nomenclature. We will notify users of any major, score-impacting changes, compatibility details, and suggested validation procedures.

5. **Export Results:** By default, matching cases are **not** permanently stored on the TxPredictor™ server. If you want to keep the results of your match run, click the **Get PDF** button in the bottom right corner to generate and download a printer-friendly summary of the analysis. The PDF mirrors the layout of the results screen for easy cross-reference (fig. 46). You may save this PDF locally for internal laboratory documentation and to support your standard reporting procedures.



PIRCHE® stem cell transplant report for patient ID: Patient435

TxPredictor

Created by hilary.mehler@pirche.com

Patient / Donor ID	A*	B*	C*	DRB1*	DQB1*	PIRCHE-T1	PIRCHE-T2
Patient435	01:01 02:01	08:01 15:01	07:01 03:04	03:01 04:01	02:01 03:02		
Donor567	01:01 02:01	08:01 15:01	07:01 03:03	03:01 04:01	02:01 03:02	0.0	4.0
Donor254	01:01 02:01	08:01 15:01	07:01 03:04	03:01 04:01	02:01 03:01	3.0	12.0
Donor372	01:01 02:01	08:01 44:02	07:01 05:01	03:01 04:01	02:01 03:02	4.0	9.0

Environment: www.pirche.com/pirche (RUO), Server: v4.5.104
Prediction Pipeline: netchop-3.1_netMHCpan-2.4_netMHCIIpan-3.0_IMGT-3.47, Time: 2026-03-14T16:33:01.272Z
Binding Class I: 500.0 nM, Binding Class II: 1000.0 nM, Cleavage p: 0.5, Imputation p: 0.99, Snowflake p: 0.26, Snowball rank: 0.68
HLA Presenter: A, B, C, DRB1, Count: 4
HLA Loci Presented: A, B, C, DRB1, DQB1, DPB1, Count: 6

Page 1 of 1

Figure 46: To permanently save your HSCT match run's data, you can export it as a PDF.

8.2 HSCT Search Profile

This module is designed to screen a patient's genotype against a virtual population of potential stem cell donors to assess the availability of immunologically favorable matches. The analysis generates a frequency profile of 9/10-matched donors based on haplotype frequencies, calculating the PIRCHE scores for each potential patient-donor pair. In addition to evaluating a theoretic donor landscape, the module facilitates the identification of allelic mismatches that minimize alloreactive risk when a 10/10 match is unavailable. A custom population (*e.g.* registry donor pool) may be uploaded as described in section 7.3.6.

To start this module, select **HSCT** → **Search Profile** from the Top Navigation Bar.

8.2.1 Input Screen

1. **Select Donor Population:** Under Settings, select the reference population to be used to generate the virtual donor panel to screen the patient against (fig. 47). *Note:* The default selection for your institution may be configured under **Settings** → **Organization Settings** (section 6.1.4).

2. **Enter Patient Data:** In the Patient section, enter the unique ID and high-resolution HLA typing for the patient (fig. 47). See section 6.2 for input requirements and methods.

3. **Verify Prediction Pipeline:** Select the prediction pipeline to use for analysis (fig. 47). *Note:* The default selection for your institution may be adjusted under **Settings** → **Organization Settings** (section 6.1.4).

4. **Run Analysis:** Click the **Search Profile** button at the bottom right (fig. 47). The results screen will load automatically.

Expected Processing Time: Given that the HSCT Search Profile module matches entire donor pools, processing time is increased compared to the HSCT Donor Selection module; however, response times typically remain under one minute.

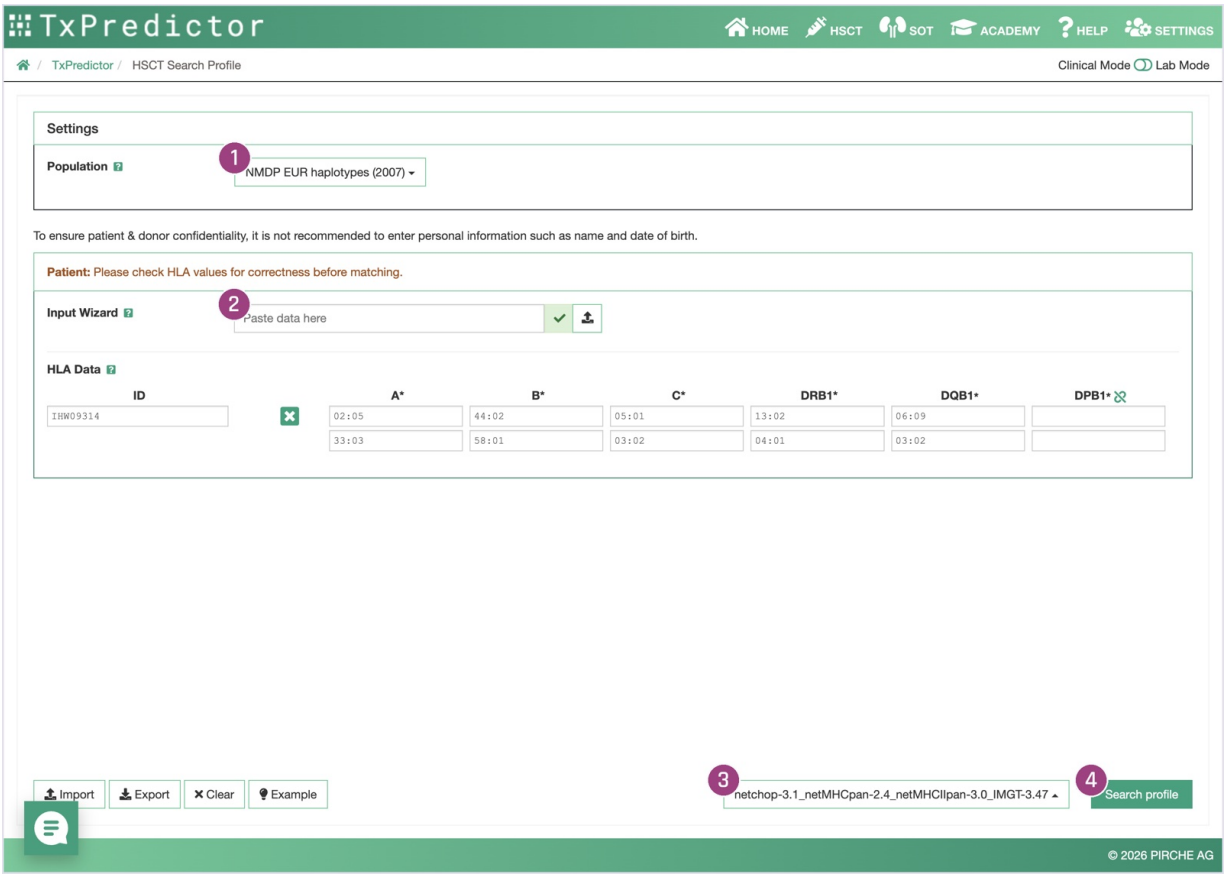


Figure 47: Verify Population, patient HLA typings, and the prediction pipeline before clicking Search Profile to submit analysis

8.2.2 Results Screen

The Search Profile Results screen provides a statistical projection of the virtual donor registry, filtered by the system to show only potential 9/10-matched genotypes. The following steps walk through the primary components of the results interface (fig. 48):

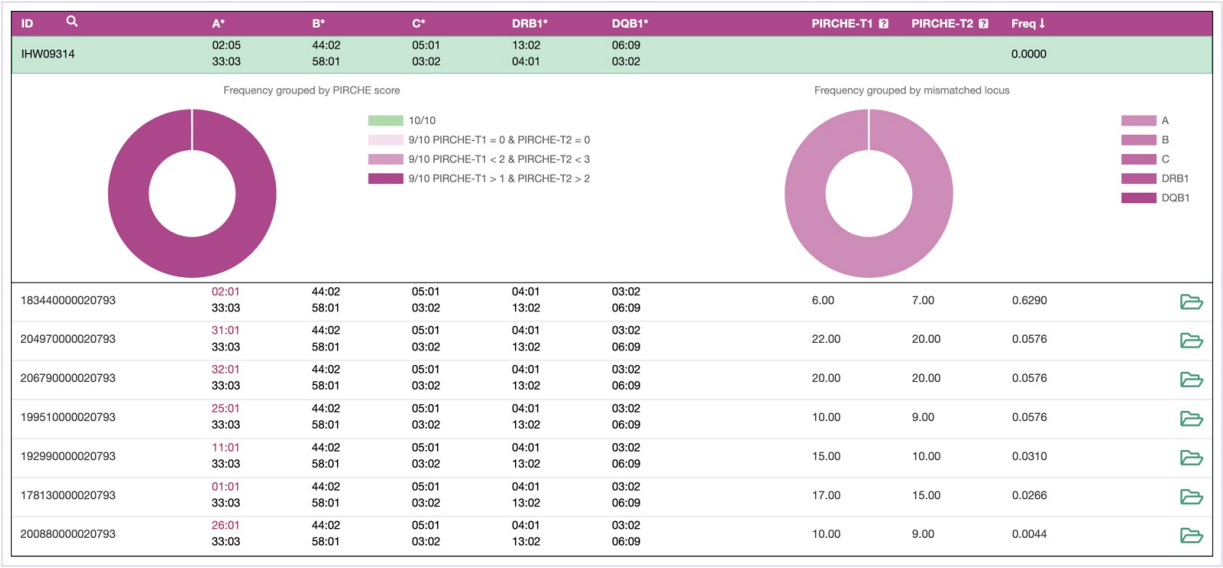
1. **Donor Frequency Charts:** Situated below the patient header, two doughnut charts visualize the cumulative frequency of potential 9/10-matched donors. The chart to the left categorizes donors by PIRCHE scores (section 3.5), while the chart to the right groups by the mismatched HLA locus. Interactive legends allow for the dynamic exclusion of specific categories.

2. **Donor List (9/10 Matches):** Donors are listed individually. The interactive header at the very top allows you to exclude mismatches per locus and sort by PIRCHE scores or frequency. Each row contains, from left to right:

    - **HLA Typing:** The HLA typing is displayed with the mismatched allele colored with red font.
    - **PIRCHE-T1:** The number of unique core peptides presented by shared HLA Class I molecules.
    - **PIRCHE-T2:** The number of unique core peptides presented by shared HLA Class II molecules.
    - **Frequency:** The frequency of the specific genotype appearing per one million individuals in the virtual population. *Note:* The frequency displayed in the patient header represents the probability of identifying a 10/10-matched donor.
    - **Expand Details:** *(Optional)* To visualize the specific mismatched epitopes driving the molecular mismatch scores, click the Folder button next to any donor row in the summary table. This expands the row to reveal a detailed data panel for the patient-donor pair as described in section 8.1.2.

3. **Metadata:** Located at the bottom left of the screen, the metadata displays the exact calculation parameters applied to the current case. This information supports standard laboratory QA procedures, compliance audits, and troubleshooting by documenting:

    - **Calculation Parameters:** The specific prediction pipeline (algorithm and database versions) alongside the underlying algorithmic thresholds applied.
    - **System Information:** The environment (*e.g.* RUO), server version, and a precise server timestamp.
    - **Evaluated Loci:** A summary of the specific presenter and presented HLA loci used for the calculation.



ID	A*	B*	C*	DRB1*	DQB1*	PIRCHE-T1	PIRCHE-T2	Freq ↓
IHW09314	02:05 33:03	44:02 58:01	05:01 03:02	13:02 04:01	06:09 03:02			0.0000
183440000020793	02:01 33:03	44:02 58:01	05:01 03:02	04:01 13:02	03:02 06:09	6.00	7.00	0.6290
204970000020793	31:01 33:03	44:02 58:01	05:01 03:02	04:01 13:02	03:02 06:09	22.00	20.00	0.0576
206790000020793	32:01 33:03	44:02 58:01	05:01 03:02	04:01 13:02	03:02 06:09	20.00	20.00	0.0576
199510000020793	25:01 33:03	44:02 58:01	05:01 03:02	04:01 13:02	03:02 06:09	10.00	9.00	0.0576
192990000020793	11:01 33:03	44:02 58:01	05:01 03:02	04:01 13:02	03:02 06:09	15.00	10.00	0.0310
178130000020793	01:01 33:03	44:02 58:01	05:01 03:02	04:01 13:02	03:02 06:09	17.00	15.00	0.0266
200880000020793	26:01 33:03	44:02 58:01	05:01 03:02	04:01 13:02	03:02 06:09	10.00	9.00	0.0044

Figure 48: Visualize genotype frequencies by total PIRCHE score and mismatched locus. A complete list of 9/10-matched genotypes is provided below.

Version numbers: TxPredictor™ server versions use a three-part format (*e.g. 4.5.104*). The first two fields (major platform and matching algorithm versions) can impact match result calculations, while the third introduces non-impacting features or fixes. Similarly, predictor versions (*e.g. Frost 1.1*) use two fields: the first indicates major updates affecting scores, and the second covers minor, non-impacting fixes and extensions. The IPD/IMGT HLA version denotes the source protein sequence and nomenclature. We will notify users of any major, score-impacting changes, compatibility details, and suggested validation procedures.

9 Batch Matching

To support analysis of large cohorts (*e.g.* for a research study or retrospective analysis), there are dedicated Batch Matching modules. These are designed for high-throughput processing (*i.e.* *many to many*), allowing you to query datasets for evaluation of PIRCHE-T1, PIRCHE-T2, PIRCHE-B, Risk Profile, and Tmem. This section details the technical requirements for the Batch Analysis modules.

To start these modules, open **SOT** or **HSCT** (depending on application) from the Top Navigation Bar, select **Batch Modules (CSV)** and select the desired module from the sub-menu.

Input and Examples: The module utilizes a CSV-formatted string input. To streamline file preparation, use the Example button located at the bottom left of the interface. This provides a template for the selected parser (Input Parser v2 is the current standard; the legacy Input Parser v1 is slated for deprecation). It serves as a structural guide for aligning raw data with the required input format and can be processed immediately to evaluate the expected output.

General Formatting Rules: To ensure the parser correctly identifies patients and donors, adhere to the following structure:

- **Case Separation:** Each unique case (one patient and multiple potential donors) must be separated by a line containing only a comma. Do not include a comma after the final case in the dataset.
- **Hierarchy:** Within a case, the first row is considered to be the Patient, followed by one or more Donors on subsequent lines.
- **ID Requirements:** Unique IDs are required for **every** individual. Duplicate IDs within a single batch will prevent the request from being processed.
- **Locus Prefix:** The locus must be included to fully define the allele (*e.g.* *A*02:01*). The order of alleles on a line **does not** matter.
- **Loci Consistency:** Because PIRCHE scores are cumulative and not normalized, it is highly recommended that all individuals have the same configuration of provided loci within a dataset. *Note:* The Batch Matching modules **will not** check for consistency.

Loci Requirements and Customizing Imputation: TxPredictor™ enforces a specific set of loci as a minimum requirement. See section 6.2 for required loci, imputation of missing loci, and loci not considered covered by imputation. HSCT modules strictly require high-resolution inputs (*i.e.* at least 2-field). Conversely, in SOT

modules, a reference population for imputation can be selected globally in the Imputation Settings panel at the top of the screen. An override population can be defined per individual, which is used instead of the reference population. To specify a population for a specific individual, append #[ID] to the end of the typing string. Population IDs can be looked up in the Help box of the Imputation Settings.
Note: If an incorrect population ID is provided, TxPredictor™'s imputation may fail for the affected individual without a system alert. In such instances, the specific patient-donor pair is omitted from the final results to allow the remainder of the batch to proceed without interruption.

Example: `ptA,A*01:01,A*03:01,B*17,B*35...DQB1*05:01#11` *(where 11 is the population ID)*

Serology Nomenclature: To resolve ambiguities between molecular and serology inputs not distinguished by the previous parser, a new default input data parser was introduced:

- **Parser v2 (Current):** Serologic antigen data should be entered directly (*e.g. DQ7* or *DR11*). Use this parser for all new projects. *(Default)*

- **Parser v1 (Legacy):** Deprecated and supported only for backwards compatibility. Requires the locus and antigen name separated by an asterisk (*e.g. DQB1*7* or *DRB1*11*).

Exporting Results: Once the analysis is complete, results are displayed on-screen. Use the Export button to download a CSV file containing detailed results. Large requests may require processing as asynchronous job, storing the data on the TxPredictor™ server, where the user can download the results once the job is completed. The result data contains a metadata header, total PIRCHE scores, loci-specific PIRCHE scores, and matching details:

- **PIRCHE-T1:** the actual pHLA (core, presenter, binding rank, imputation frequency) separated by presenting and presented locus

- **PIRCHE-T2:** the actual pHLA (core, 15-mer, presenter, binding rank, imputation frequency) separated by presenting and presented locus

- **PIRCHE-B:** mismatched amino acid position and configuration separated by origin locus

Non-standard Workflows: These modules are intended for study workflows only. Several safety nets can be disabled to allow for the processing of diverse or non-standard requests, which – if incorrectly or unknowingly set – may corrupt molecular mismatch load calculations. *Note:* Discrepancies or "impossible" HLA typings may result in the individual being silently omitted from the final analysis rather than triggering an error. Always perform a manual audit of the typings in your exported results file to ensure all cases were processed correctly.

Research Support: To prevent accidental misconfiguration, most algorithm settings can not be changed by regular users. If you have specific research requests modifying the underlying algorithms (*e.g.* skipping mandatory loci, ignoring self-peptidomes, etc.), reach out to our support team (section 12).

Data Post-processing: The TxPredictor™ Batch Matching modules generate high-volume, machine-readable CSV datasets. Because standard spreadsheet applications impose inherent structural limits, opening these files directly can result in silent data truncation, thereby compromising the integrity of the peptide data. Consequently, we strongly advise against using conventional spreadsheet software for post-processing. To ensure accurate analysis, users should utilize dedicated statistical environments (*e.g.* R or SPSS) or apply the data conversion scripts as *e.g.* available on our public GitHub repository (https://github.com/PIRCHE/pipeline.git) to format the outputs safely.

10 Upcoming Features

10.1 Outcomes Prediction Module

The Outcomes Prediction Module (OPM) is a new module built into TxPredictor™, that transfers molecular matching scores into clinical prediction models next to various clinical baseline parameters. Its first iteration features two statistical models published by Niemann et al. [15], predicting graft survival after kidney transplantation. Graft survival estimates are put in perspective by comparing them to the population's average graft survival. The Model Copilot (fig. 49) visualizes hazards and relative rank to the reference cohort, allowing users to weigh risk factors against others. The early access program of OPM has started and interested users are encouraged to reach out (section 12).

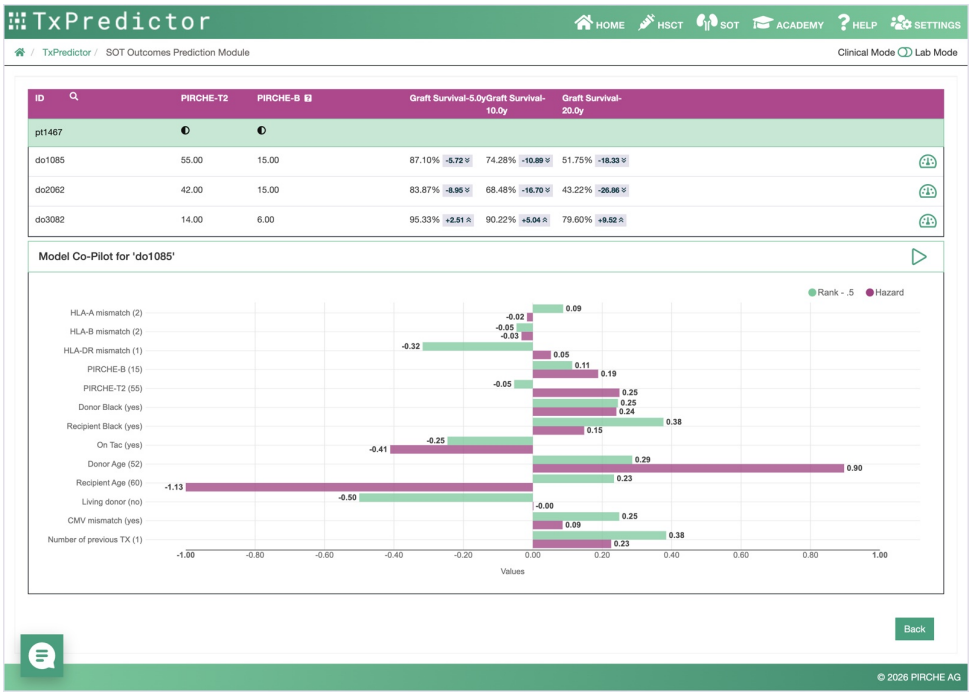


Figure 49: The addition of the PIRCHE® Outcomes Prediction Module allows you to translate molecular mismatch scores into clinical outcomes and compare them between donors.

10.2 Quality Metrics

We are dedicated to providing the transplant community with a robust, highly reliable analytical platform – specifically promoting QA. To facilitate the rigorous validation of data integrity and the matching process prior to reporting, an upcoming software update will introduce the Quality Metrics feature (fig. 50). Located on the Results screen, this feature transparently presents categorized metrics and checkpoints of the entire matching process, allowing users to evaluate its technical validity. To expedite the identification of data anomalies, these values are augmented by indicators *e.g.* suggesting further inspection.

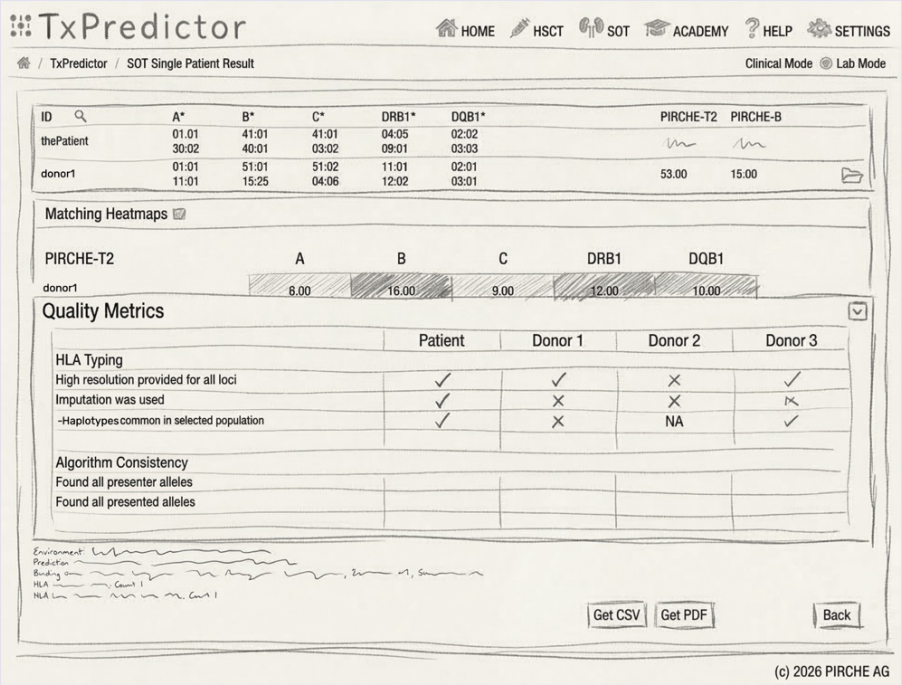


Figure 50: The Quality Metrics feature will allow users to evaluate the technical validity of a match, further supporting QA efforts.

We are continuously working to improve TxPredictor™ to better serve your analytical needs. Your feedback is a vital part of this process. If you have ideas for new features, workflow enhancements, or general improvements, we highly encourage you to reach out and share your suggestions with our team (section 12).

11 FAQ

- *What's the difference between PIRCHE and eplet matching?*
  They model different immune response pathways. Eplet matching predicts **B-cell (antibody) epitopes**, which are 3D conformational structures on intact donor HLA proteins bound directly by antibodies. In contrast, the PIRCHE algorithm evaluates **indirect T-cell epitopes**, which are linear peptide fragments presented to the recipient's T-cells (section 3.5).

- *What's the difference between eplet and Snow matching?*
  Both models evaluate B-cell epitopes but identify structural targets differently. Eplet matching relies on predefined structural configurations ("Eplets") of surface-accessible amino acids, typically within a 3 Angstrom radius. In contrast, the Snow algorithm evaluates the unique 3D **structure of individual HLA proteins** systematically by using deep learning to calculate precise, allele-specific **surface area** (Snowflake) and **localized protrusion** (Snowball), counting mismatched amino acids only if they exceed empirical thresholds for both metrics (section 3.3).

- *How does the algorithm handle ambiguous/low-resolution typing?*
  To handle low-resolution or ambiguous typing, TxPredictor™ uses a **multiple imputation algorithm** that relies on large-scale haplotype frequency tables to infer possible high-resolution genotype combinations. The system calculates the molecular mismatch scores for each potential pair and aggregates them into a final score using a **weighted average based on their statistical probability**, ensuring a reliable risk assessment despite data uncertainty (section 3.7).

- *How to impute typing in the absence of ancestry information?*
  If patient or donor ancestry is unknown, a **generic combined population** table can be used as a reliable fallback (section 3.7.2).

- *Does PIRCHE handle less than 5 loci?*
  Yes, but only for SOT. For SOT analyses, TxPredictor™ requires a **minimum of HLA-A, -B, and -DRB1** data, and will use its multiple imputation algorithm to infer the missing HLA-C and -DQB1 loci (section 3.7). However, for HSCT, imputation is not used due to the critical nature of allele-level matching, meaning a minimum of 5 loci (HLA-A, -B, -C, -DRB1, and -DQB1) is strictly required.

- *Does PIRCHE handle more than 5 loci?*
  Yes, TxPredictor™ supports extended PIRCHE analyses beyond the minimum 5 loci, allowing the **optional inclusion of HLA-DRB3/4/5, -DQA1, -DPA1, and -DPB1**. However, high-resolution (protein-level) typing is strictly required for any of these additional loci. Importantly, because PIRCHE scores are not normalized, incorporating more loci will generally increase the calculated molecular mismatch load. Therefore, you must use a consistent set of loci across your workflow, as a 5-loci analysis cannot be directly compared to a 6- or 11-loci analysis.

- *Can IMGT or prediction pipeline versions affect scores?*
  Yes. The algorithms fetch source HLA protein sequences from the IPD-IMGT/HLA database, meaning continuous prediction pipeline releases and IMGT upgrades can potentially impact match result calculations. To maintain workflow consistency, institutions can lock their analysis to a specific default prediction pipeline version in their Organization Settings (section 6.1.4). Furthermore, TxPredictor™ will notify users of any major score-impacting changes, compatibility details, and suggested validation procedures when new versions are introduced.

- *Are my match results permanently saved on the TxPredictor™ server?*
  No, by default, matching cases are not permanently stored on the server. To keep a record of your match runs, you must download a PDF.

- *Can we integrate TxPredictor™ directly with our Laboratory Information Management System (LIMS)?*
  Yes, TxPredictor™ offers an **Application Programming Interface (API)** that acts as a secure digital bridge to your local software (section 6.3.4).

- *What should I use for the Patient or Donor ID?*
  To ensure strict patient and donor confidentiality, the entry of personal identifiable information (PII)—such as names or dates of birth—is strictly prohibited. Always use de-identified codes that can only be mapped back to the patient record internally by the laboratory users (e.g., a LIMS-generated ID).

- *What is the best way to input my HLA data?*
  TxPredictor™ is designed to scale with your laboratory. You can choose between manual entry with built-in validation, automated file parsing, or complete system integration via API. We are happy to assist your team in determining and implementing the workflow that best fits your infrastructure (section 6.2).

- *What are the cutoffs to use?*

  In statistical models, molecular mismatch load scores are typically treated as continuous variables. Consequently, while lower scores generally correlate with reduced risk, any individual molecular mismatch inherently carries some degree of immunological risk. However, specific clinical or analytical applications may require the establishment of discrete acceptability thresholds. Section 4.5 references recently published literature utilizing current prediction pipelines, which may serve as an evidence-based baseline for defining custom thresholds.

12 Support and Contact

We offer several resources within the application to help you find answers or connect with our support team.

12.1 Self-Service Resources

Find answers, tutorials, and journal articles directly inside the app.

- **Help Boxes**: Throughout the application, you will see question mark buttons. Click one to show context-specific support that provides details about the specific feature it is located near.
- **Help**: Navigate to the ”Help” page from the Top Navigation Menu for central access to **Frequently Asked Questions (FAQ)** and comprehensive reference materials regarding core application features.
- **Academy**: Access the ”Academy” for in-depth learning resources, including:
  - Literature Compass
  - Publication List
  - Webinars

12.2 Direct Support

If you can’t find what you need in our resources, our support team is ready to assist you.

- **Chat Support:** Use the chat tool found at the bottom-left corner of the application to send our team a message. We monitor this channel closely and will respond as quickly as possible.
- **Email Support:** Reach out to our team anytime at support@pirche.com.

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- **Snow:** The Snow algorithm is currently patent pending. PIRCHE AG and University Medical Center Utrecht have jointly filed a patent application covering the prediction of an alloimmune response against allele-specific solvent-accessible and protruding amino-acid mismatches. (PCT/EP2024/058372)