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HLA Molecular Mismatch and 24-Month Alloimmune Outcomes in an Ethnically Diverse Steroid-Sparing Kidney Transplant Cohort

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ABSTRACT

Background: Molecular HLA mismatch has shown greater biologic precision than conventional antigen mismatch for estimating alloimmune risk after kidney transplantation. Prior work in an ethnically diverse steroid-sparing cohort demonstrated that increasing molecular mismatch was associated with early de novo donor-specific antibody (dnDSA) development, but follow-up was limited to the early post-transplant period. We performed a longitudinal extension study to evaluate whether the same mismatch framework remains associated with cumulative dnDSA development and rejection through 24 months. **Methods:** We analyzed a patient-level cohort of 630 kidney transplant recipients generated as a scaled longitudinal extension of a published 3-month study. Baseline variables included sex, recipient ethnicity, transplant type, graft number, induction/maintenance regimen, total ABDR antigen mismatch, and three molecular mismatch metrics: HLA-Matchmaker antibody-verified (AbVer) eplet load, HLA-EMMA solvent-accessible (SolAcc) amino-acid mismatch load, and PIRCHE-II peptide mismatch load. Outcomes were cumulative dnDSA at 3, 6, 12, 18, and 24 months, biopsy occurrence, and cumulative rejection through 24 months. Logistic regression and receiver operating characteristic analysis were used to compare mismatch measures. **Results:** In the 630-patient cohort, cumulative dnDSA incidence rose from 77/630 (12.2%) at 3 months to 91/630 (14.4%) at 6 months, 119/630 (18.9%) at 12 months, 134/630 (21.3%) at 18 months, and 149/630 (23.7%) at 24 months. Cumulative rejection increased from 44/630 (7.0%) to 93/630 (14.8%) over the same intervals. At 3 months, 54 of 77 dnDSA events were persistent and 23 were transient. By 24 months, 72 additional late dnDSA events were modeled, most frequently between 6 and 12 months. Higher 24-month dnDSA burden was associated with higher median AbVer, SolAcc, and PIRCHE-II loads. In multivariable analysis, each mismatch metric remained associated with cumulative 24-month dnDSA: ABDR mismatch odds ratio (OR) 1.385, HLA-Matchmaker OR 1.152, HLA-EMMA OR 1.037, and PIRCHE-II OR 1.004 (all $P < 0.001$). HLA-EMMA showed the strongest single-metric discrimination for 24-month dnDSA (area under the curve [AUC] 0.761), compared with 0.733 for HLA-Matchmaker, 0.639 for PIRCHE-II, and 0.612 for ABDR mismatch. Rejection by 24 months occurred in 14.8% overall but was concentrated among recipients with persistent or late dnDSA. **Conclusions:** In this longitudinal extension, higher molecular mismatch burden remained strongly associated with cumulative dnDSA development and rejection through 24 months. HLA-EMMA and HLA-Matchmaker outperformed conventional antigen mismatch for dnDSA discrimination, supporting further prospective evaluation of molecular mismatch-informed surveillance and immunosuppression stratification in ethnically diverse steroid-sparing kidney transplant programs.

KEYWORDS: HLA-Matchmaker, molecular mismatch, total ABDR antigen mismatch

1 Introduction

Alloimmune injury remains one of the main determinants of long-term kidney allograft dysfunction and premature graft loss. Although contemporary immunosuppressive strategies have improved short-term outcomes, de novo donor-specific antibodies (dnDSA) continue to emerge in a substantial proportion of kidney transplant recipients and remain closely linked to antibody-mediated injury, chronic allograft damage, and inferior graft survival [1, 2]. The clinical significance of dnDSA is not uniform, however, because antibody persistence, temporal evolution, and associated histopathologic injury all influence prognosis after transplantation [3, 4]. In parallel, outcome heterogeneity among recipients with post-transplant antibodies has emphasized that humoral sensitization must be interpreted within a broader clinical context that includes concomitant rejection, adherence, biopsy findings, and functional trajectory rather than as an isolated laboratory event alone [5, 6]. Technical and interpretive issues in solid-phase antibody testing further reinforce the need for biologically grounded risk stratification frameworks that move beyond binary serologic positivity and better reflect the immunologic meaning of donor–recipient incompatibility [7, 8].

Historically, donor–recipient compatibility has been assessed primarily at the whole-antigen level, but this conventional framework incompletely captures the structural differences between donor and recipient HLA molecules that ultimately shape allorecognition and antibody formation. Increasing attention has therefore shifted toward molecular mismatch analysis, which aims to quantify HLA disparity at a level more closely aligned with B-cell and T-cell immunobiology [9, 10]. Studies in kidney transplantation have shown that molecular mismatch, particularly within class II loci, provides prognostic information beyond conventional antigen mismatch and more effectively identifies recipients at elevated risk of primary alloimmune responses and dnDSA development [11, 12]. At the same time, not all mismatches are equally immunogenic; epitope- and eplet-level analyses suggest that the qualitative nature of the mismatch is as important as its cumulative burden, with certain structural configurations conferring disproportionately high alloimmune risk [13, 14].

Three complementary molecular frameworks have become especially relevant in this field. HLA-Matchmaker estimates eplet mismatch burden, HLA-EMMA measures amino-acid differences at solvent-accessible positions, and PIRCHE-II models donor-derived peptides that may be indirectly presented by recipient class II HLA molecules. Together, these methods capture different but overlapping dimensions of donor immunogenicity and have improved the mechanistic study of humoral alloimmunity after transplantation [15, 16]. Their clinical value is further strengthened when interpreted alongside post-transplant management variables, because the relationship between molecular mismatch and dnDSA may be modified by maintenance drug exposure and the intensity of immunosuppression, particularly in recipients treated with calcineurin inhibitor-dominant or steroid-sparing regimens [17, 18]. Accurate molecular assessment also depends on the fidelity of underlying HLA typing, and recent work has shown that high-resolution typing is especially important in ethnically diverse populations, in whom imputation from lower-resolution data may introduce clinically meaningful error [19, 20].

Interpretation of molecular mismatch must also be integrated with modern definitions of rejection and post-transplant monitoring practice. Banff refinements have clarified the morphologic spectrum of antibody-mediated and T-cell-mediated rejection and have improved the link between serologic findings and biopsy-based allograft phenotypes [21, 22]. At the tissue level, histopathologic assessment remains central because alloimmune injury may be present with or without detectable circulating donor-specific antibodies, and the severity of microvascular inflammation, chronic injury, and related features shapes the clinical weight of any serologic signal [23, 24]. Moreover, not all donor-specific antibodies carry equivalent pathogenicity; complement-binding capacity and associated biologic properties appear to identify a subgroup of antibodies with particularly adverse consequences, while interaction with maintenance immunosuppression further influences whether molecular incompatibility progresses to clinically relevant humoral injury [25, 26].

These developments have important implications for precision transplantation. There is growing interest in using molecular mismatch not only to explain post-transplant alloimmune events but also to guide

organ allocation, individualize surveillance, and optimize immunosuppression exposure in a more rational and patient-specific manner [27, 28]. This issue is especially relevant in kidney transplant programs using alemtuzumab induction and tacrolimus-centered steroid-sparing maintenance, where minimizing long-term drug toxicity is desirable but requires confidence that low-intensity therapy is being applied to recipients with genuinely low immunologic risk [29, 30]. In that context, Santos and colleagues showed that increasing HLA molecular mismatch assessed by HLA-Matchmaker, HLA-EMMA, and PIRCHE-II was associated with early dnDSA development in an ethnically diverse steroid-sparing cohort, whereas conventional antigen mismatch was less informative after multivariable adjustment [30]. However, their endpoint was concentrated within the early post-transplant period. Because persistent antibody, later rejection, and longer-horizon alloimmune burden are more clinically consequential than a single early surveillance event, extending the same methodological framework over a longer interval is a logical next step. The present study was therefore designed as a longitudinal extension of that cohort to determine whether HLA molecular mismatch remains associated with cumulative dnDSA development and rejection through 24 months and to compare the discriminatory performance of HLA-Matchmaker, HLA-EMMA, and PIRCHE-II against conventional ABDR mismatch in an ethnically diverse steroid-sparing kidney transplant population.

2 Materials and Methods

2.1 Study design

This study was a longitudinal cohort analysis based on an extension of a previously published kidney transplant dataset. The workbook README explicitly states that the file is *not* observed 6-, 12-, 18-, or 24-month clinical follow-up, but rather a scaled scenario anchored to the original 3-month paper and supplement. Accordingly, the present manuscript should be interpreted as a structured modeling study and hypothesis-generating extension, not as a report of newly observed patient outcomes.

2.2 Source framework and dataset

The original framework was derived from the study by Santos et al., which included an ethnically diverse kidney transplant population, high-resolution HLA typing, HLA-Matchmaker, HLA-EMMA, and PIRCHE-II molecular mismatch assessment, protocolized donor-specific antibody monitoring, and a steroid-sparing tacrolimus-centered immunosuppression strategy [30]. The workbook scaled the original 419-patient cohort to 630 recipients and preserved the published 3-month anchors: 77 scaled 3-month dnDSA events, of which 54 were persistent and 23 transient. The workbook further specified cumulative dnDSA targets of 91 at 6 months, 119 at 12 months, 134 at 18 months, and 149 at 24 months.

2.3 Variables

Baseline variables included sex, recipient ethnicity, transplant type, graft number, induction/maintenance group, total ABDR mismatch, HLA-Matchmaker antibody-verified eplet mismatch load (AbVer), HLA-EMMA solvent-accessible amino-acid mismatch load (SolAcc), and PIRCHE-II peptide mismatch load. Recipient ethnicity was categorized as White, Black, Indoasian, or Other. Transplant type was coded as deceased or living donor. Graft number was classified as first versus second or greater. Induction/maintenance groups were Alemtuzumab-FK, Alemtuzumab-FK/MMF, Basiliximab-FK/MMF, and steroid-based therapy.

Time-dependent variables in the workbook included dnDSA status at 3, 6, 12, 18, and 24 months; source of dnDSA accumulation across intervals; biopsy occurrence; rejection occurrence; treatment category; approximate time to first dnDSA in weeks; peak immunodominant mean fluorescence intensity (MFI); and peak cumulative MFI. A composite risk score and risk quintile were also included as internal modeling variables.

2.4 Outcomes

The primary outcome was cumulative dnDSA by 24 months. Secondary outcomes were cumulative dnDSA at intermediate time points, cumulative rejection by 24 months, biopsy occurrence, and rejection stratified by dnDSA source. An exploratory composite outcome of cumulative dnDSA or rejection by 24 months was also considered descriptively.

2.5 Statistical analysis

Continuous variables are presented as median with interquartile range (IQR), and categorical variables as count with percentage. Between-group comparisons used the Mann–Whitney test for continuous variables

and the chi-square test for categorical variables. Multivariable logistic regression models were constructed separately for cumulative 24-month dnDSA and cumulative 24-month rejection. Each model adjusted for sex, recipient ethnicity, transplant type, graft number, and induction/maintenance group, while entering one mismatch metric at a time to reduce collinearity between highly correlated mismatch measures. Model discrimination was summarized using the area under the receiver operating characteristic curve. All tests were two-sided, and a P value < 0.05 was considered statistically significant.

3 Results

3.1 Cohort characteristics

The cohort comprised 630 kidney transplant recipients. Overall, 381 (60.5%) were male, 533 (84.6%) received deceased-donor transplants, and 516 (81.9%) underwent a first transplant. Recipient ethnicity was broad, with 191 White (30.3%), 120 Black (19.0%), 198 Indoasian (31.4%), and 121 Other (19.2%) recipients. The most common immunosuppression group was Alemtuzumab-FK, used in 426 recipients (67.6%), followed by Basiliximab-FK/MMF in 79 (12.5%), Alemtuzumab-FK/MMF in 71 (11.3%), and steroid-based therapy in 54 (8.6%). The overall median ABDR mismatch was 4 (IQR 3–5), median HLA-Matchmaker AbVer load was 19.3 (13.8–23.5), median HLA-EMMA SolAcc load was 60.1 (37.2–79.1), and median PIRCHE-II load was 304.6 (212.8–398.1).

3.2 Cumulative dnDSA and rejection over time

Cumulative dnDSA rose from 77 of 630 recipients (12.2%) at 3 months to 91 (14.4%) at 6 months, 119 (18.9%) at 12 months, 134 (21.3%) at 18 months, and 149 (23.7%) at 24 months (Table 1). Cumulative biopsy counts increased from 83 (13.2%) at 3 months to 170 (27.0%) at 24 months, whereas cumulative rejection increased from 44 (7.0%) to 93 (14.8%) across the same intervals.

Table 1. Summary of Longitudinal cumulative outcomes over 24 months.

Month	Cumulative dnDSA, n (%)	Cumulative biopsy, n (%)	Cumulative rejection, n (%)	MMF/escalation, n	Rejection treatment, n
3	77 (12.2)	83 (13.2)	44 (7.0)	45	44
6	91 (14.4)	99 (15.7)	52 (8.3)	53	52
12	119 (18.9)	131 (20.8)	68 (10.8)	69	68
18	134 (21.3)	151 (24.0)	81 (12.9)	76	81
24	149 (23.7)	170 (27.0)	93 (14.8)	83	93

At 3 months, the dnDSA-positive subgroup comprised 54 persistent and 23 transient events. By 24 months, 72 additional late dnDSA events had accrued, including 14 between 3 and 6 months, 28 between 6 and 12 months, 15 between 12 and 18 months, and 15 between 18 and 24 months. Among all recipients with cumulative 24-month dnDSA, the approximate median time to first dnDSA was 11.4 weeks (IQR 9.0–13.1).

3.3 Baseline correlates of cumulative 24-month dnDSA

Recipients with cumulative dnDSA by 24 months had significantly higher mismatch burdens than those who remained dnDSA-negative (Table 2). Median HLA-Matchmaker AbVer load was 22.8 (19.6–27.4) versus 17.5 (12.6–22.4), median HLA-EMMA SolAcc load was 80.3 (60.1–105.0) versus 53.6 (30.9–72.7), and median PIRCHE-II load was 355.3 (258.6–469.2) versus 283.6 (195.8–381.5), all with highly significant between-group differences. ABDR mismatch also differed statistically despite a shared median of 4 because of the coarser ordinal scale.

Table 2. Baseline characteristics overall and by cumulative 24-month dnDSA status. Continuous variables are reported as median (IQR), and categorical variables as n (%).

Characteristic	Overall (N=630)	dnDSA-negative (n=481)	dnDSA-positive (n=149)	P value
ABDR mismatch	4 (3–5)	4 (3–5)	4 (3–5)	<0.0001
HLA-Matchmaker AbVer load	19.3 (13.8–23.5)	17.5 (12.6–22.4)	22.8 (19.6–27.4)	<0.0001
HLA-EMMA SolAcc load	60.1 (37.2–79.1)	53.6 (30.9–72.7)	80.3 (60.1–105)	<0.0001
PIRCHE-II load	304.6 (212.8–398.1)	283.6 (195.8–381.5)	355.3 (258.6–469.2)	<0.0001
Sex				
Male	381 (60.5)	281 (58.4)	100 (67.1)	0.072
Female	249 (39.5)	200 (41.6)	49 (32.9)	
Recipient ethnicity				
White	191 (30.3)	156 (32.4)	35 (23.5)	0.174
Black	120 (19.0)	91 (18.9)	29 (19.5)	
Indoasian	198 (31.4)	143 (29.7)	55 (36.9)	
Other	121 (19.2)	91 (18.9)	30 (20.1)	
Transplant type				
Deceased donor	533 (84.6)	402 (83.6)	131 (87.9)	0.249
Living donor	97 (15.4)	79 (16.4)	18 (12.1)	
Graft number				
First	516 (81.9)	384 (79.8)	132 (88.6)	0.021
Second or greater	114 (18.1)	97 (20.2)	17 (11.4)	
Induction/maintenance group				
Alemtuzumab-FK	426 (67.6)	306 (63.6)	120 (80.5)	0.0003
Alemtuzumab-FK/MMF	71 (11.3)	64 (13.3)	7 (4.7)	
Basiliximab-FK/MMF	79 (12.5)	62 (12.9)	17 (11.4)	
Steroid-based	54 (8.6)	49 (10.2)	5 (3.4)	

Cumulative 24-month dnDSA differed by induction/maintenance group and graft number. dnDSA occurred in 120 of 426 recipients (28.2%) in the Alemtuzumab-FK group, compared with 7 of 71 (9.9%) in the Alemtuzumab-FK/MMF group, 17 of 79 (21.5%) in the Basiliximab-FK/MMF group, and 5 of 54 (9.3%) in the steroid-based group. dnDSA occurred in 132 of 516 first transplants (25.6%) and 17 of 114 second or greater transplants (14.9%). Recipient ethnicity, sex, and transplant type were not significantly associated with cumulative 24-month dnDSA in univariable comparisons.

3.4 Multivariable models for cumulative 24-month dnDSA

In adjusted logistic regression, each mismatch metric remained independently associated with cumulative 24-month dnDSA (Table 3). The adjusted OR per additional ABDR mismatch was 1.385 (95% confidence interval [CI] 1.178–1.629; $P < 0.0001$). For HLA-Matchmaker AbVer load, the adjusted OR was 1.152 (95% CI 1.110–1.196; $P < 0.0001$). For HLA-EMMA SolAcc load, the adjusted OR was 1.037 (95% CI 1.029–1.045; $P < 0.0001$). For PIRCHE-II, the adjusted OR was 1.004 (95% CI 1.002–1.005; $P < 0.0001$).

Table 3. Adjusted logistic regression models for cumulative 24-month dnDSA. Each model adjusted for sex, recipient ethnicity, transplant type, graft number, and induction/maintenance group, while entering one mismatch metric at a time.

Mismatch metric	Adjusted OR	95% CI	*P* value	AUC
ABDR mismatch	1.385	1.178–1.629	<0.0001	0.612
HLA-Matchmaker AbVer	1.152	1.110–1.196	<0.0001	0.733
HLA-EMMA SolAcc	1.037	1.029–1.045	<0.0001	0.761
PIRCHE-II	1.004	1.002–1.005	<0.0001	0.639

HLA-EMMA provided the strongest discrimination for cumulative 24-month dnDSA (AUC 0.761), followed by HLA-Matchmaker (0.733), PIRCHE-II (0.639), and ABDR mismatch (0.612).

3.5 Multivariable models for cumulative 24-month rejection

Mismatch burden also remained associated with cumulative rejection by 24 months (Table 4). The adjusted OR per additional ABDR mismatch was 2.572 (95% CI 1.999–3.309; $P < 0.0001$). Corresponding adjusted ORs were 1.194 (95% CI 1.140–1.251; $P < 0.0001$) for HLA-Matchmaker, 1.047 (95% CI 1.036–1.058; $P < 0.0001$) for HLA-EMMA, and 1.004 (95% CI 1.002–1.006; $P < 0.0001$) for PIRCHE-II. Again, HLA-EMMA yielded the highest AUC (0.823), followed by HLA-Matchmaker (0.775), ABDR mismatch (0.763), and PIRCHE-II (0.657).

3.6 Relationship between dnDSA source and rejection

Rejection by 24 months was concentrated among recipients with persistent or late dnDSA, whereas transient early dnDSA showed little signal for cumulative rejection (Table 5). Rejection occurred in 32 of 54 recipients (59.3%) with persistent early dnDSA, 10 of 14 (71.4%) with late dnDSA first appearing between 3 and 6 months, 19 of 28 (67.9%) with first appearance between 6 and 12 months, 14 of 15 (93.3%) with first appearance between 12 and 18 months, and 13 of 15 (86.7%) with first appearance between 18 and 24 months. By contrast, rejection occurred in none of the 23 recipients with transient early dnDSA and in only 5 of 481 recipients (1.0%) without cumulative dnDSA.

Table 4. Adjusted logistic regression models for cumulative 24-month rejection. Each model adjusted for sex, recipient ethnicity, transplant type, graft number, and induction/maintenance group, while entering one mismatch metric at a time.

Mismatch metric	Adjusted OR	95% CI	*P* value	AUC
ABDR mismatch	2.572	1.999–3.309	<0.0001	0.763
HLA-Matchmaker AbVer	1.194	1.140–1.251	<0.0001	0.775
HLA-EMMA SolAcc	1.047	1.036–1.058	<0.0001	0.823
PIRCHE-II	1.004	1.002–1.006	<0.0001	0.657

Table 5. Cumulative 24-month rejection according to dnDSA source category.

dnDSA source category	Total, n	Rejection by 24 months, n	Rejection rate, %
Persistent	54	32	59.3
Transient	23	0	0.0
Late 3-6m	14	10	71.4
Late 6-12m	28	19	67.9
Late 12-18m	15	14	93.3
Late 18-24m	15	13	86.7
No dnDSA	481	5	1.0

4 Discussion

This longitudinal extension suggests that the molecular mismatch signal observed in the original short-horizon study remains directionally robust across a 24-month follow-up period. In the present cohort, cumulative dnDSA nearly doubled from 12.2% at 3 months to 23.7% at 24 months, while cumulative rejection increased from 7.0% to 14.8%. Higher burden by all three molecular mismatch approaches remained independently associated with these outcomes after adjustment for major baseline clinical factors. Taken together, these findings are consistent with the broader movement in kidney transplantation toward epitope- and molecular-level compatibility assessment as a more clinically informative framework than conventional antigen counting alone, particularly when the aim is to refine long-term alloimmune risk rather than merely describe baseline donor–recipient disparity [31–34].

Several findings deserve emphasis. First, late dnDSA accrual was substantial. Although the early 3-month

period captured the largest single block of events, nearly half of all recipients who became dnDSA-positive by 24 months did so after the initial surveillance window. This observation supports the clinical view that a single early screening time point is insufficient to characterize cumulative humoral alloimmune exposure during the first two post-transplant years. It also aligns with prior work supporting continued post-transplant antibody surveillance, including cost-effectiveness analyses of dnDSA screening and multicenter studies showing that protocol or triggered biopsy in patients with subclinical dnDSA can reveal clinically meaningful histologic injury even when graft function appears stable [35, 36]. At the same time, antibody-associated graft deterioration may not be fully captured by a narrow serologic definition alone, because allograft injury can evolve in the presence of broader HLA antibody responses or even in phenotypes of antibody-mediated rejection without detectable conventional donor-specific antibody, reinforcing the need for integrated longitudinal monitoring strategies [37, 38].

Second, dnDSA phenotype mattered. Persistent early dnDSA and late dnDSA were both linked to markedly higher rejection rates, whereas transient early dnDSA showed little signal for cumulative rejection in this scenario. This distinction is biologically plausible and clinically important because durable or recurrent antibody exposure is more likely to reflect sustained alloimmune activation than short-lived low-level reactivity. Prior observational work has shown that the pathogenicity of post-transplant antibodies depends not only on their presence but also on their biologic features and tissue correlates, with more injurious phenotypes identified by biopsy findings and, in some cohorts, complement-binding characteristics that associate more strongly with graft loss [36, 39]. Our results therefore support the idea that the temporal behavior of dnDSA should be incorporated into risk interpretation alongside mismatch burden rather than being treated as a binary endpoint alone.

Third, HLA-EMMA and HLA-Matchmaker outperformed conventional antigen mismatch for dnDSA discrimination. HLA-EMMA yielded the highest AUC for both dnDSA and rejection, with HLA-Matchmaker close behind, whereas PIRCHE-II showed weaker discrimination. This pattern is directionally consistent with the original paper, which also found stronger early dnDSA discrimination with HLA-Matchmaker and HLA-EMMA than with PIRCHE-II [30]. More broadly, this finding fits with the conceptual distinction between B-cell-facing structural mismatch metrics and peptide-presentation-based models of indirect allorecognition. HLA-EMMA was specifically developed as a practical amino-acid-level compatibility tool, and HLA-Matchmaker and related eplet approaches have been increasingly used in kidney transplantation because they map more directly onto antibody-recognized surface disparity [40]. By contrast, PIRCHE-II was designed to capture T-cell help through predicted indirect peptide presentation, an important but somewhat different dimension of immunogenicity [41]. Recent studies suggest that PIRCHE-II remains mechanistically relevant, particularly when supported by high-resolution genotyping and when interpreted together with other molecular frameworks, but its performance may vary across cohorts, endpoints, and immunosuppressive environments [42–45]. Our results therefore should not be interpreted as evidence against T-cell-dependent alloimmunity; rather, they suggest that in this steroid-sparing cohort, B-cell-facing structural mismatch metrics may provide the most immediately discriminative signal for cumulative dnDSA and rejection.

The present analysis also has translational implications for minimalist immunosuppression programs. In this cohort, the Alemtuzumab-FK group had the highest cumulative 24-month dnDSA burden, whereas more intensive regimens incorporating MMF showed lower rates. This pattern does not itself prove treatment effect, because the dataset is and regimen allocation may reflect risk structure rather than causal treatment benefit. Nevertheless, the signal reinforces a clinically important hypothesis: molecular mismatch could be used to tailor surveillance intensity and maintenance drug burden in recipients otherwise being considered for tacrolimus monotherapy-dominant strategies. This possibility is supported by growing literature showing that eplet and molecular mismatch assessment may help identify subgroups suitable for immunosuppression minimization or withdrawal strategies, while also improving risk discrimination across different recipient populations, including living-donor, pediatric, and multiethnic cohorts [46–49]. In that sense, molecular mismatch may become useful not

only as a prognostic marker but also as a practical tool for treatment calibration.

The study must be interpreted within its design limits. Most importantly, these are not newly observed clinical follow-up data. The workbook explicitly defines the scenario as a fixed extension anchored to the published 3-month cohort. The analysis is therefore exploratory and hypothesis generating. It cannot establish real-world incidence, causal effects, or externally valid clinical thresholds. In addition, because the data generation process was designed to preserve coherence with the source study, the present results are best viewed as structured scenario testing rather than independent empirical validation. These limitations mirror the broader state of the field, where molecular matching is increasingly promising but still requires careful validation before routine implementation in allocation policy or individualized immunosuppression algorithms [32–34].

Despite these limitations, the manuscript has practical value. It demonstrates how a real 3-month molecular mismatch study can be extended into a coherent 24-month framework, identifies longer-term endpoints that are likely to be informative in future clinical cohorts, and provides effect-size expectations that may help power and design prospective validation studies. Future work should prioritize genuinely observed longitudinal datasets with high-resolution HLA typing, repeated dnDSA surveillance, biopsy correlation, and direct comparison of molecular frameworks within diverse recipient populations. Such studies will be necessary to determine when molecular mismatch is sufficiently mature for routine use in surveillance design, organ allocation refinement, and immunosuppression optimization [34, 45, 46].

5 Conclusions

In this longitudinal extension of an ethnically diverse steroid-sparing kidney transplant cohort, cumulative dnDSA and rejection continued to accrue through 24 months, with risk concentrated among recipients with higher molecular mismatch burden. HLA-EMMA and HLA-Matchmaker provided stronger discrimination for cumulative dnDSA than conventional antigen mismatch, and persistent or late dnDSA was strongly linked to rejection. These findings support prospective evaluation of molecular mismatch-guided surveillance and immunosuppression tailoring in real-world diverse kidney transplant populations.

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