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## *Biomarker Qualification Letter of Intent (LOI)*

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Submission Date: 13 Aug 2018

### Drug Development Need

Transplantation has minimal novel drug development to improve graft and patient survival. In part, this is due to excellent short-term outcomes with the standard of care (**SOC**) [i.e. 1-year graft rejection 10-15%, graft and patient survival >92% with calcineurin inhibitor (CNI) + antiproliferative combination therapy].<sup>1,2</sup> Hence development of new immunosuppressive drugs focusing on the average treatment effect in the overall population requires phase 3 clinical trials lasting  $\geq 5$  years and relatively large sample sizes to demonstrate superiority to SOC. For companies to re-engage in transplant drug development, there is an *urgent need for qualified biomarkers* to allow for efficient drug trials with a small sample size and of a short duration.<sup>1,2</sup>

Unfortunately, long-term outcomes have seen minimal improvement – a *major unmet need requiring targeted drug development*.<sup>1</sup> Indeed, up to 25% of patients are under immunosuppressed, despite receiving SOC, evidenced by the formation of *de novo* donor specific antibodies (DSA) [defined by solid phase single antigen bead assay<sup>2</sup>], graft rejection [defined by biopsy proven T cell-mediated rejection (TCMR) and/or antibody-mediated rejection (ABMR) as classified by the Banff criteria<sup>4</sup>], which results in premature graft loss [defined by estimated glomerular filtration rate (eGFR) <15 ml/min/1.73m<sup>2</sup>, return to dialysis, or re-transplantation].<sup>2,3</sup> The SOC is also limited by significant rates of metabolic and neurologic adverse events, infection, and cancer resulting in a poor quality of life and premature death.<sup>2</sup>

Apart from desensitization drug studies, all randomized control trials (RCTs) in kidney transplantation exclude patients at risk for immune memory based on pre-transplant testing for the presence of DSA to the donor's mismatched HLA molecules.<sup>5</sup> Nevertheless, patients in these RCTs have varying degrees of risk for primary alloimmune events.<sup>2,5</sup> How to accurately quantify this risk is an *unmet need* leading to large sample size RCTs due to patient heterogeneity. We propose to qualify **prognostic biomarkers**, determined at the time of transplant to categorize kidney transplant patients as high, intermediate, or low risk for *de novo* DSA, graft rejection, and graft loss. As a **drug development tool**, they are envisioned to enable **enrichment in phase 2 and 3 RCTs**.<sup>6</sup>

## Biomarker Information

- **Biomarker Name: HLA-DR/DQ MOLECULAR MISMATCH SCORE**
- **Biomarker Description:** A computational measurement of the degree of gene loci HLA-DRB1/3/4/5 and HLA-DQA1/DQB1 molecular sequence differences between a kidney donor and the transplant recipient.<sup>7-9</sup>

To calculate the HLA-DR/DQ molecular mismatch score the donor and recipient DNA must be HLA typed at the allele level (i.e. 4-digit) for the HLA-DRB1/3/4/5 and HLA-DQA1/DQB1 gene loci. Publicly available computational software (e.g. HLA DRDQDP Matching, version 2.0) inputs the donor and recipient 4-digit HLA typing to generate individual HLA-DR and HLA-DQ molecular mismatch scores. Pre-determined risk thresholds set for a composite HLA-DR/DQ molecular mismatch score are then applied to assign the transplant recipient into one of three prognostic biomarker risk categories (low, intermediate or high) for post-transplant *de novo* HLA-DR/DQ donor specific antibodies (DSA), graft rejection, and graft loss.

- **Biological Rational:** The recipient's immune response to the donor organ is initiated after recognition of the transplanted allograft as non-self. It accomplishes this through the B-cell Receptor (BCR) that directly binds mismatched amino acids on the surface of the donor's HLA (B-cell epitope) or the T-cell Receptor (TCR) that recognize processed donor mismatched HLA allopeptides (T-cell epitope) expressed by recipient antigen presenting cells.<sup>10</sup> While mismatched donor HLA Class I (HLA-A, -B, -Cw) and Class II (HLA-DR, -DQ, -DP) molecules can all be immune targets, the HLA-DR/DQ molecular mismatch is the dominant HLA driver of allorecognition by the recipient's immune system that consistently correlates with post-transplant alloimmune events (i.e. *de novo* DSA, graft rejection, and graft loss).<sup>9</sup>

## Context of Use

**Prognostic biomarkers** (i.e. determined at the time of transplant in conjunction with baseline testing to rule-out preformed alloimmunity) categorizing kidney transplant patients as high, intermediate, or low risk for *de novo* DSA, graft rejection and graft failure, with categories to be used independently, in pairs or triplet risk categories to **enrich phase 2 and 3 clinical trials with patients based upon risk category in studies evaluating novel drugs.**

## Biomarker Measurement (Analytical)

*A major advantage of this biomarker is that HLA typing is already performed on all donors and recipients in accredited histocompatibility laboratories around the world. As such all programs are capable of developing this biomarker building upon their existing HLA typing standard operating procedures that meet regulatory standards.*

**HLA-DR and HLA-DQ Allele Level Sequencing:** Kidney donor and transplant recipient genomic DNA is used as source material to determine the allele level HLA sequence. Sequence-specific oligonucleotide (SSO) probe based HLA typing kits (One Lambda, Canoga Park, CA, 510(k) BK160018, BK120024; LABType™ XR Class II DRB1 typing test (Catalog ID RSSOX2B1), LABType™ SSO Class II DRB3,4,5 typing test (Catalog ID RSSO2345), and LABType™ SSO Class II DQA1/DQB1 typing test (Catalog ID RSSO2Q)) are employed and supplemented by sequence-specific primer (Micro SSP™, One Lambda) HLA DNA typing trays as required to achieve allele level (4-digit) HLA typing.<sup>9</sup>

For SSO HLA typing kits, target DNA is polymerase chain reaction (PCR)-amplified using a biotin labelled group-specific primer. The biotinylated PCR product is then denatured and allowed to re-hybridize to complementary DNA probes conjugated to fluorescently coded microspheres. The reaction is washed to remove unbound DNA, and then phycoerythrin (PE)-conjugated streptavidin (SAPE) is used to label the biotinylated DNA bound to the microspheres. A Luminex instrument (LABScan 3D™, One Lambda, Luminex® FLEXMAP 3D®, Luminex Inc, Austin,

Tx, 510(k) K121399) identifies and records the fluorescent intensity of the PE on each microsphere. The assignment of DNA sequence is then based on the reaction pattern compared to patterns associated with published HLA gene sequences using HLA Fusion™ software (One Lambda, 510(k) BK160017).

**HLA-DR and HLA-DQ Molecular Mismatch Analysis:** The HLA allele level sequence data generated above for a given donor-recipient combination is entered into the publicly available software [**HLA DRDQDP Matching program (version 2.0; <http://www.epitopes.net>)**] to analyze for sequence differences between donor and recipient HLA-DRB1/3/4/5 and HLA-DQA1/DQB1 loci.<sup>7,9,11,12</sup> Specifically it identifies patches (3 angstrom radius) of polymorphic amino acids on the HLA molecule surface (referred to as “eplets” by the program developers) and reflects the central part of the entire epitope that an antibody would bind (**detailed methods found in ref. 11 and 12**). The number of donor-recipient eplet mismatches at the HLA-DR and DQ loci are then calculated to generate an individual HLA- DR and HLA-DQ molecular mismatch score, which is used for constructing the prognostic biomarker for each individual kidney transplant recipient [see Biomarker Measurement (Clinical) below].<sup>7,9</sup>

#### **Background for the HLA DRDQDP Matching program:**

“The construction of the eplet repertoire is based on polymorphic amino acid residues on the HLA molecular surface. Their locations are easily determined with three dimensional models of class II molecules. The Entrez Molecular Modeling Database of the National Center for Biotechnology Information stores on its Web site (<http://www.ncbi.nlm.nih.gov/Structure>) an extensive collection of crystallographic structures of HLA molecules that can be viewed with the Cn3D structure and sequence alignment software program.”<sup>12</sup>

The biological rationale for the use of HLA eplet identification by the computational software is on the basis of two underlying principles: (1) The immune system recognizes and develops antibodies against nonself-antigens or more specifically, the epitopes on those antigens, while ignoring self-antigens/epitopes; (2) Antibody specificity is largely determined by a small number of polymorphic amino acids near the center of the epitope that bind the third complementary determining region of the antibody variable heavy chain. Comparing the amino acid sequences between donor and recipient alleles allows for the identification and quantification of these theoretical eplet differences.<sup>10-12</sup>

## **Biomarker Measurement (Clinical)**

- *Characterization of Biomarker for COU:* The goal is to assign all kidney transplant recipients at the time of transplant to a prognostic biomarker risk category for a primary alloimmune events based on the HLA-DR/DQ molecular mismatch score.
- *Calculation/Modeling/Construction of Biomarker into a Decision Tool:* While HLA-DR or HLA-DQ molecular mismatch scores (i.e. eplet mismatches) can be used as a continuous variable in risk assessment, it was determined (via receiver operator curve (ROC) analysis) that there are three major risk categories for post-transplant *de novo* HLA-DR or HLA-DQ DSA development: **Low** – 0 eplet mismatch; **Intermediate** – 1 to 11 eplet mismatches; and **High** – >11 eplet mismatches (**detailed in ref. 9**). The HLA-DR molecular mismatch cut-off of >11 eplet mismatches has a sensitivity of 90% and an AUC 0.73 for predicting *de novo* HLA-DR DSA, and the HLA-DQ molecular mismatch cut-off of >11 eplet mismatches has a sensitivity of 94% and an AUC of 0.72 for predicting *de novo* HLA-DQ DSA.<sup>9</sup>

To move the individual HLA-DR and HLA-DQ molecular mismatch scores into a **prognostic biomarker**, a *combined (or composite)* HLA-DR/DQ molecular mismatch score is developed based on the greatest risk associated with the patient’s individual HLA-DR and HLA-DQ mismatch scores. For example, if the HLA-DR molecular mismatch score was 0 eplet mismatches (i.e. low risk) but the HLA-DQ mismatch score was >11 eplet mismatches (i.e. high risk) then the patient overall was said to be at high risk for a *de novo* Class II DSA (in this case an HLA-DQ DSA). Applying this logic, all patients can be broadly categorized as: **Low Risk** – HLA-DR and HLA-DQ molecular mismatch are both 0 eplet mismatches; **Intermediate Risk** – one or both of the individual HLA-DR or HLA-DQ molecular mismatch are 1-11 eplet mismatches, but neither are >11 eplet

mismatches; and **High Risk** – one or both of the individual HLA-DR and HLA DQ molecular mismatch are >11 eplet mismatches.

- *Expected Distribution of Decision Criteria for COU:* The distribution of the combined (or *composite*) HLA-DR/DQ molecular mismatch scores in a consecutive unselected patient cohort of adult and pediatric kidney transplant recipients from both living and deceased donors (n=596) is as follows: **Low Risk** – 14.4%, **Intermediate Risk** – 17.6%, and **High Risk** – 68.0%.
- *Decision Criteria Cut-offs and Application to COU:* Once a prognostic risk category is assigned then risk categories to be used independently, in pairs or triplet risk categories to **enrich phase 2 and 3 clinical trials with patients based upon risk category** in studies evaluating novel drugs. For example, high risk patients could be enrolled in RCTs evaluating novel immunosuppressive drugs versus SOC (i.e. an enrichment strategy for the primary clinical outcome of *de novo* DSA, graft rejection and graft loss).
- *Clinical Validation:* The HLA-DR/DQ molecular mismatch score (derived from HLA DRDQDP Matching software) has been evaluated in three independent cohorts of patients:
  1. ! *A prospective consecutive cohort of unselected kidney transplant recipients.*<sup>7,9</sup> This cohort is used (a) to derive the optimized risk cut-offs for HLA-DR or HLA DQ molecular mismatch score correlating with *de novo* HLA-DR or HLA-DQ DSA respectively; (b) to determine in multivariate models, other independent factors associated with *de novo* DSA, graft rejection or graft loss; (c) to determine the modulatory effect of HLA-DR/DQ molecular mismatch scores on the levels of tacrolimus required to prevent *de novo* HLA-DR/DQ DSA formation – low/intermediate risk patients tolerated lower tacrolimus levels without developing a *de novo* HLA-DR/DQ DSA, whereas high risk patients with the same frequency below these tacrolimus levels as the low/intermediate risk patients experienced increased rates of *de novo* DSA.
  2. ! *A prospective cohort of patients who were monitored for early medication non-adherence with electronic pill-cap monitoring* (i.e. gold standard to assess adherence) and retrospectively analyzed to determine the HLA-DR/DQ molecular mismatch scores.<sup>8</sup> This cohort demonstrates that pre-determined HLA-DR/DQ molecular scores in the high risk category interact synergistically with medication non-adherence to increase the risk of graft rejection and graft loss.
  3. ! *The NIH Clinical Trials in Organ Transplantation (CTOT)-09 RCT.*<sup>13</sup> This RCT applying **the current standard** (e.g. first transplant, living donor, low PRA, adult and Caucasian recipient) **to indicate “low risk”** for post-transplant primary alloimmune events, proved the current standard held no prognostic utility to identify those at low risk for withdrawing CNI immunosuppression – the study was terminated by the Drug Safety Monitoring Board as the CNI withdrawal arm hit stopping rules for graft rejection and *de novo* DSA events. A post-hoc analysis of the HLA-DQ molecular mismatch score determined that all patients in the CNI withdrawal arm who developed a *de novo* HLA-DQ DSA were in the HLA-DQ molecular mismatch score high risk category, as compared to patients who remained *de novo* HLA-DQ DSA free off CNI who were predominantly in the low/intermediate risk categories.<sup>13</sup>
- *Benefits and Risks of Applying Clinical Decision Tool:* To date, evaluation of novel drugs focus on the average treatment effect in the overall kidney transplant population requiring phase 3 clinical trials lasting  $\geq 5$  years and relatively large sample sizes to demonstrate superiority to SOC.<sup>1,2</sup> This is due in part to the lack of robust prognostic biomarkers to define the pre-transplant risk for primarily alloimmune events resulting in the inclusion of very heterogeneous populations at risk. Use of the HLA-DR/DQ molecular mismatch score as a prognostic marker should significantly address this issue to improve RCT efficiency (enrichment strategy).

The risk to the patient of misclassification is related to assigning them to a low/intermediate risk category when in fact the individual is at high risk – this would be especially true in CNI substitution trials. Conversely, in patients misclassified as high risk when in fact they are at low/intermediate risk may lead to inclusion in trials of increased immunosuppression when in fact they don't require it. However, the **current standard** of categorization of immune risk in clinical transplant RCTs is already putting patients at both of these described risks. To mitigate misclassification, ongoing studies are enhancing the accuracy of the prognostic biomarker.

The general risk to applying the HLA-DR/DQ molecular mismatch score as a prognostic biomarker enrichment strategy in drug development RCTs relates to qualifying a drug in only a sub-population of kidney transplant patients when in fact there is a benefit of the drug for the entire population versus SOC. This risk can be mitigated by validating the prognostic utility of the biomarker in a prospective RCT that uses a biomarker-stratified design (i.e. randomly assigning to high/intermediate/low risk groups to the investigational therapy).<sup>4</sup>

- *Describe Knowledge Gaps, Limitations and Assumptions:* It is not clear if an HLA-DR/DQ molecular mismatch score and thresholds will be equally prognostic in all races. While there is no reason to presuppose it will not (the process of allorecognition is not race specific) it nonetheless needs to be proven as the majority of the data to date has been generated in Caucasian, Indigenous American, or Asian transplant recipients.

Most transplant recipients are also mismatched for Class I HLA (i.e. HLA-A, -B, -Cw) molecules in addition to HLA-DR/DQ. Therefore, in using a molecular mismatch score only focused on HLA-DR/DQ one is assuming that the information derived from a similar evaluation of the Class I HLA mismatches does not appreciably prognosticate the primary alloimmune risk. Supporting this assumption, in our natural history cohort study noted above, the frequency of isolated Class I driven primary alloimmune responses leading to graft loss is very low over the first 10 years post-transplant.<sup>9</sup>

## Supporting Information

### Summary of existing preclinical or clinical data

The **current approach** to prognosticate primary alloimmune risk related to HLA is to consider whole molecule (i.e. antigen) level mismatches between the kidney donor and the transplant recipient. Over the years studies have found a correlation between donor-recipient HLA mismatch (e.g. HLA DR mismatch) with post-transplant primary alloimmune outcomes and death-censored graft survival.<sup>2</sup> Indeed, this is why HLA DR matching is still awarded points in the current United Network for Organ Sharing (UNOS) kidney allocation algorithm in the USA.

However, a 1 DR or 1 DQ whole molecule mismatch may represent a wide range of HLA-DR or HLA-DQ molecular mismatches (e.g. eplet mismatches).<sup>7,9</sup> Because whole molecule mismatch assessment for HLA-DR or HLA-DQ can each only be scored as a 0, 1, or 2 mismatch, it has limited utility to prognosticate post-transplant primary alloimmune events at the individual level. By comparison, in kidney transplantation a recipient's level of HLA-DR/DQ molecular mismatch (i.e. HLA-DR/DQ eplet mismatch) score retains more granular data and as such:

- (1) Is a strong independent correlate with HLA-DR/DQ *de novo* DSA, graft rejection, transplant ! glomerulopathy, and graft loss.<sup>2,7,9</sup> !
- (2) Interacts synergistically with medication nonadherence correlating with graft rejection and graft loss.<sup>8</sup>
- (3) Modulates the tacrolimus trough level required to prevent *de novo* DSA development.<sup>9</sup>
- (4) Predicts CNI minimization tolerability at an individual level for the risk of *de novo* DSA formation.<sup>13</sup>

### Summary of planned studies to support the biomarker and COU

Ongoing work by our group is continuing to develop new strategies to refine the accuracy of determining the HLA-DR/DQ molecular mismatch score to derive more accurate risk categories as a prognostic marker for primary alloimmune events. In addition, investigator-initiated biomarker-stratified RCTs are being planned to validate the prognostic utility of the HLA-DR/DQ molecular mismatch score.

### Alternative/comparator approaches

Alternative computational tools for HLA-DR/DQ molecular mismatch assessment are available. As recently reported by our group these tools are highly correlated with one another and perform equally well as prognostic biomarkers of risk for *de novo* HLA-DR/DQ DSA in multivariate models.<sup>14</sup>

## Previous Qualification Interactions and Other Approvals

- Letter of Support (LOS) issued for this biomarker: **None**
- Discussed in a Critical Path Innovation Meeting (CPIM): **None**
- Previous FDA Qualification given to this biomarker with DDT Tracking Record Number: **None**
- Qualification submissions to any other agencies with submission number: **None**
- Prior clearances or approvals: **None**
- Prior or current Regulatory submissions: **None**

## Attachments

### Publications Referenced

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