Ixazomib for Desensitization (IXADES)

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CLINICAL STUDY PROTOCOL

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Ixazomib for Desensitization (IXADES)

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PROTOCOL SUMMARY

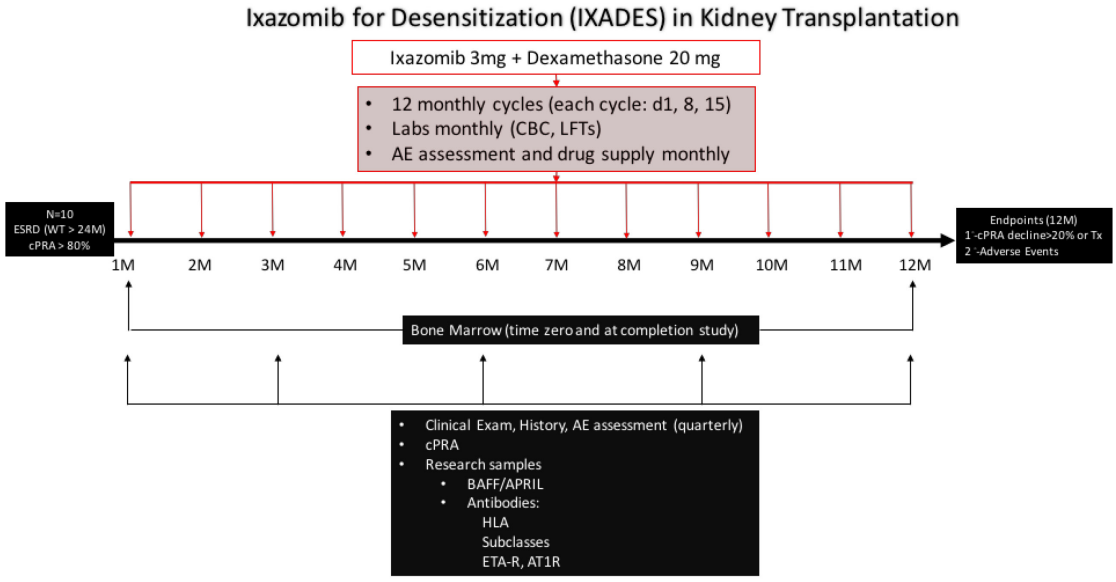
This is a pilot exploratory, proof of concept, open-label, single-center phase II investigator initiated clinical trial entitled IXAzomib for DESensitization (IXADES). The purpose of the study is (1) to examine the safety and efficacy of ixazomib for desensitization of highly sensitized kidney transplant candidates and (2) to conduct mechanistic studies to address the role of HLA and non-HLA antibodies, T and B cell phenotypes, and BAFF/APRIL in immune monitoring of sensitized kidney transplant candidates (Figure 1).

Specific Aim 1. To determine the safety and efficacy of ixazomib as a desensitization strategy. There is currenty no effective desensitization strategy for highly sensitized patients defined as calculated Panel of Reactive Antibodies (cPRA) ≥ 80%. For this study, 10 highly sensitized kidney transplant candidates on the waitlist for more than 24 months will receive ixazomib 3 mg (and dexamethasone 20 mg) on days 1, 8, and 15 of a 28 cycle for 12 months. The primary objective is to evaluate the safety (distal neuropathy, thrombocytopenia, and gastrointestinal symptoms) and efficacy (decline in cPRA > 20%) of ixazomib. The secondary efficacy endpoint is transplantation rate within 12 months of therapy.

Specific Aim 2. Identify immune indices which predict the course of disease and/or response to treatment in highly sensitized patients. Mechanistic studies will use bone marrow and blood obtained from subjects in Aim 1 to determine the effect of treatment on immune regulation and reconstitution after therapy. Since the bone marrow microenvironment produces BAFF/APRIL and supports plasma cell maturation, we will determine the effect of therapy on the generation of BAFF/APRIL by bone marrow mesenchymal stem cells and the survival of bone marrow-derived plasma cells after desensitization. We specifically propose to:

- Identify if bone marrow plasma cells, IgG subsets, and levels including free light chains, and circulating BAFF/APRIL predict outcomes.
- Determine if treatment is effective in downregulating circulating BAFF/APRIL and anti-HLA, endothelin-1 type A receptor (ETAR), angiotensin type 1 receptor (AT1R), and complement fixing C1q antibodies.

Figure 1. Protocol Summary



Study Title: IXAzomib for **DES**ensitization (**IXADES**)

Phase: II

Number of Patients: 10

Study Objectives

The primary objective is to evaluate the efficacy of ixazomib in desensitizing highly sensitized kidney transplant recipient, defined as a decline in calculated PRA (cPRA) > 20%, or successful transplantation within 12 months.

The secondary objective is to determine the toxicity and tolerability of ixazomib with particular focus on distal neuropathy, thrombocytopenia, and gastrointestinal symptoms.

Overview of Study Design:

We will examine the safety and efficacy of ixazomib for desensitization of highly sensitized kidney transplant candidates and conduct mechanistic studies to address the role of IgG subclasses, IgG complement binding capacity, non-HLA antibodies, T and B cell phenotypes, and BAFF/APRIL in immune monitoring and regulation.

- Primary efficacy and safety endpoint:
  - Efficacy endpoint: A decline in cPRA > 20%.
  - Safety endpoint: infections, malignancies, hematological complications including leucopenia, anemia, and thrombocytopenia, cardiovascular complications and events, distal neuropathy, gastrointestinal symptoms, and immunosuppression-related adverse effects
- Secondary efficacy endpoint: Successful transplantation within 12 months
- Tertiary/Exploratory Endpoints/Correlataive studies: Defining a marker of disease activity in sensitized patients will have a significant impact on the future of desensitization protocols. Although DSA, cPRA, CDC and T cell and B cell flow crossmatch have been used to characterize the severity of sensitization, these assays are labor intensive and expensive. The proposed *plasma BAFF/APRIL* ELISA assays can be performed in 2-3 hours (1). We will determine whether these cytokines can reflect the immune response and predict response to desensitization and ultimately transplant outcome. *In addition*, we propose studies to address the effect of ixazomib on (a) *bone marrow derived BAFF/APRIL* generated by bone marrow-mesenchymal stem cells, knowing that these stromal cells play a key role during the maturation activation of plasma cells, (b) *bone marrow-derived antibody secreting cells (plasma cells)* (2) and (c) plasma *IgG subclasses, anti-HLA, ETAR, AT1R, and C1q antibody* response to treatment (3, 4).

Study Population:

Patients will be recruited from our center. All patients must be **(1)** Male or female patients 18-70 years of age **(2)** Able to provide informed consent **(3)** Female patients who are postmenopausal for at least 1 year before the screening visit, or are surgically sterile, or If they are of childbearing potential, agree to practice 2 effective methods of contraception, at the same time, from the time of signing the informed consent form through 90 days after the last dose of study drug, OR agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception.) **(4)** Male patients, even if surgically sterilized (ie, status post-vasectomy), must agree to one of the following: Agree to practice effective barrier contraception during the entire study treatment period and through 90 days after the last dose of study drug, or Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception.) **(5)** Patients must be highly sensitized with a cPRA ≥ 80% **(6)** Be active on the waitlist for kidney transplantation > 24 months to confirm their inability to receive a deceased donor transplant because of their sensitization status **(7)** Patients must meet the following clinical laboratory criteria (a) Absolute neutrophil count (ANC) $\geq 1{,}000/mm^3$ and platelet count $\geq 75{,}000/mm^3$. Platelet transfusions to help patients meet eligibility criteria are not allowed within 21 days before study enrollment (b) Total bilirubin $\leq 1.5 \times$ the upper limit of the normal range (ULN) (c) Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leq 3 \times$ ULN.

<u>Exclusion criteria</u>: Patients will be excluded from the study based on the following criteria: **(1)** Female patients who are lactating or have a positive serum pregnancy test during the screening period **(2)** Major surgery requiring hospitalization within 6 months before enrollment **(3)** Infection requiring systemic antibiotic therapy or other serious infection within 14 days before study enrollment. **(4)** Evidence of current uncontrolled cardiovascular conditions, including uncontrolled hypertension, uncontrolled cardiac arrhythmias, symptomatic congestive heart failure, unstable angina, or myocardial infarction within the past 6 months **(5)** Systemic treatment, within 14 days before the first dose of ixazomib, with strong CYP3A inducers (rifampin, rifapentine, rifabutin, carbamazepine, phenytoin, phenobarbital), or use of St. John's wort. **(6)** Any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of treatment according to this protocol **(7)** Inability to take oral medication **(8)** Diagnosed or treated for another malignancy within 2 years before study enrollment or previously diagnosed with another malignancy and have any evidence of residual disease. Patients with nonmelanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection. **(9)** Grade 2 or greater peripheral neuropathy **(10)** Participation in other desensitization therapies or investigational drug clinical trials, including those with other investigational agents not included in this trial, within 6 months of the start of this trial and throughout the duration of this trial **(11)** Patients that have previously been treated with ixazomib, or participated in a study with ixazomib whether treated with ixazomib or not **(12)** Active or treated infection for HIV, HCV or HBV **(13)** History of liver cirrhosis, biopsy confirmed **(14)** Elevated transaminases (greater than 3 times the upper limit of normal) **(15)** Known hypersensitivity to ixazomib **(16)** Active substance abuse by self-report or medical record **(17)** Electrocardiography evidence of acute ischemia or active conduction system abnormalities

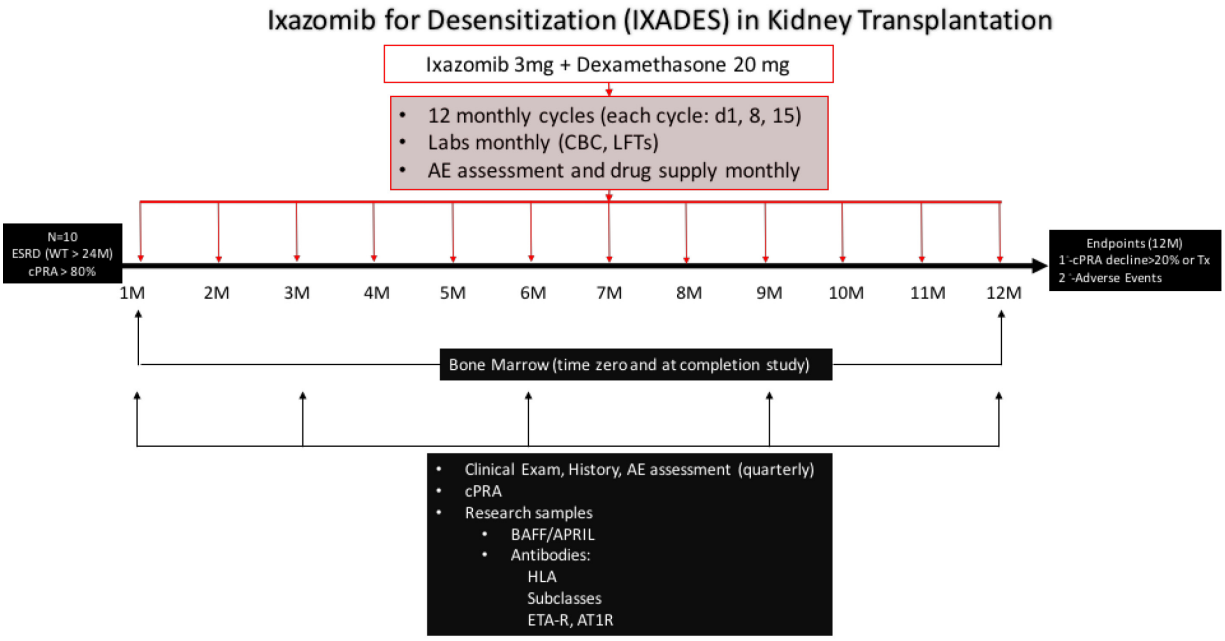
Duration of Study: Patients will be on study for one year, plus 1 month. For those transplanted, data on rejection, graft and patient survival will be collected for 6 months.

STUDY OVERVIEW DIAGRAM *(optional)*

Table 1-1 IXADES Treatment Protocol

28-Day Cycle (a 4-week cycle)								
	Week 1		Week 2		Week 3		Week 4	
	Day 1	Days 2-7	Day 8	Days 9-14	Day 15	Days 16-21	Day 22	Days 23-28
Ixazomib (NINLARO) 3 mg	✔		✔		✔			
Dexamethasone 20 mg	✔		✔		✔			

Figure 1. Study Overview Diagram



SCHEDULE OF EVENTS

Table 2-1 (a)-Schedule of events (first six cycles)

Procedures Performed	Screening (</= 30 days)	Cycle 1 (< /= 3 months after screening)			Cycle 2			Cycle 3			Cycle 4			Cycle 5			Cycle 6		
Treatment Days (+6, -3)		1	8	15	29	36	43	57	64	71	85	92	99	113	120	127	141	148	155
Ixazomib and Dexamethasone		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Medical History	X	X									X			X			X		
Physical Exam	X	X*									X								
Vital Signs (T, P, R, BP)	X	X			X			X			X			X			X		
AE Assessment		X			X			X			X			X			X		
Electrocardiogram (ECG)	X																		
Echocardiodiogram	X																		
Medication History	X	X			X			X			X			X			X		
Drug Accountability					X			X			X			X			X		
Pregnancy Test (where appropriate)	X																		
CBC	X	X			X			X			X			X			X		
LFTs	X	X		X	X		X	X	X		X			X			X		
cPRA		X									X								
BAFF/APRIL		X									X								
DSA Antibodies		X									X								
Bone Marrow Aspiration		X[1]																	

*Physical examination to be completed at visit 1, if not completed during screening visit.

1 Bone Marrow Aspiration must be done before drug administration and at one of the following endpoints: one month (+6,- 3 days) after the end of the 12 month study, cPRA decline > 20%, time of transplant, or time of subject withdrawal from the study.

Table 2-1 (b)-Schedule of events (last six cycles)

Procedures Performed	Cycle 7			Cycle 8			Cycle 9			Cycle 10			Cycle 11			Cycle 12			Final visit 1M after last dose	ETV or Unscheduled Visit
Treatment Days	169	176	183	197	204	211	225	232	239	253	260	267	281	288	295	309	316	323	373	
Ixazomib and dexamethasone	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Medical History	X									X									X	
Physical Exam	X									X									X	
Vital Signs (T, P, R, BP)	X			X			X			X			X			X			X	X
AE Assessment	X			X			X			X			X			X			X	X
Electrocardiogram (ECG)	X																		X	
Echocardiogram	X																		X	
Medication History	X			X			X			X			X			X			X	X
Drug Accountability				X			X			X			X			X			X	X
Pregnancy Test (where appropriate)																				
CBC	X			X			X			X			X			X			X	
LFTs	X			X			X			X			X			X			X	
cPRA	X									X									X	
BAFF/APRIL	X									X									X	
DSA Antibodies	X									X									X	
Bone Marrow Aspiration																			X[1]	X[1]

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LIST OF ABBREVIATIONS AND GLOSSARY OF TERMS

AE: Adverse Event
AL: Amyloid Light Chain
AMR: Antibody Mediated Rejection
APRIL: A Proliferation-Inducing Ligand
AUC: Area under the Curve
BAFF: B cell activating factor
C4d: component of complement
CBC: Complete Blood Count
CNI: Calcineurin Inhibitor
cPRA: calculated Panel Reactive Antibody
CSA: Cylosporine A
CYP: Cytochrome P
DLT: Dose-Limiting Toxicity
DMC: Data Monitoring Committee
DSA: Donor Specific Antibodies
ESRD: End-stage renal disease
FDA: Food and Drug Administration
GI: gastrointestinal
H&P: History and Physical
HLA: Histocompatibility antigens
ICTR: Institute for Clinical and Translational Research
IgG: Immunoglobulin G
IV: Intravenous
IXADES: IXAzomib for DESensitization
LFTs: Liver Function Tests
MTD: Maximum Tolerated Dose
NDMM: Newly Diagnosed Multiple Myeloma
PK: Pharmacokinetics
PO: Oral (by mouth)
RP2D: Recommended Phase 2 Dose
RRMM: Relapse/Refractory Multiple Myeloma
SMS: Study Monitoring Service
TEAE: Treatment-Emergent Adverse Event
TLR: Toll-like Receptors
ULN: Upper Limit of Normal

1. BACKGROUND AND STUDY RATIONALE

1.1 Scientific Background

1.1.1 Disease Under Treatment

The disease under treatment is sensitization in kidney transplant candidates. One of the major challenges in the current era of organ transplantation is to find suitable organs for highly sensitized patients defined as a cPRA > 80%. The cPRA may be calculated at https://optn.transplant.hrsa.gov/resources/allocation-calculators/cpra-calculator/. These patients have developed antibodies against major histocompatibility (HLA) antigens due to previous transplantation, blood transfusion, or pregnancy. Despite some success, current desensitization protocols are limited because of their complexity and lack of effective agents against plasma cells, which actually produce anti-HLA antibodies. As a result, many patients die on dialysis prior to receiving a transplant (5, 6).

The two approaches for helping highly sensitized patients are: 1) To increase the chance of finding a crossmatch negative donor, or 2) To remove the pre-existing antibodies using desensitization protocols (7-11). Emerging evidence suggests that strategies to improve transplant rates in highly sensitized patients enhance survival rates and the quality of life, while reducing costs compared to chronic dialysis (12, 13). Current desensitization protocols combine Rituximab (anti-CD20 monoclonal antibody) to deplete B cells, and plasmapheresis plus intravenous immunoglobulins (IVIG) to block or remove preformed DSA (7-11). Since no agent previously used in transplantation has the adequate ability to inhibit mature plasma cells, there has been no effective way of reducing or preventing antibody production besides targeting B cells. Therefore, despite some success, contemporary desensitization protocols are limited by their inability to desensitize 30 to 90% of patients (5, 8, 14). For example, using a preconditioning regimen similar to the one used at our institution combining plasmapheresis, IVIG and Rituximab, the Mayo Clinic Transplant Program was unable to desensitize 15/29 patients who had an CDC crossmatch titer ≥ 1:8 (8). Similarly, Marfo et al demonstrated that pretransplant desensitization with high dose IVIG and rituximab was not successful in sensitized kidney transplant candidates with cPRA levels higher than 90% (5). It is for this reason that we propose a pilot desensitization study targeting the cells responsible for generating alloantibodies using ixazomib (Ninlaro®), a second generation proteasome inhibitor, recently approved therapy for multiple myeloma.

There is conflicting data regarding the effectiveness of bortezomib, a first generation proteasome inhibitor, in desensitization for kidney transplant candidates (15, 16). The Cincinnati group reported a positive experience (26.3% responders) while the Mayo group did not show a favorable safety and efficacy profile. However, none of this studies addressed the effect of bortezomib on target bone marrow plasma cells. In addition, clinical and preclinical studies suggest that ixazomib has a superior safety and efficacy profile than bortezomib (17, 18). Ixazomib has a shorter proteasome dissociation half-life and improved pharmacokinetics, pharmacodynamics, and antitumor activity compared with bortezomib. This is important as there is a correlation between greater pharmacodynamic responses and improved antitumor activity (18). In a double-blind, placebo-controlled, phase 3 trial of 722 patients with relapsed, refractory (including to bortezomib), or relapsed and refractory multiple myeloma, the addition of ixazomib to a regimen of lenalidomide and dexamethasone was associated with significantly longer progression-free survival; the additional toxic effects with this all-oral regimen were limited (17). Lastly, The use of this oral drug is also significantly more convenient for patients on chronic dialysis who need dialysis therapy three times per week.

Our long-term goal is to improve the quality-of-life and survival of kidney transplant recipients. Our specific hypothesis for this proposal is that ixazomib can significantly reduce DSA production and allow for kidney transplantation in highly sensitized patients. Ixazomib is a second generation proteasome inhibitor, recently approved by the FDA for the treatment of multiple myeloma. We further hypothesize that specific molecular and cellular indices can be predictive of treatment response and toxicity. These hypotheses are based on preliminary findings demonstrating that: (a) The first-generation proteasome inhibitor, bortezomib (Velcade®), is an effective option for the treatment and prevention of antibody mediated rejection (AMR) in clinical settings; (b) Ixazomib prevents acute AMR in a robust preclinical model of kidney transplantation by downregulating B cell activation, antigen presentation, T cell and B cell signaling, and microcirculation inflammation; (c) IgG subclasses, IgG complement binding capacity, and serum B cell activating factor (BAFF) and APRIL (a proliferation-inducing ligand) are associated with AMR and graft loss in kidney transplantation.

Based on these observations and the critical need to define effective desensitization strategies, we propose a pilot exploratory, proof of concept, open-label, single-center phase II clinical trial entitled **IXA**zomib for **DES**ensitization (**IXADES**). We will examine the safety and efficacy of ixazomib for desensitization of highly sensitized kidney transplant candidates and conduct mechanistic studies to address the role of IgG subclasses, IgG complement binding capacity, non-HLA antibodies, T and B cell phenotypes, and BAFF/APRIL in immune monitoring and regulation.

1.1.2 Ixazomib (MLN9708)

1.2 Preclinical Experience

There is a need for new immunosuppression strategies to minimize calcineurin inhibitor (CNI) toxicity while effectively preventing antibody-mediated rejection (AMR). We have been the first group to examine the impact of ixazomib on AMR in kidney transplantation. We tested the efficacy of an investigational proteasome inhibitor, ixazomib, alone and in a CNI minimization strategy in a rat kidney transplant model of transfusion-elicited acute AMR. Nonsensitized (naïve) and sensitized allograft recipients were randomized into 4 treatment groups (8 groups total, n = 3 to 6 in each group) and treated for 1 week. Groups included: no treatment, full dose cyclosporine (CsA, 10 mg/kg per day), ixazomib (0.25 mg/kg on days -5, -2 and +2) alone, and half dose CsA (5 mg/kg per day) + ixazomib (19).

Compared to untreated animals, ixazomib alone or in combination with ½ dose CsA reduced donor-specific antibody, intragraft transcripts for chemokines CCL-21 and CXCL-13, and CD19 expression in both sensitized and naïve transplants. Compared to full dose CsA, the CNI minimization strategy with ixazomib inhibited AMR and allograft injury as evidenced by reduced C4d staining in peritubular capillaries, microcirculation inflammation, splenic plasma cells, circulating B cell activating factor, and intragraft transcripts for major histocompatibility complex class II, Toll-like receptors (TLR-1, TLR-10, and TLR-12) and CCL-21 and CXCL-13 in sensitized animals, indicating downregulation of B cell activation, antigen presentation and T-cell and B-cell signaling.

These studies suggest that CNI minimization strategies including ixazomib are effective to prevent AMR including in sensitized kidney allograft recipients. Clinical studies are needed to determine the role of novel proteasome inhibitors for the prevention and treatment of AMR.

1.3 Clinical Experience

Ixazomib has been evaluated as an oral single agent in phase 1 studies that have included patients with advanced solid tumors, lymphoma, relapse/refractory MM (RRMM), and relapsed or refractory light-chain (AL) amyloidosis and demonstrated early signs of activity. Ongoing studies continue to investigate both single-agent ixazomib and ixazomib in combination with standard treatments. Based on encouraging preliminary data observed in patients with MM requiring systemic treatment, 2 phase 3 trials in newly diagnosed MM (NDMM) (C16014) and RRMM (C16010) patient populations are currently evaluating ixazomib in combination with Revlimid and Dexamethasone (RevDex) versus placebo/RevDex. Both trials are combining ixazomib at a weekly dose of 4.0 mg on Days 1, 8, and 15 in a 28-day cycle to a standard dose of lenalidomide with a weekly dexamethasone dose of 40 mg. In addition, clinical pharmacology studies have evaluated drug-drug interactions with ketoconazole, clarithromycin, and rifampin, as well as the effect of food, renal impairment, and hepatic impairment on the PK of ixazomib. Studies evaluating the safety and pharmacokinetics (PK) of ixazomib alone (in Japanese patients) and in combination with lenalidomide and dexamethasone in Asian adult patients (including Japanese patients) with a diagnosis of RRMM are ongoing.

As of 27 March 2013, preliminary clinical data is available for a total of 653 patients across 13 studies. The emerging safety profile indicates that ixazomib is generally well tolerated. The adverse events (AEs) are consistent with the class-based effects of proteasome inhibition and are similar to what has been previously reported with VELCADE though the severity of some, for example peripheral neuropathy, is less. While some of these potential toxicities may be severe, they can be managed by clinical monitoring and standard medical intervention, or, as needed, dose modification or discontinuation.

Fatigue was the most common AE reported among 384 patients treated in the oral (PO) studies (47%). Other common AEs reported in the pooled intravenous (IV) and PO safety populations include nausea, thrombocytopenia, diarrhea, and vomiting. Rash is also a commonly reported treatment-emergent event; however, there is some variety in its characterization and causality resulting in different preferred terms to describe it. A high-level term outline of rash events includes rashes, eruptions and exanthems NEC; pruritus NEC; erythemas; papulosquamous conditions; and exfoliative conditions. The dose escalation phases of most trials reported in the IB have now completed enrollment, and gastrointestinal (GI) symptoms were the common dose-limiting toxicities (DLTs) when the use of prophylactic anti-emetics was not permitted per protocol. In the expansion cohorts or phase 2 cohorts (as per each study), the incidence and severity of GI symptoms was mitigated by the use of the lower maximum tolerated dose (MTD)/recommended phase 2 dose (RP2D) (as per each study) and standard clinical usage of anti-emetics and/or antidiarrheal medications as deemed appropriate. Prophylactic use of anti-emetics has not been required as with other agents but (as outlined in Section 6.7) has been used according to standard practice and are effective.

The most frequent (at least 20%) treatment-emergent adverse events (TEAEs) reported with the PO formulation pooled from single-agent studies (n = 201) irrespective of causality to ixazomib, include nausea (53%), fatigue (51%), diarrhea (44%), thrombocytopenia (34%), vomiting (38%), decreased appetite (32%), fever (21%), and anemia (21%). The most frequent (at least 20%) TEAEs reported with the PO formulation pooled from combination trials (irrespective of the combination) (n = 173), irrespective of causality to ixazomib, include diarrhea (47%), fatigue (44%), nausea (38%), peripheral edema (35%), constipation (33%), insomnia (29%), thrombocytopenia (28%), anemia (26%), vomiting (26%), neutropenia (25%), back pain (24%), pyrexia (23%), peripheral edema (21%, each), fever (20%), cough (20%), hypokalemia (20%),

neutropenia (20%), and upper respiratory tract infection (20%). Overall rash of all grades is reported in approximately 50% of patients and is more common when ixazomib is given in combination with lenalidomide where rash is an overlapping toxicity.

Additional detailed information regarding the clinical experience of ixazomib may be found in the IB, including information on the IV formulation.

1.4 Pharmacokinetics and Drug Metabolism

After oral dosing, absorption of ixazomib is rapid with a median first time to maximum observed plasma concentration (T_{max}) of approximately 1 hour postdose. The plasma exposure (AUC) of ixazomib increases in a dose-proportional manner over a dose range of 0.2 to 10.6 mg based on population PK analysis. The absolute oral bioavailability (F) of ixazomib is estimated to be 58% based on population PK analysis. A high-fat meal reduced ixazomib C_{max} by 69% and AUC_{0-216} by 28%. This indicates that a high-fat meal decreases both the rate and extent of absorption of ixazomib. Therefore, ixazomib should be dosed at least 2 hours after food or 1 hour before food.

The steady-state volume of distribution of ixazomib is large and is estimated to be 543 L based on a population PK model. Based on in vitro plasma protein binding measurements on samples from clinical studies (Studies C16015 and C16018), ixazomib is highly bound to plasma proteins (99%). Ixazomib concentrations are higher in whole blood than in plasma, indicating extensive partitioning of ixazomib into red blood cells, which are known to contain high concentrations of the 20S proteasome.

Metabolism appears to be the major route of elimination for ixazomib. In vitro studies indicate that ixazomib is metabolized by multiple cytochrome P450 (CYP) and non-CYP proteins. At concentrations exceeding those observed clinically (10 µM), ixazomib was metabolized by multiple CYP isoforms with estimated relative contributions of 3A4 (42.3%), 1A2 (26.1%), 2B6 (16.0%), 2C8 (6.0%), 2D6 (4.8%), 2C19 (4.8%), and 2C9 (<1%). At 0.1 and 0.5 µM substrate concentrations, which are closer to clinical concentrations of ixazomib following oral administration of 4 mg ixazomib, non-CYP mediated clearance was observed and seemed to play a major role in ixazomib clearance in vitro. These data indicate that at clinically relevant concentrations of ixazomib, non-CYP proteins contribute to the clearance of ixazomib and no specific CYP isozyme predominantly contributes to the clearance of ixazomib. Therefore, at clinically relevant concentrations of ixazomib, minimal CYP-mediated DDIs with a selective CYP inhibitor would be expected.

Ixazomib is neither a time-dependent inhibitor nor a reversible inhibitor of CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4/5. Ixazomib did not induce CYPs 1A2, 2B6, and 3A4/5 activity or corresponding immunoreactive protein levels. Thus, the potential for ixazomib to produce DDIs via CYP isozyme induction or inhibition is low.

Ixazomib is not a substrate of BCRP, MRP2 and OATPs. Ixazomib is not an inhibitor of Pgp-, BCRP, MRP2, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2K. Ixazomib- is unlikely to cause or be susceptible to clinical DDIs with substrates or inhibitors of clinically relevant drug transporters.

The geometric mean terminal halflife- ($t_{1/2}$) of ixazomib is 9.5 days based on population PK analysis. For both IV and oral dosing, there is an approximately average 3fold accumulation (based on AUC) following the Day 11 dose for the -twiceweekly- schedule and a 2fold accumulation (based on AUC) following the Day 15 dose for the -onceweekly- schedule.

Mean plasma clearance (CL) of ixazomib is 1.86 L/hr based on the results of a population PK analysis. Taken together with the blood-to-plasma AUC ratio of approximately 10, it can be inferred that ixazomib is a low clearance drug. Using the absolute oral bioavailability (F) estimate of 58% (also from a population PK model), this translates to an apparent oral plasma clearance (CL/F) of 3.21 L/hr. The geometric mean renal clearance for ixazomib is 0.119 L/hr, which is 3.7% of CL/F and 6.4% of CL estimated in a population PK analysis. Therefore, renal clearance does not meaningfully contribute to ixazomib clearance in humans. Approximately 62% of the administered radioactivity in the ADME study (Study C16016) was recovered in the urine and 22% of the total radioactivity was recovered in the feces after oral administration. Only 3.2% of the administered ixazomib dose was recovered in the urine as unchanged ixazomib up to 168 hours after oral dosing, suggesting that most of the total radioactivity in urine was attributable to metabolites.

The PK of ixazomib was similar with and without co-administration of clarithromycin, a strong CYP3A inhibitor, and hence no dose adjustment is necessary when ixazomib is administered with strong CYP3A inhibitors. Consistently, in a population PK analysis, coadministration of strong CYP1A2 inhibitors did not affect ixazomib clearance. Therefore, no dose adjustment is required for patients receiving strong inhibitors of CYP1A2. Based on information from the clinical rifampin DDI study, ixazomib -C_{max} and $AUC_{0\text{-last}}$ were reduced in the presence of rifampin by approximately 54% and 74%, respectively. Therefore, the co-administration of strong CYP3A inducers with ixazomib is not recommended.

Mild or moderate renal impairment (CrCL ≥ 30 mL/min) did not alter the PK of ixazomib based on the results from a population PK analysis. As a result, no dose adjustment is required for patients with mild or moderate renal impairment. In a dedicated renal impairment study (C16015), unbound $AUC_{0\text{-last}}$ was 38% higher in patients with severe renal impairment or ESRD patients requiring dialysis as compared to patients with normal renal function. Accordingly, a reduced starting dose of ixazomib is appropriate in patients with severe renal impairment or ESRD requiring dialysis. Pre- and post-dialyzer concentrations of ixazomib measured during the hemodialysis session were similar, suggesting that ixazomib is not readily dialyzable, consistent with its high plasma protein binding (99%).

The PK of ixazomib is similar in patients with normal hepatic function and in patients with mild hepatic impairment, as defined by the National Cancer Institute Organ Dysfunction Working Group (total bilirubin <1.5 times the upper limit of normal [ULN]), based on the results from a population PK analysis. Consequently, no dose adjustment is required for patients with mild hepatic impairment. In a dedicated PK study in patients with moderate (total bilirubin >1.5 to 3 times the ULN) or severe (total bilirubin >3 times the ULN) hepatic impairment (Study C16018), unbound dose-normalized $AUC_{0\text{-last}}$ was 27% higher in patients with moderate or severe hepatic impairment as compared to patients with normal hepatic function. Therefore, a reduced starting dose of ixazomib is appropriate in patients with moderate or severe hepatic impairment.

There was no statistically significant effect of age (23-91 years), sex, body surface area (1.2-2.7 m^2), or race on the clearance of ixazomib based on the results from a population PK analysis.

Further details on these studies are provided in the IB.

1.5 Clinical Trial Experience Using the Oral Formulation of Ixazomib

As of 27 March 2013, a total of 507 patients with differing malignancies (multiple myeloma, AL amyloidosis, nonhematologic cancers, and lymphoma) have been treated in studies evaluating the oral ixazomib formulation. These patients have been treated with different doses of ixazomib either as a single-agent treatment (in 201 patients) or in combination with currently clinically available treatments (in 306 patients). Information regarding the ongoing studies, patient populations, and doses investigated is included in Table 3-1.

Table 3-1-1 Clinical Studies of Oral Ixazomib

Trial/ Population	Description	Doses Investigated
C16003 RRMM N = 60	PO, TW, single agent	0.24-2.23 mg/m^2 TW MTD: 2.0 mg/m^2 DLT: rash, thrombocytopenia Closed to enrollment
C16004 RRMM N = 60	PO, W, single agent	0.24-3.95 mg/m^2 W MTD: 2.97 mg/m^2 DLT: rash, nausea, vomiting, diarrhea Closed to enrollment
C16005 NDMM N = 65	PO, W, combination with LenDex 28-day cycle	1.68-3.95 mg/m^2 W MTD: 2.97 mg/m^2 DLT: nausea, vomiting, diarrhea, syncope RP2D[a]: 4.0 mg fixed (switched to fixed dosing in phase 2, equivalent to 2.23mg/m^2) Closed to enrollment
C16006 NDMM N = 20	PO, TW (Arm A- 42 day cycle) and W (Arm B- 28 day cycle), combination with Melphalan and Prednisone	Arm A[a]: 3-3.7-mg fixed dose TW DLT: rash, thrombocytopenia, subileus Arm B[a]: 3-5.5-mg fixed dose, W DLT: Esophageal ulcer nausea, vomiting, hematemesis, thrombocytopenia, ileus, neurogenic bladder MTD = 3.0 mg
C16007 RRAL N = 27	PO, W, single agent	4-5.5-mg fixed dose[a] W DLT: thrombocytopenia, diarrhea, dyspnea, acute rise in creatinine, cardiac arrest MTD: 4.0 mg W
C16008 NDMM N = 64	PO, TW, combination with LenDex 21-day cycle	3.0-3.7mg fixed -dose[a] W MTD: 3.0 mg Closed to enrollment
C16009 Solid tumors, Lymphomas N = 54	PO, W, single agent	5.5mg fixed -dose[a] W
C16010 RRMM N = 200	PO, W, with LenDex versus placebo-LenDex	4.0 mg W
C16011 RRAL N = 4	PO, W, with Dex versus physician's choice of a Dex-based regimen	4.0 mg W

Table 3-1-1 Clinical Studies of Oral Ixazomib

Trial/ Population	Description	Doses Investigated
C16013 RRMM N = 9	PO, W, with LenDex	4.0 mg W
C16014 Symptomatic MM N=701	PO, combination with LenDex	ixazomib 4.0 mg or matching placebo on Days 1, 8, and 15, plus Len 25 mg on Days 1-21 (10 mg if low creatinine clearance, with escalation to 15 mg if tolerated) and Dex 40 mg (or 20 mg if >75 years old) on Days 1, 8, 15, and 22
C16015 Symptomatic MM with normal renal function or severe renal impairment N=28	PO, combination with Dex	Part A: ixazomib 3.0 mg on Day 1 Part B: ixazomib 4.0 mg on Days 1, 8, and 15, plus Dex 40 mg (or 20 mg if >75 years old) on Days 1, 8, 15 and 22 of a 28-day cycle
C16017 RR follicular lymphoma N=58	PO, W	4.0, 5.3, and 7.0 mg, W Treatment at RP2D once determined.
C16018 Advanced solid tumors or hematologic malignancies with varying degrees of liver dysfunction N=45	Part A: PO, Day 1 of 15-day cycle Part B: PO, W	1.5 mg (severe hepatic impairment), 2.3 mg (moderate hepatic impairment), or 4.0 mg (normal hepatic function)
TB-MC010034 RRMM N = 10	PO, W	4.0 mg, W Single agent: 4.0 mg Combination with Rd

Abbreviations: RRAL = Relapsed and/or refractory Primary systemic light chain (AL) amyloidosis; BSA = body surface area; Dex=dexamethasone; DLT = dose-limiting toxicity; IV = intravenously; LenDex = lenalidomide plus dexamethasone; MTD = maximum tolerated dose; NDMM = newly diagnosed multiple myeloma; PO = orally; RR= relapsed and/or refractory; RRAL= relapsed and/or refractory systemic light chain amyloidosis RRMM = relapsed and/or refractory multiple myeloma; TBD = to be determined; TW = twice weekly; W = weekly; RP2D= recommended phase 2 dose.

Note that blinded data from pivotal Studies C16010 and C16011 are not included.

a Approximate BSA and fixed dosing equivalence: 3 mg~ equivalent to 1.68 mg/m^2 BSA dosing; 4.0 mg ~ equivalent to 2.23 mg/m^2 BSA dosing; and 5.5 mg~ equivalent to 2.97 mg/m^2 BSA dosing.

Overview of the Oral Formulation of Ixazomib

The emerging safety profile indicates that ixazomib is generally well tolerated. The adverse events (AEs) are consistent with the class-based effects of proteasome inhibition and are similar to what has been previously reported with VELCADE though the severity of some, for example peripheral neuropathy, is less. While some of these potential toxicities may be severe, they can be managed by clinical monitoring and standard medical intervention, or, as needed, dose modification or discontinuation.

In the 4 ongoing studies (C16003, C16004, C16007, and C16009) investigating single-agent oral ixazomib in patients with differing malignancies (multiple myeloma, AL amyloidosis, nonhematologic cancers, and lymphoma), a total of 201 patients have been treated as of 27 March 2013. These patients have been treated with different doses of ixazomib as they are all phase 1 trials. An overview of the most frequent (at least 10%) AEs occurring in the pooled safety population from single-agent oral ixazomib Studies (C16003, C16004, C16007, and C16009) is shown in Table 3-2.

Table 3-2 Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Single-Agent Studies

Primary System Organ Class Preferred Term	Oral Single Agent Total n = 201 n (%)
Subjects with at Least One Adverse Event	197 (98)
Gastrointestinal disorders	160 (80)
Nausea	106 (53)
Diarrhoea	88 (44)
Vomiting	77 (38)
Constipation	46 (23)
Abdominal pain	33 (16)
General disorders and administration site conditions	151 (75)
Fatigue	103 (51)
Pyrexia	51 (25)
Oedema peripheral	27 (13)
Asthenia	31 (15)
Nervous system disorders	92 (46)
Headache	29 (14)
Dizziness	26 (13)
Neuropathy peripheral	21 (10)
Metabolism and nutrition disorders	107 (53)
Decreased appetite	64 (32)
Dehydration	37 (18)
Blood and lymphatic system disorders	98 (49)
Thrombocytopenia	68 (34)

Table 3-2 Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Single-Agent Studies

Primary System Organ Class Preferred Term	Oral Single Agent Total n = 201 n (%)
Anaemia	42 (21)
Neutropenia	29 (14)
Lymphopenia	20 (10)
Skin and subcutaneous tissue disorders	90 (45)
Rash macular[a]	23 (11)
Musculoskeletal and connective tissue disorders	93 (46)
Back pain	24 (12)
Arthralgia	28 (14)
Respiratory, thoracic and mediastinal disorders	78 (39)
Cough	28 (14)
Dyspnoea	30 (15)
Infections and infestations	89 (44)
Upper respiratory tract infection	31 (15)

Source: Ixazomib Investigator's Brochure Edition 7

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities, version 15.0.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator.

a Note that rash maculopapular and rash macular represent the 2 most common terms used to describe rash.

As of 27 March 2013, there are 5 studies actively enrolling patients with multiple myeloma to investigate oral ixazomib in combination with standard combination regimens. The most frequent (at least 10%) AEs occurring in the pooled safety population from Studies C16005, C16006, C16008, and C16013 are shown for all grades (Table 3). Note that in combination trials, related is defined as related to any study drug in the combination regimen.

Table 3-3 Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Combination Studies

Primary System Organ Class Preferred Term	Total Oral Combo Agent (5/6/8/13) n = 173 n (%)
Subjects with at Least One Adverse Event	163 (94)
Gastrointestinal disorders	139 (80)
Nausea	65 (38)

Table 3-3 Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Combination Studies

Primary System Organ Class Preferred Term	Total Oral Combo Agent (5/6/8/13) n = 173 n (%)
Diarrhoea	81 (47)
Vomiting	51 (29)
Constipation	57 (33)
General disorders and administration site conditions	132 (76)
Fatigue	76 (44)
Pyrexia	39 (23)
Oedema peripheral	61 (35)
Asthenia	20 (12)
Nervous system disorders	115 (66)
Headache	28 (16)
Dizziness	34 (20)
Neuropathy peripheral	45 (26)
Metabolism and nutrition disorders	91 (53)
Decreased appetite	25 (14)
Hypokalaemia	34 (20)
Blood and lymphatic system disorders	88 (51)
Thrombocytopenia	49 (28)
Anaemia	45 (26)
Neutropenia	43 (25)
Lymphopenia	20 (12)
Skin and subcutaneous tissue disorders	102 (59)
Rash maculopapular[a]	29 (17)
Rash macular[a]	22 (13)
Musculoskeletal and connective tissue disorders	99 (57)
Back pain	42 (24)
Pain in extremity	31 (18)
Arthralgia	22 (13)
Respiratory, thoracic and mediastinal disorders	80 (46)
Cough	36 (21)
Dyspnoea	26 (15)
Infections and infestations	92 (53)
Upper respiratory tract infection	35 (20)

Table 3-3 Most Common (At Least 10% of Total) Treatment-Emergent Adverse Events in Oral Combination Studies

Primary System Organ Class Preferred Term	Total Oral Combo Agent (5/6/8/13) n = 173 n (%)
Psychiatric disorders	73 (42)
Insomnia	50 (29)

Source: Ixazomib Investigator's Brochure Edition 7

Abbreviations: MedDRA = Medical Dictionary for Regulatory Activities, version 15.0.

Subject Incidence: A subject counts once for each preferred term. Percentages use the number of treated subjects as the denominator.

Data from ongoing blinded pivotal trials (C16010) are not included.

a Note that rash maculopapular and rash macular represent the 2 most common terms used to describe rash.

The clinical experience with ixazomib also shows early signs of antitumor activity as evidenced by at least a 50% reduction in disease burden in some patients and prolonged disease stabilization in others across all ongoing trials. The antitumor activity has been seen with single-agent ixazomib, when combined with established therapies, and across the malignancies studied (advanced solid tumors (20), non-Hodgkin's disease, Hodgkin's disease (21), relapsed and/or refractory multiple myeloma (22, 23), relapsed or refractory systemic light chain amyloidosis (24), and newly diagnosed multiple myeloma (25-27) to date.

Though additional data are needed to characterize the clinical benefit of this drug, the emerging data supports the ongoing development of ixazomib.

1.6 Relapsed and/or Refractory Multiple Myeloma

The early development of ixazomib in patients with RRMM involves 2 studies (C16003 and C16004) with similar objectives, but each investigated 1 of the 2 dosing schedules commonly used with the first-in-class proteasome inhibitor, VELCADE.

Study C16003 is an open-label, dose escalation, phase 1 study of ixazomib dosing on a twice-weekly schedule on Days 1, 4, 8, and 11 of a 21-day cycle in adult patients with RRMM (28, 29). Study C16004 is an open-label, dose escalation, phase 1 study of ixazomib dosing on a weekly schedule on Days 1, 8, and 15 of a 28-day cycle in adults patients with RRMM (30-32) Both studies have now completed enrollment. The DLTs in Study C16003 were rash macular and thrombocytopenia and the DLTs in C16004 were nausea, diarrhea, vomiting, and erythema multiforme.

In the dose escalation component of both studies, patients had multiple myeloma that had relapsed following at least 2 lines of therapy that must have included bortezomib, thalidomide (or lenalidomide), and corticosteroids. In both studies, when the MTD was established, cohorts of patients representing the heterogeneous patient population currently seen in clinical practice were to be enrolled into 1 of 4 expansion cohorts, including a relapsed and refractory cohort, a carfilzomib cohort, a proteasome inhibitor-naïve cohort, and a VELCADE-relapsed cohort. Final study results are currently being analyzed, but preliminary data suggest that ixazomib has anti-tumor activity in heavily pretreated MM patients, with durable responses/disease control, and is generally well tolerated. Please refer to the ixazomib IB and SMA for further information.

1.7 Newly Diagnosed Multiple Myeloma (NDMM)

Multiple research paths are being explored in patients with NDMM with a focus on evaluating ixazomib in combination with agents commonly used across treatment settings. The development of ixazomib in combination with lenalidomide and dexamethasone (LenDex) in patients with NDMM who are transplant eligible or ineligible involves 2 studies (C16005 and C16008) with similar study designs except for a few key differences, namely the schedules of ixazomib and dexamethasone. Ixazomib is also being evaluated in combination with melphalan and prednisone (MP) for patients who are not transplant eligible due to age or coexisting morbidity (in Study C16006).
All 3 studies are phase 1/2, with phase 1 focusing on safety and phase 2 on efficacy (and further characterization of safety). Please refer to the ixazomib IB and SMA for further information.

1.8 Clinical Trial Experience Using the Intravenous Formulation of Ixazomib

See the IB for descriptions of the 2 studies that investigated IV ixazomib in advanced solid tumors and advanced lymphoma (Studies C16001 and C16002, respectively).

1.9 Study Rationale

One of the major challenges in the current era of organ transplantation is to find suitable organs for highly sensitized patients. These patients have developed antibodies against major histocompatibility (HLA) antigens due to previous transplantation, blood transfusion, or pregnancy. Despite some success, current desensitization protocols are limited because of their complexity and inability to reduce donor specific antibodies (DSA) in 30-90% of patients, and the absence of effective agents against plasma cells, which actually produce the HLA alloantibodies. As a result, many patients die on dialysis prior to receiving a transplant.

Our long-term goal is to improve the quality-of-life and survival of kidney transplant recipients. Our specific hypothesis for this proposal is that ixazomib (Ninlaro®) can significantly reduce DSA production and allow for kidney transplantation in highly sensitized patients. Ixazomib is a second generation proteasome inhibitor, recently approved by the FDA for the treatment of multiple myeloma. We further hypothesize that specific molecular and cellular indices can be predictive of treatment response and toxicity. These hypotheses are based on preliminary findings demonstrating that: (a) The first-generation proteasome inhibitor, bortezomib (Velcade®), is an effective option for the treatment and prevention of antibody mediated rejection (AMR) in clinical settings; (b) Ixazomib prevents acute AMR in a robust preclinical model of kidney transplantation by downregulating B cell activation, antigen presentation, T cell and B cell signaling, and microcirculation inflammation; (c) IgG subclasses, IgG complement binding capacity, and serum B cell activating factor (BAFF) and APRIL (a proliferation-inducing ligand) are associated with AMR and graft loss in kidney transplantation.

Based on these observations and the critical need to define effective desensitization strategies, we propose a pilot exploratory, proof of concept, open-label, single-center phase II clinical trial entitled **IXA**zomib for **DES**ensitization (**IXADES**). We will examine the safety and efficacy of ixazomib for desensitization of highly sensitized kidney transplant candidates and conduct mechanistic studies to address the role of IgG subclasses, IgG complement binding capacity, non-HLA antibodies, T and B cell phenotypes, and BAFF/APRIL in immune monitoring and regulation.

Sensitization in kidney transplant candidates. Alloantibodies (anti-HLA antibodies) arise through previous transplants, blood transfusions, and pregnancy. Currently, 39% of patients on the active kidney transplant waitlist are sensitized, evidenced by a Panel Reactive Antibody (PRA) ≥ 1% (33). Of these, nearly 15,000 are highly sensitized, which means that they have a PRA ≥ 80% (33). Transplant rates vary by PRA, ranging from 143.0 per 100 active waitlist years for candidates with a PRA of less than 1% to only 6.9 for those with a PRA of 98% or higher (33). Median waiting time for kidney transplantation in highly sensitized patients approaches 12 years, which is more than 3 times than for non-sensitized patients (33). As a result, a significant number of highly sensitized patients die before receiving a transplant, outlining the critical importance of desensitization strategies.

***Desensitization protocols*.** The two approaches for helping highly sensitized patients are: 1) To increase the chance of finding a crossmatch negative donor, or 2) To remove the pre-existing antibodies using desensitization protocols (7-11). Emerging evidence suggests that strategies to improve transplant rates in highly sensitized patients enhance survival rates and the quality of life, while reducing costs compared to chronic dialysis (12, 13). Current desensitization protocols combine Rituximab (anti-CD20 monoclonal antibody) to deplete B cells, and plasmapheresis plus intravenous immunoglobulins (IVIG) to block or remove preformed DSA (7-11). Since no agent previously used in transplantation has the adequate ability to inhibit mature plasma cells, there has been no effective way of reducing or preventing antibody production besides targeting B cells. Therefore, despite some success, contemporary desensitization protocols are limited by their inability to desensitize 30 to 90% of patients (5, 8, 14). For example, using a preconditioning regimen similar to the one used at our institution combining plasmapheresis, IVIG and Rituximab, the Mayo Clinic Transplant Program was unable to desensitize 15/29 patients who had an CDC crossmatch titer ≥ 1:8 (8). Similarly, Marfo et al demonstrated that pretransplant desensitization with high dose IVIG and rituximab was not successful in sensitized kidney transplant candidates with cPRA levels higher than 90% (5). It is for this reason that we propose a pilot desensitization study targeting the cells responsible for generating alloantibodies using ixazomib, a recently approved therapy for multiple myeloma.

Ixazomib (Ninlaro®, Takeda-Millennium Pharmaceuticals, Inc). In kidney transplantation, the first generation proteasome inhibitor bortezomib (Velcade®) has been utilized as part of desensitization (16) and AMR treatment strategies (14, 34-37). Ixazomib is a second generation **oral** proteasome inhibitor, recently approved for the treatment of multiple myeloma (38-43). Ixazomib is administered as a citrate ester, ixazomib citrate, that rapidly hydrolyzes under physiological conditions to its biologically active form, ixazomib The active form potently, reversibly and selectively inhibits the proteasome (39-41). It is **more effective** than earlier generation proteasome inhibitors in targeting plasma cells **with improved side effect** profile (38, 44-49). Having a more potent, less toxic proteasome inhibitor that does not need to be delivered intravenously and can be used in patients with reduced kidney function will be clinically advantageous (46). Despite the absence of data on the use of ixazomib in clinical transplantation (only FDA approved in November 2015), we have been the only group to demonstrate that the drug is safe and effective for the treatment of antibody mediated rejection (AMR) in a robust preclinical model (19). More specifically, it is unknown whether ixazomib depletes plasma cells, prevents their generation or both. For example, the effects of treatment on differentiated bone marrow-derived $CD138^+$ **plasma cells** in clinical transplantation are unknown. These antibody producing cells thrive in a microenvironment rich in BAFF and APRIL generated by stromal mesenchymal stem cells (MSCs). We propose to test the safety and efficacy of this drug in a phase II trial for desensitization.

***BAFF/APRIL and Desensitization*.** BAFF and APRIL are homologues and members of the tumor

necrosis factor superfamily (50). They are produced by neutrophils, macrophages, dendritic cells and stromal mesenchymal stem cells in the bone marrow (1, 50, 51). Both cytokines are potent activators and survival factors for B cells and plasma cells. Through their receptors BAFF-R (BAFF receptor), TACI (transmembrane activator and calcium modulator and cyclophilin ligand interactor) and BCMA (B cell maturation protein), BAFF/APRIL activate the prosurvival NF-kappaB and MEK signaling pathways (1, 50, 51). In animal models, excess BAFF leads to the development of autoimmune disorders including lupus nephritis, while APRIL deficient mice lack proper activation and maturation of plasmablasts (50, 51). High levels of BAFF/APRIL are detected in the serum of patients with autoimmune disease, which in some studies correlate with disease activity (50, 51). BAFF and APRIL inhibitors such as Belimumab (Lymphostat-B) and TACI-Fc fusion protein (Atacicept) have been recently used in phase II and III clinical trials of lupus and rheumatoid arthritis (50, 51). Consistent with these data, serum BAFF levels are increased in kidney transplant recipients undergoing AMR, where this cytokine may play an important role in the immune response to the allograft (1, 52). We hypothesize that in highly sensitized patients, BAFF/APRIL generated by bone marrow-derived mesenchymal stem cells (BM-MSCs) play a key role in the survival and maturation of donorspecific- plasma cells and that serum BAFF/APRIL levels have an important clinical value in predicting and monitoring treatment response.

Immunoglobulins and Desensitization. Terasaki et al. identified HLA antibodies in the serum of patients after transplantation nearly 45 years ago (53). Preexisting (54-56) and de novo circulating antibodies (dnDSA) (57, 58) have been shown to compromise renal allograft survival. These antibodies may be directed against HLA or non-HLA molecules on endothelial cells including angiotensin II type 1 receptor (AT1R), or endothelin type A receptor (ETAR) (59-61). It is important to note that currently available HLA antibody tests are qualitative and have not been cleared by the FDA for quantitative measurements (62). In addition, *the critical issue for patient care is discerning which of the detected antibodies are pathogenic as many patients with donor specific antibodies prior and after transplantation have stable allograft function and experience no rejection*.

For example, patients with dnDSA at the time of biopsy have worse graft survival than those with preexisting DSA, indicating a more pathogenic role for dnDSA (63). Similarly, it has been proposed that the assessment of the complement-binding capacity of donor-specific anti-HLA antibodies by the C1q assay may be useful in identifying patients at high risk for kidney-allograft loss (64). However, we have recently conducted studies demonstrating that the C1q binding activity by DSA largely reflects differences in antibody strength, questioning the biologic significance of this test (65). It has also been proposed that among IgG subclasses, IgG3 may have a more pathogenic role in AMR (66, 67) but the role of immunoglobulin subclasses in desensitization and kidney transplantation remains unknown. To address these questions, we propose to identify risk stratification strategies and to determine the pathogenic role and specificity of anti-HLA, non-HLA-DSA and complement fixing antibodies in desensitization.

Future Biomedical Research. Subjects will be asked in the consent form if they agree to allow a portion of their bone marrow aspiration sample to be used for a future research project that will involve groiwing the mesenchymal stromal cells in Dr. Djamali's laboratory. If subjects do not achieve desensitization during their participation in this research study, they may be offered injection with their mesenchymal stromal cells, in an effort to achieve desensitization in a future research study.

1.10 Potential Risks and Benefits

Please refer to the current ixazomib IB and SMA for a complete list of the risks of ixazomib. The clinical benefit of ixazomib continues to be studied in a comprehensive and global development plan that involves studies sponsored by Millennium. Ixazomib appears to show a significant impact on the production of allo antibodies in preclinical studies of AMR. The preliminary findings are favorable when considering historical and currently available therapies for the patient populations evaluated. Though additional data are needed to characterize the clinical benefit of this drug, the emerging data supports expanded development of ixazomib for the treatment of patients with advanced malignancy.

This study will be conducted in compliance with the protocol, good clinical practice (GCP), applicable regulatory requirements, and International Conference on Harmonisation (ICH) guidelines.

Compliance with eligibility criteria: The PI will sign-off on the eligibility checklist for each subject prior to study drug dispensation.

The risks to subjects in this trial will be minimized by compliance with eligibility criteria, clinical monitoring, adherence to protocol contraception requirements and investigator guidance regarding specific safety areas.

The specific safety areas that will be closely monitored in this subject population are;

Ixazomib: thrombocytopenia, diarrhea, fatigue, nausea, vomiting, rash, risk of reactivation of herpes infection, neutropenia, fluid deficit, hypotension, posterior reversible encephalopathy syndrome (PRES), and transverse myelitis.

Bone Marrow Aspiration Risks: This procedure may be very painful; however, the pain only lasts for about 15 to 30 seconds. The hip area may be sore for a day or two. It is possible, but not likely that the subject could get an infection or have a large amount of bleeding. In very rare cases, people might have an allergic reaction to the local anesthesia. The allergic reaction could include rash/hives, flushing of the face, itching, wheezing and tightness in the throat.

For subjects that are on warfarin, INR levels will be checked prior to the BMA, and pressure will be applied for

- 10 minutes after the procedure if INR 1.5-2
- 15 minutes after the procedure if INR 2-2.5
- 20 minutes after the procedure if INR > 2.5

Warfarin dosing will not be adjusted. Blood Draw Risks: The risks of taking blood include pain, a bruise at the point where the blood is taken, redness and swelling of the vein and infection, and a rare risk of fainting.

Overall, the balance of benefit and risk supports the proposed clinical study to evaluate the potential of ixazomib to significantly reduce DSA production and allow for kidney transplantation in highly sensitized patients.

Subject Remuneration
Subjects will receive $100 after Visit 1 and will receive $20 per visit for visits 2 - 13. Payment will be made by check approximately 6 weeks after each study visit.

2. STUDY OBJECTIVES

2.1 Primary Objectives

Specific Aim 1. To determine the safety and efficacy of ixazomib as a desensitization strategy. 10 highly sensitized kidney transplant candidates on the waitlist for more than 24 months will receive ixazomib 3 mg (and dexamethasone 20 mg) on days 1, 8, and 15 of a 28 cycle for 12 months. The primary objective is to evaluate the safety (distal neuropathy, thrombocytopenia, and gastrointestinal symptoms) and efficacy (decline in cPRA > 20%) of ixazomib. The secondary efficacy endpoint is transplantation rate within 12 months of therapy.

2.2 Secondary Objectives

Specific Aim 2. Identify immune indices which predict the course of disease and/or response to treatment in highly sensitized patients. Mechanistic studies will use bone marrow and blood obtained from subjects in Aim 1 to determine the effect of treatment on immune regulation and reconstitution after therapy. Since the bone marrow microenvironment produces BAFF/APRIL and supports plasma cell maturation, we will determine the effect of therapy on the generation of BAFF/APRIL by bone marrow mesenchymal stem cells and the survival of bone marrow-derived plasma cells after desensitization. We specifically propose to:

- Identify if bone marrow plasma cells, IgG subsets, and circulating BAFF/APRIL predict outcomes.
- Determine if treatment is effective in downregulating circulating BAFF/APRIL and anti-HLA, endothelin-1 type A receptor (ETAR), angiotensin type 1 receptor (AT1R), and complement fixing C1q antibodies.
- Identify immunological indices which predict treatment toxicity and responders vs. non-responders.

In summary, sensitization is a significant clinical and immunological problem in kidney transplantation. In a unique effort involving the University of Wisconsin's Divisions of Nephrology, Transplantation, and Hematology-Oncology (clinical core), The University of Michigan (Plasma cell core), and The Terasaki Foundation Laboratory (DSA core), coordinated by the UW Office of Clinical Trials, an experienced clinical research organization, we propose the planning of a pilot phase II safety and efficacy trial of ixazomib for desensitization of highly sensitized kidney transplant candidates. We further outline mechanistic studies to determine the pathogenesis of alloantibody generation and define novel biomarkers and surrogate endpoints for a future Clinical Trial Implementation grant (NIAID PAR-13-149). The results are expected to have a positive impact as proposed studies are crucial steps towards developing improved therapies and monitoring options to reduce the long-term impact of sensitization.

3. STUDY ENDPOINTS

3.1 Primary Endpoints (safety and efficacy)

Primary efficacy and safety endpoint:

- Efficacy endpoint: A decline in cPRA > 20%.
- Safety endpoint: infections, malignancies, hematological complications including leucopenia, anemia, and thrombocytopenia, cardiovascular complications and events, distal neuropathy, gastrointestinal symptoms, and immunosuppression-related adverse effects.

3.2 Secondary Endpoints (efficacy endpoint)

Secondary efficacy endpoint: Successful transplantation in 12 months

3.3 Tertiary/Exploratory Endpoints

Correlataive studies: Defining a marker of disease activity in sensitized patients will have a significant impact on the future of desensitization protocols. Although DSA, cPRA, CDC and T cell and B cell flow crossmatch have been used to characterize the severity of sensitization, these assays are labor intensive and expensive. The proposed *plasma BAFF/APRIL* ELISA assays can be performed in 2-3 hours (1). We will determine whether these cytokines can reflect the immune response and predict response to desensitization and ultimately transplant outcome. *In addition*, we propose studies to address the effect of ixazomib on (a) *bone marrow derived BAFF/APRIL* generated by bone marrow-mesenchymal stem cells, knowing that these stromal cells play a key role during the maturation activation of plasma cells, (b) *bone marrow-derived antibody secreting cells (plasma cells)* (2) and (c) plasma *IgG subclasses, anti-HLA, ETAR, AT1R, and C1q antibody* response to treatment (3, 4).

BAFF/APRIL studies (Djamali lab and Platt lab):

- These studies will be performed at time zero, prior to initiation of therapy, then every three months for 12 months.
- To define the range of BAFF/APRIL in sensitized patients, we will determine baseline levels and end of study levels in the sera and marrow obtained from peripheral blood and bone marrows of all 10 patients prior to treatment initiation. Higher BAFF/APRIL levels may be an indication of higher sensitization status.
- To determine whether serum BAFF/APRIL levels predict the level of sensitization, we will assess the correlation between BAFF/APRIL and other markers of sensitization in all 10 patients prior to treatment initiation. Using appropriate parametric and nonparametric statistical analyses we will determine whether BAFF or APRIL can independently predict cPRA, DSA and B cell flow crossmatch channel shift in case of transplantation to demonstrate the clinical value of these tests in defining sensitization intensity.
- To determine whether serum BAFF/APRIL levels predict treatment response, we will first determine if baseline serum BAFF/APRIL levels were higher in patients who failed to reach the primary or secondary endpoints. Next we will examine the correlation between BAFF/APRIL levels and outcomes using Pearson correlation coefficient analyses. Last, the effect of serum BAFF/APRIL on endpoints will be measured using Kaplan-Meier and Cox proportional-hazards models.
- To determine whether ixazomib therapy inhibits BAFF/APRIL generation by stromal BM-MSCs, we will determine if ixazomib inhibits BAFF/APRIL generation by BM-MSCs *in vivo*

and *in vitro* by (a) determining the difference in the production of BAFF/APRIL by BM-MSCs at enrollment and after study completion and (b) by measuring the direct effects of ixazomib on BAFF/APRIL generation in MSC cultures from pretreatment samples.

Antibody Secreting Studies (Platt lab):

- These studies will be performed at time zero prior to initiation of therapy, and at 12 months unless patients receive a kidney transplant. 10-60 mL of marrow will be collected from the iliac crest either in the transplant clinic or in the operating room right before the kidney transplant procedure.
- To determine the effect of treatment on bone marrow derived $CD138^+$ plasma cells, we will (a) determine the difference in the percentage of bone marrow plasma cells at enrollment and after study completion (kidney transplantation or failure of desensitization) and (b) measure antibody secretion against the tetanus antigen and the three HLA peptides the patient is most sensitized against (c) conduct similar analyses to BAFF/APRIL studies described above in order to determine the level of sensitization and prediction to treatment response. These studies will be performed in collaboration with the laboratories of Dr. Platt and Cascalho laboratory as described previously (2, 37).

Antibody assays (Terasaki Research Foundation Laboratory)

- These studies will be performed at time zero, prior to initiation of therapy, then every three months for 12 months.
- As mentioned earlier, the critical issue for patient care is discerning which of the detected antibodies are pathogenic. In addition, the role of immunoglobulin subclasses in desensitization and kidney transplantation remains unknown. To address these questions, we propose to identify risk stratification strategies and to determine the pathogenic role and specificity of IgG subclasses, anti-HLA, non-HLA-DSA and complement fixing antibodies in desensitization.

4. STUDY DESIGN

4.1 Overview of Study Design

Recruitment

Two avenues of recruitment will be followed.

- The first option is when the physician of a patient who has a cPRA < 80% and has been on the kidney transplant list for over 24 months, believes the patient is an eligible candidate for this study, the patient's physician or other clinical personnel involved in the care of the patient will describe the study to the patient. If the individual is interested, has provided permission for their name and contact information to be shared with the study team, and agrees to be contacted by the study team, the study team may contact the individual either in person (at this visit) or via telephone or e-mail (after this visit). If study personnel are available to meet with the potential subject in clinic, the study personnel will explain the study thoroughly and ask the potential subject if they are interested in hearing more information. If so, the study personnel will continue and review the consent form, answering questions and concerns. If the patient is comfortable with the study, they can sign the consent form at that time or they may take the consent form home for further review and finalize consent at a later date. If the study team is not available to meet with the potential subject in the clinic, the study team will follow-up by telephone. If the study discussion occurs via phone, the informed consent document and authorization form will be mailed to the potential subject for review.
- The second option for potential subject identification is to pre-screen highly sensitized patients (cPRA ≥ 80%) on the kidney transplant waitlist (> 24 months) in the Transplant

Database. If a potential subject is identified, the subject will be sent a letter of introduction that includes information about the study and informs them that they will receive a phone call from a member of the study team to discuss the details of the study, and provides contact information to opt out of receiving the phone call.

Use of E-mail: E-mail will be used as a means of communicating with subjects two different ways;
1. E-mail may be used to schedule the initial study visit. After the subject has had the study introduced to them through a physician or other clinical personnel involved in their clinical care, the study team will e-mail them using the template provided in the recruitment section.
2. E-mail may be used throughout the course of the study for the purpose of scheduling study visits and notifying subjects of other study-related information.

Study Procedures

Screening Visit

The screening visit procedures will occur within a 30-day window prior to the Visit 1, Cycle 1 window (3-month window after screening). The following procedures will occur at the screening visit;

- Physical exam
- Medical history (including most recent cPRA results prior to enrollment)
- Vital Signs (Temperature, pulse, respirations, and blood pressure)
- Medication History
- Pregnancy Test (Serum HCG) for females of child-bearing potential
- CBC
- LFTs

Re-screening

Subjects who fail screening may be considered for re-screening if, in the opinion of the principal investigator, their clinical status has changed such that they may now be eligible for study participation per all eligibility criteria. At the discretion of the principal investigator, selected tests, such as lab tests, will not be repeated if previous screening results remain valid per the protocol-defined window for that test.

Visits: Patients will only receive one month's supply of ixazomib and dexamethasone and will return monthly for resupply. At each visit, they will be assessed for AEs by the study team. They will be examined by the principal investigator 5 times (please see tables 2-1 a-b and Figure 1, below): Days 1, 85, 169, 253, and 373 +6, - 3).

- Day 1: Prior to treatment initiation. H&P, Labs, Bone Marrow Aspirate
- Day 85 + 6, - 3: Prior to treatment dose. H&P, Labs
- Day 169 + 6, - 3 Prior to treatment dose. H&P, Labs
- Day 253 + 6, - 3 Prior to treatment dose. H&P, Labs
- Day 373 + 6, - 3 One month after treatment completion, H&P, Labs, Bone Marrow Aspirate
- Study drug will be dispensed at each month's in-person visit, prior the initiation of the next cycle, and after reviewing the AEs and monthly labs.

- Liver Function tests will be conducted twice a month (+ 6 /- 3 days) for the first 3 months, then monthly thereafter. The complete blood count (CBC) will be checked monthly. cPRA, BAFF/APRIL, DSA and antibodies will be checked every three months (5 times total). Bone marrow aspirates will be collected at the beginning and at one of the following endpoints: one month (+6, - 3 days) after the end of the 12 month study, cPRA decline > 20%, time of transplant, or time of subject withdrawal from the study. ECG and echocardiograms will be done at the baseline, 6 months, and end of the study visits.
- During the first three months of study participation, the bi-weekly Liver Function Tests may be done at the subject's local lab. The local lab will process the sample and send the result to the study team.
- Additional visits may be required if the subject has to hold treatment and needs to return to the clinic for blood draws to confirm eligibility to continue. Please see Table 4-3, Dose Adjustments for Hematologic Toxicities for dose adjustment plan.
- Data on acute rejection, graft and patient survival will be collected for six months after surgery for those subjects who receive a successful kidney transplant.

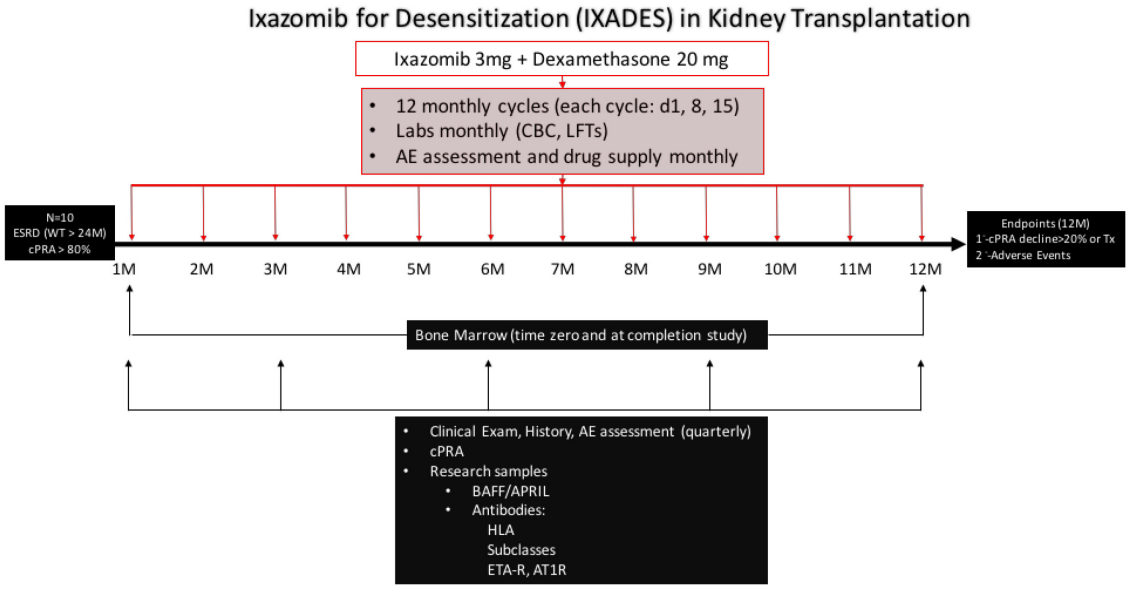
Unscheduled Visits: Subjects may have unscheduled visits due to dose adjustments based on lab results, an acute illness of undetermined cause, for other reasons, or at the discretion of the Investigator. Unscheduled visits may include any of the following procedures;

- Adverse Event Assessment
- Medication History
- Drug Accountability
- Physical Exam
- Vital Signs (Temperature, Pulse, Respirations, Blood Pressure)
- Lab work (CBC, LFTs, cPRA, BAFF/APRIL, DSA Antibodies)
- Bone Marrow Aspiration

Early Termination Visit: All subjects who end study participation prior to the end of the scheduled treatment will undergo an early termination visit within one week from study discontinuation. The early termination visit will consist of the following procedures;

- Adverse Event Assessment
- Medication History
- Drug Accountability
- Physical Exam
- Vital Signs (Temperature, Pulse, Respirations, Blood Pressure)
- Lab work (CBC, LFTs, cPRA, BAFF/APRIL, DSA Antibodies)
- Bone Marrow Aspiration (bone marrow aspiration will be done within 48 hours of transplant)

Figure 1. Study Overview Diagram



4.2 Number of Patients

A total of 10 patients will be enrolled in this pilot clinical trial. We currently have more than 40 patients that qualify for the study based on the inclusion criteria described below. A patient will be considered enrolled after the first day of ixazomib treatment.

4.3 Duration of Study

Participants will be followed for the entire length of treatment, plus one month. All subjects, including those that receive a a kidney transplant and those that do not, will continue to be monitored for one month following the last dose of study drug to monitor for and address potential adverse events from treatment (Final visit on day 373 + 6, - 3). Thereafter, dialysis nephrologists will routinely follow the patients. For those tansplanted, data on acute rejection, graft and patient survival will be collected for 6 months after surgery.

5. STUDY POPULATION

The study population will include all highly sensitized kidney transplant candidates on the waitlist for more than 24 months at UW. A preliminary query of the Transplant Database indicates more than 40 candidates who are eligible for this study. This study will enroll 10 subjects.

5.1 Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be enrolled in the study:

1. Male or female patients 18-70 years of age.
2. Able to provide informed consent.
3. Female patients who are postmenopausal for at least 1 year before the screening visit, or are surgically sterile, or If they are of childbearing potential, agree to practice 2 effective methods of contraception, at the same time, from the time of signing the informed consent

form through 30 days after the last dose of study drug, OR agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception.)

4. Male patients, even if surgically sterilized (ie, status post-vasectomy), must agree to one of the following: Agree to practice effective barrier contraception during the entire study treatment period and through 30 days after the last dose of study drug, or Agree to practice true abstinence when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence (eg, calendar, ovulation, symptothermal, post-ovulation methods] and withdrawal are not acceptable methods of contraception.)
5. Patients must be highly sensitized with a cPRA ≥ 80%
6. Be active on the waitlist for kidney transplantation > 24 months to confirm their inability to receive a deceased donor transplant because of their sensitization status.
7. Patients must meet the following clinical laboratory criteria:
    - Absolute neutrophil count (ANC) $\geq$ 1,000/mm^3 and platelet count $\geq$ 75,000/mm^3. Platelet transfusions to help patients meet eligibility criteria are not allowed within 3 days before study enrollment.
    - Hemoglobin higher than 6 g/dL
    - Total bilirubin ≤ 1.5 $\times$ the upper limit of the normal range (ULN).
    - Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) $\leq$ 3 $\times$ ULN.

5.2 Exclusion Criteria

Exclusion criteria: Patients will be excluded from the study based on the following criteria:
(1) Female patients who are lactating or have a positive serum pregnancy test during the screening period
(2) Major surgery requiring hospitalization within 6 months before enrollment
(3) Infection requiring systemic antibiotic therapy or other serious infection within 14 days before study enrollment
(4) Evidence of current uncontrolled cardiovascular conditions, including uncontrolled hypertension, uncontrolled cardiac arrhythmias, symptomatic congestive heart failure, unstable angina, or myocardial infarction within the past 6 months
(5) Systemic treatment, within 14 days before the first dose of ixazomib, with strong CYP3A inducers (rifampin, rifapentine, rifabutin, carbamazepine, phenytoin, phenobarbital), or use of St. John's wort
(6) Any serious medical or psychiatric illness that could, in the investigator's opinion, potentially interfere with the completion of treatment according to this protocol
(7) Inability to take oral medication
(8) Diagnosed or treated for another malignancy within 2 years before study enrollment or previously diagnosed with another malignancy and have any evidence of residual disease. Patients with nonmelanoma skin cancer or carcinoma in situ of any type are not excluded if they have undergone complete resection
(9) Grade 2 or greater peripheral neuropathy according to NCI Common Terminology Criteria for Adverse Events (CTCAE)
(10) Participation in other desensitization therapies or investigational drug clinical trials, including those with other investigational agents not included in this trial, within 6 months of the start of this trial and throughout the duration of this trial
(11) Patients that have previously been treated with ixazomib, or participated in a study with ixazomib whether treated with ixazomib or not
(12) Active or treated infection for HIV, HCV or HBV

(13) History of Liver cirrhosis, biopsy confirmed
(14) Elevated transaminases (greater than 3 times the upper limit of normal)
(15) Known hypersensitivity to ixazomib
(16) Active substance abuse by self-report or medical record
(17) Electrocardiography evidence of acute ischemia or active conduction system abnormalities

6. STUDY DRUG

6.1 Description of Investigational Agents

Ixazomib Capsules. Provided by Millennium-Takeda. The ixazomib drug product is provided in strengths of 3.0-, and 2.3-mg and 0.5-, and capsules as the active boronic acid. The different dose strengths are differentiated by both capsule size and color as described below: *For this study, we will initiate therapy with the 3 mg capsules according to the package insert information for patients with end-stage renal disease.* For additional details, please see the ixazomib IB.

Dose Strength	Capsule Size	Capsule Color
3.0 mg	Size 3	Light gray
2.3 mg	Size 2	Light pink
0.5 mg	Size 3	Dark green

Dexamethasone tablets, Dexamethasone Tablets USP are available for oral administration containing either 0.5 mg, 0.75 mg, 1 mg, 1.5 mg, 2 mg, 4 mg or 6 mg of dexamethasone USP. *For this study, we will use the 20 mg dose (5 tablets of 4 mg each)*. For additional information please refer to the attached IB for dexamethasone.

6.2 Study Drug Administration

- Ixazomib and dexamethasone will be taken based on the information in the package insert.
- The starting dose of ixazomib will be 3 mg taken orally on days 1, 8, and 15 of a 28-day cycle. The dose of dexamethasone will be 20 mg taken orally on the same days as ixazomib.
- Patients will take ixazomib and dexamethasone for twelve (12) 28-day cycles.
- Ixazomib should be taken once a week on the same day and at approximately the same time for the first three weeks of a four week cycle. Ixazomib should be taken at least one hour before or at least two hours after food. The whole capsule should be swallowed with water. The capsule should not be crushed, chewed or opened. If an ixazomib dose is delayed or missed, the dose should be taken only if the next scheduled dose is ≥ 72 hours away. A missed dose should not be taken within 72 hours of the next scheduled dose. A double dose should not be taken to make up for the missed dose. If vomiting occurs after taking a dose, the patient should not repeat the dose. The patient should resume dosing at the time of the next scheduled dose.
- Ixazomib and dexamethasone should not be taken at the same time, because dexamethasone should be taken with food and ixazomib should not be taken with food.
- Ixazomib and dexamethasone will be dispensed monthly by the PRC
- Diaries/logs will be used by patients to track their doses.

Treatment Plan and Laboratory Assessment. Participants will undergo a 12-month treatment

regimen of ixazomib-dexamethasone on days 1, 8, and 15 of a 28-day cycle, for 12 cycles (Table 1-1 below). The dose of ixazomib (3 mg) is based on packet information for patients with reduced kidney function and will be taken at least one hour before or at least two hours after food. The dose of dexamethasone is 50% lower than the dose recommended in the package insert to reduce the possibility of metabolic complications in ESRD.

Table 1-1 IXADES Treatment Protocol for desensitization

28-Day Cycle (a 4-week cycle)								
	Week 1		Week 2		Week 3		Week 4	
	Day 1	Days 2-7	Day 8	Days 9-14	Day 15	Days 16-21	Day 22	Days 23-28
Ixazomib (NINLARO) 3 mg	✔		✔		✔			
Dexamethasone 20 mg	✔		✔		✔			

Duration of Treatment and Follow-up. All participants, those that receive a kidney transplant during the defined period and those that do not will be followed for the entire length of treatment, plus one month following the last dose of the study drug. All subjects will be asked to return for a final study visit approximately one month following the last dose of study drug. During this final study visit, subjects will undergo physical examination, bone marrow aspirate collection, CBC, LFT check, ECG and echocardiogram.

Bone Marrow Aspirate Collection Procedure: Subjects will be given the option of having the bone marrow aspirate performed under conscious sedation or local anesthetic only. The bone marrow aspirate will be collected in a transplant clinic room. If the bone marrow aspiration is done under conscious sedation, subjects will be given instructions to fast for six hours before the procedure (medications are allowed) and not to drive themselves home or operate dangerous equipment after the procedure.

Measurement of effect. We will determine the effect of ixazomib by measuring cPRA, whether patients receive a kidney transplant while participating in the study, antibody strength by MFI, and the toxicity (measured by the incidence of adverse events)of the treatment quarterly up to the end of the study.

Ixazomib Administration

All protocol-specific criteria for administration of study drug must be met and documented before drug administration. Study drug will be dispensed only to eligible patients by a member of the study team. Patients should be monitored for toxicity, as necessary, and doses of ixazomib should be modified as needed to accommodate patient tolerance to treatment; this may include symptomatic treatment, dose interruptions, and adjustments of ixazomib dose (see Section 6.3).

Capsules of ixazomib will also be referred to as study drug. Study drug will be supplied by Millennium as capsules 0.5, 2.3, and 3.0 mg ixazomib. The prescribed administration of ixazomib doses in this study is 9 mg ixazomib in a 28 day cycle. Patients should be instructed to swallow ixazomib capsules whole, with water, and not to break, chew, or open the capsules. Ixazomib should be taken on an empty stomach (no food or drink) at least 1 hour before or at least 2 hours after food. Each capsule should be swallowed separately with a sip of water. A total of approximately 8 ounces (240 mL) of water should be taken with the capsules.

Missed doses can be taken as soon as the patient remembers if the next scheduled dose is 72 hours or more away. A double dose should not be taken to make up for a missed dose. If the patient vomits after taking a dose, the patient should not repeat the dose but should resume dosing at the time of the next scheduled dose.

Ixazomib Destruction

Investigational ixazomib (expired or end of study) should be destroyed on site according to the institution's standard operating procedure. We will document removal and destruction on drug accountability logs.

Dexamethasone: 20 mg of dexamethasone (5 tablets of 4 mg each) will be taken with food on days 1, 8, and 15 of a 28 day cycle (same days as ixazomib).

6.3 Dose-Modification Guidelines

The ixazomib dose reduction steps are 3 mg -> 2.3 mg -> 1.5 mg (Table 4-1), and the dose modification guidelines are provided in the IB and Table 4-2 and 4-3 below.

Treatment monitoring and dose modifying strategies. Liver Function Tests will be conducted twice a month for the first 3 months, then monthly thereafter. The Complete Blood Count (CBC) will be checked monthly. They will undergo clinical evaluation at baseline and every three months until the end of the study. Ixazomib dose modifying strategies will be implemented as outlined in the IB.

6.3.1 Recommended Ixazomib Criteria for Beginning or Delaying a Subsequent Treatment Cycle & Dose Modifications for Treatment Associated Toxicity

Treatment with ixazomib will use a cycle length of 28 days. For a new cycle of treatment to begin, the patient must meet the following criteria:

- ANC must be ≥ 1,000/mm^3.
- Platelet count must be ≥ 75,000/mm^3.
- All other nonhematologic toxicity (except for alopecia) must have resolved to ≤ Grade 1 or to the patient's baseline condition

If the patient fails to meet the above-cited criteria for initiation of the next cycle of treatment, dosing should be delayed for 1 week. At the end of that time, the patient should be re-evaluated to determine whether the criteria have been met. If the patient continues to fail to meet the above-cited criteria, delay therapy and continue to re evaluate. The maximum delay before treatment should be discontinued will be 3 weeks or at the discretion of the Principal Investigator.

For dosing recommendations upon recovery, refer to Table 642 and Table 4-3.

Table 4-1 Ixazomib Dose Adjustments

Dose Level	Dose (mg)
Starting Dose	3.0 mg

First Level Dose Reduction	2.3 mg
Second Level Dose Reduction	1.5 mg
Third Level Dose Reduction	Discontinue

Once ixazomib is reduced for any toxicity, the dose may not be re-escalated.

Dosage adjustments for hematologic toxicity are outlined in Table 4-2.

Table 4-2 I**xazomib** Dose Adjustments for Hematologic Toxicities

Criteria	Action
Dose Modifications for Treatment Cycles	
• Delay of > 2 weeks in the start of a subsequent cycle due to lack of toxicity recovery as defined in Section 6.3.1 • ANC < 1.0 × 10^9/L, platelet count < 75 × 10^9/L, or other nonhematologic toxicities > Grade 1 or not to the patient's baseline condition	• Hold ixazomib until resolution as per criteria Section 6.3.1. • Upon recovery, reduce ixazomib 1 dose level. • The maximum delay before treatment should be discontinued will be 3 weeks or at the discretion of the PI.

Treatment modifications due to ixazomib-related AEs are outlined in Table 4-3.

Table 4-3 **Ixazomib** Treatment Modification (Delays, Reductions, and Discontinuations) Due to Adverse Events (Non-Hematologic Toxicities)

Adverse Event (Severity)	Action on Study Drug	Further Considerations
Peripheral Neuropathy:		
Grade 1 peripheral neuropathy	• No action	Grade 1 signs and symptoms: asymptomatic; without pain or loss of function; clinical or diagnostic observations only [14]
New or worsening Grade 1 peripheral neuropathy with pain or Grade 2	• Hold study drug until resolution to Grade ≤ 1 or baseline	Grade 2 signs and symptoms: Moderate symptoms; limiting instrumental activities of daily living (ADL) [14]

Table 4-3 **Ixazomib** Treatment Modification (Delays, Reductions, and Discontinuations) Due to Adverse Events (Non-Hematologic Toxicities)

Adverse Event (Severity)	Action on Study Drug	Further Considerations
New or worsening Grade 2 peripheral neuropathy with pain or Grade 3	• Hold study drug until resolution to Grade ≤ 1 or baseline • Reduce study drug to next lower dose upon recovery	Grade 3 signs and symptoms: severe symptoms; limiting self-care ADL; assistive device indicated [14]
New or worsening Grade 4 peripheral neuropathy	• Discontinue study drug	
Grade 2 Rash	• Symptomatic recommendations as per section 6.7	The investigator and project clinician may discuss considerations for dose modifications and symptom management.
Grade 3 nonhematologic toxicity including hepatic and cardiac judged to be related to study drug	• Hold study drug until resolution to Grade < 1 or baseline	Symptomatic recommendations noted in Section 6.7
If not recovered to < Grade 1 or baseline within 4 weeks	• Reduce study drug 1 to next lower dose upon return to < Grade 1 or baseline	
Subsequent recurrence Grade 3 that does not recover to < Grade 1 or baseline within 4 weeks	• Hold study drug until resolution to Grade < 1 or baseline • Reduce study drug to next lower dose	Monitor closely, take appropriate medical precautions, and provide appropriate symptomatic care
Grade 4 nonhematologic toxicities including hepatic and cardiac judged to be related to study drug	• Consider permanently discontinuing study drug	Exceptions are cases in which the investigator determines the patient is obtaining a clinical benefit

Once ixazomib is reduced for any toxicity, the dose may not be re-escalated.

6.3.2 Recommended Dose Modifications for dexamethasone Treatment Associated Toxicity

The primary complications of dexamethasone requiring a dose reduction or discontinuation of therapy will be severe allergic reaction, psychosis, self-reported or diagnosed infection or

hyperglycemia requiring hospitalization. In case of severe allergic reaction or psychosis we will discontinue dexamethasone therapy. Otherwise, dexamethasone will be reduced or discontinued in accordance with clinical guidelines.Re-escalation is allowable.

6.4 Excluded Concomitant Medications and Procedures

The following medications and procedures are prohibited during the study.
Systemic treatment with any of the following metabolizing enzyme inducers should be avoided, unless there is no appropriate alternative medication for the patient's use (Rationale: If there were to be a drug-drug interaction with an inducer, ixazomib exposure would be decreased):

- Strong CYP3A inducers: rifampin, rifapentine, rifabutin, carbamazepine, phenytoin, and phenobarbital

The following medicinal products and procedures are prohibited during the study.

- Excluded dietary supplements include St. John's wort
- Platelet transfusions to help patients meet eligibility criteria are not allowed within 3 days prior to study drug dosing for any dosing day

6.5 Permitted Concomitant Medications and Procedures

The following medications and procedures are permitted during the study:

- Antiemetics, including 5-HT3 serotonin receptor antagonists, may be used at the discretion of the investigator.
- Loperamide or other antidiarrheal should be used for symptomatic diarrhea at discretion of the investigator. The dose and regimen will be according to institutional guidelines. Intravenous fluids should be given to prevent volume depletion.
- Growth factors (eg, granulocyte colony stimulating factor [G-CSF], granulocyte macrophage-colony stimulating factor [GM-CSF], recombinant erythropoietin) are permitted. Their use should follow published guidelines and/or institutional practice; however, alternative usage may be reviewed with the Millennium Clinical or Medical Representative . Erythropoietin will be allowed in this study. Their use should follow published guidelines and/or institutional practice.
- Patients should be transfused with red cells and platelets as clinically indicated and according to institutional guidelines as long as "3 days prior to study drug dosing for any dosing day" – per excluded procedures in seciton 6.4 above.
- Antiviral therapy such as acyclovir may be administered if medically appropriate.
- Concomitant treatment with bisphosphonates will be permitted, as appropriate.
- Patients who experience worsening neuropathy from baseline may be observed for recovery, and have dose reductions/delays as indicated in the protocol, and any supportive therapy or intervention may be initiated as appropriate at the discretion of the investigator.
- Supportive measures consistent with optimal patient care may be given throughout the study.

6.6 Precautions and Restrictions

Fluid deficit Fluid deficit should be corrected as per standard of care before initiation of treatment and during treatment.

Pregnancy

It is not known what effects ixazomib has on human pregnancy or development of the embryo or fetus. Therefore, female patients participating in this study should avoid becoming pregnant, and male patients should avoid impregnating a female partner. Nonsterilized female patients of reproductive age group and male patients should use effective methods of contraception through defined periods during and after study treatment as specified below.

Female patients must meet 1 of the following:

- Postmenopausal for at least 1 year before the screening visit, or
- Surgically sterile, or
- If they are of childbearing potential, agree to practice 2 effective methods of contraception from the time of signing of the informed consent form through 90 days after the last dose of study drug, or
- Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods] and withdrawal are not acceptable methods of contraception.)

Male patients, even if surgically sterilized (ie, status postvasectomy) must agree to 1 of the following:

- Practice effective barrier contraception during the entire study treatment period and through 90 days after the last dose of study drug, or
- Agree to practice true abstinence, when this is in line with the preferred and usual lifestyle of the subject. (Periodic abstinence [eg, calendar, ovulation, symptothermal, postovulation methods for the female partner] and withdrawal are not acceptable methods of contraception.)

6.7 Preparation, Reconstitution, and Dispensing

Ixazomib is an anticancer drug and as with other potentially toxic compounds caution should be exercised when handling ixazomib capsules.

Dexamethasone is a corticosteroid drug and will be provided by the study sponsor through the UW-Pharmaceutical Research Center (PRC).

Subjects will be dispensed one cycle of ixazomib and dexamethasone at each visit.

Ixazomib: Subjects will be dispensed a wallet/carton of 3 capsules in each wallet/carton.

Dexamethasone: Subjects will be dispensed 15 4mg tablets in an amber bottle per cycle.

6.8 Packaging and Labeling

The study drug ixazomib capsules will be provided by Millennium. The study drug will be labeled and handled as open-label material, and packaging labels will fulfill all requirements specified by governing regulations.

The capsules are individually packaged using cold-form foil-foil blisters that are in a child-resistant carton. There are 3 capsules in each wallet/carton.

Dexamethasone 4mg tablets will be provided by the UW-PRC. For each 20 mg dose, 5 tablets will be prepared in a small package. Three packages of 20 mg total dexamethasone tablets will

be used for each cycle. The PRC will provide treatment for one cycle at a time. The package will be provided to subjects at each study visit.

6.9 Storage, Handling, and Accountability

Upon receipt at the investigative site, ixazomib should remain in the blister and carton provided until use or until drug is dispensed. The container should be stored at the investigative site refrigerated (36°F to 46°F, 2°C to 8°C). Ensure that the drug is used before the retest expiry date provided by Millennium. Expiry extensions will be communicated accordingly with updated documentation to support the extended shelf life.

In countries where local regulations permit, ixazomib capsules dispensed to the patient for take-home dosing should remain in the blister packaging and refrigerated as noted above until the point of use. The investigative site is responsible for providing the medication to the patient in the correct daily dose configurations. Comprehensive instructions should be provided to the patient in order to ensure compliance with dosing procedures. Patients who are receiving take-home medication should be given only 1 cycle of medication at a time. Patients should be instructed to store the medication refrigerated (36°F to 46°F, 2°C to 8°C) for the duration of each cycle. Patients should be instructed to return their empty blister packs to the investigative site, rather than discarding them. Reconciliation will occur accordingly when the patient returns for their next cycle of take-home medication. Any extreme in temperature should be reported as an excursion and should be dealt with on a case-by-case basis.

Ixazomib will be handled with due care. Patients should be instructed not to chew, break, or open capsules. In case of contact with broken capsules, raising dust should be avoided during the clean-up operation. The product may be harmful by inhalation, ingestion, or skin absorption. Gloves and protective clothing should be worn during cleanup and return of broken capsules and powder to minimize skin contact.

The area should be ventilated and the site washed with soap and water after material pick-up is complete. The material should be disposed of as hazardous medical waste in compliance with federal, state, and local regulations.

In case of contact with the powder (eg, from a broken capsule), skin should be washed immediately with soap and copious amounts of water for at least 15 minutes. In case of contact with the eyes, copious amounts of water should be used to flush the eyes for at least 15 minutes. Medical personnel should be notified. Patients are to be instructed on proper storage, accountability, and administration of ixazomib, including that ixazomib is to be taken as intact capsules.

Dexamethasone 4mg tablets will be provided by the UW-PRC. For each 20 mg dose, 5 tablets will be prepared in a small package. Three packages of 20 mg total dexamethasone tablets will be used for each cycle. The PRC will provide treatment for one cycle at a time. The package will be provided to subjects at each study visit.

6.10 Study Drug Compliance

Study drug will be administered or dispensed only to eligible patients by the investigator, identified sub-investigator(s), or study coordinators. The appropriate study personnel will maintain records of study drug receipt and dispensing. The study personnel will monitor the following compliance

criteria quarterly for subjects to remain in the study (i.e. subjects <75% study drug compliance will be re-educated). If they fall below 75% study drug compliance again, they will be withdrawn.

6.11 Treatment Assignment *(if applicable)*

N/A

6.12 Termination of Treatment and/or Study Participation

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator also has the right to withdraw patients from the study for any of the following reasons:

- If study drug is discontinued due to AE
- Protocol violation
- Lost to follow-up
- Study terminated
- Non-compliance as defined above in section 6.10

Patients who are withdrawn from the study will not be replaced. The primary reason for patient's withdrawal from the study should be recorded in the source documents and CRF. Subjects will complete an early termination visit.

6.14 Specimen Handling and Confidentiality

Samples that will be sent to the Terasaki Research Foundation Laboratory and University of Michigan will be labeled with the study ID number, date of service and subject age. Researchers at outside institutions will not have access to the key that links the code to identifiable information. Any leftover samples that are not used for the analysis will be destroyed after the study is over. The Terasaki Research Foundation Lab will perform analysis of HLA-DSA, IgG subclasses, ETA-R and AT1-R antibodies in 10 subjects at 5 time points (50 samples total).

6.15 Bone Marrow Studies

Mechanistic studies on B cells and plasma cells (University of Michigan) and mesenchymal stromal cells (UW) witll be conducted to better understand the effect of treatment on desensitization. Dr. Cascalho and Dr. Platt at UM will participate in the research by processing bone marrow studies (ASCs, BAFF/APRIL). All subjects will be enrolled in the IXADES study at the University of Wisconsin-Madison. University of Michigan will not be involved with subject recruitment or subject interaction. The samples that will be sent to the University of Michigan will be labeled with a study ID number and will contain; date of service, and subject age. Dr. Cascalho and Dr. Platt will process the samples in the lab and perform mechanistic studies to achieve the aims of the research. Dr. Cascalho and Dr. Platt will analyze the bone marrow samples, provide the results to the study team at UW, and assist with writing the manuscript. Dr. Cascalho and Dr. Platt will not have access to the key to the code. The UW study team will communicate with the study team at the University of Michigan once per month through phone calls or e-mails.

A portion of marrow aspirates will be saved at UW for future research studies related to sensitization or transplantation. In addition, the samples may be used in a future therapeutic study to reinject mesenchymal stromal cells in subjects who did not achieve desensitization

through the current study. Samples will be banked at UW for an indefinite period of time. Subjects may withdraw their permission at any time.

7. STATISTICAL AND QUANTITATIVE ANALYSES

7.1 Statistical Methods

More than 50% of patients with CDC T cell crossmatch titers ≥ 1:8 and/or cPRA > 80% fail to respond to desensitization (5, 8). In this pilot exploratory, proof of concept, open-label, single-center phase II clinical trial, we hypothesize that our study will have a higher rate of transplantation. Dr. Brad Astor, PhD, epidemiologist and biostatistician in the division of nephrology, will perform the statistical analyses. ***Primary*** efficacy (decline in cPRA > 20%) and safety (discontinuation of therapy due to adverse events) endpoint will be analyzed using the exact binomial test. The ***secondary*** endpoint (time to transplantation) will be assessed using Kaplan-Meier analyses and log rank tests. Baseline characteristics will be compared between participants with and without the primary end point using the 2-sample t test for continuous variables and the Fisher's exact test for categorical variables. Relationships between serum BAFF/APRIL, antibodies, and baseline cPRA will be assessed using the Pearson correlation coefficient. The influence of serum BAFF/APRIL, antibodies, and bone marrow plasma cells on end points of interest will be investigated using Kaplan-Meier curves and Cox proportional-hazards models.

7.1.1 Determination of Sample Size

The primary outcome will be considered as a dichotomous outcome (decline in cPRA of >20% or not). We will test the hypothesis that the success rate (i.e., the % that have a decline in cPRA>20%) is 0% using the exact binomial test (Figure 2a) (5). The figure shows the lower and upper 95% confidence limits resulting from any observed success rate. For example, if we observe success in 3/10 (30%), the resulting 95% confidence interval (CI) is (6.7%, 65.2%). Any observed success demonstrates that the success rate of the proposed treatment is >0. Figure 2b represents the power curve, that is, the probability of observing any success, given different values of true success rate. For example, we would have > 80% chance of observing at least one success if the true success rate is > 0.2.

Figure 2a. Sample Size Analysis

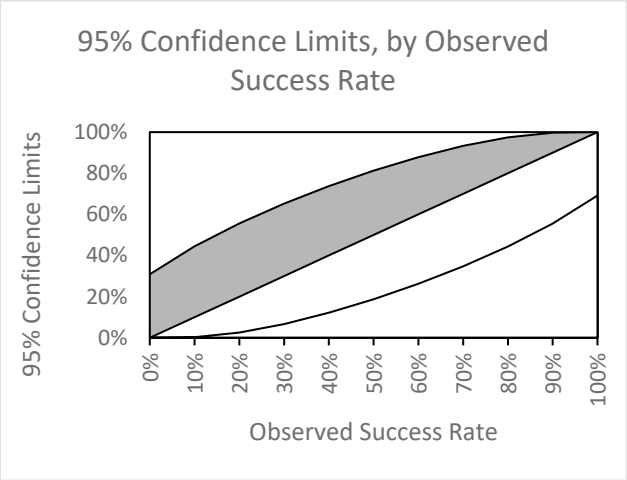
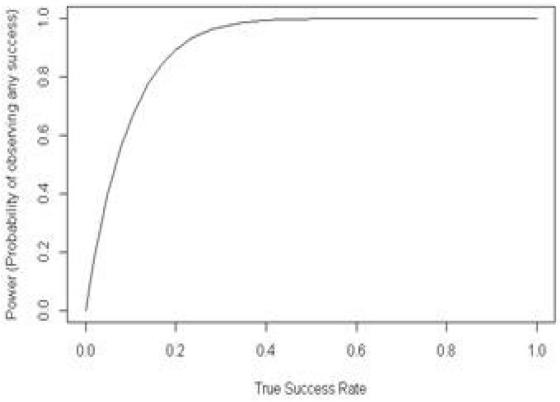


Figure 2b. Power curve



7.1.2 Randomization and Stratification

N/A

7.1.3 Populations for Analysis

All patients enrolled will be included in the safety analysis.

7.1.4 Procedures for Handling Missing, Unused, and Spurious Data

All available data will be included in the data listings and tabulations. Where appropriate, imputations of values for missing data will be performed using last observation carried forward and specified in the Statistical Analysis Plan. All data recorded on the CRF will be included in the data listings that will accompany the clinical study report.

7.1.5 Demographic and Baseline Characteristics

Demographic and baseline characteristics in all enrolled patients will be reported in a table format. These will include age, gender, race, ethnicity, cause of ESRD, history of sensitization (transfusion, previous transplantation, pregnancy), type of renal replacement therapy, baseline cPRA, CBC, LFTs, and medications.

7.1.6 Efficacy Analysis

Primary outcome will be a decline in cPRA > 20%. Secondary outcome will be kidney transplantation within 12 months of therapy. These outcomes will be assessed using paired t-Test, Kaplan-Meier methods and log rank tests, where appropriate. In addition, we will analyze the change in HLA antibody strength by comparing the MFI values pre and post therapy.

7.1.7 Pharmacokinetics/Pharmacodynamics/Biomarkers *(if applicable)*

N/A

7.1.8 Safety Analysis

Together with the UW ICTR Data Monitoring Committee (DMC) we will develop a detailed a data and safety monitoring plan (DSMP) which will ensure ongoing safety of participating patients commensurate with the level of risk and complexity of the protocol treatment to minimize risks, integrity of trial data and scientific validity of the trial. The development of the DSMP will be guided by the NIH Policy for Data and Safety Monitoring and by the FDA Guidance on the Establishment and Operation of Clinical Trial Data Monitoring Committees. The plan will make certain that review of the conduct of research is rigorous, independent and objective, interim safety and efficacy data is collected and presented and progress towards achieving study goals is made. It will cover policies and procedures for reporting adverse events to the DSMB, IRB, and the FDA.

The safety analysis will focus on infections, malignancies, hematological complications including leucopenia, anemia, and thrombocytopenia, cardiovascular complications and events, distal neuropathy, gastrointestinal symptoms, and immunosuppression-related adverse effects. Patients will have a physical exam 5 times (beginning and end of the study, and quarterly during the study) following enrollment: Days 1, 85, 169, 253, and 373 (+ 6, - 3 for all visits except for visit 1).

- Day 1: Prior to treatment initiation. H&P, Labs, Bone Marrow Aspirate
- Day 85 + 6, - 3: Prior to treatment dose. H&P, Labs
- Day 169 + 6, - 3 Prior to treatment dose. H&P, Labs
- Day 253 + 6, - 3 Prior to treatment dose. H&P, Labs
- Day 373 + 6, - 3 After treatment completion, H&P, Labs, Bone Marrow Aspirate
- At each of these visits, the patients will undergo a medical history and physical exam, reviewing the adverse event data, vital signs, monthly labs, changes in patients' health status and medications.
- Study drug will be dispensed at each month's in-person visit, prior the initiation of the next cycle, and after reviewing the AEs and monthly labs.
  Liver Function Tests (LFTs) will be conducted twice a month for the first 3 months, then monthly thereafter. The Complete Blood Count (CBC) will be checked monthly.

7.1.9 Interim Analysis

We will utilize the UW ICTR Data Monitoring Committee (DMC) to oversee the study and is described further in section 9.1 *S*ource documents may be reviewed to allow the DMC to independently judge whether the overall integrity and conduct of the protocol remain acceptable based on data provided and reported by the Principal Investigator. The DMC will make recommendations to the Principal investigator that could include actions of continuation, modification, suspension or termination.

7.1.10 Study Stopping Rules

Subject Stopping Rules

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator also has the right to withdraw patients from the study for any of the following reasons:

- If study drug is discontinued due to AE

  If a subject is withdrawn due to an adverse event, the dose adjustment, modification and withdrawal procedures related to adverse events described in section 6.3 of the clinical protocol will be followed.
- Protocol violation
- Lost to follow-up
- Study terminated
- Non-compliance

Study Stopping Rules

Serious Adverse Events (SAEs) as described below, will be reviewed to determine if additional risk could be posed to the subject in question or others participating in the study. If it is determined that a serious adverse event, new information, or an unanticipated event could pose a potential risk to subjects,the DMC and/or the investigator may decide to stop the study.

8. ADVERSE EVENTS

Adverse drug reactions such as thrombocytopenia, diarrhea, fatigue, nausea, vomiting, and rash have been associated with ixazomib treatment.

8.1 Definitions

8.1.1 Adverse Event Definition

Adverse event (AE) means any untoward medical occurrence in a patient or subject administered a pharmaceutical product; the untoward medical occurrence does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not it is related to the medicinal product. This includes any newly occurring event, or a previous condition that has increased in severity or frequency since the administration of study drug. AEs will be assessed at study visits and during the final follow-up study visit. In addition, data on rejection, graft, and patient survival will be collected for 6 months following kidney transplantation.

An abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered by the investigator to be a clinically significant change from baseline. These AEs will be entered into the OnCore system.

8.1.2 Serious Adverse Event Definition

Serious AE (SAE) means any untoward medical occurrence that at any dose:

- Results in **death.**

- Is **life-threatening** (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- Requires inpatient **hospitalization or prolongation of an existing hospitalization** (see clarification in the paragraph below on planned hospitalizations).
- Results in **persistent or significant disability or incapacity**. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- Is a **congenital anomaly/birth defect**.
- Is a **medically important event (Tables 4-2 and 4-3)**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent 1 of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, grade 4 neuropathy, cardiac, or hepatic toxicity, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (eg, prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term *severe* is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as *serious*, which is based on patient/event outcome or action criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm^3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations.

8.2 Procedures for Reporting Serious Adverse Events

AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. For serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

AEs which are serious must be reported to Millennium Pharmacovigilance (or designee) from the first dose of study drug through 30 days after administration of the last dose of ixazomib. Any SAE that occurs at any time after completion of ixazomib treatment or after the designated follow-up period that the sponsor-investigator and/or sub-investigator considers to be related to any study drug must be reported to Millennium Pharmacovigilance (or designee). In addition, new primary malignancies that occur during the follow-up periods must be reported, regardless of causality to study regimen, up to one month after the last dose of the investigational product, starting from the first dose of study drug. All new cases of primary malignancy must be reported to Millennium Pharmacovigilance (or designee).

Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).
Since this is an investigator-initiated study, the principal investigator Dr. Arjang Djamali, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor- investigator's EC or IRB.

Regardless of expectedness or causality, all SAEs (including serious pretreatment events) must also be reported in English to Millennium Pharmacovigilance (or designee):

> **Fatal and Life Threatening SAEs** within 24 hours of the sponsor-investigator's observation or awareness of the event
>
> **All other serious (non-fatal/non life threatening) events** within 4 calendar days of the sponsor-investigator's observation or awareness of the event

See below for contact information for the reporting of SAEs to Millennium Pharmacovigilance.
The sponsor-investigator must fax or email the SAE Form per the timelines above. A sample of an SAE Form will be provided.
The SAE report must include at minimum:

- **Event term(s)**
- **Serious criteria**
- **Intensity of the event(s):** Sponsor-investigator's or sub-investigator's determination. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version specified in the protocol, as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.
- **Causality of the event(s):** Sponsor-investigator's or sub-investigator's determination of the relationship of the event(s) to study drug administration.

Follow-up information on the SAE may be requested by Millennium.

In the event that this is a multisite study, the sponsor-investigator is responsible to ensure that the SAE reports are sent to Millennium Pharmacovigilance (or designee) from all sites participating in the study. Sub-investigators must report all SAEs to the sponsor-investigator so that the sponsor-investigator can meet his/her foregoing reporting obligations to the required regulatory agencies and to Millennium Pharmacovigilance, unless otherwise agreed between the sponsor-investigator and sub-investigator(s).

Relationship to all study drugs for each SAE will be determined by the investigator or sub-investigator by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the study drug(s)?

Sponsor-investigator must also provide Millennium Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to the study product(s), as soon as possible but no later than 4 calendar days of such communication.

SAE and Pregnancy Reporting Contact Information
US & Canada

Fax Number: 1-800-963-6290
Email: TakedaOncoCases@cognizant.com

Rest of World
Fax #: 1 202 315 3560

E-mail: TakedaOncoCases@cognizant.com
Suggested Reporting Form:

- SAE Report Form (provided by Millennium)
- US FDA MedWatch 3500A:

http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm

- Any other form deemed appropriate by the sponsor-investigator

8.3 Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

If a woman becomes pregnant or suspects that she is pregnant while participating in this study or within 90 days after the last dose, she must inform the investigator immediately and permanently discontinue study drug. The sponsor-investigator must immediately fax a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee (see Section 8.2). The pregnancy must be followed for the final pregnancy outcome.
If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately fax a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee (see Section 8.2). Every effort should be made to follow the pregnancy for the final pregnancy outcome.
Suggested Pregnancy Reporting Form:

- Pregnancy Report Form (provided by Millennium)

9. SAFETY MONITORING

9.1 Safety Monitoring

The investigator is responsible for monitoring the safety of subjects who have enrolled in the study. For this study, adverse events will be solicited from subjects to identify an occurrence or worsening of an unintended sign or symptom, related to the ingestion of the study drug. The study team will ask subjects prior to each cycle if they experienced any new medical related signs or symptoms since taking the study drug. Subjects will be asked about any adverse events they may have experienced again monthly, prior to each cycle, and at the five study visits. Only the adverse events considered related (possibly, probably or definitely) to the ingestion of the study drug will be entered into and managed in the ICTR OnCore system quarterly.

At each visit, subjects will be assessed for AEs by the study team. They will be examined by the principal investigator 5 times (please see tables 2-1 a-b and Figure 1, below): Days 1, 85, 169, 253, and 373 + 6, - 3).

- Day 1: Prior to treatment initiation. H&P, Labs, Bone Marrow Aspirate
- Day 85 + 6, - 3: Prior to treatment dose. H&P, Labs
- Day 169 + 6, - 3 Prior to treatment dose. H&P, Labs
- Day 253 + 6, - 3 Prior to treatment dose. H&P, Labs
- Day 373 + 6, - 3 After treatment completion, H&P, Labs, Bone Marrow Aspirate
- Study drug will be dispensed at each month's in-person visit, prior the initiation of the next cycle, and after reviewing the AEs and monthly labs.

- Patients will undergo monthly CBC and bi-weekly liver function tests (LFTs) for the first three months. After the first 3 months, CBCs and LFTs will be done monthly. cPRA, BAFF/APRIL, DSA and antibodies will be checked every three months (5 times total). Bone marrow aspirates will be collected at the beginning and at one of the following time points: one month (+ 6, - 3 days) after the end of the 12 month study, cPRA<80%, time of transplant, or time of subject withdrawal from the study.
- Additional visits may be required if the subject has to hold treatment and needs to return to the clinic for blood draws to confirm eligibility to continue. Please see Table 4-3, Dose Adjustments for Hematologic Toxicities for dose adjustment plan.

Based on the subject population, it is anticipated that the laboratory results that will be abstracted from the EMR for study purposes will be abnormal. The PI will not make clinically significant/non-clinically significant determinations of these results, as this subject population is expected to be abnormal and clinically significant hence their current clinical treatment. All reportable events related to the study protocol and its associated procedures will be reported to the IRB. It is the responsibility of the Investigator to promptly notify the Institutional Review Board of all unanticipated problems involving risk to human research subjects. Reportable events, such as protocol noncompliance, serious adverse events, new information, and unanticipated problems will be reported to the IRB and DMC in accordance with the UW IRB guidelines.

We will utilize the UW ICTR Data Monitoring Committee (DMC) to oversee the study. The UW ICTR DMC is comprised of experienced members (core plus ad hoc) with expertise required to oversee this study. In providing oversight for the conduct of this study, the ICTR DMC will meet annually during the five year time period in which subjects will have any study procedures. At these annual meetings, the DMC members will review protocol-specific reports created by statisticians using data pulled from the ICTR OnCore clinical research management system. These standard reports will include an overview of study objectives, a review of actual and projected accrual rates, an evaluation of patient demographics, a summary of the number and seriousness of adverse events, and an analysis of the primary and secondary outcomes. The safety data that will be reviewed includes the number and seriousness of infections, malignancies, hematological complications including leucopenia, anemia, and thrombocytopenia, cardiovascular complications and events, distal neuropathy, gastrointestinal symptoms, and immunosuppression-related adverse effects. Additional specific safety areas that will be closely monitored are listed in Section 1.10.

Source documents may be reviewed to allow the DMC to independently judge whether the overall integrity and conduct of the protocol remain acceptable based on data provided and reported by the Principal Investigator. The DMC will make recommendations to the Principal investigator that could include actions of continuation, suspension, modification or termination.

The boundary for excess harm will equal observed harm which, in the judgment of the DMC, is excessive. We will report all AEs and SAEs into OnCore for review by the DMC and the IRB oversight committee UW Madison Health Sciences IRB (HS-IRB) in accordance with the HS-IRB reporting guidelines.

9.2 IND Monitoring Plan

The principal investigator will follow the FDA regulations in 21 CFR 312 applicable to sponsor responsibilities, particularly Subpart D. This includes:

- Filing protocol amendments when required by 21 CFR 312.30;
- Notifying the FDA and participating investigators of adverse events as required in 21 CFR 312.32;
- Filing annual reports with the FDA as required by 21 CFR 312.33;
- Record keeping requirements of 21 CFR 312.57; and
- Promptly reporting as required in 21 CFR 312.56(b) to the FDA and all participating investigators of significant new adverse effects or risks with respect to the study drug.

The Investigator will permit trial-related monitoring, audits, IRB review and regulatory inspection(s) by providing direct access to source data and study documents.

9.3 ICTR STUDY MONITORING SERVICE (SMS)

While many institutions involved in clinical research conduct various types of quality assurance reviews and audits, University of Wisconsin Institute for Clinical and Translational Research (ICTR) is one of a few institutions to offer independent Study Monitoring Services, a robust academic equivalent to the industry Contract Research Organization (CRO) standards for ongoing study monitoring.

The sponsor-investigator has contracted with ICTR Study Monitoring Service (SMS) for the proposed study to provide ongoing monitoring services to conduct and follow-up of monitoring visits throughout the life cycle of the study. Refer to the Study Monitoring Plan.

For this study, UW ICTR SMS personnel will conduct a Site Initiation Visit (SIV) and ongoing Interim Monitoring Visits (IMVs), either on-site, remotely, centrally or a combination thereof, throughout the duration of the study. During IMVs, the monitors will review study materials, including but not limited to: regulatory files, consent forms, case report forms, and drug accountability logs. UW ICTR SMS will review FDA communications to ensure compliance with applicable FDA IND regulations, guidelines, and institutional policies. UW ICTR SMS personnel will conduct a Close-Out Visit (COV) upon completion of the study at the study site.

Monitoring will consist of all (100%) of the study-related records representing 50-75% of those enrolled at a minimum. SMS personnel could increase the percentage of study or subject records to be reviewed if warranted by the ongoing monitoring findings, resulting in a partial or full review of up to 100% of the study-related subject records. Based on the plan to review approximately 50-75% of the subject records, it is anticipated that the following monitoring visit schedule will implemented; 1 SIV, 1 IMV following the enrollment of the first subject(s), followed by approximately 8-9 IMVs through the end of the study, planned on an approximate quarterly basis. The frequency of the IMVs could increase based on subject enrollment and preivous monitoring visit findings, but will not decrease.

The SMS will review the accuracy and completeness of completed consent and HIPAA documents for each enrolled subject. For approximately 50-75% of enrolled subjects, the SMS will evaluate the completeness and accuracy of the data collected and conduct a comparison with the electronic data captured, resulting in electronic queries with resolution or validation. In addition, the SMS staff will verify adherence to protocol eligibility criteria, verify documentation for the accountability and administration of the study product, verify documentation of study procedures, and verify that FDA required SAE reports were appropriately submitted.

The study monitor(s) will work closely with the ICTR DMC statistician and the study statistician to conduct periodic central data reviews, with follow-up conducted by the study monitors for any data discrepancies identified.

9.4 DATA MANAGEMENT SYSTEM

9.4.1 Data Collection Tools

The critical data collected to address the study objectives will be electronically captured and managed in the clinical trials management software, OnCore, managed by the Institute for Clinical and Translational Research (ICTR). The study specific data collection tools will be developed and maintained with the assistance of the ICTR OnCore data management team, in consultation with the ICTR Study Monitoring Service and the ICTR Data Monitoring Committee to ensure the collection and recording all subject considered critical to answer the study objectives. It is the Investigator's responsibility to ensure that these are properly, legibly and fully completed and signed where appropriate. The data collection forms are used to record study data and are an integral part of the study and subsequent reports.

9.4.2 Data management Tool

The critical data collected to address the study objectives will be electronically captured and managed in the clinical trials management software, OnCore, managed by the Institute for Clinical and Translational Research (ICTR). The ICTR OnCore data management team will work with the investigator and study team to develop the data collection tools, both in paper and electronic format, to collect the relevant, applicable study data. The ICTR OnCore data management team will also work with the study team to identify and implement quality assurance features at the time of data entry (i.e. use of minimum/maximum limits, constraints and branching logic to require or skip data based on preceding data entered). These features will reduce the amount of data entry error at the time of entry.

OnCore is a sophisticated, web-based study and data management system that: a) ensures secure, easy data entry at multiple sites; b) integrates multiple data sources; c) provides controlled, secure access to sensitive data using role-based access control; d) provides workflow automation; and e) allows export and reporting of data for Data and Safety Monitoring Boards and biostatisticians.

In addition to being a secure web-based system with restricted access, the ICTR OnCore system provides an audit trail from the moment the data is first entered, revised and locked. The ICTR OnCore system also provides the functionality to the SMS staff to electronically query data, the study team to electronically respond, resulting in a final state of validation prior to the locking of study data. The various states (i.e. Started, Completed, Queried, Ameneded, Validated) are built in the system to allow easy navigation as well as an assessment of the state of the data at any given moment.

10. ETHICAL CONSIDERATIONS AND COMPLIANCE WITH GMP

10.1 Statement of Compliance

This trial will be conducted in compliance with the protocol, current Good Clinical Practices (GCP), adopting the principles of the Declaration of Helsinki, and all applicable regulatory requirements.

Prior to study initiation, the protocol and the informed consent documents will be reviewed and approved by an appropriate ethics review committee or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are implemented.

10.2 Informed Consent

The informed consent form is a means of providing information regarding the trial to a prospective participant and allows for an informed decision about participation in the study. All participants in the IXADES study must read, sign and date a consent form prior to participation in the study, taking study drug and/or undergoing any study-specific procedures.

The informed consent form must be updated or revised whenever important new safety information is available, whenever the protocol is amended, and/or whenever any new information becomes available that may affect a patients' participation in the trial.

A copy of the informed consent will be given to a prospective participant for review. The Study Investigator or a designee of the Investigator will review the consent and answer any questions that the potential participant may have. The participant will be informed that their participation is voluntary and they may withdraw from the study at any time, for any reason.

10.3 Privacy and Confidentiality

Subject privacy and confidentiality will be respected throughout the study. Each study participant will be assigned a sequential subject identification number specific to the given study (i.e., IXA001 – IXA-010). These numbers rather than names will be used to collect, store and report participant information. Contact with subjects will be conducted in private exam or procedure rooms to ensure subjects' privacy. No information other than information needed specifically for the study will be collected.

The ICTR data management team will work with investigators to collect only relevant, applicable study data, which will be entered in a secure web-based system with restricted access.

11. ADMINISTRATIVE REQUIREMENTS

11.1 Product Complaints

A product complaint is a verbal, written, or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact Millennium (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Millennium Quality representative.

For Product Complaints,

- Phone: 1-844-ONC-TKDA (1-844-662-8532)
- E-mail: GlobalOncologyMedinfo@takeda.com
- FAX: 1-800-881-6092
- Hours: Mon-Fri, 9 a.m. – 7 p.m. ET

Product complaints in and of themselves are not AEs. If a product complaint results in an SAE, an SAE form should be completed and sent to Millennium Pharmacovigilance

12. APPENDICES

12.1 Eastern Cooperative Oncology Group (ECOG) Scale for Performance Status

Grade	Description
0	Normal activity. Fully active, able to carry on all predisease performance without restriction
1	Symptoms but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (eg, light housework, office work)
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

Source: Oken MM, Creech RH, Tormey DC, Horton J, Davis TE, McFadden ET et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. Am J Clin Oncol 1982; 5 (6):649-55.

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