[INVESTIGATIONAL DRUG CODE/everolimus]

Clinical Study Protocol IIRP-1426

A prospective, randomized, single center pilot study comparing patient and graft survival, adverse events and tolerability of Zortress® (everolimus) versus Rapamune® (sirolimus) in combination with low dose Neoral® (cyclosporine) dosed by C2 monitoring, in deceased and living donor renal transplant recipients under a Thymoglobulin® (antithymocyte globulin) and rapid steroid induction protocol.

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List of abbreviations

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
b.i.d.	twice a day
CRF	Case Report/Record Form
CRD	Clinical Research and Development
CPO	Country Pharma Organization
CRO	Contract Research Organization
CSR	Clinical Study Report
ECG	Electrocardiogram
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
IMS	Integrated Medical Safety
i.v.	intravenous(ly)
IRB	Institutional Review Board
IVRS	Interactive Voice Response System
o.d.	once a day
p.o.	oral(ly)
REB	Research Ethics Board
SAE	serious adverse event

Glossary of terms

Assessment	A procedure used to generate data required by the study
Control drug	A study drug used as a comparator to reduce assessment bias, preserve blinding of investigational drug, assess internal study validity, and/or evaluate comparative effects of the investigational drug
Enrollment	Point/time of patient entry into the study; the point at which informed consent must be obtained (i.e. prior to starting any of the procedures described in the protocol)
Investigational drug	The study drug whose properties are being tested in the study; this definition is consistent with US CFR 21 Section 312.3 and is synonymous with “investigational new drug.”
Medication number	A unique identifier on the label of each medication package in studies that dispense medication using an IVR system
Patient number	A number assigned to each patient who enrolls in the study. When combined with the center number, a unique identifier is created for each patient in the study.
Stage	A major subdivision of the study timeline; begins and ends with major study milestones such as enrollment, randomization, completion of treatment, etc.
Period	A minor subdivision of the study timeline; divides phases into smaller functional segments such as screening, baseline, titration, washout, etc.
Premature patient withdrawal	Point/time when the patient exits from the study prior to the planned completion of all study drug administration and assessments; at this time all study drug administration is discontinued and no further assessments are planned
Randomization number	A unique identifier assigned to each randomized patient, corresponding to a specific treatment arm assignment
Stop study participation	Point/time at which the patient came in for a final evaluation visit or when study drug was discontinued whichever is later
Study drug	Any drug administered to the patient as part of the required study procedures; includes investigational drug and any control drugs
Study drug discontinuation	Point/time when patient permanently stops taking study drug for any reason; may or may not also be the point/time of premature patient withdrawal
Variable	Information used in the data analysis; derived directly or indirectly from data collected using specified assessments at specified timepoints

Protocol synopsis

Title of study: A prospective, randomized, single center pilot study comparing patient and graft survival, adverse events and tolerability of Zortress® (everolimus) versus Rapamune® (sirolimus) in combination with low dose Neoral® (cyclosporine) dosed by C2 monitoring, in deceased and living donor renal transplant recipients under a Thymoglobulin® (antithymocyte globulin) and rapid steroid induction protocol.

Purpose and rationale: Our center's renal transplant immunosuppression protocol has consisted of Thymoglobulin and steroid induction for 5 doses only, with early initiation of Rapamune® (sirolimus) and then Neoral for nearly a decade now. We do observe a significant amount of adverse events in our patient population, with many of these potentially related to the sirolimus. The combination of "half-dose" cyclosporine (early C2 levels around 800 to 1000 ng/ml) allows us to minimize calcineurin inhibitor toxicity and its combination with the mTOR drug sirolimus provides excellent protection against acute rejection in our renal transplant recipients. Our earlier experience with everolimus and the recent publications describing renal recipients' outcomes with this drug, suggest to us that our patients may do better on everolimus than sirolimus. For our center, this pilot study would be very straightforward as the only difference in medication from our current well established protocol would be the randomization to either Rapamune®, our current standard of care, or Zortress®, the study drug.

Objectives: The primary objective of this pilot study will be to determine equivalency of Zortress® as compared to Rapamune® when used in our de novo immunosuppression regimen following renal transplantation. The primary endpoint will be a composite endpoint of graft survival (non-death censored) and biopsy proven acute rejection at 1 year. The primary outcome of immunosuppressive protection would be studied in our Thymoglobulin and rapid steroid discontinuation protocol, with "half-dose" Neoral as described above.

Secondary outcomes would include factors involved with adverse events and tolerability and would include: renal graft survival (non-death censored) and biopsy proven acute rejection at 6 months, 2 and 3 years, as well as the number and grade of acute rejection episodes and responsiveness to treatment, serum creatinine and estimated GFR, results of renal biopsies at 12 months post-transplant to look for histological evidence of graft injury, early and late wound complications, infections including CMV, EBV and BK infections as monitored by serum PCR testing, lipid panel testing, particularly triglycerides, cardiac events such as acute myocardial infarctions or Congestive Heart Failure episodes, and tolerability of the Neoral and mTOR combination as determined by the patients who required conversion off of the intended randomized drug combination, with attempt to document why the patient did not tolerate the drug combination.

Exploratory objectives would include molecular markers indicative of kidney and vascular endothelial function.

Population: The patient population will be all adults (18-75 years of age) who receive a living donor or deceased donor renal transplant as a first or subsequent renal transplant procedure only (no extra-renal organ transplant recipients). We will invite any patient who would normally be considered for our current Sirolimus®/ Neoral® protocol with Thymoglobulin® and steroid induction to consent for this pilot study and then undergo randomization post-operatively if the transplant surgeon anticipates normal early graft function. We would propose to enroll a total of 60 patients.

Inclusion/Exclusion criteria:

- Inclusion Criteria
    - Primary renal transplant recipients between ages 18 and 75 years of age.
    - Females capable of becoming pregnant must have a negative pregnancy test prior to transplantation and practice an effective form of birth control for the duration of the study.

- Exclusion Criteria
    - Total cholesterol > 300 mg/dl or triglycerides > 400 mg/dl despite lipid lowering therapy
    - Pre-existing bone marrow suppression (WBC < 3000, platelets < 100,000)
    - Positive crossmatch or ABO incompatible
    - Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive serum human chorionic gonadotrophin laboratory test (>5 mIU/mL)
    - Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, including women whose career, lifestyle, or sexual orientation precludes intercourse with a male partner and women whose partners have been sterilized by vasectomy or other means, UNLESS they are using two birth control methods. The two methods can be a double barrier method or a barrier method plus a hormonal method.
        - Adequate barrier methods of contraception include: diaphragm, condom (by the partner), intrauterine device (copper or hormonal), sponge or spermicide. Hormonal contraceptives include any marketed contraceptive agent that includes an estrogen and/or a progestational agent.
        - Reliable contraception should be maintained throughout the study and for 12 weeks after study drug discontinuation.
        - Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or six months of spontaneous amenorrhea with serum FSH levels > 40 mIU/mL and estradiol < 20 pg/mL] or have had surgical bilateral oophorectomy (with or without hysterectomy) at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.
    - Active infection (HBV, HIV)
    - Malignancy (except for adequately treated squamous or basal cell skin carcinoma) unless patient has written clearance from an Oncologist or if patient has had no malignancy for at least 2 years prior to the transplant
    - Allergy or intolerance to sirolimus, everolimus, cyclosporine, or ATG

Investigational and reference therapy: Zortress® bid for goal levels of 3 to 8 ng/ml by approved testing methods; Rapamune® goal levels of 10 to 18 ng/ml by Architect assay, or 8 to 12 by LC-MS methodology; Neoral® goal levels of 800 to 1000 ng/ml by immunoassay until 3 months Post-transplant, 600-800 ng/ml 3-6 months post-transplant, 400-600 ng/ml 6-12 months post-transplant, and 400 ng/ml > 12 months post-transplant. Induction agents: Thymoglobulin 1.25 mg/kg for 4 to 5 doses adjusted for white blood count and platelet count, as well as appropriate levels of the two maintenance immunosuppressive agents, and steroids given as methylprednisone IV for two doses, then oral prednisone at: 500 mg, 200 mg, 125 mg, 50 mg, and 25 mg.

Study design: Randomized, single center parallel-group, open-label study.

Pilot study with exploratory objectives.

Eligibility: All primary or retransplant recipients of deceased donor or living donor grafts with negative crossmatch who are felt to have good expectations for renal function recovery after surgery will be offered entry into the study.

Treatment scheme is as described above, with Solumedrol 500 mg IV in OR as induction, then 5 doses of Thymoglobulin starting morning of POD 1 at 1.25 mg/dose (rounded to nearest 25 mg) with

rapid steroid taper over five doses. The mTOR inhibitor drug will be started on POD 0 and Neoral introduced as renal function recovers, usually starting on POD 2.

Measurement/Assessments: as described above, graft and patient survival per standard definitions, clinically indicated biopsies for determination of acute cellular or antibody mediated rejection, renal function as estimated from serum Cr based on MDRD, clinic visits for wound assessment, standard of care labs for renal function, bone marrow function (WBC and platelet count), liver function, lipid panel, donor specific antibody determination during any significant increase in serum Cr, drug levels routinely monitored for need of number of dose adjustments and compliance, Emergency Room and hospitalizations, as well as unscheduled clinic and other physician visits. For the pilot study, we would perform scheduled CMV, EBV and serum BK PCRs monthly for the first year, then quarterly or with any viral symptoms.

Potential study participants will be approached for consent immediately prior to transplantation by the transplant surgeon or appointed representative if they meet the inclusion criteria above and do not fit the exclusion criteria above. If the transplant candidate agrees to participate he/she will be categorized as a living or deceased donor kidney recipient category and as primary or subsequent kidney transplant recipient. The candidate will then be randomized, in a 2:1 fashion, to either the Zortress or Rapamune study arm. Following transplantation all recipients will receive daily Thymoglobulin doses of 1.25 mg/kg starting on POD #1 for 4 to 5 doses adjusted for white blood count and platelet count. All recipients will be administered steroids as methylprednisone IV for two doses, then oral prednisone at: 500 mg, 200 mg, 125 mg, 50 mg, and 25 mg and will receive delayed Neoral® on POD #2-3 dosed to achieve serum levels of 800 to 1000 ng/ml by immunoassay until 3 months post-transplant, 600-800 ng/ml 3-6 months post-transplant, 400-600 ng/ml 6-12 months post-transplant, and 400 ng/ml > 12 months post-transplant. Zortress® will be started on POD #0 for Zortress study arm recipients, administered bid and dosed to achieve target levels of 3 to 8 ng/ml by approved testing methods; Rapamune® study arm recipients will receive Rapamune once daily, dosed to achieve target levels of 10 to 18 ng/ml by Architect assay, or 8 to 12 by LC-MS methodology. Neoral and Rapamune/Zortress levels will be monitored twice weekly for the first 3 months, once weekly for months 3-6, every other week months 6-12, and monthly thereafter. This pilot study is estimated to take one year for enrollment and three years from the date of last patient enrollment for completion of last patient visit, then several months for completion of paper work. This will be about 4.5 to 5 years.

Efficacy assessments:

- Incidence of graft loss in the first post-transplant year
- Incidence of patient death in the first post-transplant year
- Incidence of acute rejection at 1 year post-transplant

Other assessments:

- Incidence of non-death censored graft loss at 6 months, 2 and 3 years after transplantation
- Number and grade of acute rejection episodes at 6 months, 1, 2, and 3 years after transplantation
- Tolerability of Zortress at 1 year post-transplant
- Estimated GFR at 6 months, 1, 2, and 3 years after transplantation
- Incidence of CMV, EBV and BK infections 1 year after transplantation
- Results of 12 month surveillance biopsies
- Lipid panels and use of lipid lowering medications at 1 year after transplant
- Incidence of MI and CHF at 1 and 3 years after transplant

- Incidence of wound infection at 1 year after transplantation
- Incidence of lymphocele at 1 year after transplantation
- Plasma concentrations of S-adenosyl homocysteine (SAH) and S-adenosyl methionine (SAM)Plasma concentrations of endothelial dysfunction markers (symmetric and asymmetric dimethyl arginine (SDMA, ADMA), L-arginine, L-citrulline, L-ornithine cysteine, and homocysteine over the observation period

- Plasma concentrations of bioactive lipids (HODEs, HEPEs, HETEs, and prostaglandins) over the observation period
- Plasma protein patterns

Data analysis: The incidence of graft loss, patient death, and acute rejection will be compared by chi squared analyses. Patient and graft Kaplan-Meier survival curves will be compared by log rank test. We hypothesize there will be no statistically significant difference between the treatment arms. As this is a pilot study, it was not statistically powered to detect subtle differences in outcome. All continuous variables such as serum creatinine, cGFR, serum lipids, WBC, platelets, etcetera will be compared by student's t test. Any other dichotomous variables will be compared by chi squared analysis.

1 Background

Two prospective randomized trials (A2306 and A2307) have demonstrated that everolimus combined with steroids and low dose Neoral with (A2307) or without (A2306) basiliximab induction result in excellent 1 year outcomes (1). In 2002 we adopted an immunosuppressive regimen using Rapamune and reduced dose Neoral without steroids using induction therapy with Thymoglobulin. Since employing this regimen our acute rejection rate within the first year post-transplant has been between 7% and 10% for any given calendar year. However, many patients need to have their Rapamune discontinued due to drug related side effects or compliance difficulties related to the noon dosing (when most other medications are taken at 8 am and 4 pm). We feel strongly that use of an alternative drug to Rapamune in such cases is necessary. Use of Zortress in this setting would be ideal, however, no data currently exists concerning the use of Zortress with Neoral in a steroid free maintenance immunosuppression regimen following Thymoglobulin induction. Thus we propose this pilot study primarily to verify Zortress efficacy using our established immunosuppressive protocol. Secondarily we are interested in characterizing the Zortress drug related side effect profile to enable us in the future to exploit any favorable differences between Zortress and Rapamune.

2 Purpose and rationale

Our center's renal transplant immunosuppression protocol has consisted of Thymoglobulin and steroid induction for 5 doses only, with early initiation of Rapamune® (Sirolimus) and then Neoral for nearly a decade now. We do observe a significant amount of adverse events in our patient population, with many of these potentially related to the Sirolimus. The combination of "half-dose" cyclosporine (early C2 levels around 800 to 1000 ng/ml) allows us to minimize calcineurin inhibitor toxicity and its combination with the mTOR drug Sirolimus provides excellent protection against acute rejection in our renal transplant recipients. Our earlier experience with Everolimus and the recent publications describing renal recipients' outcomes with this drug, suggest to us that our patients may do better on Everolimus than Sirolimus (1,2). For our center, this pilot study would be very straightforward as the only difference in medication from our current well established protocol would be the randomization to either Rapamune®, our current standard of care, or Zortress®, the study drug.

3 Objectives

3.1 Primary objectives

The primary objective of this pilot study will be to determine equivalency of Zortress® as compared to Rapamune® when used in our de novo immunosuppression regimen following renal transplantation. The primary endpoint will be a composite endpoint of graft survival (non-death censored) and biopsy proven acute rejection at 1 year. The primary outcome of immunosuppressive protection would be studied in our Thymoglobulin and rapid steroid discontinuation protocol, with "half-dose" Neoral as described above.

3.2 Secondary objectives

Secondary outcomes would include factors involved with adverse events and tolerability and would include: renal graft survival (non-death censored) and biopsy proven acute rejection at 6 months, 2 and 3 years, as well as the number and grade of acute rejection episodes and responsiveness to treatment, serum creatinine and estimated GFR, results of renal biopsies at 12 months post-transplant to look for histological evidence of graft injury, early and late wound complications, infections including CMV, EBV and BK infections as monitored by serum PCR testing, lipid panel testing, particularly triglycerides, cardiac events such as acute myocardial infarctions or Congestive Heart Failure episodes, and tolerability of the Neoral and mTOR combination as determined by the patients who required conversion off of the intended randomized drug combination, with attempt to document why the patient did not tolerate the drug combination. All objectives are exploratory in nature as this is a pilot study.

3.3 Exploratory objectives

4 To explore the kidney and vascular endothelial function assessed by a panel of molecular markers over the observation period. Study design

Randomized, single center parallel-group, open-label study.

Pilot study with exploratory objectives.

Eligibility: All primary or retransplant recipients of deceased donor or living donor grafts with negative crossmatch who are felt to have good expectations for renal function recovery after surgery will be offered entry into the study.

Treatment scheme is as described above, with Solumedrol 500 mg IV in OR as induction, then 5 doses of Thymoglobulin starting morning of POD 1 at 1.25 mg/dose (rounded to nearest 25 mg) with rapid steroid taper over five doses. The mTOR inhibitor drug will be started on POD 0 and Neoral introduced as renal function recovers, usually starting on POD 2.

Measurement/Assessments: as described above, graft and patient survival per standard definitions, clinically indicated biopsies for determination of acute cellular or antibody mediated rejection, renal function as estimated from serum Cr based on MDRD, clinic visits for wound assessment, standard of care labs for renal function, bone marrow function (WBC and platelet count), liver function, lipid panel, donor specific antibody determination during any significant increase in serum Cr, drug levels routinely monitored for need of number of dose adjustments and compliance, Emergency Room and hospitalizations, as well as unscheduled clinic and other physician visits. For the pilot study, we would perform scheduled CMV, EBV and serum BK PCRs monthly for the first year, then quarterly or with any viral symptoms.

A subset of patients will have coded plasma samples sent to clinical research and development at the University of Colorado. These biomarker samples will be used to assess vascular endothelial dysfunction and inflammation markers as well as kidney dysfunction markers.

Plasma samples are collected and stored at The Ohio State University Wexner Medical Center tissue typing lab per standard of care.

5 Population

The patient population will be all adults (18-75 years of age) who receive a living donor or deceased donor renal transplant as a first or subsequent renal transplant procedure only (no extra-renal organ transplant recipients). We will invite any patient who would normally be considered for our current Sirolimus®/ Neoral® protocol with Thymoglobulin® and steroid induction to consent for this pilot study and then undergo randomization post-operatively if the transplant surgeon anticipates normal early graft function. If there were significant concerns for prolonged graft dysfunction, then the patients will not be invited into the pilot study. Invited patients would include anyone currently not on steroids prior to transplantation, and those with no known intolerance to any of the drugs mentioned. Patients must also be willing to perform the expectations of the study protocol reliably.

This will be a single center pilot study performed at The Ohio State University Comprehensive Transplant Center. Our center performs approximately 100 to 110 living donor and 90 to 100 deceased donor renal transplants per year. If we assume that at least 150 patients per year qualify for the study, then a conservative estimate of 75 enrolled patients a year is possible. We would propose to enroll a total of 60 patients over 12 months, and then follow these patients for a complete 3 years. We will randomize 2:1 Zortress to Sirolimus using a random number generator.

5.1 Inclusion criteria

Patients eligible for inclusion in this study have to fulfill all of the following criteria at the time of enrollment:

1. Patients must give written informed consent before any assessment is performed.
2. Primary renal transplant recipients between ages 18 and 75 years of age.
3. Females capable of becoming pregnant must have a negative pregnancy test prior to transplantation and practice an effective form of birth control for the duration of the study and 12 weeks after discontinuation of the study drug if applicable.

5.2 Exclusion criteria

- Exclusion Criteria at the time of enrollment:
  - Total cholesterol > 300 mg/dl or triglycerides > 400 mg/dl despite lipid lowering therapy
  - Pre-existing bone marrow suppression (WBC < 3000, platelets < 100,000)
  - Positive crossmatch or ABO incompatible

- Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive serum human chorionic gonadotrophin laboratory test (>5 mIU/mL)
- Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, including women whose career, lifestyle, or sexual orientation precludes intercourse with a male partner and women whose partners have been sterilized by vasectomy or other means, UNLESS they are using two birth control methods. The two methods can be a double barrier method or a barrier method plus a hormonal method.
    - Adequate barrier methods of contraception include: diaphragm, condom (by the partner), intrauterine device (copper or hormonal), sponge or spermicide. Hormonal contraceptives include any marketed contraceptive agent that includes an estrogen and/or a progestational agent.
    - Reliable contraception should be maintained throughout the study and for 12 weeks after study drug discontinuation.
    - Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or six months of spontaneous amenorrhea with serum FSH levels > 40 mIU/mL and estradiol < 20 pg/mL] or have had surgical bilateral oophorectomy (with or without hysterectomy) at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.
- Active infection (HBV, HIV)
- Malignancy (except for adequately treated squamous or basal cell skin carcinoma) unless patient has written clearance from an Oncologist or if patient has had no malignancy for at least 2 years prior to the transplant
- Allergy or intolerance to sirolimus, everolimus, cyclosporine, or ATG

6 Treatment

6.1 Investigational and control drugs

Zortress will be started on POD #0 and initially dosed at 0.75 mg BID (12 hours apart) dosed simultaneously with Neoral and the dose adjusted thereafter to achieve a target trough serum level between 3 ng/ml and 8 ng/ml. Sirolimus will be dosed per our standard approach, specifically started on POD #0 at 5 mg/d, decreasing to 3 mg/d on POD #2, and adjusted thereafter at achieve a target trough serum level of 10 to 18 ng/ml by Architect assay, or 8 to 12 by LC-MS methodology.

6.2 Treatment arms

Patients will be assigned to one of the following "n" treatment arms in a ratio of 2:1; Zortress or Sirolimus started on POD 0, with Solumedrol 500 mg IV in OR as induction, then 5 doses of Thymoglobulin starting morning of POD 1 at 1.25 mg/dose (rounded to nearest 25 mg) with rapid steroid taper over five doses. Neoral will be introduced as renal function recovers, usually starting on POD 2. We will enroll a total of 60 patients.

- Zortress and Neoral, with Thymoglobulin induction and perioperative steroids
- Rapamycin and Neoral, with Thymoglobulin induction and perioperative steroids

6.3 Treatment assignment

Randomization will be stratified by type of donor kidney received (living or deceased donor kidney) and primary versus retransplant recipient. Randomization will be performed using a random number generator.

6.4 Treatment blinding

This is an open-label study and thus there will be no blinding.

6.5 Treating the patient

Patients enrolled in this study will receive our normal standard of post-transplant follow up care.

6.5.1 Patient numbering

As this is an open-label study there will be no blinding and thus no need for patient identifying numbers as part of a blinding strategy.

6.5.2 Dispensing the study drug

As this is an open-label study utilizing currently commercially available drugs, the drugs under study will be dispensed utilizing standard operating procedures.

6.5.3 Study drug supply, storage and tracking

As this is an open-label study utilizing currently commercially available drugs, special arrangements for study drug supply, storage and tracking will not be necessary.

6.5.4 Permitted study drug dose adjustments and interruptions

Zortress and Sirolimus dose adjustments and interruptions will be allowed and will be made at the discretion of the treating physician. All drug adjustments and interruptions will be recorded and monitored for this study, which requires little additional effort as this is already our current practice.

6.5.5 Study drug discontinuation and premature patient withdrawal

As we currently do for Sirolimus, Zortress dosing will be decreased or discontinued when the transplant physician deems appropriate due to deleterious side effects or the presence of infection or malignancy where a reduction in overall immunosuppression in desirable.

6.5.6 Early study termination

Early study termination will be considered if there is a statistically significant difference in primary outcome between the 2 study arms and it is the opinion of the treating physicians that this is likely due to differences in efficacy

7 Visit schedule and assessments

Table 7-1 lists all of the assessments and indicates with an “x” the visits when they are performed.

7.1 Table 7-1

Assessment schedule:

Visit number	1 Screening/ Baseline	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Time of Visit		Day 0	Day 1	Day 2	Day 3	Day 4	Day 5[1]	Day 6[1]	Day 7[1]	Wk 2	Mo 1	Mo 2	Mo 3	Mo 6	Mo 12	Mo 18	Mo 24	Mo 36
Inclusion/Exclusion criteria	X																	
Informed consent	X																	
Physical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Demographics	X																	
Medical History	X																	
Pregnancy Test[2]	X																	
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hematology		X			X				X	X	X	X	X	X	X	X	X	X
Chemistry		X			X				X	X	X	X	X	X	X	X	X	X
Lipid panel	X										X	X	X	X	X	X	X	X
24 hour urine													X	X	X	X	X	X
Alloscreen	X														X			
Flow Crossmatch	X														X			
HGB A1C															X		X	X
Kidney Biopsy	X														X			
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Cyclosporine level[3]					X				X	X	X	X	X	X	X	X	X	X
Everolimus level[3]					X				X	X	X	X	X	X	X	X	X	X
Optional sub study: Plasma for Biomarkers[4]	X				X				X	X	X	X	X	X	X	X	X	X

Legend:

1: If still inpatient

2: Females capable of childbearing

3: Cyclosporine and Zortress levels are monitored twice weekly for 0-3 months post-transplant, every two weeks for 3-12 months post-transplant, and monthly greater than 1 year post-transplant.

4: Plasma for biomarkers will be assessed at the University of Colorado in a subset of patients using existing plasma samples.

Patients will be followed as per the standard of care at this transplant center (The Ohio State University Medical Center, Division of Transplantation). Additional study procedures include a protocol biopsy at month 12 and some additional laboratory work at screening/baseline and at months 6, 12, 24, and 36.

7.2 Information to be collected on screening failures

No information will be collected on patients failing the screening process.

7.3 Patient demographics/other baseline characteristics

Patient demographics are routinely captured within our transplant database and as such will be available for analysis. Demographic data collection includes, but is not limited to, name, age, gender, race, cause of renal failure, years on dialysis, sensitization status (PRA),

7.4 Treatment exposure and compliance

Zortress serum trough levels will be monitored twice weekly for the first 3 months after transplant, once weekly between months 3 and 6, every other week between months 6 and 12, and monthly thereafter. Compliance with Zortress can be determined based on serum trough levels.

7.5 Efficacy

7.5.1 Efficacy assessment 1

- Incidence of graft loss in the first post-transplant year
- Incidence of patient death in the first post-transplant year
- Incidence of biopsy proven acute rejection at 1 year post-transplant
- Tolerability of Zortress at 1 year post-transplant
- Renal function at 1 year post-transplant

8 Safety monitoring

8.1 Adverse events

Adverse events that the investigators consider to be clinically significant and associated with the use of Zortress® or Rapamune® will be recorded at each study visit. Serious adverse events will be reported to Novartis within 24 hours of discovery or notification. An adverse event will be considered serious if it results in any of the following outcomes:

- Results in death,
- Is life threatening (an adverse event is considered "life-threatening: if, in the view of the investigator, its occurrence places the subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death),
- Results in persistent or significant disability/incapacity, or substantial disruption of the ability to conduct normal life functions,
- Results in congenital anomaly, or birth defect,
- Requires inpatient hospitalization or leads to prolongation of hospitalization (hospitalization for treatment/observation/examination caused by AE is to be considered as serious).
- Other medically important events

Follow-up information for serious adverse events will be collected, recorded, and reported to Novartis.

Adverse events that meet IRB reporting requirements will be submitted to the IRB for review.

8.2 Serious adverse event reporting

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided informed consent and until 30 days after the patient has stopped study participation (defined as time of last dose of study drug taken or last visit whichever is later).

Any SAEs experienced after this 30 day period should only be reported to Novartis if the investigator suspects a causal relationship to the study drug.

8.3 Pregnancies

To ensure patient safety, each pregnancy in a patient on study drug must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

9 Data review and database management

9.1 Data collection

Patient data will be collected/retained within our transplant patient database as per our normal operating procedure and thus immediately available and retrievable for subsequent analyses. The data is housed within the Ohio State University Medical Centers Transplant server located in a locked, secure facility.

9.2 Database management and quality control

Permanent patient electronic records will be stored and maintained on the Transplant server housed in a locked, secure facility. Study patient data will be subject to the same quality controls already in place for all patients treated by the Ohio State University Medical Centers Transplant Program.

10 Data analysis (Any or all sections may be included based on type of study)

10.1 Analysis of the primary objective(s)

10.1.1 Variable

The primary endpoint consists of non-death censored graft survival and biopsy proven acute rejection at 1 year post-transplant. These events will be directly measured and not calculated.

10.1.2 Statistical hypothesis, model, and method of analysis

The primary endpoint will be a composite endpoint of graft survival (non-death censored) and biopsy proven acute rejection at 1 year.

Secondary endpoints at 6 months, 1, 2 and 3 years will include: graft survival and patient survival/death at 2 and 3 years, rate and grade of acute cellular rejection, rate of acute antibody mediated rejection, rate of new donor specific antibody formation, estimated CrCl by MDRD, lipid control as determined by requirement for anti-lipid medications and average levels with and without medications, ability to maintain initial immunosuppressive medications and reason for switch if required, wound complications as determined by need for wound packing, infection treatment with antibiotics, presence of hernia on exam and requirement for hernia repair, as well as presence of symptomatic lymphocele, and New Onset Diabetes After Transplant as determined by need for new oral or sustained Insulin medication.

Statistical analysis will be performed on SPSS or STATA software with dichotomous variables evaluated by Chi-Squared analysis, continuous variables by Student's T-Test, non-parametric outcomes will be assessed by Wilcoxon rank sum test (Mann-Whitney U test). Kaplan-Meier survival curves with log rank tests will be used to determine a difference in graft and patient survival over time.

Safety data will include patient and graft survival, biopsy proven acute rejection, and hospital admissions.

10.1.3 Handling of missing values/censoring/discontinuations

Patients lost to follow-up or discontinued from the study will be censored from further analysis at the time of their withdrawal from the study.

10.2 Analysis of secondary objectives

10.2.1 Efficacy (secondary)

Secondary endpoints at 6 months, 1, 2 and 3 years will include: graft survival and patient survival/death at 2 and 3 years, rate and grade of acute cellular rejection, rate of acute antibody mediated rejection, rate of new donor specific antibody formation, estimated CrCl by MDRD, lipid control as determined by requirement for anti-lipid medications and average levels with and without medications, ability to maintain initial immunosuppressive medications and reason for switch if required, wound complications as determined by need for wound packing, infection treatment with antibiotics, presence of hernia on exam and requirement for hernia repair, as well as presence of symptomatic lymphocele, and New Onset Diabetes After Transplant as determined by need for new oral or sustained Insulin medication.

Statistical analysis will be performed on SPSS or STATA software with dichotomous variables evaluated by Chi-Squared analysis, continuous variables by Student's T-Test, non-parametric outcomes will be assessed by Wilcoxon rank sum test (Mann-Whitney U test). Kaplan-Meier survival curves with log rank tests will be used to determine a difference in graft and patient survival over time.

10.2.2 Safety

Safety will be assessed by reviewing hospital admissions and graft and patient survival on a monthly basis as we currently do for our center's quality assurance reviews. Any apparent differences in outcome showing a disadvantage to the patient's in the Zortress group will be reviewed by our Renal Transplant surgical and medical directors and they will decide if the study needs to be halted or amended.

10.3 Sample size calculation

Due to the fact that the sample size is 60 patients, this will be a pilot study that is exploratory in nature, and therefore not powered to show statistical difference.

10.4 Power for analysis of critical secondary variables

Due to the fact that the sample size is 60 patients, this will be a pilot study that is exploratory in nature, and therefore not powered to show statistical difference.

10.5 Interim analysis

Interim analyses will be performed at 6 months post-transplant to assure patient safety. We expect to demonstrate non-inferiority of the primary endpoint for patients in the Zortress study arm.

11 Ethical considerations

11.1 Regulatory and ethical compliance

This clinical study was designed and shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including European Directive 2001/20/EC, US Code of Federal Regulations Title 21, and Japanese Ministry of Health, Labor, and Welfare), and with the ethical principles laid down in the Declaration of Helsinki.

11.2 Informed consent procedures

Eligible patients may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC/REB-approved informed consent, or, if incapable of doing so, after such consent has been provided by a legally acceptable representative of the patient. In cases where the patient's representative gives consent, the patient should be informed about the study to the extent possible given his/her understanding. If the patient is capable of doing so, he/she should indicate assent by personally signing and dating the written informed consent document or a separate assent form. Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the patient source documents.

Women of child bearing potential should be informed that taking the study medication may involve unknown risks to the fetus if pregnancy were to occur during the study and agree that in order to participate in the study they must adhere to the contraception requirement for the duration of the study. If there is any question that the patient will not reliably comply, they should not be entered in the study.

11.3 Responsibilities of the investigator and IRB/IEC/REB

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board (IRB/IEC/REB) before study start. A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC/REB must be given to Novartis before study initiation.

11.4 Publication of study protocol and results

Study data in preparation for publication will be made available to Novartis for review prior to submission.

12 Protocol adherence

12.1 Protocol Amendments

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by Novartis, Health Authorities where required, and the IRB/IEC/REB. Only amendments that are required for patient safety may be implemented prior to IRB/IEC/REB approval. Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any patient included in this study, even if this action represents a deviation from the protocol.

13 References

1. Vitko S, Tedesco H, Eris J, Pascual J, Whelchel J, Magee J, et al. Everolimus with optimized cyclosporine dosing in renal transplant recipients: 6-month safety and efficacy results of two randomized studies. Am J Transplant 2004; 4: 626–635