CLINICAL INVESTIGATOR SPONSORED TRIAL PROTOCOL

TITLE:	**Combination Therapy with the Proteasome Inhibitor Carfilzomib for the Antibody-Mediated Rejection Diagnosis in Lung Transplantation Trial (PICARD-Lung)**
Protocol number:	**PRO15010152**
Study drug:	**Carfilzomib (Kyprolis®)**
Primary investigator:	**John F. McDyer, MD**
Co-investigators:	**Adriana Zeevi, PhD** **Matthew R. Morrell, MD**
Sponsor:	**John F. McDyer, MD**
DATE:	**09/20/2018**

CONFIDENTIAL

This is an investigator sponsored trial that contains confidential information. It is intended solely for use by Amgen, the clinical investigator team, and study support structure and must not be disclosed to any other party. This material may be used only for the evaluation and conduct of the aforementioned clinical investigation and any other use requires consent from the primary investigator.

TABLE OF CONTENTS

1.1 PROTOCOL FINALIZATION

TITLE:	**Combination Therapy with the Proteasome Inhibitor Carfilzomib for the Antibody-Mediated Rejection Diagnosis in Lung Transplantation Trial (PICARD-Lung)**
Protocol number:	**PRO15010152**
Study drug:	**Carfilzomib (Kyprolis®)**
Primary investigator:	**John F. McDyer, MD**
Co-investigators:	**Adriana Zeevi, PhD** **Matthew R. Morrell, MD**
Sponsor:	**John F. McDyer, MD**
DATE:	**09/20/2018**

This protocol was finalized on the date shown above.

1.2 PROTOCOL ACCEPTANCE SIGNATURE PAGE

TITLE:	**Combination Therapy with the Proteasome Inhibitor Carfilzomib for the Antibody-Mediated Rejection Diagnosis in Lung Transplantation Trial (PICARD-Lung)**
Protocol number:	**PRO15010152**
Study drug:	**Carfilzomib (Kyprolis®)**
Primary investigator:	**John F. McDyer, MD**
Co-investigators:	**Adriana Zeevi, PhD** **Matthew R. Morrell, MD**
Sponsor:	**John F. McDyer, MD**
DATE:	**02/20/2018**

I agree to conduct the study in accordance with the current protocol.

_____ _____

John F. McDyer, MD, Principal Investigator Date

1.3 PROTOCOL SYNOPSIS

TITLE:	**Combination Therapy with the Proteasome Inhibitor Carfilzomib for the Antibody-Mediated Rejection Diagnosis in Lung Transplantation Trial (PICARD-Lung)**
Protocol number:	**PRO15010152**
Study drug:	**Carfilzomib (Kyprolis®)**
Phase:	**phase II**
Patient enrollment:	**30**
Indication:	**Antibody mediated rejection after lung transplantation**
Sponsor:	**John F. McDyer, MD**
DATE:	**09/20/2018**

Objectives

1: To test the hypothesis in a prospective interventional clinical trial that addition of carfilzomib to conventional therapy will result in depletion of the immunodominant DSA.

2: To test the hypothesis in a prospective interventional clinical trial that addition of carfilzomib to conventional therapy will result in improved or stabilized allograft function.

3: To test the hypothesis that lung transplant recipients with AMR have effector B cell/plasma cell populations that can be identified and characterized, and that carfilzomib alters these immune mechanisms.

Study design

Phase II, prospective, open-label, single-arm active treatment, single center pilot clinical trial of the addition of carfilzomib to conventional therapy for AMR after lung transplantation.

Outcome measures

Primary endpoint: absolute change in DSA strength, titer, and complement fixation from day 1 to day 42.

Secondary endpoints:

Absolute change in DSA strength, titer, and complement fixation from day 1 to day 90 and from day 42 to day 90

Absolute change in forced expiratory volume in 1 second (FEV1) from day 1 to day 42 and to day 90.

Presence or absence of pathologic changes consistent with AMR on transbronchial biopsy from day 1 to day 42.

Patient death at any time after day 1 attributable to AMR.

Safety endpoints:

Non-lung irreversible end-organ failure (e.g., end-stage renal disease) any time after day 1 attributable to carfilzomib.

Incidence of adverse effects (AE) requiring dose-modification any time during days 1-16.

Incidence of any AE at any time after day 1 as graded by the NIH Common Terminology Criteria for Adverse Events (CTCAE) (appendix B) criteria.

Incidence of hypogammaglobulinemia any time after day 1.

Incidence of culture-proven *de novo* infection any time after day 1.

Diagnosis of systemic inflammatory response syndrome (SIRS) any time during days 1-16

Patient death at any time after day 1 to day 90 attributable to carfilzomib.

Retrospective control group:

A 1:1 retrospective control group including lung transplant recipients from the University of Pittsburgh with Antibody Mediated Rejection will be created to describe differences in the endpoints using the CFZ-based regimen vs. historic standard of care.

Safety plan

Patients will be enrolled according to the inclusion and exclusion criteria. Patients will provide informed consent to participate in this clinical trial. In the informed consent process, patients will be educated about the purpose for carfilzomib and the potential adverse.

A study data safety monitoring board (DSMB) will be established. The DSMB will meet once yearly to review study progress, to ensure appropriate enrollment milestones are being met, and to review the adverse event reporting. The DSMB will meet on an ad-hoc basis to review any SAEs.

The DSMB may terminate the study only after at least 15 patients have been enrolled. The DSMB may only terminate the study for safety reasons in which the perceived risks of continuing to provide carfilzomib to upcoming patients who may be enrolled outweighs the potential benefit to the patient and the allograft. The DSMB may not terminate the study for any reason related to carfilzomib efficacy.

Study treatment

Carfilzomib dosing schedule: Carfilzomib will be administered on the multiple myeloma schedule consisting of 20 mg/m^2 on days 1 2, 8, 9, 15, and 16 (one cycle) to constitute one therapeutic cycle. On PLEX/IVIG days, carfilzomib will be given in between PLEX and IVIG to avoid inadvertent blockade of the Fcγ receptor. Premedications for carfilzomib administration will be given as follows: acetaminophen 650 mg PO, diphenhydramine 25 mg PO, ondansetron 4 mg PO, prednisone 40 mg PO or methylprednisolone 40 mg IV, all at least 30 minutes prior to and not more than 6 hours before carfilzomib, unless otherwise directed on the part of one of the investigators if believed to be in the best interests of patient care. Preinfusion hydration with a one-time bolus of 250 mL 0.9% sodium chloride will be given at least 30 minutes prior to and not more than 6 hours before carfilzomib to minimize the nephrotoxic AE. Postinfusion hydration with a one-time bolus of 250 mL 0.9% sodium chloride may be given immediately after carfilzomib to minimize the nephrotoxic AE.

Concomitant therapy and clinical practice

Conventional therapy for AMR: Therapeutic plasma exchange (PLEX) 1.5 plasma volumes replaced with 5% albumin or fresh frozen plasma for 8 sessions every-other day. Total intravenous immunoglobulins (IVIG) 100 mg/kg IV after each PLEX with the exception of the final PLEX where IVIG 500 mg/kg will be given. On day 16, if the IgG level is below 700 mg/dL, an additional dose of IVIG 500 mg/kg will be administered. If the IgG result is found to be 700ml/dL or higher, the IVIG 500mg/kg dose will not be administered. Premedications will be given for IVIG as follows: acetaminophen 650 mg PO and diphenhydramine 25 mg PO both at least 30 minutes prior to and not more than 6 hours before IVIG, unless otherwise directed on the part of one of the investigators if believed to be in the best interests of patient care. On days where IVIG and CFZ are given together, only one set of premedications will be administered. Conventional therapy requires an inpatient hospital stay. Patients will receive a tunneled PLEX catheter and peripherally inserted central catheter (PICC) inserted by interventional radiology in the routine clinical care of these patients prior to receiving any conventional or carfilzomib therapy.

Women of childbearing potential must either agree to abstain from sexual intercourse or use an effective birth control method during treatment and for an additional 30 days after the last dose of carfilzomib. Men who are sexually active with women of childbearing potential must either agree to abstain from sexual intercourse or use a condom with spermicide during treatment and for an additional 30 days after the last dose of carfilzomib, and the female partner should consider using an effective method of birth control.

Statistical methods

Data will only be analyzed in aggregate at study conclusion for the final enrolled patient on the efficacy and safety outcomes.

Descriptive statistics will be used to describe baseline and study characteristics of patients. Univariate parametric and nonparametric assessments, where appropriate, will be used to assess change from baseline in the efficacy and safety outcomes. Kaplan-Meier method will be used to describe freedom from endpoints and mortality in the patients treated with carfilzomib. Kaplan-Meier method will be used to visually represent the time to events and overall event rate in the patients in this pilot, not to compare events to any other group.

2 BACKGROUND

2.1 Background and significance

The lack of improvement in long-term allograft survival, despite improved short-term outcomes, remains the most pressing challenge in the lung transplantation field. Lung transplant recipients fare poorly relative to other solid organs with 30% incidence of chronic rejection at 2.5 years, 75% at 10 years, and only 55% of recipients surviving five years, primarily due to bronchiolitis obliterans syndrome (BOS).[1,2] This problem is compounded by major gaps in our understanding of the immune mechanisms underlying chronic rejection.

Donor specific antibodies have a reported incidence of 10-40% in lung transplantation, and DSA both precedes and is associated with an increased risk of BOS.[3-6] Further, pre-transplant sensitization in lung transplant recipients was found to correlate with worse survival.[7] In addition to BOS, other studies have shown that DSA is associated with severe or recurrent acute cellular rejection (ACR) and lymphocytic bronchiolitis (LB), two forms of cellular rejection that are major risk factors for BOS.[8,9] Moreover, detection of DSA with clinical antibody mediated rejection (AMR) in lung transplant recipients is difficult to treat and is associated with higher morbidity compared to ACR.20 Several studies have shown an association between the development of anti-HLA Abs and/or DSA, BOS, and decreased survival.[4,5,8-10] One such paper shows that anti-HLA antibodies are associated with ACR and lymphocytic bronchiolitis, two forms of cellular rejection that are major risk factors for BOS.[8] We further investigated the relationship between DSA and BOS in 445 LT recipients at the University of Pittsburgh, over a 7-year period (2003-2010). As shown in figure 1, DSA was associated with significantly reduced freedom from BOS and decreased survival in LT recipients followed for 3.4 ±1.6 yrs. Furthermore, we found that the risk of BOS was significantly increased in the DSA+ group compared to the non-DSA group using a Cox proportional hazards model (HR 6.32; 95% CI 4.34-9.18; p<0.001).

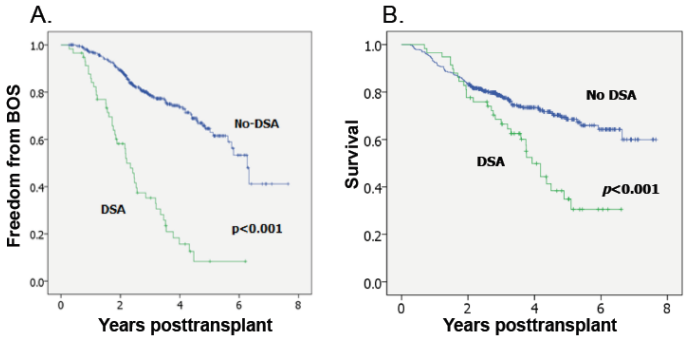

Figure 1: Freedom from BOS and Mortality in DSA vs. no DSA in lung transplantation recipients from UPMC.

Moreover, the presence of high-grade BOS (BOS stage 2 or 3) was significantly increased in the DSA+ versus the no-DSA group. Together, these data indicate that development of DSA is significantly associated with accelerated, high-grade BOS in lung transplantation recipients, along with diminished patient survival. Thus, DSA and AMR are emerging as a formidable challenge in lung transplantation.

Contrary to lung transplantation, the evidence and agreement supporting the AMR are robust in renal transplantation. That said, the majority of the principles learned in renal transplantation are applicable to the lung. One such lesson is the impact of functional phenotype of DSA, ergo whether the DSA is able to fix complement at peripheral blood concentrations in vitro. A novel solid phase Luminex single antigen bead (SAB) assay was developed which allows the detection of complement fixing HLA antibodies with high sensitivity and specificity (Luminex-C1q).[11] In renal transplantation, several small studies suggest that C1q-binding DSA correlates with transplant glomerulopathy (TG) and irreversible renal allograft failure.[12] In particular, persistent complement binding DQ-specific DSA is associated with a 30% lower 5-year allograft survival. In a recent large study in a cohort of 1016 renal transplant recipients, co-authored by co-investigator Dr. Adriana Zeevi, the presence of DSA and C1q-binding donor-specific anti-HLA antibodies at 1 year, or at the time of acute rejection within 1 year, was correlated with 5 year allograft survival (Figure 2). Patients with complement-binding DSA had significantly reduced 5-year allograft survival (54%) compared to those with non-complement binding DSA (93%). C1q binding of *de novo* DSA was independently associated with graft loss whether it was detected in samples taken at the one year protocol biopsies or in samples taken during acute rejection in the first-year post-transplantation.

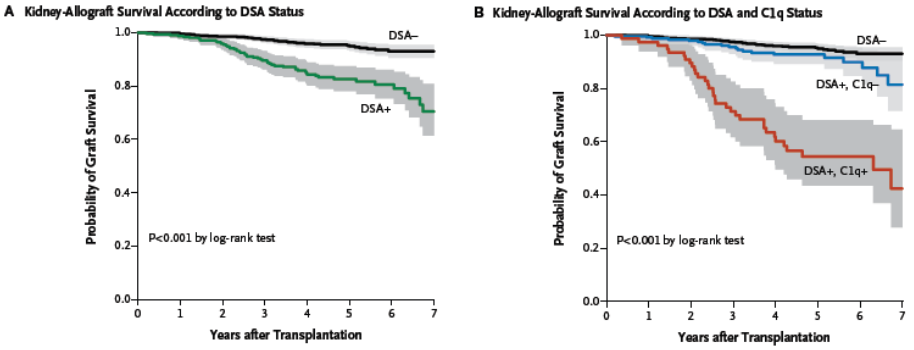

Figure 2: Kaplan-Meier curve of probability of graft survival based on DSA phenotype. Panel A: conventional Luminex-IgG vs. no DSA. Panel B: Luminex-IgG and C1q vs. no DSA.

2.2 Study rationale

The optimal treatment for DSA in lung transplant recipients remains unclear. In a recent study, 65 lung transplant recipients who developed DSA were treated preemptively before the development of BOS with either IVIG alone or IVIG/rituximab.[13] On follow-up evaluation, persistence of DSA despite therapy was associated with increased BOS (Figure 3B). However, recipients treated with IVIG alone had persistence of DSA comparable to those treated with IVIG/rituximab (35% vs.38%, respectively). Thus, while there was no untreated control group, these data suggest that the addition of rituximab therapy did not enhance DSA clearance and had no significant effect on freedom from BOS (Figure 3A) compared to IVIG alone. These data indicate that either IVIG alone or IVIG/rituximab therapy results in substantial persistence of DSA in lung transplant recipients (35%), and is associated with significantly increased progression to BOS.

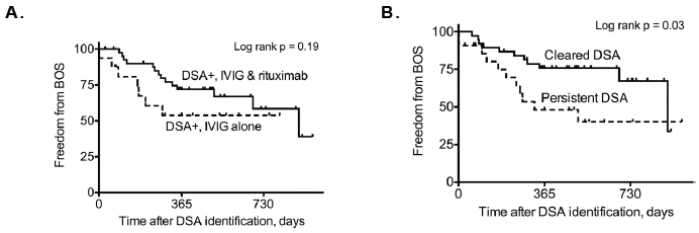

Figure 3. Freedom from BOS in lung transplant recipients with DSA treated with IVIG vs. IVIG/rituximab and in patients who cleared vs. persisted DSA.

Since rituximab appears to add little to conventional therapy, proteasome inhibition with bortezomib alone and in combination with conventional therapy was investigated in 6 renal transplant recipients. Bortezomib-based therapy effectively treated 6 patients with refractory combined AMR/ACR and stably reduced DSA by at least 50% (figure 4, next page).[14] Moreover, a single cycle of Bortezomib alone was as effective as Bortezomib combined with PLEX and IVIG or rituximab in reducing de novo DSA.[15] Of 11 patients (bortezomib alone), 4 had a 50% decrease in DSA, while 7 had complete remission (CR; MFI<1000) which lasted >2 years in 3/7.41 Patients with stable CR, had a lower serum CR at 14 months than those with DSA relapse –adding credence to the notion that successful treatment of DSA may alter outcome. Importantly proteasome inhibitors were well tolerated, and neither overall IgG nor IgG levels for childhood immunizations were changed.[16,17] The relatively specific loss of DSA after proteasome inhibitor therapy may relate to their predominant effect on the most active antibody responses, with residual DSA binding to the allograft. Interestingly, recent evidence in vitro indicates that proteasome inhibitors induce B cell apoptosis in addition to the effects on plasma cells and therefore, may impact and expand cell populations targeted by anti-CD20 based therapies.[18]

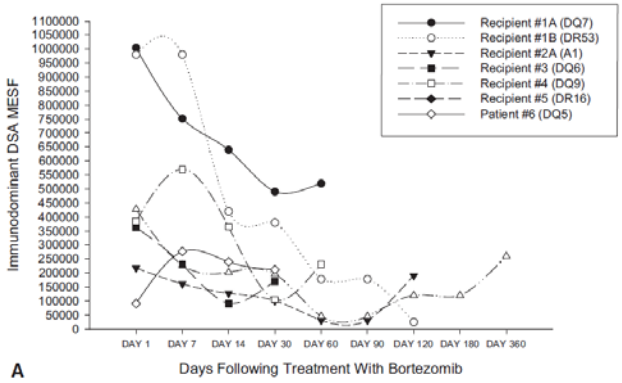

Figure 4. Reduction of immunodominant DSA following Bortezomib therapy in 6 renal transplant recipients

Carfilzomib has mechanistic advantages over bortezomib. Carfilzomib has irreversible activity at the proteasome and an extraordinarily short half-life which eliminates concerns of subsequent removal by PLEX. Moreover, carfilzomib has been effective in studies of bortezomib-refractory multiple myeloma.[19-21] As such, the PICARD-Lung investigators conducted a small pilot study of combination carfilzomib-based therapy in 7 lung transplant recipients who met the diagnostic criteria for AMR and required therapy and had previously failed other conventional regimens for AMR consisting of PLEX, IVIG, and rituximab or bortezomib. LT recipients with AMR were treated with CFZ-based therapy (8 1.5x volume plasma exchanges (PE) every-other day, 1.2 g/kg total immunoglobulin, and 6 doses of CFZ 20 mg/m2 on days 1, 2, 8, 9, 15, and 16). Luminex IgG and

C1q DSA in neat serum, PFTs, and biopsy (TBBx) were done at baseline and repeated 4-6 weeks post-therapy. 7 patients received CFZ-based therapy. Patients 1-4 were refractory to rituximab-based therapy; patient 4 was refractory to both rituximab and bortezomib-based therapy. FEV1 at AMR diagnosis was significantly reduced from baseline (-19.8%, $p<0.01$) and oxygen requirements worsened in all patients. DSA were of moderate to strong strength by IgG and 6 of 7 fixed complement by C1q (MFI>500) at baseline (table 1). TBBx in patients 2 and 5 were diffusely positive for pericapillary C4d; patient 5 had diffuse neutrophilic capillaritis, and patient 4 had minimal cellular rejection (A1). All pathologic findings of AMR resolved after therapy. DSA IgG MFI declined significantly ($p=0.02$) and 6 of 7 DSA became non-complement fixing after therapy (table). Oxygen requirements improved in all patients; 5 patients recovered to discharge with stable allograft function; patient #6 remains chronically ventilated. FEV1 data were available in 6 patients and either stabilized or rose in 5 of the 6 patients (figure 5). One patient died due to end-stage BOS, transient thrombocytopenia occurred in 2 patients which recovered, acute kidney injury occurred in 3 patients and recovered in 2 patients; patient 5 was critically ill during treatment and remains dialysis dependent. Thus, carfilzomib-based therapy appears useful for AMR after lung transplantation in this small cohort.

Table 1. Change in strength of immunodominant DSA by IgG and functional phenotype after carfilzomib-based therapy.

Patient	Immunodominant DSA	IgG pre	IgG post	C1q pre	C1q post
1	DQB1*04:02/DQA1*04:01	18105	9796	1152	10
2	DQB1*02/ DQA1*05	13941	9511	2832	97
3	DQB1*07/DQA1*05	7793	1878	6568	10
4	DR53	3004	2359	10	10
5	DQB1*03:02/DQA1*03:02	3182	593	20691	10
6	DQB1*03:01/DQA1*05:05	744	5296	19442	28783
7	DQ7	5020	1957	14282	30

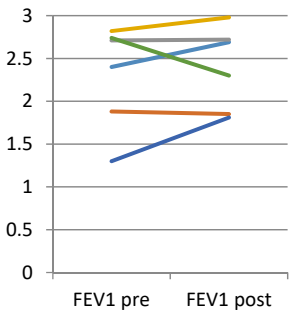

Figure 5. FEV1 before and by C1q after treatment with combined carfilzomib-based therapy.

Considering the recent studies by Witt[22] and Snyder[3] demonstrating the difficulty and frequent failure of various regimens to treat AMR in LT recipients, these preliminary data showing an impact using a carfilzomib-based treatment regimen for established DSA responses with allograft dysfunction are indeed encouraging.

As a result of these positive preliminary findings, expansion of the sample size is required to confirm both the efficacy of combination therapy with carfilzomib as well as the safety of such an approach in this population.

3. OBJECTIVES

3.1 Objectives

Short-term outcomes after lung transplantation are improved; however, long-term outcomes remain poor and this is a major challenge for the field. Progressive allograft dysfunction due to

the clinical diagnosis of bronchiolitis obliterans syndrome, or chronic rejection, limits both patient survival and quality of life. The impact of donor-specific anti HLA-antibodies (DSA) and AMR on the early development and progression of BOS have been well-described.[3,13,22] In addition to these data, our preliminary data show a dramatic reduction in lung function in the presence of DSA, accelerated diagnosis of phenotypically more advanced BOS, and early mortality. At present, targeting this pathway represents a viable approach to impact these key risk factors, DSA and AMR, and decrease expression of chronic rejection, graft loss, and patient death. Additionally, major gaps in our knowledge of the antibody-mediated phenotype of BOS exist. While early evidence of clinical benefit appears to exist in our preliminary data, this needs to be validated with larger patient numbers. Moreover, the underlying mechanisms of potential benefit remain to be elucidated.

Conventional therapy for this problem, including plasma exchange and intravenous immunoglobulins, are limited and targeted at removal of circulating antibody; not at the antibody-producing apparatus, the plasma cell.[13,23] Thus, **our hypothesis is that the combination of conventional therapy with plasma-cell targeted therapy using the proteasome-inhibitor carfilzomib in symptomatic lung transplant recipients with AMR will improve/stabilize allograft function and result in depletion of the immunodominant DSA.** In this proposal, we will conduct a pilot phase II open label observational trial in lung transplant recipients to test whether addition of carfilzomib to conventional therapy improves short-term outcomes after the diagnosis of antibody-mediated rejection, and to identify the cellular mechanisms that support these clinical benefits. Clinical and immune phenotyping of study subjects will advance our understanding of anticipated therapeutic responses. Specifically, cellular mechanistic studies to detect immune system changes that will enable us to phenotype LTRs with AMR and determine or predict risk and outcomes, such as BOS. Patients who have met the clinical diagnosis of AMR requiring therapy will be targeted for enrollment in the study and treated with the open-label carfilzomib-based combination protocol.

To execute this study, we have established the **Combination Therapy with the Proteasome Inhibitor Carfilzomib for the Antibody-Mediated Rejection Diagnosis in Lung Transplantation (PICARD-Lung) Trial Study Group.** This clinical trial will be executed at the University of Pittsburgh Medical Center leveraging the expertise and prowess of one of the highest-volume clinical lung transplant services worldwide, the McDyer immunology lab, and the Zeevi immunohistochemistry and histocompatibility labs.

1: To test the hypothesis in a prospective interventional clinical trial that addition of carfilzomib to conventional therapy will result in depletion of the immunodominant DSA. In this trial, lung transplant recipients will be treated with the carfilzomib-based combination protocol and screened with Luminex single antigen bead testing to identify DSA burden by IgG and functional status by C1q before and after treatment.

2: To test the hypothesis in a prospective interventional clinical trial that addition of carfilzomib to conventional therapy will result in improved or stabilized allograft function. In this trial, lung transplant recipients will be treated with the carfilzomib-based combination protocol and screened with pulmonary function testing to identify whether forced expiratory volume in 1 second returns to pre-AMR diagnosis level.

3: To test the hypothesis that lung transplant recipients with AMR have effector B cell/plasma cell populations that can be identified and characterized, and that carfilzomib alters these immune mechanisms. In this trial, we will determine the immunologic phenotype

of these patients and describe functional immune system changes in memory and effector cells, plasma cells, and respective pathologic and immunohistochemical correlates relevant to AMR.

The PICARD-Lung investigators and the study center are highly experienced clinical and translational investigations in lung transplantation. Successful completion of these specific aims will establish the safety and efficacy of combination therapy with carfilzomib/PLEx/IVIG for AMR after lung transplantation and elucidate important clinical and immunologic phenotypes and mechanisms associated with these outcomes. Thus, the results of this trial will advance treatment efforts for AMR, which are desperately needed, as well as provide important insights into the link between AMR and BOS, the latter of which significantly limits long-term outcomes in lung transplant recipients. Lastly, we fully anticipate this study will lay the foundation for future clinical and basic investigation, perhaps at the multi-center level, to further delineate the role and immune effects of carfilzomib in the treatment of AMR and the prevention of BOS.

4 STUDY DESIGN

Phase II, prospective, open-label, single-arm active treatment, single center pilot clinical trial of the addition of carfilzomib to conventional therapy for AMR after lung transplantation.

Study site: University of Pittsburgh Medical Center, Pittsburgh PA USA

Conventional therapy for AMR: Therapeutic plasma exchange (PLEX) 1.5 plasma volumes replaced with 5% albumin or fresh frozen plasma for 8 sessions every-other day. Total intravenous immunoglobulins (IVIG) 100 mg/kg IV after each PLEX with the exception of the final PLEX where IVIG 500 mg/kg will be given. On day 16, if the IgG level is below 700 mg/dL, an additional dose of IVIG 500 mg/kg will be administered. If the IgG result is found to be 700ml/dL or higher, the IVIG 500mg/kg dose will not be administered. Premedications will be given for IVIG as follows: acetaminophen 650 mg PO and diphenhydramine 25 mg PO both at least 30 minutes prior to and not more than 6 hours before IVIG, unless otherwise directed on the part of one of the investigators if believed to be in the best interests of patient care. On days where IVIG and CFZ are given together, only one set of premedications will be administered. Conventional therapy requires an inpatient hospital stay. Patients will receive a tunneled PLEX catheter and peripherally inserted central catheter (PICC) inserted by interventional radiology in the routine clinical care of these patients prior to receiving any conventional or carfilzomib therapy.

Carfilzomib dosing schedule: Carfilzomib will be administered on the multiple myeloma schedule consisting of 20 mg/m^2 on days 1, 2, 8, 9, 15, and 16 to constitute one therapeutic cycle. On PLEX/IVIG days, carfilzomib will be given in between PLEX and IVIG to avoid inadvertent blockade of the Fcγ receptor. Carfilzomib will be delivered via central access IV over 30 minutes (+15 minutes); slowed to 60 minutes (+15 minutes) if infusion reactions occur. Carfilzomib will be diluted in a total of 80 mL dextrose 5% water (D5W) for administration. Premedications for carfilzomib administration will be given as follows: acetaminophen 650 mg PO, diphenhydramine 25 mg PO, ondansetron 4 mg PO, prednisone 40 mg PO or methylprednisolone 40 mg IV, all at least 30 minutes prior to and not more than 6 hours before carfilzomib, unless otherwise directed on the part of one of the investigators if believed to be in the best interests of patient care. Preinfusion hydration with a one-time bolus of 250 mL 0.9% sodium chloride will be given at least 30 minutes prior to and not more than 6 hours before carfilzomib to minimize the nephrotoxic AE. Postinfusion hydration with a one-time bolus of 250 mL 0.9% sodium chloride may be given immediately after carfilzomib to minimize the nephrotoxic AE

4.1 Rationale

Carfilzomib is marketed for multiple myeloma and this IST represents an off label use of carfilzomib; thus, the criteria for phase II designation have been met. This prospective, open-label, single-arm active treatment trial will establish the rate of response to carfilzomib (in addition to conventional therapy) for AMR after lung transplantation. This information will be necessary for use in planning further larger dual-arm placebo or active therapy controlled clinical trials. This trial will enroll 30 patients at the University of Pittsburgh Medical Center. The volume of lung transplants performed and the rate of diagnosis of AMR at UPMC will allow for adequate enrollment of study patients within the 3-year study period.

The dosing strategy of carfilzomib will follow that of the multiple myeloma schedule as described below in section 5.3. This dosing strategy has been well-studied in patients with myeloma (see trial designations below) and has proven to result in an acceptable AE profile for the patients with AMR after lung transplantation. In addition, our preliminary off label experiences with this dosing strategy have yielded an acceptable AE profile in lung transplant recipients with AMR.

Trial designations: Carfilzomib has been evaluated in 2 completed Phase 1 studies (PX-171-001 and PX-171-002), 5 completed Phase 1b/2 studies (PX-171-003, PX-171-004, PX-171-005, PX-171-006, and PX-171-008), and in 1 completed, Phase 2, expanded-access study (Study 2011-002, Carfilzomib Multiple Myeloma Expanded Access Protocol [C-MAP]) that was co-sponsored by Amgen and the Multiple Myeloma Research Foundation.

Carfilzomib is currently being evaluated in 2 ongoing Phase 1 studies (CFZ001 [Renal] and CFZ002 [Hepatic]), 5 ongoing Phase 1b/2 studies (PX-171-007, PX-171-010, 2012-002 [CHAMPION 1], 2012-003 [CHAMPION 2], and CFZ004 [small cell lung cancer]), 4 ongoing Phase 3 studies (PX-171-009 [ASPIRE], PX-171-011 [FOCUS], 2011-003 [ENDEAVOR], and 2012-005 [CLARION]), and 2 ongoing Phase 1/2 studies sponsored by Ono Pharmaceutical Co, Ltd in Japan (ONO-7057-01 and ONO-7057-02).

Data are available from the following 10 company-sponsored Phase 1,1b, and 2 studies: PX-171-001, PX-171-002, PX-171-003, PX-171-004, PX-171-005, PX-171-006, PX-171-007, PX-171-008, PX-171-010 (ongoing), and 2012-002, (CHAMPION 1, ongoing). Of these, 9 studies enrolled multiple myeloma subjects. Study PX-171-007 also enrolled subjects with lymphoma and solid tumors. Study PX-171-008 enrolled solid tumor subjects only. Data also are available from a company-sponsored, Phase 2, expanded-access study (Study 2011-002 [C-MAP]) and 2 ongoing Phase 1/2 Ono-sponsored studies (ONO-7057-01 and ONO-7057-02). Furthermore, after the IB data cutoff date of 10 July 2014, a pre-planned interim analysis was performed for the Phase 3 Study PX-171-009 (ASPIRE) on 01 August 2014 and a final analysis was performed for the Phase 3 Study PX-171-011 (FOCUS) on 11 August 2014.

Five Phase 3 studies have been or are being conducted. Safety and efficacy results are available for 2 completed studies, Study PX-171-009 (ASPIRE) and Study PX-171-011 (FOCUS); subjects are still being followed for safety in both studies and for final OS in Study PX-171-009

(ASPIRE). Summaries for 3 ongoing studies, Study 2011-003 (ENDEAVOR), Study 2012-005 (CLARION), and Study CFZ014 (A.R.R.O.W.)

4.2 Investigators

Primary investigator: **Dr. John McDyer** (PI), Director of lung transplant translational research, Associate Professor of Medicine, works closely with Drs. Zeevi, and Morrell, as part of the DSA/AMR working group at UPMC, and is an expert in clinical lung transplantation, mechanisms of rejection, host defense and lung T cell immunity, and will lead Aim 3.

Co-investigators: **Dr. Adriana Zeevi**, Professor of Pathology, Surgery and Immunology at the University of Pittsburgh and Director of the UPMC Tissue Typing Laboratory. She is an established NIH investigator and has published extensively in the area of anti-HLA antibodies and immune monitoring in solid organ transplants. Dr. Zeevi also works closely with Drs. McDyer, and Morrell as part of a DSA/AMR working group in LT at UPMC. All the assays proposed are available in her laboratory and she has extensive research resources and space to perform all the proposed studies. **Dr. Matthew Morrell,** Assistant Professor of Medicine, works closely with Drs. McDyer, and Zeevi on the DSA/AMR working group at UPMC, is an expert in clinical lung transplantation, and conducts excellent clinical science on the topic of DSA and lung transplant in the UPMC cohort. **Dr. Joseph Pilewski**, Associate Professor of Medicine. One of the focuses of his clinical interest is lung transplantation, with special interest in patients with suppurative lung diseases like CF. He has been Medical Director of the Lung Transplant Program at the University of Pittsburgh Medical Center since 2004. **Dr. Bruce Johnson**, Assistant Professor of Medicine. His practice focuses on the pre and post-transplant care of lung transplant recipients. He has been actively and continuously managing these patients since 1991 and is the senior clinician in lung transplantation at UPMC. **Dr. Silpa Kilaru,** Clinical Assistant Professor of Medicine. Her clinical interest is focused primarily on lung transplantation, namely taking care of patients both before and after lung transplant. She spent the majority of her time rotating on the inpatient lung transplant service, seeing patients in clinic, and serving as the attending in the bronchoscopy suite. **Carlo Iasella, PharmD, BCPS** and **Cody A. Moore, PharmD, MPH, BCPS** are transplant pharmacists assisting the PI with execution of the study.

Study definitions

4.3.1 Antibody strength (MFI = mean fluorescent intensity by Luminex-IgG single antigen bead (SAB) assay).
Low strength = MFI < 2000 units
Moderate strength = MFI 2000-8000 units
High strength = MFI > 8000 units

4.3.2 Antibody titer
Low titer = MFI < 2000 units at 1:4 dilution
High titer = MFI > 8000 units at 1:16 dilution

4.3.3 Functional DSA phenotypes
Complement fixing DSA = MFI > 500 units in neat serum by Luminex-C1q
Non-complement fixing DSA = MFI < 500 units in neat serum by Luminex-C1q

4.3.4 Kinetic DSA phenotypes
Early DSA = present within 6 months post-transplant
Late DSA = present after 6 months post-transplant

4.3.5 Immunologic DSA phenotypes
Memory response = sensitized pre-transplant with DSA pattern (MFI < 1000 units)
de novo response = not sensitized with no detectable pre-transplant DSA pattern

4.3.6 Antibody-mediated rejection (AMR) diagnosis (must have first 2 criteria)
1. Presence of DSA
2. Allograft dysfunction (absence of another identifiable cause, except cellular rejection (ACR))
    Fall of > 10% in FEV1, or
    Fall of > 30% in FEF 25/75, or
    New onset hypoxia/hypoxemia requiring supplemental oxygen, or
    Need for mechanical ventilation
3. **With or without** pathologic findings consistent with AMR
    Capillaritis
    Endothelialitis
    C4d pericapillary deposition in > 50% of biopsy

4.3.7 AMR phenotypes
Probable AMR alone = DSA, allograft dysfunction, no AMR pathologic findings, ACR grade ≤ A1

Definite AMR alone = DSA, allograft dysfunction, AMR pathologic findings, ACR grade ≤ A1

Mixed rejection = DSA, allograft dysfunction, with or without AMR pathologic findings, ACR grade ≥ A2

4.3.8 Bronchiolitis obliterans syndrome (BOS) diagnosis[24]
Baseline FEV1 defined as mean of 2 best FEV1 post-transplant
BOS 0 = FEV1 > 80% of baseline
BOS 0p = FEV1 81-90% of baseline and/or FEF25/75 ≤ 75% of baseline

BOS 1 = FEV1 66-80% of baseline
BOS 2 = FEV1 51-65% of baseline
BOS 3 = FEV1 ≤ 50% of baseline

4.3.9 Hypogammaglobulinemia, total serum IgG < 700 mg/dL

4.4 Study Endpoints

4.4.1 Primary endpoint

The primary endpoint of this study is the absolute change in DSA strength, titer, and complement fixation from day 1 to day 42. DSA strength will be determined by Luminex single-antigen bead testing in the neat serum and quantitated in units of mean fluorescent intensity. DSA titer will be determined by Luminex single-antigen bead testing in the diluted serum to 1:16 and quantitated in units of mean fluorescent intensity. DSA complement fixation will be determined by C1q-based Luminex single-antigen bead testing in the neat serum and quantitated in units of mean fluorescent intensity.

A 1:1 retrospective control group including lung transplant recipients from the University of Pittsburgh with Antibody Mediated Rejection will be created to describe differences in the endpoints using the CFZ-based regimen vs. historic standard of care.

4.4.2 Secondary endpoints

Secondary endpoints of this study include the following:

4.4.2.1 Absolute change in DSA strength, titer, and complement fixation from day 1 to day 90 and from day 42 to day 90. DSA strength will be determined by Luminex single-antigen bead testing in the neat serum and quantitated in units of mean fluorescent intensity. DSA titer will be determined by Luminex single-antigen bead testing in the diluted serum to 1:16 and quantitated in units of mean fluorescent intensity. DSA complement fixation will be determined by C1q-based Luminex single-antigen bead testing in the neat serum and quantitated in units of mean fluorescent intensity.

4.4.2.2 Absolute change in forced expiratory volume in 1 second (FEV1) from day 1 to day 42 and to day 90. FEV1 will be quantitated via UPMC PFT lab standard methods on routine pulmonary function testing obtained in the routine clinical care of these patients.

4.4.2.3 Presence or absence of pathologic changes consistent with AMR on transbronchial biopsy from day 1 to day 42. Pathologic changes consistent with AMR will be defined as follows: Complement-4d fixation on >50% (diffuse) of the capillaries in the biopsy by standard immunoperoxidase method, capillaritis by standard hematoxylin and eosin (H&E) stain; or endothelialitis by standard H&E stain.

4.4.2.4 Patient death at any time after day 1 attributable to AMR.

A 1:1 retrospective control group including lung transplant recipients from the University of Pittsburgh with Antibody Mediated Rejection will be created to describe differences in the endpoints using the CFZ-based regimen vs. historic standard of care.

4.4.3 Safety outcomes

Safety outcomes of the study include the following:

4.4.3.1 Non-lung irreversible end-organ failure (e.g., end-stage renal disease) any time after day 1 attributable to carfilzomib.

4.4.3.2 Incidence of adverse effects (AE) requiring dose-modification (see section 5.3.1.2) any time during days 1-16.

4.4.3.3 Incidence of any AE at any time after day 1 as graded by the NIH Common Terminology Criteria for Adverse Events (CTCAE) (appendix B) criteria, as follows:

Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL (Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.).

Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL (self-care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden).

Grade 4: Life-threatening consequences; urgent intervention indicated.

Grade 5: Death related to AE.

4.4.3.4 Incidence of hypogammaglobulinemia any time after day 1.

4.4.3.5 Incidence of culture-proven *de novo* infection any time after day 1.

4.4.3.6 Diagnosis of systemic inflammatory response syndrome (SIRS) any time during days 1-16; requiring at least 2 of the following 4 criteria:

Body temperature: < 36 or > 38 degrees Celsius

Tachycardia: > 90 beats per minute

Tachypnea: > 20 breaths per minute

White blood cells: < 4000 or > 12000 cells/mm^3

4.4.3.7 Patient death at any time after day 1 attributable to carfilzomib.

A 1:1 retrospective control group including lung transplant recipients from the University of Pittsburgh with Antibody Mediated Rejection will be created to describe differences in the endpoints using the CFZ-based regimen vs. historic standard of care.

4.4.4 Exploratory outcomes

Exploratory outcomes of the study include the following:

4.4.4.1 Identification of antibody producing cells (APCs) from peripheral blood mononuclear cells (PBMCs), such as activated B cells and plasmablasts in patients before and during carfilzomib therapy, compared to LTRs without AMR and not receiving carfilzomib. This will be done using a combination of techniques including MHC class I and II tetramers, overlapping peptides to key MHC that are targeted by DSA responses, and an ELISPOT assay (so-called ex vivo studies).

4.4.4.2 Expansion of B cells from PBMC using a cytokine/anti-CD40 ligand cocktail as well as EBV transformation. We will then determine whether these B cell stimuli lead to detectable levels of in vitro alloantibody (compared to serum anti-HLA Abs detected by the HLA laboratory). Using carfilzomib in vitro, we will also determine whether it decreases the expansion of these APCs and blocks alloantibody production.

4.5 Safety plan

Patients will be enrolled according to the inclusion and exclusion criteria established in section 5.3. Patients will provide informed consent to participate in this clinical trial. In the informed consent process, patients will be educated about the purpose for carfilzomib and the potential adverse effects as described in section 6.1.1. Adverse effects will be recorded and graded according to the CTCAE as described above in section 4.4.3. Serious adverse effects (SAE) will be reported timely as required and established in section 6.4.

A study data safety monitoring board (DSMB) will be established consisting of 3 members who represent non-funded non-investigators with no conflicts of interest regarding carfilzomib or the IST funding agency. The study investigators will invite qualified individuals to participate in the DSMB. The DSMB members will possess either PharmD, PhD, or MD qualifications and have a working knowledge of lung transplantation and AMR. The DSMB will be chaired by one of the three members as determined by the DSMB membership. The DSMB will meet once yearly to review study progress, to ensure appropriate enrollment milestones are being met, and to review the adverse event reporting. The DSMB will meet on an ad-hoc basis to review any SAEs.

The DSMB may terminate the study only after at least 15 patients have been enrolled. The DSMB may only terminate the study for safety reasons in which the perceived risks of continuing to provide carfilzomib to upcoming patients who may be enrolled outweighs the potential benefit to the patient and the allograft. The DSMB may not terminate the study for any reason related to carfilzomib efficacy.

4.6 Ethical considerations

There are no identifiable ethical considerations that would preclude the execution of this clinical trial. Patients cannot receive placebo since this IST represents a single-arm active-treatment trial.

4.7 Administrative structure

The Division of Pulmonary, Allergy, and Critical Care Medicine within the University of Pittsburgh School of Medicine employs substantial research infrastructure to successfully execute this IST. This infrastructure constitutes the UPMC Pulmonary Research Core. Working with the University of Pittsburgh Office of Research who will coordinate the expenditure of research funds and the execution of trial contracts, the research core will be responsible for all clinical trial interventional and regulatory support.

4.7.1 UPMC Pulmonary research core

The UPMC Pulmonary Research Core will be responsible for the support of this IST. The research core study coordinator will be alerted by one of the investigators of a patient who will be enrolled in the study. This coordinator will execute all paperwork required for enrollment in the clinical trial, establish patient linkage codes, support the data collection required by the IST, and collect any study blood obtained outside the routine clinical care of the patient, such as blood for PBMC isolation to support the exploratory outcomes.

4.7.2 Data management plan

All study data will be collected using REDcap. The pulmonary research core study nursing staff will be responsible for entering all data to be collected as required by this IST. This database will only contain linkage codes to identify the study patients. A separate file containing the protected health information of the study participants along with their linkage codes will be maintained. These files will be stored on secured research servers behind the University of Pittsburgh's research firewall. Data will only be accessible by protected passwords by study investigators and support personnel.

4.7.3 Investigational drug pharmacy

All study drug will be prepared and dispensed by the University of Pittsburgh Investigational Drug Service in accordance with the drug handling and dispensing practices outlined in appendix C. The investigational drug service charges a dispensing fee associated with each dose in the drug cycle. This fee is included in the study budget.

4.8 Compliance with laws and regulations and the institutional review board

This study will be conducted in accordance with the U.S. Food and Drug Administration (FDA) regulations, the International Conference on Harmonisation (ICH) E6 Guideline for Good Clinical Practice (GCP), and all applicable local, state, and federal laws.

This study will undergo full prospective institutional review board (IRB) evaluation by the University of Pittsburgh IRB prior to any patient enrollment to ensure scientific merit and all appropriate patient protections and information protections are in place.

5 Materials and methods

5.1 Patients

5.1.1 Patient selection

Patients will be eligible for enrollment if they have met the diagnostic criteria for AMR as described in section 4.3.6. This involves the identification of DSA via Luminex SAB technology prior to day 1. Any patient who meets the diagnostic criteria after study initiation will be screened for enrollment. Patients who satisfy the inclusion and exclusion criteria will be approached to provide informed consent and enroll in the study.

A 1:1 retrospective control group including lung transplant recipients from the University of Pittsburgh with Antibody Mediated Rejection will be created to describe differences in the endpoints using the CFZ-based regimen vs. historic standard of care.

5.1.2 Inclusion criteria

Adult lung transplant recipients ≥ 18 years of age who meet the diagnostic criteria for AMR as described in 4.3.6 who have underwent PFT testing unless intubated and transbronchial biopsy prior to enrollment. Patients who have not experienced improvement or regression are candidates for a second cycle of therapy on the protocol.

5.1.3 Exclusion criteria

Study exclusion criteria are as follows:

5.1.3.1 Exclusion related to disease

Direct contraindications or previous intolerances to any component of the standard of care regimen including PLEX, 5% human albumin, 5% gammagard S/D or 10% gammagard liquid

5.1.3.2 Exclusion related to general health

Leukopenia defined as WBC < 2000
Neutropenia defined as ANC < 1000
Thrombocytopenia defined as platelets < 75000 x 10^6
Known Child-Pugh B/C cirrhosis
Total bilirubin > 4
ALT > 90
Known systolic heart failure with LVEF < 40%
Known pulmonary hypertension
Any uncontrolled comorbid condition
Pregnant women (negative serum pregnancy test required within 7 days prior to study drug administration for women of childbearing potential)
Breastfeeding women

5.1.3.3 Exclusion related to risk for infections

Ongoing bacterial or fungal or viral infection that is life-threatening
Active cytomegalovirus disease

Active varicella zoster infection

5.1.3.4 Exclusion related to medications

Previous intolerance to carfilzomib
Concurrent use of another proteasome inhibitor (e.g., bortezomib)

5.2 Treatment assignment

This is an open-label single-arm active-treatment study; thus, patients will be assigned to carfilzomib therapy in the order in which they are enrolled.

5.3 Study treatment

Conventional therapy for AMR: Therapeutic plasma exchange (PLEX) 1.5 plasma volumes replaced with 5% albumin or fresh frozen plasma for 8 sessions every-other day. Total intravenous immunoglobulins (IVIG) 100 mg/kg IV after each PLEX with the exception of the final PLEX where IVIG 500 mg/kg will be given. On day 16, if the IgG level is below 700 mg/dL, an additional dose of IVIG 500 mg/kg will be administered. If the IgG result is found to be 700ml/dL or higher, the IVIG 500mg/kg dose will not be administered. Premedications will be given for IVIG as follows: acetaminophen 650 mg PO and diphenhydramine 25 mg PO both at least 30 minutes prior to and not more than 6 hours before IVIG, unless otherwise directed on the part of one of the investigators if believed to be in the best interests of patient care. On days where IVIG and CFZ are given together, only one set of premedications will be administered. Conventional therapy requires an inpatient hospital stay. Patients will receive a tunneled PLEX catheter and peripherally inserted central catheter (PICC) inserted by interventional radiology in the routine clinical care of these patients prior to receiving any conventional or carfilzomib therapy.

Conventional therapy rationale: PLEX serves two important roles in this regimen: first, to remove circulating injurious DSA, and second, to turn on the plasma cell apparatus due to depletion in order to enhance selection of highly active cells by the proteasome inhibitor. IVIG also serves two purposes: first, to provide putative Ig replacement to decrease infectious complications from PLEX, and second, to blunt the rebound antibody response due to depletion. Investigational carfilzomib will be given to enrolled patients between PLEX and IVIG on PLEX days, and alone on non-PLEX days. The rationale to insert carfilzomib between PLEX and IVIG is to avoid any derangements to the Fcγ receptor (FcγR) by IVIg and allow carfilzomib unfettered access to the highly active plasma cells.

5.3.1 Investigational therapy

Carfilzomib (in addition to conventional therapy). Please refer to appendix D, investigators brochure for additional information regarding carfilzomib and clinical trial data.

5.3.1.1 Dosage, administration, and storage

Carfilzomib dosing schedule: Carfilzomib will be administered on the multiple myeloma schedule consisting of 20 mg/m^2 on days 1 2, 8, 9, 15, and 16 (one cycle) to constitute one therapeutic cycle. On PLEX/IVIG days, carfilzomib will be given in between PLEX and IVIG to avoid inadvertent blockade of the Fcγ receptor. Carfilzomib will be delivered via central access IV over 30 minutes (+15 minutes); slowed to 60 minutes (+15 minutes) if infusion reactions occur.

Carfilzomib will be diluted in a total of 80 mL dextrose 5% water (D5W) for administration. Premedications for carfilzomib administration will be given as follows: acetaminophen 650 mg PO, diphenhydramine 25 mg PO, ondansetron 4 mg PO, prednisone 40 mg PO or methylprednisolone 40 mg IV, all at least 30 minutes prior to and not more than 6 hours before carfilzomib, unless otherwise directed on the part of one of the investigators if believed to be in the best interests of patient care. Preinfusion hydration with a one-time bolus of 250 mL 0.9% sodium chloride will be given at least 30 minutes prior to and not more than 6 hours before carfilzomib to minimize the nephrotoxic AE. Postinfusion hydration with a one-time bolus of 250 mL 0.9% sodium chloride may be given immediately after carfilzomib to minimize the nephrotoxic AE

Carfilzomib may be administered for 1-2 complete cycles in the study. A second cycle may be given for lack of resolution of AMR or for continued detection of DSA in the absence of allograft improvement. If > one cycle is needed, the patient must still meet the enrollment criteria prior to the second cycle, and must not have had improvement after the first cycle or have experienced regression from initial improvement to qualify for a second study cycle. If > two cycles are desired, these must be given outside of the study. If > two cycles are desired, the third cycle must not be given within 90 days of day 1 of the first cycle. If the third cycle begins within this 90 day period, this will constitute a protocol violation.

5.3.1.2 Dosage modification for drug related toxicity

AES grade III or IV that have not reduced to grade II or less at the time that the next dose is due and are considered related to the study drug will be subject to dose modification or hold.

Toxicity	Parameters	Dose adjustment
Neutropenia	ANC < 1000	Withhold dose until > 1000 and reduce next scheduled dose from 20 mg/m2 to 15 mg/m^2 If reduced dose tolerated, dose may be escalated back to 20 mg/m2 at the discretion of the investigators.
Thrombocytopenia	< 50,000	Reduce next scheduled dose from 20 mg/m2 to 15 mg/m^2 If reduced dose tolerated, dose may be escalated back to 20 mg/m2 at the discretion of the investigators.
	< 25,000	Withhold dose until > 25,000 and reduce next scheduled dose to 15 mg/m^2 If reduced dose tolerated, dose may be escalated back to 20 mg/m2 at the discretion of the investigators.
Peripheral neuropathy	Affecting ADLs	Hold until resolved to baseline and reduce next scheduled dose to 15 mg/m^2 If reduced dose tolerated, dose may be escalated back to 20 mg/m2 at the discretion of the investigators.
Nephrotoxicity	SCr > 2 x baseline or eGFR reduced by 50%	Hold until resolved to baseline and reduce next scheduled dose to 15 mg/m^2 If reduced dose tolerated, dose may be escalated back to 20 mg/m2 at the discretion of the investigators.

Hepatotoxicity	Mild to moderate liver dysfunction: defined as 2 consecutive values, at least 28 days apart, of: (1) Total Bili (>33% direct) . 1x ULN to < 3xULN OR (2) An elevation of AST and/or ALT with normal bilirubin Grade 3 elevation in ALT and/or AST (>5xULN) Grade 3 elevation in total bilirubin **Drug-Induced hepatotoxicity (attributable to carfilzomib**	25% dose reduction. Dose may be re-escalated if liver function tests return to normal (baseline) and drug-induced hepatotoxicity is excluded. Hold until resolved to baseline. Monitor any abnormality weekly. Resume carfilzomib with a 25% dose reduction (15 mg/m^2) if drug-induced hepatotoxicity is excluded. If reduced dose tolerated, dose may be escalated back to 20 mg/m2 at the discretion of the investigators. Hold Carfilzomib until resolution to baseline. Monitor total bilirubin and direct bilirubin weekly. Upon resolution of total bilirubin to normal, resume carfilzomib dosing with a 25% dose reduction if drug-induced hepatotoxicity is excluded. **Discontinue carfilzomib.**
Posterior Reversible Encephalopathy Syndrome (PRES)		If PRES is suspected, hold carfilzomib. Consider evaluation with neuroradiological imaging, specifically MRI, for onset of visual or neurological symptoms suggestive of PRES. If PRES is confirmed, permanently discontinue carfilzomib. If the diagnosis of PRES is excluded, carfilzomib administration may resume at same dose, if clinically appropriate.
Thrombotic Microangiopathy (TMA)		If the diagnosis is suspected, hold treatment and manage per standard of care including plasma exchange as clinically appropriate. If TMA is confirmed and related to carfilzomib or pomalidomide, permanently discontinue drug treatment. If the diagnosis of TMA is excluded, treatment may resume if clinically appropriate.
Other grade 3 or 4 toxicity (considered related to study drug)		Hold until resolved to baseline and reduce next scheduled dose to 15 mg/m^2 If reduced dose tolerated, dose may be escalated back to 20 mg/m2 at the discretion of the investigators.

5.3.2 Concomitant therapy

Conventional therapy for AMR: Therapeutic plasma exchange (PLEX) 1.5 plasma volumes replaced with 5% albumin or fresh frozen plasma for 8 sessions every-other day. Total intravenous immunoglobulins (IVIG) 100 mg/kg IV after each PLEX with the exception of the final PLEX where IVIG 500 mg/kg will be given. On day 16, if the IgG level is below 700 mg/dL, an additional dose of IVIG 500 mg/kg will be administered. If the IgG result is found to be 700ml/dL or higher, the IVIG 500mg/kg dose will not be administered. Premedications will be given for IVIG as follows: acetaminophen 650 mg PO and diphenhydramine 25 mg PO both 30 minutes prior to IVIG, unless otherwise directed on the part of one of the investigators if believed to be in the best interests of patient care. Conventional therapy requires an inpatient hospital stay. Patients will receive a tunneled PLEX catheter and peripherally inserted central catheter (PICC) inserted by interventional radiology in the routine clinical care of these patients prior to receiving any conventional or carfilzomib therapy.

Contraception

Female:
Women of childbearing potential must either agree to abstain from sexual intercourse or use an effective birth control method during treatment with carfilzomib. Given that carfilzomib was clastogenic in the in vitro chromosomal aberration test in peripheral blood lymphocytes, as a precaution, females of childbearing potential and/or their male partners should use effective contraception methods or abstain from sexual activity during and for 30 days after treatment with carfilzomib. If pregnancy occurs during this time, patients should be apprised of the potential hazard to the fetus. Kyprolis (carfilzomib) should only be used during pregnancy if the potential benefits to the mother outweigh the potential risks to the fetus.

Based on its mechanism of action and findings in animals, carfilzomib can cause fetal harm when administered to a pregnant woman. Carfilzomib caused embryo-fetal toxicity in pregnant rabbits at doses that were lower than in subjects receiving the recommended dose. Carfilzomib administered to pregnant rats and rabbits during the period of organogenesis was not teratogenic at doses up to 2 mg/kg/day in rats or up to 0.8 mg/kg/day in rabbits. If carfilzomib is used during pregnancy, or if the subject becomes pregnant while taking this drug, she should inform the investigator or study staff immediately. The investigator should notify Amgen of the pregnancy and discuss follow-up with the subject. It is not known if carfilzomib will reduce the efficacy of oral contraceptives. Due to an increased risk of venous thrombosis associated with carfilzomib, subjects currently using oral contraceptives or a hormonal method of contraception associated with a risk of thrombosis should consider an alternative method of effective contraception.

Male:
Males of reproductive potential should be advised to avoid fathering a child while being treated with carfilzomib. The potential for carfilzomib to be transferred via semen and its effect on sperm are unknown. Male subjects treated with carfilzomib and/or their female partners (if of childbearing potential) should use effective contraceptive methods or abstain from sexual activity while treated with carfilzomib and refrain from donating sperm while on carfilzomib and for 90 days after treatment. If pregnancy occurs during this time, patients should be apprised of the potential hazard to the fetus, study drug should be interrupted, and a serum pregnancy test

performed. Study drug administration may resume if result is negative and study drug be discontinued, if positive.

Male subjects should be advised to inform the investigator or study staff immediately in the event that their female partner becomes pregnant during the study. Upon receipt of this information, the investigator should notify Amgen of the pregnancy and discuss follow-up regarding the pregnancy outcome with the subject.

Breastfeeding:
If a woman breastfeeds during the study, she must inform the investigator or study staff immediately. The investigator should notify Amgen that the subject has breastfed the infant and discuss follow-up with the subject.

It is not known whether Kyprolis is present in human breast milk. Due to the potential for adverse effects in nursing infants from carfilzomib, a decision should be made whether to discontinue nursing or to discontinue Kyprolis, taking into account the potential benefit of carfilzomib to the mother.

No studies of carfilzomib have been conducted in breastfeeding women. Carfilzomib should not be used during breastfeeding. Breastfeeding women and women planning on breastfeeding may not participate in clinical trials with carfilzomib.

A Woman of Childbearing Potential (WOCBP)
Any female who has experienced menarche and is not postmenopausal (i.e., has had menses at any time in the preceding 12 consecutive months).

Highly effective method of contraception / birth control include:
- Combined (estrogen and progestogen) hormonal methods: pills, vaginal ring, or skin patch
- Single hormonal methods (progestogen) to stop release of the egg from the ovary: [pills, shots/injections, implants (placed under the skin by a healthcare provider)]
- Intrauterine device (IUD)
- Intrauterine hormonal-releasing system (IUS)
- Surgery to tie both fallopian tubes (bilateral tubal ligation/occlusion)
- Your male partner has had a vasectomy and testing shows there is no sperm in the semen
- Sexual abstinence (not having sex)

Barrier Contraceptive as defined in ICH (M3)
A contraceptive device that physically prevents sperm from entering the endometrial cavity and fallopian tubes (e.g. male condom, female condom or diaphragm).

5.3.3 Excluded therapy

The following medications must be discontinued prior to enrollment:
Eculizumab (Soliris)
Rituximab (Rituxan)
Cyclophosphamide IV (Cytoxan)

5.4 Study assessments

See appendix B for full listing of study data collection.

Study therapy will be given as an inpatient. Screening patients for AMR as defined above in section 4.3 for enrollment in the trial may occur as an inpatient or outpatient. Patients will undergo a minimum 16 day hospitalization for conventional AMR therapy and for study carfilzomib. Patients may return to the clinic for standard of care visits on days 28 and 42 and 90 (2 and 4 and 10 weeks post therapy completion) for assessment as described below.

Patients will be followed in the study until day 90. There are no follow-up visits as part of this research study, however, if patients return to their standard of care routine visits, a medical record review will be performed to obtain this data from patients' medical records, whenever available. Patients will be subsequently followed by their treating physician under routine clinical care for recurrent AMR until they expire and any available clinical information related to their standard lung transplant care will be obtained from medical records up to 360 days.

For patients who prematurely discontinue therapy, all future planned study and routine clinical care assessments will be completed unless the patient expires. An assessment for the purpose for premature discontinuation will be taken at the time of study discontinuation.

The University of Pittsburgh laboratories will be used to assess all samples. DSA samples will be processed by the University of Pittsburgh Histocompatibility Lab under the direction of co-investigator Dr. Zeevi. PBMC isolation, and exploratory testing samples will be process by the research lab of co-principal investigator Dr. McDyer.

5.4.1 Definition of study assessments

Standard of Care procedures:

History and Physical Exam: A complete patient history and physical exam includes the evaluation of the head, ears, eyes, nose, and throat (HEENT), cardiovascular, dermatological, musculoskeletal, respiratory, gastrointestinal, and neurologic systems. Changes from baseline abnormalities will be recorded at each subsequent physical examination. New or worsened abnormalities will be recorded as AEs if deemed to be related to study therapy.

Complete blood count: white blood cell count, hemoglobin, hematocrit, and platelet count.
WBC differential: absolute neutrophil, lymphocyte, eosinophil, and monocyte counts.

Complete metabolic panel (CMP): sodium, potassium, chloride, bicarbonate, blood urea nitrogen, serum creatinine, glucose, calcium, magnesium, phosphorus, AST, ALT, Alkaline phosphatase, and total bilirubin levels.

Pulmonary function tests: forced expiratory volume in 1 second, forced vital capacity, forced expiratory flow.

Biopsy: transbronchial forceps biopsy.

Luminex SAB: whole blood draw for processing by the HLA lab.

Luminex C1q-SAB: whole blood draw for processing by the HLA lab.

Complement 3 and 4: whole blood draw for processing by the UPMC basic laboratory.

Research Procedures:

Pregnancy test: all women of childbearing potential will have a serum pregnancy test within 7 days prior to study drug administration.

Total IgG and IgG subclasses: whole blood draw for processing by the HLA lab. This samples will only be obtained if patient is coming to their standard of care visit on days 28, 42 and 90 (±14 days).

Blood for PBMC: whole blood draw for processing by the McDyer lab. This sample will only be obtained if patient is coming to their standard of care visit on days 28, 42 and 90 (±14 days).

5.5 Screening and pretreatment assessments

All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before the first infusion of carfilzomib. Written informed consent for participation in the study must be obtained before performing any study-specific screening tests or evaluations. Informed consent forms will be maintained at the study site. Refer to Section 4, table 4 for specific delineations.

5.6 Assessments during treatment

All visits must occur within ± 14 days of the scheduled data, unless otherwise noted. Assessments scheduled on the day of study drug administration should be performed prior to study drug infusion, unless otherwise noted. We will collect results from medical records from procedures done as Standard of Care (biopsies, PFTs, bronchoscopies, physical exam, lab results, PLEX and IVIG). Standard of Care procedures will not be done solely for research purposes.

5.7 Study completion

Patients discontinued from the study early (dropout) will be asked to return to the clinic within 30 days after the last infusion of carfilzomib for a follow-up visit.

5.8 Follow-up assessments

Ongoing AEs thought to be related to carfilzomib will be followed until the event has resolved to baseline, the event is assessed by the investigators to be stable, the patient is lost to follow up, the patient withdraws consent, or when it is determined the AE is not related to study drug.

5.9 Patient discontinuation

Patients will be removed from study continuation if the toxicities of therapy are deemed by the investigators to outweigh the potential benefit of therapy. Such toxicities may include severe thrombocytopenia (platelets < 10,000 x 10^6), acute kidney injury requiring renal replacement therapy, or severe hepatitis (ALT > 1000), or others deemed relevant to the individual patient by the investigators.

If patients discontinue study drug early, these patients may be replaced in the study by patients who are likely to complete at least 1 cycle of carfilzomib.

The investigators have the right to discontinue a patient from the study for any medical condition that the investigator determines may jeopardize the patient's safety if he or she continues in the study or if the investigator determines discontinuation is in the best interest of the patient.

5.10 Study discontinuation

Amgen may discontinue the study prematurely for the following reasons: the incidence or severity of AEs in this study indicates a potential serious health hazard to patients, if enrollment is sufficiently poor, or if data recording is inaccurate or incomplete.

5.11 Post-trial access

No post-trial access of carfilzomib will be available to patients under the auspices of the study. Additional off label marketed carfilzomib may be given to patients outside of the study if necessary under the routine clinical care of the patient; however, such administration must not occur before day 90. If such administration occurs prior to day 90, this will reflect a protocol violation.

5.12 Assay methods

All blood draws will be acquired with usual clinical techniques. Luminex SAB and C1q-SAB and IgG subclass identification will be performed using usual clinical techniques as directed by the HLA lab under the CLIA approved standard practice. All routine labs (CBC, WBC differential, CMP) will be performed using usual clinical techniques as directed by the basic laboratory under the CLIA approved standard practice. PBMC isolation and subsequent exploratory testing will occur under usual investigatory techniques as directed by Dr. McDyer.

5.13 Statistical methods

Data will only be analyzed in aggregate at study conclusion for the final enrolled patient on the efficacy and safety outcomes.

Descriptive statistics will be used to describe baseline and study characteristics of patients. Univariate parametric and nonparametric assessments, where appropriate, will be used to assess change from baseline in the efficacy and safety outcomes. Kaplan-Meier method will be used to describe freedom from endpoints and mortality in the patients treated with carfilzomib. Kaplan-Meier method will be used to visually represent the time to events and overall event rate in the patients in this pilot, not to compare events to any other group.

5.13.1 Analysis of the conduct of the study

Enrollment, protocol violations, and discontinuations from the study will be summarized by patient.

Demographic and baseline characteristics will be summarized using means, standard deviations, medians, interquartile ranges. All summaries will be presented by group.

Study drug administration data will be listed by dose level, and any dose modifications will be flagged. Means and standard deviations will be used to summarize the total dose of carfilzomib received. All summaries will be presented by group.

5.13.2 Safety analyses

All AE data will be listed by patient, cycle, and dose. All AE occurring after informed consent is given will be summarized by event, system, and grade. All SAE will be listed and summarized separately.

5.13.3 Determination of sample size

The sample size for this trial is based on perceived appropriate enrollment of 10 patients per year for 3 consecutive years. Since this is a single-arm, active-treatment study, no such calculation of statistical power is needed to quantitate the sample size.

5.14 Data quality assurance

The Pulmonary Research Core will be responsible for data management of this trial, including quality checking of the data. Data will be collected and aggregated in REDCap. Investigators and support personnel from the research core will be responsible for entering the needed data into REDCap. In the event of discrepant data, clarification will be performed by the primary investigators.

6 Assessment of safety

Safety assessments will consist of monitoring and recording adverse events (AE) and serious adverse events (SAE) as defined in this section; measurement of protocol-specified hematology and basic chemistry laboratory values, measurement of protocol-specified vital signs, and other non-protocol specific tests that are deemed critical to the safety evaluation of the patient.

Investigators are responsible for reporting any SAE to Amgen as described below. Amgen will be responsible for reporting SAEs to the appropriate regulatory body.

6.1 Possible risks and discomforts

6.1.1 Carfilzomib Risks

Carfilzomib is approved by the U.S. Food and Drug Administration (FDA) to be used only in certain U.S. patients with relapsed and refractory multiple myeloma that have tried and failed other therapies. It has not been approved to be used for any other disease or condition. In this study, carfilzomib is an investigational study drug because it is not approved for use in all patients in the United States, and it is not approved by some regulatory authorities (the agencies that are responsible for approving the use of a medicine in a country such as the European Medicines Agency and Health Canada).

6.1.2 Adverse effect (AE) definition

An AE is any unfavorable and unintended sign, symptom, or disease temporarily associated with the use of an investigational medicinal product or other protocol-imposed intervention, regardless of attribution.

This includes the following:

AEs not previously observed in the patient that emerge during carfilzomib therapy (days 1through 16, and 28, 42, 90), including signs or symptoms associated with AMR that were not present prior to the AE reporting period.

Complications that occur as a result of protocol-mandated interventions.

AEs that occur prior to the assignment of study treatment that are related to a protocol-mandated intervention.

Preexisting conditions judged by the investigators to have worsened in severity or frequency or changed in character during carfilzomib therapy (days 1through 16, and 28, 42, 90).

6.1.3 Serious adverse effect (SAE) definition

Death

Life threatening experience defined as any adverse experience that places the subject, in the view of the Investigator, at immediate risk of death at the time of occurrence; i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

Requires prolongation of the study hospitalization.

Results in persistent or significant disability/incapacity.

Congenital anomaly/birth defect in the offspring of an exposed subject.

Important medical events that may not result in death, be life-threatening, or require hospitalization, may be considered an SAE, when, based upon appropriate medical judgment, it jeopardizes the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

All AEs that do not meet these criteria should be regarded as non-serious AEs.

The terms serious and severe are not synonymous. Severity refers to the intensity of an AE; thus, severe AEs may not represent serious AEs. SAEs include only the above items that are based on patient or event outcome or action criteria associated with events that pose a threat to the patient's life or vital functions and require reporting to appropriate regulatory agencies as defined in this section.

6.1.4 All previously reported AEs related to carfilzomib from clinical trials

Organ system	All adverse reactions, frequency (%)	Grade 3 and above adverse reactions, frequency (%)	Serious adverse reactions, frequency (%)
Blood and lymphatic system disorders			
Anemia	45.9	22.5	1.9
Thrombocytopenia	34.9	23.2	1.6
Neutropenia	23.8	15.7	0.6
Lymphopenia	12.5	9.9	-
Leukopenia	10.2	4.3	0.1
Febrile neutropenia	2.2	1.8	1.6
Cardiac disorders			
Cardiac failure	4.4	2.9	2.8
Myocardial infarction	0.8	0.6	0.6
Cardiac arrest	0.6	0.6	0.6
Myocardial ischemia	0.4	0.3	0.3
Eye disorders			
Vision blurred	5.1	< 0.1	-
Cataract	2.6	1	0.1
Gastrointestinal disorders			
Diarrhea	33.7	2.3	0.8
Nausea	31.9	0.9	0.1
Constipation	18.5	0.2	-
Vomiting	17.1	0.6	0.2
Abdominal pain	10.6	1.2	0.6
Dyspepsia	5.8	0.1	-
Toothache	2.5	0.1	-
General disorders and administration site conditions			
Fatigue	43.6	5.9	< 0.1
Pyrexia	28.2	1.6	2.9
Edema	21.8	0.6	< 0.1
Asthenia	12	1.6	0.3
Chills	10.6	0.1	0.1
Pain	7	1.3	0.6
Multiorgan failure	0.5	0.5	0.4
Infusion site reactions	0.2	-	-
Hepatobiliary disorders			
Hepatic failure	0.1	< 0.1	< 0.1
Infections and infestations			
Respiratory tract infection	26.2	3.1	2
Pneumonia	12.5	9.3	9.7
Nasopharyngitis	9.9	< 0.1	< 0.1
Bronchitis	7.3	1	1.1
Urinary tract infection	7	1.3	0.9

Influenza	3.7	0.3	0.4
Sepsis	1.8	1.7	1.5
Viral infection	1.7	-	< 0.1
Investigations			
Increased blood creatinine	15.6	2.3	0.5
Decreased creatinine clearance	1.8	0.2	-
BUN increased	3.4	1.1	-
AST increased	6.8	2	0.1
ALT increased	6	2.4	0.1
C-reactive protein	1.3	0.3	-
Metabolism and nutrition disorders			
Hypokalemia	15.8	4.7	0.3
Decreased appetite	15.1	0.3	-
Hyperglycemia	13.3	4.3	0.3
Hypomagnesemia	10.2	0.6	-
Hypocalcemia	9.7	2.4	0.2
Hypophosphatemia	9.4	5.6	< 0.1
Hyponatremia	7.7	4.3	0.3
Hypercalcemia	7.3	2.7	1.9
Hyperuricemia	5.9	1.5	-
Hyperkalemia	5.1	1.4	0.2
Hypoalbuminemia	5.1	2	< 0.1
Dehydration	4	1	0.7
Tumor lysis syndrome	0.7	0.6	0.6
Musculoskeletal and connective tissue disorders			
Back pain	19	2.8	0.8
Muscle spasms	15.9	0.5	-
Arthralgia	14.5	1	0.1
Pain in extremity	12.8	1	0.2
Musculoskeletal pain	9.8	1.5	0.1
Musculoskeletal chest pain	8.8	0.4	0.1
Muscular weakness	6.2	1.1	0.3
Myalgia	5.7	0.3	-
Nervous system disorders			
Headache	20.4	1.1	0.3
Dizziness	11.6	0.6	< 0.1
Neuropathy	8.7	1	< 0.1
Parestheisa	8.3	0.2	< 0.1
Hypoesthesia	6.9	0.1	-
Psychiatric disorders			
Insomnia	16.1	0.7	-
Anxiety	6.6	0.3	-
Renal and urinary disorders			

Acute kidney injury	5.9	4.5	4.4
Renal failure	3.4	1.6	0.9
Renal impairment	1.6	0.6	0.3
Respiratory, thoracic and mediastinal disorders			
Dyspnea	27.6	4	2.1
Cough	24.2	0.3	0.1
Epistaxis	7.9	0.4	0.2
Oropharyngeal pain	7.8	-	-
Dysphonia	2.6	-	-
Pulmonary edema	1.6	1	0.9
Pulmonary embolism	1.5	1.3	1.3
Skin and subcutaneous tissue disorders			
Rash	8	0.5	0.3
Pruritis	5.9	< 0.1	-
Erythema	4.5	-	-
Hyperhidrosis	3.9	-	-
Vascular disorders			
Hypertension	14.9	4.4	0.6
Hypotension	5.6	1.5	0.9
Deep venous thrombosis	3.1	1.2	1.1

6.2 Methods and timing for capturing and assessing safety parameters

The investigators are responsible for ensuring that all AEs will be recorded on the AE form and SAEs are recorded on the SAE form. All reportable SAE will be reported on the FDA MedWatch forms and reported to Amgen as defined in this section.

6.2.1 Adverse event reporting period

After informed consent is obtained but before any administration of study drug, only SAEs due to protocol mandated interventions will be recorded. Any AE or SAE at any time after the first infusion of carfilzomib is initiated until day 16, and on days 28, 42, 90 ±14 days must be recorded by the investigators and reported to Amgen as defined in this section. After this period, only SAEs deemed to be related to study drug will be recorded and reported.

Serious Adverse Event Reporting Contact Information:
Amgen Global Safety
Toll-free #:1-888-814-8653
For countries where the U.S. toll-free # cannot be used: +44-20-7136-1046
Email (Only for sponsors with a secure email connection with Amgen):
svc-ags-in-us@amgen.com

6.2.2 Eliciting adverse events

A consistent methodology of non-directive questioning for eliciting AEs at all patient evaluation time points will be adopted.

This will include the following two questions directed to study subjects:

How have you felt since your last infusion of carfilzomib?

Have you had any new or changed health problems since you were last seen?

These questions will be asked in person at days 28, 42 and 90 ±14 days, or by phone if patient does not return to their standard of care visit at these time points.

6.2.3 Assessment of severity and causality of adverse events

Investigators will seek information on AEs and SAEs at each patient contact. All AEs and SAEs, whether reported by the patient or noted by authorized study personnel, will be recorded in the patient's medical record and reported on the AE or SAE forms and reportable SAE on the FDA MedWatch form.

For each AE and SAE recorded, the investigators will make an assessment of seriousness and severity based on the CTCAE criteria, and causality to carfilzomib.

6.3 Procedures for recording adverse events

All AEs will be recorded on the AE form . Only one AE will be reported on one form. For multiple AEs, multiple forms will be used. Study investigators will be responsible for codifying and keeping electronic copies of the form in the study database in protected files on the Pitt Research servers.

The Investigator-sponsor must be notified of the occurrence of any SAE within 24 hours of the investigator, designee, or site personnel's knowledge of the event. The Investigator-sponsor is responsible for notifying the appropriate health authorities (HAs), ethics committees (ECs), and investigators, of any expedited, annual, or other periodic safety reports in accordance with applicable regulations. Any safety report submission will cross reference the Amgen investigational new drug (IND) or clinical trial approval (CTA) number.

The Investigator is also responsible for notifying the local ECs in accordance with local regulations. Additionally, the Investigator-sponsor is responsible for reporting SAEs to Amgen.

SAE Reporting by Investigator-sponsor to Amgen

The Investigator-sponsor must inform Amgen in writing by e-mail or fax at the contact information listed below for all SUSARs that are judged as reasonably related to the Amgen study drug. Site will transmit the final CIOMS of that event to Amgen within twenty-four (24) hours of submitting the report to the applicable regulatory authority.

For regulatory reporting purposes, an event of "Death, Cause Unknown" from the study shall be processed as a SUSAR. All forms must be completed and provided to Amgen in English.

The Individual Case Safety Report (ICSR) may be referred to as an individual safety report or SAE Report, including Pregnancy Exposure Reports and Follow up Reports. The ICSR must be as complete as possible, at a minimum including event reference number, protocol name and number, investigator contact information, specific patient identifiers (e.g., initials, patient number, date of birth or age, or gender), the name of the suspect Study Drug, the date and dosage(s) of exposure, event, the date(s) of event, country of event, "Serious" Criteria, Relationship/causality of Study Drug, Hospitalization history for the event, Event status/outcome, Relevant history (including

diagnostics, laboratory values, radiographs, concomitant medications, and event treatment, and narrative summary.

Sponsor shall be responsible for collecting all SAEs and Pregnancy and Lactation Exposure Reports and will exercise commercially reasonable due diligence to obtain follow-up information on incomplete SAE or Pregnancy and Lactation Exposure Reports. In the event that the Company requires clarification or further information on individual SAE or Pregnancy and Lactation Exposure Reports, Company will not contact non-party investigators directly, but will route all such inquiries through Site for forwarding to such investigator(s). Site will be responsible to ensure such inquiries are completed and timely provided to Company.

Information not available at the time of the initial report (e.g., an end date for the SAE, discharge summaries, lot numbers, relevant laboratory values, scan data and autopsy reports) which are received after the initial report must be documented on a follow-up form, and submitted to Amgen in the same timelines as outlined above. Sponsor shall be responsible for obtaining follow-up information for the SAEs and demonstrate diligence in attempting to obtain such information by, among other things, maintaining written records of such attempts.

Other aggregate analysis including reports containing safety data generated during the course of the study is to be submitted to Amgen at the time the sponsor ISS submits to anybody governing research conduct i.e. RA, IRB etc. Final study report including unblinding data when applicable and reports of unauthorized use of a marketed product to be submitted to Amgen at the time the sponsor ISS submits to anybody governing research conduct i.e. RA, IRB etc. but not later than one calendar year of study completion.

Sponsor will provide an annual IND report to Amgen. Reports containing safety data generated during the course of the study is to be submitted to Amgen at the time the sponsor submits to anybody governing research conduct, i.e. regulatory authorities and IRBs. Sponsor will support reconciliation of all ICSRs at the end of the study at a minimum.

6.3.1 Diagnosis

If known, a diagnosis should be recorded on the AE form rather than individual signs and/or symptoms. However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE. If a diagnosis is subsequently established, it should be reported as follow up information.

6.3.2 Persistent or recurrent AEs

A persistent AE is one that extends continuously, without resolution between patient evaluation time points. Such events will only be recorded once unless severity has increased since initial recording. If a persistent AE becomes more severe, it shall be recorded again.

A recurrent AE is one that occurs and resolved between patient evaluation time points and subsequently occurs again. All recurrent AEs shall be recorded again.

6.3.3 Abnormal laboratory values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs. If the lab is a sign of a disease, only the diagnosis needs to be recorded. If the lab is not a sign of a disease, then the laboratory value or the designated term should be recorded (e.g., transaminitis or ALT 1750). Repeated observations of the same lab abnormality from patient contact time point to time point need not be recorded again unless they have worsened in severity (e.g., serum creatinine of 1.8 for 2 lab draws will only be recorded once).

6.3.4 Deaths

Deaths meet the criteria for SAE and all rules and regulations regarding reporting as defined in this section must be fulfilled. All deaths, irrespective of cause, will be recorded as SAEs and will be reported to the Sponsor. This includes death due to progression of AMR. Cause of death, if known, shall be recorded on the FDA Medwatch form. If deaths are due to progression of AMR, progression of study disease shall be recorded as a contributing factor. If deaths are due to study therapy, such notation shall be recorded as a contributing factor. If death occurs, it shall be noted on the FDA Medwatch form whether this death was expected or unexpected. After study completion (day 90), deaths due to progression of AMR shall only be recorded for the purposes of data analysis in the study.

6.3.5 Preexisting medical conditions

A preexisting medical condition is one that is present at the time of informed consent. Such conditions shall be recorded in the study database.

A preexisting condition will only be recorded as an AE or SAE if the frequency, severity, or character of the condition worsens during the study. When recording such events, the investigators will make note of the characteristics that have worsened (e.g., increased systolic blood pressure for preexisting hypertension).

6.3.6 Worsening antibody mediated rejection

Worsening or progression of AMR shall not be recorded as an AE or SAE. These data will be captured as efficacy assessment data only.

6.3.7 Prolonged hospitalization

Any AE that results in prolonging of the study hospitalization shall be documented on the FDA Medwatch form as an SAE and be subject to all the rules and regulations of reporting as described in this section.

6.3.8 Pregnancy

<u>Pregnancy inclusion criteria</u>

Women of childbearing potential must have a negative serum pregnancy test within the 7 days prior to study drug administration.

Women of childbearing potential must either agree to abstain from sexual intercourse or use an effective birth control method during treatment and for an additional 30 days after the last dose of carfilzomib. Men who are sexually active with women of childbearing potential must either agree to abstain from sexual intercourse or use a condom with spermicide during treatment and for an additional 30 days after the last dose of carfilzomib, and the female partner should consider using an effective method of birth control.

Pregnancy exclusion criteria

Women who are pregnant and/or breast feeding

6.3.8.1 Pregnancy reporting

Subjects will be instructed to notify the investigator as soon as possible after learning becoming pregnant or the pregnancy of a partner during treatment of or up to 30 days following the last study drug administration.

Pregnancy Reporting by Investigator-sponsor to Amgen

Report Pregnancy and potential infant exposure including Lactation, within ten (10) calendar days of Sponsor awareness. Provide to Amgen the SAE reports associated with pregnancy. SUSARs are to be reported within twenty-four (24) hours of submitting the report to the applicable regulatory authority.
Amgen Drug Safety and Pharmacovigilance Contact Information:

- Drug Safety Reporting Fax
- Toll-free US 888-814-8653
- Toll US 805-480-9205

Drug Safety Reporting by secure e-mail can be established upon request

If the patient becomes pregnant while taking an Amgen drug, the drug will be immediately discontinued.
The investigator will discuss the risks and concerns of investigation drug exposure to a developing fetus and counsel the subject and/or pregnant partner (or ensure that such counseling is provided).
Pregnancies will be followed through the outcome of the pregnancy.

Newborns should be followed for a minimum of 12 weeks.

The Investigator will complete a Pregnancy Monitoring Form and report the information regarding the pregnancy, outcome, and status of the newborn as appropriate.

6.4 Procedures for reporting serious adverse events

Provide SAE reports on MedWatch forms for mandatory reporting

Drug Safety Reporting Fax

- Toll-free US 888-814-8653
- Toll US 805-480-9205

Drug Safety Reporting by secure e-mail can be established upon request

6.4.1 Reporting requirements for serious adverse events

The Investigator-sponsor must be notified of the occurrence of any SAE within 24 hours of the investigator, designee, or site personnel's knowledge of the event.

SAE Reporting by Investigator-sponsor to Amgen

The Investigator-sponsor must inform Amgen in writing by e-mail or fax at the contact information listed below for all SUSARs that are judged as reasonably related to the Amgen study drug. Site will transmit the final CIOMS of that event to Amgen within twenty-four (24) hours of submitting the report to the applicable regulatory authority.

6.5 Follow-up of patients after adverse events

All SAEs regardless of relationship to CFZ must be followed to resolution or to stabilization if improvement or resolution is not expected.

7 Investigator requirements

7.1 Study initiation

Before the start of the study and any study-related procedures the following documents must be signed by the co-principal investigators. A copy must be retained both by Amgen and the study site team.

US FDA Form 1572: Statement of investigator signed by the co-primary investigators. The names of the co-investigators must appear on this form.

Current curriculum vitae and evidence of licensure for all investigators.

Complete financial disclosure forms as required by FDA Form 1572 for all investigators.

Written University of Pittsburgh IRB statement of approval and compliance of the protocol and informed consent form.

Investigator brochure receipt signed and dated by the co-principal investigators.

Protocol acceptance form signed and dated by the co-principal investigators.

7.2 Study completion

The following data and materials will be submitted to Amgen upon the conclusion of the study.

Laboratory findings, clinical data, and all special test results from screening through the end of the study period for all study subjects.

Completed drug accountability forms.

Copies of any protocol amendments and IRB approval notifications.

Signed and dated protocol amendment acceptance forms.

Summary of the study prepared by the co-principal investigators (IRB summary close letter).

Updated financial disclosure forms for all investigators.

7.3 Informed consent form

The informed consent forms for the study must be approved by the University of Pittsburgh IRB.

Consent forms must be signed by the patient or legal designee before his/her participation in the study. A signed copy of the informed consent form will be inserted into the medical record and retained with the study investigators files. A copy of the informed consent form will be given to the patient or legal designee.

Consent forms must be available for review by the sponsor at any time.

Consent forms should be revised whenever there are changes to the procedures outlined in the informed consent form or when new information is available that may affect the willingness of patients to participate.

7.4 Communication with the IRB

This protocol, the informed consent forms, and any information to be given to the patient must be submitted to the IRB by the co-principal investigators for review and approval before the study is initiated. In addition, any patient recruitment materials must be approved by the IRB.

The principal investigator is responsible for providing written summaries of the status of the study to the IRB at least annually in accordance with all IRB policies and procedures. Investigators are also responsible for promptly informing the IRB of any protocol changes or amendments and any unanticipated problems involving risk to patients or others.

Any other IRB requirements not delineated in this section must be fulfilled by the co-primary investigators.

7.5 Study monitoring requirements

Site visits may be conducted by an authorized Amgen representative to inspect the study data, patient records, and AE reports. The co-primary investigators permit Amgen, IRB, FDA, or other respective relevant health authorities to inspect study facilities and records relevant to this study.

7.6 Source data documentation

Study monitors may perform ongoing source data verification to confirm that critical protocol data are entered accurately and completely in the study database.

Source documents are defined as the location in the official medical record or other credible source of information where patient data are recorded and documented for the first time.

Source documents will never be destroyed or altered.

7.7 Study medication accountability

All study drug required for completion of this study will be provided by Amgen. The UPMC investigational drug service (IDS) pharmacy will acknowledge receipt of the drug by returning the relevant documentation forms indicating shipment content and condition. Damaged supplies will be replaced.

Accurate records of all study drug received at, dispensed from, returned to and disposed of by the UPMC IDS pharmacy shall be recorded.

7.8 Disclosure of data

Patient medical information obtained by this study is confidential and may only be disclosed to third parties as permitted in the informed consent form signed by the patient or legal designee as required by law.

Medical information may be given to a patient's personal physician or other appropriate healthcare provider for purposes of patient treatment and welfare.

Data generated by this study must be available for inspection upon request by representatives of the FDA or other relevant regulatory agencies and the study sponsor.

7.9 Retention of records

US FDA regulations (21 CFR 312.62[c]) and the ICH guideline for GCP require that records and documents pertaining to the conduct of this study and distribution of investigational drug, including AE forms, consent forms, lab results, and medication inventory records be retained by the co-principal investigators for 2 years after the formal discontinuation of the study and the FDA is notified.

No records shall be disposed of without the permission of Amgen. Written notification shall be provided to Amgen for transfer of any records to another party or location.

8 References

1. Weigt SS, Wallace WD, Derhovanessian A, et al. Chronic allograft rejection: epidemiology, diagnosis,pathogenesis, and treatment. Semin respir crit care med 2010;31:189-207.
2. Yusen RD, Christie JD, Edwards LB, et al. The Registry of the International Society for Heart and Lung Transplantation: thirtieth adult lung and heart-lung transplant report-2013. J Heart Lung Transplant 2013;32:965-78.
3. Snyder LD, Wang Z, Chen DF, et al. Implications for human leukocyte antigen antibodies after lung transplantation: a 10-year experience in 441 patients. Chest 2013;144:226-33.
4. Sundaresan S, Mohanakumar T, Smith MA, et al. HLA-A locus mismatches and development of antibodies to HLA after lung transplantation correlate with the development of bronchiolitis obliterans syndrome. Transplantation 1998;65:648-53.
5. Jaramillo A, Smith MA, Phelan D, et al. Development of ELISA-detected anti-HLA antibodies precedes the development of bronchiolitis obliterans syndrome and correlates with progressive decline in pulmonary function after lung transplantation. Transplantation 1999;67:1155-61.

6. Palmer SM, Davis RD, Hadjiliadis D, et al. Development of an antibody specific to major histocompatibility antigens detectable by flow cytometry after lung transplant is associated with bronchiolitis obliterans syndrome. Transplantation 2002;74:799-804.
7. Hadjiliadis D, Chaparro C, Reinsmoen NL, et al. Pre-transplant panel reactive antibody in lung transplant recipients is associated with significantly worse post-transplant survival in a multicenter study. J Heart Lung Transplant 2005;24:S249-54.
8. Girnita AL, McCurry KR, Iacono AT, et al. HLA-specific antibodies are associated with high-grade and persistent-recurrent lung allograft acute rejection. J Heart Lung Transplant 2004;23:1135-41.
9. Girnita AL, Duquesnoy R, Yousem SA, et al. HLA-specific antibodies are risk factors for lymphocytic bronchiolitis and chronic lung allograft dysfunction. Am J Transplant 2005;5:131-8.
10. Smith MA, Sundaresan S, Mohanakumar T, et al. Effect of development of antibodies to HLA and cytomegalovirus mismatch on lung transplantation survival and development of bronchiolitis obliterans syndrome. J Thorac Cardiovasc Surg 1998;116:812-20.
11. Chin C, Chen G, Sequeria F, et al. Clinical usefulness of a novel C1q assay to detect immunoglobulin G antibodies capable of fixing complement in sensitized pediatric heart transplant patients. J Heart Lung Transplant 2011;30:158-63.
12. Yabu JM, Higgins JP, Chen G, Sequeira F, Busque S, Tyan DB. C1q-fixing human leukocyte antigen antibodies are specific for predicting transplant glomerulopathy and late graft failure after kidney transplantation. Transplantation 2011;91:342-7.
13. Hachem RR, Yusen RD, Meyers BF, et al. Anti-human leukocyte antigen antibodies and preemptive antibody-directed therapy after lung transplantation. J Heart Lung Transplant 2010;29:973-80.
14. Everly MJ, Everly JJ, Susskind B, et al. Bortezomib provides effective therapy for antibody- and cell mediated acute rejection. Transplantation 2008;86:1754-61.
15. Everly MJ, Terasaki PI, Trivedi HL. Durability of antibody removal following proteasome inhibitor-based therapy. Transplantation 2012;93:572-7.
16. Perry DK, Burns JM, Pollinger HS, et al. Proteasome inhibition causes apoptosis of normal human plasma cells preventing alloantibody production. Am J transplant 2009;9:201-9.40.
17. Everly MJ, Terasaki PI, Hopfield J, Trivedi HL, Kaneku H. Protective immunity remains intact after antibody removal by means of proteasome inhibition. Transplantation 2010;90:1493-8.
18. Mulder A, Heidt S, Vergunst M, Roelen DL, Claas FH. Proteasome inhibition profoundly affects activated human B cells. Transplantation 2013;95:1331-7.
19. Jagannath S, Vij R, Stewart AK, et al. An open-label single arm pilot phase II study (PX-171-003-A0) of low-dose, single agent carfilzomib in patients with relapsed and refractory multiple myeloma. Clin Lymphoma Myeloma Leuk 2012;12:310-8.
20. Siegal DS, Martin T, Wang M. A phase 2 study of single-agent carfilzomib (PX-171-003-A1) in patients with relapsed and refractory multiple myeloma. Blood 2012;120:2817-25.
21. Vij R, Siegal DS, Jagannath S, et al. An open-label, single-arm, phase 2 study of single-agent carfilzomib in patients with relapsed and/or refractory multiple myeloma who have been previously treated with bortezomib. Br J Haematol 2012;158:739-48.
22. Witt CA, Gaut JP, Yusen RD, et al. Acute antibody-mediated rejection after lung transplantation. J Heart Lung Transplant 2013;32:1034-40.
23. Takemoto SK, Zeevi A, Feng S, et al. National conference to assess antibody-mediated rejection in solid organ transplantation. Am J Transplant 2004;4:1033-41.
24. Estenne M, Maurer JR, Boehler A, et al. Bronchiolitis obliterans syndrome 2001: an update of the diagnostic criteria. J Heart Lung Transplant 2002;21:297-310.

9 Appendices

9.1 Appendix A: Study Flowcharts

Study Procedures		Intervention Phase ±14 days																Testing Phase ±14 days			Post-study Fol			
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	28	42	90	120	150	180	210
Screening/Enrollment	x																							
Standard of Care Procedures																								
H&P		x																						
Progress note			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x
PLEX 1.5 volumes		x		x		x		x		x		x		x		x								
Premeds for IVIG		x		x		x		x		x		x		x		x	x							
IVIG 100 mg/kg		x		x		x		x		x		x		x										
IVIG 500 mg/kg																x	X*							
Medrol 750-1000 mg IV		x	x	x	*only given if concomitant ACR*																			
CBC w/differential		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
CMP		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Total IgG		x															x	x	x	x	x	x	x	
Complement 3 & 4		x															x	x	x	x			x	
Luminex IgG-SAB		x															x	x	x	x	x	x	x	x
Luminex C1q-SAB		x															x	x	x	x	x	x	x	x
PFTs		x																x	x	x	x	x	x	x
TB Biopsy		x																	x					*clini*
Study Specific Procedures																								
Premeds for CFZ		x	x						x	x						x	x							
CFZ 20 mg/m2		x	x						x	x						x	x							
CFZ AE assess			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
CFZ response assess																		x	x	x				
IgG-SAB subclasses**		x															x	x	x	x				
Blood for PBMC**		x															x	x	x	x				

Progress notes indicate inpatient admission or clinic visits within ±14 days of the stated days, unless the patient is unable to present due to unforeseen circumstances (e.g., death, hospitalization at another facility, etc.). Standard of care procedures will not be done solely for research purposes.

Study ends at the day 90 visit. Post-study follow-up will continue for all patients alive at day 90 to 1 year post enrollment through a medical records review.

Study CFZ AE assessment will be conducted either in-person or via telephone (day 1 through 16, and 28, 42 and 90

*** Dose will be administered only If IgG level is below 700 mg/dL.**

**** Research samples will only be obtained if subject is coming to their standard of care visit on days 28, 42 and 90.**

Appendix B: Study data collection summary

Baseline characteristics

1. Lung transplant indication: interstitial lung disease, chronic obstructive pulmonary disease, cystic fibrosis, scleroderma, sarcoidosis, pulmonary arterial hypertension, other (specify)
2. Transplant type: single, bilateral
3. Date of transplant
4. Time after transplant to enrollment
5. Age at transplant
6. Age at enrollment
7. Gender: male, female
8. Race: Caucasian, African American, Native American, Hispanic, other (specify)
9. Induction immunosuppression
10. Maintenance immunosuppression at enrollment
11. De novo DSA vs. preformed DSA prior to transplant
12. PFTs at enrollment (if not intubated): FEV1, FVC, FEF25/75
13. Bronchiolitis obliterans syndrome grade at enrollment
14. Daily oxygen use (Y/N)
15. CMV donor/recipient status: mismatch, recipient positive, negative
16. EBV donor/recipient status: mismatch, recipient positive, negative
17. HSV donor/recipient status: mismatch, recipient positive, negative
18. VZV donor/recipient status: mismatch, recipient positive, negative
19. Chronic infections at enrollment (specify)

Study characteristics

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	28	42	90
WBC	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x
ANC	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x
ALC	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x
Hematocrit	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x
Platelets	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x
BUN	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x
Creatinine	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x
Total IgG	x															x	x	x	x
IgG 1	x															x	x	x	x
IgG 2	x															x	x	x	x
IgG 3	x															x	x	x	x
IgG 4	x															x	x	x	x
Complement 3	x															x	x	x	x
Complement 4	x															x	x	x	x
DSA MFI neat	x															x	x	x	x
DSA MFI 1:16	x															x	x	x	x
DSA MFI EDTA	x															x	x	x	x
DSA MFI C1q neat	x															x	x	x	x
DSA MFI AHG-C1q neat	x															x	x	x	x
DSA MFI C1q 1:16	x															x	x	x	x
DSA MFI C1q EDTA	x															x	x	x	x

DSA IgG subtype	x															x	x	x	x
FEV1	x																x	x	x
FVC	x																x	x	x
FEF 25/75	x																x	x	x
ACR A grade	x																	x	
ACR B grade	x																	x	
ACR C grade	x																	x	
AMR findings (Y/N)	x																	x	
Blood for PBMC (Y/N)	x															x	x	x	x
PLEX (Y/N)	x		x		x		x		X		x		x		x				
IVIG 100 mg/kg (Y/N)	x		x		x		x		X		x		x						
IVIG 500 mg/kg (Y/N)															X**				
Carfilzomib 20 mg/m^2 (Y/N)	x	x						x	X						x	x			
Other carfilzomib dose and reason	x	x						x	X						x	x			
Medrol (Y/N)	x	x	x																
Medrol dose	x	x	x																
AE assessment	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x
Death	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x
AE/SAE	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x
Cultures*	x	x	x	x	x	x	x	x	X	x	x	x	x	x	x	x	x	x	x

*Cultures: bacterial, viral, fungal, AFB, cytology. Positive cultures will be recorded at any time if they are taken.

**** Dose will be administered only If IgG level is below 700 mg/dL.**