A Phase II open label study of Oral Doxycycline administered as an adjunct to plasma cell directed therapy in light chain (AL) amyloidosis.

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Abbreviations:

AL amyloidosis- immunoglobulin light chain amyloidosis
H&P- history & physical
CBC- complete blood count
β-HCG- Beta human chorionic gonadotropin
KPS- Karnofsky Performance Status
LVEF- left ventricular ejection fraction
NT-proBNP- N-terminal pro-Brain Natriuretic Peptide
BID- twice daily
CyBorD- Cyclophosphamide/Bortezomib/Dexamethasone
CyBorP- Cyclophosphamide/Bortezomib/Prednisone
Mel/Dex- Melphalan/Dexamethasone

Synopsis

A Phase II open label study of Oral Doxycycline administered as an adjunct to plasma cell directed therapy in light chain (AL) amyloidosis.

PRIMARY OBJECTIVES:

1. Determine the efficacy of adjunctive doxycycline in addition to specific anti plasma cell therapy in patients with AL amyloidosis in improving amyloid organ response at 1 year.
2. Assess the safety of doxycycline + anti-plasma cell chemotherapy regimen in AL amyloidosis.

SECONDARY OBJECTIVES:

1. To assess rates of hematologic response.
2. To assess rates of mortality at 1 month, 6 months and 1 year.
3. To measure patient reported outcomes at baseline, 3, 6, 9 and 12 months.
4. To evaluate biomarkers associated with anti-amyloid response.

STUDY DESIGN:

This is a phase II study of doxycycline for 1 year, used as an adjunct to plasma cell directed therapy, to improve amyloid organ response.

PATIENT ELIGIBILITY CRITERIA:

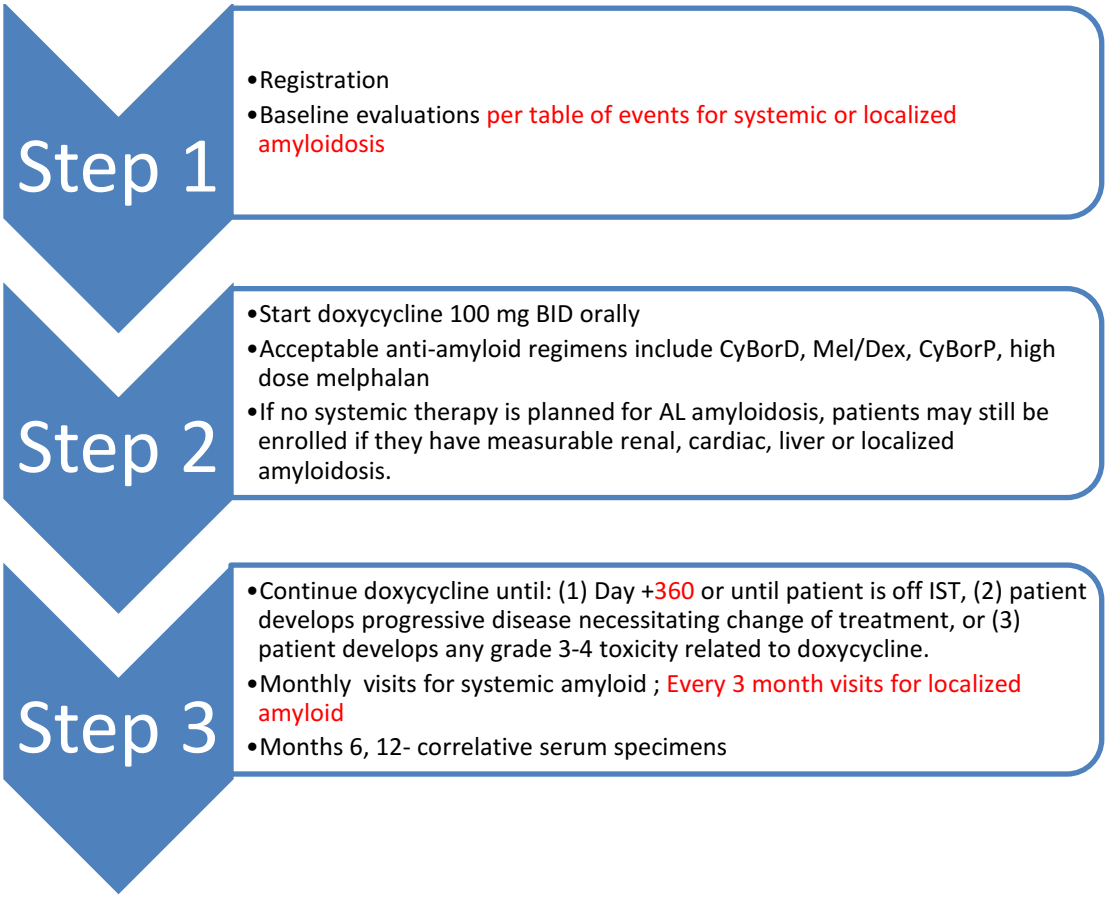
Inclusion Criteria:

1. Patients with biopsy proven AL amyloidosis.
2. Patients ≥ 18 years of age are eligible.
3. Patient must provide informed consent.
4. All patients must have measurable amyloid organ involvement of a vital organ (heart, liver, kidneys). Localized amyloidosis will be eligible as long as the amyloid involvement is radiologically measurable.
5. A negative pregnancy test will be required for all women of child bearing potential. Breast feeding is not permitted.
6. Patients with history of intolerance or allergic reactions with doxycycline will not be eligible.
7. Patients who have previously been taking doxycycline will be eligible as long as there is no contraindication to stay on doxycycline 100 mg BID for 1 year in the opinion of the treating physician.
8. Creatinine clearance of >25 ml/min.

Exclusion Criteria:

1. Patients with severe malabsorption syndrome precluding absorption of oral agents will be excluded.
2. Known intolerance or allergic reactions with doxycycline.
3. Previous chemotherapy for AL amyloidosis.

Patient Treatment Plan



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1. INTRODUCTION:

1.1 Amyloidosis:

The amyloidoses are a diverse group of protein misfolding diseases wherein proteins aggregate and form fibrillar deposits (amyloid) in tissues.[1] Examples of amyloid diseases include Alzheimer's disease, hereditary transthyretrin-associated familial amyloid polyneuropathy, dialysis related amyloidosis, AA amyloidosis with chronic systemic inflammatory states and immunoglobulin-derived AL amyloidosis. Human amyloid-associated illnesses pose a therapeutic challenge since amyloid is a long-lived protein and there are no approved drugs demonstrated to disrupt pre-formed amyloid.

AL amyloidosis is a malignant plasma cell disease characterized by the formation of amyloid from immunoglobulin light chains produced by clonal plasma cells.[1] The amyloid deposits into vital organs such as the heart, liver, kidney, and nerves resulting in inexorable systemic decline in function and culminating in death. Of the 27 known proteins resulting in amyloidosis in humans, AL amyloidosis is the most common and the most rapidly fatal with a median survival of 6 months in advanced disease.[2] The drugs most frequently used to treat AL amyloidosis eradicate cells that produce AL amyloid protein but have no effect on pre-formed amyloid (see below).

1.2 Tetracyclines:

The tetracyclines are a well-established family of broad-spectrum antimicrobials first isolated from *Streptomyces aureofaciens* in 1947 from soil screening.[3] Semisynthetic tetracyclines were developed for antibacterial use, of which doxycycline is one. Their antibiotic effect is primarily mediated by binding to the bacterial ribosome and inhibiting protein synthesis. Separate from their anti-microbial effect, tetracyclines, including doxycycline, possess the ability to inhibit members of the matrix metalloproteinase (MMP) family of endopeptidases. The MMPs are zinc-dependent proteases involved in a gamut of physiological and pathophysiological processes such as embryogenesis, tissue remodeling, inflammation and tumor invasion.[4] It is hypothesized that overproduction of MMPs can result in AL renal and cardiac damage.[5,6] High levels of MMPs appear to correlate with diastolic dysfunction and clinical manifestations of AL cardiomyopathy.[6] Doxycycline-induced inhibition of MMPs appears to be beneficial in conditions associated with pathologic MMP-mediated proteolysis of the extracellular matrix, including cardiac remodeling, periodontitis, arthritis and cancer.[7-9]

Owing to its lipophilicity, doxycycline also concentrates in organs at sites of injury including gums in gingivitis, brain in meningitis and the myocardium in infarcts.[10]

1.3 Doxycycline effects in Amyloidosis:

The first report of the anti-amyloidogenic activity of doxycycline was suggested in a study of Alzheimer's disease.[11] Forloni, et al, showed that co-incubation of tetracyclines with β 1-42 synthetic peptide, which is highly represented in Alzheimer amyloid deposits, resulted in a) marked reduction of amyloid fibril formation, b) inhibition of amyloid aggregation and c) de-fibrillogenic effect against pre-formed amyloid fibrils.[11] Cardoso, et al, tested various tetracyclines and showed doxycycline to be the most effective of the family in disrupting transthyretrin amyloid fibrils after incubation.[12] This group also showed that the anti-amyloid effect is independent of the amyloid precursor protein.[13] Doxycycline also disrupted amyloid in animal models, including a familial amyloid polyneuropathy transgenic mouse model.[14] Further studies showed that tetracyclines also produced de-structuration of β2-microglobulin in dialysis related amyloidosis[15] In AL amyloidosis, Ward, et al, showed that doxycycline can inhibit amyloid fibril aggregation and can destroy preformed amyloid in vitro and in a transgenic murine AL model.[16]

1.4 Clinical experience with Doxycycline:

Doxycycline is well-tolerated and safe, and is widely used in clinical practice for antibacterial prophylaxis, community acquired pneumonia and chronic obstructive pulmonary disease. It is also efficacious in unusual infections such as Lyme disease, cholera, syphilis, plague and malaria.[10] The side-effect profile of doxycycline is well-studied. Prolonged doxycycline administration at doses of 100 -200 mg daily for 6-24 months is well tolerated.[17-19] Commonly described adverse events with doxycycline use are cutaneous photosensitivity and self-limiting non-specific gastrointestinal symptoms.[18,19] Within our oncology practice, doxycycline is commonly used in patients with solid tumors and hematologic malignancies for anti-microbial prophylaxis in the setting of chemotherapy-induced neutropenia, particularly in patients with penicillin allergies with good tolerance.[17,20]

1.5 Clinical data of Doxycycline in Amyloidosis:

There are limited but positive data suggesting an anti-amyloid efficacy of doxycycline in humans. Montagna, et al, treated 3 patients with severe painful arthropathy related to β2-microglobulin dialysis related amyloidosis with resolution of arthropathy in all three patients.[21] Kumar, et al, reviewed a large cohort of AL patients who underwent stem cell transplantation for therapy, and received a year of doxycycline treatment for anti-bacterial prophylaxis. They found that among patients who had a hematologic response to stem cell transplantation, those patients on doxycycline prophylaxis had a higher survival rate than those on penicillin G prophylaxis.[20] This study also suggested the tolerability and safety of doxycycline administration in AL amyloidosis patients even in the critical post-transplant setting.[20] Doxycycline is currently being studied in patients with familial amyloid polyneuropathy.

1.6 Limitations of current AL amyloidosis therapy:

The current paradigm of AL amyloid treatment is focused upon eradicating the underlying malignant plasma cell clone with cytotoxic agents. This produces a hematologic response by clearing circulating immunoglobulin light chains which would have eventually been deposited into amyloid. Treatments range from high dose therapy with autologous stem cell transplantation in eligible patients, or anti-myeloma chemotherapy regimens such as melphalan/dexamethasone.[22] Newer anti-myeloma agents such as thalidomide,[23,24] lenalidomide,[25-28] pomalidomide,[29] and bortezomib[30,31] have excellent anti-plasma cell efficacy in amyloidosis but are also associated with organ and tissue toxicity making their use in clinical practice challenging, particularly in advanced AL disease.[32] Treatment of AL amyloidosis thus remains a frustrating journey for patients and their treating physicians, where clinically tenuous and frail patients with compromised vital organ function are treated with cytotoxic chemotherapy that often rapidly clears light chains from serum (i.e. hematologic response) but does not rapidly improve organ function. Many of these patients succumb to organ failure despite hematologic response. Indeed, in many patients with advanced stage cardiac amyloidosis, use of newer anti-myeloma drugs such as lenalidomide and bortezomib causes an initial worsening in cardiac biomarkers and even death.[25,30,32] Patients with advanced stage amyloidosis have a 40% risk of mortality in the first year following diagnosis, a number that has not changed since the 1970s (Figure 1) [2] despite the

availability of more effective anti-myeloma chemotherapy that have clearly improved myeloma outcomes during the same time frame.[33]

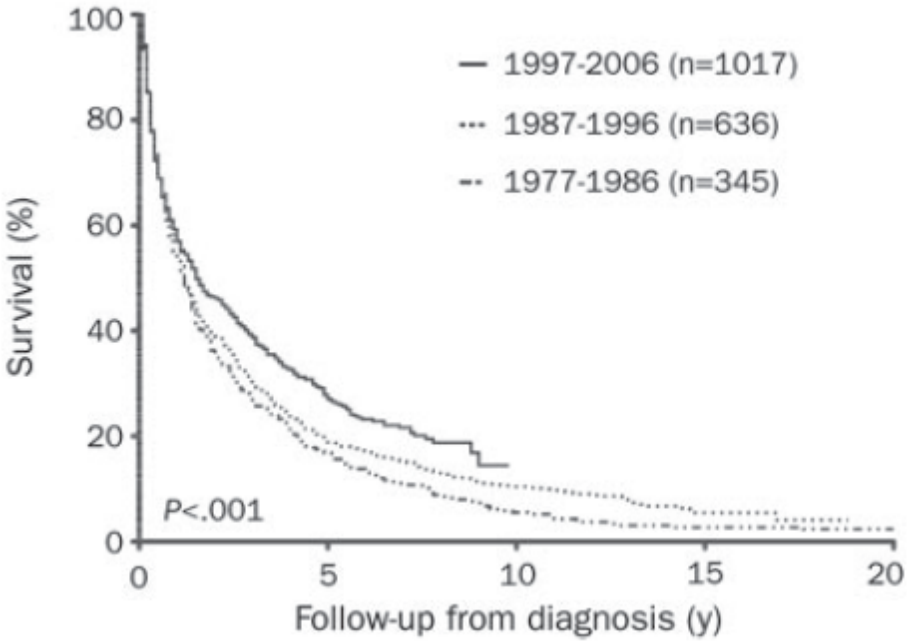


Figure 1. Overall survival of AL amyloidosis divided over three time periods. This figure shows that in the first year following diagnosis, 40% of patients die with no change in the three time periods.[2]

As outlined above, there is a clear need to identify treatments that do more than eradicate the malignant clone (the primary objective of anti-myeloma therapies) to improve the high early mortality that occurs even while on "effective" chemotherapy. Thus, consideration of therapies that complement current cytotoxic anti-amyloid treatment by hastening organ response is required. The compelling preclinical data of doxycycline's potential to benefit amyloidosis support prospective investigation of testing the use of doxycycline for AL amyloidosis treatment. The current study aims to generate key safety and efficacy data of doxycycline administration to AL amyloidosis patients. Our patients will potentially benefit from a novel mechanism of anti-amyloid effect in conjunction with anti-plasma cell chemotherapy with minimal additional risks and side-effects and, perhaps, even an added benefit of simultaneous anti-microbial activity.

1.7 Study Rationale:

Organ response to anti-plasma cell therapy in AL amyloidosis lags behind hematologic response as chemotherapy has no effect on pre-formed amyloid.[32] Doxycycline has pleiotropic, de-fibrillogenic and inhibitory effects on amyloid fibrils shown to be beneficial in in vitro, in murine models and other preclinical studies. We will prospectively evaluate the safety and efficacy of doxycycline in AL amyloidosis patients.

2. OBJECTIVES

2.1 Primary Objectives:

1. Determine the efficacy of adjunctive doxycycline in addition to specific anti plasma cell therapy in patients with AL amyloidosis in improving amyloid organ response at 1 year.
2. Assess the safety of doxycycline + anti-plasma cell chemotherapy regimen in AL amyloidosis.

2.2 Secondary Objectives:

1. To assess rates of hematologic response.
2. To assess rates of amyloid organ response at months 6 and 12.
3. To assess rates of mortality at 1 month, 6 months and 1 year.
4. To measure patient reported outcomes at baseline, 3, 6, 9 and 12 months.
5. To evaluate biomarkers associated with anti-amyloid response.

3. PATIENT ELIGIBILITY:

3.1 Inclusion Criteria:

1. Patients with biopsy proven AL amyloidosis.
2. Patients ≥18 years of age are eligible.
3. Patient must provide informed consent.
4. All patients must have measurable amyloid organ involvement of a vital organ (heart, liver, kidneys). Localized amyloidosis will be eligible as long as the amyloid involvement is radiologically measurable.
5. A negative pregnancy test will be required for all women of child bearing potential. Breast feeding is not permitted.
6. Patients who have previously been taking doxycyline will be eligible as long as there is no contraindication to stay on doxycycline 100 mg BID for 1 year in the opinion of the treating physician.
7. Creatinine clearance of >25 ml/min.

3.2 Exclusion Criteria:

1. Patients with severe malabsorption syndrome precluding absorption of oral agents will be excluded.
2. Known intolerance or allergic reactions with doxycycline.
3. Previous chemotherapy for AL amyloidosis.

4. REGISTRATION PROCEDURE:

4.1 Registration

- All source documents that support eligibility including a signed informed consent/HIPAA and signed eligibility checklist, should be available review and eligibility verification.
- At the point of registration, the research nurse or data manager will register the patient in the electronic database, including demographic, consent and on-study information. The patient will be assigned a unique sequence number for the study.

5. TREATMENT PLAN:

This is a phase II study of doxycycline as an adjunct to anti-amyloid treatment in patients with AL amyloidosis.

5.1 Administration schedule to Patients

1. Doxycycline will be administered at dose of 100mg orally twice daily. Baseline blood and urine laboratory testing must be completed within 28 days prior to starting doxycycline.
2. Patients who have previously been taking doxycycline will be eligible as long as there is no contraindication to take doxycycline (200mg/day) and continue for 1 year in the opinion of the treating physician.
3. Patients undergoing chemotherapy for AL amyloidosis can receive one of following regimens:
    a. Melphalan/Dexamethasone (Palladini regimen)[22]
    Oral Melphalan 0.22 mg/kg + Dexamethasone 40 mg , Days 1-4, every 28 days
    b. Cyclophosphamide/Bortezomib/Dexamethasone.[34]

Cyclophosphamide 300 mg/m^2 + Bortezomib 1.3 mg/m^2 + Dexamethasone 40 mg, days 1, 8, 15 every 28 days. Any combinations of these medications will be allowed based on treating clinician's judgement.

c. High dose Melphalan/Stem Cell rescue.[35]
   IV Melphalan 140-200 mg/m^2 followed by autologous stem cell infusion
d. Other chemotherapy regimens will be permitted after consultation with study PI. Dose modifications in treatments will be allowed per opinion of the treating physicians.

4. Antibiotic prophylaxis in patients undergoing high dose melphalan will be given according to institutional guidelines. Routine antibacterial prophylaxis will be substituted with doxycycline. If clinically indicated, additional antibacterial coverage will be provided depending on physician judgment.
5. Temporary interruption (≤10-14days) of doxycycline is permitted in patients unable to tolerate oral intake secondary to severe nausea, vomiting, mucositis etc. In case doxycycline therapy is interrupted, the time interval should be recorded.
6. Patients with severe macroglossia from amyloid involvement will have the option of using doxycycline oral suspension.
7. Toxicity will be evaluated according to CTCAE v4.0.
8. Hematologic response following treatment will be recorded at monthly intervals until chemotherapy is stopped or no longer indicated.
9. Organ response will be recorded at 6 and 12 months using RECIST criteria.[36]
10. Overall survival will be measured at 1 month, 6 months, 12 months, and yearly until five years.
11. If chemotherapy is switched due to inadequate hematologic response, continued doxycycline use will be permitted.
12. Patients on warfarin will be monitored as doxycycline may enhance anticoagulant effect of warfarin.
13. Patients on antacids containing aluminum, calcium or magnesium and iron-containing preparations can impair absorption of doxycycline. Doxycycline administration will be 2 hours before or after any of these medications. Since dairy products also contain

calcium, patients will be counselled to avoid dairy products 2 hours before and after doxycycline ingestion.

5.2 Dose modification

1. Patients experiencing a grade 3-4 hematological or non-hematological toxicity (as specified in CTCAE v4.0) thought to be related to doxycycline will be removed from the study permanently. The following dose modifications for doxycycline will be permitted : Dose lowering down to 50 mg BID may be permitted if patient cannot tolerate 100 mg BID due to side effects.

5.3 Duration of therapy

Doxycycline will be continued until one of the following criteria is met:

-Patient has completed 1 year of doxycycline therapy

-Patient develops any grade 3-4 toxicity related to doxycycline use.

Patients experiencing an amyloid hematologic relapse can continue doxycycline at the discretion of treating physician as long as there is no organ worsening.

Patients who discontinue prior to 1 year of doxycycline therapy should have an End of Treatment visit.

5.4 Data Safety Monitoring Plan

This study will be reviewed by the Medical College of Wisconsin Cancer Center Data Safety Monitoring Committee (MCW CC DSMC). A summary of the MCW CC DSMC activities are as follows:

- Review the clinical trial for data integrity and safety
- Review all unexpected grade 3, and all grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. Review all DSM reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

A copy of the MCW CC Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee

will review reports from the study PI twice annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary.

Any available DSMC letters will be submitted to the IRB of record as required.

6. MEASUREMENT OF EFFECT:

6.1 Visit Schedule:

The schedule for the study is shown in the table below.

Study Visit	Target Day
Baseline	Day -1 to -42
Month 1	Day 0
Month 2	Day 30 ± 5
Month 3	Day 60 ± 7
Month 4	Day 90 ± 10
Month 5	Day 120 ± 10
Month 6	Day 150 ± 10
Month 7	Day 180 ± 15
Month 8	Day 210 ± 15
Month 9	Day 240 ± 15
Month 10	Day 270 ± 21
Month 11	Day 300 ± 21
Month 12	Day 330 ± 28
End of study treatment	Day 360 ± 30

*After 12months, patients will be followed at least yearly for survival, amyloid relapse and cause of death for 5 years post treatment.

6.2 Amyloid Response Assessment:

Amyloid hematologic response assessment will be undertaken at monthly intervals using serologic studies with a serum protein electrophoresis, immunofixation electrophoresis and free light chain analysis. Monthly troponin-T, NT-pro BNP, alkaline phosphatase, creatinine will also be obtained per clinical physician's judgment.

Formal organ assessments will be undertaken at months 6 and 12. For patients with cardiac involvement, an echocardiogram will be repeated in addition to troponin-T and NT-pro BNP. For patients with liver involvement, an abdominal ultrasound and alkaline phosphatase will be done. For patients with renal involvement, a 24 hour urinary protein with urine protein electrophoresis and immunofixation electrophoresis will be checked.

For patients with localized amyloidosis, radiologic testing with a CT scan will be conducted.

Hematologic Response Grading[37](See Appendix A)

Complete response (CR) – Negative serum/urine immunofixation with normal FLC ratio

Very Good Partial Response (VGPR)- Difference between involved and uninvolved FLCs (dFLC) < 40 mg/L

Partial Response (PR)- dFLC decrease > 50%

No Response (NR)- less then PR.

Organ Response Grading[32](See Appendix A)

Heart

NT-proBNP response >30% and > 300 ng/L decrease in patients with baseline NT-proBNP ≥650 ng/L (Patients with progressively worsening renal function cannot be scored for NT-proBNP progression).

Improvement by 2 NYHA classes without an increase in diuretic use or in echocardiographic wall thickness.

≥ 2 mm reduction in the interventricular septal thickness by echocardiogram or
Improvement of ejection fraction by ≥20%.

Liver

≥50% decrease in an initially elevated alkaline phosphatase level, or
Decrease in liver size by at least 2 cm by ultrasound.

Kidney

50% reduction in 24-hour urine protein excretion (at least 0.5 g/day) without worsening of creatinine or creatinine clearance by 25% over baseline.

7. STUDY PARAMETERS (Study Calendar):

7.1 Patient Study Calendar:

The table below summarizes the patient clinical assessments over the course of the study.*

Systemic Amyloidosis		**Months**												
	Baseline^	**1^**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**End of Treatment (within 30 days)**
Inclusion & Exclusion criteria	X													
Informed consent	X													
H & PE[1]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECOG performance status	X			X			X			X			X	X
NYHA class	X						X[8]						X[8]	X[8]
Complete Blood Count with differential	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serum chemistries panel[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Amyloid subtype#	X													
$Beta_2$ microglobulin	X													
Myeloma screening panel[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Bone marrow aspirate/biopsy	X[7]													
B-HCG serum pregnancy test [5]	X													
Bone survey[11]	X[7]													
2D echocardiogram (IVS + LVEF)	X						X[8]							X[8†]
Troponin-T, NT-proBNP	X	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8]	X[8†]
Abdominal Imaging	X[7]						X[9]							X[9†]
24 hour urinary protein with UPEP/immunofixation	X[10]			X[10]			X[10]			X[10]				X[10 †]
Toxicity assessment		X	X	X	X	X	X	X	X	X	X	X	X	
Research specimens [6]	X							X						X[†]
Patient questionnaire (Appendix B)	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Notes

H & PE= history and physical examination

ECOG- Eastern Co-operative Oncology Group performance score

NYHA- New York Heart Association Class

2D echocardiogram- Two-dimensional transthoracic echocardiogram

IVS- interventricular septal thickness

LVEF- left ventricular ejection fraction

^ Baseline and Month 1 can occur within 7 days without the need to repeat the Month 1 testing.

If a patient has had amyloid subtyping performed in the past, this need not be repeated again.

† May be performed anytime during cycle 12 or within 30 days of the last dose of doxycycline

[1]History is only required at baseline.

[2]Vital signs: blood pressure, pulse rate, respiratory rate and temperature.

[3]Serum chemistries panel: electrolytes, BUN, ALT, AST, creatinine, bilirubin, alkaline phosphatase, LDH, albumin, uric acid. Electrolytes to include sodium, potassium, chloride, carbon dioxide, and calcium.

[4]Myeloma screening panel includes serum protein electrophoresis, immunofixation electrophoresis, free light chain assay

[5]Females of reproductive potential only.

[6]Peripheral blood specimens: draw 6 mL in green top (heparin containing) tube at indicated time points and send to the Tissue Bank at MCW after processing. Samples should be labeled with patient study number, date of collection, time point (baseline, 6 months or End of Treatment and MCW/FH IRB PRO number.

[7] May be performed within 3 months of baseline

[8]Cardiac assessment will be followed only if baseline cardiac involvement

[9]Abdominal imaging will be performed only if there is a suspicion of hepatic or splenic involvement based on hepatomegaly or splenomegaly on physical examination or labs (increased alkaline phosphatase greater than or equal to twice the upper limit of normal) and repeated only if there is baseline organ involvement

[10]Urinary studies: A urine protein/creatinine ratio which has ~100% concordance to 24 hour urinary protein will be allowed to test for baseline renal involvement. Urinary studies will be repeated only if baseline renal involvement. The urine test will remain consistent for a given patient, eg. if baseline urinary test was 24 hour urinary protein, the follow up tests for that patient will also be 24 hour urinary protein.

[11] Not required if ≤10% plasma cells in bone marrow

Classic localized amyloidosis						
	Baseline^	**1^**	**4**	**7**	**10**	**End of Treatment (within 30 days)**
Inclusion & Exclusion criteria	X					
Informed consent	X					
H & PE[1]	X	X	X	X	X	X
Vital signs[2]	X	X	X	X	X	X
ECOG performance status	X		X	X	X	X
Complete Blood Count with differential	X	X	X	X	X	X
Serum chemistries panel[3]	X	X	X	X	X	X
Amyloid subtype#	X					
$Beta_2$ microglobulin	X[8]					
Myeloma screening panel[4]	X	X[8]	X[8]	X[8]	X[8]	X[8]
B-HCG serum pregnancy test [5]	X					
Troponin-T, NT-proBNP	X[8]					
24 hour urinary protein with UPEP/immunofixation	X[8*]					
Bone marrow aspirate/biopsy	X*					
2D echocardiogram (IVS + LVEF)	X*					
Abdominal Imaging	X*					
Radiologic imaging[7]	X			X		X†
Toxicity assessment		X	X	X	X	
Research specimens [6]	X			X		X†
Patient questionnaire (Appendix B)	X	X	X	X	X	X

Notes

H & PE= history and physical examination

ECOG- Eastern Co-operative Oncology Group performance score

^ Baseline and Month 1 can occur within 7 days without the need to repeat the Month 1 testing.

† May be performed anytime during cycle 12 or within 30 days of the last dose of doxycycline

*Bone marrow evaluation, 2D echocardiogram, Abdominal Ultrasound and 24 hour urine protein test may be held based on the treating physician's judgment.

If a patient has had amyloid subtyping performed in the past, this need not be repeated again.

[1]History is only required at baseline.

[2]Vital signs: blood pressure, pulse rate, respiratory rate and temperature.

[3]Serum chemistries panel: electrolytes, BUN, ALT, AST, creatinine, bilirubin, alkaline phosphatase, LDH, albumin, uric acid. Electrolytes to include sodium, potassium, chloride, carbon dioxide, and calcium.

[4]Myeloma screening panel includes serum protein electrophoresis, immunofixation electrophoresis, free light chain assay.

[5]Females of reproductive potential only.

[6]Peripheral blood specimens: draw 6 mL in green top (heparin containing) tube at indicated time points and send to the Tissue Bank at MCW after processing. Samples should be labeled with patient study number, date of collection, time point (baseline, 7 months or End of Treatment) and MCW/FH IRB PRO number.

[7]Radiologic imaging: as clinically indicated

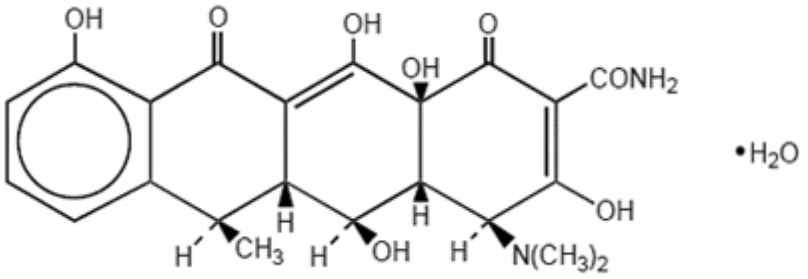
[8] Per MD discretion

8. Drug Formulation and Procurement

8.1 Doxycycline monohydrate

8.1.1 Drug description

Doxycycline is a synthetic antibacterial drug derived from oxytetracycline and available for oral administration. Doxycycline monohydrate has the empirical formula of C22H24N2O8.H2O and a molecular weight of 462.46. The chemical designation for doxycycline is 4-(Dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro-3,5,10,12,12a pentahydroxy-6-methyl-1,11-dioxo-2-naphthacenecarboxamide monohydrate. It is a light yellow crystalline powder and very slightly soluble in water. It has a high degree of lipod solubility and a low affinity for calcium binding. It is highly stable in normal human serum. It is available in syrup, capsule, oral suspension and tablet formulations. Inert ingredients in the tablet formulation are ethylcellulose; hypromellose; magnesium stearate; microcrystalline cellulose; propylene glycol; sodium lauryl sulfate; talc; titanium dioxide; Yellow 6 Lake. Inert ingredients for the oral suspension formulation are: carboxymethylcelluose sodium; Blue 1; methylparaben; microcrystalline cellulose; propylparaben; raspberry flavor; Red 28; simethicone emulsion; sucrose.



8.1.2 Pharmacodynamics

Tetracyclines are readily absorbed and bound to plasma proteins in varying degree. They are concentrated by the liver in the bile, and excreted in the urine and feces at high concentrations and in a biologically active form. Doxycycline is virtually completely absorbed after oral administration.

8.1.3 Pharmacokinetics

Doxycycline is virtually completely absorbed after oral administration. Following a 200 mg dose, normal adult volunteers averaged peak serum levels of 2.6 mcg/ml of doxycycline at 2 hours, decreasing to 1.45 mcg/ml at 24 hours. Excretion of doxycycline by the kidney is about 40%/72 hours in individuals with normal renal function. This percentage excretion may fall as low as 1-5%/72 hours in individuals with severe renal insufficiency (creatinine clearance below 10 ml/min). Studies have shown no significant difference in serum half-life of doxycycline (range 18-22 hours) in individuals with normal and severely impaired renal function.

8.1.4 Contraindications

Hypersensitivity to any Component of this Medication

Pregnancy

Nursing Mothers

Children under 8 years

Active Clostridium difficile associated disease

8.1.5 Drug Interactions

Interacting Agents	Prescribing Recommendations
Antacids containing aluminum, calcium, or magnesium and iron-containing preparations can impair absorption	Avoid doxycycline administration for 2 hours before and after any of these medications.
Barbiturates, carbamazepine, and phenytoin increase the half-life of doxycycline	No dose modifications recommended.

8.1.6 Adverse Events:

Gastrointestinal side-effects

Anorexia, nausea, vomiting, diarrhea, glossitis can occur. Rare instances of esophagitis and esophageal ulcerations have been reported in patients receiving capsule and table forms of the drug. Most of these patients took the medications immediately before going to bed.

Cutaneous reactions

Photosensitivity manifested by an exaggerated sunburn reaction has been observed. Patients should be advised against direct sunlight exposure and to use adequate sun protection with sunscreen. Uncommon skin reactions include toxic epidermal necrolysis, Stevens-Johnson syndrome, erythema multiforme, maculopapular and erythematous rashes and exfoliative dermatitis.

Renal toxicity

An increase in blood urea nitrogen from an anti-anabolic action has been reported from doxycycline.

Immune

Hypersensitivity reactions including urticarial, angioneurotic edema, anaphylaxis, anaphylactoid purpura, serum sickness, pericarditis, exacerbation of systemic lupus erythematosus and drug rash with eosinophilia and systemic symptoms (DRESS) syndrome have been rarely reported.

Hematologic

Hemolytic anemia, thrombocytopenia, neutropenia and eosinophilia have been reported.

Dental

The use of tetracyclines during tooth development (last half of pregnancy, infancy and childhood to the age of 8 years) may cause permanent discoloration of teeth (yellow-gray-brown). Enamel hypoplasia has also been reported.

Clostridium difficile associated diarrhea (CDAD)

CDAD has been reported with the use of nearly all antibacterial agents, including doxycycline, and may range in severity from mild diarrhea to fatal colitis.

8.2 How supplied and Storage/Handling

Doxycycline monohydrate is available in salmon colored film-coated tablets containing doxycycline monohydrate equivalent to 100 mg of doxycycline, bottles of 50 (NDC 0069-0990-50)

Doxycycline monohydrate for oral suspension is available as a raspberry-flavored, dry powder for oral suspension. When reconstituted, each 5 ml contains doxycycline monohydrate equivalent to 25 mg of doxycycline: 2 oz (60 ml) bottles (NDC 0069-0970-65)

All products are to be stored below 86ºF (30ºC) and dispensed in tight, light-resistant containers (USP).

8.3 Methods of procurement

The Froedtert Hospital Departments of Pharmacy will order Doxycycline 100 mg and receive drug specifically for this study. The CTO (at MCW) will be billed for the cost of the drug which will be paid for by study funds. The drug will be dispensed per patient (1 month supply at a time for systemic amyloidosis; 3 month supply at a time for local amyloidosis) and labeled according to more stringent state or federal law. Drug accountability and inventory records will be created and maintained within the Investigational Drug Services.

9. STATISTICAL CONSIDERATIONS

The study is a single-arm phase II trial evaluating the safety and efficacy of doxycycline used in adjunct to anti-amyloid treatment to improve organ response in patients with AL amyloidosis. The primary objective of this study is to evaluate the cumulative organ response at 1 year. The incidence of organ response to conventional chemotherapy at 1 year is approximately 20-25%. At the time of publication the results of the study will be retrospectively compared against AL amyloidosis patients undergoing chemotherapy alone at our institution in the last 5 years.

For this phase II study, we will use an exact single-stage phase II design. We will consider the "experimental" regimen of doxycycline with conventional anti-amyloid treatment to be no more effective than conventional anti-amyloid treatment alone, the true probability of organ response at 1 year is no less than 25% (p0). We will assume that the new experimental regimen is worthy of further study of the true probability of organ response greater than 50% (p1). In statistical terms, we are testing the null hypothesis H_0: $p \leq 0.25$, versus the alternate H_1: $p \geq 0.5$, where p is probability of organ response. Our power analysis indicated that we will need 26 patients to have 80% power to detect the designed difference at 5% significance level. We will increase the sample size by 15% to 30 in order to account for patients with advanced disease who may die prior to response. Patients dying prior to response are a competing risk event. The probability of response will be calculated using the cumulative incidence curve to accommodate for competing risks. Six month and twelve month cumulative incidence rates of response with 95% confidence intervals will be reported. Point-wise test will be considered to compare response rates of study patients to response rates under standard treatment using historical controls from published literature.

In the final analysis, if 11 or more of the patients develop organ response we will reject the null hypothesis.

We anticipate that a maximum of 30 patients will be accrued to the study. Descriptive statistics (i.e. means, standard deviations, 95% confidence intervals for continuous variables, and frequencies for discrete data) will be computed for all correlative laboratory parameters. Patients who receive <1 month of doxycycline therapy and withdraw from the study will be replaced.

Safety/Stopping Rules:

1. Development of grade 3-4 adverse events deemed to be related to doxycycline in 6 or more patients, will mandate halting further patient accrual until review by DSMB.

Accrual Estimate: 26-30 patients. Patients experiencing mortality within 30 days of starting treatment due to disease progression, will be replaced to fully assess 1 year amyloid organ response. However, all patients will be included in toxicity and survival analysis.

Accrual Period : Approximately 12-24 months

Follow-Up Period : One year to evaluate incidence of amyloid (hematologic and organ) response and relapse pattern. Five years for survival outcomes, relapse, and mortality.

10. ADVERSE EVENT REPORTING REQUIREMENTS

10.1 Definitions

The following are definitions of adverse events as defined by 21CFR312.32.

Types of Adverse Events

Adverse Event means any untoward medical occurrence associated with the use of a drug in humans, whether or not consider drug related.

Life-threatening adverse event or life-threatening suspected adverse reaction: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

Serious adverse event or serious suspected adverse reaction: An adverse event or suspected adverse reaction is considered "serious" if, in the view of the investigator or sponsor, it results in any of the following outcomes:

- Death,
- A life-threatening prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- A congenital anomaly/birth defect.

- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent of the outcomes listed above.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. "Reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, it is not consistent with the risk information currently described.
Unexpected also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Adverse Event Grading

Grade	Description
0	**No AE** (or within normal limits).
1	**Mild**; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	**Moderate**; minimal, local or noninvasive intervention (e.g., packing cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).

3 **Severe** or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.

4 **Life-threatening** consequences; urgent intervention indicated.

5 **Death** related to AE

Adverse Event Attribution

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE *is clearly **NOT** related* to the intervention
	Unlikely	The AE *is **doubtfully related*** to the intervention
Related to investigational agent/intervention	Possible	The AE ***may be related*** to the intervention
	Probable	The AE ***is likely related*** to the intervention
	Definite	The AE ***is clearly related*** to the intervention

Reporting of Adverse Events

Because all of the study transplant recipients will be receiving potentially toxic preparative therapy, significant regimen related toxicity is expected. A study specific toxicity CRF will be designed to capture information regarding these expected events. Unexpected adverse events will be reported throughout the study.

Unexpected Adverse Events

Unexpected Grades 3-5 (severe, life threatening, disabling, or fatal) require expedited reporting and will be submitted to the DSMC within 5 calendar days of discovery for review. If the Grade 3-5 event is determined to be an unanticipated problem, the event will be forwarded to the MCW/FH IRB for review as required by their policy. Unexpected adverse events, regardless of severity, will be reported to the DSMC and reviewed on a quarterly basis.

Expected Adverse Events

Grade 4 (non hematologic) and Grade 5 (fatal) expected adverse events require expedited reporting and will be reported to the DSMC within 5 calendar days for review. Expected Grade 4 hematologic and GI toxicity adverse events do not require expedited reporting. Expected adverse events that are being captured on the study toxicity form will be reported at the time of the form's scheduled due date.

All DSMC reports and recommendations will be submitted to the IRB for their review.

Adverse Events Occurring after the End of the Study

Follow-up of AEs

Any unexpected AEs ongoing at the time of study discontinuation will be followed until resolution or stable for at least 2 months.

FDA Reporting Procedures

Commercial Agents: Commercial agents are those agents not provided under an IND but obtained instead from a commercial source. In some cases an agent obtained commercially may be used for indications not included in the package label. The following procedures should be followed to determine if an adverse is reportable to the FDA:

Refer to the pharmaceutical section of the protocol to determine if an agent is investigational or commercial.

- **WHAT TO REPORT:** An unexpected, life-threatening (Grade 4) or unexpected, fatal (Grade 5) adverse event with an attribution of possible, probable or definite.

- **WHEN TO REPORT:** These events should be reported within (7) working days.

- **WHERE TO REPORT:** These adverse events with commercial agents must be reported to the FDA using the MedWatch form. A copy of the MedWatch form can be

obtained from theFDA's MedWatch web site at www.fda.gov/medwatch. You can mail the reports to the address below or fax it 1-800-332-0178.

MedWatch
5600 Fishers Lane
Rockville, MD 20852-9787

11. PATIENT CONSENT AND PEER REVIEW STATEMENT

11.1 Subject Information and Informed Consent

Written informed consent must be obtained from the subject prior to study participation.

The informed consent document must be signed and dated by the subject and properly witnessed (if applicable) before initiation of any study procedures including any change in medication or initiation of study drug dosing.

Subjects must be consented in accordance with all local regulatory and legal requirements. This process must include a verbal explanation of the nature, scope, and possible consequences of the study provided in plain language. The information should be presented by the investigator unless a designee is permitted by local regulations. The potential study subject should be encouraged to ask questions about the study.

The informed consent document must be prepared in accordance with GCP guidelines and with local regulatory and legal requirements. A copy of the signed consent form will be given to the subject and the original document must be safely archived by the investigator so that the forms can be retrieved at any time for monitoring, auditing, and inspection purposes.

The informed consent will be updated as appropriate (e.g., due to protocol amendment or if significant new safety information that may be relevant to consent of the subjects becomes available). If the informed consent is revised, it is the investigator's responsibility

to ensure that an amended consent form is reviewed and signed by all subjects subsequently entered into the study and those currently in the study.

12. PATIENT REPORTED OUTCOMES ASSESSMENT

We will collect patient-reported health quality of life in this study.

At baseline, at each subsequent study visit and End of Treatment visit, the study coordinator will ask patients to complete a questionnaire to report on common domains of health-related quality of life using the Patient Reported Outcomes Measurement Informations System (PROMIS) Global Health instrument (**See Appendix B**), a 10-item standardized patient-reported outcome measure that provides global ratings of physical function, fatigue, pain, emotional distress, social health, as well as perceptions of general health that cut across these key domains.[38]
We will describe the patient experience over the course of the year.

13. CORRELATIVE SPECIMENS

We will collect serum from patients at baseline, months 6 and 12. These specimens will be stored in the Tissue Bank at MCW. We will perform serum inflammatory markers known to be associated with doxycycline use[8] to identify possible biomarkers of amyloid response to doxycycline. We will obtain MMP-2, MMP-7, MMP-8, MMP-9 and TIMP-1 (tissue inhibitor of metalloproteinase-1) levels in all patients at baseline, months 6 and 12 of doxycycline use. To measure MMP activity, zymograms will be performed on sera from all the patients. Briefly, zymography is a simple sensitive and functional assay to analyze MMP activity. Proteins are separated by electrophoresis utilizing SDS-PAGE gels containing the according MMP substrate, MMP2 and 9 are gelatinases therefore the gel will contain gelatin. The gel for MMP7 will contain casein and for MMP8 collagen-I. The MMP activity will be measured by the degradation of the substrate and therefore the inability of Coomassie Blue to stain the gel at the molecular weight appropriate spot. The TIMP-1 expression will be measured by ELISA utilizing TIMP-1 ELISA kit (abcam #ab100651).[39,40] Briefly, ELISA is a test that uses antibodies and color change to identify an antigen in a liquid substrate measured with a microplate reader. These tests will be performed in the Weihrauch Laboratory at MCW using techniques that have already been optimized and validated in human sera and myocardial interstitial fluid.[41-45] Descriptive statistics will be computed for all the correlative laboratory parameters. If a significant difference

is seen in these biomarkers at 6 and 12 months, and organ response is seen (i.e. positive study), these results will be used to for further hypothesis generating questions, and phase III design.

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15. APPENDIX A

Hematologic response definition

Response type	Abbreviation	Criteria
Complete response	CR	Negative serum and urine IFE and Normal serum immunoglobulin κ/λ FLC ratio
Very good partial response	VGPR	dFLC<40 mg/L[b]
Partial response	PR	dFLC decrease of greater than equal to 50%[b]
No response	NR	Less than a partial response
Progression	Prog	From CR, any detectable monoclonal protein or abnormal free light chain ratio (light chain must double) From PR, serum immunoglobulin free light chain increase of 50% which also must be to a level of greater than 100 mg/L, Or a 50% increase in serum M protein which must also be to a level greater than 5 g/L, Or a 50% increase in urine M protein which also must be to a level greater than 200 mg/day (a visible peak must be present)

Organ response criteria

Organ system	Involvement[b]	Improvement
Kidney	24-hour urine protein > 0.5 g/day, predominantly albumin	50% reduction in 24-hour urine protein excretion (at least 0.5 g/day) without worsening of creatinine or creatinine clearance by 25% over baseline
Heart	Echo: mean wall thickness > 12 mm, no other cardiac cause	*New:* NT-proBNP response[c] (> 30% and > 300 ng/L decrease in patients with baseline NT-proBNP ≥ 650 ng/L) *Remaining* Improvement by 2 NYHA classes without an increase in diuretic use or in wall thickness *Removed 2011* ≥ 2 mm reduction in the interventricular septal (IVS) thickness by echocardiogram, or Improvement of ejection fraction by ≥ 20%
Liver	Total liver span > 15 cm in the absence of heart failure or alkaline phosphatase > 1.5 times institutional upper limit of normal	≥ 50% decrease in an initially elevated alkaline phosphatase level, or Decrease in liver size by at least 2 cm (radiographic determination).

16. APPENDIX B

PRO ASSESSMENT

Participant ID _____ **Date** _____

Please answer the questions as best you can, thinking about your daily life in the past 7 days.

If you have any comments about these questions, please provide them in the textbox provided at the end of this set of questions.

	Excellent	Very good	Good	Fair	Poor
In general, would you say your health is:	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
In general, would you say your quality of life is:	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1
In general, how would you rate your physical health?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

	Completely	Mostly	Moderately	A little	Not at all
To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

	Without any difficulty	With a little difficulty	With some difficulty	With much difficulty	Unable to do
Are you able to do chores such as vacuuming or yard work?	☐	☐	☐	☐	☐
Are you able to go up and down stairs at a normal pace?	☐	☐	☐	☐	☐
Are you able to go for a walk of at least 15 minutes?	☐	☐	☐	☐	☐
Are you able to run errands and shop?	☐	☐	☐	☐	☐

	Excellent	Very good	Good	Fair	Poor
In general, how would you rate your mental health, including your mood and your ability to think?	☐ 5	☐ 4	☐ 3	☐ 2	☐ 1

In the past 7 days…

	Never	Rarely	Sometimes	Often	Always
How often have you been bothered by emotional problems such as feeling anxious, depressed or irritable?	1	2	3	4	5

	Never	Rarely	Sometimes	Often	Always
I felt fearful					
I found it hard to focus on anything other than my anxiety					
My worries overwhelmed me					
I felt uneasy					

	Never	Rarely	Sometimes	Often	Always
I felt worthless					
I felt helpless					
I felt depressed					
I felt hopeless					

	Excellent	Very good	Good	Fair	Poor
In general, how would you rate your satisfaction with your social activities and relationships?	5	4	3	2	1
In general, please rate how well you carry out your usual social activities and roles. (This includes activities at home, at work and in your community, and responsibilities as a parent, child, spouse, employee, friend, etc.)	5	4	3	2	1

In the past 7 days…

	Never	Rarely	Sometimes	Often	Always
I have trouble doing all of my regular leisure activities with others	☐	☐	☐	☐	☐
I have trouble doing all of the family activities that I want to do	☐	☐	☐	☐	☐
I have trouble doing all of my usual work (include work at home)	☐	☐	☐	☐	☐
I have trouble doing all of the activities with friends that I want to do	☐	☐	☐	☐	☐

During the past 7 days…	Not at all	A little bit	Somewhat	Quite a bit	Very much
I feel fatigued	☐	☐	☐	☐	☐
I have trouble starting things because I am	☐	☐	☐	☐	☐
In the past 7 days…					
How run-down did you feel on	☐	☐	☐	☐	☐
How fatigued were you on average?	☐	☐	☐	☐	☐
How much were you bothered by your fatigue on	☐	☐	☐	☐	☐
To what degree did your fatigue interfere with your physical	☐	☐	☐	☐	☐

In the past 7 days…	Never	Rarely	Sometimes	Often	Always
How often did you have to push yourself to get things done because of your fatigue?	☐	☐	☐	☐	☐
How often did you have trouble finishing things because of your	☐	☐	☐	☐	☐

In the past 7 days…	Very poor	Poor	Fair	Good	Very good
My sleep quality was.....	☐	☐	☐	☐	☐

In the past 7 days…	Not at all	A little bit	Somewhat	Quite a bit	Very much
My sleep was refreshing.....	☐	☐	☐	☐	☐
I had a problem with my sleep	☐	☐	☐	☐	☐
I had difficulty falling asleep	☐	☐	☐	☐	☐

How would you rate your pain on average?.....	☐ 0 No pain	☐ 1	☐ 2	☐ 3	☐ 4	☐ 5	☐ 6	☐ 7	☐ 8	☐ 9	☐ 10 Worst imaginable pain

In the past 7 days…	Not at all	A little bit	Somewhat	Quite a bit	Very much
How much did pain interfere with your day to day activities?	☐	☐	☐	☐	☐
How much did pain interfere with work around the home?	☐	☐	☐	☐	☐
How much did pain interfere with your ability to participate in social activities? .	☐	☐	☐	☐	☐
How much did pain interfere with your household chores?	☐	☐	☐	☐	☐