Immunotherapy for the treatment of breast cancer related upper extremity lymphedema (BCRL) protocol # CQBX258X2203T

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

Principal Investigator/Department:	Babak Mehrara, MD	Surgery/Plastic & Reconstructive Service
Co-Principal Investigator(s)/Department:	Joseph Dayan, MD Kimberly Van Zee, MD Jacqueline Bromberg, MD, PhD	Surgery/Plastic & Reconstructive Service Surgery/Breast Service Medicine/Breast Medicine Service
Investigator(s)/Department:	Andrea Barrio, MD Raghu Parsad Kataru, PhD Shari Goldfarb, MD Maura Dickler, MD Shanu Modi, MD Elizabeth Comen, MD Neil Iyengar, MD Ayca Gucalp, MD Tiffany Traina, MD Sarat Chandarlapaty, MD Mario Leitao, MD Sharlynn Tuohy, PT, DPT, MBA Ting-Ting Kuo, PT, DPT, MBA, WCS, CLT Jaennette Zucker, PT, DPT Laryssa Buoneto, PT Andrea Pusic, MD Evan Matros, MD Peter Cordeiro, MD Joseph Disa, MD Colleen McCarthy, MD Monica Morrow, MD Tari King, MD Mary Gemignani, MD Virgilio Sacchini, MD Alexandra Heerdt, MD Lisa Sclafani, MD Hiram Cody, MD George Plitas, MD Debra Capko, MD Mahmoud El-Tamer, MD Melissa Pilewski, MD Elyn Riedel, MA Melissa Lee-Teh, RPh Svetlana Kleyman, MD Andrew Weinstein, MD Geoffrey Hespe Gabriela Garcia Nores, MD Jessie Yu, MD	Surgery/Breast Service Surgery/Plastic & Reconstructive Service Medicine/Breast Medicine Service Medicine/Breast Medicine Service Medicine/Breast Medicine Service Medicine/Breast Medicine Service Medicine/Breast Medicine Service Medicine/Breast Medicine Service Medicine/Breast Medicine Service Medicine/Breast Medicine Service Surgery/GYN Service Neurology Neurology Neurology Neurology Surgery/Plastic & Reconstructive Service Surgery/Plastic & Reconstructive Service Surgery/Plastic & Reconstructive Service Surgery/Plastic & Reconstructive Service Surgery/Plastic & Reconstructive Service Surgery/Breast Service Surgery/Breast Service Surgery/Breast Service Surgery/Breast Service Surgery/Breast Service Surgery/Breast Service Surgery/Breast Service Surgery/Breast Service Surgery/Breast Service Surgery/Breast Service Surgery/Breast Service Surgery/Breast Service Epidemiology and Biostatistics Pharmacy Surgery/Plastic & Reconstructive Service Surgery/Plastic & Reconstructive Service Sloan Kettering Institute Sloan Kettering Institute Sloan Kettering Institute

Consenting Professional(s)/Department:	Babak Mehrara, MD	Surgery/Plastic & Reconstructive Service
	Joseph Dayan, MD	Surgery/Plastic & Reconstructive Service
	Kimberly Van Zee, MD	Surgery/Breast Service
	Jacquiline Bromberg, MD	Medicine/Breast Medicine Service
	Andrea Barrio, MD	Surgery/Breast Service
	Shari Goldfarb, MD	Medicine/Breast Medicine Service
	Maura Dickler, MD	Medicine/Breast Medicine Service
	Shanu Modi, MD	Medicine/Breast Medicine Service
	Elizabeth Comen, MD	Medicine/Breast Medicine Service
	Neil Iyengar, MD	Medicine/Breast Medicine Service
	Ayca Gucalp, MD	Medicine/Breast Medicine Service
	Tiffany Traina, MD	Medicine/Breast Medicine Service
	Sarat Chandarlapaty, MD	Medicine/Breast Medicine Service

Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

Memorial Sloan-Kettering Cancer Center
1275 York Avenue
New York, New York 10065

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

Study Title: Immunotherapy for the treatment of breast cancer related upper extremity lymphedema (BCRL)

Duration: All study participants will be followed for 7 months

Background:

Over the past decade, improved understanding of the pathologic processes that regulate a variety of chronic disorders has made it possible to devise targeted treatment options that greatly reduce the morbidity and treatment costs of these diseases. The purpose of this proposal is to develop a similar strategy for patients who suffer from breast cancer related lymphedema (BCRL) using a monoclonal antibody targeting the pathological process of this disease. This proposal is a direct bench to bedside translation of NIH funded research completed in our laboratory elucidating the pathological mechanisms of lymphedema.[1-5]

Lymphedema is a common and morbid complication of breast cancer treatment developing in 1 in 3 women who undergo axillary lymph node dissection.[6,7] Lymphedema also occurs in approximately 4-9% of women who undergo sentinel lymph node biopsy[8,9] and conservative estimates suggest that 5-7 million Americans suffer from this disorder.[10] In these patients, damaged lymphatic circulation results in lifelong swelling and fibroadipose tissue deposition in the affected extremity. These pathological changes lead to impaired quality of life, functional deficits, and development of severe infections necessitating hospital admission for intravenous antibiotics.[11] However, despite the fact that lymphedema is common and morbid, treatments for this disorder are palliative and rely exclusively on compression to prevent swelling and progression of symptoms. Importantly, there have been few advancements in the treatment of this disorder, and to date, there are no proven targeted treatment options designed to address the pathologic process using a mechanistic approach. The proposed study is designed to address these shortfalls and aims to translate our preclinical findings in the laboratory elucidating the cellular and molecular mechanisms of lymphedema.

Using a variety of animal models, as well as tissue biopsy specimens obtained from patients with BCRL, we have previously shown that CD4+ cells play an important role in the pathology of this disease by regulating tissue fibrosis and lymphatic dysfunction.[1-5] We have shown that similar to other fibroproliferative disorders, differentiation of naive CD4+ cells to the T-Helper 2 (Th2) phenotype is necessary for this pathology. More importantly, we have shown that targeted blockade of Th2 differentiation using monoclonal antibodies (mAbs) directed against interleukin 4 (IL4) and interleukin 13 (IL13) markedly decreases the pathological changes of lymphedema in a mouse model resulting in significantly decreased fibrosis, decreased adipose deposition, and improved lymphatic function.[3] This is important because IL4/IL13 blockade using mABs is currently clinically used in phase I and phase II studies as an experimental treatment for a variety of fibroproliferative disorders including pulmonary fibrosis, keloids, liver fibrosis, kidney fibrosis, asthma, eosinophilic esophagitis, among others. These once a month treatments have been well tolerated in both animal and human studies with no major adverse events reported. Thus, the use mABs for the treatment of lymphedema would be similar to FDA approved treatment options for other chronic disorders such as plaque psoriasis and rheumatoid arthritis. These pharmaceutical treatments are a paradigm shift in the treatment of lymphedema providing a non-compressive and targeted therapeutic option for

patients who suffer from this disease. If the current trial is successful, our plan would be to proceed with multicenter trials for phase II and III clinical trials.

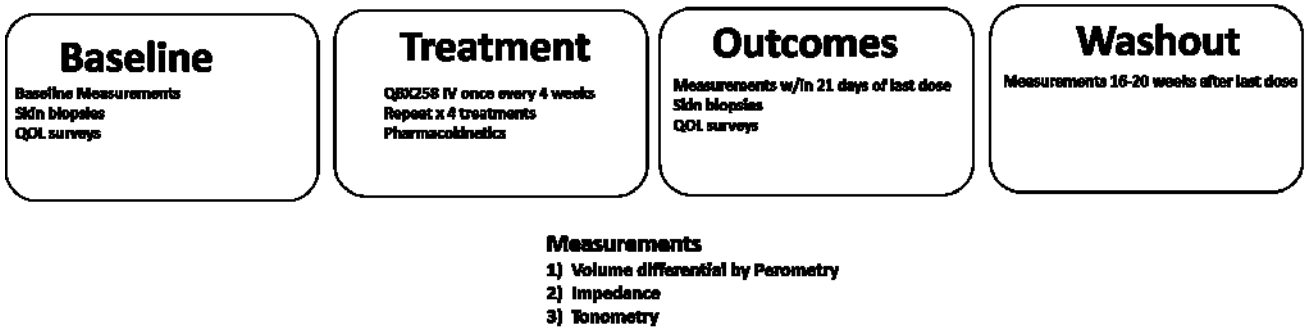
Study Objectives: Our **primary objective** is to evaluate the efficacy of QBX258 in reducing arm volume excess in women with stage I-II BCRL. QBX258 is a compound developed by Novartis Corporation and is a combination of 2 antibodies that inhibit the bioactivity of IL4 and IL13.

Our **secondary objectives** are to analyze tolerability and pharmacokinetics of QBX258, evaluate the efficacy of drug therapy in decreasing fluid content and fibrosis of the affected extremity, determine how this treatment changes quality of life measures using validated quality of life surveys, and to elucidate the histological changes in lymphedematous tissues by analyzing skin biopsies obtained before and after treatment.

Patient Population: Women ages 18 to 70 who suffer from unilateral stage I or II BCRL as defined by the America Society of Lymphology (spontaneously reversible or irreversible lymphedema in which the tissue has a spongy consistency with mild to moderate pitting and mild to moderate fibrosis of the skin) with a minimum volume excess of 300 mL as compared with the normal upper extremity.

Study Design: This will be a single arm, open label pilot study designed to test the efficacy of QBX258, a combination of 2 humanized monoclonal antibodies that inhibit the bioactivity of interleukin 4 and interleukin 13, for the treatment of stage I or II BCRL. Patients who meet the inclusion criteria will be recruited to the study and baseline lymphedema measurements will be performed by a licensed physical therapist. Patients will then be treated with QBX258 once every 4 weeks for 4 treatments followed by repeat measures performed within 21 days of the final treatment dose. Washout measurements, based on the known pharmacokinetics of monoclonal antibody treatment, will be performed 16-20 weeks after the final dose of QBX258 to analyze changes after withdrawal of treatment (treatment half life is 4 weeks, therefore 16-20 weeks after last treatment is sufficient for complete clearance). During the course of treatment and washout periods, patients will continue their usual routine for lymphedema care including the use of skin care products and compression garments according to their pre-treatment directions. However, to avoid potential confounding effects, patients will avoid the use of intermittent pneumatic compression devices, manual lymphatic massage by a physical therapist, laser treatment, or treatment with high compression short stretch bandages during the study period. It is important to note that although these modes of treatment are used commonly for lymphedema, the standard of care for the treatment of this disease is skin care and compression garments. In fact, a recent review of the literature has shown that these adjunctive treatments have no statistically significant benefit in the treatment of lymphedema in the vast majority of well controlled studies when compared to standard therapy.[12]

The plan and schema for the study are summarized below.



2.0 OBJECTIVES AND SCIENTIFIC AIMS

Primary objective:

- Evaluate the efficacy of QBX258 in reducing arm volume excess in women with unilateral stage I or stage II BCRL with a minimum volume difference of 300 mL between the normal and lymphedematous limb.

Secondary objectives:

- Evaluate the efficacy of QBX258 in decreasing fluid content of the affected extremity (bioimpedance measurements)
- Examine changes in skin elasticity/fibrosis in response to treatment (skin tonometry).
- Examine how this treatment changes quality of life measures using validated quality of life surveys (ULL-27)
- Examine the histological changes in lymphedematous tissues as compared with normal tissues by analyzing skin biopsies obtained before and after treatment.
- Analyze tolerability of QBX258 treatment
- Analyze pharmacokinetics of QBX258 treatment

3.0 BACKGROUND AND RATIONALE

Targeted monoclonal antibody treatments have revolutionized the treatment of a variety of chronic disorders. Over the past decade, improved understanding of the pathologic processes that regulate a variety of disabling chronic disorders has made it possible to devise targeted treatment options that greatly reduce the morbidity and treatment costs of these diseases. For example, patients with rheumatoid arthritis, chronic plaque psoriasis, ankylosing spondylitis, inflammatory bowel disease, and psoriatic arthritis have achieved significant treatment benefits with reduced pain, improvements in disease status, decreased hospitalization, and reduced need for chronic steroid use. The purpose of this proposal is to develop a similar strategy for patients who suffer from breast cancer related lymphedema (BCRL) using a monoclonal antibody that is targeted to the pathological process of this disease.

Lymphedema is a common and morbid complication of cancer treatment. Lymphedema is a dreaded complication of breast cancer treatment occurring in 33-50% of patients who undergo lymph node dissection.[7] Although the number of axillary lymph node dissections performed has decreased with introduction of sentinel biopsy procedures, even these relatively minor injuries to the lymphatic system cause lymphedema in 4-9% of patients.[8,9] As a result, it is estimated that 5-6 million Americans suffer from lymphedema and as many as 25-50,000 new cases are diagnosed annually.[10] The incidence of lymphedema is likely to increase due to increased rates of obesity, use of radiation therapy, and an aging population since these variables independently increase the risk of this complication.[13-15] In addition, improved cancer treatment will also increase the burden of lymphedema as the development of this complication and survival are directly correlated.

Patients with lymphedema complain of pain, heaviness, and decreased arm function. These changes signficantly decrease patient reported quality of life and are a significant source of anxiety and depression.[16,17] Some patients develop frequent and often severe infections of the affected extremity requiring hospitalization and antibiotic therapy.[11,18] In fact, many patients consider their lymphedema as worse than their cancer diagnosis because the latter was finite and resolved with treatment while lymphedema is chronic and progressive.

Treatment of lymphedema is palliative. Despite the fact that lymphedema is common and a major source of morbidity, there are no proven preventative or curative options. Surgical and medical treatments have been reported, however, the results of these studies have been variable and in most cases disappointing. As a result, treatment is palliative in nature with the hope of preventing disease progression rather than achieving a cure. Patients are required to constantly wear tight fitting, uncomfortable compression garments designed to prevent fluid accumulation. Physical therapy treatments are also performed in some cases and are time intensive (4-6 week treatment course for an hour a day and 24/7 wrapping with bandages) and expensive (more than $10,000/year by one conservative estimate).[17] These facts, together with denial of service by many insurance companies for garments or therapy, leads to significant rates of non-compliance and disease progression.

Etiology of lymphedema remains unknown. Development of effective treatments for lymphedema has been hampered by the fact that the etiology of this disorder remains unknown. It is unclear for instance, why an identical operation leads to lymphedema in some patients and not others. Similarly, it is unknown why even trivial lymphatic injury in the form of sentinel lymph node biopsy can result in significant lymphedema. Perhaps the most perplexing aspect of lymphedema is the fact that progressive arm swelling in most cases occurs in a delayed fashion, usually 1-5 years after surgery.[19] Thus, although lymphatic injury is clearly the initiating event, the pathologic events that follow and lead to the development of lymphedema in a subset of patients remain unknown. This gap in our knowledge serves as a significant barrier to the development of targeted, rational treatment methods for this disabling and common disorder.

Fibrosis is a key regulator of pathology in lymphedema. Recent studies in our lab have shown that fibrosis is a key pathologic process and may serve as a "second hit" following lymphatic injury ultimately leading to the development of lymphedema.[1,2,4,20] This hypothesis is supported by the fact that fibrosis is a hallmark of lymphedema clinically and progression of disease is characterized by deposition of fibroadipose tissues and replacement of collecting lymphatic vessels with proliferative smooth muscle cells with luminal obliteration.[21] In addition, it is well recognized that fibrosis is a common cause of end-organ failure in a number of other organ systems including liver, lung, skin, kidney, and heart suggesting that lymphedema may simply represent "end-organ failure" of the lymphatic system due to fibrosis.[22] Fibrosis also provides a rationale for the delayed development of lymphedema since the progressive abnormal collagen deposition necessary to cause organ dysfunction takes time to accumulate. This hypothesis is also supported by preclinical studies in our lab demonstrating that interventions designed to decrease tissue fibrosis in lymphedema potentially increase lymphatic function and decrease the pathological consequences of lymphatic injury in a variety of mouse models.[1-5,20,23]

Lymphedema results in chronic CD4+ cell inflammation. Based on our hypothesis that lymphedema is fibrosis related end-organ failure of the lymphatic system, our lab has spent the last 6 years studying the mechanisms that regulate fibrosis in lymphedema. Using mouse models and clinical biopsy specimens obtained from patients who suffer from BCRL, we have shown that lymphedema, similar to other fibroproliferative diseases, results in chronic tissue inflammation.[3] We have shown that the majority of inflammatory cells that are present in lymphedematous tissues are CD4+ cells and that the severity of lymphedema positively clinically correlates with the degree of CD4+ cell inflammation.[3] In addition, we have shown that similar to other fibrotic disorders, CD4+ cells present in lymphedematous tissue have a mixed T-helper 1 (Th1), T-helper 2 (Th2) phenotype.[2,5] This is important because previous studies have shown that Th2 cells produce profibrotic cytokines such as interleukin 4 (IL4), interleukin 13 (IL13), and transforming growth factor beta 1 (TGF-B1). These molecules activate a diverse array of pathways that increase collagen synthesis, decrease matrix turnover and remodeling thus resulting in replacement of functional parenchyma with scar.[22]

CD4+ cell inflammation and differentiation to Th2 phenotype plays a key role in the pathology of lymphedema. We have shown that depletion of CD4+ cells with neutralizing antibodies or loss of CD4+ cells in CD4 knockout mice prevents development of lymphedema in our mouse model.[5,23] More importantly, we have shown that blockade of Th2 differentiation with neutralizing antibodies directed against IL4 or IL13 not only prevents development of lymphedema after lymphatic injury but also can reverse pathological changes in established lymphedema.[3] Animals treated with just 3 doses of antibodies demonstrated marked reversal of tissue fibrosis, had significantly increased lymphatic function, and decreased chronic inflammation. More recently, in collaboration with our colleagues at MD Anderson Cancer Center, we have compared tissue biopsy samples before and 6 months after lymphatic bypass surgery (a treatment that decreases lymphatic fluid stasis) and have found that subjective and objective improvements with these surgical treatments correlate with decreased CD4+ cell inflammation, decreased fibrosis, and decreased hyperkeratosis (a clinical hallmark of lymphedema).[24]

Taken together, these preclinical and clinical studies strongly suggest that the pathology of lymphedema is related to chronic CD4+ cell inflammation, differentiation to Th2 phenotype, and tissue fibrosis. Therefore, the use of antifibrotic treatments represents a novel and targeted treatment of this common and morbid complication of cancer treatment. This ***paradigm shift*** in the treatment of lymphedema provides numerous applications and novel treatment strategies for a disease that has previously been primarily treated with dubious preventative measures and palliative treatment options.

Th2 responses are involved in other fibroproliferative disorders. Despite differences in parenchyma and function, the mechanisms that regulate fibrosis in a variety of organ systems including the lung, liver, kidney, and skin are largely conserved and dependent in large part on Th2 cytokines. The aggregate of a large number of studies has led to the proposal of the Th2 paradigm hypothesis by Wynn and colleagues postulating that Th2 responses, as we have observed in lymphedema, are a major pathologic response in fibroproliferative disorders.[22,25] These studies have led to a application of Th2 blockade in phase I and II clinical trials for the treatment other fibrotic disorders including pulmonary fibrosis, keloids, eosinophilic esophagitis, and asthma among others. These studies have focused on the use of monoclonal antibodies against IL4 or IL13 (or both) since the use of targeted immunotherapy has been highly successful in a variety of diseases including breast cancer (herceptin), melanoma, psoriasis, rheumatoid arthritis, among others. Therefore, based on this rationale in this protocol we propose to use a monoclonal IL4/IL13 neutralizing antibody for the treatment of lymphedema.

Blockade of Th2 responses is oncologically safe. T cell resposes in general, and Th2 differentiation in particular are known regulators of breast cancer tumorigenesis.[26,27] Previous studies have shown that breast tumors are infiltrated by Th2 cells that strongly express IL4 and IL13 and that these cytokines prime tumor development by directly interacting with tumor cells, inhibiting dendritic cell responses, and regulating expression of cancer cell differentiation markers.[26,28,29] Other studies have shown that activation of IL4/IL13 signaling pathways increases breast cancer invasion and propensity for lung metastasis.[30] In addition, several studies have shown that blockade of IL4 and IL13 with neutralizing antibodies (similar to the approach proposed in this application) inhibit tumor growth and metastasis in a variety of tumor types including breast cancer.[26,27] These findings have led some authors to propose that blockade of Th2 responses may be a viable strategy as either a mainstream or alternative method for the treatment of breast and other cancers.[31] This conclusion is further supported by other studies demonstrating that blockade of Th2 responses increases tumor surveillance by immune cell types in a variety of tumors.[32-34] Finally, in addition to the wealth of experimental data cited above supporting our hypothesis that blockade of Th2 responses does not increase the risk of breast cancer development, growth, or metastasis, there has been no evidence of carcinogenic potential for QBX258 or its component antibodies in long-term repeat dose toxicity or preclinical primate studies (performed up to 6 months in duration). These facts together lead us to conclude that the use of QBX258 and resultant IL4/IL13 blockade in breast cancer survivors is oncologically safe.

Volume measurements are the gold standard method by which efficacy of treatment for BCRL is measured. Previous studies have shown that arm volume measurements over short periods of time (as long as 1 year) remain relatively stable unless the patient experiences an acute exacerbation resulting from an infection or cellulitis. For example, Shaw and colleagues in a study on the effect of weight loss on BCRL found very little spontaneous changes in limb volumes in patients relegated to the control (i.e. no weight loss) group.[35] Previous studies have also shown that conservative measures that patients use such as compression garments or self massage also do not spontaneously decrease arm volumes; rather, these approaches are designed to prevent progression of disease.[36] Therefore, spontaneous decreases in arm volumes without a clinically signficant intervention is unusual.

Numerous studies have shown that volume measurements have high intrarater/interrater and test-retest reliability (intraclass correlation coefficient=0.997) of perometer measurements[37,38] able to detect even relatively modest changes in arm volume with a high degree of sensitivity and specificity. Therefore, we expect that changes in arm volume measurements will be a sensitive primary outcome measure to analyze therapeutic interventions for BCRL. In addition, based on a comprehensive review of the literature on previous studies reporting positive outcomes in lymphedema treatment using various treatment modalities (e.g. laser, pneumatic pump devices), we have found that reductions in arm volumes of 20-30% after treatment are considered to be a clinically significant endpoint.[35,39-49] Based on this analysis, we conclude that novel treatments such as Th2 blockade as proposed in this trial would be clinically relevant if they can decrease arm volumes by 20-30%.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This will be a single arm, open label design pilot study, aiming to test the efficacy of QBX258, a combination of two fully human monoclonal antibodies that neutralize the biologic activity of interleukin 4 and interleukin 13 (IL4/IL13), for the treatment of stage I or II breast cancer related upper extremity lymphedema (BCRL). Patients with stage I or II BCRL (n=22) who meet the inclusion and exclusion criteria (see below) will be recruited to the study and baseline lymphedema measurements will be performed. Based on these measurements, women with a minimum volume excess of 300 mL between the normal and lymphedematous arm and at least 6 months postop from axillary lymph node dissection will be eligible for the study. We have elected to limit the study to patients with stage I or II lymphedema since previous studies have shown that antifibrotic strategies are most effective in early stage disease (i.e. when end organ failure is not complete). We have chosen not to include patients with latent (stage 0) lymphedema since these patients typically have very little difference in arm volumes between the lymphedematous and normal limbs thus making it difficult to analyze the relative effectiveness of our treatment on our primary outcome measure (volume reduction). Similarly, we have chosen not to include patients with end stage (stage III) lymphedema since it is unlikely that the pathologic changes that have occurred in these cases (usually over the course of many years) will be reversed with the short term treatment as proposed in this study.

Once enrolled, patients will be treated once every 4 weeks for 4 treatments with QBX258 followed by analysis of our primary and secondary outcome measures within 21 days of the last dose of antibody treatment. We will also evaluate outcome measures after a 16-20 week washout period to analyze the effect of treatment withdrawal on recurrence of symptoms. During the course of the study, patients will continue the use of standard therapy for lymphedema (compressive garments, aerobic exercise, weight lifting) according to their pre-treatment directions. However, to avoid potential confounding effects of manual lymphatic massage or intense physical therapy, these interventions will not be performed in the active treatment period.

4.2 Intervention

In preclinical studies we have shown that blockade of Th2 cellular differentiation using IL4 or IL13 antibodies markedly decreases the pathological effects of lymphedema and improves lymphatic function.[3,5] Therefore, the objective of this study is to determine if a similar treatment approach is effective for the treatment of BCRL, the most common cause of lymphedema in the United States and Western countries. Based on our inclusion and exclusion criteria (see below) **women with stage I or II BCRL with a minimum volume excess of 300 mL** and at least 6 months postop from axillary lymph node dissection **will be recruited for treatment with QBX258 a monoclonal IL4/IL13 neutralizing antibody for once every 4 weeks for 4 treatments.** Objective/subjective measures of treatment as well as the pharmacokinetics and tolerability of treatment will be evaluated.

Primary objective:

1) Arm volume calculations. These measurements will be performed using a perometer (Volumerter; Bosl Medizintechnick, Aschen Germany), a non-invasive infra red scanner that reproducibly measures the diameter of the extremity at multiple points and calculates arm volumes using the truncated cone formula. The perometer has been shown to have excellent intrarater/interrater and test-retest reliability (intraclass correlation coefficient=0.997) with a greater than 95% specificity for lymphedema.[37,38] Analysis of arm volume is considered the “gold-standard” for measurement of fluid and adipose accumulation in patients with lymphedema and is used in most lymphedema studies to evaluate response to treatment. Duplicate measurements will be performed at baseline following enrollment (0-2 weeks prior to initiation of treatment). Measurements will be performed between the hours of 8AM and 5PM and will be performed by a licensed physical therapist at MSKCC. Volume excess will be calculated as compared with the normal upper extremity. Duplicate arm volumes will be re-measured within 21 days after last dose of QBX258. Finally, washout measurements will be performed 16-20 weeks following the last dose of QBX258.

Secondary objectives:

1) Bioimpedance measurements will be performed using the ImpediMed L-DexTM U400 device. This device is FDA approved for measurement of tissue dielectric constant and calculates the fluid content of tissues using differential transmission of low voltage electrical current.[50] Bioimpedance has very high sensitivity (100%) and specificity (98%) for lymphedema as compared with limb volume measurements.[51] This approach is widely used commercially in consumer electronics as a means of calculating body fat composition and has no reported side effects. The relative fluid content of the affected extremity is calculated by comparing the ratio of the normal and lymphedematous limbs using single use gel pads similar to those used for an EKG. Bioimpedance measurements have been shown to be highly sensitive for detecting changes in fluid content in patients with stage I and II lymphedema.[50] Bioimpedance measurements will be performed at the same sessions as the volumetric analysis.

2) Skin fibrosis/elasticity will be assessed using a non-invasive device (Elastimeter; Delfin Technologies). Skin tonometry measurements are a sensitive means of analyzing response to treatment and progression of disease in patients with lymphedema.[40,52] The ICC for test-retest and interrater reliability of tonometry ranges between 0.69-0.88 and tonometry is considered to have very high reliability.[53] The Elastimeter is a battery operated device that measures the force required to indent the skin at a given location. The indentation that is measured is minor and monitored with built in force sensors that measure skin elasticity based on a low level deformation force that enables instantaneous measurements. There is no change in skin structure and the force is applied for less than 3 seconds. Skin fibrosis/elasticity measurements will be performed at the same sessions as the volumetric analysis.

3) Quality of life survey (ULL 27) will be administered at the same sessions as volumetric analysis. The ULL27 is validated for patients with lymphedema, is simple to complete, and enables us to track patient reported outcomes of lymphedema.[54,55] The scale is highly precise, accurate, and sensitive to changes in symptoms. The ULL 27 is comprised of 27 items divided into three dimensions: 'physical' (15 items), 'psychological' (seven items) and 'social' (five items). The recall period is short (previous 4 weeks) enabling us to compare pre and post treatment quality of life measures. In addition, the ULL27 has been shown to have a high degree of sensitivity for quantifying clinical improvements in lymphedema symptoms. The questionnaire and scoring methods are appended to this protocol.

4) Histologic outcomes. Histologic analysis of tissue changes after targeted therapy is an important part of this proposal as it will enable us to perform mechanistic analysis of response to Th2 blockade. We have performed this analysis previously on nearly 200 patients in collaboration with our colleagues at Stanford University, MD Anderson, and University of Chicago and have shown that the biopsy procedure is safe (no adverse effects including infections or wound healing complications in any of the patients), simple to perform in an outpatient setting, and extremely well tolerated (i.e. no need for pain medications). We have published this protocol and results from this analysis in 5 peer reviewed manuscripts.[2,3,24,56,57] This analysis has enabled us to validate our preclinical models and, as proposed in the current study, will enable us to analyze fibrotic and inflammatory responses to treatment. Because of the important mechanistic information that will be provided by the biopsies, the pre and post treatment biopsy procedures will not be optional.

5 mm punch biopsies will be obtained from the affected and normal arms. The exact location of the biopsy will be determined based on physical exam and will correspond to the location judged to have the most edema, fibrosis, and hyperkeratosis. The hand will not be used for biopsy sites. The same location on the arm will be biopsied in both normal and affected limbs. Histologic analysis will include fibrosis (collagen deposition, scar index), inflammation (CD4+ cells, Th2 populations, macrophages), hyperkeratosis, and mast cells. Skin biopsies will be performed at baseline after enrollment (0-2 weeks prior to initiation of treatment) and after conclusion of treatment (within 21 days after last dose of QBX258) under sterile conditions by Dr. Mehrara or Dr. Dayan in the plastic surgery outpatient office. We will use the protocol that we have previously used in our collaborations with Stanford University, MD Anderson, and University of Chicago.[2,3,24,56,57] Briefly, patients will be treated with a dose of antibiotics (Keflex 500mg or clindamycin 900mg PO if allergic to PCN) 1 hour before procedure. A 5 mm punch biopsy will be performed under local anesthesia from the exact location in the normal and lymphedematous upper extremity as previously reported. The resulting wound will be closed with a single 5-0 suture and standard wound care instructions will be provided. The patient will be seen in followup 3-21 days to assess proper wound healing.

5) Tolerability of treatment. In general, treatment with QBX258 as well as its individual component parts (VAK694 and QAX576) in both preclinical and clinical studies have been well tolerated (see section 11 Toxicology). There have been no serious adverse effects directly related to either QBX258 or its component monoclonal antibodies. In addition, minor to moderate adverse effects have all resolved spontaneously without long-term sequelae. An objective of this study will be to continue these observations and report SAEs or AEs if they were to develop.

6) Pharmakokinetics (PK) of QBX258. A number of clinical and primate animal studies have evaluated the PK of QBX258 component parts (VAK694 and QAX576). In addition, the PK of QBX258 has been analyzed in one clinical study as well as long term primate animal studies. Therefore, a secondary aim of this study is to analyze the PK of QBX258 treatment.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

- QBX258 is the combination of two antibodies (VAK694 and QAX576) developed by Novartis Corporation to inhibit bioactivity of IL4 and IL13. VAK694 is a fully human monoclonal IgG1k antibody (mAb) with a specificity and high affinity for IL4 (KD= 7pM). QAX576 is a fully human IgG1k mAB directed against IL13 (KD = 139pM). The drug (QBX258) as well as drug administration equipment (e.g. pumps, tubing) and pharmacy instructions will all be supplied by Novartis.
- QBX258 as a combined injection of QAX576 and VAK694 is administered IV and has been tested in rhesus monkeys, marmoset monkeys, and humans.
- The Investigational New Drug Application (IND) has been submitted by Novartis Corporation and approved to proceed by the FDA (IND #107,646). Memorial Sloan Kettering Cancer Center will cross reference this IND held by Novartis Corporation.

Dose Calculation

The doses of the combination antibody components of QBX258 to be used in the current study are based on previous/ongoing clinical trials with VAK694 (3mg/kg) and QAX576 (6mg/kg).

In a clinical study of QBX258, sequential i.v. doses of VAK694 (3 mg/kg) and QAX576 (0, 0.3, 1, 3 and 6 mg/kg, 1 hour after VAK694) were administered on a single occasion. As expected for IgG1 type antibodies, the mean systemic clearances for VAK694 and QAX576 were low (4.1 mL/day/kg and 3.0 mL/day/kg, respectively). The systemic clearance for VAK694 was not modified upon QAX576 administration, and that for QAX576 was consistent with values observed in studies where QAX576 was administered alone. The mean terminal phase half-life for VAK694 was 23.1 days and not significantly affected upon QAX576 administration. The mean half-life for QAX576 was also as expected (22.8 days) and in the range of values previously observed when QAX576 was administered alone. Given in combination on one occasion, VAK694 and QAX576 demonstrated typical IgG1 type kinetics and very similar profiles as compare to when given alone. Because they also demonstrated linear PK behaviour, no pharmacokinetic interactions are expected as well when these two entities will be administered together on multiple occasions.

Both VAK694 (3mg/kg) and QAX576 (6mg/kg) in the doses chosen for the current study have been generally safe and well tolerated in other clinical trials for treatment periods up to 6 months. For both antibodies, adverse events have been mild to moderate and have resolved spontaneously without sequelae. There have been no study drug-related SAEs for VAK694. SAEs that have been suspected to be related to QAX576 include a possible drug interaction with acetaminophen and an occurrence of Ramsay-Hunt syndrome. There were no adverse event with an expected causal relationship and no fatal adverse events with a suspected causal relationship. No significant immunogenicity has been observed.

Preparation and packaging

QAX576: The drug product is a white, solid lyophilisate in 10 mL colorless glass vials with rubber stopper and aluminum flip-off cap to be reconstituted with water for injection (WFI). The powder for solution for infusion/injection contains QAX576 in a formulation of histidine (pH 6.0 ± 0.5), sucrose, glycine and Polysorbate 80. The formulation does not contain a preservative as it is to be used for single-dose administration only. It is a solid lyophilisate powder for solution for infusion/injection. It is reconstituted with 1.0 mL or 3.4 mL sterile water for injection to yield a solution of 150 mg/mL or 50 mg/mL QAX576, respectively, and is used for single dose administration only.

VAK694: 150 mg is a lyophilisate to be reconstituted with 1.0 mL or 3.4 mL sterile water for injection to yield a solution of 150 mg/mL or 50 mg/mL VAK694, respectively. The lyophilisate is formulated with histidine (pH 6.0 ± 0.5), sucrose, arginine hydrochloride and polysorbate 20. The formulation does not contain a preservative as it is to be used for single-dose administration only. It is a lyophilisate to be reconstituted with 1.0 mL or 3.4 mL sterile water for injection to yield a solution of 150 mg/mL or 50 mg/mL VAK694, respectively.

QAX576 and VAK694 are dosed together as a single infusion. Note that VAK694 and QAX576 Powder for Solution for Infusion are to be reconstituted separately. The resulting VAK694 and QAX576 Concentrates for Solution for Infusion have to be diluted into the same infusion bag sequentially to yield ready-to-use QBX258 Solution for Infusion.

Reconstituted VAK694 and QAX576 Powder for Solution for Infusion should not be mixed with each other before injection of the Concentrates for Solution for Infusion into the infusion bag. Investigational Product QAX576 and VAK694, will be supplied by Novartis Drug Supply Management as patient specific open label bulk medication.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

We will use the following inclusion and exclusion criteria to identify potential study candidates.

6.1 Subject Inclusion Criteria

- Women 18-70 with unilateral stage I or II BCRL
- Volume difference of at least 300 mL between the normal and lymphedematous limb based on perometry evaluation
- BMI of 18-30
- No current evidence of breast cancer
- At least 6 months postop from axillary lymph node dissection
- Women of childbearing potential must be willing to use a highly effective form of contraception for the duration of the trial and for 18 weeks (5 half-lives) after the last dose of treatment

6.2 Subject Exclusion Criteria

- Bilateral lymphedema or history of bilateral axillary lymph node dissection
- Recent history of cellulitis in the affected extremity (within last 3 months)
- Recurrent breast cancer or other malignancy
- Current (within last month) use of chemotherapy for breast or other malignancy
- Currently receiving adjuvant trastuzumab (Herceptin)
- Current (within last 3 months) use of radiation for breast or other malignancy
- Recent (within last month) or current intensive MLD and/or short stretch bandage use
- Unstable lymphedema (i.e. worsening symptoms/measurements in the past 3 months)
- Pregnant or nursing (lactating) women
- Stage III lymphedema
- Patients that take acetaminophen (paracetamol) chronically, i.e. more than 1 g/day for more than 3 out of 7 days, or more than 2 g/ day for more than 1 day out of 7 days
- Use of other investigational drugs at the time of enrollment, or within 30 days or 5 half-lives of enrollment, whichever is longer
- History of hypersensitivity to any of the study drugs or to drugs of similar chemical classes (e.g. monoclonal antibodies, polyclonal gamma globulin, polysorbates).

7.0 RECRUITMENT PLAN

Potential research subjects will be identified by treating physicians in the Plastic Surgery, Breast Surgery, Breast Medicine, and rehabilitation medicine services. If the treating physician is also an investigator on the protocol, then s/he will discuss the study with the patient. Otherwise the patient will be referred to a study investigator for evaluation. It is estimated that more that 30% of women who undergo axillary lymph node dissection for the treatment of breast cancer go on to develop lymphedema. Because these procedures are performed commonly (approximately 150/year despite the widespread use of sentinel lymph node biopsy) a large number of patients go on to develop lymphedema. In addition, because lymphedema is a lifelong condition and our treatments for breast cancer have increased survival, a large number of breast cancer survivors suffer from lymphedema thus providing a large pool of patients who are eligible for the trial. Therefore, patients will be identified as they present to the individual services. To increase awareness of the trial, the clinical trial will also be listed on clinicaltrials.gov. Because breast cancer is most commonly diagnosed in women, we will focus our recruitment efforts to women.

During the initial assessment, the patient may be asked to provide certain health information that is necessary for the recruitment and enrollment process. Specifically, the diagnosis, current treatments, stability, and symptoms of lymphedema will be queried. In addition, the patient will be asked about the current status of their disease, history of lymphedema related infections, history of fibroproliferative disorders (e.g. pulmonary fibrosis, cirrhosis, keloids), history of axillary surgery, and the current treatment plan for their lymphedema. The investigator/research staff may also review portions of her medical records at MSKCC in order to further assess eligibility. They will use the information provided by the patient and/or medical record to confirm that the patient is eligible and to contact the patient regarding study enrollment. If the patient is ineligible for the research study, the research staff will destroy all information collected on the patient during the initial conversation and medical records review, except for any information that must be maintained for screening log purposes. If the woman agrees to study participation, a consenting individual (listed below in section 15.1) will obtain informed consent from the patient until a goal of 22 eligible patients are entered into the study in order to have 20 evaluable patients.

In the majority of cases, we expect that the initial contact with prospective subjects will be conducted by the treatment team or investigators/research staff working in consultation with the treatment team. The recruitment process outlined in this application presents no more than minimal risk to the privacy of potential subjects and minimal PHI will be maintained as part of a screening log. As a result, we request a *(partial)* limited waiver of authorization in order to (1) review medical records to identify potential research subjects and obtain information relevant to the enrollment process; (2) Discuss potential enrollment with patients; (3) handling of PHI contained within those records and provided by the potential subjects; and (4) maintaining information in a screening log of patients approached (if applicable).

Once a suitable candidate is identified and volume difference of at least 300 mL between the normal and lymphedematous limbs is confirmed using perometry, the patient will be offered the opportunity to join the study. The discussion process will include the rationale for the study including the pathological processes involved, the goals of the study, the length of the study, measurements an analyses to be performed, the expected time commitment and number of clinic visits. Investigators will discuss with the patient that the proposed trial is a short-term treatment rather than a cure for lymphedema and that we expect that the symptoms of lymphedema will recurr once treatment has been terminated. The risks of the treatment including potential adverse reactions, transfusion/infusion reactions, and hypersensitivity reactions will be discussed in detail with the patient. In addition, we will discuss the time commitment of the protocol and schedule for followup visits, measurements, biopsies, and blood draws.

8.0 PRETREATMENT EVALUATION

The pretreatment evaluation will aim to quantify the objective and subjective changes of lymphedema. This analysis will include:

1) **Physical Examination** will be performed to evaluate lymphadenopathy, evidence of cancer recurrence, and general physical health. This will include vital signs as well as height/weight measurements.

2) **Urine Pregnancy test** will be administered prior to enrollment into the study and prior to each monthly drug treatment. Although QBX258 treatment has not exhibited evidence of maternal toxicity in multiple preclinical trials with marmoset monkeys, there was a trend towards an increased rate of spontaneous abortions in these studies. However, fetuses that were born to monkeys treated with QBX258 had normal fetal developmental parameters and there was no evidence of teratogenicity. Therefore, patients capable of childbearing recruited to the study will be required to use highly effective birth control measures whilst participating in the QBX258 trial and for at least 18 weeks (5 half-lives) after the last dose of QBX258.

3) **Arm volume measurements** will be performed in duplicate at baseline using a perometer (Pero-Systems). Measurements will be performed by a licensed physical therapist at the out patient physical therapy office located at 53rd and Madison Avenue between the hours of 8AM and 3PM. The perometer is a non-invasive infrared scanner that measures the circumference of the upper extremity at multiple points. Volumes are then calculated using the truncated cone formula. This analysis is highly reproducible and accurate avoiding the need for traditional volume measurements (fluid displacement) or manual limb circumference measurements. Volume excess will be calculated as compared with the normal (i.e. contralateral) upper extremity.

4) **Bioimpedance measurements** will be performed to compare the normal and lymphedematous limbs using the ImpediMed L-DexTM U400 device. The ImpediMed device is FDA approved and is used to estimate the fluid content of tissues based on resistance to transmission of electrical current. These measurements are simple to perform and are highly accurate in analyzing changes in early stage lymphedema. Bioimpedance measurements will be performed in duplicate at the same time as volume measurements.

5) **Skin fibrosis** will be assessed at the same session as arm volume measurement using a tonometer (Elastimeter; Delfin Techonolgies). Duplicate measures of a point located 10 cm above and 10 cm below the olecranon process on the dorsal and ventral surfaces of the limb will be recorded. Skin fibrosis resulting from hyperkeratosis and fibrosis is a characteristic feature of lymphedema.

6) **Quality of life survey (ULL27**) will be administered upon enrollment to quantify the subjective measures of lymphedema. These questionnaires are validated lymphedema specific surveys and are simple to perform and have been used in previous lymphedema trials performed at MSKCC. The questionnaires will be administered within 6 weeks enrollment and prior to the start of therapy.

7) **Histologic analysis**. A defining feature of lymphedema is skin fibrosis and inflammation. We have previously compared skin biopsy specimens from the normal and lymphedematous limbs of patients to analyze inflammatory changes and, more recently, to evaluate tissue changes that occur after lymphedema surgery. These biopsies have been performed in over 200 patients under IRB approved protocols by our collaborators at Stanford University, MD Anderson Medical Center, and The University of Chicago and have been well tolerated with minimum pain, no surgical complications, and most importantly no postoperative infections.

The procedure will be performed using strict sterile protocol and a single pre-procedure dose of antibiotics. A 5 mm punch biopsy will be obtained from the volar surface of the affected and normal arms. The exact location of the biopsy will be determined based on physical exam and will correspond to the location judged to have the most edema, fibrosis, and hyperkeratosis. The hand or wrist will not be used for biopsy sites. The same location on the arm will be biopsied in both normal and affected limbs. The biopsy will then be fixed and embedded for histological analysis. This pretreatment biopsy specimen will be compared with post-treatment biopsies to analyze treatment efficacy for decreasing inflammation and fibrosis using our previously published protocols. Skin biopsies (both normal and lymphedematous arms) will be performed before and after treatment.

Histologic analysis of biopsy specimens will be performed using our previoulsy published reports to analyze inflammation and tissue fibrosis.[1-5,20,57] Briefly, CD4+ and Th2 cells will be identified and quantified using immunohistochemical staining according to our previously published protocols.[3] In addition, we will analyze the degree of tissue fibrosis using collagen staining and analysis of Sirius Red Birifringence (scar index) according to our previously published methods. It is important to note that in our previous studies we have shown that the severity of lymphedema clinically is positively correlated with the number of CD4+ cells, the number of Th2 cells, and the degree of tissue fibrosis. In addition, in other clinical studies comparing pre and postoperative tissue biopsy samples in patients who underwent lymphovenous bypass procedures (a surgical treatment that aims to bypasses the obstructed lymphatic system to the venous circulation) we have found that the severity of CD4+ cell inflammation and fibrosis decreased signficantly after surgery and that this response correlarted with symptomatic improvement.[24]

8) **Routine Blood draw**. A pretreatment complete blood count and differential will be performed within one month of starting the trial to establish baseline values for complete blood count, basic metabolic panel, and liver enzymes.

9) **Research Blood draw**. A pretreatment PK analysis measurement blood draw will be performed.

10) **Urine Analysis** will be performed to rule out pre-existing urinary conditions.

9.0 TREATMENT/INTERVENTION PLAN

Once patients are registered for the trial, they will be treated with QBX258 (VAK694 3mg/kg and QAX576 6mg/kg) delivered via peripheral intravenous injection once every 4 weeks (± 1 week) for 4 treatments. Intravenous access will be limited to the non-lymphedematous limb and will be performed using standard techniques. Infusions will be performed at the Breast Center (BAIC, 66th street) and patients will monitored at the infusion center. The infusions will take approximately 2 hours and patients will be monitored for 2 hours after the completion of infusion. As a result, we expect a total time of approximately 4 hours for each infusion session. Infusions will be ordered by medical oncologists listed on the protocol.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

Overview of study:

Study Phase	Screening	Baseline	Treatment/FU				Outcome	Washout
Weeks	-	-2 to 0	0	4	8	12	12-15	28(±3)-32 (±3)
Inclusion/Exc.	x	x						
Demographics	x	x						
Physical Exam		x					x	x
Pregnancy Test		x	x	x	x	x		
Evaluation of acute transfusion reaction			x	x	x	x		
Pharmacokinetics		x	x	x	x	x	x	
Height		x						
Weight		x					x	x
Temperature		x	x	x	x	x	x	x
Vital signs		x	x	x	x	x	x	x
ECG		x					x	
CBC		x					x	
Metabolic panel		x					x	
Liver Enzymes		x					x	
Urine Analysis		x					x	
Arm Volumes		x					x	x
Bioimpedance		x					x	x
Tonometry		x					x	x
QOL questionnaire		x					x	x
Skin Biopsy		x					x	
Adverse event Check		x	x	x	x	x	x	x

Pharmacokinetics

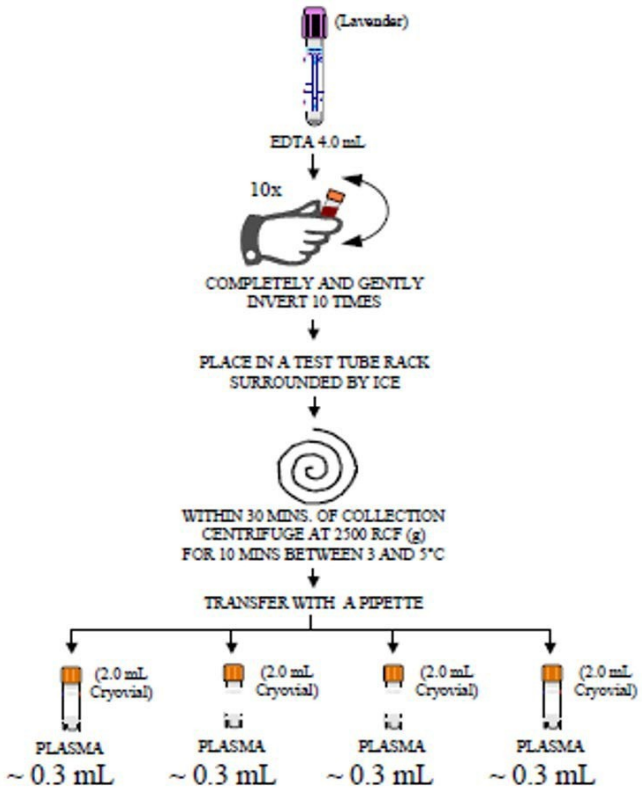
In order to calculate the pharmacokinetics of both component antibodies of QBX258, blood draws will be performed from study subjects according to the following schedule:

Initial day of dosing: Blood draw will be performed within 30 minutes after the conclusion of infusion ± 5 minutes

Day 15 (±3 days) after 1st dose

Days 29, 43, 57, 71, 85, 99, 113, and 141 (± 3 days) after 1st dose; Day 183 after 1st dose (± 10 days)

Blood samples will be taken either by direct venipuncture or an indwelling cannula inserted in the normal (i.e. non-lymphedematous) hand or forearm vein. At the specified time points, 4ml of blood will be collected in tubes with EDTA as an anticoagulant to obtain 4 aliquots of at least 0.3ml of plasma. All samples will be given a unique sample number and a collection number (de-identified of all patient information). The actual sample collection date and time will be entered on the PK blood collection page. Samples will be kept upright at -70 degrees celcius on site and sent in batch shipments as necessary to AtlanBio for analysis (Z.I. de Brais - 1 rue Graham Bell, 44600 Saint Nazaire; France). The plan for PK blood collection is summarized in the diagram below.



Evaluation of acute infusion reaction and tolerability of QAX258.

The risk of anaphylaxis or anaphylactoid reactions is present but is minimized due to fact that QAX576 and VAK694 are fully human monoclonal antibodies. Anaphylactic reactions are much less common to human mAbs than mouse, chimeric or humanized mAbs.[58]

Although very rare, if these reactions occur, they usually do so after the first or any subsequent intravenous administration of the drug. Therefore, **vital signs will be assessed after initiation of infusion and repeated every hour after infusion for a total of 2 hours**. Patients will be closely monitored and will be evaluated by staff trained in the management of infusion reactions and anaphylaxis.

As with any drug, there is a small chance that subjects will experience an allergic reaction to the medicine, such as an allergic skin rash, or hives. There may be unknown or unforeseeable risks. We do not anticipate that the combination of both antibodies poses a high risk of allergic reactions based on data with the individual components. Nevertheless, patients will be **contacted by telephone within 96 hours after administration of each dose** of QX258 and queried regarding allergic reactions or systemic symptoms including rash, hives, fevers, nausea, vomiting, malaise, headaches, arthralgias, urticaria, and palpitations. The telephone contact will be performed by the study RSA. This contact information (person calling and patient reports) will be recorded in a log created for each patient at the onset of the study. The log will be kept on a secure server. Patients will be instructed to call or contact study investigators if symptoms develop after this period. In addition, patients will be queried about these symptoms prior to initiation of 2nd, 3rd, and 4th infusions. The patient specific log will be updated at these time points by the study RSA. This plan is based on the experience with other FDA monoclonal antibody treatments (e.g. Remicade®). Patients who report symptoms or potential adverse effects will be evaluated in person and possible relationship to the study will be assessed.

Examination of biopsy sites and wound healing

Biopsy sites in both the lymphedematous and normal limbs will be assessed by a consenting physician or licensed practitioner 3-21 days after the procedure to ensure that it is healing appropriately and rule out evidence of infection. Any potential infections will be reported and treated with antibiotics.

Pregnancy test

Given the potential reproductive toxicity of Th2 blockade (see section below on toxicity), a urine pregnancy test will be administered to volunteers of childbearing age upon enrollment and before each infusion.

Outcome measures of lymphedema treatment

Outcome measures of lymphedema will be assessed as outlined above and include limb volume measurements, bioimpedance, skin fibrosis/elasticity, QOL surveys, and histologic analysis. Volumetric analysis, bioimpedance, skin fibrosis/elasticity, and QOL surveys will be assessed within 21 days of the last dose of QBX258 and after a washout period of 16-20 weeks. In addition, the patients weight will be checked at each time point.

11.0 TOXICITIES/SIDE EFFECTS

Identifying and reporting of toxicity or complications

We will utilize the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 for grading of all adverse events. A copy of the CTACE V4.03 can be downloaded from the CTEP home page (http://evs.nci.nih.gov/ftp1/CTCAE/About.html). All investigators and treatment areas will have access to a copy of the CTCAE V4.03

Definition of an Adverse event: Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of unrelated, unlikely, possible, probable, or definite). [CTEP, NCI Guidelines: Adverse Event Reporting Requirements. January 2005;http://ctep.cancer.gov/reporting/adeers.html])

Definition of an SAE: Any adverse experience occurring during any part of protocol treatment and 30 days after that results in any of the following outcomes:

- Death;
- A life-threatening adverse drug experience;
- Inpatient hospitalization or prolongation of existing hospitalization;
- A persistent or significant disability/incapacity;

Important medical events that may not result in death, be life threatening, or require hospitalization may be considered an SAE, when, based upon medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in the definition. Any pregnancy occurring on study will be reported as a medically significant event.

All AEs and SAEs will be reported to the IRB and sponsoring institution as outlined in section 17.2 below. In the event of a grade III or IV SAE, the study will be halted until causality is determined.

Patients who experience AE or SAE will be managed by the Breast medical oncologists and plastic surgery attendings listed on the protocol. Any potential wound healing issues or complications will be addressed by plastic surgery and consultation with Breast Medicine as necessary. In the event that hospital admission is required for management of an SAE, patients will be admitted to the Plastic Surgery Service with close consultation and management with the Breast Medical Oncology Service.

Potential toxicity related to QBX258 treatment in previously conducted trials: QBX258 has been shown to be well tolerated in both preclinical studies and human trials. In animal studies using Rhesus monkeys treated with escalating doses of QBX258 up to a dose of 100mg/kg, there were no QBX258 treatment-related deaths. There were no QBX258-related clinical signs or changes in bodyweight, food consumption, ophthalmic examinations, ECG, heart rate or blood pressure. There were no effects on clinical pathology, hematology, clinical chemistry and urinalysis investigations. There were no effects either on peripheral blood lymphocyte immunophenotyping parameters or on immune function (generation of a primary and secondary T cell-dependent IgM and IgG antibody response to KLH). There were no effects on organ weight or in macroscopic or microscopic observations, including no effects on extended histopathology of lymphoid organs.

Similarly, in the first study of QBX258 in humans, sequential intravenous doses of VAK694 (3mg/kg) and QAX256 (up to 6mg/kg) on a single occasional were safe and well tolerated. In a different study of 40 subjects with bronchial asthma, QBX258 treatment was well tolerated with no significant imbalance of adverse events between patients treated with QBX258 and placebo. A single serious adverse effect was reported for a subject who was hospitalized following a snake bite and required hospitalization. A second patient had an increase in CPK, however this was not suspected to be associated with the drug. Most other adverse events (e,g. headache, etc) were mild or moderate and resolved spontaneously.

The potential on-target risk of QBX258 is further mitigated by experience with other biological compounds that targeted the actions of IL-4 and IL-13 in humans. For example, trials using antibodies to IL4Ra[59] and IL-13[60] have been reported to be well tolerated without significant adverse events.

Potential toxicity related to individual components of QBX258 (VAK694 and QAX576). Because QBX258 is a combination of 2 separate monoclonal antibodies (VAK694 and QAX576), the known toxicity of these individual components and the combination drug are listed below.

VAK694 has been well tolerated when administered to human subjects in doses ranging from a single infusion of 0.1mg/kg to healthy individuals to four sequential infusions of 3mg/kg given every 4 weeks to subjects with seasonal allergic rhinitis. There have been no study drug related SAEs. Adverse effects have been mild to moderate and have resolved spontaneously. No significant immunogenicity has been observed.

- Doses as high as 100mg/kg/dose (more than 30 times the dose administered in humans) administered once weekly for 4 weeks in cynommolgus monkeys were well tolerated with and no treatment or dose level related effects were observed. There were no clinical signs indicative of neurobehavioral effects or quantitative electrocardiographic changes. There were no effects on the injection site, body weight, ophthalmology, electrocardiography, hematology, clinical biochemistry, immunochemistry, urinalysis, or organ weights. There were no spontaneous tumors.
- As of July 2013, 3 VAK694 studies have been completed with one study in healthy volunteers and two studies in patients with seasonal rhinitis. A total of 61 subjects have received VAK694. Single doses up to 1mg/kg in healthy volunteers, multiple doses up to 1mg/kg weekly x 3 to asymptomatic patients with allergic rhinitis, and multiple doses of

3mg/kg every 4 weeks x 4 in patients with seasonal allergic rhinitis were safe and well tolerated.

- There has been a single SAE in patients treated with VAK694 (hospitalization for acute appendicitis) which was not considered to be related to the study drug. Adverse events have been mild to moderate and have resolved spontaneously without sequela. These included once case of nasal congestion, once case of pharyngolaryngeal pain, 1 patient with a headache, and 1 case of traumatic injury of the knee joint.

QAX576 has been generally safe and well tolerated in doses up to 6mg/kg every three weeks or 10mg/kg every 4 weeks up to 6 months. SAEs suspected to be related to QAX576 include a possible drug interaction with acetaminophen and an occurrence of Ramsay-Hunt Syndrome. There were no adverse events with an expected causal relationship and no fatal adverse events with a suspected causal relationship.

- Doses as high as 100mg/kg/twice weekly for up to 13 weeks to marmoset monkeys (>15 fold of doses to humans) intravenously had no significant toxicological or local tolerability effects. There was no evidence of immunotoxcity. There were no drug related changes in body weight, food consumptions, ophthalmoscopy, electrocardiography, colorectal pathology, clinical chemistry or urinalysis, organ weight, or macroscopic organ changes. There was also no maternal toxicity however, the incidence of prenatal loss was increased modestly.
- As of December 2012, 10 clinical trials with QAX576 have been completed or terminated after enrolling some patients; 3 in healthy volunteers and 7 in patients. The trials consisted of allergic rhinitis, pulmonary fibrosis, keloid, and other Th2 related disorders. A total of 168 patients have participated in phase I programs and 227 in phase 2 programs. The programs have included both women and men in ages ranging from 18-80.
- In a study of 24 healthy volunteers, There were no clinically significant changes noted on safety assessments including vital signs, blood chemistries, hematology, urinalysis, physical examinations and electrocardiogram measurements at all dose levels. No SAEs were reported in this study. A total of 2 adverse effects were reported: one subject experienced a mild headache and an upper respiratory infection which was not felt to be related to QAX576. In another study, one subject experienced a severe episode of migraine that was not suspected to be related to QAX576. One patient developed mild arthralgia which resolved spontaneously. No immunogenicity signal was detected and no positive antibodies to QAX576 were reported up to 28 days after the study drug administration.
- In a multiple dose study in patients with pulmonary fibrosis secondary to systemic sclerosis, five serious adverse events were reported in 2 patients. One patient experienced hypotension with mild tachycardia, and pneumonia post bronchoscopy. Another patient suffered an episode of pseudo-bowel obstruction. These SAEs were judged by the principal investigator as not to be related to the study drug.
- In a double blind placebo controlled study of asthma, a patient injured his back after the second dose of study medicated and was prescribed paracetamol. Afterwards the patient presented with some adverse events which lead to hospitalization for the treatment of potential paracetamol overdose.

Potential toxicity related to Th2 blockade

Infection. Published studies in mice deficient in either cytokine alone do not demonstrate an increased susceptibility to parasitic diseases, reproductive abnormalities, or alterations in humoral response.[61] Nevertheless, mice that lack both IL-4 and IL-13 clear intestinal infection with helminthes more slowly than wild-type mice or mice that lack either cytokine alone.[62] Given that patients enrolled in our study are unlikely to suffer from parasitic diseases, we feel that this is a low risk potential toxicity.

Reproductive toxicity. No reproductive abnormalities have been described in mice lacking IL-4 and IL-13.[62] While the risk of embryofetal toxicity with VAK694 is presently unknown, preliminary data from embryo-fetal development (EFD) and combined EFD pre-/post-natal development (PPND) studies with QAX576 in the marmoset do not exclude an adverse impact of IL-13 suppression on the pregnancy outcome. Furthermore, data in animals and in humans support a role for Th2 cytokines, including IL-13 and IL-4, in the maintenance of pregnancy.[63] No teratogenic effects of QAX576 have been observed. No overt phenotypic abnormalities have been observed in mice in which both IL-4 and IL-13 have been disrupted.[62] Nevertheless, a teratogenic effect of therapeutic neutralization of both IL-4 and IL-13 cannot be excluded. For these reasons, we will exclude pregnant and lactating women from participation in the trial. In addition, we will carefully monitor volunteers for pregnancy before and during the trial. Female subjects of child-bearing potential must use highly effective measures of contraception (see below) whilst participating in the QBX258 trial and for at least 18 weeks (5 half-lives) after the last dose QBX258. Women who are considered post-menopausal and not of child bearing potential i.e. if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) will not be required to use contraception.

Highly effective contraception methods are defined as:

- Total abstinence: when this is in line with the preferred and usual lifestyle of the subject. [Periodic abstinence (e.g. calendar, ovulation, symptothermal, post ovulation methods) and withdrawal are not acceptable methods of contraception], or
- Documented history of sterilization: surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks before taking study treatment.
- Documented history of male partner sterilization or
- Use of a combination of any two of the following (a+b or a+c, or b+c):
  a. Use of oral, injected or implanted hormonal methods of contraception (in case of use of oral contraception women should have been stable on the same pill for a minimum of 3 months before taking study treatment),
  b. Placement of an intrauterine device (IUD) or intrauterine system (IUS),
  c. Barrier methods of contraception: Condom or Occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/vaginal suppository.

Liver toxicity. In a mouse model of acetaminophen-induced liver toxicity, in which liver damage is associated with increased circulating IL-13, pre-treatment with a neutralizing antibody to IL-13 exacerbated the extent of liver injury.[64] Similar findings were obtained in IL-13 knockout mice that received high doses of acetaminophen. These data suggest a possible hepatoprotective role for IL-13, and ingestion of acetaminophen (paracetamol) will be limited in this clinical study. To date, liver toxicity has not been seen in QAX576 human clinical trials, however, patients enrolled in this study will be advised to avoid using acetaminophen until the trial has completed.

Potential complications related to biopsy

Biopsy of lymphedematous and normal arms are an important aspect of this application as these procedures will enable us to determine if Th2 blockade is successful in reversing the pathologic tissue changes associated with lymphedema. In addition, these studies are critical for performing mechanistic studies that may further our studies and understanding of lymphedema. We have performed these procedures in over 200 patients in collaboration with our colleagues at Stanford University, MD Anderson Cancer Center, and the University of Chicago. No adverse events have been reported in these studies and all wounds have healed uneventfully without evidence of infection, delayed healing, pain, or lymph leak. As a result, we expect that we will have similar results in the current proposal. If adverse effects (i.e. delayed healing, infection, lymph leak etc) are identified, then these will be reported immediately to the principal investigators and the IRB and appropriate treatments including antibiotic treatment (PO or IV depending on severity of infection), debridement, and further management will be instituted as necessary.

Potential toxicity or complications related to measurements

Measurements performed in this study (perometry, bioimpedance, tonometry) are all non-invasive and we do not expect to experience any AEs. Similarly, the patient reported outcome questionnaires administered in this study have been validated and used in hundreds of patients without any significant adverse events.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Patients will be considered evaluable if they provide both a baseline and follow-up arm volume measurements. If biopsy data or time points from PK analysis measurement are not available at followup due to patient desire to avoid additional surgery or a missed blood draw appointment, then the remainder of the outcomes will be analyzed. Similarly, if impedance, tonometry, or QOL data are not available, then other measures available will be analyzed. Therapeutic volume changes in the arm will be calculated using the methods published by Anderson et al (2000).[65] Briefly, the difference in volume measurments between the normal and lymphedematous arms at baseline (i.e volume excess) will be compared to the volume differential after drug treatment and following the washout period using the following formula:

$\underline{(V_L\text{-}V_N)_B - (V_L\text{-}V_N)_F}$

V_L = Volume of the lymphedematous arm
V_N = Volume of the normal arm
B= Baseline
F = Follow up

13.0 CRITERIA FOR REMOVAL FROM STUDY

Patients will be removed from the study according to the following criteria:

- Significant toxicity, persistent AE or SAE related or unrelated to the treatment.
- If at any time the patient is diagnosed or develops recurrent breast cancer or suspicious changes on laboratory or radiological testing suggestive of malignancy.
- Subject non-compliance with infusions, measurements/analysis at the appropriate time intervals.
- Voluntary wish of the subject to withdraw from the protocol.
- Development of cellulitis or infection of the affected extremity.
- Significant progression of lymphedema volume changes during treatment (greater than 30% increase in limb volume as assessed by perometry).
- Unexpected pregnancy or non-compliance with the use of birth control methods

The study will be halted in the event of a grade 3 or 4 SAE until investigation of causality with drug treatment is concluded. At this point if the SAE is not thought to be caused by drug treatment, then the treatment plan will be reviewed with the company and the IRB to decide if the study should be restarted.

14.0 BIOSTATISTICS

This is a prospective, longitudinal, open label pilot study designed to evaluate the efficacy of QBX258, a monoclonal antibody treatment designed to block biologic activity of interleukin 4 (IL4) and interleukin 13 (IL13). Our primary end point is volume changes as measured by perometry. Our secondary endpoints include pharmacokinetics, bioimpedance measurements, skin tonometry, the ULL27 lymphedema questionnaire, and histologic studies.

Sample size calculation: Based on a review of the literature, reductions of 20-40% in the extent of excess lymphedema volume before and after various interventions such as weight loss or cold laser have been reported. [35,39-49] However, these interventions have not gained widespread clinical incorporation due to compliance issues (weight loss) and time intensive nature of the therapies. However, it is commonly accepted that a 30% reduction to any potential therapy for lymphedema is a good clinical response and worthy of additional study. We will therefore consider an average decrease of 30% in arm volume excess in the cohort as a therapeutic response. Previous studies have also shown that the minimum measurable change in volume is 60-75 cc.[66] Therefore, based on these findings we have used a minimum volume excess of 300 cc between the normal and lymphedematous arms as an inclusion criterion measurable. Thus, a excess volume reduction in a patient who has a volume excess of 300 cc prior to treatment would be equal to a 90cc reduction, which is greater than the minimal measurable change of 60-75cc ().

Using data from the weight loss study sited in the Background section in which perometer measuments were used to calculate limb volumes before and after weight loss, we assume a baseline mean excess lymphedema volume of 802ml and a standard deviation of 325 for the difference in pre and post treatment excess volume. With a sample size of 20 patients, we can detect a 30% reduction in excess volume (236ml difference between pre and post treatment) with 90% power and 5% type I error. Assuming a 10% drop out rate, we will therefore plan on accruing 22 patients to meet our study goal.

We expect to accrue approximately two patients per month and will complete accrual within one year.

Analysis of primary objective: Our primary aim is to establish the difference in the extent of excess lymphedema volume before and after treatment with QBX258. The extent of excess lymphedema volume is defined as the difference between the volume of the lymphedematous arm and the normal arm at each time point (i.e. Prior to treatment; after treatment, and after washout). Since the volume measurement at each time point is taken in duplicate, the average between the duplicate measurements will be used to calculate the excess lymphedema volume; see section 12.0). The difference in the extent of lymphedema between time points will be assessed with paired two-sided t-tests comparing the post-treatment and washout measurements with the pre-treatment measurements and the post-treatment measurements with the washout measurements. In addition, we will analyze the effect of potential confounding variables such as pre treatment BMI on volume reduction using analysis of covariance (ANCOVA) with the arm volume difference after completion of treatment as the outcome variable and baseline volume difference and BMI as covariates. We will report a two-tailed p-value and a 95% confidence interval for the difference between groups. Data will be presented graphically to facilitate analysis.

Analysis of secondary objectives:

Tolerability will be evaluated using toxicity data and will be tabulated by severity. ULL-27 questionnaire responses will be graded and scored according to previously published methods (see Appendix A). Changes in each symptom scale (physical, psychological and social) as well as the overall score will be compared pre and postn treatment using paired two-sided t-tests. The bioimpedance and tonometry measurements yield continuous variables and will be analyzed using paired t-tests. Similarly, histologic analysis of the biopsy specimens will yield data on the number of CD4+ cells, the number of Th2 cells, and the degree of tissue fibrosis, all of which are also continuous variables. These variables will be compared pre and post treatment using paired t-tests. For phamacokinetic analysis, mean volume of distribution at steady state (V_{ss}), mean total systemic clearanceand the mean distribution half-life ($t_{1/2\alpha}$) will be calculated using published methods.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures.

During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

This is an open label trial and randomization will not be required.

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study and will be responsible for project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol study team. The data collected for this study will be entered into a secured database (Clinical Research Database, CRDB) at Memorial Sloan-Kettering Cancer Center. Source documentation will be available to support the computerized patient record.

We will administer the ULL27 questionnaire via either paper forms in private areas of waiting rooms at the MSKCC 60th street Plastic Surgery offices, the 64th Street Breast Cancer Center, or the Department of Rehabilitation Medicine located at 53rd and Madison Avenues. The QOL survey will be completed prior to initiation of therapy, at the conclusion of therapy, and after a washout period as outlined above and will be brought to the patients by the study RSA and collected after completion. The data collected in this manner will be entered into a secured database (Clinical Research Database, CRDB) at Memorial Sloan-Kettering Cancer Center. Source documentation will be available to support the computerized patient record and will be kept in a secure locked cabinet.

16.1 Quality Assurance

Registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study and potential problems will be brought to the attention of the study team for discussion and action.

Random-Sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year and more frequently if indicated.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) plans at Memorial Sloan-Kettering Cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled "Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials" which can be found at: http://mskweb2.mskcc.org/irb/index.htm.

Clinical trials are monitored by a number of different mechanisms to ensue data safety and quality. Institutional processes are in place for quality assurance (e.g. protocol monitoring, compliance and data verification audits, therapeutic responses, and staff education on clinical research). In addition, there are departmental procedures for quality control as well as two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center's Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for it's level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed and the monitoring procedures will be established at the time of protocol activation.

17.0 PROTECTION OF HUMAN SUBJECTS

Prior to the enrollment of each patient, the risks, benefits and objectives of the study will be reviewed with the participant, including a discussion of the possible toxicities and side effects. Financial costs and burdens of the trial will be reviewed in detail including a detailed discussion of the tests/measurements that will be performed. The tests and measurements will be the financial responsibility of the study. The cost of traveling to and from the center for evaluation will be the financial responsibility of the patient. Blood work analysis as well as analysis of EKG and other monitoring tests will be provided at no cost to the patient. Every effort will be made to keep study records private.

Neither the patient's name nor anything else that could identify the patient will be used in any reports or publications that result from this study. Trained staff at Memorial Hospital and the Institutional Review Board at Memorial Hospital may review medical records if necessary. The patient may terminate participation in the study at any time during the trial. In the event that a toxicity or complication arises as a result of the study, the patient will be evaluated and treated at no cost to the patient unless it is determined that the adverse effect is not related to the study drug in which case treatment costs will be billed to the patients insurance.

17.1 Privacy

MSKCC's Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.2 Serious Adverse Event (SAE) Reporting

Any SAE will be reported to the IRB/PB as soon as possible but no later than 5 calendar days. The IRB/PB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office at sae@mskcc.org. The report will contain the following information:

Fields populated from CRDB:

- Subject's name (generate the report with only initials if it will be sent outside of MSKCC)
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The PI's signature and the date it was signed are required on the completed report.

17.2.1

SAEs (as defined above) will be reported to the sponsoring company (Novartis Corp) in a deidentified manner within 15 business day of the event as outline the companies standard operating procedures. This report will include:

- Protocol number and title
- The date the adverse event occurred
- The adverse event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject's condition
    - Indication if the subject remains on the study
    - If an amendment will need to be made to the protocol and/or consent form.

The SAE will also be reported to the FDA through the IND Office and that report will include the FDA assigned IND number and name.

18.0 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form.

Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

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20.0 APPENDICES

- Appendix A - QOL Survey
- Appendix B – QOL scoring
- Appendix C – Laboratory Requisition Forms