### 1.1.6. Plans for This Study

This study will address safety, feasibility and toxicity, and plan for the investigation of clinical (or immunological) responses. As mentioned above, we have published results of a study finding widespread clinical regressions in breast cancer. Results from the current study will possibly support or preclude expansion to Phase II and Phase III studies under this IND.

At the initiation of dosing in this study, the Food and Drug Administration (FDA) requested that the first three patients be dosed sequentially, with at least 2 weeks between patients, to evaluate the safety of SV-BR-1-GM. This phase of the study was completed as of 27 September 2017 and no safety issues related to SV-BR-1-GM were noted that would limit continued dosing in these patients or preclude dosing additional patients. With this phase of the study completed, the second phase of the study commenced where patients can be enrolled and dosed simultaneously. This marks the transition from the Phase I to the Phase IIa part of the study.

In addition, one patient developed a fatal serious adverse event on study. The patient was a 54-year-old woman with metastatic breast cancer involving one breast, including cutaneous metastases, and the pleura with a large pleural effusion. Prior treatment had included neo-adjuvant docetaxel, carboplatin, trastuzumab, pertuzumab and capecitabine. She came in for cycle 2 of cyclophosphamide. She had evidence of a clinical response in her cutaneous metastases. She was scheduled to have a pleurex catheter placed for chronic, recurrent pleural effusion. Breathing was at baseline (mild dyspnea). She received cyclophosphamide. Two days later the patient presented to clinic with complaint of weakness and shortness of breath, worsening over the last 2 days. The patient had not had much to eat or drink, this is typical of when she has had chemo before, similar to cycle 1 of cyclophosphamide. Urine appeared very concentrated and 1 liter of normal saline was administered in clinic along with oxygen. On exam she had a blood pressure of 113/70s, pulse 90s. Her left pleural effusion (on physical exam) was ~1/2 lung field. She had trace bipedal edema, which she also noted following her cycle 1 dose. She was advised to go to the emergency room to have the pleural effusion drained. The patient received SV-BR-1-GM following which she was stable with continued dyspnea and sent to the emergency room. In the emergency department, the patient was evaluated, and her initial intake vital signs showed a temperature of 35.4°C, a pulse of 71, a blood pressure of 97/72, a respiratory rate of 14, with 98% oxygen saturation on room air. On physical exam, she was noted to be distressed and tachycardic with decreased breath sounds on her left, consistent with the known malignant pleural effusion. Labs were remarkable for hyperkalemia, lactic acidosis, hypoproteinemia, transaminitis and leukocytosis. An initial chest x-ray was read as “Near complete white out of the left hemithorax…Heart size cannot be accurately assessed.” ECG findings included sinus tachycardia and low voltage QRS. Approximately 3 hours after her initial arrival to the emergency department, the subject went into pulseless electrical activity (PEA) arrest. CPR was initiated, the patient was intubated, and a left chest tube was placed. During the code, a limited transthoracic echocardiogram was obtained. With only subcostal windows, the patient was noted to have global left ventricular hypokinesis (ejection fraction estimated at 10%), “RV collapse during systole in the presence of a circumferential small volume pericardial effusion.” The patient had return of circulation with support of an epinephrine drip. A CT scan (without IV contrast) was obtained that was read as having “near complete consolidation of both lungs…Moderate to large pericardial effusion.” The patient remained on vasopressor medications in the ICU; however, she continued to decline clinically and ultimately

2. To gather data in support of further investigations blood and urine will be stored in a tissue repository for future analysis. Studies anticipated will include, but are not limited to histocompatibility characterization, levels of circulating cytokines antibodies and cell mediated immune responses.
3. To measure the quality of life (QOL) of participants and the effect the biological agent may have on the subjective sense of well-being. To measure changes in weight, performance status, and pain.

### 1.2.3. Rationale

A recent review describes a number of studies using cancer cell targeted immunotherapies genetically modified to produce various cytokines, including GM-CSF [75]. These reports add to the understanding of safety and tolerability for GM-CSF-transfected whole-cell targeted immunotherapies and reinforce the notion that the method of GM-CSF introduction appears to have several important advantages over subcutaneous injections of the exogenous cytokine: a) it is less labor-intensive, b) it is less likely to cause the side-effects associated with systemic GM-CSF injections, c) local cytokine concentration is more stable and long-lasting, and d) cytokine is directly released to activate APC at the site of targeted immunotherapy injection.

Thus, given the current data regarding GM-CSF secreting breast cancer cell line SV-BR-1-GM with several desirable features developed in our laboratory, we propose this clinical investigation. SV-BR-1-GM will be injected intradermally to potentiate activation of *in-situ* APC. Interferon-alpha-2b (Merck) will be used subsequently as an adjuvant, since it serves as a "danger signal" and has been shown to facilitate the maturation of APC precursors into functionally active dendritic cells [11]. Patients will be premedicated with low-dose cyclophosphamide because of its effect on T-regulatory cell activity [8, 9, 10, 76] and potential synergism with the targeted immunotherapy process by fostering cytokine responses, induction of MHC antigens on tumor cells or other mechanisms not yet identified [77].

### 1.2.4. Hypothesis

Based on preliminary results of the sponsor's previous studies of SV-BR-1-GM, it is hypothesized that this biological agent will be safe, well-tolerated, and clinically efficacious for metastatic breast cancer patients.

#### 1.2.4.1. Success Measures

Success of the study in meeting the above hypothesis will be defined according to traditional FDA criteria. We will investigate the likelihood of objective responses and will apply the combined RECIST/iRECIST criteria as recently studied [78]. The key to success at this stage will be to compile an adequate pool of data to merit FDA recognition of "proof of principle" sufficient to warrant expansion to the next level of study (i.e. traditional Phase II and/or Phase III).

To this end, while the core success measure is safety and lack of toxicity, any of the following may be applied as Success Measures:

1. Objective clinical response as defined by RECIST 1.1 criteria or iRECIST criteria in 25% of patients.
2. Improvement in quality of life in 50% or more patients as evidenced by significant change in 1 or more scales in the SF-36 questionnaire.
3. Prolongation of disease-free and overall survival as compared with historical controls from reports of other salvage therapies in the published literature.

Evidence of development or amplification of immune responses, especially if correlating with prolongation of survival.

are considered clinically meaningful, require therapy (e.g., hematologic abnormality that requires transfusion), or require changes in the study drug(s).

Adverse events that begin or worsen after informed consent should be recorded on the Adverse Events page of the CRF. Conditions that were already present at the time of informed consent should be recorded on the Medical History page of the CRF. Adverse event monitoring should be continued for at least 30 days after the last dose of study drug. Adverse events (including laboratory abnormalities that constitute AEs) should be described using a diagnosis whenever possible rather than by individual underlying signs and symptoms. When a clear diagnosis cannot be identified, each sign or symptom should be reported as a separate AE.

Adverse events will be assessed according to the CTCAE version 4.03. The occurrence of AEs should be sought by nondirective questioning of the subject during the screening process after signing the ICF and at each visit during the study. Adverse events may also be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. As far as possible, each AE should be evaluated to determine:

- The severity grade (CTCAE Grade 1 to 5).
- Reasonable possibility that the AE is related to the study treatment: unrelated (no) or related (yes).
  - Note that when SV-BR-1-GM is used in combination with other agents (e.g. cyclophosphamide, interferon-α), the relationship to study drug can be assessed for SV-BR-1-GM alone, the other study medications alone, or the combination of agents.
- Start and end dates, unless unresolved at final examination.
- Action taken with respect to study drug (e.g., none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable).
- Outcome (e.g., not recovered/not resolved, recovered/resolved, recovering/resolving, recovered/resolved with sequelae, fatal, unknown).
- Whether it is serious, as per serious adverse event (SAE) definition provided in Section 8.3.1.

Unlike routine safety assessments, SAEs are monitored continuously and have special reporting requirements, see Section 8.3.2.

All AEs should be treated appropriately. If a concomitant medication or nondrug therapy is given, this action should be recorded on the AE and Prior/Concomitant medications pages of the CRF.

Once an AE is detected, it should be followed until it has resolved or until it is judged to be permanent; assessment should be made at each visit (or more frequently if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Disease progression should not be regarded or reported as an AE itself, unless it is associated with a separate AE.

- Social reasons and respite care, in the absence of any deterioration in the subject's general condition.
- Any SAEs that are expected due to the condition being treated, including if the SAE is a primary outcome measure, or where there has been a clear agreement with regulators not to consider these as SAEs, provided the information is collected elsewhere.

The Sponsor will notify FDA and all participating investigators in an IND safety report of potential serious risks identified, from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after determination that the information qualifies for reporting, as delineated in CFR 312.32 (available at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.32). Unexpected fatal or life-threatening suspected adverse reaction reports. The Sponsor will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the Sponsor's initial receipt of the information. To ensure subject safety, every SAE, regardless of suspected causality, occurring after the subject has signed the ICF and up to the last study visit, or up to 30 days after the subject has stopped study treatment, whichever is later, must be reported to the sponsor (or designee) within 24 hours of learning of its occurrence. Any SAEs experienced after this period should be reported to the sponsor (or designee) only if the investigator suspects a causal relationship to the study drug. Recurrent episodes, complications, or progression of the initial SAE must be reported as the follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. Previously planned (before providing informed consent) surgeries should not be reported as SAEs unless the underlying medical condition worsens over the course of the study.

Information about all SAEs is collected and recorded on the Serious Adverse Event Report Form. The investigator must assess and record the relationship of each SAE to each specific study drug (if there is more than 1), complete the SAE Report Form in English, and send the completed, signed form by fax within 24 hours to the sponsor or its designee. The investigator must assess if there is a reasonable possibility that the SAE is related to the study treatment: unrelated (no) or related (yes).

Note that when SV-BR-1-GM is used in combination with other agents (e.g. cyclophosphamide, interferon-α, ipilimumab, pembrolizumab), the relationship to study drug can be assessed for SV-BR-1-GM alone, the other study medications alone, or the combination of agents.

Serious AEs related to unblinded comparator drugs or concomitant medications/drug delivery systems are reported directly to the manufacturers of those drugs/devices in accordance with the package insert.

The telephone and facsimile number of the sponsor's contact persons, specific to the study, are listed in the investigator folder provided to each site. The original copy of the SAE Report Form and the fax confirmation sheet must be kept with the CRF documentation at the study site.

Follow-up information is sent to the same person to whom the original SAE Report Form was sent, using a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. Each recurrence, complication, or progression of

No funds have been provided or set aside in case of injury to a subject. Each subject (primarily their insurance or Medicare) will be responsible for usual and customary fees regarding the procedures, supplies used in this study, and office visits, with the exception of the experimental therapy. There will be no charges for preparation or administration of SV-BR-1-GM, but there will be routine charges for an office visit, imaging, laboratory tests, etc.

### 4.6.4. Adverse Events of Special Interest

An Adverse Event of Special Interest (AESI) is to be reported on the SAE form and timelines for submission and processing are expected to be the same as a reported SAE. Adverse Events of Special Interest include the following:

- New or worsening Autoimmune Disease
- Major cutaneous reactions at the inoculation sites (e.g., ulcers, necrosis)
- Allergic reactions to SV-BR-1-GM
- Cardiac events

## 4.7. BENEFIT TO SUBJECTS & OTHERS

This is a Phase I/IIa safety and efficacy study of an experimental treatment that has not yet been proven. No direct benefits to subjects can be anticipated. Even though this experimental treatment is based on current medical science theories, it is not possible to predict the outcome in advance. Possible benefits may include improvement in immune function or possible tumor regression, or possible improvement in time to progression or survival after treatment. However, each person is unique and results are not predictable.

# 5. STUDY PROCEDURES, METHODS, AND MATERIALS

## 5.1. RECRUITMENT

It is expected that patients will sometimes be referred by clinicians who know the sponsor or affiliated investigators, or they may self-refer after an internet search. The sponsor will refer all patient inquiries to an appropriate principal investigator.

Potential participants will be informed that this program is experimental and that an evaluation on site is necessary to make the final decision on whether a patient qualifies for the program. In addition, each potential participant is to be clearly informed that travel costs and other expenses associated with the screening, eligibility, and study procedures will not be compensated without pre-approval of the Sponsor. Prospective participants will be denied participation if all eligibility criteria are not met or, if after screening and evaluation the principal investigator or the patient's personal physician feels some other therapy is more appropriate.

## 5.2. CONSENT PROCESS

a) The investigational trial site staff will explain the study, its procedures, and the consent documents to patients.
b) Potential participants will be given an opportunity to read the consent documents, to have their questions answered to their satisfaction, and to defer a decision, perhaps to discuss study participation with their physician or significant others.
c) It will be carefully explained to participants that they may withdraw from participation at any time, without prejudice or jeopardy to their standard medical care.
d) In all cases, prior to initiation of treatment, participants are again queried regarding their understanding of the program, or any unresolved issues. A copy of the consent form will be provided to the participant, and this is to be noted within the electronic case report form (eCRF).

## 5.3. SCREENING PROCESS

Written informed consent will be obtained before any screening procedures are initiated. Study staff may explain the study to participants and ascertain presumptive eligibility. Multiple visits may be conducted to complete all required procedures if necessary. Due to the delay in receiving the results of tests, the final eligibility decision cannot be made during the participant’s initial screening visit.

a) After review of all screening information, an entry will be made in the Screening & Enrollment Log for all participants who consent to be in the study, whether or not they qualify after the screening procedures.

b) If any participant is determined to meet preliminary eligibility criteria, and expresses willingness to continue with the study, she will proceed. Detailed instructions, study-specific procedures and study calendar will again be explained to qualified participant.

11. CEA and CA 27.29 (or CA 15-3 if preferred by the Investigator).
12. Brain scan MRI or CT technique if clinical indication of neurological symptoms.
13. Serum beta-HCG pregnancy test within 7 days before starting treatment for women of childbearing potential.
14. Immunohistological and FISH evaluation, if not already performed by a referring physician, of HER2/neu expression from whatever previous biopsy was diagnostic of cancer metastases.
15. If tumor tissue is available for additional testing, it may be sent for evaluation of additional markers such as PDL1, PRAME, HLA-A, HLA-B, HLA-C, HLA-DR, HLA-DP, and/or HLA-DQ.
16. If malignant fluids (e.g. pleural fluid, ascites) or tumor tissue (e.g. from biopsies) are available, unused or unneeded portions may be collected and archived for future research, provided nothing is done to compromise good clinical practice for the intended diagnostic evaluation. Possible research may involve characterization studies (e.g. antigenicity, genomic evaluations, transcriptome or proteome analyses) or establishment of cell lines.

During the initial phase of experimental therapy, participants will get SV-BR-1-GM injected and closely observed about every 2 weeks at the investigational sites. Subsequent inoculations occur at monthly intervals. Data will be recorded on eCRF for safety monitoring and/or relevant toxicity evaluation.

#### 5.5.2.2. Immunological/Allergy Monitoring

**Anergy Evaluation:** Antigen preparations and suppliers as determined by availability: Candida 0.10 ml (Candin: Niesen Biosciences, San Diego, CA. nielsenbio.com). The erythema and induration reaction will be measured and captured in the EDC at approximately 2 days (to coincide with the Interferon-alpha-2b injection appointment).

**Delayed-type hypersensitivity (DTH) reaction - tumor-specific:** An aliquot of 1.0 (± 0.2) x $10^6$ non-transfected irradiated SV-BR-1 tumor cells or 1.0 (± 0.2) x $10^6$ irradiated SV-BR-1-GM cells, and, if available, irradiated autologous tumor cells, prepared identically as original source of SV-BR-1 (BriaTest™), each in 0.1ml of LRS will be injected intra-dermally into the patient's arm on the day of SV-BR-1-GM injection.

At the time of testing, the cutaneous tumor-cell injection will be observed for the possibility of immediate hypersensitivity reactions, and the patient will be monitored for 20 minutes or more before proceeding with full dose SV-BR-1-GM injection. Any evidence of immediate hypersensitivity, such a urticaria, will preclude administration of full dose SV-BR-1-GM. The erythema and induration reaction will also be measured approximately 2 days later (to coincide with the Interferon-alpha-2b (Merck) injection appointment) and captured in the EDC.

#### 5.5.2.3. Cutaneous Monitoring Criteria

Immediate hypersensitivity is defined as a 2x increase of the two largest measured diameters of the initial injection wheal, occurring within 20 minutes, or the development of systemic symptoms of wheezing, additional zones of urticaria remote from the injection site, or generalized pruritus. Immediate hypersensitivity precludes further SV-BR-1-GM treatment on

Because immunological therapies may sometimes require a lengthy time interval to observe response, our concept emphasizes observation over a period of several months. The key evaluation is that which occurs two weeks after the last SV-BR-1-GM in the initial treatment phase. This evaluation, which occurs at month 3, is to identify if there is progressive tumor growth refractory to current treatment. Patients will continue 6 months of treatment, the decision to cease or continue treatment occurs after the 6-month re-evaluation.

Measurable lesions (together with immunological indices) will be evaluated after about 3 months from initiation of therapy and every 3 months of treatment. Performance status and quality of life will be documented regularly using the ECOG/WHO performance scale and the widely used, validated [74] SF-36 questionnaire.

For a list of potential procedures to be performed, see the Procedure and Form Itemization in section 8.

The final visit will be the off-treatment visit, (at the conclusion of approximately 6-12 months of experimental treatment or whenever the patient comes off study).

## 5.6. STUDY VISITS & PROCEDURES

For a detailed itemization of study visits and procedures, see section 8, “Procedure Itemization”

## 5.7. DATA COLLECTION

Relevant study data will be collected on each subject and entered into an Electronic Data Capture (EDC) system. The URL for the EDC system is https://login.medrio.com. Supported operating systems are Microsoft Windows XP, Microsoft Windows 2000, Microsoft Windows 7 and 8, Microsoft Vista, and Mac OS X. Microsoft Internet Explorer (version 9 and later), Firefox (version 27 and later), Chrome (version 30 and later), and Safari (version 9.0 and later) are acceptable web browsers.

Medrio software supports compliance with regulations such as 21 CFR Part 11, Annex 11, EU Safe Harbor, Good Clinical Practice, and HIPAA. Procedural controls include electronic audit trails of all changes to study data, electronic signatures, data encryption, and access restrictions to Protected Health Information (PHI). Access to the Medrio EDC system will only be granted to study personnel after training is conducted. User accounts are permission based which grants limited access and functionalities dependent upon permissions granted by study administrator. All data that goes into and out of the server is encrypted using 128-bit Secure Sockets Layer (SSL) and 1024-bit RSA public keys. The servers, hosted by Medrio, are housed in a fully redundant N+1 data center with redundant power, cooling, and network connectivity. All servers reside behind Cisco firewalls and advanced intrusion detection/prevention systems. Medrio performs nightly backups to locations both on-site and off-site and maintains redundant hardware.
Data collection will begin at the signing of informed consent. All data must be submitted within 72 working hours of the visit in which data was collected. Edit checks (often called queries)

will fire in real time as data is being entered to ensure quality data is provided. In addition to edit checks, manual queries will be generated by Data Managers and Monitors. Sites will have 10 working days to address all edit checks and manually created queries. Source Data Verification (SDV), Data Management Review and electronic signature by site PI will all take place prior to locking data.

## 5.8. CONFIDENTIALITY& RECORDS

### 5.8.1. General Precautions

The confidentiality of subjects will be protected. Names and other identifying information will not appear on any study forms, except the Informed Consent, HIPAA Authorization and the Enrollment Log, where subject names will be matched to their unique Study Number.

Subsequent study forms and any laboratory tests will be identified only by a unique Study Number assigned to each subject, which is recorded on the EDC (see section 9.1.6).

The source documents (which meet the record-keeping requirements of §312) are maintained and protected in a fireproof file cabinet in a locked room. Written research records will be kept in a locked room, in a locked file cabinet accessible only to the principal investigator and only those members of staff with specific project responsibilities. Research charts will not be labeled by patient name but rather by unique identification code. Records will be maintained for at least 7 years after completion of the drug investigation.

Research data will be stored and analyzed by computer. The research team members will be the only people to have access to the computer file, which is protected with a confidential password. Computer records are not totally immune to review by unauthorized persons, (hackers), but will be protected by password and unique ID of each patient.

Research records may be also be reviewed by government organizations such as the Food and Drug Administration, the Institutional Review Board, and other persons who are required to watch over the safety and effectiveness of medical products and therapies or the conduct of research. If the research record is reviewed by any of these groups, there may be a need to examine the entire medical record and/or hospital charts. Under certain circumstances, Congress or a court order could obtain research records. While every effort will be taken to keep information confidential, under special circumstances, this could mean public disclosure.

### 5.8.2. Study Information Leaving the Offices of the Investigator

All identifying information will be stripped from study records provided to the Study Monitor. Data on a subject generated by other physicians and by the Principal Investigator will be provided to the Study Monitor only after being de-identified. No consent forms or other documents identifying the subjects in any way will be provided to the Study Monitor, except to be provided at the site during a monitoring visit. The Study Monitor will have access to all study records to verify proper conduct of the study.

## 7.3. RESPONSE DEFINITIONS

Both objective tumor response (CR or PR) and immunological response will be considered as evidence of activity. Criteria for clinical response have been described in detail in the discussion of RECIST and iRECIST. Three tests will be used for evaluation of SV-BR-1-specific immunological response. Serum antibody levels against live SV-BR-1 cells will be measured by cell suspension ELISA. T-cell mediated response will be measured by delayed type hypersensitivity (DTH) skin test and by flow cytometry. The latter test is designed to calculate the frequency of peripheral blood T cells producing interferon-gamma in response to stimulation with monocytes pulsed with SV-BR-1 cell lysate. For cell suspension ELISA and flow cytometry assay, a 1.5-fold increase from pre-SV-BR-1-GM to post-SV-BR-1-GM level is considered evidence of response [87]. For DTH, an induration of >5mm in diameter is commonly considered evidence of reaction [83], although erythema has been considered informative in one recent report [88]. If a patient has either a clinical response or an immunological response on any of the three measures, he/she will be considered to have a biological response. In addition, data may later be analyzed using the recently published iRECIST criteria [89].

These criteria for response (as well as an alpha error rate of 0.10) are being used because this is an early trial of a new regimen that is expected to be well tolerated and is being used in patients who have failed the standard therapeutic approaches. Their survival time is expected to be limited under any circumstances, and it is important not to miss a possibly active treatment regimen. Insofar as the patients for this regimen will have very advanced cancer, the absence of toxicity will be considered very significant and will encourage consultation with the FDA for advice for planning further investigations in patients with more favorable prognoses and more robust immune capabilities.

Also, early termination can occur based on toxicity, as described earlier in the toxicity section.

## 7.4. DATA ANALYSIS

A variety of statistical analyses will be performed to assess the relationship between clinical response, immunological response, and possible prognostic factors. The appropriate statistical test will be determined by the bio-statistician.

Multiple regression and/or Cox regression will be performed to identify factors predictive of response if the number of subjects entered into the study so permits. This may include logistic regression when using response as the endpoint and Cox regression when using survival time. Other parametric and nonparametric tests will be used as appropriate to evaluate relationships of interest. For all tests, criterion for statistical significance will be set at $p < 0.05$, two-tailed test.

The principal investigator will review clinical and laboratory data at the times of reimaging and restaging or earlier as needed. Protocol deviations will be entered onto a form to be included in the regulatory binder. Missing data, patient absence or other non-compliance will be documented in a Note to File, copied to the IRB, and included in the annual report unless safety concerns warrant prompt FDA filing as per the criteria for Serious Adverse Events.

# 8. PROCEDURE AND FORM ITEMIZATION

## Cycle I

| Day | Procedure |
|---|---|
| Within 2-4 weeks before starting Initial Treatment Phase (ITP) | Get blood tests and imaging tests. HLA profile if not done already; Serologic Markers; Urinalysis.<br>Blood /specimens for research.<br>Baseline physical and medical history.<br>Decision about eligibility.<br>SF-36 "Quality of Life" form.<br>Explanation of program, questions answered, study enrollment forms reviewed and signed. (Section 9 for forms)<br>Cardiac assessments: baseline ECG, cardiac troponin (cTn), NT-proBNP and an estimation of left ventricular ejection fraction (LVEF) by either cardiac echo or MUGA scan. |
| 2-3 days before starting ITP | Get routine blood tests. Serologic Markers; Urinalysis.<br>Blood/specimens for research.<br>Abbreviated physical exam (only if not performed within the past 2 weeks).<br>Beta-HCG serum pregnancy test for women of childbearing potential within 7 days of first SV-BR-1-GM.<br>SF-36 "Quality of Life" form.<br>Have low-dose cyclophosphamide (Cytoxan) premedication (to possibly enhance immune response). |
| Day 0 | **Start of Initial Treatment Phase (ITP)**<br>Anergy test. (to occur with cycles 1, 3, 6, 9, 12)<br>Have DTH (~$10^6$ BriaTest™ or SV-BR-1-GM) and anergy (Candin) skin test injections in forearm. Evaluate for immediate hypersensitivity to BriaTest™ or SV-BR-1-GM.<br>Receive injections of full dose SV-BR-1-GM into skin of right and left thighs and two places on upper back (unless allergy to skin test is seen). |
| Day 2 & 4 (±1) | Evaluation of skin tests (anergy to Candin and DTH to BriaTest™ or SV-BR-1-GM) and of SV-BR-1-GM injection sites.<br>Interferon-alpha-2b (Merck) injection into SV-BR-1-GM sites on day 2 (± 1 day) and 4 (± 1 day) to possibly enhance immune response. |

## Cycle 2

| Day | Procedures |
|---|---|
| Day 11 or 12 | Brief physical and update of medical history. |

**Step 4: Screening Tests & Procedures**

***Is the subject eligible to be in this study, based on the results of:***

**Focused medical & social history**

□ Yes □ No □ N/A

Date done:

**Complete history physical exam & ECOG scale**

□ Yes □ No □ N/A

Date done:

**Blood tests**

□ Yes □ No □ N/A

Date done:

**Serum beta-HCG pregnancy test (within 7 days of SV-BR-1-GM start date)**

□ Yes □ No □ N/A

Date done:

**Immunohistological evaluation**

□ Yes □ No □ N/A

Date done:

**MRI or mammogram of the breast**

□ Yes □ No □ N/A

Date done:

**CT scan chest, abdomen and pelvis**

□ Yes □ No □ N/A

Date done:

**CA 125**

□ Yes □ No □ N/A

Date done:

**CEA, CA 27.29 (or CA 15-3)**

□ Yes □ No □ N/A

Date done:

**Urinalysis**

□ Yes □ No □ N/A

Date done:

**HL-A profile**

□ Yes □ No □ N/A

Date done:

**SF-36 Questionnaire**

□ Yes □ No □ N/A

Date done:

**Chest X-ray**

□ Yes □ No □ N/A

Date done:

died. No autopsy was performed. The Investigator and the Sponsor both noted the event was unlikely related to study drug.

## 1.2. STUDY DESIGN

This study incorporates elements of Phase I design such as toxicity and immunological/allergy monitoring, which will measure the overall tolerability of the experimental therapy. In addition, Phase II elements such as clinical efficacy - determined as RECIST and iRECIST criteria - will be studied.

### 1.2.1. Study Purpose

The purpose of this study is to gather preliminary data to evaluate the safety, tolerability, and feasibility of targeted immunotherapy for advanced breast cancer with a special consideration for CNS metastasis using allogeneic breast tumor cell line transfected with the GM-CSF gene (SV-BR-1-GM). This experimental therapy will be evaluated in a small initial cohort of 25-40 subjects with qualifying stage IV breast cancer. SV-BR-1-GM is inoculated by intradermal injections with subsequent local injection of low-dose Interferon-alpha-2b (Merck).

### 1.2.2. Study Objectives

As this design incorporates elements of a preliminary safety/toxicity study, clinical response, duration of response, and survival are not primary endpoints of the protocol, but such data will be monitored for possible support of further research plans. Similarly, quality of life (QOL) will be assessed using a standardized questionnaire [73, 74] (See SF-36 Health Survey)

To evaluate the performance of the experimental therapy, the following objectives will be assessed.

#### 1.2.2.1. Primary Objectives

To evaluate the number, frequency, duration, and relation of toxicity events to SV-BR-1-GM, as defined by CTCAE and additional tests as described in section 5.5.2.

#### 1.2.2.2. Secondary Objectives

1. To evaluate tumor response:
   a. Objective response rate (ORR), defined as complete response (CR) or partial response (PR) per RECIST and iRECIST response criteria
   b. Non-progressive rate, defined as CR, PR or stable disease (SD) per RECIST and iRECIST
   c. Durability of response, by evaluating those patients eligible to complete the optional treatments from 9-12 months.

#### 1.2.2.3. Exploratory Objectives

1. To assess immune responses to SV-BR-1-GM, and to recall antigens, if any, as measured by DTH skin tests and/or other immunological tests.

# 2. STUDY PLAN

## 2.1. TRIAL SITES

The investigational sites will screen patients and enroll within trial. The treatments will be administered to patients at the investigational sites. All scans will be obtained at the investigational sites and evaluated by the site PI. All blood tests will be performed at that the investigational sites or using major CLIA certified national laboratories. The results will be entered into the EDC.

## 2.2. STUDY POPULATION

### 2.2.1. Subjects

The subjects will be females age 18 or older, who are diagnosed with metastatic breast cancer and qualify via the inclusion and exclusion criteria. A sufficient number of subjects will be enrolled to assure a minimum of 10 for initial safety analysis and then expanded to 25-40 to complete the primary, secondary and exploratory end points. Patients who do not reach the initial on treatment tumor assessment may be replaced at the Sponsor's discretion.

### 2.2.2. Source of Subjects

New subjects may be enrolled into this study from several sources, including but not limited to:

1. From the investigational treatment sites, as qualifying patients present and are referred.
2. From physician referrals to the trial.
3. From the NCI-PDQ website.
4. From patient advocacy organizations or any other pertinent agencies.

### 2.2.3. Recruitment of Subjects

(See also section 5.1 for more details.) IRB-approved recruitment materials may be used to approach potential subjects during the screening period, as they present to the investigational treatment sites. These recruitment materials will also be provided to potential referring physicians, at their request. The consent form and attached study calendar; a brochure of frequently asked questions and answers; the enrollment forms are included in section 9.

## 2.3. ELIGIBILITY CRITERIA

### 2.3.1. Inclusion Criteria

1. Have histological confirmation of breast cancer with recurrent and/or metastatic lesions via investigational site.

**** Patients with new or progressive breast cancer metastatic to brain will be eligible provided:

a. There is no need for steroids and patients have not had steroids at least 2 weeks
b. No individual tumor size is >50 $mm^3$

## 4.4. LABORATORY TEST ABNORMALITES

### 4.4.1. Definitions and Reporting

Laboratory abnormalities that constitute an AE in their own right (are considered clinically meaningful, induce clinical signs or symptoms, require concomitant therapy, or require changes in study drug), should be recorded on the AE page of the CRF. Whenever possible, a diagnosis rather than a symptom should be provided (e.g., anemia instead of low hemoglobin). Laboratory abnormalities that meet the criteria for AEs should be followed until they have returned to normal or an adequate explanation of the abnormality is found. When an abnormal laboratory test result corresponds to a sign or symptom of a previously reported AE, it is not necessary to separately record the laboratory test result as an additional event.

Laboratory abnormalities that do not meet the definition of an AE should not be reported as AEs. A Grade 3 or 4 (severe) AE, as per CTCAE, does not automatically indicate an SAE unless it meets the definition of serious, as defined in Section 8.3.1, and/or per the investigator's discretion. A dose interruption or adjustment for the laboratory abnormality may be required (see Section 5.6) and should not contribute to the designation of a laboratory test abnormality as an SAE.

## 4.5. SERIOUS ADVERSE EVENTS

### 4.5.1. Definitions and Reporting

A SAE is defined as an event that meets 1 of the following criteria:

- Is fatal or life-threatening (i.e., immediate risk of dying).
- Results in persistent or significant disability or incapacity.
- Constitutes a congenital anomaly or birth defect.
- Is clinically meaningful (i.e., defined as an event that jeopardizes the subject or requires potential medical or surgical intervention to prevent 1 of the outcomes listed above). Considered meaningful by the investigator as an important medical event that may not result in death, be life-threatening, or require hospitalization, but may be considered a SAE when, based upon appropriate medical judgment, it may jeopardize the subject or may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition.
- Requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is a result of:
  - Routine treatment or monitoring of the studied indication not associated with any deterioration in condition. Elective or preplanned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the ICF.
  - Treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission.

the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the subject continued or withdrew from study participation, or if study drug was interrupted or discontinued.

If the SAE is not previously documented in the IB for the study drug (new occurrence) and is thought to be related to the sponsor's study drug, a sponsor's associate may urgently require further information from the investigator for reporting to health authorities.

The sponsor or its designee may need to issue an Investigator Notification (IN) to inform all investigators involved in any study with the same drug that this SAE has been reported. Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC, or as per national regulatory requirements in participating countries.

## 4.6. RESPONSE TO ADVERSE EVENTS

All adverse events will be recorded on the Adverse Event Form. Development of treatment related Grade IV toxicity will truncate all new subject accrual until further review by the medical monitor, IRB and FDA as appropriate. Treatment-related Grade III allergy/hypersensitivity will truncate further inoculations to any particular subject. The first three patients will be enrolled sequentially, with at least 2 weeks between patients. After 10 patients are enrolled a safety analysis will be performed. If no significant toxicity has been reported, an additional cohort of 15-30 participants (to a total of 25-40) may be enrolled to evaluate the primary, secondary and exploratory end points.

If any subjects show an unanticipated abnormality on any lab assay or screening procedure, clinical management of their condition will take precedent. Results of testing will be provided to their physician(s) with appropriate medical release.

### 4.6.1. During SV-BR-1-GM Administration

1. ***Pain and/or anxiety***: Subjects will be closely monitored for their reaction to SV-BR-1-GM administration, including any pain at the injection site, general anxiety or discomfort. Appropriate rest periods will be offered, and subjects always have the right to withdraw from the study at any time and/or refuse an injection.
2. ***Other adverse events***: Unanticipated adverse events should be managed as clinically appropriate, and recorded on the Adverse Event Form.

### 4.6.2. After the SV-BR-1-GM Administration

After SV-BR-1-GM inoculation, the subject will be monitored for 60 minutes for any adverse reaction to the SV-BR-1-GM. Unanticipated adverse events should be managed as clinically appropriate, and recorded on the Adverse Event Form.

### 4.6.3. In Case of Injury

c) If any participant is determined to be ineligible, she will be informed by the study staff of the reason(s) for ineligibility.

## 5.4. WITHDRAWAL and TERMINATION CRITERIA

### 5.4.1. Individual Subject Withdrawal Criteria

1. Criteria - Any individual subject can be withdrawn from the study if any of the following occurs:
   a) The subject may request at any time to withdraw from the study, with no prejudice to her medical care at the principal investigator's practice.
   b) The subject is unable to reliably adhere to the study procedures.
   c) The subject exhibits extreme anxiety during the treatment.
   d) Staff members are unable to contact the subject or proxy to collect post-procedure information will account as withdrawal.

2. After providing 'adequate trial' with sufficient time and doses, experimental therapy will be terminated in a subject with significantly progressive disease.

3. In addition, experimental therapy will be terminated in a subject at the discretion of the sponsor or principal investigator if:
   a) The patient/subject demonstrates evidence of progressive disease as per RECIST and iRECIST criteria.
   b) Grade III or IV toxicity of any kind occurs, or
   c) Autoimmune disease (validated) of any toxicity level.

4. Subjects MUST be withdrawn from dosing for any of the following:
   a) Any grade 3 or greater NCI CTC v. 4.03 toxicity at least possibly related to study agent.
   b) Any grade 2 or greater hypersensitivity reaction.
   c) If LVEF drops >20% or is below facility limits of normal or the patient becomes symptomatic of CHF and requires treatment. LVEF assessment may be repeated once at the discretion of the Investigator with the approval of the Sponsor.

### 5.4.2. Study Termination Criteria

The study will be terminated if any of the following occur:
   a) Any death at least possibly related to the study drug.
   b) 2 or more grade 4 events at least possibly related to the study drug.

If either of these occur, recruitment will be halted and dosing of all subjects paused while the case(s) are investigated. Following discussions with the IRB/Ethics Committee and with regulatory authorities, the protocol may be amended. The study may be restarted only with the approval of the IRB/Ethics Committee and regulatory authorities.

that visit, will be treated as appropriate in the clinical judgment of the clinician, and evaluation by an allergist will be encouraged. Further SV-BR-1-GM inoculations are still permissible contingent on recommendations by a board-certified allergist

The presence of an immediate reaction to tumor SV-BR-1-GM may not imply absence of desired delayed-type cell-mediated immune responses. It is not known whether further tumor SV-BR-1-GM immunization might or might not down-regulate the Type I hypersensitivity, analogous to clinical allergy desensitization.

Delayed type hypersensitivity will be assessed at about 2 days after injection. The two largest diameters of induration and erythema will be measured in our clinic by the PI, nurse, or other clinical staff, and each type of response will be evaluated independently. While most investigators consider induration as the relevant response, a recent paper involving melanoma demonstrated improved survival was highly correlated to skin test responses as measured by erythema [84]

#### 5.5.2.4. Tumor and SV-BR-1-GM Specific Immunological Response

**Serum antibodies to SV-BR-I whole cell antigens, and to HER2/ neu:** The antibody titers will be determined by ELISA according to methods described in laboratory SOPs.

**Peripheral blood antigen-reactive T cells to SV-BR-I cell:** The antigen reactive T cells will be determined by flow cytometry or by other tests (e.g. ELISpot).

#### 5.5.2.5. Collection of Biological Specimens for Ongoing Research

Biological specimens will be collected from patients who provide additional consent using the form in section 9.1.3. Collection will take place during study visits according to the schedule in Table 2 and will take place in the offices of investigational institutions. Whenever possible, specimen collection will take place during the same venipuncture procedures as for the toxicity studies, so that only one blood draw procedure need be performed during any visit. Urine will be collected via urine specimen cup using the clean catch method.

Specimens to be collected include:

- 1 red top (~8ml), 1 purple top (~8 ml), and 3 yellow top (citrate) vacutainer tubes (3x ~8 ml).
- 2 CellSave Preservative Tubes (https://www.cellsearchctc.com/product-systems-overview/cellsave-preservative-tubes) (~10 ml each) – One tube may be collected at the Investigator's discretion if necessary to protect patient safety.
- Urine specimen cup (~100ml or larger).

Phlebotomy of peripheral blood will be collected by credentialed staff (RN, LVN, etc.) according to community practice. Serum (approximately 8 ml) will be collected in commercially available vacuum tubes (red top) used for serum collection, purple top tube (approximately 8ml) used for plasma and an additional amount will be collected in commercially available citrate vacuum tubes for harvest of peripheral blood lymphocytes (approximately 24ml) and for evaluation of

## 5.9. COMPENSATION

Subjects will not be paid for their participation. Compensation for travel or other expenses associated with study procedures require pre-approval from the Sponsor. Patients (or their insurance, e.g. Medicare) are expected to be responsible for all usual and customary fees associated with office visits, study tests and procedures, and follow-up costs, as is customary for national cancer trials.
There will be no fee attached to the preparation or quality control testing of the SV-BR-1-GM, but standard fees for doctor visits and nurse visits will apply.

## 5.10. SPECIMEN LABELING INFORMATION

Laboratory specimens and test results will be coded only with a unique identifying number (i.e. "Study Number"), date of collection, and the protocol number. The Consent Forms and Enrollment Log will be the only places on which subject names will be linked to their Study Number. (Names and other identifying information of screening failures will not be recorded.). Biological specimens will be stored according to the sponsor's laboratory SOPs, and used for ongoing research only as specified in the supplemental enrollment form, "Consent for the Use of Blood or Tissue." (See section 9)

| | |
|---|---|
| | Get routine blood tests.<br>SF-36 "Quality of Life" form.<br>Low-dose cyclophosphamide (Cytoxan) |
| Day 14 | Have DTH (~$10^6$ BriaTest™ or SV-BR-1-GM) skin test injections in forearm. Evaluate for immediate hypersensitivity. Any evidence of immediate hypersensitivity precludes dosing with SV-BR-1-GM.<br>Receive injections of SV-BR-1-GM into skin of right and left thighs and two places on the upper back. |
| Day 16 & 18 (±1) | Evaluation of skin tests (DTH to BriaTest™ or SV-BR-1-GM). Evaluation of SV-BR-1-GM injection sites.<br>Interferon-alpha-2b (Merck) injection into SV-BR-1-GM sites on 2 days (± 1 day) and 4 days (± 1 day) after SV-BR-1-GM to possibly enhance immune response. |

## Cycle 3

| Day | Procedures |
|---|---|
| Day 25 or 26 | Brief physical and update of medical history.<br>Get routine blood tests.<br>SF-36 "Quality of Life" form.<br>Low-dose cyclophosphamide (Cytoxan). |
| Day 28 | Have DTH (~$10^6$ BriaTest™ or SV-BR-1-GM) and anergy (Candin) skin test injections in forearm. Evaluate for immediate hypersensitivity to BriaTest™ or SV-BR-1-GM. Any evidence of immediate hypersensitivity to BriaTest™ or SV-BR-1-GM precludes dosing with full dose SV-BR-1-GM.<br>Anergy test (Candin) is to occur with cycles 1, 3, 6, 9, and 12.<br>Receive injections of SV-BR-1-GM into skin of right and left thighs and two places on the upper back. |
| Day 30 & 32 (±1) | Evaluation skin tests (anergy to Candin and DTH to BriaTest™ or SV-BR-1-GM) and of SV-BR-1-GM injection sites<br>Interferon-alpha-2b (Merck) injection into SV-BR-1-GM sites on 2 days (± 1 day) and 4 days (± 1 day) after SV-BR-1-GM to possibly enhance immune response. |

## Cycle 4

| Day | Procedures |
|---|---|
| Day 53 or 54 | Brief physical and update of medical history.<br>Get routine blood tests.<br>SF-36 "Quality of Life" form.<br>Low-dose cyclophosphamide (Cytoxan). |
| Day 56 | Have DTH (~$10^6$ BriaTest™ or SV-BR-1-GM) skin test injections in forearm. Evaluate for immediate hypersensitivity. Any evidence of immediate hypersensitivity precludes dosing with full dose SV-BR-1-GM. |

**Isotope bone scan, and/or selected bone X-rays**

□ Yes □ No □ N/A

Date done:

**Brain scan MRI or CT technique**

□ Yes □ No □ N/A

Date done:

Explain any non-eligibility factors, exceptions, or deviations from protocol (notify the IRB):

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**Step 5: Eligibility Status**

□ Eligible Final eligibility status

□ Ineligible

If ineligible, what was the reason?

**Step 6: Signatures**

| | **Principal Investigator** | **Person who collected information on this form**. |
|---|---|---|
| **Printed Names** | | |
| **Signatures** | | |
| **Date / Time** | | |

**Step 7: FILE the original form in the study files for this participant.**