A Pilot Trial of a WT1 Analog Peptide Vaccine in Patients with Multiple Myeloma following Autologous Stem Cell Transplantation

MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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

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1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This a pilot study investigating the immunological response and safety of a multi-valent WT1 peptide vaccine administered as post remission therapy in patients with multiple myeloma following autologous stem cell transplantation. The vaccine consists of native and synthetic WT1-derived peptides and the immune adjuvant Montanide ISA 51 VG. Sargramostim (GM-CSF) will be used to increase immunogenicity. The primary study endpoint is immunologic response at 12-14 weeks post initial vaccination. Secondary study endpoints include safety, disease-free and overall survival, as well as WT1 expression on plasma cells by immunohistochemistry (IHC) and minimal residual disease as assessed by RT-PCR measurement of WT1 transcript in the bone marrow.

2.0 OBJECTIVES AND SCIENTIFIC AIMS

2.1 Primary Objective:

To assess T-cell response 12-14 weeks after the initial WT1 peptide vaccine in patients with multiple myeloma following autologous stem cell transplantation.

2.2 Secondary Objectives:

2.2.1 To confirm the toxicity profile of the WT1 peptide vaccine

2.2.2 To describe the WT1 expression on malignant plasma cells by IHC.

2.2.3 To estimate the proportion of patients with minimal residual disease as measured by flow cytometry of marrow specimen.

2.2.4 To estimate the disease-free and overall survival

3.0 BACKGROUND AND RATIONALE

The Wilms' tumor gene, WT1, was first identified in childhood renal tumors, but WT1 is also highly expressed in multiple other hematologic malignancies and solid tumors, and, in particular, mesothelioma.[1-3] The WT1 cDNA encodes a protein containing four Kruppel zinc fingers and contains a complex pattern of alternative splicing resulting in four different transcription factors. Each WT1 isoform has different DNA binding and transcriptional activities and can positively or negatively regulate various genes involved in cellular proliferation, differentiation, apoptosis, organ development and sex determination. WT1 is normally expressed in tissues of the mesodermal origin during embryogenesis including the kidney, gonads, heart, mesothelium and spleen. In normal adult tissues, WT1 expression is limited to low levels in the nuclei of normal CD34+ hematopoietic stem cells, myoepithelial progenitor cells, renal podocytes and some cells in the testis and ovary. The low level of expression has not caused toxicity in any reported trials.

More recently, WT1 has been described as an oncogene. WT1 is overexpressed in a number of hematologic malignancies including up to 70% of acute myelogenous leukemia (AML), acute lymphoblastic leukemia (ALL), chronic myelogenous leukemia (CML), and myelodysplastic syndrome.[4] A high level of WT1 by leukemic blasts in AML is associated with poor response to chemotherapy, a greater risk of disease relapse, and reduced probability of extended disease-free survival. For these reasons, WT1 expression serves as a prognostic marker. Several research groups are using quantitative PCR methods to monitor disease response and minimal residual disease.[5]

The target protein. The strong expression of WT-1 protein in mesothelioma and myeloid neoplasms makes these cancers rational targets for first therapeutic attempts. The expression is so high in mesothelioma that pathologists routinely use immunohistochemical stains for WT-1 to help distinguish epithelial mesothelioma from other cancers. In three different pathology series, the rate of WT-1 nuclear staining ranged from 72% to 93%. In AML, WT1 expression can be used to monitor disease progression or relapse.[6] One investigator reported that WT1 transcript numbers paralleled other molecular markers used for minimal residual disease monitoring. In a series of patients monitored prospectively, increased WT1 expression always indicated impending hematologic relapse even in patients lacking other molecular markers. Normal WT1 transcript copy numbers in treated leukemia patients were associated with persisting complete remission.[7]

Our group has recently identified WT1 as a target antigen on malignant plasma cells in patients with relapsed multiple myeloma. [8-10]See details under 'Rational for Myeloma' below.

The use of a T cell vaccine strategy. Although WT-1 is not a cell surface protein and therefore is not a target for an antibody-based therapy, it is processed and presented on the cell surface. For this reason, WT-1 is an attractive target for T cell immunotherapy either by active (vaccine) or adoptive WT1-specificT-cell infusion approaches.[11]

The use of analog peptides. WT1 protein is a self-antigen, and, as a result, breaking tolerance is a potential problem for effective vaccination. Our strategy to circumvent the poor immunogenicity and potential tolerance of tumor-associated peptides is to design synthetic analog peptides that will be more immunogenic. Such peptide analogues could generate an immune response that not only recognizes the immunizing epitopes, but also cross-reacts with the original native peptides; this is known as a heteroclitic response. By using computer prediction analysis, we designed a large number of synthetic peptides derived from WT-1 protein sequences in which single or double amino acid substitutions were introduced into the peptides at key HLA-A*0201 binding positions.[12] Peptides predicted to bind with high affinity to HLA- A*0201 molecules were directly assayed for their ability to stabilize MHC class I A*0201 molecules on the surface of TAP negative T2 cell line. The new peptides could stabilize MHC class I A*0201 molecules better than native sequences. Avidly binding peptides were then assayed in an antigen specific T-cell expansion in vitro system for ability to elicit HLA-restricted, peptide-specific CTL responses using purified T cells from healthy donors. Two synthetic analog peptides generated more effective immune responses than the native peptides. In addition, CD8+ T cells stimulated with the new synthetic peptides displayed heteroclitic features and cross-reacted with the native WT-1 peptides and also were able to mediate peptide specific cytotoxicity. Importantly, T cells stimulated with the new synthetic peptides cross-reacted with the native WT1 peptide sequence and were able to kill HLA matched CML blasts. This validates the expression and possibility of killing tumor in this system. Other groups have validated the same native sequence we use as a target.[13]

Stimulating CD4 T cells. Peptides capable of stimulating CD4+ response are necessary for inducing long-term T-cell memory. WT1 peptides are capable of stimulating a peptide-specific CD4+ response that can recognize WT1+ tumor cells in multiple HLA-DR.B1 settings. Using cross priming experiments, it was shown that WT1 peptide is presented on the surface of mesothelioma tumor cells and could be recognized by the T cells stimulated by the individual WT1DR peptides.[14] The vaccine includes 3 peptides designed to stimulate common HLA-DR expressing cells in addition to 1 peptide for HLA-A*0201 cells. Therefore, the relevant HLA alleles include HLA-DR B- 1, 4, 7, 11, and 15 (and possibly others), which together with A*0201 should cover most patients. Assuming the peptides are immunogenic to one of these HLA types, immunologic responses should be seen in a large fraction of the cohort.

Summary of preclinical data. Analog heteroclitic WT1 peptides with increased immunogenicity can be synthesized and are potential vaccine candidates. To these peptides we have added 3 longer WT1 sequences that are capable of inducing CD4 responses in vitro. The 4 peptides along with the

immunological adjuvants Montanide and GM-CSF constitute our vaccine that was taken into human trials in AML and mesothelioma. The structural Formulas (amino acid sequences) of the vaccine administered are:

Name of Peptide	Sequence
WT1-A1	YMFPNAPYL
WT1-122A1 long	SGQAYMFPNAPYLPSCLES
WT1-427 long	RSDELVRHHNMHQRNMTKL
WT1-331 long	PGCNKRYFKLSHLQMHSRKHTG

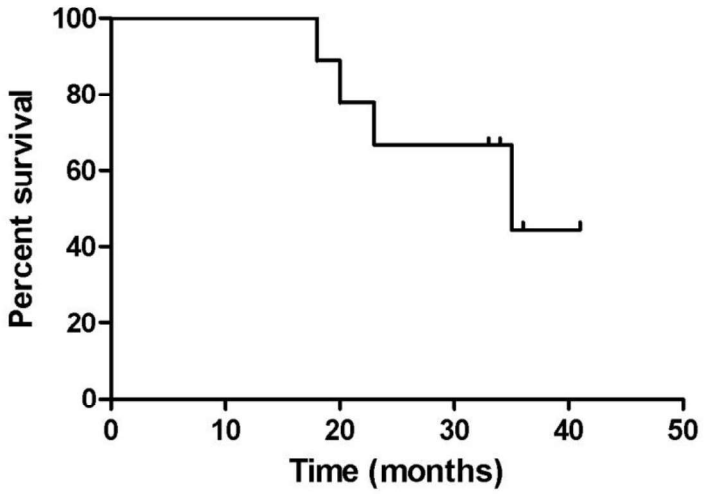
Experience with other WT1 vaccines in humans elsewhere: The use of WT1-derived peptides for vaccination of AML and certain carcinomas have also been reported in Japan and Germany.[15,16] Oka and colleagues in Japan conducted a Phase I clinical study of immunotherapy targeting the WT1 protein in patients with leukemia, MDS, lung cancer, or breast cancer.[11] Patients were intradermally injected with an HLA-A*2402-restricted, natural, or modified 9-mer WT1 peptide emulsified with Montanide ISA 51 VG UFCH adjuvant at 0.3, 1.0, or 3.0 mg per body at 2-week intervals. Twenty-six patients received one or more WT1 vaccinations, and 18 of the 26 patients completed WT1 vaccination protocol with three or more injections of WT1 peptides. Toxicity consisted only of local erythema at the WT1 vaccine injection sites in patients with breast or lung cancer or acute myeloid leukemia with adequate normal hematopoiesis. Severe leukocytopenia occurred in patients with myelodysplastic syndrome with abnormal hematopoiesis derived from WT1-expressing, transformed hematopoietic stem cells. Twelve of the 20 patients for whom the efficacy of WT1 vaccination could be assessed showed clinical responses such as reduction in leukemic blast cells or tumor sizes and/or tumor markers. A clear correlation was observed between an increase in the frequencies of WT1-specific cytotoxic T lymphocytes after WT1 vaccination and clinical responses. It was therefore demonstrated that WT1 vaccination could induce WT1-specific cytotoxic T lymphocytes and result in cancer regression without damage to normal tissues in the clinical setting. Trials including patients with renal cancer, myeloma and glioblastoma have also been conducted.[15] Letsch and colleagues in Germany reported the preliminary findings from their study vaccinating 14 patients with AML or MDS with an HLA-A2 restricted WT1 peptide with KLH and GM-GSF adjuvants. The vaccine augmented IgM responses in 6 of 6 patients at week 26 or 30, but no CD4 T-cell or IgG responses were noted.[17] The NIH is also conducting a trial with the native A0201 peptide mixed with another myeloid antigen peptide (PR1) in patients with AML. After vaccination, the emergence of PR1 or WT1(+) CD8(+) T cells was associated with a decrease in WT1 mRNA expression as a marker of minimal residual disease, suggesting a vaccine-driven antileukemia effect.[18]

MSKCC WT1 peptide vaccine clinical trial: At MSKCC, we conducted a pilot trial in AML and Mesothelioma to determine the immunogenicity and safety of vaccination with our WT1 heteroclitic peptides in humans (IRB# 06-085).[19,20] As described above, in order to broaden the immune response in the setting of varied HLA subtypes, four different peptides were included in the vaccine. Peptides were suspended in Montanide adjuvant, and GM-CSF was administered at the injection site. Patients were required to have neoplasms that showed immunohistochemical staining for WT1 in greater than 10% of cells. All HLA subtypes were eligible. The vaccine was well tolerated except for one patient with a grade 2 allergic reaction, who was removed from study.

In one arm of the study, we evaluated 10 patients with mesothelioma with unresectable or relapsed disease and had received no more than one prior pemetrexed containing chemotherapy regimen and patients with NSCLC, either stage III or IV after completion of initial treatment with surgery and/or chemotherapy and/or radiation therapy. Six out of nine patients tested demonstrated CD4 T-cell proliferation to WT-1 specific peptides, and all five HLA A*0201 patients tested mounted a CD8 T-cell response. Vaccination induced polyfunctional T cell responses, with up to 4 cytokines produced documented. Median survival in the MPM patients is 11+ months with five of the 9 alive. One patient with MPM has no progression 18 months after completion of 12 vaccinations.

In the other arm we evaluated 9 AML patients in remission and expected to receive no additional therapy. Seven of 8 tested patients responded with CD4 responses to the long peptides and 3 of 3 HLA-A*0201 patients responded to the class 1 peptide. Median overall survival (See Figure1B) was approximately 35 months for the group as a whole. The median disease free survival was not reached for this small group of patients.

Figure 1B



These results are superior to the published AML outcome data.[21-23] The AML overall survival rate in the U.S. from 1996 – 2002 was 21.7%. Age appears to be a major determinant in patient outcomes with the oldest populations deriving little benefit from many of the dose intensive therapies introduced in the last few years. Appelbaum et al reported a median overall survival of 18.8 months in patients younger than 56 years old, 9.0 months in patients 56-65 years old and 6.9 months in patients 66-75 years of age.[23] The median age of patients treated on the pilot study was 64 years old so the outcomes for this study group compare favorably with published data from the youngest cohort who have the best outcomes. Caution however, needs to be exercised in interpreting these results, as there may be a bias with regard to patient selection in the study group. The results are however, intriguing enough to warrant further study in a clinical trial examining the role of vaccination as a viable treatment for multiple myeloma, which is the rationale for the current study.

Rationale for Multiple Myeloma: Multiple Myeloma (MM) is a malignant disease of plasma cells that accumulate in bone marrow, leading to bone destruction and bone marrow failure. [24,25] The disease accounts for approximately 10% of all hematologic malignancies, with an estimated 20,000 new MM diagnoses in 2007, and about 11,000 projected to die of the disease this year. Despite recent advances with novel drugs and high-dose chemotherapy with autologous stem cell transplantation (auto SCT)[26-28] the improvement of the natural history of most patients with symptomatic myeloma has been limited. Therefore, posttransplantation strategies that induce myeloma-specific immune responses are warranted to improve their long-term outcome.

MM cells were also recently shown to overexpress WT1. The expression of WT1 in bone marrow correlates with numerous prognostic factors, including disease stage and M protein ratio.[29] MM cells are highly susceptible to perforin-mediated cytotoxicity by WT1-specific cytotoxic T lymphocytes (CTL), and WT1 expression is sufficient to induce WT1-specific IFN-y production by CTL.[30] Clinical responses have also been reported with WT1 peptide-based immunotherapy. Following immunization with a synthetic WT1 peptide, reductions in the myeloma disease-load in bone marrow and level of M protein in the urine were observed, along with bone scintigram

improvement. This partial response to vaccination correlated with expansion of functional WT1-specific CTL and migration of WT1-specific T cells to the bone marrow. [31]

In our own studies, we found an association between the emergence of WT1-specific T cells and graft-versus-myeloma effect in pts with relapsed and high-risk cytogenetics undergoing T-cell depleted transplants. In correlative studies, we evaluated the significance of WT1-specific cellular immune responses following T-cell depleted hematopoietic stem cell transplantation (TCD HSCT) and donor lymphocyte infusion (DLI) in these patients. WT1-specific T-cell frequencies were determined in peripheral blood and bone marrow specimens by staining for intracellular IFN-γ production in response to WT1 peptides and/or by tetramer analyses, where available.

Of 20 patients evaluated, all patients exhibited low frequencies of WT1-specific T-cell responses pre TCD HSCT. Ten of these patients received DLI post TCD HSCT. All 10 patients developed increased WT1-specific T cell responses post DLI. These increments in WT1-specific T-cell frequencies were associated with reduction in specific myeloma markers. Four patients with increasing M protein post TCD HSCT achieved durable complete remissions post DLI in the absence of GvHD and are currently 18, 23, 24 and 40 months post allo BMT. Long-term evaluation of these patients demonstrated fluctuations in persisting WT1-specific T-cell frequencies following DLI. [8,9]

Importantly, our studies by immunohistochemical analyses of WT1 and CD138 co-staining in bone marrow specimens demonstrated consistent co-expression within malignant plasma cells **(Figure I).** In 6 patients tested, WT1 expression in the bone marrow correlated with the extent of malignant plasma cell infiltration. In contrast, no WT1 expression was observed when disease was absent.

We examined the frequencies of WT1-specific T cells in 24 patients with multiple myeloma. All of these patients had long-lasting disease and had undergone several courses of chemotherapy and autologous stem cell transplantation. T-cell frequencies were examined in freshly isolated peripheral blood specimen from 20 patients by IFN-γ assay. MHC-tetramer analyses were performed in 4 patients for whom freshly isolated samples were not available. WT1-specific IFN-γ production was detected in all patients tested **(Figure II A-B).** Non-pulsed PBMC were used as a control to measure baseline activation of the T cells prior to peptide pulsing. We observed a significant 4.7-fold increase in the percentage of cells producing IFN-γ in response to WT1-pulsed PBMC compared with non-pulsed control targets. In contrast, we did not detect significant WT1 reactivity in healthy donor T-cell products **(Figure II A).** By calculating the absolute numbers of WT1-specific T cells, we determined a mean of 14.6 WT1-specific T cells per microliter of PB (range 0.1–56.78 cells/microliter) **(Figure II B).** [9,10] These pre-existing WT1-specific T-cell frequencies in patients with myeloma post autologous transplant provide a strong rational of testing the above WT1 vaccine to enhance specific immune responses in patients with multiple myeloma following autologous transplantation.

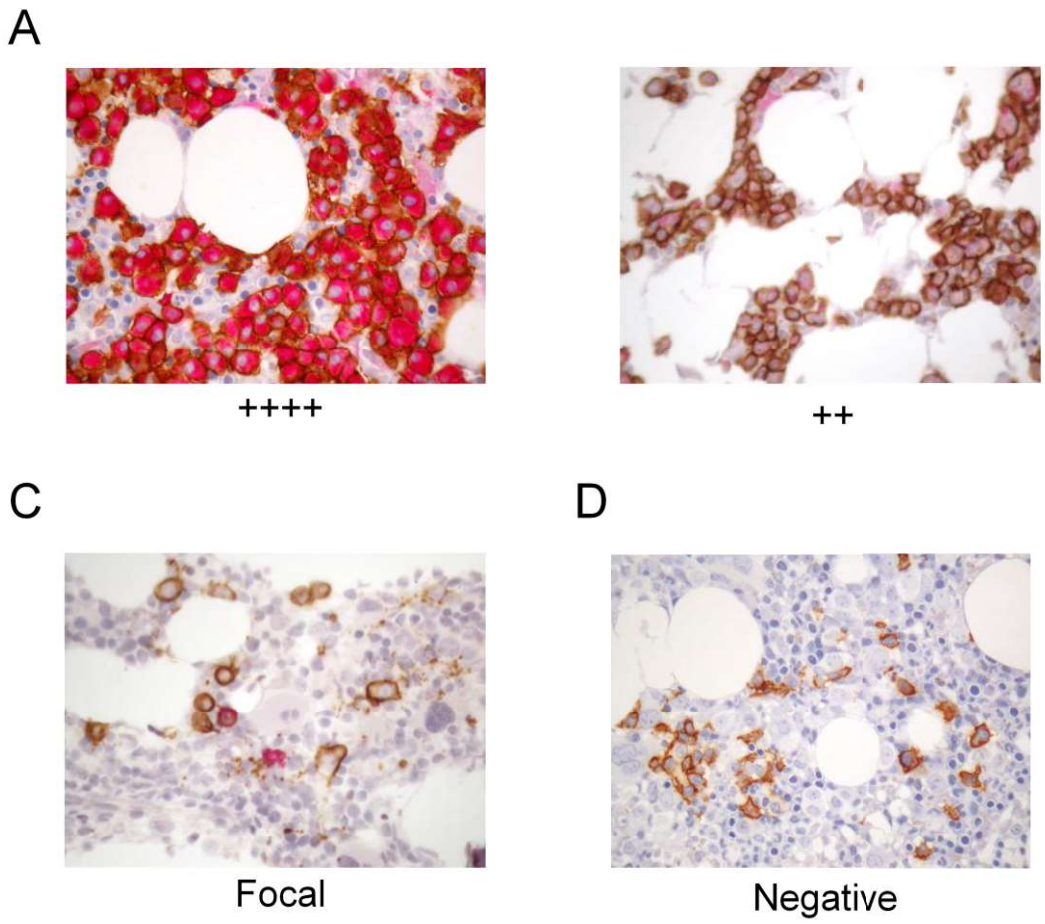


Figure I: WT1 is expressed in malignant plasma cells in the BM of MM patients. Paraffin-fixed BM biopsies from MM patients were double stained with monoclonal antibodies to CD138 (MI15; DAB, brown) and WT1 (6F-H2; nFu, red). Immunohistochemical analysis of WT1 expression was performed and biopsies were graded negative, focal, +, ++, +++ or ++++ based on the percentage of $CD138^+$ plasma cells staining positive for WT1. Representative biopsy stains shown. (A) ++++ >75% of $CD138^+$ plasma cells stain positive for WT1 (B) ++ >25-30% of $CD138^+$ cells are $WT1^{+.}$ (C) Focal, <5% of $CD138^+$ cells are $WT1^+$ (D) Negative, no $CD138^+$ are positive for WT1; (20x magnification).

B

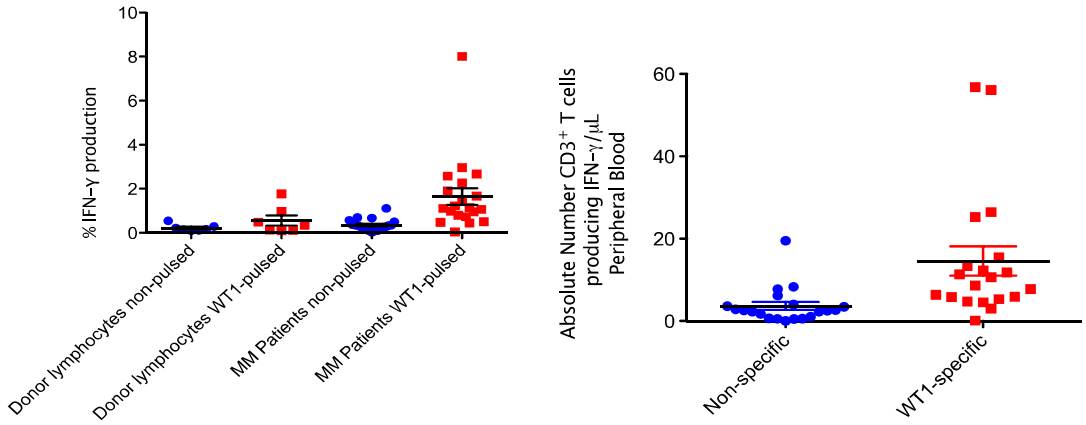


Figure II. WT1-specific T-cells are detected in MM patients post autologous stem cell transplantation. Frequencies of WT1-specific T cells in the PB of MM patients were quantified by intracellular IFN-γ assay. (A) Percentage of CD3+ cells in healthy donors and MM patients producing IFN-γ in response to non-pulsed autologous PBMC or PBMC pulsed with WT1 peptide pools. (B) Absolute numbers of CD3+ cells producing IFN-γ in response to non-pulsed or WT1 peptide pool-pulsed PBMC were quantified by multiplying the percentage of cells producing IFN-γ by the patient's absolute lymphocyte count.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a pilot study evaluating immunologic response of the WT1 peptide vaccine in patients with multiple myeloma following autologous stem cell transplantation. Twenty patients with a diagnosis of myeloma who have completed autologous stem cell transplantation will be enrolled in this study. Patients will be vaccinated with a preparation of WT1 derived native and synthetic peptides plus the immunologic adjuvant Montanide ISA 51 VG (Seppic Pharmaceuticals, Fairfield, N.J.) and Sargramostim (GM-CSF; Bayer Healthcare Pharmaceuticals, Seattle, WA).

4.2 Intervention

Vaccinations will be initiated 12-22 days following autologous stem cell transplantation. Patients will receive 6 vaccinations over 10 weeks. Early toxicity will be assessed at weeks 2 and 4. Routine toxicity assessments will continue throughout the trial. Immune responses will be evaluated in blood and/or marrow pre autologous stem cell transplantation, pre vaccination (baseline) and at 2-4 weeks following the vaccination schedule by intracellular interferon-γ assay of CD3+, CD4+ and CD8+ T lymphocytes and by WT1 MHC tetramer staining, if available for the patients HLA-type. Immunohistochemical analyses of WT1 and CD138 co-staining in bone marrow specimens as well as minimal residual disease (MRD) will also be assessed by RT-PCR for WT1 transcript in bone marrow aspirates will be assessed at 2-4 weeks following the vaccination schedule.

Patients who are clinically stable (no active infection with fevers and no cardiovascular or respiratory compromise) and have not had disease recurrence may continue with up to 6 more vaccinations administered approximately every month. In that case, patients will be re-evaluated with a bone marrow / immunologic studies 2-4 weeks after the completion of all vaccinations.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

WT1 Vaccine: The vaccine contains 2 WT1 derived peptides (to stimulate CD8+ responses) and 2 WT1 peptides (to stimulate CD4+ responses) and one may stimulate both:

The structural Formulas (amino acid sequences) of the vaccine administered are:

Name of Peptide	Sequence
WT1-A1	**YMFPNAPYL**
WT1-122A1 long	**SGQAYMFPNAPYLPSCLES**
WT1-427 long	**RSDELVRHHNMHQRNMTKL**
WT1-331 long	**PGCNKRYFKLSHLQMHSRKHTG**

5.1 Drug Product: WT-1 vax will be manufactured at AmbioPharm, Inc. The four peptides are provided in a sterile solution with phosphate buffered saline to produce the vaccine product. Each vial contains 0.4 mg/ml of each peptide in a total volume of 0.7 ml (overfill of 40%). Vialing under GMP conditions and sterility testing will be performed by University of Iowa Pharmaceuticals. It will be shipped directly to individual sites at -80° C. The vaccine emulsion will be individually prepared prior to use. This will require mixture of the peptide solution with the immunologic adjuvant Montanide ISA 51 VG.

Formulation: WT-1 vax is emulsified with Montanide ISA 51 VG by pharmacists in response to a physician order to treat a protocol patient. 4 peptides (200µg each, total 800 µg) will be mixed at a 1:1 ratio with Montanide ISA 51 VG as an emulsion in Phosphate buffered saline to a total volume of 1 ml. The peptide solution and Montanide ISA 51VG will be provided in separate vials. The WT1 peptides are supplied at a concentration of 0.4 mg/ml in 0.7 ml vials to use 0.5 ml only. The peptides are stored at -70º - -80º C. The peptide solution will comprise 50% (.50ml) of the mixture and Montanide will be the remaining 50% (.50ml). The peptide and Montanide mixture will then be vortexed in a Fisher Scientific vortex machine running at highest speed (>3000rpm) for 12 minutes with the use of an attachment. This preparation will take place in the research pharmacy. The emulsified solution should be administered to the patient within 2 hours of preparation. The dose, 1ml of the emulsion, will be drawn up into a 1-3 ml syringe and the dose will then be injected into the patient subcutaneously.

Dose: 200 µg was chosen as the dose because it is within the range of safe and active doses used by others. Peptide vaccines have generated immune and clinical responses within a wide range of doses (100-2000 µg injected) without clear evidence of dose- response relationships. Higher doses have the theoretical possibility of stimulating lower affinity TCRs on T cells and making a reduced response.

Route: Subcutaneous

Storage: Vials of WT1 vax should be stored at or below -70° C until use.

IND: This vaccine will be administered under an IND held by Sellas Life Sciences.

5.2 Montanide ISA 51 VG: A water-in-oil (w/o) emulsion with immunoadjuvant activity. Montanide ISA 51 VG appears to act by enhancing the immune system's cytotoxic T-lymphocyte (CTL) response against antigen(s) in vaccines. The surfactant mannide monooleate in Montanide ISA 51 VG contains vegetable-grade oleic acid derived from olive oil. The adjuvant is stored between 4ºC and 40ºC. preferably 15º-20ºC. Current shelf life in ampule is 5 years. See above for instructions on its use in this study.

Sargramostim (GM-CSF): Sargramostim (GM-CSF) will be administered at a dose 70 μg (140 μl) as a subcutaneous injection at the site of vaccination on day -2 (± 1 day) and day 0. Sargramostim (GM-CSF) is available as a liquid and is commercially available from Bayer Pharmaceuticals. It is formulated as a sterile, preserved (1.1% benzyl alcohol) injectable solution (500 μg/ml) in a vial. The liquid may be stored for up to 20 days at 2-8 º C once the vial has been entered. After 20 days, the remaining solution in the vial should be discarded. If required, other prescribing information for Sargramostim (GM-CSF) can be found on the MSKCC formulary intranet website. The 250 mcg powder for injection formulation may be used if the 500 mcg/mL formulation is not available.

GM-CSF has been used in a number of vaccines studies as an immunologic adjuvant with the presumed benefit mediated through effects on dendritic and other antigen-presenting cells. Several human vaccine studies in melanoma and other human malignancies have reported both encouraging immunologic and clinical results. More recently, two melanoma vaccine studies have reported a deleterious effect of GM-CSF when used as a vaccine adjuvant.[32,33] While it is important to be cognizant of such a potential negative effect, this study uses a different vaccine with a different dose of GM-CSF in a different disease. Other peptide vaccines studies in AML have used GM-CSF as an adjuvant and no negative results from the use of this agent have been reported.[14,34,35] There is an extensive body of literature detailing GM-CSF use in AML and it has been approved in the U.S. for use in AML to promote granulocyte recovery following the administration of chemotherapy.[36-38]

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

- Symptomatic multiple myeloma, ISS stage 1-3 with confirmed diagnosis of multiple myeloma at MSKCC
- Patients must be eligible to undergo autologous stem cell transplantation by standard institutional criteria
- Patients must have documented WT1 positive disease. For purpose of this study, this is defined as detectable presence of WT1 expression by immunohistochemistry or by WT1 transcript via RT-PCR on a bone marrow or other plasma cell-related biopsy specimen prior to autologous stem cell transplantation. Bone marrow or other biopsy specimen from time of diagnosis from patients diagnosed at MSKCC or outside hospital may be requested for assessment of WT1 expression by IHC
- Age $\geq$ 18 years
- Karnofsky performance status $\geq$ 50%
- Hematologic parameters:
    - Absolute neutrophil count (ANC) $\geq$ 1000/μl
    - Platelets > 50k/ μl
- Biochemical parameters:
    - Total bilirubin $\leq$ 2.0 mg/dl
    - AST and ALT $\leq$ 2.5 x upper limits of normal

- Creatinine ≤ 2.0 mg/dl

6.2 Subject Exclusion Criteria

- Pregnant or lactating women
- Patients with active infection requiring systemic antimicrobials
- Patients taking systemic corticosteroids
- Patients with serious unstable medical illness
- Concurrent malignancies

7.0 RECRUITMENT PLAN

The study will be conducted at Memorial Sloan-Kettering Cancer Center. Twenty patients with multiple myeloma who are planned to undergo autologous stem cell transplant will be enrolled and it will take approximately 2 years to complete the study. Every effort will be made to include women and minorities in the research study.

Potential research subjects will be identified by a member of the patient's treatment team, the protocol investigator, or research team at Memorial Sloan-Kettering Cancer Center. If the investigator is a member of the treatment team, they will screen their patient's medical records for suitable research study participants and discuss the study and their potential for enrolling in the research study. Potential subjects contacted by their treating physician will be referred to the investigator / research staff of the study.

During the initial conversation between the investigator / research staff and the patient, the patient may be asked to provide certain health information that is necessary to the recruitment and enrollment process. The investigator / research staff may also review portions of their 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 turns out to be 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.

8.0 PRETREATMENT EVALUATION

The following tests are required prior to beginning of the conditioning regimen for autologous transplant:

- Bone marrow biopsy or other plasma cell-related biopsy specimen with relevant diagnostic tests including:
  - IHC for CD138
  - WT1 expression

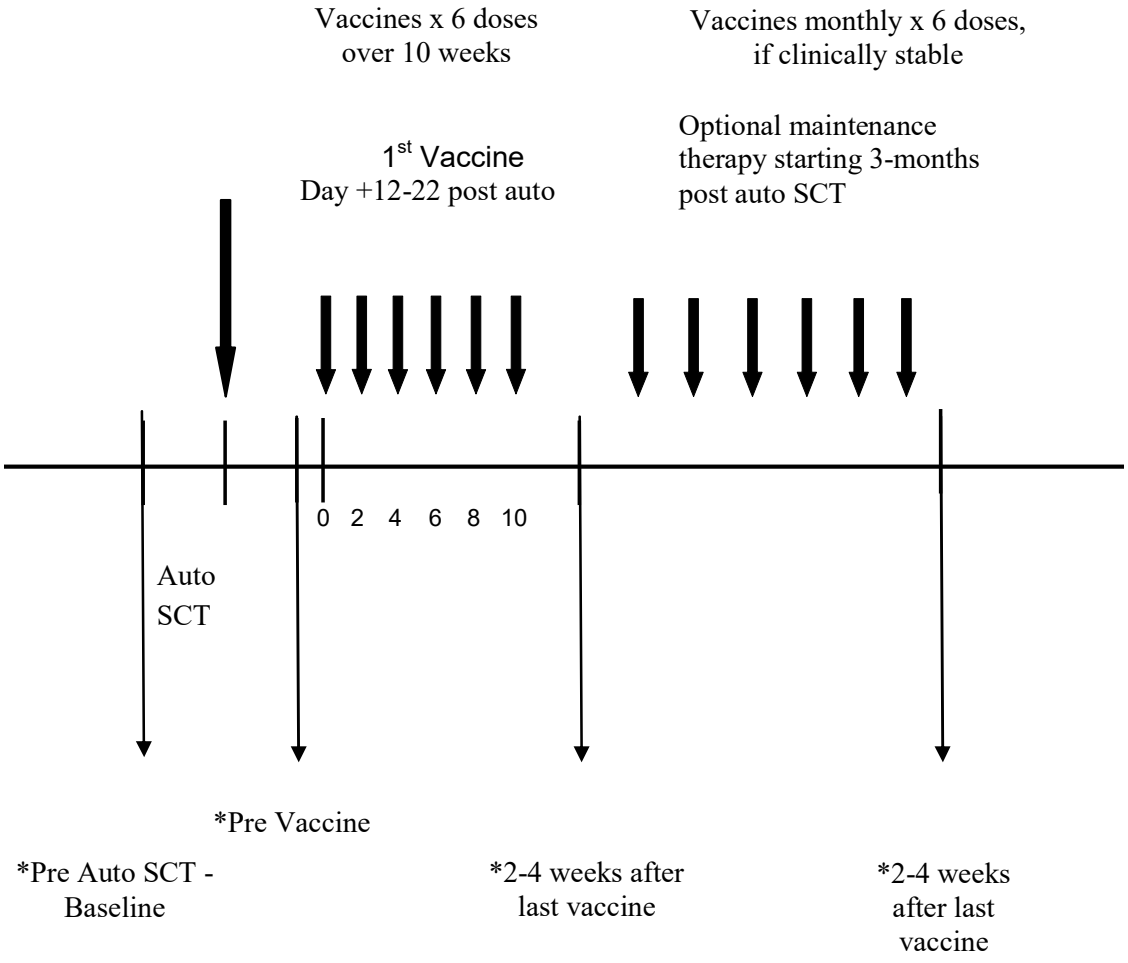
  Note: Bone marrow or other biopsy specimen from time of diagnosis from patients diagnosed at MSKCC or outside hospital may be requested for assessment of WT1 expression by IHC.

- Pre- SCT bone marrow aspirate and biopsy
- Optional aspirate specimen for immulogical responses
- Blood for immunological responses

9.0 TREATMENT/INTERVENTION PLAN

- Patients can be vaccinated as inpatients or outpatients. The vaccine will be administered by medical personnel
- Six vaccinations of the WT1 peptide preparation (1.0 ml of emulsion) will be administered on weeks 0, 2, 4, 6, 8, and 10. All vaccinations will be administered subcutaneously with vaccination sites rotated among extremities
- Injection sites will be pre-stimulated with Sargramostim (GM-CSF 70 μg) injected subcutaneously on days -2 (± 1 day) and 0 of each vaccination. Note: during each vaccination, the Sargramostim (GM-CSF) and the vaccine emulsion will be administered to the same anatomical site. The treating healthcare professional will mark this site and it will be noted in a site injection log
- Patients will be observed for at least 30 minutes after vaccination
- Patients, who are clinically stable (no active infection with fevers and no cardiovascular or respiratory compromise) and have not had disease progression, may receive up to 6 more vaccinations administered appropriately every month
- The use of post-stem cell transplant maintenance therapy is allowed starting 3 months or more after transplant.

Schema Of Vaccination and Evaluation



- Bone Marrow
  - WT1 PCR
  - IHC WT1
  - Not done at pre vaccine time point
- Immune responses

10.0 EVALUATION DURING TREATMENT/INTERVENTION

10.1 Correlative Studies

Peripheral blood, bone marrow, and/or plasma cell-related biopsy specimens will be collected from patients prior to autologous stem cell transplantation.

The obtained samples will be utilized to:

1. To analyze the development of WT1 antigen-specific immune responses in patients with MM undergoing autologous stem cell transplantation followed by WT1 analog peptide vaccine

2. Ascertain the Wilms tumor 1 antigen (WT1) expression on myeloma cells and to comparatively validate results by immunohistochemistry from bone marrow biopsies and/or plasma cell-related biopsies for monitoring of residual disease.

Blood and bone marrow will also be drawn at time intervals post vaccinations as detailed in 'Schedule of study assessment'.

The obtained specimen will be processed as follows:
Bone marrow biopsy and/or plasma cell-related biopsy specimens will be split: 1 piece will be fixed in 10% neutral buffered formalin with consecutive processing as paraffin-embedded standard specimens for routine morphological diagnostics and for immunohistochemical analysis. The corresponding remaining piece will be fixed in RNAlater solution for later RNA extraction. Specimen will be submitted to Dr. Achim Jungbluth, Schwartz 631 for analysis of the WT1 antigen profile by immunohistochemistry.

Protein expression analysis for WT antigens will be done by immunohistochemistry (IHC) as follows: Monoclonal antibodies to CD138/Syndecan, co-express WT1 when staining WT1 mAB 6F-H2 will employed by the study specified research lab on S-631. All IHC will be performed on formalin-fixed paraffin embedded tissues employing established detection and antigen-retrieval techniques as described previously. [39-41]

Quantitative analysis of mRNA expression for WT1 antigen will be performed with a '7500 Fast Real-Time PCR System' by Applied Biosystems (Foster City, CA).Total RNA is isolated from patient samples collected in EDTA using a phenol / chloroform extraction method. RNA purity is confirmed by absorbance at 260 nm. The reverse transcription reaction was adapted from protocols supplied by Applied Biosystems.[14,42] Briefly, reaction conditions were 2 min at 50ºC, 10 min at 95ºC followed by 50 cycles of 15 s at 95ºC and 60 s at 60ºC. Each reaction is done in triplicate and discrepancies > 1 Ct in one of the wells were excluded. The quantitative reverse transcription- PCR and fluorescence measurements are made on the

Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems, Foster City, CA). ABL expression is used as the endogenous cDNA quantitative control for all samples.

Immune responses of antigen-specific T-cell response against WT1 protein will be performed by intracellular IFN-γ analyses and/or MHC-tetramer analyses as previously described. [43,44]

10.2 **Immunological Response**

Approximately 60 – 90 ml peripheral blood (4-16 green top tubes) will be drawn before auto SCT, vaccination initiation and on week 12. Additionally**, an optional** 3ml of marrow in EDTA will be obtained before auto SCT and on week 12. Optional peripheral bloods can be drawn, at the investigator's discretion, at timepoints outlined in the study table below. These samples will be delivered to:

Guenther Koehne, M.D., Ph.D.
Memorial Sloan-Kettering Cancer Center
Mortimer B Zuckerman Research Center: Z1645
415 E 68th St.
New York, NY 10065

The samples of patient's blood and optional marrow obtained at baseline and bloods and marrow obtained at week 12-14 will be tested for WT1-specific T-cell responses by intracellular interferon-γ responses and/or MHC-tetramer analyses. EliSpot and proliferation assays may also be performed, if indicated. Patients who receive more than 6 vaccinations will also be tested 2 weeks after their last planned vaccination.

Bone marrow samples from patients will be analyzed for WT1 transcript via RT-PCR at timepoints outlined in the study table. Samples (approximately 3 ml) will be collected in EDTA. Each sample will also be sent to Guenther Koehne, MD, PhD at the laboratory location indicated above.

Bone marrow biopsy or plasma cell-related biopsy specimens are collected at MSKCC but for WT1 antigen expression by immuno histochemistry will be processed by:

Achim Jungbluth, MD
1250 First Avenue
Schwartz S-631
New York, NY 10065

Patients continuing on study following the initial vaccinations will have blood drawn for the laboratory evaluations listed below.

10.3 Research Non-Billable

The following tests are research studies, which will not be billed to patients or insurers because they are being done solely for research purposes: Tests listed under correlative studies include: MHC tetramer analyses, intracellular interferon gamma analyses, IHC for WT1 and WT1 RT-PCR.

10.2 Clinical course: Refer to study table below:

Procedure	Prior to SCT	Days post Vaccine initiation						
		0	**14**	**28**	**42**	**56**	**70**	**91**
Window (days)			(+/-) 2	(+/-) 2	(+/-) 2	(+/-) 2	(+/-) 2	(+/-) 7
History, medications review		X[a]	X	X	X	X	X	X
Physical exam		X[a]	X	X	X	X	X	X
CBC with diff		X[a]	X	X	X	X	X	X
Comprehensive panel		X[a]	X	X	X	X	X	X
Urinalysis		X[a]						
Bone marrow biopsy and/or plasma cell-related biopsy with confirmed WT1 positive disease	X							
Bone marrow aspirate and biopsy	X							X
Optional immunology bone marrow aspirate[d]	X							X
Informed consent	X							
Immunology bloods	X	X[a]	X[c]	X[c]	X[c]	X[c]	X[c]	X
GM-CSF[b]		X	X	X	X	X	X	
Vaccine		X	X	X	X	X	X	
Toxicity Assessment		X	X	X	X	X	X	X

[a]Test required prior to first vaccination
[b] Sargramostim (GM-CSF) will be injected subcutaneously two days (±1) before and on the day of each vaccination
[c]Optional immunology bloods (4-16GTT); to be collected at the investigator's discretion
[d]Immunology BM aspirate (3mL in EDTA) will be drawn at this time point

Procedure	Additional Monthly dosing if clinically stable						
	1	**2**	**3**	**4**	**5**	**6**	**End of dosing**
Window (days)	(+/-) 7	(+/-) 7	(+/-) 7	(+/-) 7	(+/-) 7	(+/-) 7	2-4 weeks post vaccine
History, medications review	X	X	X	X	X	X	X
Physical exam	X	X	X	X	X	X	X
CBC with diff	X	X	X	X	X	X	X
Comprehensive panel	X	X	X	X	X	X	X

Urinalysis							
Bone marrow aspirate and biopsy							X
Optional immunology bone marrow aspirate[d]							X
Immunology bloods	X[c]	X[c]	X[c]	X[c]	X[c]	X[c]	X
RT-PCR WT1							X
GM-CSF[b]	X	X	X	X	X	X	
Vaccine	X	X	X	X	X	X	
Toxicity Assessment	X	X	X	X	X	X	X

- History and physical examination at baseline, at weeks 0, 2, 4, 6, 8, 10, and 12 -14 and monthly thereafter as indicated above

- CBC and comprehensive panel at baseline and at weeks 0, 2, 4, 6, 8, 10, and 12-14 and monthly thereafter as indicated above

- Bone marrow aspirate at baseline and at week 12 -14

- For patients who are clinically stable and have not had disease recurrence may receive up to six more vaccinations administered approximately monthly. In that case, patients re-evaluated with history and physical exam, CBC and comprehensive panel prior to each vaccination. Bone marrow and immunologic studies repeated at the completion of vaccination

- Following completion of vaccination, patients will be followed at regular intervals (approximately every 1-3 months) in the outpatient setting and undergo standard follow-up. This will include CBC's and /or bone marrow aspirations as clinically indicated

11.0 TOXICITIES/SIDE EFFECTS

11.1 Expected Toxicities

WT1 Peptide
- mild inflammation at injection sites
- occasional fever
- rare patients - allergic reactions have been observed

Montanide ISA 51 VG
- mild inflammation at injection sites
- occasional fever
- rare patients - allergic reactions have been observed

Sargramostim (GM-CSF; at standard doses of 250 μg/m2/ day for 5 to 14 days)
- edema
- fluid retention
- headache
- myalgia

- arthralgia
- dyspnea
- allergic reactions
- Patients with pre-existing renal and / or hepatic disorders may demonstrate elevations in serum creatinine or bilirubin / transaminases respectively

11.2 Potential Risks

Autoimmune or hypersensitivity reactions to components of the vaccine or skin test antigens are possibilities. There have been no autoimmune or hypersensitivity reactions reported in patients vaccinated with other WT1 peptides.

In a prior pilot study performed with this vaccine, definite related toxicities were minimal (≤ grade 2) and generally consisted of local irritation, swelling, redness, tenderness, or pruritus at the site of vaccine administration for 1 to 3 days. No significant pulmonary, renal or hematologic toxicity was noted. One patient developed a delayed grade 2 allergic reaction to the vaccine consisting of generalized urticaria and a description of perceived laryngeal spasm approximately 1-2 hours after the administration of the 5th vaccine. She was treated with antihistamines and observation in the emergency room and the symptoms quickly resolved. However, in the interest of patient safety she received no further vaccinations and was taken off the study. She suffered no further episodes and remains in CR 41+ months after her leukemia diagnosis. Another patient developed a localized hypersensitivity reaction to the GM-CSF adjuvant consisting of pain, erythema and edema approximately 2 days following Day -2 administration of the adjuvant prior to vaccine #9. The vaccine was held on Day 0 and the patient had resolution of the symptoms within 2 weeks. However, a recurrence of the symptoms took place when the patient was re-challenged with GM-CSF at an alternative site and the patient was therefore unable to continue with the therapy as outlined in the trial.

11.3 Criteria for Toxicity

Toxicity will be graded in accordance with Common Toxicity Criteria, version 4.0 (CTCAE 4.0) developed by the National Cancer Institute, June 10th, 2003 (http://ctep.cancer.gov). The only toxicities captured outside of the SAEs reported will be all Grade 1-5 toxicites deemed definitely, probably, or possibly related to the vaccine portion of the study.

11.4 Dose Adjustments for the Vaccine

11.41 Expected adverse events from the vaccine include local erythema, edema and itching. These are usually mild and may be observed after each administration of the vaccine. These side effects will be treated symptomatically and will not require treatment interruptions or discontinuation.

11.42 In patients who develop a Grade 2 allergic reaction consisting of generalized urticaria or evidence of systematic allergic reaction, vaccination will be discontinued and the patient will be taken off study.

11.43 Patients who develop Grade 3 or 4 toxicity that is considered probably related to the vaccine or any of its components will be taken off study.

11.44 Patients who develop evidence of autoimmune disorders or anaphylactic reaction considered to be possibly related to the vaccine will be taken off study.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

12.1 Immune response

Immune responses to WT1 peptides will be measured by intracellular interferon-γ production assay and MHC tetramer analyses, if available for patient's HLA-type.

T cell responses ex vivo: Heparinized peripheral blood (60 - 90 ml) will be drawn prior to treatment and at week 12 during treatment as indicated in Section 10.0. Additional samples will be drawn at the completion of vaccination for those who continue with vaccination after the initial 12 weeks. Peripheral blood lymphocytes (PBLs) will be tested for intracellular interferon-γ production and MHC tetramers, if available for patient's HLA-type, as described.

T cell interferon production responses will be measured ex vivo at each time-point after challenge with the vaccinating peptide; an irrelevant peptide and no peptide will be used as a control for T-cell stimulation to determine the background activity. MHC tetramers analyses will also be performed at each time point, if they were available for patient's HLA type at baseline. A positive response is defined as a two fold increase in WT-1 specific tetramers at 12-14 weeks over the baseline level. This assay is very specific and generating a specific CD8+ WT1 population may be evidence of the immunologic effect of the vaccine. The assays performed in Dr Koehne's laboratory, may also be performed and confirmed in Dr. Scheinberg's laboratory and/or in the Ludwig Immune Monitoring Facility.

RT-PCR for WT1: For purposes of this study, the ability to detect WT-1 transcript is indicative of minimal residual disease. There is ample literature to support this, particularly with increasing WT1 levels predicting overt clinical relapse. A biologic effect on the target of vaccination would be of interest to the investigators and stable or decreasing transcript levels may be interpreted as evidence of immune effect on minimal residual disease.

12.2 Clinical course:

Bone marrow evaluation will be performed pre autologous stem cell transplantation, at week 12 and at the completion of vaccination. In addition, bone marrow examination may be repeated as clinically indicated.

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the patient develops recurrent disease he / she will be taken off study and any remaining vaccinations will be discontinued. The patient will continue to be followed for overall survival.

If at any time the patient refuses to comply with study procedures or withdraws informed consent, he/she will be taken off study.

If at any time the patient develops unacceptable toxicity he / she will be removed from study.

If at any time the patient is found to be ineligible for the protocol a designated in the section on Criteria for Patient / Subject Eligibility (i.e., change in diagnosis), the patient will be removed from study.

14.0 BIOSTATISTICS

This is a pilot study of WT1 peptide vaccine in patients with a history of multiple myeloma undergoing autologous stem cell transplantation followed by WT1 analog peptide vaccination. The primary endpoint is detectable WT1 T-cell response in the blood and marrow 12 -14 weeks following initial vaccination. A positive response is described in section 12.1.

We will utilize a Simon's minimax two-stage design to investigate the primary objective. For the design of the study, a 5% detectable WT1 T-cell response 12-14 weeks following vaccination would be considered unpromising, while a 25% response would be considered promising for further investigation. Using a type I and type II error of 0.10, this trial will accrue a maximum of 20 patients. In the first stage, 13 patients will accrue on the study. If no patient achieves a response, the trial will close due to a lack of efficacy; otherwise, an additional seven patients will accrue. At the end of the trial, if at least three out of the 20 patients achieve a response, the WT1 vaccination will be considered promising.

While unanticipated, this trial also includes early stopping for the adverse events outlined in sections 11.42, 11.43, and 11.44. In the event that two in the first seven patients, three in the first 17 patients, or four anytime have one of these events, the trial will be terminated. If the true unknown toxicity rate is 5%, the probability of declaring the intervention unsafe is 8%. If the true toxicity rate is 25%, the probability of declaring the intervention unsafe is 87%.

In addition to the primary endpoint, there are a number of secondary objectives in this trial.

1. Kaplan- Meier methodology will be used to estimate overall survival and disease-free survival.

2. Frequency of toxicities will be tabulated according NCI Common Toxicity Criteria.

3. Descriptive statistics and graphical displays will explore immunologic response based on CD3+, CD4+ and CD8+ intracellular T cell interferon-γ production assay and WT1 tetramer staining.

4. The proportion of patients that have stable, decreasing levels of WT1 or convert to negative WT1 by RT-PCR will be estimated.

It is anticipated that 1-2 patients per month will be accrued to the study and the study accrual will be completed in approximately 2 years.

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

Not applicable.

16.0 DATA MANAGEMENT ISSUES

A research Study Assistant (RSA) will be assigned to the study. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization and coordinate the activities of the protocol study team.

The data collected for this study will be entered into a secure database. Source documentation will be available to support the computerized patient record.

16.1 Quality Assurance

Weekly 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 period 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, 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://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Office of Clinical Research. The MSKCC Data and

Safety Monitoring Plans can be found on the MSKCC Intranet at: *http://mskweb2.mskcc.org/irb/index.htm.*

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (*e.g.*, protocol monitoring, compliance and data verification audits, therapeutic response, and staff education on clinical research QA) and departmental procedures for quality control. Additionally, two institutional committees 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 its 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

Risks: The standard of care for patients eligible for this study is generally surveillance for disease recurrence. Administration of the WT1 vaccine has the potential of causing toxicities that would not occur if no treatment was administered.

Benefits: Administration of the Wt1 vaccine will potentially improve relapse rates or time to progression when compared to observation alone.

Possible toxicities / side effects : The potential toxicities were surmised for the prior pilot study and other similar vaccine studies. Toxicities may result from the WT1 peptide themselves, from the Montanide adjuvant, or from Sargramostim (GM-CSF). The most likely toxicity is redness or swelling at the site of injection. Other possible toxicities include bone or muscle aches, fever, headache, swelling in the feet. Less common but potentially serious toxicities include allergic reactions, elevated liver function blood tests, elevated kidney function blood tests, or shortness of breath.

Costs: Patients will be charged for physician visits, routine laboratory, radiologic, and pathologic studies required for monitoring of their condition. Patients will not be billed for the vaccine and GM-CSF. Patients will not be charged for immunologic testing or the RT-PCR for WT1.

Alternatives: Alternative treatment options include observation, or participation in other investigational studies.

Inclusion of Children in Research: This protocol does not include children because there is no experience administering this vaccine in this patient population, the number of children is limited and the majority of children are already accessed by a nationwide pediatric cancer research network. This statement is based on exclusion 4b of the NIH Policy and Guidelines on the

Inclusion of Children as Participants in Research Involving Human Subjects.

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

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant's last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 'Reporting of Serious Adverse Events', the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows:

For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

For all other trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to sae@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject's initials
- 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
- The grade of the 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
- If the SAE is an Unanticipated Problem

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

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office

All patients will be followed for safety and toxicity related to the study. Potentially serious toxicities are an expected part of transplant therapy. The reportable serious adverse events (SAEs) will be defined according to the current MSKCC Adult and Pediatric BMT Adverse Event Reporting Guide.

17.2.1 SAE Reporting to Sellas Life Sciences

Sellas Life Sciences must be notified via fax of any SAE within one business day. The CRDB SAE report should be faxed to PPD pharmacovigilance (PVG) at 1-888-529-3580

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

Not applicable.