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**Title:** A randomized phase II trial of Interferon + GM-CSF versus K562/GM-CSF vaccination in CML patients achieving a complete cytogenetic response to frontline tyrosine kinase inhibitor therapy

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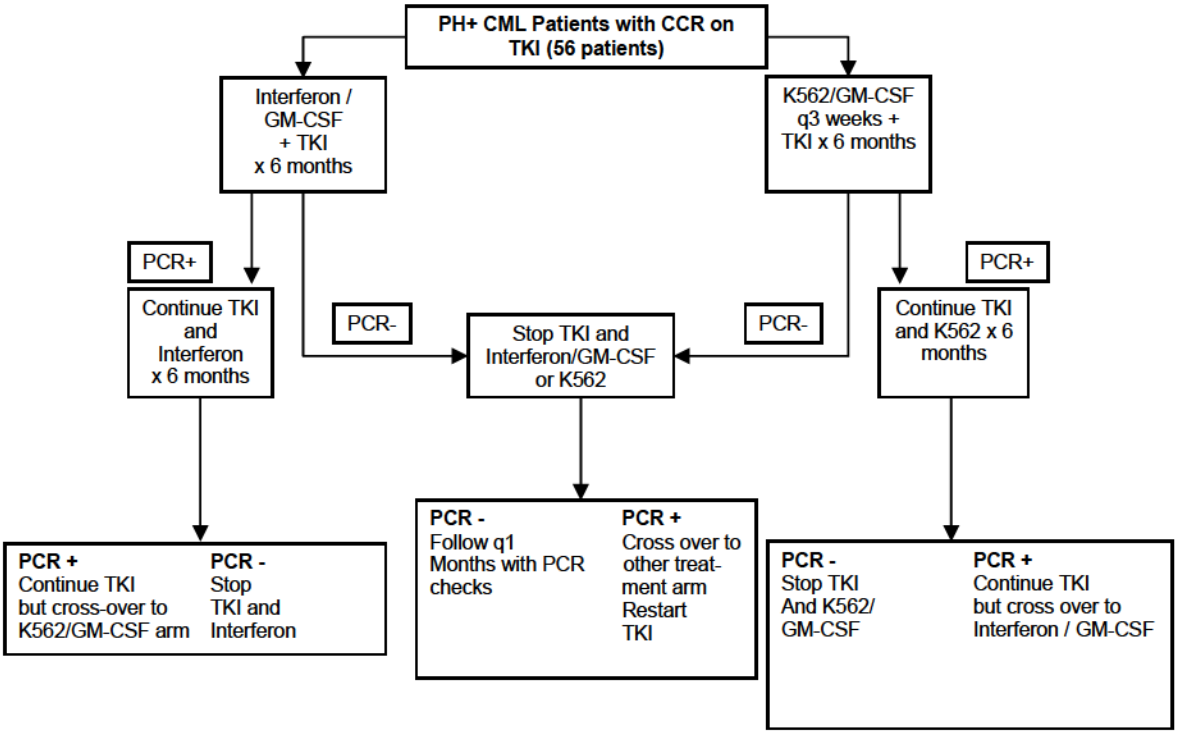
## SCHEMA


PH+ CML Patients with CCR on TKI (56 patients)
Interferon / GM-CSF + TKI x 6 months
K562/GM-CSF q3 weeks + TKI x 6 months
PCR+
Continue TKI and Interferon x 6 months
PCR-
Stop TKI and Interferon/GM-CSF or K562
PCR-
PCR+
Continue TKI and K562 x 6 months
PCR +
Continue TKI but cross-over to K562/GM-CSF arm
PCR -
Stop TKI and Interferon
PCR -
Follow q1 Months with PCR checks
PCR +
Cross over to other treat-ment arm Restart TKI
PCR -
Stop TKI And K562/ GM-CSF
PCR +
Continue TKI but cross over to Interferon / GM-CSF

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## 1. OBJECTIVES

1.1. To determine whether sequential, combination therapy can improve clinical responses of single agent tyrosine kinase inhibitor (TKI) for patients with Ph+ CML in chronic phase in cytogenetic remission. The primary clinical endpoint of the study will be progression-free survival at one year of molecular complete remissions obtained on combination therapy following discontinuation of all therapy. The secondary clinical endpoint will be the rate of molecular CR in each of two treatment arms described as follows:

All subjects will be treated with TKI and will have achieved a complete cytogenetic remission. Subjects will then be randomized to receive either Study Arm A) interferon-α + GM-CSF or Study Arm B) a series of K562/GM-CSF vaccinations in combination with frontline TKI. All subjects achieving a molecular CR after a minimum duration of combination therapy will then have the combination discontinued. Subjects who do not achieve a molecular CR after 1 year of combination therapy are eligible to cross over to the other study arm.

The study will be modified based on the results of the planned interim analysis. Individual Study Arms will continue to accrue and treat as indicated by the analysis. The study in its current format will continue should the planned interim analysis indicate both Study Arms remain viable as effective treatments.

1.2 Secondary measures of response to therapy will include: time to PCR negativity after randomization, disease free survival, and percent molecular complete remissions at one year within each treatment arm.

1.3 Toxicities will be described for each arm of the combination therapy.

1.4 Patient motivation for trial enrollment will be investigated through in-depth interviews.

## 2. BACKGROUND

2.1 <u>Chronic Myelogenous Leukemia:</u>

Chronic myelogenous leukemia (CML) is a malignant clonal hematopoetic stem cell disorder with defined t(9;22) chromosomal rearrangement of the breakpoint-cluster region(BCR)/ Abelson leukemia virus (ABL) causing constitutive tyrosine kinase activation. This chromosomal abnormality is termed the Philadelphia Chromosome (Ph+). Resultant deregulated intracellular signaling

pathways leads to uncontrolled cell growth, resistance to apoptosis, and subsequent elevation of peripheral blood counts and bone marrow hyperplasia.[1] CML occurs at the level of hematopoietic stem cells and, like their normal counterparts, CML stem cells undergo orderly differentiation. Thus, the bulk of the leukemic mass in CML consists of differentiated blood cells, whereas the rare cells responsible for disease maintenance resemble normal hematopoietic stem cells. CML takes form in three different clinical phases including chronic phase, accelerated phase, and blast crisis indicating a complex biologic transformation that has been under intense investigation. With the known presence of the BCR-ABL defect, a biologically directed treatment was developed to target this mutation and now TKIs dominate the current treatment paradigm. Although, TKIs have been a major scientific breakthrough, resistance and resolution of effect upon withdrawal has indicated that TKIs are not the sole panacea for CML treatment.

## 2.2 Historical Treatment of CML:

### 2.21 Hydroxyurea and Busulfan:

Historically, treatment for CML has been focused on cytoreduction with agents such as busulfan and hydroxyurea. A seminal study published by Hehlmann, et al with the German CML Study Group published in 1994 evaluated Ph+ CML treated with interferon compared to the standard regimens of hydroxyurea or busulfan. In the Busulfan group, complete hematologic remission was seen in 22.6 %, partial response in 68.8% and minimal response in 8.6%. There were no complete cytogenetic responses, 1 major cytogenetic response, and 3 minimal cytogenetic responses. In the Hydroxyurea group, complete hematologic remissions were slightly higher at 38.7%, partial remissions 51%, and no response in 10%. Cytogenetic responses were slightly increased in the hydroxyurea group compared to busulfan with 1 complete, 1 major/minor, and 4 with no cytogenetic response.[2] Although it is evident that there is a significant hematologic response to treatment with both hydroxyurea and busulfan, the infrequency of complete cytogenetic remissions and corresponding survival curves showed that these agents were not curative. Theoretically, these agents decreased the bulk of the expanded more mature leukemia clones. However, as no significant cytogenetic remissions were seen, the more primitive leukemic progenitors were likely not affected by the treatment and allow re-expansion of the primitive leukemic clone upon withdrawal of the drug.

### 2.22 Interferon-alfa:

Interferon alpha is a cytokine produced by a variety of cells in the body with multiple effects including inhibition of cellular growth and proliferation as well as effects on immune effectors cells including T,B, NK and dendritic cells[3]. Common side effects include leukopenia, thrombocytopenia, anemia and flu-like symptoms including fever, chills, myalgias, malaise, nausea, vomiting, and diarrhea [4]. After scientific review of greater than 30 uncontrolled observational studies in the 1980's, interferon alfa became more widely used in treatment of CML chronic phase. The largest example study was completed at MD Anderson by Talpaz et al between 1981 and 1988 evaluating 96 patients treated with interferon-alpha showing a 72% hematologic remission and 19% complete cytogenetic remission (CCR)[5]. This study prompted larger scale studies to further evaluate response.
The results of 4 randomized controlled studies in the 1990's, as reviewed by Silver et. al, comparing interferon alpha to the standard hydroxyurea or busulfan treatment for CML, confirmed the above mentioned observational results. Although the details of the four studies are beyond the scope of this protocol background, the overall results showed a 5 year survival of 50-59% in the interferon arm compared to 29% in the busulfan arm and 44% in the hydroxyurea arm. Cytogenetic responses, including partial and complete, ranged from 7 – 19 %[2] with the prolongation of survival by interferon alpha [6,7]. Moreover, about half of the patients who achieve a CCR appear to remain in CCR after stopping interferon and may in fact be cured[8,9]. Thus, interferon appears to change the natural history of CML, suggesting it affects CML stem cells. Although there were some differences between the studies, it is clear that those patients who responded the best to interferon alpha were with early phase or low level disease.[10] Several studies, including our own[11,12], confirm that interferon specifically targets CML stem cells in vitro. Understanding the biology of this impact is important in assessing the utility of interferon as a potential agent for cure in CML.

## 2.23 Interferon-alfa + Sargramostim

As illustrated above, interferon alpha can induce a hematologic remission in approximately 60-80% of patients and a cytogenetic complete remission in approximately 30% of patients. Studies have shown that the combination of Sargramostim and Interferon alfa enhances the effects of interferon against CML stem cells in vitro[12], and is able to improve the cytogenetic response clinically. We found that the combination induces terminal differentiation of CML stem cells[12], and Chen et al showed that the combination generated

malignant antigen presenting cells with the capacity for potent T cell stimulation [13]. Early clinical studies by Cortes et al with the combination of Interferon alfa and Sargramostim in doses of 30-60mcg/m2/day showed improvement in cytogenetic response rate in 30% of the twelve patients studies[14]. Clinical studies at Johns Hopkins in 1998 – 2002 tested the combination of Interferon-alfa + Sargramostim therapy at doses of 125micrograms/m2/day for 6 months in patients with chronic phase CML. The results were impressive and showed an actuarial probability of achieving a major cytogenetic response of 74% at one year. This indicates that Sargramostim improves response to the already documented benefit of interferon-alfa and will thus be used as one arm of this study.

2.24 Imatinib, Dasatinib, and Nilotinib:

Imatinib mesylate, Dasatinib, and Nilotinib are all three FDA approved for the frontline treatment of chronic phase CML. This class of agents functions as selective and potent inhibitors of the protein tyrosine kinase of the BCR-ABL fusion protein. These agents also target other tyrosine kinases, including platelet-derived growth factor (PDGF) and c-kit expressed in normal hematopoetic cells. The initial Phase I trial using Imatinib by Druker et al in patients with CML chronic phase who had failed interferon alpha showedremarkable responses with 53 of the 54 patients showinga complete hematologic response and 17 of 54 patients (31%) showed a cytogenetic response. [15] Subsequent Phase II testing in 1999 evaluated use of imatinib in patients in both blast crisis and chronic phase. A study of 260 patients with CML by Sawyers et al, 229 with documented CML in blast crisis, yielded an overall hematologic response rate of 52% and cytogenetic response rate of 16% [16]. In a Phase II study of imatinib in patients in chronic phase who had failed interferon alfa, even more astounding results of 95% hematologic response and 60% major cytogenetic responses (40% complete) occurred [17]. These studies led to the eventual Phase III IRIS trials comparing imatinib with interferon plus cytarabine. At median follow-up analysis at 19 months, complete hematologic response was seen in 95% of patients, and major cytogenetic responses in 87% with 76% complete cytogenetic responses [18].

The second generation TKIs, dasatinib and nilotinib, were developed to address the development of Imatinib resistance and Imatinib intolerance. Recent reports note that they are efficacious as upfront therapy as each has been studied in randomized fashion to standard dose Imatinib 400 mg daily. In the DASISION trial, newly diagnosed patients in chronic phase CML were randomized to Dasatinib 100mg daily (n = 259) or imatinib 400 mg daily (n = 260). Primary results

showed patients randomized to Dasatinib were more likely to achieve a complete cytogenetic remission at 12 mos and more likely to achieve a major molecular response at 12 mos.(Kantarjian, et. al., Blood, Feb 2, 2012; 119(5) 1123-29) Results were similar for patients randomize to nilotinib 300mg twice a day or 400 mg twice a day as opposed to Imatinib 400 mg daily on the ENESTnd study with both Nilotinib arms achieving complete cytogenetic remissions and major molecular responses more frequently.(Kantarjian, et. al., Lancet Oncology, September 2011; 12(9): 841-51) Despite the earlier and deeper responses seen with the second generation TKIs, overall survival differences have not been seen.

Although the TKIs have shown outstanding results in terms of hematologic and cytogenetic responses in the setting of an acceptable side effect profile, they have not yielded cures and resistance has emerged. Interestingly, despite the frequent occurrence of complete cytogenetic responses, most studies have shown that most patients treated with TKIs do not become RT-PCR negative for the BCR-ABL transcript and remain with low level residual disease. Additionally, resistance has emerged through mechanisms such as gene amplification of the BCR-ABL transcript, point mutations, and increased expression of MDR1[19]. With the significant paucity of complete molecular remission defined as RT-PCR negativity, and relapse upon withdrawal of imatinib, it appears that imatinib is not eliminating the CML stem cell. Laboratory studied by several groups [20,21], including our own[12], confirmed that imatinib has little if any activity against CML stem cells in vitro.

For purposes of this study, 'frontline TKI therapy' will be defined as treatment with any of the FDA-approved first-line TKIs (i.e., imatinib, dasatinib, or nilotinib) being used as the patient's primary therapy or as subsequent therapy in the setting of intolerance to a prior TKI.

2.25 Bone Marrow Transplantation and Immunologic Responsiveness of CML:

Allogeneic bone marrow transplantation is the only curative treatment of CML. The overall five year survival in patients undergoing transplant is approximately 50-60%, but with that survival comes a treatment related mortality of 20-30%. The collective experience with transplantation in patients with CML has shown that delay of transplant to greater than one year from diagnosis, higher Sokal score, increased age, and complete T cell depletion of the donor bone marrow are all associated with decreased disease free survival. [22] In the past, T cell depletion was accompanied by decreased graft versus host disease and increased relapse speaking to the immunotherapy nature of

transplant. Further research has substantiated the importance of T cell mediated immunotherapy in the setting of re-induction of remission in CML relapse with infusion of donor lymphocytes[23,24]. These findings of decreased graft versus host disease but increased relapse with T cell depletion, increased relapse in syngeneic transplants, and the induction of remission with donor lymphocyte infusion (DLI) establishes the impact of T cell immunity in treatment of the malignant clones and provides a framework for other types of adoptive immunotherapy with T cell manipulation with vaccine strategies.

## 2.3 K562/GM-CSF Vaccine:

### 2.31 Immunologic and Clinical Effect of Cancer Vaccines:

Tumor cell-based vaccine strategies seek to enhance the immunogenicity of tumor cells by modifying them in vitro to express immunomodulatory cytokines.[25-32] Dr. Levitsky's group at Johns Hopkins Hospital (JHH) and many others have demonstrated the generation of T-cell mediated systemic anti-tumor immunity capable of eradicating a small, pre-established tumor burden in certain murine models following vaccination. The vaccination site has been shown to contain an influx of eosinophils, activated macrophages,[30] and dendritic cells (DCs),[33] with complete destruction of the vaccinating tumor cells within three to five days.[34] GM-CSF producing tumor vaccines act by recruiting and activating professional antigen presenting cells (APCs) which process antigens liberated from the irradiated tumor cells at the vaccine site and migrate to the draining lymph nodes. Here, processed antigen is presented to tumor-specific T-cells leading to their activation.

APCs are critical in the priming phase of this response. This is underscored by demonstration that tumor-specific $CD8^+$ cytotoxic T-lymphocytes (CTL) are actually primed by bone marrow derived APCs that have processed exogenous tumor antigen in response to vaccination ("cross-priming"), rather than by tumor presentation of endogenous antigen.[35] Significantly, this provides a rationale for the use of allogeneic tumor cells as a source of antigen in clinical settings where collection of autologous tumor is not feasible. Many tumor-associated antigens identified to date are not patient specific, but rather are common to the type of tumor. [36] Since host APCs initiate the T-cell response to such vaccines, it is not necessary to "match" the HLA of the allogeneic tumor cell with that of the patient; instead antigens are liberated from the irradiated cell lines, captured, processed, and presented by host APCs in an autologous HLA-restricted fashion to host T-cells.

The efficacy of GM-CSF transduced tumor vaccines has been demonstrated in multiple animal models of melanoma, lung cancer, colon cancer, renal cell cancer,[30] prostate cancer, [37]acute leukemia, [38] and B-cell lymphoma. [39]In addition, early clinical trials with GM-CSF expressing vaccines have tested patients with renal cell carcinoma, [40] prostate cancer, [41] melanoma, [42]and non-small cell lung cancer and pancreatic cancer [43]. The clinical data available to date reveal minimal toxicities (local swelling and tenderness at the vaccine site) at doses as high as $5x10^8$ cells per injection, with tumor cells secreting up to 1000 ng GM-CSF/$10^6$ cells/24 hours (i.e. up to 500 micrograms of GM-CSF/day), with serum GM-CSF levels peaking 3 days post vaccine and becoming undetectable by 7 days. In vitro assays have demonstrated the induction of tumor-specific cytotoxic lymphocyte activity [42] and the appearance of high titer antibody to the vaccinating cell population. [41]

2.32 <u>K562/GM-CSF as a universal GM-CSF producing Bystander Cell:</u>

Dr. Levitsky's group transfected the K562 cell line, derived from a CML patient in blast crisis, with a plasmid vector encoding human GM-CSF. K562/GM-CSF grows well in serum-free media, stably expresses > 1000 ng of GM-CSF / $10^6$ cells/ 24 hours, and is easily expanded to large numbers for vaccine production.

Clinical trials performed at Johns Hopkins initially used the K562/GM-CSF vaccine as a "bystander" cell together with irradiated autologous tumor in patients with multiple myeloma and acute myeloid leukemia. In those studies, analysis of vaccine biopsy sites, serum GM-CSF levels, and serial white blood cell counts indicate that GM-CSF production is sustained for at least 3-5 days post vaccination.

The theoretical benefit to the vaccine in patients with CML is immune response to the common myeloid antigens between the K562 cell line and the CML tumor cells. Four interesting candidate antigens are described below that are abundantly represented in the K562/GM-CSF vaccine with other potential yet unidentified tumor antigens.

<u>BCR/ABL fusion protein</u>: The BCR-ABL fusion protein was among the first targets to be evaluated in CML. Because the breakpoint in BCR can occur in at least three sites (resulting in a 190kd, 210kd, and 230kd fusion protein) and between different exons (for p210, the breakpoint can occur between exons b2 and b3 or between exons b3 and b4), immune recognition of the unique amino acid sequences at the junction had to be evaluated individually. Such approaches have identified both MHC class I and II epitopes from the fusion protein. [44-50] While T-cell clones raised against some of these failed to recognize

endogenously processed antigens on CML blasts, other candidates did recognize endogenous peptides in an HLA restricted fashion. Importantly, direct evidence that one such peptide (HLA–A3 restricted) could be identified from the surface of CML blasts by mass spectroscopy confirmed the legitimacy of this strategy.[49]

Proteinase-3: Proteinase-3 is a constituent of myeloid granules and is over-expressed roughly ten-fold in myeloid leukemic cells [51]It is also an auto-antigen in Wegener's granulomatosis. Barrett's group initially identified an HLA-A0201 restricted epitope of proteinase-3 (named PR-1) [52,53] and demonstrated that T-cells specific for PR-1 could kill HLA-A0201$^{+}$ leukemia cells and inhibit leukemia colony formation *in vitro*. Using HLA-A0201 tetramers loaded with PR-1, Molldrem and colleagues showed a remarkable correlation between increased frequency of PR-1 specific CD8$^{+}$ CTL and remission status of CML patients treated with interferon-< or allogeneic BMT,[54] whereas patients treated with chemotherapy alone did not have detectable PR-1 specific CTL.

Wilms Tumor–1 (WT-1): WT-1 gene on chromosome 11 encodes a zinc-finger transcription factor expressed during embryogenesis in both time and tissue specific manners in regions associated with hematopoesis [55]. Whereas inactivation of *WT-1* underlies tumorigenesis in Wilms tumor, the high expression of wild-type WT-1 in leukemia[57-61], and in many solid tumors (including ovarian, gastric, colon, lung, breast, and mesothelioma) suggests an oncogenic rather than a tumor suppressor function in these diseases. Indeed there is a correlation between leukemia remission status and the production of antibodies against WT-1[62]. Studies of tumor growth inhibition with anti-sense oligonucleotides [63,64] and growth promoting effects of constitutively expressed WT-1 through gene transfection[65] have made WT-1 an attractive target in several cancers.

Survivin: Survivin is a protein encoded by chromosome 17 that associates with microtubules early in mitosis and regulates cell division as well as counteracts apoptosis. [66] The anti-apoptotic gene, survivin, is selectively over-expressed in a wide variety of tumor types [67,68] including leukemias.[69,70] This member of the inhibitor of apoptosis gene family is expressed during fetal development, but its expression is limited in terminally differentiated tissues (although it may play a role in cell cycle regulation of mitotically active cells). Like BCR/ABL and WT-1, survivin is an attractive target because it may contribute to the malignant phenotype by inhibiting apoptosis. Intracellular staining of survivin on our K562/GM-CSF cell line revealed abundant protein expression compared to normal bone marrow cells

2.33 K562/GM-CSF Vaccination in patients with CML on imatinib:

Currently, a trial is ongoing at JHH using K562/GM-CSF vaccine as an allogeneic source of CML tumor cells in patients with high risk CML in combination with imatinib. Preliminary results within this study have been quite encouraging. Of 5 patients who were initially FISH +, three have become FISH negative and 1 PCR negative. Of the patients who were initially FISH negative but PCR +, 3 of 12 have become PCR negative.

2.34 Vaccine preparation

The clinical grade K562/GM-CSF vaccine is manufactured and provided by Cell JH CPGT. The vaccine is derived from a master bank of a high-expression GM-CSF producing clone. The master cell bank is the source material for all working clinical lots. The vaccine has undergone extensive regulatory testing and screening for contaminating pathogens (including mycoplasma and adventitious viruses), and is stable and adapted to serum free medium. It is generated under GMP conditions, irradiated with 10,000 rads, frozen under controlled-rate conditions (with dimethyl sulfoxide, human serum albumin, and Pentaspan® as cyroprotectant) and stored in vapor-phase liquid nitrogen. The clinical lot will be shipped overnight in vapor-phase liquid nitrogen vessels to our Cell Therapy Laboratory where it will be stored.

2.35 Vaccine Schedule:

Patients will continue to take their TKI once CCR has been achieved. Vaccination will begin at that time with a dose of 1 X 10^8 cells distributed over 3 limbs on a schedule of every three weeks for a total of 6 months. If a molecular complete remission is not achieved at the end of the first 6 months, the patient will continue on their TKI and receive an additional 6 months of vaccines at 3 week intervals. At that point if no molecular complete response is achieved, there will be a cross-over to the other treatment arm. If molecular complete remission (PCR negativity) is achieved at the end of the initial 6 months of vaccination, the patient's TKI and the vaccinations will be discontinued.

2.36 Rationale for Vaccine Dose and Schedule:

The dose and schedule of K562/GM-CSF in this study are based on prior dose escalation studies using GM-CSF transduced tumor cells. [40-42] In these studies, in which conversion of DTH skin testing was used as a positive outcome, 1 x $10^8$ tumor cells/ vaccination led to

conversion in most patients, whereas 1 x $10^7$ cells had a conversion rate of <50%. Additionally, mouse studies showed that administration of a given dose of vaccine distributed over multiple sites improved systemic immunity.[71]

There is very little data available that pertains to the potential benefit of serial vaccinations and/vaccination boosts. Animal models have been largely unhelpful in this regard because of the relatively rapid kinetics of the growth and progression of mouse tumors, precluding an examination of dosing intervals of a therapeutic cancer vaccine. In the trials using K562/GM-CSF cells as a "bystander" source of GM-CSF together with autologous tumor (AML and myeloma) vaccine dosing has been performed at three-week intervals with no evidence of cumulative toxicities. Based on theoretical grounds, it takes between 5-10 days for T and B cells responses to peak, which is followed by a contraction phase and the generation of stable memory T and B cell populations. There is some evidence that immunization during the peak response may be deleterious, leading to activation induced cell death (AICD). Three weeks is likely to be well beyond this peak response. Most cancer vaccine protocols use intervals of anywhere between two and four weeks, with some studies employing a late "boost", although evidence in support of this is lacking.

2.4 Rationale:

The highly effective and rapid responses of CML patients treated with TKIs have been profound. There are currently 3 TKIs FDA-approved for the upfront treatment of chronic phase CML, Imatinib, Dasatinib, and Nilotinib. The disappointing clinical features of each of these agents have been the development of resistance and the expected relapse upon withdrawal of the agent from a PCR-undetectable state. Again, both findings point to the limited impact of these agents on the CML stem cell.

With the premise that biology should dictate treatment, understanding the biology of CML offers such treatment options. In review of the studies discussed above, it is evident that although interferon alpha has a less favorable side effect profile and only 13% occurrence of complete cytogenetic remissions, the time to remission and duration of response [8] suggest the possible target of the rare quiescent stem cell. In contrast, the studies with imatinib by O'Brien et al [18] with the rapidity of both hematologic and cytogenetic responses indicate a target of the more committed progenitors that make up the bulk of the tumor. Further support of this argument is evidence showing resistance of the CML stem cell to all TKIs in vitro studies [72] . Additionally, many patients show relapse upon withdrawal of their TKI, even those with transient

PCR negativity, indicating the continued presence of the CML stem cell in the setting of TKI treatment [73].

Given the above limitations with TKI treatment, many questions remain. Is it safe or ethical to withdraw TKIs at the time of complete molecular response given the history of documented relapse in that setting? With the findings of increasing TKI resistance, what are other treatment options to move towards cure of CML outside of the transplant setting? Why is PCR negativity not enough to document cure of CML?

We hypothesize that TKIs are crucial for debulking the differentiated CML progenitors and other tools are needed to target the CML stem cell. Based on studies described above, Interferon-alpha may play a role in eradicating the CML stem cell. In addition, the exciting results in a current study at JHH looking at vaccination with K562/GM-CSF in patients with CML in combination with imatinib described above have hinted at the vaccine potentially targeting the CML stem cell as well. Moreover, there are now several reports of discontinuation of TKIs in responding CML patients. Although many, if not most patients, eventually relapse, all rapidly went back into remission with restarting their TKI[73-75]. Thus, although the patient numbers are small, it appears that TKIs can be safely stopped in complete molecular remission, without emergence of resistance.

With the historical data with interferon and GM-CSF treatment and the preliminary pilot data with the K562/GM-CSF vaccine trial in CML patients, we proposed the following Phase II randomized study. Ph+ CML patients in chronic phase achieving cytogenetic complete response (CCR) while on TKIs will be randomized to either treatment with interferon 2 alfa + Sargramostim or K562/GM-CSF vaccination sequence. Following molecular complete response, treatment arm and the patient's TKI will be discontinued and progression free survival at one year after combination treatment cessation, defined as maintenance of molecular complete remission, will be assessed separately in each arm.

## 3. PATIENT SELECTION

### 3.1 Eligibility Criteria

3.11 Ph+ CML. The diagnosis of chronic phase CML based on cytogenetic detection of the Ph chromosome and/or detection of the BCR-ABL rearrangement by molecular analysis (recombinant DNA analysis of the BCR-ABL fusion gene, fluorescence *in situ* hybridization, or polymerase chain reaction detection of the BCR-ABL hybrid mRNA).

3.12 Documentation of complete cytogenetic response (CCR) by conventional cytogenetics or FISH analysis on a frontline TKI with stable dosing.

3.13 Age $\geq$ 18 years

3.14 Negative pregnancy test if applicable

3.15 ECOG performance status of 0, 1, or 2

3.16 Patients must have normal organ function as defined by Cr $\leq$ 2.0, Bilirubin $\leq$ 2.0, and AST/ALT $\leq$ 2.5 X institutional upper limit of normal.

3.17 Life expectancy greater than 24 months

3.18 Written consent by the participant

3.2 Exclusion Criteria

3.21 Any phase of CML other than chronic phase

3.22 History of bone marrow or other transplantation.

3.23 Treatment with hydroxyurea, busulfan, cytoreductive agents other than frontline TKI, or an investigational agent within 28 days of registration. Patients who are on alpha-interferon as primary therapy are not eligible.

3.24 History of an active malignancy or prior malignancy except for the following: patients greater than 5 years out from their diagnosis and or treatment, patients with local malignancies who have undergone localized therapy felt to be curative (e.g., colposcopy resection of in situ cervical carcinoma, surgically resected non-melanoma skin cancer, local irradiation, surgery, or ablative radioactive iodine treatment for local thyroid cancer). Patients who have undergone such interventions should be greater than one year from therapy without evidence of recurrence.

3.25 Any other disease requiring long-term corticosteroids or immunosuppressants.

3.26 Patient may not have been resistant to any other prior TKI therapy. Prior TKI therapy must only have been discontinued due to intolerance.

3.3 Inclusion of Women and Minorities

Both men and women and members of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

All patients will be registered with the Central Registration Office using the Clinical Research Management System (CRMS) after obtaining signed informed consent.

5. TREATMENT PLAN

5.1 Agent Administration

5.11 Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for K562/GM-CSF vaccine, TKIs, and interferon alfa are described in Section 7. No dose modification exists for the K562/GM-CSF vaccine. Appropriate dose modifications for TKI and interferon alfa will be described below. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

At time of enrollment all patients will be on a stable standard dose of their frontline TKI and in a cytogenetic complete remission (CCR) as

documented by conventional metaphase cytogenetics or FISH analysis. At that point, patients will be treated on Study Arm A and receive interferon-alpha + GM-CSF.

Arm A: Interferon-alfa + Sargramostim:

The addition of interferon-alfa to the patient's standard, stable TKI dose will follow a dose escalation pattern. Patients will initially receive interferon alpha at doses of 1-2 Million units/day + 125 mcg/$m^2$ of Sargramostim per day, both given subcutaneously. Dosing of interferon alpha will be rounded to the nearest 0.5 Million units to simplify dosing. The dose of IFN will be escalated every 1 – 2 weeks by increments of 1 million units/day to a maximum of 10 million units/day or the highest dose tolerable. Additionally, Sargramostim dosing will be rounded to the nearest 50 mcg to simplify dosing. Hematologic parameters monitored to determine this highest dose allowable are ANC>500, platelets >20K. Flu-like symptoms are common during the first few weeks of IFN therapy, usually respond to acetaminophen, and should not be an indication for stopping interferon. Consideration should also be given to treating depressive symptoms associated with IFN with an antidepressant such as fluoxetine or paroxetine.

Upon initiating therapy, subjects should follow-up for physical exam and blood work every 3 weeks +/- 1 week allowing for holiday and other schedule conflicts. At the discretion of the PI, patients may be allowed to extend their follow-up interval from every 3 weeks (+/- 1 week) to every 6 weeks (+/- 1 week), given they are tolerating the medications without complications. They will still be required to get blood work done locally every 3 weeks (+/- 1 week).

The combination of TKI and IFN + Sargramsotim will be continued concomitantly for a minimum of 6 months. If, at the end of the 6 month period, a molecular complete remission has been achieved as defined as BCR-ABL PCR negativity confirmed by two assays separated by at least 1 month, TKI and IFN + GM-CSF will be discontinued and monitoring will ensue. If, at the end of 6 months, BCR-ABL PCR remains positive, an additional 6 months of imatinib and interferon + Sargramostim will be given. If at any time during months 6 – 12 of combination therapy the subject achieved a molecular complete response that is confirmed by two assays separated by at least 1 month, the combination therapy will be discontinued and monitoring initiated as outlined below. At the end of 12 months of combination therapy, patients will be considered to have completed Study Arm A and will then be followed per standard of care on single agent TKI.

Once both TKI and IFN + Sargramsotim are stopped, PCR will be monitored as outlined below to assess for maintenance of or loss of molecular complete remission. If molecular complete remission is lost after cessation of TKI and IFN + Sargramsotim, the patient's prior TKI will be re-initiated. If molecular complete remission is maintained for > 36 months, the patient monitoring may be decreased to every 6 months at the discretion of the study PI.

If intolerability of IFN + Sargramsotim occurs during treatment on Study Arm A, patients will be discontinued from the study and return to standard of care monitoring and following once all toxicities have resolved to pre-study baseline. Intolerability is defined as any of the following: consistent 1) hypotension with lowest possible dose; 2) arrhythmia or acute cardiac event; 3) intolerable headaches or mental impairment; 4) grade four hematologic toxicity not responsive to dose modification; 5) elevation of liver function tests > 3 times normal; 6) acute and persistent renal failure; 7) recurrent and life-threatening electrolyte abnormalities.

Dose decreases will be made for IFN in the same framework as the escalation schema described above. If intolerability at certain doses, dose will be decreased every 1 – 2 weeks by increments of 1 Million units/day to maintain the maximum dose tolerable.

Arm B: K562/GM-CSF Vaccination series:

Study Arm B is not available to newly accrued and enrolled subjects based on the interim analysis directing all new subjects to the combination of Interferon + GM-CSF. However, the addition of K562/GM-CSF vaccination series to the patient's standard, stable dose of TKI for patients currently enrolled and receiving Study Arm B or receiving K562/GM-CSF vaccination per crossover will be as follows.

All patients will be vaccinated with 1 x $10^8$ cells intradermally per dose. Depending on the cell concentration in the specific lot of vaccine used (2 X $10^7$ or 5 X $10^7$ cells in 1 ml), the vaccine syringes will be prepared as described below:

- 2 X $10^7$ cells per vial in 1 ml: 5 vials with contents distributed in 9 or 10 syringes, each syringe containing approximately 1.0 X $10^7$ cells in approximately 0.5 ml.
- 5 X $10^7$ cells per vial in 1 ml: 2 vials with contents distributed in 4 or 5 syringes, each containing 2.5 X $10^7$ cells in approximately 0.5ml

Those administrating the vaccine will record:

a. Clinical lot number
b. Date of vaccine administration
c. Number of syringes dispensed
d. Total vaccine dose
e. Vaccination sites

Patients will be observed for 60 minutes following the first vaccination and 30 minutes following subsequent vaccinations, for any adverse reactions. Patients will also be asked to complete worksheets of symptoms and events.

Vaccinations will occur every three weeks for 8 cycles for a minimum of 6 months with concomitant TKI. If a molecular complete remission occurs during this period, the TKI will be continued until the vaccine series is complete. If, at the end of the 6 month period, a molecular complete remission has been achieved as defined as BCR-ABL PCR negativity confirmed by two assays separated by at least 1 month (+/- 1 week), TKI and vaccinations will be discontinued and monitoring will ensue. If, at the end of the initial 6 months, PCR remains positive, an additional 6 months of TKI and vaccinations will be given. If at anytime during months 6 – 12 of combination therapy the subject achieved a molecular complete response that is confirmed by two assays separated by at least 1 month, the combination therapy will be discontinued and monitoring initiated. At the end of 12 months of combination therapy, patients that remain PCR positive are eligible to cross over to the IFN + GM-CSF arm.

After a molecular complete remission, once both TKI and vaccinations have been stopped, PCR will be monitored as outlined below to assess for maintenance or loss of molecular complete remission. If molecular complete remission is lost within the first twelve months after cessation of TKI and vaccinations, the patients prior TKI will be re-initiated and the patient will be allowed to cross-over to Study Arm A. If molecular complete remission is maintained > 36 months, the patient monitoring may be decreased to every 6 months at the discretion of the study PI.

Eligibility requirement for cross-over include any of the following: 1) Lack of molecular complete remission at the end of 12 months of combined TKI + K562/GM-CSF vaccinations; 3) Loss of molecular complete remission within the first 12 months after cessation of TKI and vaccine series; 4) intolerability of vaccination as defined as allergy to vaccine.

Planned monitoring for patients who achieve a complete molecular remission and have discontinued their study treatments and TKI will include: PCR measure of bcr-abl monthly for 3 consecutive months

followed by every other month for 2 consecutive measures, followed by every third month through at least 36 months following TKI discontinuation. For patients who remain with an undectable PCR > 36 months from discontinuation, monitoring may be decreased to every 6 months at the discretion of the study PI.

PCR positive result will be defined as confirmed presence of bcr-abl above the level of quantification of the assay (~0.05-0.1 copies per 1000 abl) confirmed by repeat testing at least 1 month following the initial positive test. Patients with borderline positive tests will undergo repeat testing 1 month sooner than their scheduled testing.

5.12 Cross-Over Options:

Following the planned interim analysis, there is no longer a planned cross-over for subjects. However, patients currently enrolled and undergoing treatment per their initial study arm will be eligible for cross-over as described: Cross-over options will occur at set time-points as outlined in the Schema. On either treatment arm, if a molecular complete remission is not achieved after a full 12 months of combination therapy, (TKI + Vaccine or TKI +Interferon/Sargramostim), the patients will be eligible for cross-over. In addition, if a patient loses their established molecular complete remission once the treatment arm and TKI are discontinued, the patient's prior TKI will be restarted and the patient will be allowed to cross-over to the other treatment arm. In order to be eligible to cross-over, all subjects must meet the eligibility criteria for study enrollment listed in Section 3.1 above.

Cross-over options are being allowed because studies have shown that interferon alpha up-regulates the expansion of specific cytotoxic T-cells. Thus, in patients who have been on the vaccine arm and have possibly developed cytotoxic T cells specific to the vaccine and thus CML cells, interferon may serve as a tool to expand that population. Likewise, in the patients initially on interferon, the effects on cytotoxic T cell influence may carry over to augment an immune response to the vaccine series.

5.2 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of TKIs with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any

agent known to affect or with the potential to affect selected P450 isoenzymes.

Because the K562/GM-CSF vaccine and Interferon alpha are immune modulatory treatments, immunuo-suppressents in the form of steroids, cyclosporine, imuran, cellcept, sirolimas, tacrolimus and others should not be administered during this clinical trial. Any questions regarding study eligibility for such subjects will be addressed by the Principal Investigator.

5.3 Assessments of trial enrollment motivation and assessment of apprehension regarding discontinuation of therapy

In effort to better understand individual patient's motivations for enrolling on this trial, their emotions surrounding the discontinuation of all therapy, and the tolerability of the adjuvant treatments, we will also conduct interviews to capture this data. Post-enrollment and prior to starting their assigned adjuvant therapy, patients will be interviewed following the "CML Interview Guide" included in Appendix C. Patients will also undergo a final CML Interview at the completion of the study. Dr. Holly Taylor, a study co-investigator from the Department of Health Policy and Management, Bloomberg School of Public Health, and the Berman Institute of Bioethics, will conduct the interviews. These interviews will be tape recorded and then transcribed for analysis. A total of 8 subjects from each study arm, ideally alternating every other patient enrolled per arm, will be included in this data collection plan. Patients enrolled in the in-depth interview portion will be assigned a unique number identifier. Once the second in-depth interview is completed, any personal identifying information connecting the patient to the unique identifying number will be destroyed.

5.4 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue as follows. In the both treatment arms, the initial treatment phase will be 6 months. If molecular complete remission is achieved in either arm at that point, the treatment arm and imatinib will be discontinued and monitoring as outlined above with be initiated. If molecular complete remission is not achieved after the initial 6 months of combination treatment an additional 6 months of combination treatment will occur. The total duration of therapy will be patient dependent with regards to response criteria. The minimum duration of combined therapy will be 6 months. The maximum duration of the study would be a the summary of the following: a total of 12 months of combination therapy, observation if molecular complete remission obtained, and possibility of cross-over to other study arm if loss of

molecular complete remission occurs within the twelve months of observation for a total of 24-36 months of expected therapy.

Treatment will be discontinued if:

- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse events(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

## 5.5 Duration of Follow Up

Patients will be followed for at least12 months after removal from study or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution of the AE and / or its stabilization followed by at least 12 months of standard of care as above.

* Per FDA guidelines, observation for vaccine studies is generally 15 years. However, considering the minimal risk of the K562/GM-CSF cellular vaccine, the FDA has granted permission to decrease the 15 year observational period for IND# 11284, to 12 months after removal from study or until death, whichever occurs first. The follow up period provides a safe environment for monitoring the patients treated under this IND for any adversities associated with the K562/GM-CSF Bystander vaccine cells, as supported by the FDA.

# 6. DOSING DELAYS/DOSE MODIFICATIONS

TKIs will initially be continued at the dose the patient was on that induced a CCR. The standard doses typically are: Imatinib from 400mg to 800 mg daily, Dasatinib 50-100mg daily, or Nilotinib 300mg-400 mg twice a day Should dose limiting toxicities detailed in Section 8.3 develop, the dosage will be altered. The change in dose will depend on the patient’s current dosage.

Interferon alpha will be dose escalated as described in Section 5 within the defined hematologic parameters. Should adverse symptoms arise that are not tolerable or dealt with by good medical practice the interferon dose will decrease in the same manner as the escalation. If the patient doesn’t tolerate the minimal required dose then they will be

given the option of stopping the trial or crossing over to the vaccination arm.

There are no dose modifications for the vaccine arm.

## 7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

### 7.1 Routine Adverse Event Reporting

Definition and reporting of adverse events

Adverse Event: any new, undesirable medical occurrence or worsening of a pre-existing condition that occurs as a result of a treatment.

Serious Adverse Event: any adverse drug experience occurring at any dose that is fatal, is life threatening, requires hospitalization, or results in a persistent or significant disability or incapacity.

Unexpected Adverse Event: any adverse event that is not explicitly described in the drug package insert, IND, protocol, or consent form.

Toxicities should be described according to the National Cancer Institute (NCI) Common Toxicity Criteria, version 3.0, which can be accessed and downloaded via the website: http://ctep.cancer.gov/reporting/ctc.html.

- Any unexpected, either life-threatening or fatal event that occurs between the time of registration and 30 days after completion of the vaccination series must be reported to the principal investigator or designee within one working day of discovery or notification. Adverse events will be recorded on a standard Adverse Event Report form.

- Any unexpected, either life-threatening or fatal event must be reported within 3 working days to the Johns Hopkins CRO via an Adverse Event Report form, as well as to the Institutional Review Board (IRB), Institutional Biosafety Committee, Federal Drug Administration (FDA), and National Institutes of Health Recombinant DNA Advisory Committee (NIH-RAC). Otherwise, any unexpected serious/severe adverse event must be reported within 10 working days. The principal investigator is responsible for notifying these committees.

- The principal investigator will report any serious side effects to the IND holder who will in turn report any serious or unexpected

adverse events to the FDA as stated in standard guidelines. Any death from any cause while a patient is receiving protocol treatment, or up to 30 days after the last dose of protocol treatment, or any death which occurs more than 30 days after the last protocol treatment but is felt to be possibly treatment related, must also be reported to the above committees by the principal investigator.

7.2 FDA reporting Guidelines will be followed for serious and unexpected adverse events.

- Calendar-Day Telephone or Fax Report

  The Sponsor-Investigator is required to notify the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the investigator to be possibly related to the use of K562/GM-CSF vaccine. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be telephoned or faxed to the FDA and (specify name of company supplying study drug, if appropriate) within 7 calendar days of first learning of the event. Each telephone call or fax transmission should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever is responsible for the review of the IND.

- 15 Calendar-Day Written Report
  The Sponsor-Investigator is also required to notify the FDA and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of K562/GM-CSF. An unexpected adverse event is one that is not already described in the Investigator Brochure.

- Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

- Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA and all participating investigators within 15 calendar days of first learning of the event. The FDA prefers these reports on a MedWatch 3500 Form but alternative formats are acceptable (e.g. summary letter).

7.3 Adverse Event List For Imatinib and Interferon:

See adverse event/side effect information in Section 8.0 Pharmaceutical information.

## 8. PHARMACEUTICAL INFORMATION

### 8.1 GM-CSF:

Other Names
Granulocyte-macrophage colony stimulating factor, rHu GM-CSF, Leukine, Sargramostim

Classification
Colony stimulating factor; cytokine

Mode of Action
Primarily affects the proliferation, differentiation, and activation of granulocytes and macrophages by inducing partially committed progenitor cells. It is also capable of activating mature granulocytes and macrophages.

Availability
Commercially available.

Side Effects
1. Most frequently reported: Mild to moderate fever, asthenia, chills, headache, nausea, diarrhea, musculoskeletal pain, and injection site reaction.
2. Gastrointestinal: Nausea, vomiting, anorexia, diarrhea, stomatitis, liver function abnormalities.
3. Cardiovascular: Pericardial effusion, pericarditis, chest pain, cardiac dysrhythmia, hypotension, hypertension, peripheral edema, heart failure, peripheral edema.
4. Respiratory: Dyspnea, cough, pleural effusion
5. Dermatologic: Rash, urticaria, alopecia.
6. Neurologic: Headache, dizziness
7. Hematologic: Leukocytosis, eosinophilia, thrombocytopenia, splenomegaly.
8. Renal: Transient increase in creatinine, hyponatremia
9. Other: Vascular leak syndrome (fluid retention, weight gain, edema, polyserositis), first dose effect (fever, facial flushing, hypotension, transient loss of consciousness, headache, tachycardia, pulmonary dysfunction).

Additional considerations

There are theoretical risks of leukemic proliferation and self-renewal in clinical trials involving growth factors for myeloid leukemias. Myeloid leukemia cells and their precursors express receptors for colony-stimulating factors, and these factors support the in vitro survival, proliferation, and differentiation of these cells. [76-79] Conversely, GM-CSF could potentiate several anti-tumor mechanisms mediated through induction of differentiation, increasing Fc receptors on monocytes, antibody mediated cellular cytotoxicity, [78] improved phagocytosis, and enhanced secretion of secondary cytokines (IL-1,TNF) and interferon by monocytes.[78,80,81] There are no strong clinical data supporting the efficacy of the potential antileukemic effects of growth factors, although the safety of such approaches has been examined in phase II studies. A number of CML patients have received GM-CSF after bone marrow transplantation, and increased risk of leukemic relapse has not been reported [82,83]. In recipients of T-cell depleted allografts, actuarial probability of relapse at 18 months was in fact lower in GM-CSF treated patients, with a trend toward better survival. [84]

8.2 K562/GM-CSF vaccine

The experience with K562/GM-CSF to date has shown it to be safe and well tolerated. Elevated levels of GM-CSF can be detected in the serum for 3-5 days post vaccination, and this is accompanied by a transient rise in the white blood cell, neutrophil, and absolute eosinophil counts. Toxicity is generally limited to myalgias and grade 1-2 injection site erythema, induration, tenderness, and localized pruritus. Most patients experience local vaccine site reactions that last for approximately 1 week; induration has ranged from 0.5 cm to as great as 20 cm. In other trials of GM-CSF producing vaccines, systemic reactions have included fever, headache, rash, generalized pruritus, and malaise.

As with any vaccine, there is a possibility of a serious allergic reaction.

As with any investigational drug, other adverse reactions may develop that have not yet been described.

There is a theoretical risk of autoimmune disease reactivation or development with GM-CSF producing vaccines. In the case of CML, an immune response against autologous tumor cells could in principle lead to leukopenia or anemia. In the multiple myeloma vaccine trial, induction of immune responses to autologous tumor cells has been demonstrated in vitro. Importantly, all multiple myeloma patients who have undergone autologous stem-cell transplant followed by

vaccination have had normal kinetics of engraftment. There has been no serological or clinical evidence of autoimmune disease to date arising in recipients of the K562/GM-CSF vaccine in the previous AML, CML and Myeloma trials.

8.2.1 Availability

The clinical grade K562/GM-CSF vaccine is manufactured and produced by our GMP facilities at Johns Hopkins. The vaccine is derived from a master bank of a high-expression GM-CSF producing clone. The master cell bank is the source material for all working clinical lots.

8.3 Interferon Alfa:

Other Names:
Interferon alpha 2a, Roferon - A

Classification:
Immunologic Agent

Mode of Action:
Following activation, multiple effects can be detected including induction of gene transcription. Inhibits cellular growth, alters the state of cellular differentiation, interferes with oncogene expression, alters cell surface antigen expression, increases phagocytic activity of macrophages, and augments cytotoxicity of lymphocytes for target cells.

Storage:

- Injection Powder for Solution: 18 Million U

- Injection Solution: 3 Million U/ML, 6 Million U/ML, 9 Million U/0.9 ML, 36 Million U/ML
- Kit: 3 Million IU/0.5 ML, 6 Million IU/0.5 ML, 9 Million IU/0.5 ML

Dose specifics
SubQ, I.M.: 9 million units/day, continue treatment until disease progression

Availability
Commercially available

Drug interactions

Weakly Inhibits CYP1A2 and thus has some effects on decreasing serum concentrations and possible effects of erythropoietin, melphalan, zidovudine and may increase serum concentrations and thus toxicity of ACE inhibitors, warfarin, and possibly clozapine.

Side effects

1. Flu-like Symptoms (up to 92%)
2. Cardiovascular: Hypertension – 11%, Chest Pain – 4-11%, Edema – 11% Hypotension – 6%, SVT- <3%, acute MI - <1%
3. CNS: Fatigue – 90%, Headache – 52%, psychiatric disturbances – variable greater than 15%, irritability/insomnia – 14-15%, somnolence, lethargy, confusion , mental impairment – usually seen at doses >100 million units, Confusion – 10%
4. Hematologic: Neutropenia – 32-70%; thrombocytopenia – 22-70%; anemia 24-65%.
5. Hepatic: elevation of AST – 77-80%, elevation of LDH – 47%, elevation of bilirubin – 31%
6. GI: Anorexia – 30-70%, nausea 28-53%, vomiting 10-30%, diarrhea 22-34%, taste change – 13%, pancreatitis <5%
7. Renal: Proteinuria – 15-25%
8. Dermatologic: Rash 7-18%, alopecia – 19-22%, pruritis – 13%, urticaria – 10%
9. Pulmonary: Cough – 27%; dyspnea – 7.5%
10. Neuromuscular: weakness. Arthralgia/myalgia – 5-73%; leg cramps, paresthesia – 7%
11. Endocrine: Hypocalcemia – 10-51%, hyperglycemia 33-39%, hyperphosphatemia – 2%
12. GU: impotence – 6%, menstral irregularities
13. Life threatening side-effects: <1%

9. CORRELATIVE/SPECIAL STUDIES

See section 11.0 for details of correlative studies

10. STUDY CALENDARS:

The following are minimum core evaluations requested for study purposes. Other routine assessments should be performed in accordance with good medical practices with adherence to institutional guidelines in effect for patients.

**Study Arm A:**

| Baseline Pre-Enrollment[f] | IFN/GM-CSF Arm<br><br>Follow- | 3 month F/U on combination treatment | 6 month F/U on combination Treatment | Defined Intervals[e]<br><br>Follow-Up |
|---|---|---|---|---|

| | | | | | |
|---|---|---|---|---|---|
| | | Up Monthly[i] | | | |
| H & P | X | X | | X | X |
| Med Review | X | X | | X | X |
| Toxicity | X | X | | X | X |
| FISH Ph Chromosome[a] | X | | X | X | X |
| Study F/U | X | X | | X | X |
| Heme-8 w/ Dif | X | X | | X | X |
| Chemistry [b] | X | X | | X | X |
| PTT and INR | X | | | | |
| LDH | X | | | | |
| Blood for Immune Assays[c] | X | | X | | X |
| BCR-ABL PCR [d] | X | | X | X | X |
| PregnancyTest | X | | | | |
| CML Interview[h] | X | | | | X[g] |

**Study Arm B:**

| | Baseline Pre-Enrollment[f] | K562/GM-CSF Vaccine Arm Follow-Up q3weeks | 3 month F/U on combination treatment | 6 month F/U on combination treatment | Defined Intervals[e] Follow-Up |
|---|---|---|---|---|---|
| H & P | X | X | | X | X |
| Med Review | X | X | | X | X |
| Toxicity | X | X | | X | X |
| FISH Ph Chromosome[a] | X | | X[a] | X | X |
| Study F/U | X | X | | X | X |
| Heme-8 w/ Dif | X | X | | X | X |
| Chemistry [b] | X | X | | X | X |
| PTT and INR | X | | | | |
| LDH | X | | | | |
| Blood for Immune Assays[c] | X | | X | | X |
| BCR-ABL PCR [d] | X | | X | X | X |
| PregnancyTest | X | | | | |
| CML Interview[h] | X | | | | X[g] |

a. Philadelphia Chromosome tests will be done on peripheral blood with FISH techniques
b. Chemistry panel will include electrolytes, creatinine, liver function tests, uric acid
c. Blood for immune assays: Collect 60ml of peripheral blood in 5 heparinized tubes and 1 serum separator tube. These assays will be drawn on patients at baseline and at 3 month intervals with FISH and bcr/abl testing through 36 mos in pts who are molecularly undetectable and off all therapy at which point blood will be collected annually and at the time of loss of molecular complete remission.
d. PCR for BCR-ABL transcripts will be drawn at baseline and every 3 months for both Interferon/GM-CSF and K562/GM-CSF vaccine arms. If there are two negative tests in a row, patients may be eligible to discontinue study drug and TKI and follow the planned monitoring schedule. If TKI is stopped, PCR will be tested monthly x 3 months, every other month x 2 testings, and then every 3 months thereafter up to 36 mos of remaining off of all therapy. Beyond 36 mos, PCR testing may be decreased to every 6 mos at the discretion of the study PI.
e. Follow-up after the 6 month visit will depend on PCR status. PCR status dictates which path the patient follows in the treatment schema.
f. Baseline evaluations will need to be completed and documented within 4 weeks of starting the protocol ,with the exception of FISH testing for Ph+ chromosomes that must be negative within 12, +/- 2, weeks (this is commonly available datapoint for routine care). .
g. Final CML Interview to be administered at completion of trial
h. In-Depth CML Interview will only be conducted on a subset of all trial enrollees. 8 patients from both Study Arm A and B will be included.
i. At the discretion of the PI, patients may be allowed to extend their follow-up interval from every 3 weeks (+/- 1 week) to every 6 weeks (+/-1 week), if they are tolerating the medications well. They will still be required to get blood work done locally every 3 weeks (+/- 1 week).

# 11. MEASUREMENT OF EFFECT

## 11.1.1 Hematologic monitoring

The following will be requested:

1. CBC, differential, and comprehensive chemistries will be drawn at time periods stated above in the study calendar. See Appendix B for CML Hematologic Response Criteria.

## 11.1.2 Measurement of Minimal Residual Disease

Blood for FISH and PCR for BCR-ABL will be assessed at initiation of the trial, prior to initiating Interferon alfa + Sargramostim or vaccination series and every three months during the initial 6 month randomized treatment in both study arms. PCR will continue to be assessed every month after PCR negativity through the completion of the first 6 months of the study and thereafter once the combination therapy is discontinued until full completion of the study or until progression.

BCR-ABL transcripts will be measured with the combined quantitative real-time RT-PCR (RQ-PCR) and capillary electrophoresis approach.

Results will be designated as the expression of BCR-ABL relative to ABL expression (BCR-ABL/ABL or % BCR-ABL)[85]. Replicates typically have 0.1-0.2 log intra-assay variability.

11.1.3 Additional testing

Peripheral blood and serum samples for immune studies, described below, will be drawn at the same time as for BCR-ABL RQ-PCR and FISH up to and through at least 36 mos of being discontinued from TKI per study direction. With signed consent from the patient, tissue samples will be banked for future study.

Additional medical evaluations should be performed as the patient's condition warrants according to good medical practice.

11.2 Immunologic response assessment

Blood will be drawn for *in vitro* assays in order to quantify T-cell responses to candidate target CML-associated antigens (BCR-ABL p210, proteinase-3, WT-1, and survivin).

Peripheral blood (60 cc) will be obtained for immune correlative studies at the documented time points in the study calendar by standard venipuncture techniques. Fresh blood will be delivered to the Human Immunology Core, 4$^{th}$ Floor of CRB 1.. Mononuclear cells will be recovered by standard density centrifugation using Ficoll-Hypaque. Cells will be washed twice and frozen viably in 10% DSMO. Cells will be stored by the SAC in liquid nitrogen. Patient serum samples will also be separated and prepared from the noted samples collected for research. Serum will be stored in 1-2 mil aliquots.

These assays may include, but are not limited to the following:

11.2.1 Assays dependent on specific HLA haplotyes

These assays provide frequency analysis of T-cells specific for defined peptide epitopes for the antigen of interest. These can only be performed in patients who express HLA alleles known to bind the defined epitopes, although most of the defined epitopes are for common alleles:

HLA tetramers: For the defined MHC class I epitopes reported for the four target antigens, HLA tetramers loaded with the appropriate peptide are constructed and used for fluorescent activated cell sorter (FACS) analysis, staining for CD8 versus tetramer. To accurately

quantify relatively low frequency events, methods can be used to gate-out false positive events and minimize background staining. This approach typically detects positive events in the 0.01% to 0.05% range.

Intracellular cytokine staining (ICS) and FACS analysis of peripheral blood lymphocytes (PBL) is performed after incubation with the relevant or irrelevant peptides plus golgi blockers, staining for CD8 or CD4 versus IFN-alfa.

IFN-alfa ELISPOT assay: Changes in the frequency of CML antigen-specific T cells may occur in response to vaccination but still be below the level of detection as measured by tetramers or ICS. If insufficient signals are detected in these assays, we will use the more sensitive IFN-alfa ELISPOT assay. Peripheral blood monocyte-derived DCs are pulsed with relevant peptide versus control and incubated with T-cells, whose activation generates the release of IFN-alfa. This allows for the quantification of the frequency and intensity of specific T-cell responses.

11.2.2 Assays independent of HLA haplotype

Monocyte-derived DCs (collected during apheresis) are transduced to express the full-length antigen of interest. Host APCs process and present target peptides on host HLA molecules, allowing one to perform the following on all patients:

IFN-alfa ELISPOT assay using transduced DCs and autologous peripheral blood-derived T-cells.

Intracellular cytokine staining and FACS to analyze transduced DCs as stimulators of T-cell responses.

Because we are interested in changes in the above parameters over time for each patient (i.e. before versus after vaccination), we will not perform these assays in “real-time,” but plan to collect and viably cryopreserve lymphocytes at the indicated time points. The full set will then be run in batch to minimize assay variability across time points. Therefore, for a given patient, this data may not be generated until after the final specimen collection.

11.2.3 Humoral immunity

Studies of humoral immunity to one or more K562-associated antigens as a function of vaccination may be performed.

## 12. DATA REPORTING / REGULATORY REQUIREMENTS

12.1 Data Reporting of Adverse Effects: See Section 7.0

12.2 Clinical Trial Monitoring:

External data monitoring will be performed by the Johns Hopkins Clinical Research Office in accordance with a level III risk/complexity study, as described in the Johns Hopkins Comprehensive Cancer Center (JHCCC) Data and Safety Monitoring Plan. A Medical Expert Panel will be utilized to provide addition oversight to assure the safety of individuals on the trial. This panel will meet annually during years of ongoing protocol accrual to monitor and review the conduct of the protocol and any protocol related events. The panel will also review all severe adverse events with 1 month of the SAE submission and offer guidance on the safety and practicality of continued vaccination plan. The following individuals have agreed to participate as members of the Expert Medical Panel:

Dr. Margaret Showel, Instructor of Oncology –Leukemia expert
Dr. Leisha Emens, Assistant Professor of Oncology – Vaccine expert
Dr. Carol Ann Huff, Assistant Professor of Oncology – Clinical trials expert

FDA regulations on clinical monitoring and record keeping will be followed and is detailed in section 7.2.

## 13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This trial will be based on a Randomized Phase II trial design. Patients with Ph+ CML in chronic phase who have documented cytogenetic complete remission (CCR) on single agent TKI, will be randomized to either treatment with INF-alfa + Sargramostim or K562/GM-CSF vaccinations. Rates of molecular CR at one year for each arm will be determined. Subjects achieving a molecular CR after a minimum of 6 months of combination therapy will discontinue all therapy upon achieving molecular CR. Duration of molecular CR will be measured by progression-free survival from the time of combination therapy discontinuation.

The primary endpoint of the study is progression free survival rate at 12 months off all therapy defined as maintenance of molecular complete remission. True progression free survival off TKI is unknown as the majority of patients are continued on the drug until they progress. However, small reports of RT-PCR negative patients show progression when TKI is discontinued. Moreover, several reports now show that CML stem cells are resistant to TKI and it is unlikely that patients will remain in molecular remissions off TKI. This study is being done to assess whether or not treatment with Interferon alpha or K562/GM-CSF can target the CML cancer stem cell and thus provide for the maintenance of PCR negativity after TKI is withdrawn..

A cross-over option is also built into the protocol. Should a patient lose their molecular complete remission after TKI is withdrawn as documented by recurrence of PCR positivity on two separate assays one month apart, TKI will be re-initiated in order to regain molecular complete remission. Once molecular complete remission is regained, the patient will be given the option of crossing over to the other arm of the study. In addition, after 6 months of initial therapy with either Interferon-Sargramostim or K562/GM-CSF if there is no molecular complete remission an additional 6 months of either treatment will be given. If there is still no molecular complete remission at the completion of a total of 12 months of combination therapy the patients will be crossed over to the other treatment arm.

We are additionally analyzing data regarding patient motivation for trial enrollment and emotions regarding the cessation of therapy at protocol defined time points. Since we know little about potential subject attitudes about enrollment in trials where cessation of therapy is a requirement, a well-validated approach to use when little is known about a complex topic, and relevant variables need to be identified, is a qualitative methodology. Therefore, we will use open-ended, in-depth interviews with a small number of subjects. Qualitative studies that explore a new topic typically include a sample that provides insight into the phenomenon under study. Therefore, our aim is to select a sample of patients with a range of characteristics and experience. The analysis of the data will be constant and iterative. Responses from each CML interview will be compared to the responses gathered from the prior interview in order to identify important themes and refine future interviews. Interviews will be conducted until there is informational redundancy and no new information is expected to be gathered by conducting additional interviews.

13.2 Sample Size:

The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins is a major referral center for the treatment of patients with hematologic malignancies. Previous studies with CML patients have yielded an accrual of approximately 6 to 8 patients per year.

A total accrual time of 7 years, we anticipate a sample size of 56 patients. PFS at one year, as described by maintenance of molecular complete remission, is expected to be between 1-10% one year after stopping imatinib. We expect the addition of either Interferon alpha or K562/GM-CSF to increase this rate by 20%. We also expect about 25-50% of the enrolled patients to achieve a molecular remission and stop treatment. The table below shows estimates of the one-year PFS rate and exact 90% confidence intervals for various remission rates and one-year PFS rates. With a null one-year PFS rate as high as 10%, we will observe a significant increase in this rate if at least 4 out of 10 or 6 out of 15 patients who achieve remission also achieve one-year of PFS.

| Number of patients who achieve remission | Number of patients who achieve one-year of PFS | One-year PFS estimate | Exact 95% C.I. |
|---|---|---|---|
| 5 | 2 | 40% | [5%, 85%] |
| | 1 | 20% | [1%, 72%] |
| | 0 | 0% | [0%, 52%] |
| 10 | 4 | 40% | [12%, 74%] |
| | 2 | 20% | [3%, 56%] |
| | 1 | 10% | [0.02%, 45%] |
| | 0 | 0% | [0%, 31%] |
| 15 | 6 | 40% | [16%, 68%] |
| | 3 | 20% | [4%, 48%] |
| | 1 | 7% | [0.02%, 32%] |
| | 0 | 0% | [0%, 22%] |

An interim analysis will be conducted to test the futility of the treatment. After the 14$^{th}$ patient in each arm has reached one year of follow-up, the molecular CR rate will be estimated with a 99% confidence interval. If the upper bound of the confidence interval excludes the alternative rate of 0.35, then the trial will stop. Otherwise, the trial will continue and the remaining 0.04 of the Type I error will be used for primary endpoint analysis. If the true CR rate is 0.35, then the probability of stopping early is 0.2%.

The CML Interview will be completed with 8 patients from each study arm for a total of 16 patients. Every other patient per study arm will be offered the opportunity to participate in the in-depth CML interview. Patients will complete the interview at the beginning of the trial and at completion of the study. Once 8 subjects per arm have completed the pre and post study interview, this portion of the study will be completed.

## 13.3 Analysis of Endpoints

Primary Endpoint: Progression free survival at one year after stopping imatinib will be reported for each treatment arm with exact 95% binomial confidence intervals.

Secondary Endpoints: Median time from randomization to PCR negativity and median time from stopping imatinib to progression will be estimated in each treatment arm with 95% confidence intervals. Kaplan Meier curves for each treatment arm will also be estimated. The percent of BCR/ABL PCR negative patients at one year after stopping TKI will be reported in each treatment arm with exact 95% binomial confidence intervals.

Attitudes towards trial enrollment, cessation of therapy, and other information obtained from the in-depth interviews will be analyzed in a constant and iterative manner.

## 13.4 Reporting and Exclusions

13.41 Evaluation of toxicity. All patients will be evaluable for toxicity from the time of their first treatment with interferon alpha of K562/GM-CSF vaccinations.

13.42 Evaluation of response. All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data

## 13.5 Consenting of Non-English-Speaking Subjects

The Johns Hopkins IRB has set forth guidelines for enrollment of non-English speaking subjects on clinical trials to ensure patient safety is maximized. The following guidelines will be followed in order to consent individuals using the “short-form” procedure:

1) A translator must orally present the entire JHM IRB approved English version of the consent form to the subject in a language understandable to him/her, and the subject must be given a written translation of the "short form" consent document to read;

2) The entire consent process must be witnessed by an individual who is fluent in both English and the language understandable to the subject. The translator may serve as the witness;

3) The JHM IRB approved English version of the consent form must be signed by a consent designee authorized by the JHM IRB to obtain consent and the witness to the consent process. The translated "short form" must be signed by the subject and the witness to the consent process (see 2 above); AND

4) The subject must be given copies of both the JHM IRB approved English version of the consent form and the translated version of the "short form" consent document. The original signed English version with the original signed "short form" attached should be placed in the subject's research record and a copy of both placed in his/her medical record, if appropriate.

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APPENDIX A

Performance Status Criteria

| ECOG Performance Status Scale | | Karnofsky Performance Scale | |
|---|---|---|---|
| Grade | Descriptions | Percent | Description |
| 0 | Normal activity. Fully active, able to carry on all pre-disease performance without restriction. | 100 | Normal, no complaints, no evidence of disease. |
| | | 90 | Able to carry on normal activity; minor signs or symptoms of disease. |
| 1 | Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work). | 80 | Normal activity with effort; some signs or symptoms of disease. |
| | | 70 | Cares for self, unable to carry on normal activity or to do active work. |
| 2 | In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours. | 60 | Requires occasional assistance, but is able to care for most of his/her needs. |
| | | 50 | Requires considerable assistance and frequent medical care. |
| 3 | In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours. | 40 | Disabled, requires special care and assistance. |
| | | 30 | Severely disabled, hospitalization indicated. Death not imminent. |
| 4 | 100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair. | 20 | Very sick, hospitalization indicated. Death not imminent. |
| | | 10 | Moribund, fatal processes progressing rapidly. |
| 5 | Dead. | 0 | Dead. |

## Appendix B

## CML STAGES AND RESPONSE CRITERIA

The diagnosis of CML will be made on examination of the bone marrow biopsy and peripheral blood morphology and on the presence of the Philadelphia chromosome. Staging is based on the World Health Organization criteria (Vardiman, J.W. Blood. 2002). Staging and treatment responses are defined as follows:

Chronic phase CML: <10 % blasts in peripheral blood and bone marrow

First chronic phase: chronic phase CML without a history of accelerated or blast phase

Second chronic phase: chronic phase CML with a history of treated accelerated or blast phase

Accelerated phase: any of the following:

a. 10-19% blasts in peripheral blood or bone marrow
b. > 20% basophils in peripheral blood
c. Thrombocytopenia (platelet count < 100,000/uL) unrelated to therapy
d. Hematologic unresponsiveness to conventional therapy, with either
    - progressive leukocytosis or splenomegaly
    - thrombocytosis with platelet count >1,000,000/uL
e. Cytogenetic evolution

Blast phase: any of the following:

a. ≥ 20% blasts in peripheral blood or bone marrow
b. Extramedullary blastic infiltrates

Complete hematologic remission (CHR):

a. Wbc <10,000 per cubic millimeter
b. Platelets < 450,000 per cubic millimeter
c. < 5% peripheral blasts
d. ≤ 20% peripheral basophils
e. Absence of progressive extramedullary disease

Cytogenetic response:

a. Complete cytogenetic response: no detection of Ph chromosome in bone marrow by metaphase analysis
b. Partial cytogenetic response: detection of 1-34% Ph+ metaphases in bone marrow
c. Major cytogenetic response: complete and partial cytogenetic responses
d. Minor cytogenetic response: detection of 35-94% Ph+ metaphases in bone marrow

e. No cytogenetic response: >94% Ph+ metaphases in bone marrow

Minimal residual disease: either:

a. a complete cytogenetic response with residual PCR detectable BCR-ABL transcripts
b. a partial cytogenetic response.

APPENDIX C

CML Randomized Questionnaire

**Introduction:** The purpose of this interview is to gather information from people who have enrolled in our CML Randomized trial that is investigating whether the addition of a "CML cancer stem cell agent" to standard TKI therapy is effective in curing patients with CML. The trial randomizes patients to one of two stem cell targeting agents, Interferon which has known flu-like symptom side effects, and a vaccine strategy. The goal is to learn about patient's medical experience with CML, to investigate decision-making about clinical trial enrollment, and lastly to assess QOL parameters before, during and at the end of the trial.

The interview is meant to be like a conversation. I am going to tape the interview and someone will listen to the tape and type up what we talk about today.

I want to remind you of a couple of things before we start the interview.

-Your participation is voluntary. You can stop the interview at any time.
-You can refuse to answer any of the questions I ask you.
-Your decision to participate in the interview will not affect the care you receive at Johns Hopkins.
-The answers you give me will be kept confidential. We will be using code numbers to identify the tapes and transcripts from the interviews. Any names you mention will be deleted from the transcript.

Are you ready to begin? I am going to turn the recorder on now.

**Experience with CML**

1. When you were diagnosed with CML?

2. What did your doctor tell you about CML?

3. What treatments have you received for your CML in the past?

**Probe:** Did you experience any side effects?
Did the previous treatments work?
Did you stop those treatments? (N/A if the only treatment has been Imatinib)

4. Looking ahead 5 years from now, do you expect your CML to get better, stay the same or get worse?

5. What concerns/worries do you have about having CML?

**Previous experience with Research**

1. Have you ever enrolled in a medical research project/clinical trial?
   **If yes**, tell me about your experience.
   **If no,** have you ever been approached to enroll and said no?

2. What do you think about medical research/clinical trials?

**Assessment of Understanding of CML Randomized Trial**

1. Describe for me your understanding of why this trial is being done.

2. What if anything sounded exciting about this trial?

3. What if anything sounded scary about this trial?

4. What did you hope to get out of being in this trial?

5. What did you expect to get out of being in this trial?

6. What were your concerns about being in this trial?

7. What was the primary reason (most important reason) you decided to enroll in the trial?

**Decision-Making Process**

1. How sure did you feel about your decision to enroll?

2. Did you feel like you had enough information to make a decision? Why or why not?

3. Did your decision about whether to participate in the investigational study feel any different than making a decision about starting a standard medication?

**Decision Satisfaction**

1. How do you feel about the decision you made to be in the study? (Are you satisfied with the decision you made? Why or why not?)

2. What do you think would have happened if you had decided differently?

**Advice to Others Considering Enrollment**

1. What would you tell someone else who is trying to decide whether to be in an investigational study?

a1) What are the reasons to do it and what are the reasons not to do it?

a2) What kinds of things should they think about/consider?

**Closing**

I have been trying to find out from you what you thought the purpose of the investigational study was, what you thought you would get out of the investigational study, what you wanted to get out of the investigational study and what it was like for you to make a decision about participating in an investigational study. What was most relevant to your decision, etc. Is there anything we haven't talked about that you think I should know about?

**Demographics**

I'd like to finish up by asking you a couple of basic questions.

1. In what year were you born? _____

2. Gender Male Female

3. What is your ethnicity?
_____ Hispanic, Latin, or of Spanish descent or origin
_____ Non-Hispanic

4. What is your race? (**Mark all that apply**).
___ White
___ Black, African-American
___ American Indian or Alaska Native
___ Asian or Pacific Islander
___ Other, please specify _____

5. How would you rate your health right now?
___ Excellent
___ Very good
___ Good
___ Fair
___ Poor

Do you have any questions for me?

**Thank you for your time and thoughts**