

Phase II study of stem cell harvesting in patients with chronic myelogenous leukemia (CML) in complete cytogenetic remission after imatinib mesylate.

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A. Study Purpose and Rationale

a. Objective

This is a phase II study to evaluate the safety and efficacy of collecting stem cells in patients with chronic phase CML who have achieved complete cytogenetic remission while on imatinib mesylate (IM) therapy. The protocol will involve stimulating stem cell proliferation with human recombinant G-CSF (filgrastim) while holding IM for 5-7 days.

b. Background: CML[1-5]

CML is a clonal myeloproliferative disorder of a pleuropotent stem cell. Its hallmark is the Philadelphia (Ph) chromosome, which results from a (9;22) (q34.1,11.21) chromosomal translocation that leads to fusion of the *bcr* and *c-abl* genes. The *bcr-abl* gene product is a cytoplasmic protein with unregulated constitutive tyrosine kinase (TK) activity. This unregulated activity activates many intracellular pathways that affect cell proliferation, differentiation and apoptosis via multiple signaling pathways.

The annual incidence of CML is 1-2: 100,000 with peak incidence in the 5th – 6th decade. There are three clinical phases of CML, a chronic phase, an accelerated phase and a blastic phase. If untreated, chronic CML will progress in a median of 3.5-5 years before evolving into more aggressive phases. Various therapeutic options have been evaluated in CML, and levels of response have been defined. Hematologic response is the normalization of peripheral blood smears and differentials, Complete response (CCR) is defined as 0% Ph+ cells on bone marrow biopsy (evaluation of 20-50 cells), partial response as 1-35% Ph+ cells, major response (MCR) as complete or partial response, and minor response as 35-90% Ph+. More recently, the evaluation of minimal residual disease (MRD) as evaluated by real-time PCR levels of *bcr-abl* has been suggested as a more sensitive tool for monitoring treatment

c. Background: Chemotherapy and Interferon Therapy[6-8]

Conventional chemotherapy for CML with of hydroxyurea (HU) or busulfan induces complete hematologic response (HR) in 70 % of patients, however neither of these regimens significantly suppressed Ph+ cells in the bone marrow. A randomized trial of HU compared to busulfan in patients with chronic phase CML showed HU conferred a significant survival advantage (median 58 months vs. 45 months $p < .008$) with fewer side effects.

In the early 1980's, interferon-alpha (IFN-alpha) was introduced as a therapy for CML. In patients with chronic phase CML, IFN-alpha induces a complete HR in 70-80%. Furthermore, in chronic phase CML, 26% achieved CCR, 12% partial response, and 20 % minor response. Five randomized trial comparing IFN-alpha with conventional chemotherapy confirmed increased major cytogenetic response (6-21% vs 1-5%), and meta-analysis from data from these trials demonstrated increased 5-year survival with IFN-alpha (57% vs. 42%, $p < .00001$)

d. Background: Allogeneic Stem Cell Transplant [9-12]

Allogeneic hematopoietic stem cell transplant in chronic phase is the only known therapy to result in long-lasting *bcr-abl* negativity. The curative potential of allogeneic transplant is likely due to graft-vs.-leukemia effect. However, allogeneic transplant is associated with a high transplant-related morbidity and mortality. The best disease free survival results have occurred in younger patients and those with

early disease (chronic phase < 1 year since diagnosis). Donors can be an HLA-matched related (sibling) donor (MSD) or an unrelated matched donor (UMD). Results from various transplant databases have been summarized below.

<i>Data Base</i>	<i>Donor Type</i>	<i>n</i>	<i>Endpoint</i>	<i>Results</i>
International Bone Marrow Registry	MSD	4916	5-yr survival	57% (> 1 yr CML) 69% (<1 yr CML)
European Group for Bone Marrow Transplant	MSD	373	8-yr survival/ 8-yr disease free survival (DFS)	54%/ 47%
National Marrow Donor Program	MSD	?	5-yr DFS	67% age < 40 57% age > 40
International Bone Marrow Registry	UMD	613	5-yr survival	46% (>1-yr CML) 54% (<1-yr CML)
National Marrow Donor Program	UMD	2464	5-yr DFS	61% (age < 30) 57% (age 30-40) 46% (age > 40)

The timing of allogeneic transplant remains controversial. Both MSD and UMD allogeneic transplant have demonstrated better outcomes when transplant is performed within 12 months of diagnosis.

e. Background: Autologous Stem Cell Transplant[13-20]

The rationale behind autologous stem cell transplant (AutoSCT) is that benign progenitor cells can be harvested and used as a rescue therapy after myeloablative therapy, with the goal of prolonging survival. A meta-analysis published in 1994 of 200 patients (142 chronic phase, 30 accelerated phase and 28 blast phase or stable post blast crisis) at 8 institutions treated with AutoSCT suggested a plateau in the survival curve not observed with conventional treatments. Five-year survival was 58% for chronic phase patients, and the median survival had not been reached with 7 years follow-up. AutoSCT was associated with a high engraftment rate, lower morbidity and mortality than allogeneic stem cell transplant.

Data on 196 patients in a Genoa protocol that uses idarubicin, cytarabine, and etoposide for 5 days (ICE) or three days (mini-ICE) for mobilization has been updated through 2002. In the 60 patients treated with early chronic phase disease and not treated with IFN-alpha, CCR post Auto-SCT was 60% while CCR in 68 late chronic phase was 33%.

RT-PCR was employed in 50 Ph- collections, and a *bcr-abl/abl* ratio < 0.1 was correlated with longer duration of cytogenetic response. Notably, these early chronic phase patients received low dose IFN-alpha after AutoSCT, and about 20% were treated with IM after hematologic or cytogenetic relapse. This modified AutoSCT protocol in early chronic phase CML lead to better survival data, with a projected 5year survival of about 80%.

f. Background: Molecularly Targeted Therapy; Imatinib mesylate (IM)[21-28]

IM is a tyrosine kinase inhibitor with activity against *bcr-abl*. Phase I and Phase II studies of IM suggested improved responses in all phases of CML. A randomized phase III trial, the International Randomized Study of Interferon versus STU571 (IRIS), of 1106 newly diagnosed chronic-phase CML patients comparing IM to IFN-alpha plus cytarabine (Ara-C) enrolled patients in 2000-2001. After a median 19 month follow-up, IM arm had an estimated 18 month 97% CHR, 85% MCR, and 74% CCR. By comparison, the IFN-alpha + Ara-C arm had 56% CHR, 22% MCR, and 8.5% CCR. At 12 months,

disease progression defined as death, accelerated or blast phase CML, or loss of CHR or CCR occurred in 3.4% of the IM arm and 20.1% of the IFN-alpha + Ara-C arm. The IM regimen was better tolerated, and 318 patients crossed over to IM for due either to intolerance of the combination regimen or FDA approval of IM.

A molecular analysis of *bcr-abl* transcripts, by quantitative real-time RT-PCR, was performed 81% of IRIS patients who obtained CCR. At time of CCR, the median reduction in the IM group was 2.5 log versus 2.2 log in the combination group. At 15 months after CCR, the median decrease was 3.7 log in the IM group and 2.5 log in the combination group. The continued decrease over the 15-month period was significant in the IM group ($p < .002$). *Bcr-abl* was undetectable in 10% of patients with CCR, with lower limit of detection was 4.5-log decrease. Molecular response may also add prognostic information. In patients who had CCR and > 3 log decrease in *bcr-abl* at 12 months, 100% were progression free at 24 months compared with those with CCR and *bcr-abl* reductions < 3 log at 12 months who had 95% progression free survival at 24 month. This molecular analysis further demonstrates that IM has a rapid and continued anti-leukemic effect. Unlike the data from allogeneic SCT however, most patients on IM still have disease measurable by molecular analysis at 24 months.

g. Background: Imatinib mesylate Resistance[29-33]

While IM 400 mg daily has emerged as the preferred therapy for newly diagnosed CML patients who do not undergo transplant, some patients do not respond and there is still a risk of relapse, especially patients with advanced disease. Hypothetical mechanisms of acquired resistance include point mutations in *bcr-abl*, amplification of *bcr-abl*, enhanced expression of the multi-drug resistance gene (MDR) and increased protein binding of IM. Mutations in the kinase domain are the most commonly identified mechanism of resistance. In 121 relapsed patients evaluated in 7 papers, 72% had evidence of point mutations. The first point mutation to be described in 6/9 cases of IM resistance was a C to T change at nucleotide 1091 leading to an exchange of threonine for isoleucine (T315I), which lead to decreased IM binding. Further base pair mutations have been described. These either interfere with IM binding through steric hindrance (i.e. T315I) or conformational change that leads to decreased affinity.

h. Background: Stem Cell Mobilization and Harvesting[15, 34-37]

Various stem cell mobilization and harvesting protocols have been employed for AutoSCT in CML. An early protocol utilized filgrastim at 5mcg/kg/day in 30 patients with hematologic response on IFN-alpha (17% CCR). The goal number of progenitor cells was collected in 83% of participants, and there was no evidence of significant increases in Ph+ cells on post-harvesting bone marrow biopsies. An Italian group published various results using chemotherapy (idarubicin, cytarabine, and etoposide) as well as G-CSF 5 mcg/kg/day. Their best results were with 22 newly diagnosed patients, in whom 63% had no Ph+ cells in the apheresis product. 68% had $> 2 \times 10^6$ CD34+ /kg cells, and 14 patients went on to AutoSCT

A total of 108 patients in three publications have been evaluated in harvesting protocols for patients with CCR on IM. Drummond et al evaluated a protocol using filgrastim 10 mcg/kg/day while continuing IM on 54 chronic phase and 4 accelerated phase patients who had achieved CCR. The mean time from diagnosis was 29 months (range 3.2-118) and the mean duration of IM therapy was 9.8 months (range 3.2-27.3). A Target 2×10^6 CD34+ /kg in 40%, but no apheresis in was attempted in 15 patients due to low CD34+ counts. 84% of the apheresis samples were had no Ph+ cells by cytogenetics and/or fluorescent in situ hybridization (FISH).

Kreuzer et al evaluated the same protocol in 15 chronic phase and 3 accelerate phase patients who achieved CCR. The mean time since diagnosis in this group was 24.5 months (range 8-91) and mean time on IM 17 months (6-27). A target 2×10^6 CD34+ /kg was achieved in 72%. Kreuzer also evaluated *bcr-abl* transcript levels, and found 28% of the apheresis samples to be negative (estimated sensitivity $1:10^6$). This study also compared *bcr-abl/beta-actin* ratios before and after harvesting, and found no

significant mean difference. 1 patient with rising *bcr-abl* transcripts prior to harvesting relapsed at 6 months.

Lastly, Hui et al compared filgrastim 10 mcg/kg/day in 2 non-randomly assigned groups. In the first IM was continued (n=18; 15 chronic, 3 accelerated) vs. holding IM (n=14; 10 chronic) for 5-7 days. A target of 2×10^6 CD34+ /kg achieved in 50% of the IM continued group and 71% of the IM held group. Two of 6 patients with unsuccessful mobilizations in the IM continued group were successfully mobilized with a second attempt while holding IM. The IM group required a lower median number of aphereses, and had a statistically higher mean number of CD34+ cells harvested (2.18 vs. 3.7×10^6 /kg). In the IM continued group, there was no significant change in *bcr-abl/bcr* ratios between blood samples taken before and after mobilization. At a median 26-week follow up of 14 of these patients, there was no change in *bcr-abl* transcript level or progression of disease. The RT-PCR data from the IM held group is incomplete, but the post-mobilization mean was comparable to that of the IM continued group.

i. Rationale for Stem Cell Harvest Protocol [38]

Given the improved cytogenetic response rates with IM, the management of CML has changed since FDA approval. However, even patients with CCR have evidence of MRD and evidence of IM resistance exists. While short-term data on sustained response in chronic phase disease is promising, long-term outcome is yet to be determined. In the IM arm of the IRIS trial a projected 3.3% of patients progressed at 18 months. In the CCR group with less than 3 log decrease in *bcr-abl*, there was a 5% progression at 24 months. In serial RT-PCR evaluation of 11 patients who achieved CCR from one center in the IRIS trial, 2 (18%) had a relapse by 2003, and 2 had a sustained 1-log increase in *bcr-abl* transcript level. Therefore, approaches combining IM with other therapeutic approaches including AutoSCT may lead to better outcomes such as improved leukemia free survival and overall survival.

Early AutoSCT studies in patients that were not eligible for allogeneic SCT, had a high engraftment rates, low mortality and return to normal activity levels, regained sensitivity to medical therapy, and meta-analysis suggested a plateau in the survival curve. Various in vivo conditioning regimens were used, the most successful at purging Ph+ cells used ICE, which produced Ph- apheresis product in 63% of patients with early chronic CML.

However, improved treatment efficacy in Auto SCT might be expected with improved purging with IM. Through retroviral marking, it has been demonstrated that low levels of Ph+ cells in the transplant inoculum contribute to systemic relapse. More recent AutoSCT experience has suggested that lower levels of *bcr-abl* transcript (i.e. *Bcr-abl/abl*<0.01) correlated with a longer cytogenetic response post transplant. Given the improved CCR rates with IM, harvesting during IM induced CCR could provide a greater percentage of benign progenitor cells in the apheresis product. Auto-SCT using PHPC collected during CCR might also allow for reintroduction of IM sensitivity.

The safety and efficacy of G-CSF mobilized stem cell harvesting in patients with CCR have been described by three groups. In 58 patients, Drummond et al mobilized stem cells with G-CSF while continuing IM therapy. Overall, a sufficient number of CD34+ cells for AutoSCT were collected in 48% of the patients who continued on IM for the harvesting procedure. In the group in which IM was held, 70% of the patients were successfully harvested. However, the comparison paper was not powered to detect a significant difference in these two methods. Of the published RT-PCR data, no patient had greater than 1-log increase on *bcr-abl*/"housekeeping gene" ratio. The greatest increase was 2-fold. There was no significant rise in the serum *bcr-abl* load by PCR before and after mobilization. While these studies suggest that harvesting cells may be safe, the efficacy of these mobilization protocols was inferior to those using chemotherapy. This may be due to anti-*c-kit* effect of IM interfering with mobilization of normal stem cells.

Given the demonstrated MRD in patients on IM and possibility for resistance, strategies that incorporate combinations of treatment modalities may improve outcomes in patients with CML. Specifically, the evaluation of the safety and efficacy of patients with CCR on IM should be evaluated with clear pre-defined outcomes.

B. Hypothesis

Harvesting CD34+ stem cells in chronic CML patients who have achieved CCR on IM utilizing a collection protocol of subcutaneous filgrastim while holding IM for 5-7 days is safe and efficacious. Fewer than 10% of patients will have Ph+ cells evident on bone marrow biopsy 1 month after mobilization. Fewer than 10% of patients will have a 1-log increase in *bcr-abl/abl* ratio using RT-PCR. Target CD34+ harvests will be achieved in > 50% of patients

C. Study Design and Statistical Analysis

a. Design

This is a single group observational study of a stem cell collecting protocol.

b. Outcomes

Two primary outcomes will be evaluated. One will evaluate safety, the other efficacy.

The primary safety outcome will be evaluated by confirming CCR on a bone marrow biopsy within one month of starting the harvesting protocol to that of a bone marrow biopsy 1 month after completing the protocol. The expected number of Ph+ cells on follow biopsies is zero (maintained CCR), and the protocol will be considered safe if with $p=0.05$, greater than 90% of patients remain in CCR. Bone marrow biopsy is the standard of care in following patients with CML, the sensitivity of cytogenetic analysis is 1:20 – 1:50.

The primary efficacy outcome will be defined as the ability to collect 2×10^6 CD34+ /kg in greater than 50% of patients ($p<0.05$). The expected proportion of patients in which this yield can be collected is 0.75.

A secondary safety analysis will be performed using RT-PCR. The study will be considered safe by this secondary outcome if fewer than 10% of patients ($p<0.05$) have a 1-log increase in *bcr-abl/abl* transcripts by RT-PCR in peripheral blood samples collected on the first day of the protocol and on the final day of harvesting.

c. Statistical Analysis

Primary and secondary safety as well as efficacy will be evaluated using chi-squared analysis on proportions in single group.

d. Power Calculation

Primary safety outcome: The cut-off for safety will be set at 0.9, the expected safety is 1.0. With $\alpha = 0.05$, and power = 0.8, 46 subjects will be required.

Primary efficacy outcome: The expected proportion of patients in whom 2×10^6 CD34+ /kg cells can be collected is 75%. The protocol will be considered efficacious if >50% of patients can be successfully harvested. With $\alpha = 0.05$, and power = 0.8, 33 patients will be needed. However, since 46 patients will be evaluated for safety outcomes, the protocol will be considered efficacious as long as the observed proportion of successful harvests is > 0.71.

Secondary safety outcome: The cut-off for safety will be set at 0.9, the expected safety is 1.0. With $\alpha = 0.05$, and power = 0.8, 46 subjects will be required.

D. Study procedures[39-41]

The harvesting protocol will be performed on an outpatient basis.

Initial blood work will be drawn on initial meeting with principal investigator to evaluate exclusion criteria.

Baseline bone marrow biopsy will be performed if no documented biopsy with CCR in 1 month prior to protocol start date.

Day 1, patients will have blood drawn for RT-PCR.

Patients will receive 10 micrograms/kg/day of filgrastim for 5 days while IM is held.

Semi-permanent indwelling central catheter will be placed for apheresis under CT guidance by an interventional radiologist between days 2-4. The radiologist upon completion of the harvesting protocol will remove the catheter.

Daily CBC with differential will be drawn to monitor response to filgrastim.

Peripheral CD34+ progenitor cells will then be collected via a commercially available aphaeresis unit starting on day 5, with target apheresis of 2.0×10^6 /kg CD34+ cells.

The maximum number of harvests will be 3 as collected on consecutive days, starting on day 5.

Blood will be drawn on final day of protocol for RT-PCR analysis, as well as Chem7 and LFTs to monitor for side effects.

Stem cells harvested during IM induced CCR will be preserved using standard cryopreservation techniques and will be stored in a mechanical freezer at -80 degrees Celsius. Stored cells will allow for possible use in future AutoSCT protocols.

RT-PCR for *bcr-abl* transcripts will be performed using LightCycler technology (Roche diagnostics), primers will include b2a2, b3a2, b2a3, and b3a3, which will detect the major fusion Ph+ transcripts, as well as *abl*. Transcripts will be compared to *abl* transcripts (housekeeping gene). In 254 samples from 120 individuals, the intra-assay coefficient of variation was 0.18 and the inter-assay coefficient of variation was 0.17.

E. Study drugs

Recombinant Human (rh) G-CSF is made by genetically engineered plasmid expressed in *E. coli*. rhG-CSF is supplied as a sterile buffered protein solution at pH 4.0 which must be stored at 2 degrees Celsius. Stability of rhG-CSF at 0.25mg/ml has been demonstrated for at least 10 months under these conditions.

F. Medical devices

A semi-permanent multi-lumen central catheter will be placed for stem cell harvest.

G. Study questionnaires/data collection

History and Physical: Age, sex, date of diagnosis, duration of IM therapy, medical history, vital signs, weight, physical exam, performance status, toxicity evaluation

Laboratory/Imaging Data: CBC with differential and reticulocyte count, PT/PTT, fibrinogen, LFTs, Basic Metabolic Panel, Mg, Phos, HIV, Hepatitis serologies, EKG, Cytogenetics, *bcr-abl* RT-PCR, CXR.

Bone marrow biopsy within one month of starting protocol. bone marrow biopsy one month after completing harvest.

Peripheral blood for RT-PCR day 1, final day of harvest.

Daily CBC with differential during mobilization and harvesting, chem7 and LFTs on final day of harvest.

Data from follow-up with referring oncologist at 3-6 month intervals as well as 3 month RT-PCR will be collected to monitor patient clinical status, but will not be used for data analysis.

H. Study Subjects

46 subjects will be enrolled over 2-3 year period.

a. Inclusion criteria:

- a. Chronic CML who has achieved CCR demonstrated on bone marrow biopsy within previous 1 month,
- b. Age greater than 18
- c. >3 months of IM therapy
- d. Patient ineligible or not interested in allogeneic stem cell transplant
- e. Insurance approval for harvesting can be obtained

b. Exclusion criteria:

- a. Active infection, HIV+, HCV+
- b. History of accelerated or blast phase CML
- c. Pregnancy
- d. AST/ALT greater than 3x upper limit normal
- e. WBC<3000, platelets <100,000, >3+ myelofibrosis
- f. ECOG performance status >1
- g. Creatinine clearance > 60
- h. Unstable angina
- i. NYHA Class III-VI CHF
- j. Hemorrhagic stroke within last 6 months
- k. COPD requiring oxygen
- l. Physician determined other serious illness

I. Recruitment of Subjects

Recruitment will be through physician referral and posting of trial on Herbert Irving Comprehensive Cancer Center and NCI trial database websites

J. Confidentiality of study data

Unique subject number will identify patient data and cryopreserved stem cells. The name and subject number will be kept in a locked cabinet accessible only to the research team. No identifying information will be included any publication of this protocol. Identifying information will not be provided to the makers of Gleevec nor Neupogen (filgrastim).

K. Potential Conflict of Interest

None

L. Potential Risks

1. Risks of rhG-CSF: Side effects, if any, are usually mild and brief. The most common side effect is transient bone pain, others include discomfort and pain, swelling and redness at site of injection, muscle cramps and pain in the back of the legs, nausea, phlebitis, arthritis or psoriasis. Thrombocytopenia or reversible hepatitis may occur.
2. Risks of withholding IM: Hypothetical risk of CML relapse while holding IM
3. 12.3 Risk of central-venous line placement: arterial puncture, hematoma, pneumothorax, phlebitis, infection, thrombosis and occlusion.

M. Potential Benefits

Future possibility of AutoSCT if CML relapse, which has the possibility to extent life.

N. Alternatives

Continue IM therapy without harvesting stem cells or enrollment in other clinical trial evaluating combination of IM with other therapies.

O. Compensation and costs to subjects

None

P. Minors

None

Q. Radiation or radioactive substances

CT guided catheter placement.

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