Study Title: Validation of Card-PCR analysis through programmed imatinib interruption in PCR negative CML patients.

1. Background and introduction

1.1 Chronic Myeloid Leukemia (CML)

Chronic Myeloid Leukemia (CML) represents 7–20% of all leukemia cases, with a worldwide incidence projected at one to two per 100,000 people (1).
The incidence of CML increases with age; the median age at diagnosis is 51 years and about 30% of CML patients are 60 years of age and older (2).

CML is caused by the unregulated activity of the tyrosine kinase Bcr/Abl (3), which is formed by the fusion of exons belonging to the BCR and ABL genes, located on chromosomes 22 and 9 respectively (4). This fusion originates through a 9;22 chromosomal balanced translocation [t(9;22)(q34;q11.2)], known as the Philadelphia (Ph) chromosome (5).

Untreated CML commonly progresses through three disease phases: chronic, accelerated phase (AP) and blast crisis (BC), each corresponding to increasing leukemic blast counts and clinical severity. The chronic phase (CP), that usually lasts 5 years, is characterized by an abnormal expansion of the clonal hematopoiesis retaining an apparent normal differentiation; the AP's median duration is 3-9 months and median survival 8-18 months; while BC's median survival is 3-6 months (Enright and McGlave, 2000). Last two phases are marked by the development of a secondary acute leukemia which fatally closes the course of the disease (6).
Most patients however present in the chronic phase, characterized by splenomegaly and leukocytosis with generally few symptoms.

1.2 Imatinib (IM) Therapy

The main goal of CML therapy is the suppression of Ph+ clone in the chronic phase (CP) (7). Since Bcr/Abl represents the molecular cause of CML, the targeting of its enzymatic activity represents a truly "targeted" attempt to cancer therapy.

Over the last two decades, therapy evolved from the use of non-specific cytotoxic agents (i.e. Hydroxurea, Busulfan) (8, 9) to Interferon-α (IFN-α) (10-14) or allogeneic stem cell transplantation (allo-SCT) (16-22) and more recently to Imatinib that is now recognized as the first-line treatment of CML (15-32).
Through treatment with Tyrosine kinase inhibitors (TKIs), 5-year survival rate in patients with CML is over 90% (21, 22).

Imatinib is a competitive inhibitor of Bcr/Abl catalytic domain, which proved able to inhibit Bcr/Abl activity (33, 34), to block proliferation of Bcr/Abl + cells and to induce apoptosis in these cells in vitro and in vivo (35). Imatinib is also an inhibitor of the receptor tyrosine kinases for platelet-derived growth factor (PDGF) and stem cell factor (SCF), c-Kit and inhibits PDGF- and SCF-mediated cellular events.

Imatinib induces complete cytogenetic responses (CCyR) in more than 80% of patients with CML (36), while major molecular response (MMR: < 0,1% of BCR-ABL transcripts on the international scale) is observed in 33-90% of the patients according to treatment duration. Moreover, among patients with MMR, more than 30% of the patients show polymerase chain reaction (PCR) negativity (complete molecular response, CMR: undetectable levels of BCR-ABL mRNA transcripts by Q-RT-PCR in two consecutive blood samples of adequate quality) which cannot be detected using nested reverse transcriptase-PCR.

Undetectable BCR-ABL may not equate eradication of minimal residual disease because the sensitivity of Q-RT-PCR is limited to 4 to 5 log below the standardized baseline and significant numbers of residual leukemic cells (up to 10E7) can still remain in a patient who shows PCR negativity (37).

While the activity of imatinib in over blast crisis CML is limited (38), the main effect of imatinib therapy has been to “freeze” the evolution of the disease, allowing an “open end” chronic phase to continue, possibly for an unlimited time.

2. Study Rationale

Pilot studies showed that it is possible to interrupt imatinib treatment without experiencing relapse (36).

Goh et al (39) reported that only 2 patients on 26 didn’t relapse after discontinuation, while Mahon et al (40) reported that 30 patients on 69 still have an undetectable level of BCR-ABL transcript after a median follow up of 17 months and among patients (n 39) who relapsed, 37/39 patients lost PCR negativity within 6 months from imatinib interruption. This different percentage is related to a different period of stable state of CMR before imatinib stopping (median 7 months in the first study and at least 2 years in the second one).
To date, it is not possible to surely identify “a priori” patients able to permanently discontinue imatinib, after CMR achievement. There is no diagnostic assay that can predict the relapse after discontinuation and therefore we only rely on a “watching and wait” strategy.

Considering several models to predict CML cell proliferation, it is estimated that permanent discontinuation might be possible if a patient does not relapse within 6-8 months after suspension (40, 41).
In accordance with the CML cell doubling time, the authors calculated that if only one CML cell persists, at least 6 months are necessary to reach a number of cells detectable by RT-PCR. Therefore, after 6 months without relapse, it is possible to hypothesize the eradication of residual disease or that residual CML cells are in a quiescent state (42).

Up to now, imatinib treatment for CML is recommended to be life long.
Permanent discontinuation of imatinib, if possible, would have two important advantages:

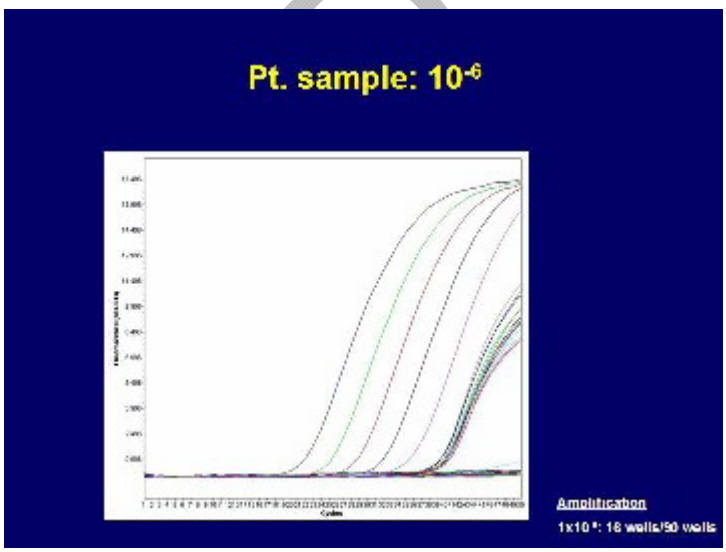
- a substantial reduction in health care spending;
- prevention of the morbidity due to imatinib side effects.

On the other hand, it is known that the discontinuation of imatinib therapy is followed by relapse in > 50% of patients. Although the relapse is reversible upon imatinib resumption (39, 40), it is theoretically possible that the disease could progress during the interruption period.

2.1 The ILTE Study

The ILTE study is an independent, academic, multicenter trial including 27 center in Europe, north/south America, Africa and Asia to present a global picture of imatinib long term effects. Overall, 832 patients, in CCyR after 2 years of treatment, were enrolled with a median of treatment of 5,8 years. Study endpoints were survival, serious adverse events (SAE), toxicities not qualifying as SAE and loss of CCyR.
This study also included among its objectives the development of a more sensitive PCR (card PCR o cPCR) to monitor residual disease.
Inside ILTE there are now 179 patients in stable PCR negativity.

A pilot study was performed on samples obtained from 30 patients, enrolled in ILTE study, who were negative by conventional PCR for at least one year, using a new much more sensitive diagnostic method, the cPCR, developed by Gambacorti-Passerini, Saglio and Kim.
In fact, using this method is now possible to obtain a cPCR sensitvity between 10E-6 and 10E-7, which is 100 times lower than actual PCRs. (Figure 1, Kim, Gambacorti- Passerini and Saglio, 43).



Among this 30 patients, only 10 were negative also in cPCR, giving a first assessment of the amount of patients whose CML could be eradicated. These numbers are in fact compatible with those obtained from a French study in which about 60% of patients relapsed within 12 months from imatinib suspension (40).

Using this method it could be therefore possible to obtain a more sensitive assessment of residual disease and to predict who, among "PCR negative" patients will not relapse following imatinib interruption.

The purpose of this study is to validate the cPCR assay in the setting of PCR negative patients that will suspend imatinib treatment.

In case of positive results, two important goals will have been achieved:

- to identify in advance patients in whom discontinuation of treatment will not be followed by molecular relapse;
- to discontinue permanently imatinib in a percentage of PCR negative patients; in this way NHS may save about 3 millions euro/year every 100 such patients.

Although this study is a continuation of the ILTE study, we would offer the possibility to stop imatinib therapy also to patients not enrolled in ILTE, but coming from other centers, if they will satisfy the eligibility criteria.

3. Objectives

3.1 Primary Endpoint

- To validate a new, more sensitive technique, cPCR, which may identify among the patients who present stable CMR, those who could be truly negative for Bcr-Abl transcripts and to evaluate its power to predict relapses after imatinib discontinuation.

3.2 Secondary Endpoints

- To evaluate in CML patients with negative PCR results since more than two years, the rate of maintenance of molecular remission, after imatinib discontinuation.
- To evaluate quality of life in CML patients after imatinib discontinuation.
- To evaluate the rate of progression/resistance in patients who relapse after imatinib discontinuation.

4. Investigational Plan

4.1 Overall Study Design

An aim of ILTE study was to set up a new molecular technique, called “Card PCR”. This system, based on an increased amount of cDNA in the well and the number of wells (from 2-3 to 96), allows to increase the replicate RQ-PCR assay, that will consequentially increase the probability to find at least one BCR-ABL molecule.

Starting from this point, we want to analyze by cPCR those CML patients, under imatinib therapy, with at least 2 years of consecutive negative conventional PCRs performed in the centers where they are followed.
The patients will sign a IC, will subsequently be tested for cPCR and will stop imatinib. Then they will be monitored with standard PCR to control their CMR status.

Collection of data will be prospective as each center will have to update the follow up of the patients. Follow up's duration will be 36 months.
At the end of follow up, a peripheral blood sample (for cPCR) will be obtained from those patients who will still have negative PCR results .

Patient's life quality before and during (months 1, 6, 12, 24, 36) imatinib discontinuation until the end of this study, will be evaluated trough a Quality of Life (QoL) questionnaire, independently of whether imatinib will be resumed.

Finally, the fraction of patients progressing or developing resistance to imatinib during or after imatinib suspension will be assessed. progression will be defined as meeting the criteria for AP or BC. Resistance will be defined as lack of CHR at 3 months or of CCyR at 6 months after resuming imatinib.

4.2 Study Population

Based on 2008 ILTE data we expect to potentially enrol potentially 180 durable PCR negative patients. According to literature we expect to find 107 molecular relapses (60%) and according to the ILTE pilot study 125 positive cPCR results(70%).
So, these numbers will allow to determine the sensitivity and specificity of cPCR to predict molecular relapse.

The target population will aslo includes CML patients, followed outside of the ILTE study who maintained CMR for at least 2 years.

5. Patient Selection Criteria

5.1 Inclusion Criteria

- Male or female patients with CML diagnosed in chronic phase and who are in CMR (as defined by their own center) since more than 2 consecutive years under imatinib therapy.
- Age > 18 years.
- Imatinib treatment with achievement of CMR.
- A minimum of 3 CMR PCR determinations to support disease status.
- Written informed consent.

5.2 Exclusion Criteria

- Patients who at discretion of referring physicians could have problems of compliance and could therefore be lost during follow up.

6. Study Procedures

After the signature of written informed consent, one 30 ml peripheral blood will be obtained from eligible patients.
Then, imatinib will be discontinued. The blood sample will be sent to the Monza Unit (Clinical Research Unit, S. Gerardo Hospital, University of Milan Bicocca, Monza, Italy) with an express courier and there the mRNA, through lysis in Triazol, will be extracted and reverse transcribed to obtain cDNA. A part of the sample will be used by Unit 1 to confirm QR-PCR and nested PCR status and the remaining one will be analysed using cPCR in the Seul Unit (division of haematology, Catholic University of Korea, Seoul, Korea). A minimum of 90 wells will be set up for each patients (20 ng cDNA/well).
cPCR value will be expressed as number of positive wells/ total wells.

During the surveillance period after discontinuation, molecular responses will be evaluated with a traditional PCR every month during the first 6 months and every 2 months thereafter. Routine blood examination and cytogenetic analyses will be done in the different units with the same schedule used before suspension. The patients will be monitored for a minimum of 36 months.

Loss of PCR negativity will be declared when two positive values in Q-PCR will be detected at least 2 weeks apart from each other, and with at least one value > 0,1%.
At the time of loss of CMR a BM aspirate will be performed before resuming imatinib to detect a possible disease progression.

Imatinib will be restarted at the same dosage used before interruption.
Hematologic relapse is based on standard hematological criteria; cytogenetic relapse is defined as presence of Ph+ metaphases among at least 20 metaphases in two consecutive cytogenetic analyses.

Patients who remain PCR negative at the end of follow up, will be submitted again to cPCR to verify the eradication of the leukemic clone.
In case of negative result they will monitored annually for 5 years with Q-PCR every 3 months.

In the end patient's life quality before, during and after imatinib discontinuation until the end of this study it will be evaluated.
This parameter will be investigated through a questionnaire: EORTC-QLQ-T30.
Data's variance analysis obtained at different times will be performed by the statistical unit.

7. Protocol Amendments and Changes in Study Conduct

Any changes to the protocol will be made in the form of an amendment.
Any change or addition to this protocol requires a written protocol amendment that must be approved by the study coordinator center and each investigator before implementation.
Amendments significantly affecting the scientific quality of the study require additional approval by the IRB at each center.

8. Data Management, assessments and follow-up

8.1 Data Collection

Investigators (or designees) must enter the information required by the protocol into Electronic Case Report Forms (E-CRF) using a site computer. E-CRF will be prepared by the statistical unit and will be distributed to each center before the initiation of the study.
The Investigator must certify that the data are complete and accurate marking a specific box at the end of E-CRF page. E-CRF completed will be sent online by investigational sites to statistical unit, while a copy of these will be kept at sites.

8.2 Quality control

Data from E-CRF are entered into the study database by statistical unit staff. Obvious errors are corrected by data management personnel. Other errors or omissions are reported to the investigational sites (queries) for resolution.

8.3 Follow Up

Follow up is at least 36 months.
Clinical evaluation, physical examination and the cytogenetic analysis will be assessed at the time of enrolment (baseline) and with the same schedule used before suspension.
Molecular responses will be evaluated with a traditional PCR every month during the first six months and every 2 months thereafter.

9. Statistical Methods

The data will be analyzed by the statistical unit.
Data will be captured using the CRF (case report form) Access files, already used by ILTE participating units.
The sensitivity and specificity of cPCR to predict molecular relapse will be assessed, as well as the overall and progression free survival of patients.

10. Ethics and Good Clinical Practice

This study must be carried out in compliance with the protocol procedures and the principles of Good Clinical Practice, as reported in D.lgs 24/06 l2003, n.211 and of the Declaration of Helsinki (Recommendations Guiding Physicians in Biomedical Research Involving Human Subjects).
The Investigator agrees when signing the protocol to adhere to the instructions and procedures described in it and thereby to adhere to the principles of Good Clinical Practice that it conforms to.

The protocol has been written, and the study will be conducted according to the ICH Harmonized Tripartite Guideline for Good Clinical Practice (ref: http://www.ifpma.org/pdfifpma/e6.pdf). The protocol will be approved by the Local, Regional or National Ethics Committees.

10.1 Ethics Committee (EC)

Before implementing this study, the protocol and the informed consent form must be reviewed and approved by the local EC. Any amendments to the protocol has to be approved by the E.C.

10.2 Informed Consent (IC)

The informed consent will be read and signed by the patient and the Investigator. A copy of the signed document will be given to the patient.

11. References

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