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TITLE: "IMATINIB LONG TERM EFFECTS (ILTE)" STUDY

STUDY NUMBER: ILTE/01

RETROSPECTIVE AND PROSPECTIVE OBSERVATIONAL STUDY

Principal Investigator and Study Coordinator: Carlo Gambacorti-Passerini[1], MD.

Scientific Board: Sarit Assouline[1], Carlo Gambacorti-Passerini[2], François Guilhot[3], Dong-Wook Kim[4], Philipp LeCoutre[5], Fabrizio Pane[6], Giuseppe Saglio[7], Maria Grazia Valsecchi[8].

Statistical Unit: Maria Grazia Valsecchi[2], Laura Antolini[2]

Data Management: Lucia Tornaghi[1], Laura Antolini[2].

1. Department of Oncology and Hematology, McGill University, Montreal, Canada
2. Department of Internal Medicine, University of Milano Bicocca, via Cadore 48, 20052 Monza, Italy
3. Department of Oncology Hematology and Cell Therapy, Clinical Research Center, University Hospital, rue de la Miletrie 2 BP 577 86021 Poitiers, Cedex, France
4. Division of Haematology, The Catholic University of Korea, Seoul, Korea
5. Clinic of Medicine Hematology and Oncology, Charitè University, Berlin, Germany
6. CEINGE – Onco-Hematology Unit, Azienda Universitaria Policlinico, University of Naples "Federico II", Via Panzini 5, 80131 Napoli, Italy
7. Clinic of Internal Medicine and Hematology, S. Luigi Hospital – University of Turin, Regione Gondole 10, 10043 Orbassano, Italy
8. Department of Internal Medicine, Statistical Unit, University of Milano-Bicocca, Via Cadore 48, 20052 Monza, Italy

SIGNATURE PAGE

STUDY NUMBER: ILTE/01

Approved by the following:

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Signature date

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Signature date

I have read this protocol and I agree to conduct this trial in accordance with all stipulations of the protocol and the Declaration of Helsinki

Prof. Carlo Gambacorti-Passerini

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Signature date

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1) INTRODUCTION

Chronic Myeloid Leukemia (CML) represents a neoplastic disease with an incidence of 1-2 cases/10^5 year (1). CML is caused by the unregulated activity of the tyrosine kinase Bcr/Abl (2), which is formed by the fusion of exons belonging to the Bcr and Abl genes, located on chromosomes 22 and 9 respectively (3). This fusion originates through a 9;22 chromosomal translocation known as the Philadelphia (Ph) chromosome (4). Since Bcr/Abl represents the molecular cause of CML, the targeting of its enzymatic activity represents a truly “targeted” attempt to cancer therapy.

The development of imatinib, the first Bcr/Abl inhibitor, indeed confirmed this hypothesis. Imatinib is a competitive inhibitor of Bcr/Abl catalytic domain, which proved able to inhibit Bcr/Abl activity (5,6), to block proliferation of Bcr/Abl+ cells, to induce apoptosis in these cells in vitro and in vivo (7), and to cure mice injected with human Bcr/Abl+ leukemic cells (8).

Following these promising preclinical results, the clinical development of imatinib represented one of the more astonishing breakthroughs in the history of medicine. Imatinib can induce Complete Cytogenetic Remissions (CCyR) in the majority of CML patients (9), with CCyR rate as high as 85% in newly diagnosed patients (10).

CML biological history is characterized by a “chronic phase” period that usually lasts 5 years, and is then followed by the transformation of the disease into an acute leukemia, known also as “blast crisis”, which is lethal in few weeks/months. While the activity of imatinib in overt blast crisis CML is rather limited (11), 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.

In fact, actual data show 83% of late chronic phase CML patients who were originally enrolled in the phase II registration protocol (protocol 110) and

achieved CCyR, to remain in cytogenetic remission after 5 years (12). More important, the curve seems to have reached a plateau in the last 12 months, especially for patients who reached a cytogenetic remission early in the course of the treatment. The presumed median duration of such responses, based on these data, exceeds 15 years. Similar results were recently reported also for newly diagnosed patients (13).

Imatinib treatment is not absolutely specific for CML cells, since other tyrosine kinases (c-KIT, PDGFR) are inhibited in addition to Abl. Although the known safety profile of imatinib is quite benign (9), several side effects and lines of evidence indicate that a long term follow-up is worth (see section 2).

While a normal marrow re-grows in the majority of treated patients, most of them remain positive for Bcr/Abl transcripts, at least up to now, when tested by the sensitive Polymerase Chain Reaction (PCR) technique, indicating the residual presence of leukemic cells in a proportion ranging from 1/100 to 1/10,000 (14). Because of this, the present treatment recommendations for CML state that treatment should be continued “indefinitely”, in spite of its high cost.

2) RATIONALE and STUDY OBJECTIVES

2.1 Rationale

The known safety profile of imatinib is quite benign and the treatment is generally well tolerated. The most common side effects are oedema, muscle cramps, diarrhoea, osteoarticular pain, skin rashes (9), asthenia; rarer toxicities include gynecomastia and liver/pancreatic damage (15, 16). Some recent some experimental data also suggested (17) that imatinib might induce cardiotoxicity. Given the need for continuous treatment, it is important to assess the long-term tolerability and side effects through a longer follow up.

In fact some adverse events may arise after several years of treatment, due to the ability of imatinib to inhibit different tyrosine kinases, including c-Kit and Platelet-Derived Growth Factor Receptor (PDGFR).

In addition Abl, the normal counterpart of the Bcr/Abl gene, is involved in growth arrest and development of apoptosis following DNA damage (18). Since imatinib blocks Abl and Bcr/Abl to a similar extent, the inhibition of Abl in normal cells could theoretically lead to decreased control over DNA damage with an ensuing propensity for cellular transformation and development of malignancies. Many cancers require several years to develop and only a long term survey can detect their onset and progression. Indeed an animal carcinogenicity study suggests a possible increase in genitourinary cancers after imatinib chronic treatment, and a retrospective analysis by a French group apparently detected an excess of prostate cancers in CML patients receiving imatinib (19).

Finally, two recent reports indicate that the discontinuation of imatinib in patients who achieved a sustained PCR negativity was not followed by disease relapse in approximately 50% of cases (20, 21). As well, a recent presentation on the molecular monitoring of patients enrolled in the IRIS trial (12), indicated that the amount of transcript decreases gradually, up to 4 years after imatinib initiation. In this report the number of patients who achieved >4 log reduction of Bcr/Abl transcript (i.e. PCR negative or almost negative) after 4 years of treatment reached the value of 41%. Therefore it would be interesting to know whether imatinib treatment could be discontinued at a certain point or whether it must be continued forever.

Additional general reasons also concur in justifying the execution of an independent survey in this case:

1. The manufacturer can efficiently survey initial registration studies; however the report of adverse events (including second cancers) in out of trial patients is notoriously very inefficient.

2. It is not known how long the manufacturer will continue to monitor and follow patients receiving imatinib and enrolled in initial trials; for some of them collection of information was already stopped. Under these conditions the ability to assess the development of long term side effects becomes unreliable.

3. The recent VIOXX case clearly epitomizes to the medical and general communities how badly needed are independent studies to identify and analyze the long term safety of medications.

2.2 Study objectives

Primary:

- ✓ To evaluate in CML patients in CCyR receiving imatinib, the occurrence of long term side effects, including the development of second cancers, using the earliest cohort of patient available.

- ✓ To identify among patients with negative PCR results, those that can be considered “true negative”, through the use of a new assay (Microfluidic Card System PCR).

Because of the relevance of these two objectives, this study has been endorsed as of “critical importance” by AIFA (Italian Drug Agency).

Secondary:

- ✓ To evaluate the incidence of specific types of cancers :
  - Prostate cancer
  - Urinary cancers
  - Lymphomas

- Ph negative Leukemias
- Other cancers

✓ To evaluate the incidence of Severe Adverse Events (SAE)

✓ To evaluate the incidence of specific types of SAE:

- Cardiovascular disease
- Pancreatitis
- Hepatic toxicity
- Myopaties
- Severe skin rashes

✓ To evaluate some non SAE side effects which could substantially affect the quality of life of patients:

- Gynecomastia
- Sexual dysfunctions
- Asthenia

✓ To evaluate the presence of SAE in offspring from treated patients during the first year of the infant's life.

✓ To set up of a new, more sensitive technique, called Microfluidific Card System PCR, which may identify among the patients who present consecutive negative PCR analyses, those who could be truly negative for Bcr-Abl transcripts.

2.3 Investigational plan

This is a multicenter, no profit, observational study involving several clinical centers.

Retrospective collection of data will be performed. All information will be fully anonymized. Informed consent will be obtained, with the exception of patients who died (see section 8.2).

The trial is composed of two phases. In the first phase retrospective information about CML patients in CCyR after two years of imatinib treatment will be collected from the database of each centre.

The observation of side effects will start after two years of treatment in order to exclude patients who did not respond to imatinib, in whom adverse events could related to represent the persistence of their leukemia. Moreover this time is sufficient to consider an observed side effect as “late”, and to allow for the conclusion that any second cancer is possibly related to the chronic treatment with imatinib.

In the second phase the collection of data will be prospective as each center will have to update the follow-up of the patients reported in the first phase.

During this phase patients in molecular remission (PCR negative) since more than one year will be identified; for this subset of patients peripheral blood samples will be obtained.

The samples will be collected by each center and will be sent to the centralized laboratory at S. Luigi Hospital (Orbassano) for the analysis by Microfluidic Card System PCR.

The trial time will be 30 months.

3) STUDY PATIENTS

3.1 Patient population

The target population includes male or female patients, followed in the involved study centers (see Appendix 3) and affected by Ph+ CML. This study population includes all consecutive patients who started imatinib between 01 September 1999 and 31 December 2004 and who were in CCyR two years after the start of imatinib therapy.
A total of 500 patients are expected to be recruited.

3.2 Eligibility criteria

1. Male or female patients with Ph+ CML
2. Age > 18 years at diagnosis
3. Imatinib treatment started between 01 September 1999 and 31 December 2004.
4. CCyR confirmed two years after the start of imatinib therapy
5. Written informed consent

3.3 Exclusion criteria

1. Patients who have received imatinib for other diseases (GIST, HES, SM, Ph+ ALL, other), and not for CML Ph+.
2. Imatinib treatment started after 31 December 2004.
3. Absence of CCyR two years after the start of imatinib therapy.

4) STUDY PROCEDURES

4.1 First phase

In this phase all centers will identify their eligible cases and report in a database the retrospective information. Data to be reported are shown in “Registration” and the “Baseline Follow up” forms (see Appendix 2). They include: demographics, date of CML diagnosis, date of beginning of imatinib, dose of imatinib in the first two years of treatment, major comorbidities, dose of imatinib in subsequent years, cytogenetic status at baseline and after 2 years from start of imatinib, date of first CCyR, previous treatments before imatinib, date of last follow up, cytogenetic status at last follow up, occurrence of one of the endpoints of interest (primary or secondary) after the first 2 years in CCR. This phase will tentatively end on Dec. 31, 2006.

4.2 Second phase

This phase will start after the retrospective data collection is performed and checked for consistency and completeness by the statistical unit. Every centre will update twice the follow-up on patients registered during the first phase: at Dec. 31, 2007 and at Dec. 31, 2008, according to the “Follow-up Form”. Data will be collected on: dose of imatinib, CCyR and PCR status, and occurrence of one of the endpoints of interest. See also the “Follow up” form.
In this phase patients who tested negative by PCR for > 1 year (evaluated by either real time PCR or nested PCR), will be identified and their peripheral blood will be drawn and sent to a central laboratory at S. Luigi Hospital. Two 15 ml peripheral blood samples will be collected by venipuncture. One sample will be obtained when the patient has exceeded 1 year of continuous

PCR negativity. The second sample will be collected at the end of the second phase (Dec. 2008) if the patient will still be in continuous PCR negativity. The blood will be collected into EDTA tubes. The tubes will be gently inverted several times to prevent clotting. The sample will be shipped at room temperature on the day of collection to S. Luigi Hospital (for extra European centers the procedure will be explained in an appropriate manual).

An express courier will be utilized (the costs of the courier will be covered by the coordinating center).

RNA and cDNA will be obtained following standard procedures and analyzed using the micro fluidic card system. The Card (7900HT Micro Fluidic Card) represents an efficient and flexible high throughput technology that simplifies the evaluation of gene expression research. It takes advantage of the use of the ABI PRISM 7900HT machine. The card will be designed for the assay configuration needed by our research project, using a specific assay-on-demand platform (Applied Byosystems). Specific sets of primers and probe are pre-loaded into the 384 wells of the Micro Fluidic Card. In particular, 192 wells will be set up for BCR/ABL determination and 192 wells for the quantitative assessment of the control housekeeping gene. The conformation of the cards will be designed in order to analyze two patients in each card. The level of negativity for BCR/ABL will be established by the number of positive wells out of 96 wells. The run of PCR will be performed following established procedures. The final value of expression will be calculated by the SDS 2.1. software. The number of negative/positive wells will allow to establish the degree of negativity.

It is expected that between 5 and 10% of enrolled patients will qualify for blood drawing.

5) PROTOCOL AMENDMENTS AND CHANGES IN STUDY CONDUCT

Any changes to the protocol will be made in the form of an amendment, which will have to be approved by the scientific board and participating centers Ethic Committee (EC) before implementation.

6) DATA MANAGEMENT

6.1 Data collection

Investigators (or designees for data collection) must enter the information required by the protocol case report forms (CRFs, appendix 2) into and ad hoc data base which will be designed by the statistical unit and will be distributed to each center for data collection.
The Investigator must certify that the data are complete and accurate marking a specific box at the end of the data reported for each patient. The completed data base on all patients reported by each center will be sent online by the investigational site to the statistical unit (while a copy of this will be kept at the site).

6.2 Quality control

The study coordinator center will perform monitoring visits. Random visits at the sites will be performed every year until the end of the trial by an authorized clinical monitor. Local investigators must cooperate with the monitor and consent direct access to source data and documents. Up to 15% of registered patients will be monitored.

The study database will be checked by the statistical unit (Responsible Unit Maria Grazia Valsecchi) for consistency and completeness. Queries will be issued to the centers. Replies will have to be reported by the centers to the clinical monitor and statistical unit.

The data analysis will be annual.

6.3 Data property

All research data and results generated during the course of the study shall be property of the Scientific Board. The data will be used to report and to publish the results to the Scientific Community. The clinical information will be anonymized and considered Confidential Information, they are protected by Italian law (D.Lgs. 196/03).

7) STATISTICAL METHODS

The data will be analyzed by the statistical unit.

The recruitment of a cohort of 500 patients is expected to approximately contribute a 1900 person/years observation. In this cohort, the expected age and sex distribution is the following: 10% with < 40 yrs, 70% with 40-69 yrs and 20% with > 70 yrs; 60% of males. Based on the background population incidence rate of all types of cancer from GLOBOCAN2002 (as published on the IARC http://www-dep.iarc.fr/globocan/database.htm website) the expected number of tumours is approximately 6 (4 in males and 2 in females). This study would have a 80% power to assess a 3-fold increase in the age standardized incidence rate of cancer in both males and females.

For specific cancer sites, the power would be generally below 80%. In particular, the study would have a 65% power to detect a 4-fold increase in prostate cancer (19).

Analysis of SAEs and non SAEs will be mostly descriptive. However, observed rates will be compared to expected rates for specific types of SAE where population incidence rates are available. For instance, based on a recent register on hospital admissions in 2005 in the province of Milan (''Ricoveri e Mortalità a Milano'', ASL Città di Milano, Zadig 2005) a comparison will be possible with the expected rate of pancreatitis.

8) 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 24/06/2003, 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.

8.1 Ethics Committee

Before implementing this study, the protocol and the informed consent form must be reviewed and approved by the local EC. Any amendment to the protocol have to be approved by the EC.

8.2 Informed consent

Each patient will be informed of the nature of the study and will give in writing her/his consent to participate to the study. The patients will be informed about the possibility that a blood sample could be collected for those with PCR negative for > 1 year

The informed consent will be read and signed by the patient and the Investigator before data collection (Appendix 1). A copy of the signed document will be given to the subject.

For patients who died before the study initiation no consent form will be obtained, being the study retrospective and the data anonymized.

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