STUDY PROTOCOL SYNOPSIS

Promoter: University Milano-Bicocca

Product: Imatinib

Title of Study: Validation of digital-PCR analysis through programmed imatinib interruption in PCR negative chronic myeloid leukemia patients.

Protocol Acronym: ISAV

Background Information and Study Rationale:

Chronic Myeloid Leukemia

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). 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), that generates the Philadelphia (Ph) chromosome (4).

Untreated CML commonly progresses through three disease phases: chronic phase (CP), accelerated phase (AP) and blast phase (BP), each corresponding to increasing leukemic blast counts and clinical severity. The chronic phase (CP), that usually lasted 2-3 years in the pre-imatinib era (5), is characterized by an abnormal expansion of the clonal hematopoiesis retaining an apparent normal differentiation; the AP's median duration is 3-9 months, while BP's median survival is 3-6 months (6). The last two phases are marked by the development of a differentiation block typical of acute leukemia which fatally closes the course of the disease (7).

Imatinib Therapy

The main goal of CML therapy is the suppression of Ph+ clone in the chronic phase (CP) (8). Since BCR/ABL translocation represents the molecular cause of CML, the targeting of its enzymatic activity represents a truly "targeted" attempt to cancer therapy. In fact, over the last two decades, the therapy evolved from the use of non-specific cytotoxic agents (i.e. hydroxurea, busulfan) (9, 10) to interferon-α (IFN-α) (11-15) or allogeneic stem cell transplantation (allo-SCT) (16-22) and more recently to imatinib, a competitive inhibitor of the BCR/ABL kinase that, with a 5-year survival rate greater than 90%, is now recognized as the first-line treatment of CML (16-26) and could allow a normal life expectancy (27).

Imatinib induces complete cytological response (CCyR) in up to 80% of patients (33) and major molecular response (MMR) in 33-90% of the patients, according to treatment duration. Moreover, approximately 1/3 of long term treated patients who are in CCyR show complete molecular response (CMR, i.e. undetectable BCR/ABL transcripts) and absence of residual sign of leukemia (27). Anyway, undetectable BCR/ABL may not equate to eradication of minimal residual disease (MRD) because the sensitivity of the standard diagnostic method, the Q-RT-PCR, is limited and significant numbers of residual leukemic cells can remain in a patient (33).

The digital-PCR assay

The development of a more sensitive Q-RT-PCR to monitor MRD was recently included among the study endpoints of the ILTE study, aimed to present a global picture of imatinib long term effects (serious adverse events (SAE), toxicities not qualifying as SAE, loss of CCyR, survival). A pilot study was performed on blood samples obtained from patients who remained negative for at least

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one year, using a new diagnostic method, the digital-PCR (dPCR), developed by Gambacorti-Passerini, Saglio and Kim (39), able to detect until 1 BCR/ABL+ cell out of 10^7 cells, which corresponds to a 100 times increased sensitivity as compared to conventional Q-RT-PCR (45). The results showed that, among 30 patients negative by conventional Q-RT-PCR for at least one year, only 10 patients (33%) were negative by dPCR. Interestingly, these numbers are compatible with those observed in a French study in which about 40% of patients were relapse-free within 12 months from imatinib suspension (27). Therefore, it is possible that dPCR, assessing with more sensitivity the presence of MRD, could better identify the patients where CML is truly eradicated.

Study Rationale

Imatinib treatment for CML is recommended to be life long, but pilot studies showed that it is possible to suspend imatinib treatment without experiencing relapse (32). According to several models predicting CML cell proliferation based on the cell doubling time, at least 6 months are necessary to reach a number of cells detectable by Q-RT-PCR if a single CML cell persists in the patient when imatinib is suspended. Therefore, after 6-8 months of imatinib suspension without relapse, it is possible to hypothesize that the disease is eradicated or that residual CML cells are in a quiescent state (38). In this condition, it is estimated that imatinib discontinuation might be permanent (36, 37, 40), allowing to achieve two important advantages:

• a substantial reduction in health care spending (about 3 millions euro/year saved every 100 such patients by the NHS)

• the prevention of the morbidity due to imatinib side effects.

Nowadays, it remains possible that during the interruption period the disease could progress in the majority of patients because it is not possible to surely identify “a priori” patients with CMR able to permanently discontinue imatinib. In fact no diagnostic assay is sensitive and specific enough to predict the disease relapse after the therapy discontinuation. Therefore, it is only possible to rely on a “watching and wait” strategy. Importantly, the relapse is reversible upon imatinib resumption (35, 36).
The present study is aimed to evaluate if the new dPCR method, able to detect a single BCR/ABL positive cell out of 10^7 cells (compared to 1 cell out of 10^4-10^5 for standard Q-RT-PCR), could predict CML reappearance after imatinib discontinuation in the setting of Q-RT-PCR negative CML patients.
In case of positive results, a rational drug suspension policy which relies on dPCR status for the decision of stopping or continuing imatinib treatment will be allowed.

Objectives:

Primary Objective

Evaluation of the power of the dPCR technique to predict disease relapses after imatinib discontinuation in CML patients with negative Q-RT-PCR results since more than 18 months.

Secondary Objectives

- Evaluation of dPCR technique specificity.
- Evaluation of the maintenance of molecular remission, after imatinib discontinuation, in CML patients with negative Q-RT-PCR results since more than 18 months.

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- Evaluation of quality of life in CML patients after imatinib discontinuation/resumption. Evaluation of progression/resistance in patients who relapse after imatinib discontinuation.

Endpoints:

Primary Endpoint:

- Negative Predicted Rate (NPV) of dPCR, i.e. the capability of the method to predict relapse-free patients.

Secondary Endpoints:

- Rate of dPCR positive relapse-free patients after 3 years follow up.
- Rate of relapse-free patients after 3 years follow up.
- Relapse free time.
- Quality of Life, as measured by EORTC-QLQ-T30 questionnaire.
- Rate of patients progressing or developing resistance after imatinib suspension.

Study Design and Methods:

In this study dPCR analysis will be performed on CML patients under imatinib therapy, with at least 18 months of consecutive negative standard Q-RT-PCR as performed in their own centers. The patients will sign an informed consent form (ICF), will subsequently be tested for dPCR and will stop imatinib. Then they will be monitored with standard Q-RT-PCR to assess the maintenance of the molecular remission. Collection of data will be prospective as each center will collect the data for 36 months. At the end of this period, a peripheral blood sample for dPCR analysis will be obtained from those patients who will still have undetectable BCR/ABL transcripts by Q-RT-PCR to verify CML eradication. Two years follow-up is then planned for patients maintaining CMR. During study, the patients with BCR/ABL transcripts detected by standard Q-RT-PCR will repeat the test not before 2 weeks and not after 2 months; in case of results confirmation and at least one BCR/ABL / BCR value >0.1%, the loss of the molecular remission will be declared and hematologic and bone marrow analyses will be performed. Such patients will immediately resume imatinib treatment at the same dosage used before interruption; progression of the disease will be evaluated as evolution to AP or BP and resistance to treatment will be defined as lack of complete hematological response at 3 months or of complete cytogenetic response at 6 months after resuming imatinib.

Patient's life quality during imatinib discontinuation/resumption will be evaluated trough a Quality of Life (QoL) questionnaire until the end of this study (for patients maintaining molecular remission at baseline and at months 1, 6, 12, 24, 36, for relapsing patient at months 1, 6, 12 after imatinib resumption).

The eligible patients will be entered between September 2011 and August 2012. Approximately 14 Centers will be involved in the study, including 5 international Centers.

Sample size

Sample size will be calculated so to provide with 80% power the alternative hypothesis that the Negative Predicted Value (NPV) of dPCR (i.e. the capability of the method to predict relapse-free patients) will exceed the one of traditional Q-RT-PCR, by more than 1.5.

$$H0(N): rNPV \leq \delta$$

$$H1(N): rNPV > \delta$$

where rNPV is the ratio of the Predicted Negative Values by the two tests respectively.

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With reference to the table below:

$$rNPV = \frac{NPV_{dPCR}}{NPV_{Q\text{-}RT\text{-}PCR}} = \frac{(n_{3+}\ n_4) / (n_{3+}\ n_4 + n_6 + n_8)}{(n_{2+}\ n_4) / (n_{2+} + n_4 + n_6 + n_8)}$$

	Relapse Free 3 years since discontinuation			Relapsed within 3 years since discontinuation		
	Q-RT-PCR at entry			Q-RT-PCR at entry		
dPCR at entry	Positive	Negative	Total	Positive	Negative	Total
Positive	n_1	n_2	$n_{1+}\ n_2$	n_5	n_6	$n_{5+}\ n_6$
Negative	n_3	n_4	$n_{3+}\ n_4$	n_7	n_8	$n_7 + n_8$
	$n_{1+}\ n_3$	$n_{2+}\ n_4$	N_{R-}	$n_{5+}\ n_7$	$n_6 + n_8$	N_{R+}

The studies estimating the relapse rate of patients after discontinuation of imatinib are still limited in number, based on a relatively low number of patients, heterogeneous by time on treatment, observation time accrued after discontinuation and therapy preceding imatinib (39, 40, 41). According to the preliminary results of the STIM study (40) that included patients in CMR for at least 24 months, 42 out of 69 patients followed for 13-30 months (median 24 months) since imatinib discontinuation relapsed. Thirty-nine percent (95% CI 28-51%) of patients were still negative at Q-RT-PCR performed at repeated occasions after discontinuation. Due to the lack of robust estimates, the sample size has been calculated assuming that $NPV_{Q\text{-}RT\text{-}PCR}$ equals to the lower limit of the 95% CI of the relapse free rate reported above i.e. 28%. If true rate of $NPV_{Q\text{-}RT\text{-}PCR}$ is higher the sample will provide the test with more power. Also, according to a pilot experience conducted within ILTE protocol, only 1/3 of patients negative by Q-RT-PCR are also negative by dPCR. Finally, n3 and n7 were assumed to be 0 because, being the dPCR method more sensitive than Q-RT-PCR, it is highly unlikely that the former turns to be negative when actually the latter is positive. With $\alpha = 0.05$, $\beta = 0.20$, $\delta = \gamma = 1.5$ under the alternative hypothesis and the above assumptions the sample size required calculated according to reference (42) is 98 patients.

Definition of Analyzed Study Populations

All enrolled patients will be evaluated. Patients who withdraw or die before 36 months from the day 1 of the study will be considered as not evaluable.

Analyses

The data will be analyzed by Milano International Oncology or designee. rNPV will be calculated as the ratio of the Predicted Negative Values by the two tests respectively . If the lower 95% confidence limit will be greater than 1.5 one would conclude that the new method is more effective in predicting negative value as compared to the traditional one.

Progression free survival computed as the day since enrolment to the day of relapse or death will be analyzed by Kaplan-Maier method. The sensitivity and specificity of dPCR to predict molecular relapse will be assessed, as well as the overall and progression free survival of patients.

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The following variables will be monitored: dPCR results, number and time of relapses (molecular, cytogenetic or hematological), presence of progression AP/BP at relapse, best response obtained after imatinib reintroduction. Quality of life will be evaluated before and after imatinib suspension and after eventual imatinib resumption.

Interim Analyses:

Interim analyses will be conducted 1, 2 and 3 years after the day of last patient enrollment.

Subject Selection:

Subject inclusion criteria:

Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Signed and dated IRB/IEC-approved Informed Consent.
2. Age > 18 years.
3. Male or female patients with CML diagnosed in chronic phase and who have been treated for more than 2 consecutive years with Imatinib therapy.
4. Achievement of CMR (as defined by their own center) sustained for at least 18 months with imatinib treatment.
5. A minimum of 3 CMR determined by Q-RT-PCR analysis to support disease status, with the last one performed within 3 calendar months prior to enrollment date.
6. Willingness and ability to comply with scheduled visits, laboratory tests and other study procedures.

Subject Exclusion Criteria**:**

The presence of any of the following will exclude a subject from study enrollment:

1. Allogenic hematopoietic stem cell transplantation.
2. Known active infections, including human immunodeficiency virus (HIV) positivity.
3. Current enrollment in another clinical trial.
4. Other severe acute or chronic medical or psychiatric condition or laboratory abnormality that may increase the risk associated with study participation or study drug administration or may interfere with the interpretation of study results and, in the judgment of the Investigator, would make the patient inappropriate for entry into this study.

Trial Product

In case of loss of molecular remission the patients will resume imatinib treatment at the same dosage used before interruption. In case of treatment resumption, imatinib will be administered as home-based treatment. Imatinib is marketed and used for the treatment of CML so it do not represent an additional cost for the study and will be supplied by the National Health Service. Imatinib is commercially available in rigid capsules of 50 mg. Imatinib is to be stored at room temperature not exceeding + 30°C and in the original packaging, out of the reach and sight of children.