Protocol Title:

Retro-prospective observational study on risk of progression in chronic phase-Chronic Myeloid Leukemia patients eligible for tyrosine kinase inhibitor discontinuation (TFR-PRO).

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Steering Committee:

Elisabetta Abruzzese MD

Alberto Alvarez MD

Sarit Assouline MD

Brian Druker MD

Patrizia Pregno MD

Carlo Gambacorti Passerini MD

Philipp leCoutre MD

Susanne Saussele MD

Coordinating Center: San Gerardo Hospital, Monza, Italy

Sponsor: University of Milano Bicocca, Italy.

SIGNATURE PAGE

Scientific coordinator of the study	
Name and Surname: Accademic title: Department: Institute: City, Country:	
Signature	Date

Principal investigator at clinical site	
Name and Surname: Accademic title: Department: Institute: City, Country:	
Signature	Date

1. SUMMARY

2. STUDY TITLE

Retro-prospective observational study on risk of progression in chronic phase-Chronic Myeloid Leukemia patients eligible for tyrosine kinase inhibitor discontinuation (TFR-PRO).

3. BACKGROUND INFORMATION AND STUDY RATIONALE

3.1. 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). This fusion originates through a 9;22 chromosomal balanced translocation [t(9;22)(q34;q11.2)], 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).

3.2. TKI therapy

The main goal of CML therapy is the suppression of Ph+ clone in the chronic phase (CP) (8). Since BCR-ABL 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 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 finally to imatinib, that with a 5-year survival rate greater than 90% is now recognized as first-line treatment of CML (16–26) and could allow a normal life expectancy (27).

Imatinib is a competitive inhibitor of the BCR-ABL kinase catalytic domain, which proved able to inhibit BCR-ABL enzymatic activity (28,29), to block proliferation and induce apoptosis of BCR-ABL+ cells both *in vitro* and in *vivo* (30). In addition, imatinib is an inhibitor of the platelet-derived growth factor (PDGF) and stem cell factor (SCF) tyrosine kinase receptors and c-Kit, and blocks the PDGF- and SCF-mediated cellular events (31).

Nowadays four additional TKIs are available to treat CML: dasatinib, nilotinib, bosutinib and ponatinib (32). Although it is acknowledged that nilotinib and dasatinib are more potent and induce faster and deeper remissions, there is no evidence of a better outcome if a second generation TKI is used first-line (33). Indeed, so far, no significant survival difference between imatinib and second generation inhibitors has been observed (34).
Imatinib and other TKIs induce complete cytogenetic response (CCyR) in up to 80%-85% of patients with CML (35), while major molecular response (MMR) is observed in 33-90% of patients according to treatment duration. Moreover, among patients with MMR, more than 30% of patients show CMR (undetectable BCR-ABL) by Q-RT-PCR and nested RT-PCR. In addition the obtainment of a Deep Molecular Response (DMR), defined as the presence of residual disease lower than 1/10000 cells (or MR4), is presently considered equivalent to CMR in terms of discontinuation outcome (see below).

Undetectable BCR-ABL or DMR 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 10^7) can still remain in a patient who shows RT-PCR negativity (35).

3.3. TKI discontinuation

3.3.1. The concept of treatment-free remission

The most recent recommendations of the NCCN and the ELN for CML propose continuation of TKI treatment indefinitely in all responding patients.
During the last decade many studies have addressed the issue of stopping TKI treatment in CP-CML patients with stable DMR (36–39), prompting the development of a new concept in the evaluation of CML patients, known as treatment-free remission (TFR). In these studies, treatment discontinuation (TD) proved successful in approximately half of the patients with DMR (40). Most studies focused on patients with excellent molecular response to their first-line TKI (usually imatinib), and report a relapse free survival (RFS) of 48-61% at 3 years of TKI discontinuation (41). Moreover TD proved a safe option, since new MMR is achieved in almost all cases with TKI rechallenge (42), being MMR defined as BCR-ABL/BCR transcripts < 0.1% on the international scale or ≥ 3-log reduction of BCR-ABL transcripts from the standardized baseline (43). MMR is considered a "safe haven" since resuming treatment if/when MMR is lost maximizes the likelihood of regaining the response and minimizes the risk of disease progression (44,45).

In these studies it was then observed that low levels of residual disease before/after TKI withdrawal did not automatically indicate CML relapse and did not preclude the possibility of remaining treatment-free. To prove this principle, a French multicenter observational study (according to stop imatinib (A-STIM)) was conducted. The criteria for stopping were less stringent than in the STIM study, and patients with occasional positive low level PCR results corresponding to MR4.5 before study entry were considered eligible. Molecular relapse was also less stringently defined as loss of MMR, which was chosen for triggering re-treatment (instead of loss of CMR), as it was also decided in studies like ISAV (39). The main conclusion drawn from these studies was to use the loss of MMR as the more appropriate trigger for restarting treatment after TKI discontinuation rather than loss of CMR. The study was also safe, as all patients were sensitive to re-treatment (46).
According to a meta-analysis reported from 15 different cohort studies, involving 509 patients who stopped imatinib with undetectable BCR-ABL transcript level, including the STIM and A-STIM studies, the overall rate of molecular relapse was 51%; after 6 months of follow up (FU), it was 41%, confirming that 80% of molecular relapses occurred in the first 6 months (47).

The feasibility of TFR after nilotinib or dasatinib was also shown. The pilot academic study STOP 2G-TKI, enrolled 60 patients in France who had received 3 years of TKI therapy, were currently receiving either nilotinib or dasatinib as frontline therapy or after imatinib, and had maintained MR4.5 with undetectable molecular residual disease for 2 years. Also patients with a prior failure to a first-line TKI were included (37). The estimated rate of TFR at 12 and 48 months was 63.3% and 53.7%, respectively. In lights of the results from this and others stopping trials with second generation TKIs, there is no proof that TFR is higher compared to imatinib. Indeed, the rate of molecular recurrence seems to be reproducible whatever the TKI used (48).
So far, no data exist regarding TD in CML patients who received treatment with bosutinib, so that the relapse rate in this context is not known.

The large EURO-SKI (European stop tyrosine kinase inhibitor) trial explored even less deep MR levels as thresholds for discontinuing imatinib, dasatinib, or nilotinib, enrolling patients who maintained MR4 for at least 1 year only. Molecular relapse-free survival for these patients was 61%

at 6 months and 50% at 24 months. No safety issues were reported. The study confirmed and validated that loss of MMR is a safe criterion for TKI retreatment, as was previously shown in the A-STIM study. This criterion increased the patient population eligible for treatment discontinuation (49).

Now treatment-free remission is an arising option in standard care and has become a possibility outside clinical trials (50). Indeed, recommendations of the most important scientific societies in different countries address this issue (34,51). The current US National Comprehensive Cancer Network (NCCN) guidelines recommend that TFR should only be attempted in patients who have been on approved TKI therapy for at least 3 years and who have been in at least MR4 for at least 2 years (51). Guidelines of the European Society for Medical Oncology (ESMO) recommend at least 5 years of TKI therapy, achievement of MR4.5, and stability of DMR (at least MR4) for at least 2 years before discontinuation (34). However it is acknowledged that less stringent criteria do not exclude successful TFR (41).

A recent meta-analysis published in 2019 provides an overview that is more similar to the current, real-practice use of TKI discontinuation. Indeed, it included 10 studies enrolling CML patients who received TKIs (imatinib, dasatinb or nilotinib in first or subsequent lines), adopting loss of MMR as definition of molecular relapse. The estimated weighted mean incidence of loss of MMR was 39% and 41% at 12 and 24 months, respectively, for patients who discontinue TKIs (52).

3.3.2. Safety of treatment-free remission

Under the aforementioned recommended conditions and with an optimal disease monitoring (defined as monthly or bimonthly PCR analysis in the initial 6 months, followed by 3-4 PCR/year (40,50), the occurrence of tumor progression (i.e. the progression to accelerated or blast phase) has been considered virtually impossible for a long time (39). Supporting this, an interesting theoretical report predicts that bi monthly monitoring for the first 6 months and 3 monthly thereafter can still be safe enough to capture recurrence (loss of MMR) before patients lose CCyR (53). Moreover, almost all patients with disease recurrence after stopping TKI will regain at least MMR on resuming their TKI. For all these reasons TFR appears globally safe (41). However, amongst many thousand patients attempting TFR from stable remissions of MMR or better, some reports exist documenting disease progression.

A lymphoid blast crisis in the French A-STIM study was the first one reported (46). This woman was diagnosed with chronic-phase CML with low Sokal score in 1996. She obtained CCyR after 6 months of IFN. IFN was interrupted in 1999 in CMR because of adverse effects. Imatinib was started in 2006 after a molecular relapse, and a second CMR was achieved in 2007. The patient was included in the A-STIM study in August 2009, and imatinib was stopped. CMR was lost 9 months later, and MMR was lost on the second assessment 1 month later. Imatinib was resumed in July 2010, and MMR was regained in October 2010. Lymphoid blast crisis was diagnosed 8.5 months after restarting imatinib. There was no evidence of mutation in the BCR-ABL gene; cytogenetic analysis showed a complex karyotype with major-route additional chromosomal aberrations, including der(22). Complete remission was achieved with the combination of nilotinib and chemotherapy (after failure of dasatinib and chemotherapy), and the patient underwent allogeneic matched unrelated-donor transplantation conditioned by total-body irradiation and cyclophosphamide in July 2011.

Other two cases were reported in the Korean Imatinib Discontinuation (KID) study. One was a patient who experienced MMR loss at 53.2 months after imatinib discontinuation, and, despite re-achieving MMR after imatinib restarting, suddenly progressed to blast crisis at 6 months after restarting the drug and, in spite of switching to dasatinib and ponatinib, she died. Another patient lost MMR at 7.4

months after imatinib discontinuation and re-achieved MMR after restarting the TKI, but progressed to accelerated phase 32 months later. The patient switched to dasatinib and was subsequently lost to follow up (54).

In the above mentioned STOP 2G-TKI observational study one patient lost MMR 5 months after first line nilotinib cessation and was found in sudden myeloid blast crisis 6 months after TKI reintroduction. No BCR-ABL mutation was found, but an inversion of chromosome 3 at karyotyping analysis. The patient underwent autologous stem cell transplantation after chemotherapy plus ponatinib and was alive in remission 29 months later (55).

In Greece, outside the context of a clinical trial, a case of blast crisis of CML after nilotinib discontinuation in a patient with previous stable DMR was published. This patient was regularly monitored and remained in DMR until 18 months off treatment, when for the first time, MR4 was lost. A subsequent sample in 2 months revealed a loss of MMR. Nilotinib was restarted and MR4 was soon again achieved. However, 6 month later, a lymphoid blast crisis and a cytogenetic and molecular clonal evolution occurred. Indeed a deletion of the short arm of chromosome 3 was found in cytogenetic analysis, while mutational analysis revealed a nilotinib resistant mutation (c.757T>C, Y253H), which was not present at the time of molecular relapse. Due to high comorbidity index the patient received low toxicity chemotherapy (high dose dexamethasone and vincristine) and ponatinib and, 3 months after the treatment, she was in DMR (42).

In a recent new analysis of the French RE-STIM study with an enlarged number of patients, one patient was reported to develop a sudden blast crisis at 4 years from the second discontinuation. The last previous molecular biology 3 months before transformation was MR4 (56).

A different but yet problematic situation was observed in the same RE-STIM study, where one patient lost MMR at 6 months from TD, but declined a TKI re-challenge. Consequently, BCR-ABL transcripts increased and a complete hematologic response was lost at 24 months. The patient was then lost to follow up (38).

An important point concerning the safety of TFR is the monitoring of the molecular remission of the disease. As mentioned above, guidelines by the most important scientific societies address the item of how to monitor these patients (34,50,51). In general, frequent monitoring is required during the first year, when most of the molecular relapses (95%) have been observed. Early relapses are characterized by an exponential increase in BCR-ABL, rising by 0.5 to 1 log per month. Thereafter less frequent assessments can be made and 3-monthly test suffice (42). Moreover recent data from the ISAV study show that in patients who discontinue imatinib and do not relapse, an increase in tumor load of approximately 1 log develops anyways over 3 years (57). Notably, a regular assessment of disease response seems having been made in many of the reported cases of CML progression after TKI discontinuation.

These results raise some concern about the safety of TKI discontinuation, since now we can no longer consider this practice as completely immune from the risk of disease progression, which drastically changes the patient situation and perspective. Nevertheless, a precise quantification of the risk of progression after TKI discontinuation has not yet been estimated.

Finally it is important to note that the exact risk of progression in this patient category which continue treatment is unclear, although considered to be very low.

4. TRIAL OBJECTIVES AND ENDPOINTS

4.1. Objectives

The main objective of this study is to investigate the safety profile of TKI discontinuation in clinical practice, with particular regard on the risk of progression after treatment discontinuation.

4.1.1. Primary Objective

- To quantify the risk of progression to accelerated phase (AP) or blast phase (BP), expressed as time adjusted rate (TAR), after TKI discontinuation in CML patients who undergo a first or subsequent TKI discontinuation attempt.

4.1.2. Secondary Objectives

- To compare the TAR of progression to AP or BP that is obtained in the target population to that obtained in a similar population of patients with the same characteristics who do not discontinue TKI treatment.
- To assess the timing of progression to AP or BP.
- To estimate the molecular relapse rate after TKI discontinuation.
- To assess the timing of molecular relapse.
- To estimate the proportion of relapsed patients who obtain a new deep molecular response (DMR) within 6-12 months of treatment resumption.

4.2. Endpoints

4.2.1. Primary Endpoint

- TAR of progression to AP or BP after TKI discontinuation. The following criteria will be used to define AP and BP (58,59):
  - **Accelerated phase**
    - 15-29% blasts in blood or bone marrow, OR
    - >30% blasts + promyelocytes (with blasts <30%) in blood or bone marrow, OR
    - ≥20% basophils in blood or bone marrow, OR
    - Persistent platelets <100 x 109/L (not related to therapy).
  - **Blast phase**
    - ≥30% blasts in blood or bone marrow
    - Extramedullary blasts proliferation, apart from spleen

4.2.2. Secondary Endpoints

- TAR of progression to AP or BP in the population of patients who do not discontinue TKI treatment.
- Progression free survival (PFS) after TKI discontinuation. PFS will be defined as time between discontinuation and progression to AP or BP.
- Rate of molecular relapse (loss of MR3 or MMR) at 12 and 24 months after TKI discontinuation.
- Relapse free survival (RFS) after TKI discontinuation. RFS will be defined as time between discontinuation and loss of MMR (i.e. molecular relapse).

- Percentage of relapsed patients who obtain a new deep molecular response (DMR) within 6-12 months of treatment resumption among all patients who restart TKI treatment because of a molecular relapse after TKI discontinuation. The following criteria will be used to define DMR (43):
  - MR4 = either (i) detectable disease with <0.01% BCR-ABL1IS or (ii) undetectable disease in cDNA with >10 000 ABL1 transcripts.
  - MR4.5 = either (i) detectable disease with <0.0032% BCR-ABL1IS or (ii) undetectable disease in cDNA with >32 000 ABL1 transcripts in the same volume of cDNA used to test for BCR-ABL1.
  - MR5 = either (i) detectable disease with <0.001% BCR-ABL1IS or (ii) undetectable disease in cDNA with >100 000 ABL1 transcripts in the same volume of cDNA used to test for BCR-ABL1.

5. INVESTIGATIONAL PLAN

5.1. Overall study design

This multicenter international retro-prospective observational study will enroll approximately 3000 CP-CML patients from at least 30 centers in 5 countries (Italy, Germany, Spain, United States, Canada).

Patients must have a history of at least 4 years of TKI treatment and at least 18 months of DMR, as performed through at least 3 molecular analyses at their own centers. Events developing in patients after the end of discontinuation and TKI resumption will be considered as linked to the discontinuation if they will develop within 36 months from the end of discontinuation. After this time they will not be considered linked to the discontinuation. This rule will apply also to subsequent TD attempts.
In case of a second or subsequent discontinuation attempt after the failure of a previous one (for molecular relapse), patients must have re-achieved a DMR with TKI therapy resumption and must keep DMR for at least 18 months before another TD.

Collection of data will be retrospective and prospective, as each center will collect the data for 24 months. Patients who discontinued before the opening of this study will contribute to the retrospective cohort, while those who will discontinue after it will contribute to the prospective cohort. Patients who discontinued before the opening of this study but will continue their discontinuation after it, will contribute to both cohorts. For patients prospectively recruited, monitoring of disease status will be performed to assess the maintenance of the molecular remission during the study period.

Patients with an atypical BCR-ABL1 fusion gene, which does not allow the use of Q-RT-PCR, will be monitored by qualitative PCR and will be analyzed separately. For these patients, negativity of nested qualitative RT-PCR will be considered a surrogate of DMR of patients monitored by Q-RT-PCR, while loss of negativity of first-round qualitative PCR will be considered a surrogate of loss of MMR (i.e. molecular relapse). Accordingly, for patients monitored by qualitative PCR, TKI resumption after TD will be provided in case of a new positivity of first-round PCR.

5.2. Study population

Approximately 3000 patients will be enrolled in the present study. Each center is expected to enroll approximately 100 patients. At least 30 centers will be involved in the study.

The target population will include all CML patients presently eligible for treatment discontinuation (>5 year of TKI treatment, >18 months of DMR) independently of whether they discontinued or not. For the time they did not discontinue they will contribute to the reference cohort, while after TD they will contribute data to the discontinuation cohort. In case of treatment resumption, the patient will still contribute to the discontinuation cohort for 36 months after treatment resumption, while he/she will contribute to the reference cohort thereafter, assuming a new TD did not occur.
CML patients who have already experienced one or more TKI discontinuation attempt before the opening of this study, will contribute to the retrospective cohort, patients who will discontinue after it will contribute to the prospective cohort, and patients who discontinued before the opening of this study but will continue their discontinuation after it will contribute to both cohorts.
As a benchmark, the study will also collect data from an analogue population of CP-CML patients, respecting the same criteria for a potential TKI discontinuation who decide against discontinuation. This second population will be useful to compare the risk of progression due to TKI discontinuation to the one in the benchmark population.

Eligible patients belonging to the prospective cohort will be entered in TFR-PRO between July 1st, 2020 and July 1st, 2021. Data from patients belonging to the retrospective cohort will be collected starting from 2011.

A previous participation in a clinical trial does not represent a reason for exclusion from the present study.

The characteristics of the eligible patients are described in section 5.

6. SUBJECT SELECTION

6.1. 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 for the prospective cohort patients.
2. Age ≥ 18 years.
3. Male or female patients with CML diagnosed in chronic phase (CP).
4. At least 4 years of TKI treatment.
5. At least 18 months of DMR.

6.2. Subject exclusion criteria

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

Allogeneic hematopoietic stem cell transplantation.

CML diagnosed in AP or BC.

7. ENROLLMENT PROCEDURES AND COLLECTION OF DATA

The informed consent for the prospective cohort should be signed and dated before starting collection of data.

Eligible patients will be enrolled via a web based application made available to the centers. The enrolment number will be sequential.

Patients who meet eligibility criteria will be studied independently of whether they decide for or against TD.

Data will be collected through the consultation of clinical diary of CML patients in each center. Information from the different participating centers will be collected on a single electronic database, created to contain all study data.

Patients will be identified through an alpha numeric code, without specific information on the general health status and removing any personal identification information, so to keep data completely anonymous.

The following data from enrolled patients will be collected:

7.1. Patient- and disease-related data

- Patient sex.
- Patient year of birth.
- Date of CML diagnosis.
- Sokal score

7.2. Therapy-related data

- Data of starting TKI therapy.
- Type of TKI used.
- Date of achievement of CCyR.
- Date of achievement of MMR.
- Date of achievement of DMR.
- Data and reason of changes in TKI therapy (intolerance, resistance, or discontinuation).
- Number and date of total/positive Q-RT-PCR before TD.
- Date of study entry, corresponding to the date of fulfilment of the eligibility criteria.
- Date of TD.
- Number of Q-RT-PCR performed and number of positive Q-RT-PCR performed after TD and recorder on a yearly basis.

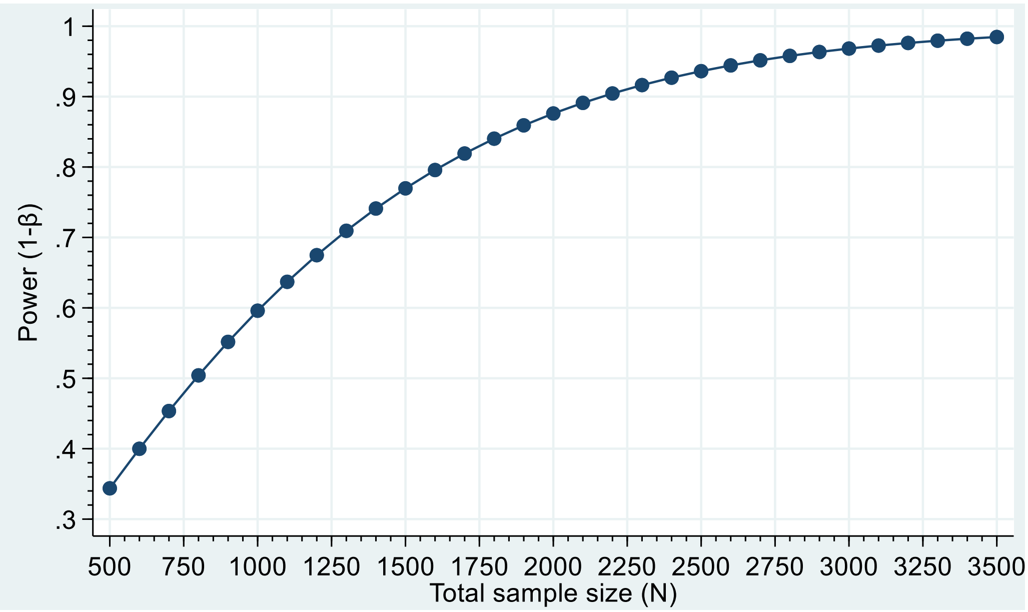
- Patients who will not discontinue will be followed in the same way.

7.3. Outcome data

- Patient's survival status at the latest FU (alive/dead).
- CML status at the latest FU (presence of MMR/CCyR/progression).
- Date and phase of CML progression (AP or BP).
- Date and value of the latest 3 Q-RT-PCR before CML progression.
- Treatment provided to patient experiencing CML progression.
- Date of molecular relapse after TD.
- Date of restart of TKI therapy after TD.
- Date in which patients who restart TKI therapy because of molecular relapse obtain a new MMR and a new DMR after treatment resumption.

8. STATISTICAL METHODS

Sample size calculations were obtained assuming a contrast between risks of progression to AP or BP of 0.01% under treatment versus 1% under discontinuation. These risks considered in a 5-years time window translate into epidemiological rates of 0.00000869 events per person-year, in other words 0.0869 events per 10000 subjects observed per one year and 0.000873 events per person-year, in other words 8.73 events per 10000 subjects observed per one year. The effect size is the rate ratio = 0.000873/0.00000869 = 100.05. The corresponding power graph is presented in figure 1 where on the x axis the total sample size is considered. Power is calculated under exponential rates and Log Rank Test.



Survival time to molecular relapse (loss of MMR) will be measured from the date where the patient satisfies the eligibility criteria to the minimum between the date of the second Q-RT-PCR assessment which defines molecular relapse and the end of the follow-up. Survival time to disease progression will be measured from the date where the patient satisfies the eligibility criteria to the minimum between the date of ascertainment of accelerated or blast phase diagnosis and the end of the follow-up. Study group classification will be defined as a binary non reversible time-varying factor where eligible patients are divided into two groups at the beginning of the follow-up depending on the presence or absence of discontinuation, and in the latter case may start discontinuation during the follow-up and thus change dynamically the study group classification.

Descriptive statistics on the main end-point will be obtained by the “Simon-Makuch” version of the Kaplan-Meier curves and log-rank test. Exponential model will be used for the calculation of confidence intervals on rates. Poisson model and Cox model will be used for data analysis allowing the shape of the hazard function to be non constant in time, accounting for the dynamic nature of the study group classification and adjusting for potential prognostics factors. Logistic regression through pseudo-observations will be used to estimate the proportion of relapsed patients who obtain a new deep molecular response (DMR) within 6-12 months of treatment resumption.

The follow-up length will be described using “reverse Kaplan-Meier” method. Rates and proportion will use the exponential model for significance and confidence interval. All the analyses and graphics will be performed by using Stata software version 16.
Age, Sokal score, duration of imatinib therapy, time from CML diagnosis to first CCyR or to TKI discontinuation and DMR duration until discontinuation of TKI will be assessed as potential prognostic factors for molecular relapse. Possible non linear effects of such factors will be included by restricted cubic splines.
Separate analyses will be performed for patients treated with second generation TKI as first line treatment versus second or subsequent line of treatment. Within the last category of patients separate analyses will be performed for patients who shifted to second generation TKI because of intolerance versus resistance to other TKIs.

9. ETHICAL / LEGAL ASPECTS:

9.1 Regulatory and ethical compliance

This clinical study was designed, shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including European Directive 2001/20/EC and US Code of Federal Regulations Title 21), and with the ethical principles laid down in the Declaration of Helsinki.

A copy of the patient informed consent must be submitted to the CE together with the protocol for written approval. Written approval of the protocol and informed consent must be obtained before starting the recruitment of patients in the study. The favorable opinion of the local Ethics Committee (EC) must be obtained before the start of the trial. The researcher must inform the EC of any amendments to the protocol.

9.2 Treatment of personal data

The personal data object of the study must be treated in compliance with the European Regulation on the Protection of Personal Data (GDPR), the Legislative Decree 196/2003 and subsequent amendments, and any other Italian law applicable to the protection of personal data.

10.DATA HANDLING AND RECORD KEEPING

Each participating site will maintain appropriate medical and research records for this trial, in compliance with Section 4.9 of the ICH E6 GCP, and regulatory and institutional requirements for the protection of confidentiality of patients. As part of participating in an Investigator-Initiated study (IIT), each site will permit authorized representatives of the sponsor(s) and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.
Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Examples of these original documents and data records include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, patients' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial.
Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site Principal Investigator. The study case report form (CRF) is the primary data collection instrument for the study. The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported in the CRFs and all other required reports. Data reported on the CRF, that are derived from source documents, should be consistent with the source documents or the discrepancies should be explained. All data requested on the CRF must be recorded. Any missing data must be explained. Any change or correction to a paper CRF should be dated, initialed, and explained (if necessary) and should not obscure the original entry. The investigator should retain records of the changes and corrections to paper CRFs.
The investigator/institution should maintain the trial documents as specified in Essential Documents for the Conduct of a Clinical Trial (ICH E6 Section 8) and as required by applicable regulations and/or guidelines. The investigator/institution should take measures to prevent accidental or premature destruction of these documents.
Essential documents (written and electronic) should be retained for a period of not less than fifteen (15) years from the completion of the Clinical Trial unless the Sponsor provides written permission to dispose of them or, requires their retention for an additional period of time because of applicable laws, regulations and/or guidelines.

Data will be input and handled through an electronic eCRF developed with REDCap Cloud platform (https://eulogin.redcapcloud.com/#cid=nph2020&act=list&studyId=362).

To protect data security and privacy, eCRF users will access exclusively via a username and password log in system. Access credentials will be generated by the study admin and communicated to the users with an automatic email generated by REDCap Cloud. The users must change the password after the first log in. The password will expire every three months. An alphanumeric code composed by the centre ID and the subject number (ex: HEITMB-001) will be assigned to all patients and data will be pseudo anonymized. To keep track of patients clinical record and subject number association, every centre will store in a separate encrypted and password protected file (protocol 256 AES) the alphanumeric code together with patients' anagraphic data; this file will be never shared with Sponsor data manager, database manager and/or statistician. No one except the study admin will be authorised to extract study data.

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