European survey on the assessment of deep molecular response in chronic phase CML patients after at least two years of therapy with tyrosine kinase inhibitors

Acronym: EUREKA

(**EU**ropean survey on the assessment of deep molecular **RE**sponse in chronic phase CML patients after at least two years of therapy with tyrosine **K**in**A**se inhibitors)

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1 Background

1.1 CML pathogenesis, diagnosis, treatment and monitoring

Chronic Myeloid Leukemia (CML) is a clonal disorder of hematopoietic stem cells resulting in marked myeloid hyperplasia in the bone marrow and peripheral blood. Clinically, the disease progresses through distinct phases referred to as chronic, accelerated, and blast crisis. CML is associated with a chromosomal translocation t(9;22)(q34;q11) detected in 95% of patients. The molecular consequence of the t(9;22) translocation is the creation of the fusion protein BCR-ABL, which is a constitutively active cytoplasmic tyrosine kinase upstream of numerous signaling pathways and necessary for initiation of leukemogenesis (*Baccarani et al.* 2007).

The goals of CML treatment are the return of blood counts to normal values, reduction and elimination of the Ph chromosome, and of BCR-ABL gene expression. The advances in understanding the biology of CML and the development of highly effective therapies have dramatically changed the natural history of the disease and the expected outcome of patients continues to improve. Prior to the advent of TKI therapy, the evaluation of hematologic and cytogenetic responses was sufficient to gauge treatment efficacy. However, with more potent TKI therapies, deeper responses are now commonly achieved, necessitating more sensitive methods of disease detection (*Cross et al*. 2012).

The European LeukemiaNet (ELN) recommends quantification of BCR–ABL transcript levels by real-time quantitative polymerase chain reaction (RQ-PCR), every 3 months during CML therapy. (*Baccarani* et al 2013)

Previous studies have reported that the comparability of molecular results between centres is often highly variable (*Müller* et al 2007) and therefore BCR-ABL transcript levels should be expressed according to the International Scale (BCR-ABL IS %) to guarantee comparability of results among different laboratories. Significant advances towards standardization of RQ-PCR for BCR-ABL have been made with the development of procedures for testing laboratories to derive conversion factors to the International Scale (*Müller et al* 2009). The establishment of a conversion factor is time-consuming, complex, expensive and only open to a limited number of laboratories at any given time. The conversion factors need to be validated over a period of time and it is essential that each testing laboratory will establish appropriate internal quality control procedures to confirm that their assay is stable over time. Furthermore it is unclear how frequently any individual CF will need to be revalidated (*Cross* et al 2009). For these reasons the standardization to the International Scale (IS) remains inconsistent. As an alternative mean for laboratories to access the IS, World Health Organization (WHO) certified reference material with BCR-ABL values assigned on the international scale was developed (*White* et al 2010).

Recently, considerable interest has focused on the achievement of undetectable levels of BCR-ABL (Complete Molecular Response, CMR) because it is now known that a proportion of patients can stop tyrosine kinase treatment and maintain remission after a prolonged period of CMR (*Mahon* et al 2010; *Ross* et al 2010). An improvement in the sensitivity of BCR-ABL detection could aid in selecting candidates who can safely cease treatment without relapse (*Branford* et al 2011).

CMR, however, is difficult to define. To consider variabilities in sensitivities, molecular response categories MR4 and MR4.5 have been defined and include both samples with BCR-ABL positive and negative results with defined sensitivities (*Cross* et al 2012).

1.1 Purpose and rationale

The purpose of this lab registry is to collect data on the standardized assessmant of molecular response in the context of real life clinical practice. Molecular data monitored with a sensitive and standardized assay do not exist so far outside of clinical trial setting.

Eligible patients have been treated with one or more TKIs for a minimum of 24 months at registry entry. According to current recommendations (*Baccarani* et al 2013), achieving a major molecular response (MMR) following 12 months treatment is considered an optimal response to therapy. Patients treated for at least 24 months are therefore most likely to have deeper molecular responses that require detection using a sensitive assay.

Based on the importance of the molecular response in the context of CML management and prognosis, accurate and reproducible molecular analyses are essential for physicians to make clinical decisions and refine treatment options. For example, the loss of a previously achieved MMR is indicative of a potential need for treatment change, as it may be related to a re-activation of the disease or the emergence of treatment-resistant mutations.

Currently, although many initiatives have been undertaken to standardize the assessment of BCR-ABL testing, standardization to the IS remains inconsistent. Furthermore, molecular monitoring is still underutilized despite improvements in education and initiatives to support the adoption of IS.

In this registry, BCR-ABL transcript levels after at least two years of TKI therapy will be evaluated for the occurrence of deeper molecular response rates and its impact on the management of patients in a clinical practice setting outside of clinical trials. Improving the monitoring of deeper and sustained molecular responses is critical for the optimal management of BCR-ABL+ CML patients. Standardized molecular monitoring is a prerequisite for any attempt of treatment discontinuation.

In summary, molecular data monitored with a sensitive and standardized assay collected in a systematic fashion do not exist so far outside of clinical trial setting.

Improving the monitoring of deeper and sustained molecular responses is critical for the optimal management of BCR-ABL+ CML patients and will assist to define the parameters for treatment discontinuation

The purpose of this lab survey is to collect data on the feasibility of the assessment of deep molecular responses in the context of real life clinical practice.

2 Objectives

Objectives and related endpoints are listed in Table 1.

Table 1 Objectives and related endpoints

Objective	Endpoint	Analysis
Primary		
To determine the proportion of patients in $MR^{4.5}$	Qualitative and quantitative BCR-ABL transcripts. Assessment of sensitivity (ABL and/or GUS transcript levels) Comparison with expected data (local lab)	Summary statistics of the primary variable will be displayed by duration and line of TKI therapy, drug and prognostic score. A 95% confidence interval (CI) of the efficacy variables will be presented.
Secondary		
Demonstrate feasibility and accuracy of deep molecular response analysis in 22 countries in Europe.	Analysis of the sensitivity of each lab. (proportion of results with 4.5 log sensitivity).	Calculation of the population with access to the EUTOS standardized molecular response analysis.
Compare expected response level (local lab) with level reported by the EUTOS lab.	Pairwise comparison of response levels reported for individual samples.	Identify potential differences of response assessment for treatment free remission studies.

3 Laboratory registry design

This is a prospective, multi-center, multinational lab registry collecting data on chronic phase BCR-ABL+ CML patients independent of the actual treatment. Data will be collected between 2014 and 2016.

This lab registry is not designed to test a formal hypothesis and therefore no formal sample size is needed. Since this is a laboratory PCR registry, each patient's visit schedule, assessments (including molecular monitoring frequency) and treatment (dose, duration and regimen) are at the discretion of the treating physician. No medication will be provided in this lab registry.

3.1 Sample and data collection process flow

A form with basic parameters describing the current status of the patient in anonymized fashion will be completed by the referring physician and should accompany the sample (see annex 1).

The laboratory performing the analysis of the sample will be responsible for inserting the accompanying form together with the results of the analysis (see annex 2) to a eCRF that will be then submitted to the database (see process flow diagram, figure 1)

3.1.1 Patients samples

Samples consist of 20 ml EDTA blood and can be sent once or repeatedly from the same individual with at least 10 weeks interval between samples over a period of 2 years. Molecular monitoring will be performed according to the routine protocols currently in use in each centre. No samples will be taken specifically for this study.

During the follow-up period, patients' BCR-ABL levels will be collected in order to evaluate the occurrence and clinical impact of deeper molecular response rates. After assessment of the response, samples will be stored in the participating lab and could be used later to test new options for response monitoring.

3.2 Sample Size

It is anticipated that a total of up to 5,000 samples from European countries will be enrolled within approximately 2 years and up to 44 laboratories will participate.

At time of registration,

- patients informed consent will be obtained by the treating physician and information will be collected on:
  - patient demographics (anonymized),
  - date of CML diagnosis,
  - type of BCR-ABL transcript,
  - initial prognostic score (EUTOS and Sokal scores)
  - past and current treatments with start and stop,
  - current molecular response according to the local laboratory.

Although there are no mandated visits in this lab registry, the ELN recommends molecular monitoring samples every 3 to 6 months (*Baccarani et al*, 2013).

Additional data regarding the ongoing treatment regimen will also be entered whenever a PCR blood sample is obtained.

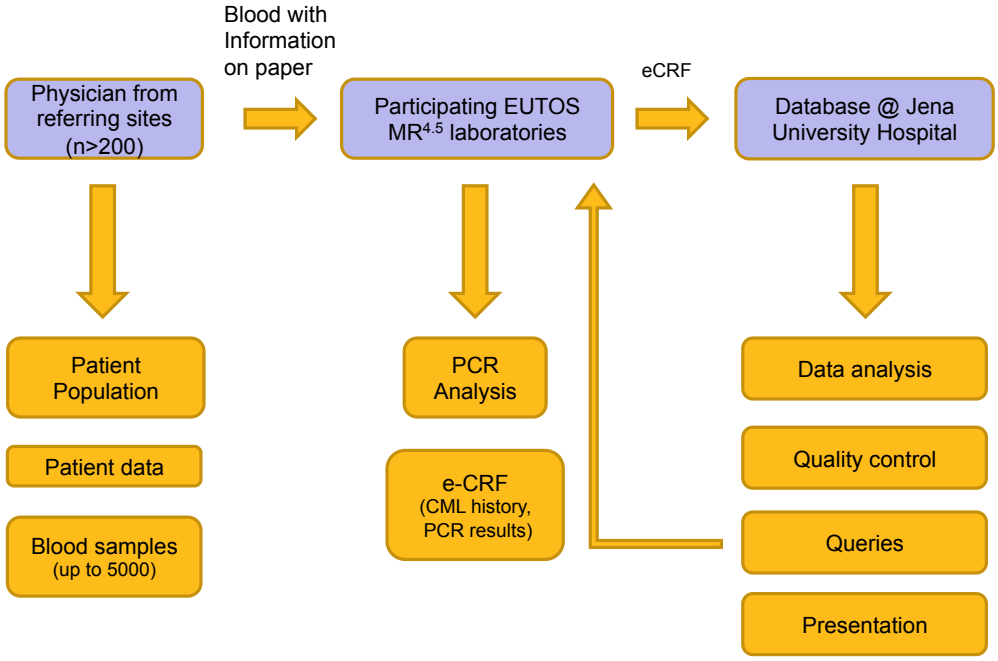

Figure 1: Flow diagram of the PCR survey

4 Population and setting

Adult chronic phase BCR-ABL+ CML patients under treatment with TKIs at any prescribed dose will be enrolled. **Patients must have received TKI treatment for a minimum of 24 months at registry entry**.

The following subpopulations of patients based on the following characteristics at registry entry will be considered:

- TKI treatments
- Duration of TKI treatment at registry entry
- EUTOS and Sokal scores
- Treatment and depth of molecular response
    - Patients in MMR
    - Patients in MR^4
    - Patients in $MR^{4.5}$
    - Patients in $>MR^{4.5}$

4.1 Inclusion criteria

1. Male or female patients ≥ 18 years of age
2. Patient with diagnosis of BCR-ABL positive CML in chronic phase (CP)
3. Patient is receiving treatment with any TKI at registry entry, as per routine clinical practice.
4. Patient has been treated with one or more TKIs for a minimum of 24 months at registry entry.
5. Written informed consent.

4.2 Data collection/measurement

This is a non-interventional lab registry and does not impose a therapy protocol, diagnostic/therapeutic procedure, or a visit schedule. Patients will be treated according to routine medical practice in terms of visit frequency and types of assessments performed and only these data will be collected as part of the lab registry.

5 Patient demographics/characteristics

5.1 Informed Consent

Written informed consent must be obtained before enrolling the patient.

5.2 Patient demographics

Patient demographics and baseline characteristics collected will include the following:

Months and year of birth, gender, ethnicity.

5.3 Medical and Disease History

Disease history includes risk scoring at diagnosis (EUTOS, Sokal), treatment and response to treatment history.

Type of BCR-ABL transcript.

Current response according to the local lab.

5.4 Medication(s) of interest

The medication(s) of interest are TKI therapy of any line.

Current and previous treatments incl. dose/regimen, resistance yes/no, and treatment duration will be collected.

5.5 Outcome of interest

5.5.1 Molecular Response

Molecular response will be assessed by Real-Time quantitative PCR (RQ-PCR). The assay used is standardized to the IS and standardized for sensitivity.

5.5.2 Safety related data

Being this lab registry non-interventional, no specific assessment will be mandated to assess safety.

6 Participating labs

Labs listed in Table 2 will participate in the survey after having passed the EUTOS certification for deep molecular response assessment.

The labs labeled by an asterisk (*) have already participated in the ENEST1st study and can start the survey immediately.

Table 2: Initial participating labs (n=33) in 22 European countries (subject to ongoing sensitivity assessment; additional laboratories are expected to join in 2014/15)

*Vienna	Austria	Dublin	Ireland
Leuven	Belgium	*Turin	Italy
Tuzla	Bosnia-Herzogovina	*Bologna	Italy
Sofia	Bulgaria	*Naples	Italy
Zagreb	Croatia	Vilnius	Lithuania
*Prague	Czech Republic	Oslo	Norway
Brno	Czech Republic	*Krakow	Poland
Veije	Denmark	*Lisbon	Portugal
*Bordeaux	France	*Bucharest	Romania
Paris	France	Ljubljana	Slovenia
Lille	France	*Barcelona	Spain
*Leipzig	Germany	Madrid	Spain
*Jena	Germany	Bern	Switzerland
*Mannheim	Germany	*London	UK
Kiel	Germany	Birmingham	UK
Thessaloniki	Greece	Salisbury	UK
Budapest	Hungary		

7 Data analysis

It is planned that the data from all participating centers will be combined, for the primary endpoint so that an adequate number of patients will be available for data analyses.

Additional analyses will be performed per country and per lab to demonstrate the concordance of expected and actual results and the sensitivity achieved in each participating lab.

7.1 Patient demographics / other baseline characteristics

Demographic and other data at the lab registry entry will be summarized descriptively. Categorical data will be presented as frequencies and percentages. For continuous data, mean, standard deviation, median, 25th and 75th percentiles, minimum, and maximum will be presented as appropriate.

7.2 Drug Exposure

Concomitant non-CML medications will not be collected during the lab registry. The proportions of patients having received various TKIs will be tabulated.

7.3 Analysis of the primary objective variable

Percentage of patients achieving deep molecular response (MR4.5) at various time points and various TKI after start of therapy.

7.4 Statistical hypothesis, and method of analysis

Primary analysis:

Summary statistics of the primary variable will be displayed by duration and line of TKI therapy, drug and prognostic score. A 95% confidence interval (CI) of the efficacy variables will be presented.

Secondary analyses:

Analysis of the results obtained in the participating lab vs. previous results achieved locally.

Achieved coverage of standardized PCR analysis in Europe (population covered vs. total population).

Achieved sensitivity of the PCR assay (overall and per individual country and individual lab).

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Annex 1
Data to be sent FROM THE REFERRING PHYSICIAN to the laboratory

(This information should accompany the blood sample)

Referring Physician Information

- Name and site of physician

Patient Information

- Patients initials, month and year of birth
- Date of informed consent
- Sex
- Ethnicity
- Type of BCR-ABL transcript at diagnosis
- EUTOS and Sokal scores at diagnosis

CML diagnosis

- Month and year of CML diagnosis
- Start and stop of first line TKI, dose (resistance yes/no)
- Start and stop of second line TKI, dose (resistance yes/no)
- Start and stop of third line TKI, dose (resistance yes/no)
- Additional CML specific therapies

CML treatment

- Current treatment (treatment and dose)
- Most recent molecular result from the local lab
- Judgement of molecular response by the physician:

  MMR
  MR4
  MR4.5
  MR5

Blood Sample Collection

- Date of sample

Annex 2
Data to be collected FROM the MR4.5 EUTOS Laboratory

The participating laboratory will be responsible for the input of
a) data from Referring Physicians form accompanying the blood sample and
b) generated data below into the EUTOS Molecular Monitoring survey eCRF

- Date of arrival of the sample in the lab
- Blood volume
- Volume cDNA used for analysis
- BCR-ABL transcripts
- Lowest positive standard
- ABL transcripts
- alternatively or in addition: GUS transcripts

- Ratio BCR-ABL/ABL
- alternatively or in addition: Ratio BCR-ABL/GUS

- BCR-ABL (IS) % derived from BCR-ABL/ABL
- alternatively or in addition: BCR-ABL (IS) % derived from BCR-ABL/GUS
- Nested PCR result
- PCR repeats used for pooled analysis

- Interpretation:
  - No MMR
  - MMR
  - MR4
  - MR4.5
  - MR5