Next Generation Sequencing (NGS) and Droplet Digital PCR mutation testing project for optimal monitoring of your CML and Ph+ ALL patients

OVERALL OBJECTIVE

Early determination of the occurence of mutations in the kinase domain of the BCR-ABL gene in CML who are in the "warning" or "failure" zone during current TKI therapy (ELN 2013 guidelines) and Ph+ ALL patients as from diagnosis.

RATIONALE

Next Generation Sequencing (NGS) and Droplet Digital PCR (ddPCR) are sensitive detection techniques which can contribute to early detection of treatment failure and therefore guide optimal treatment.

NGS will be used in CML and ALL patients to screen for mutations in the bcr-abl kinase domain. Droplet Digital PCR is an even more sensitive technique and will be used in both CML and Ph+ ALL to screen for 3 the most common mutations, representing 75% of all mutations in the bcr-abl kinase domain: T315I, E255K and Y253H. (Soverini, 2014)

INCLUSION CRITERIA

CML patients with failure or warning to their current TKI therapy - all lines of therapy (ELN guidelines 2013)
Ph+ ALL patients from diagnosis and/or at molecular relapse - all lines of therapy. Monitoring when clinically appropriate.

SAMPLE PROCEDURE

- preferred: 10 to 20 µl cDNA (at room temperature)
- or: 1 µg RNA (or at least 15 µl if < 1 µg) RNA (at – 20°C)
- or: 3 ml of EDTA (at room temperature)

SHIP TO (PREFERABLY WITHIN 48 HOURS)

IPG - Dr. Pascal Vannuffel / Dr. Céline De Rop
NGS project
Avenue George Lemaître 25
6041 Gosselies (Belgium)

PLEASE COMPLETE THIS SECTION WITH NECESSARY DATA

Patient ID: ..... Sample ID: ..... Sample date: .....

Sample source: ☐ peripheral blood ☐ bone marrow

Diagnosis: ☐ CP-CML ☐ BP-CML ☐ AP-CML ☐ Ph+ ALL

% BCR-ABL$_{IS}$ transcript level: .....

If Ph+ ALL, specify isoform of BCR-ABL: ☐ P210 ☐ P190

Prior TKI treatment(s): .....

Confirm that there is NO suspected lack of adherence ☐ Yes

Doctor: .....

E-mail: .....

Institution: .....

City: .....

Additional information if possibly relevant: .....

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IMPORTANT

- If questions, do not hesitate to call or e-mail one of the investigators
- We will also be pleased to explain the project during a Hemato Staff meeting in your hospital
- The samples will be analyzed and results will be reported to you within 2 weeks
- The analysis of samples via this Project does not substitute for the analysis of samples performed in your routine clinical practice
- The costs of sample analysis are covered by the project
- This project is supported by an unrestricted educational grant from Incyte Biosciences Benelux

Dr. Pascal Vannuffel, Molecular Biologist
Dr. Céline De Rop, Clinical Biologist
Principal Investigators

IPG - Gosselies - Tel: 071/44.71.36
Pascal.vannuffel@ipg.be - Celine.derop@ipg.be

Dr. Friedel Nollet, Molecular Biologist
Dr. Barbara Cauwelier, Clinical Biologist
Dr. Helena Devos, Clinical Biologist
Collaborative Lab

AZ St-Jan Brugge-Oostende AV, campus Brugge - Tel: 050/45.99.00
friedel.nollet@azsintjan.be - barbara.cauwelier@azsintjan.be
helena.devos@azsintjan.be

BCR-ABL inhibitor activity against BCR-ABL single mutants[1]

BCR-ABL MUTANT	PONATINIB	IMATINIB	DASATINIB	BOSUTINIB
Native	3	201	2	71
M244V	3	287	2	147
L248R	8	10000	6	874
L248V	4	586	5	182
G250E	5	1087	4	85
Y253H	**5**	**4908**	**3**	**40**
E255K	**6**	**2487**	**9**	**181**
E255V	16	8322	11	214
V299L	4	295	16	1228
T315A	4	476	59	122
T315I	**6**	**9773**	**10000**	**4228**
F317C	3	324	45	165
F317I	7	266	40	232
F317L	4	675	10	82
F317V	10	1023	104	1280
M351T	4	404	2	97
E355A	7	441	3	74
F359C	6	728	2	70
F359I	11	324	3	76
F359V	4	346	2	59
H396R	4	395	2	60
E459K	5	612	4	127

Criteria used to classify drug potency

Effective C_{ave} at rec. dose	28*	444	11	159
IC_{50} <75% of C_{ave}	<21	<333	<8	<119
IC_{50} 75-150% of C_{ave}	21-42	333-666	8-17	119-239
IC_{50} 150-300% of C_{ave}	43-84	667-1332	18-33	240-477
IC_{50} >300% of C_{ave}	>85	>1332	>33	>477

**Ponatinib 45 mg dose. Data shown as mean IC50 (nM) from 3 eparate experiments*

1. Gozgit, et al. Blood. 2013;122:abstr 3992.

ELN Guidelines 2013 (considered as common practice)

CML patients with failure or warning to their current TKI therapy - all lines of therapy

	WARNING BCR-ABL levels of	FAILURE BCR-ABL levels of
1st line TKI	>10% @ 3 months	>10% @ 6 months
	>1% @ 6 months	>1% @ 12 months
	>0.1% @ 12 months**	there after loss of MMR (2 consecutive tests), or increase of BCR-ABL levels of at least 2 fold
2nd line TKI	>10% @ 3 months	>10% @ 6 months
	>1% @ 12 months	there after loss of MMR (2 consecutive tests), or increase of BCR-ABL levels of at least 2 fold

*** To be able to perform mutation analysis, BCR-ABL level should be above 0,1%. All BCR-ABL levels expressed as IS (International Scale)*

**** No confirmed or no suspected lack of adherence (based on feedback from treating hematologist)*