Identification of IDH 1&2 mutations and SNP rs11554137 in AML using HRM

Laboratory of Molecular Haematology
Center of Human Genetics
CHU Liege- ULg

Dodémont Magali

Molecular diagnostic- Antwerpen- 13/12/11

PLAN

- **INTRODUCTION**
  - IDH and AML (Biochemistry - Oncogenic hypotheses)
  - SNP rs11554137
  - AIM of our study
- **PATIENTS and METHOD**
  - Study Group
  - Detection by High Resolution Melting Analysis (HRM)
  - Confirmation by Sequencing
- **RESULTS**
- **CONCLUSION**

INTRODUCTION

- **IDH1&2**

Function **: converts isocitrate to α-ketoglutarate**
(NADP+-dependent manner)

Subcellular localisation **: cytoplasm and peroxisom (IDH1) and mitochondria (IDH2).**

Mutation **: loss of normal enzymatic function**
⟶ neomorphic enzymatic activity
⟶ accumulation of 2-hydroxyglutarate (2HG)

INTRODUCTION

- **Accumulation of 2HG: consequences**

- Activation of HIF1a.
HIF1a activates the transcription of genes involved in angiogenesis, cell survival.

- Change in DNA and histone methylation patterns
Alteration of oncogenes or tumor suppressors expression

- Accumulation of reactive oxygen species
DNA damages

► ***Facilitate oncogenic pathways***

INTRODUCTION

- **IDH1&2 mutations in AML**

- Mutations IDH1^{R132}, IDH2^{R140} and IDH2^{R172}

- Heterozygote mutation and mutually exclusive

- Prevalence: about 17% of AML patients
  - **° IDH2 mutations appear more common than IDH1**
  - **° predominantly IDH2^{R140} rather than IDH2^{R172}**

- Most often in CN-AML (~24%)

INTRODUCTION

- Unfavorable impact outcome
⟶ especially FLT3-ITD-/NMP1+ patients.

- IDH2^{R172}: less likely to have other mutations.

- AML with or without maturation (FAB M1 or M2) and with dysplastic features.

- Relatively stable disease marker (allow MRD follow-up).

INTRODUCTION

- **SNP rs11554137** (Wagner et al.)

  - Located in codon 105 in the same exon as R132 mutation (exon4)

  - 12% of CN-AML ⟶ a frequency similar to normal subjects

  - Worse survival

  ► Potential new prognostic marker in CN-AML

INTRODUCTION

- **AIM of our study**

Develop a screening strategy for fast and sensitive detection of IDH1&2 mutation **and** SNP based on HRM followed by sequencing confirmation.

PATIENTS

- **Study group**

  - 64 *de novo* adult AML patients

  - 10 patients without AML

  ⟶ DNA was extracted from anticoagulated bone marrow or peripheral blood samples.

METHOD

- **New HRM assay adapted from *Noordermeer* publication** (bjh 2010)

  2 steps:

  - Screening for mutation and SNP using a **melting curve** based LightCycler® 480 **assay** (Roche Diagnostics).

  - Confirmation by **direct sequencing** to determine exact nucleotide change.

METHOD

- **Disadvantages of the first method**

  SNP rs11554137 is located in IDH1 primer.

  ► Risk of mismatch yielding lower PCR efficacy

  ► Inability to detect the SNP

  ↳ Design of new primers with Primer3 for HRM analysis

METHOD

- **First IDH1 HRM primers-> *Noordermeer's* primers**

Name	Primer 1	Matching/ Mismatching bases	Primer 2	Matching/ Mismatching bases	Chr.	Amplified Region	Result	SNPs
IDH1R132F_and_IDH1R132R	GGCACGGTCTTCAGAGAAGC	20/0	CAACATGACTTACTTGATCCCCATAA	26/0	2	115 bp 209113080..209113194	SNPs Found	rs11554137

A SNP in the area of hybridization of primers ⟶ PCR problem

METHOD

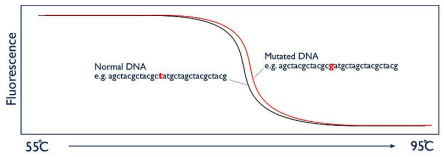
- **New IDH1 HRM Primers**
  - Check the specificty of the primers using databases:
    in silico PCR
    Ensembl
    Primer-BLAST
  - Check the absence of polymorphism using the *SNP Check* program

Name	Primer 1	Matching/ Mismatching bases	Primer 2	Matching/ Mismatching bases	Chr.	Amplified Region	Result	SNPs
IDH1_SNP_M13F_and IHH1_SNP_M13R	accaaatggcaccatacga	19/0	tcacattattgccaacatgacctt	23/0	2	158 bp 209113068.. 209113225	No SNPs Found	

METHOD

- **HRM technique**

Principle **:** melting curves shape depends on the DNA sequence. Samples with a SNP or a mutation have a different nucleotide sequence than wild-type sample, resulting in specific profiles of the melting curves.



METHOD

- **Controls**
  - **Positive controls :**
    IDH1: rs11554137 SNP is illustrated by HL60 cell line's profile IDH1^{R132} mutation is illustrated by a mutated sample (confirmed by sequencing)
    IDH2: IDH2^{R140} and IDH2^{R172} mutation is illustrated by a mutated sample
  - **Negative controls :**
    IDH1: wild-type patients (confirmed by sequencing)
    IDH2: HL60 cell line

METHOD

- **Sequencing**
  - ***IDH1*****:** M13 primers ⟶ sequence analysis is carried out on PCR products generated during the PCR step of HRM analysis.
  - ***IDH2*****:** dedicated PCR before sequencing.

RESULTS

- **HRM profiles**

IDH1

Four groups after HRM analysis :

1. Rs11554137 SNP
2. IDH1^{R132} mutation
3. Both IDH1^{R132} and rs11554137 SNP
4. Wild-type samples

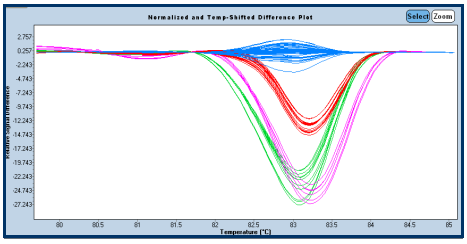
IDH2

Two clusters :

1. Mutated samples
2. Wild-type samples

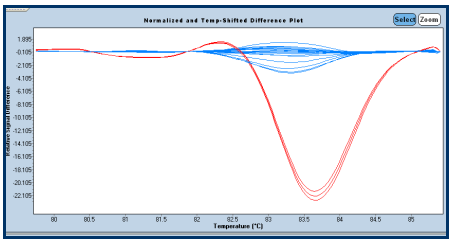
RESULTS : HRM

- ***IDH1***



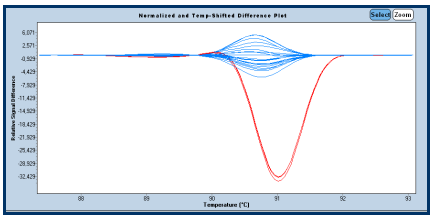
RESULTS

- ***IDH2^{R140}***



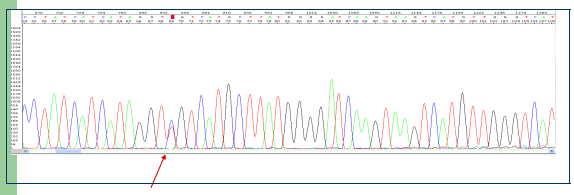
RESULTS

- ***IDH2^{R172}***



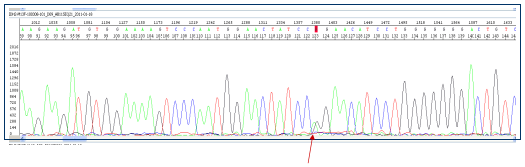
RESULTS : sequencage

- ***IDH1^{R132}***



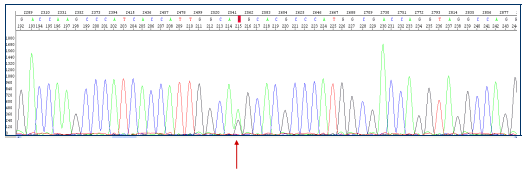
RESULTS : sequencage

- ***IDH2^{R140}***



RESULTS : sequencage

- ***IDH2^{R172}***



RESULTS

- **Results of our patients cohort analysis**

AML samples:

- 15 abnormal HRM profiles → IDH1 or IDH2 mutation ± SNP
- 7 abnormal HRM profiles → rs11554137 SNP alone

These results were confirmed by sequencing except for two patients with 'abnormal profiles' for which we were not able to confirm any mutation or SNP using direct sequencing

► Method sensibility: **96.9%**
► Limit of detection: **5%** of mutated allele for IDH1 and **3%** for IDH2.

RESULTS

	HRM	Sequencing
IDH1		
100212-0114	Abormal profil compatible with a mutation	IDH1R132G
080304-0004	Abormal profil compatible with a mutation	IDH1R132C
070816-0110	Abormal profil compatible with a mutation	IDH1R132C
070523-0011	Abormal profil compatible with a mutation +SNP	IDH1R132H SNP rs11554137
091123-0129	Abormal profil compatible with a mutation	IDH1R132C
101223-0102	Abormal profil compatible with a mutation	IDH1R132C
080617-0056	Abormal profil compatible with a mutation +SNP	IDH1R132H SNP rs11554137
110311-0022	Abormal profil compatible with a mutation	IDH1R132H
100603-0158	Abormal profil compatible with a mutation +SNP	IDH1R132C SNP rs11554137

RESULTS

	HRM	Sequencing
IDH1		
081113-0185	Abormal profil compatible with a SNP	SNP rs11554137
090807-0122	Abormal profil compatible with a SNP	SNP rs11554137
090901-0024	Abormal profil compatible with a SNP	SNP rs11554137
091028-0103	Abormal profil compatible with a SNP	SNP rs11554137
080527-0127	Abormal profil compatible with a SNP	SNP rs11554137
070118-0131	Abormal profil compatible with a SNP	SNP rs11554137
110110-0099	Abormal profil compatible with a SNP	SNP rs11554137

RESULTS

	HRM	Sequencing
IDH2 R140		
100308-0101	Abormal profil compatible with a mutation IDH2	IDH2R140Q
080429-0063	Abormal profil compatible with a mutation IDH2	IDH2R140Q
110106-0016	Abormal profil compatible with a mutation IDH2	IDH2R140Q
IDH2 R172		
070607-0060	Abormal profil compatible with a mutation IDH2	IDH2 R172K
110110-0099	Abormal profil compatible with a mutation IDH2	IDH2 R172K
110414-0091	Abormal profil compatible with a mutation IDH2	IDH2 R172K

RESULTS

- **Patients without leukemia**

No abnormal HRM profil → no mutation were found by sequencing

► **Method specificity of 100%**

RESULTS

	HRM	Sequencing
Patients without AML		
1	Wild-type profile	No mutation and SNP
2	Wild-type profile	No mutation and SNP
3	Wild-type profile	No mutation and SNP
4	Wild-type profile	No mutation and SNP
5	Wild-type profile	No mutation and SNP
6	Wild-type profile	No mutation and SNP
7	Wild-type profile	No mutation and SNP
8	Wild-type profile	No mutation and SNP
9	Wild-type profile	No mutation and SNP
10	Wild-type profile	No mutation and SNP

RESULTS

- **Comparison on the two methods**

	First method *Noordermeer & al.*	New method
Specificity	100%	100%
Sensibility	96.9%	95.3%
Detection of rs11554137 SNP	NO	YES

CONCLUSION

- **Advantages of new method**

- Detection of both mutation and SNP
- Fast and sensitive to screen patients
- Only positive HRM profiles need to be sequenced
- Rs 11554137 SNP is associated with a characteristic profile ⟶ doesn't require to be sequenced

► **Saving time and reducing cost**

THANKS

To the team of Doctor Lambert
(Laboratory of Molecular Biology
Departement of Human Genetic)
Sabine Franke
Stéphanie Gofflot
Miguel Lopez
Hélène Charlier
Monica Di Cesare
Sophie Castermans
Fanny Orban
Audrey Sinon

Laboratory of molecular haemato-oncology
Dr Frederic Lambert
Center of human genetic
Tour de Pathologie +3, B23
4000 LIEGE
BELGIUM

flambert@chu.ulg.ac.be