ALLTogether

Consortium (ATC) protocol

Cytogenetics STRATIFICATION

GFCH 13 octobre 2022

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GEN-GR

CYTO-GR

or GR-CNA group A

Good risk genetic abnormalities

Good risk cytogenetic abnormalities
- *ETV6-RUNX1*/t(12;21)(p13;q22)
- High Hyperdiploidy (51-65 chromosomes)

Good risk copy number alteration profiles
- No deletion of *IKZF1, CDKN2A/B, PAR1, BTG1, EBF1, PAX5, ETV6* or *RB1*
- Isolated deletions of *ETV6, PAX5 or BTG1*
- *ETV6* deletions with a single additional deletion of *BTG1, PAX5 or CDKN2A/B*

Inclusion :
1 à 18 ans

GEN-PR

CYTO-HR

or GR-CNA group B

Poor risk genetic abnormalities

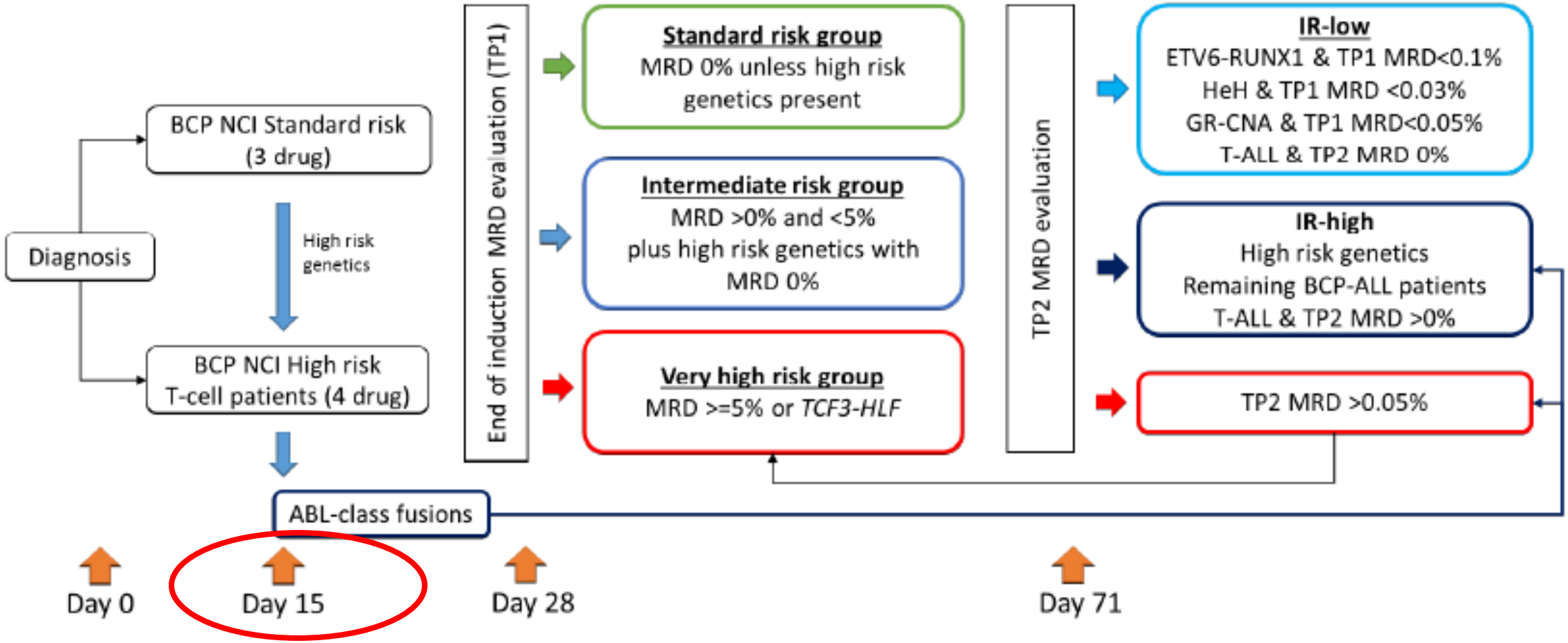
High risk cytogenetic subgroups
- t(9;22)(q34;q11)/*BCR-ABL1*
- *MLL*/11q23 translocation
- Near haploidy (<30 chromosomes)
- Low hypodiploidy / near triploidy (30-39 / 60-78 chromosomes)
- Intrachromosomal amplification of chromosome 21 (iAMP21)
- t(17;19)(q23;p13)/*TCF3-HLF*

Intermediate and poor risk copy number alteration profiles
- Any deletion of *IKZF1, PAR1, EBF1* or *RB1*
- All other copy number alteration profiles not mentioned above.

Patients are classified hierarchically with cytogenetic abnormalities taking precedence over copy number alteration profiles.

Definition of novel genetic risk groups for pediatric BCP-ALL.

Moorman AV et al, Blood, 124(9),1434-1444, 2014



MRD 0%: undetectable MRD by IG/TCR PCR;
High risk genetics: *KMT2A/MLL* fusions, near haploidy, low hypodiploidy, iAMP21.
ABL-class: *ABL1, ABL2, PDGFRB, CSF1R* fusions

GR-CNA UKALL profile:

- no deletion of *IKZF1, CDKN2A/B, PAR1, BTG1, EBF1, PAX5, ETV6, RB1*;
- isolated deletions of *ETV6, PAX5, BTG1*;
- *ETV6* deletions with a single additional deletion of *BTG1, PAX5, CDKN2A/B.*

Guidelines for screening methodology in the ALLTogether protocol

Day	Genetic aberrations/karyotypic abnormality patterns	Methods					
		Array	FISH	RT-PCR	MLPA	G/R-Banding	NGS
71	High hyperdiploidy (HeH; 51-67 chromosomes)	**X**				X	X
15	Low hypodiploidy (HoL; 30-39 chromosomes)	**X**				X	X
15	Near haploidy (NH; 25-29 chromosomes)	**X**				X	X
15	iAMP21	**X**	X^m				X
15	*KMT2A* rearrangements		**X**			X	**X**
15	*BCR-ABL1*		**X**	**X**		X	**X**
15	ABL-class fusions (*ABL1, ABL2, CSF1R, PDGFRB*)		**X**				**X**
15	*TCF3-HLF*		**X**	**X**		X	**X**
71	*ETV6-RUNX1, TCF3-PBX1**		**X**	**X**		X	**X**
29/ 71	Copy Number Alterations (Down: *IKZF1* day 29, rest day 71) *BTG1, CDKN2A/B, EBF1, ETV6, IKZF1,* PAR1, *PAX5, RB1*	**X**			**X**		X

assumed to be mutually exclusive

* Distinct subgroup, mutually exclusive with other subtypes, with dismal prognosis at relapse

CARYO/FISH pour stratification: **J15**

LAL-B

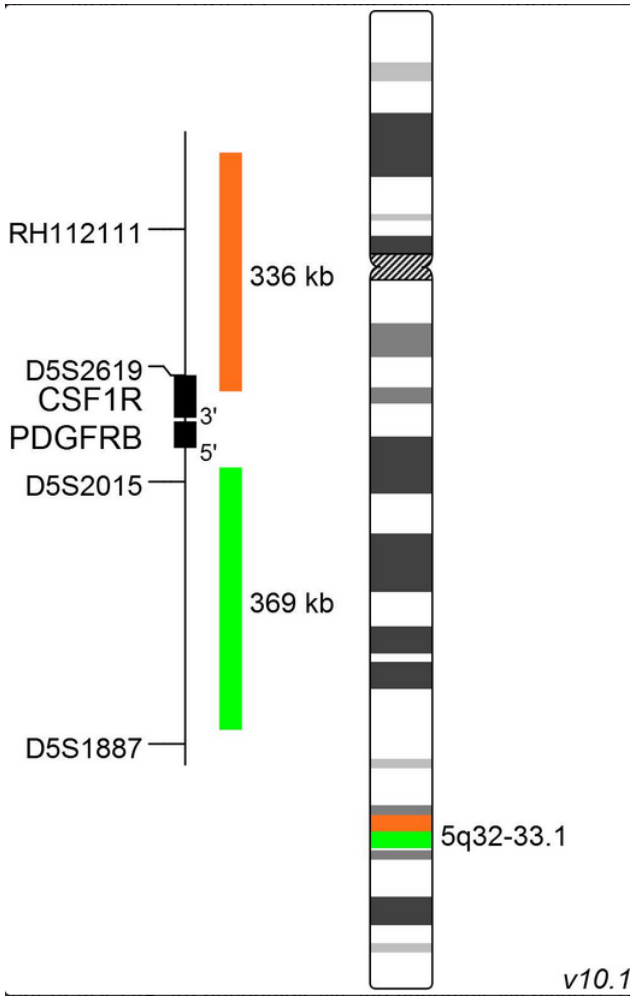
BCR-ABL1 si pos changement de protocole
TEL(ETV6)-AML1(RUNX1) *(iAMP incluse)*
HeH
Low/near hypo
KMT2A
TCF3(E2a) HLF/PBX1

ABL CLASS FUSION : PDGFRB/CSF1R/ABL1/ABL2 Nouveauté ALLtogether ++++
(JAK 2 fait partie des B-others)

LAL-T
Protocole non modifié avec génétique
BCR-ABL1
KMT2A

Remarque: Anomalie clonale pris en compte théranostic > ou égal à 20% surtout valable pour la biologie moléculaire, mais aussi en Fish….

Point Sondes de FISH:



<u>Première salve :</u>
BCR-ABL1 1er permet check **ABL1**
TEL-AML1 (+Iamp21)
KMT2A(MLL)
E2A(TCF3) 5+/- en fonction centre BM

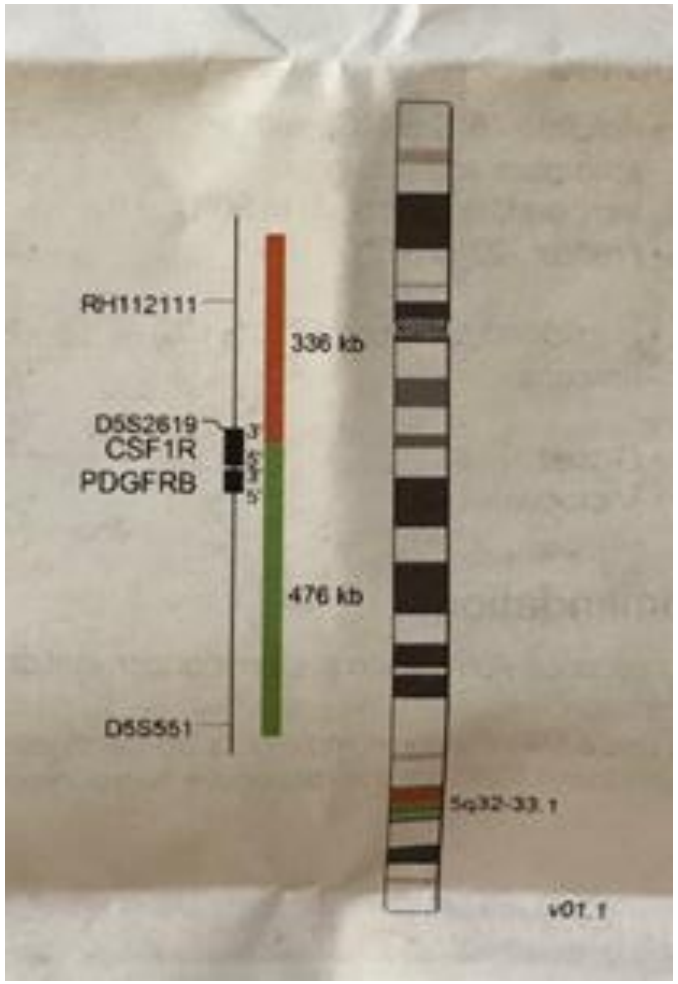
<u>2eme salve:</u>
3 signaux non trisomie -> **ABL1** break apart
ABL2 break apart
PDGFRB/CSF1R
Metasystem fait les 2 dans une: gènes collés
***CSFR1 break apart** isolée testée (cf infra) utilité?*

MAIS PDGFRB/CSF1R suffit à répondre « ABL like » à J15!

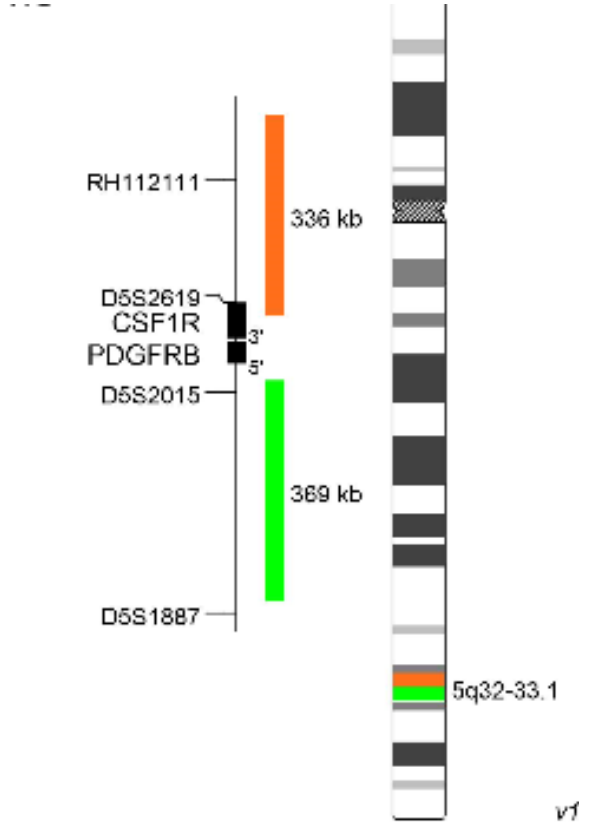
JAK2 dans les B other donc post J15

Conclusion:
Tous centres cytogénétiques ayant des LAL-B de l'enfant ALLtogether doit avoir **ABL2** break apart**,** et **PDGFRB/CSF1R** (déjà pour SMD/SMP) et **ABL1** break apart donc en théorie ce sera achat de 2 sondes à prévoir.

CSFR1 Break apart



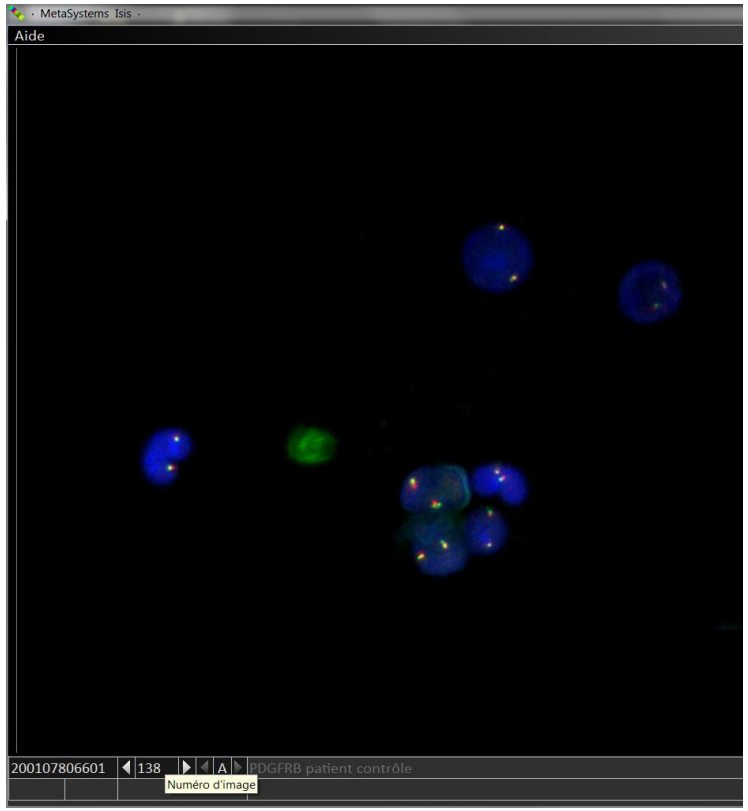
PDGFRB break apart MS



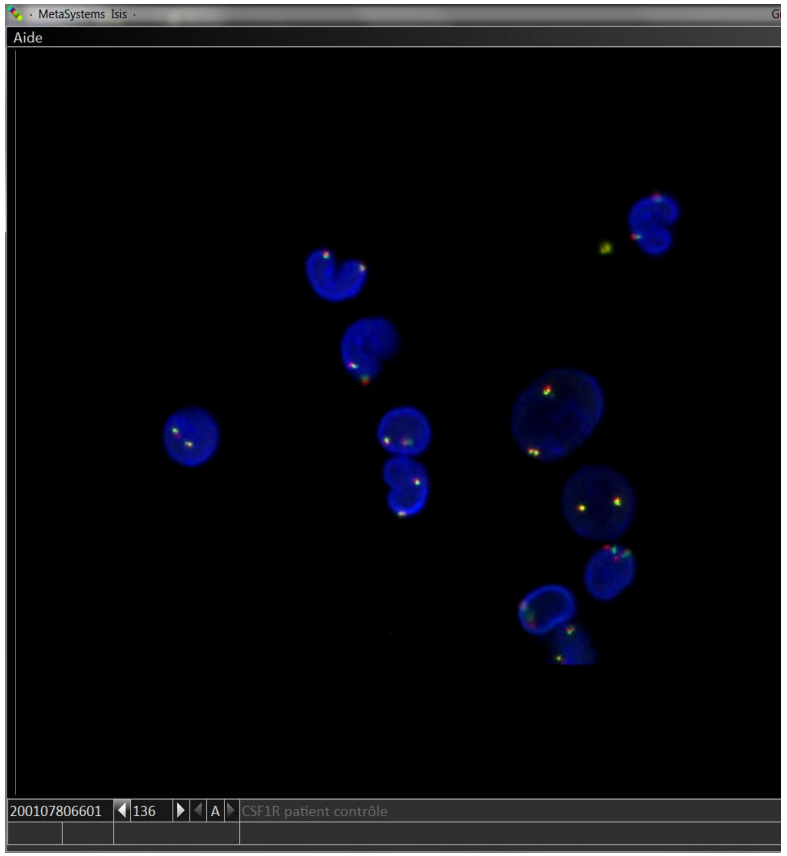
Wendy C

Patient caryotype normal (culot n°987 de 2020)

Absence de réarrangement de PDGFRB/ CSF1R et CSF1R.



sonde PDGFRB patient contrôle : normal

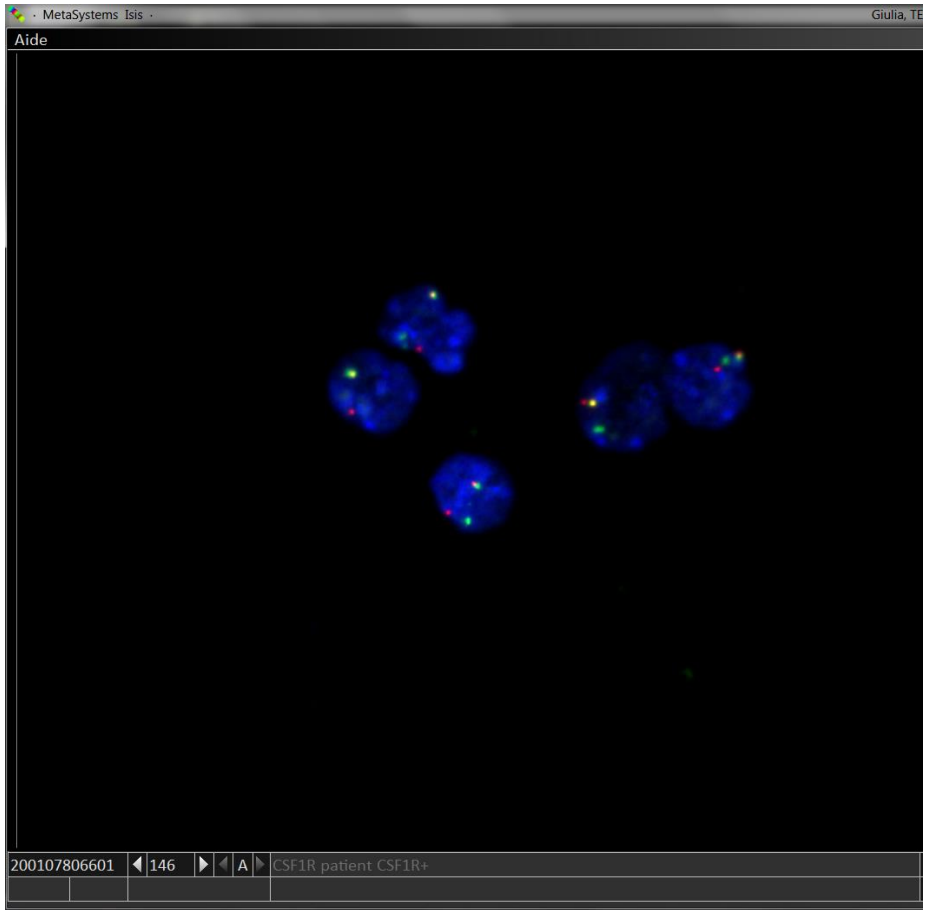


sonde CSFR1 patient contrôle : normal

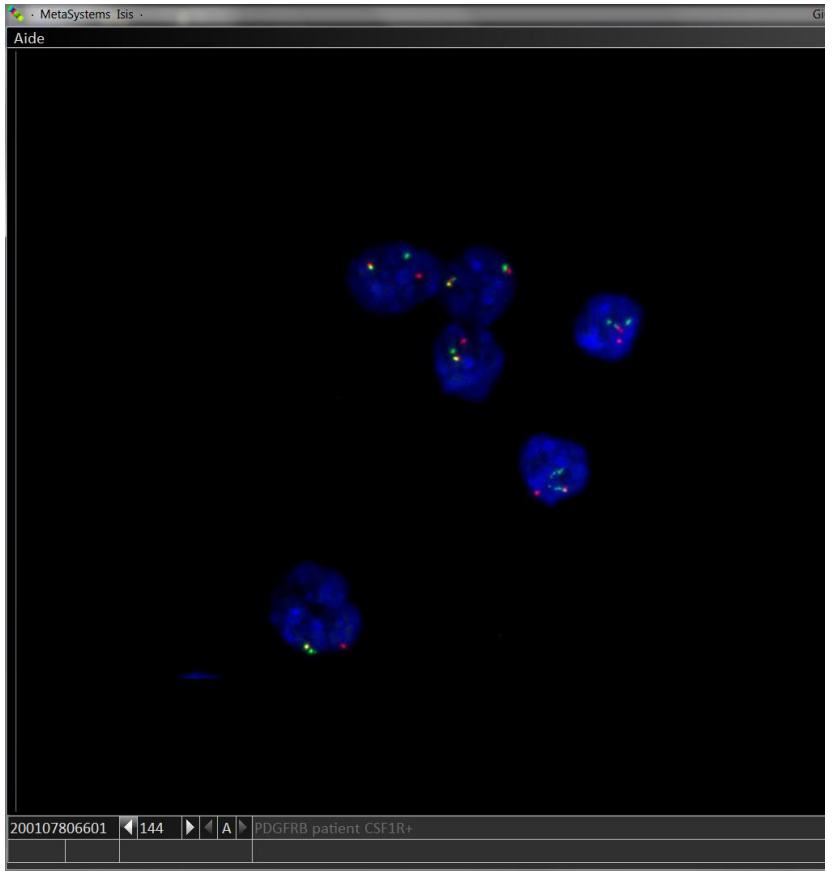
Hôpital Saint Louis Dr.Tueur, Dr.Chat, Dr.Cuccuini

Patient avec réarrangement EBF1-CSF1R en BM

Présence d'un réarrangement avec les 2 sondes PDGFRB/CSF1R et CSF1R BA.



Sonde CSFR1 remaniée sur Patient CSFR1-EB1 pos

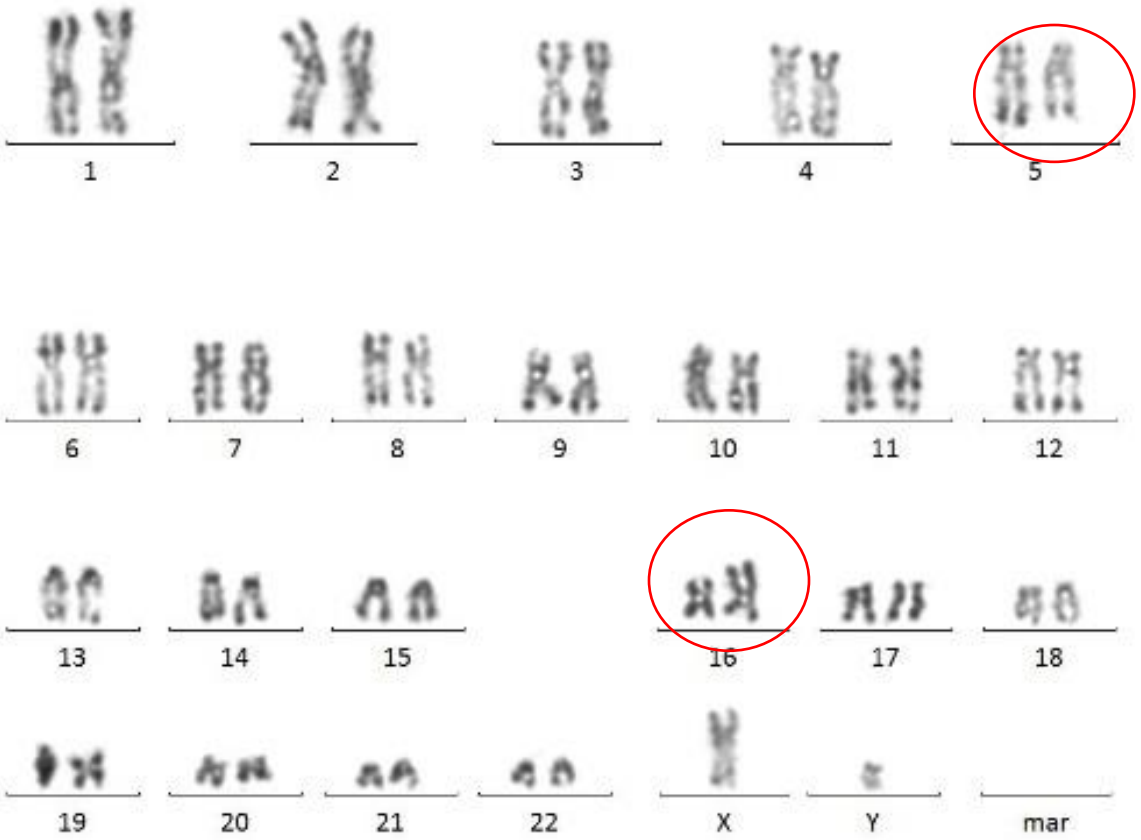


Sonde PDGFRB/CSF1R remaniée sur ce patient CSFR1-EBF1 pos

Hôpital Saint Louis Dr.Tueur, Dr.Chat, Dr.Cuccuini

Tester un patient pos PDGFRB transcrit CRAMP1-PDGFRB en BM

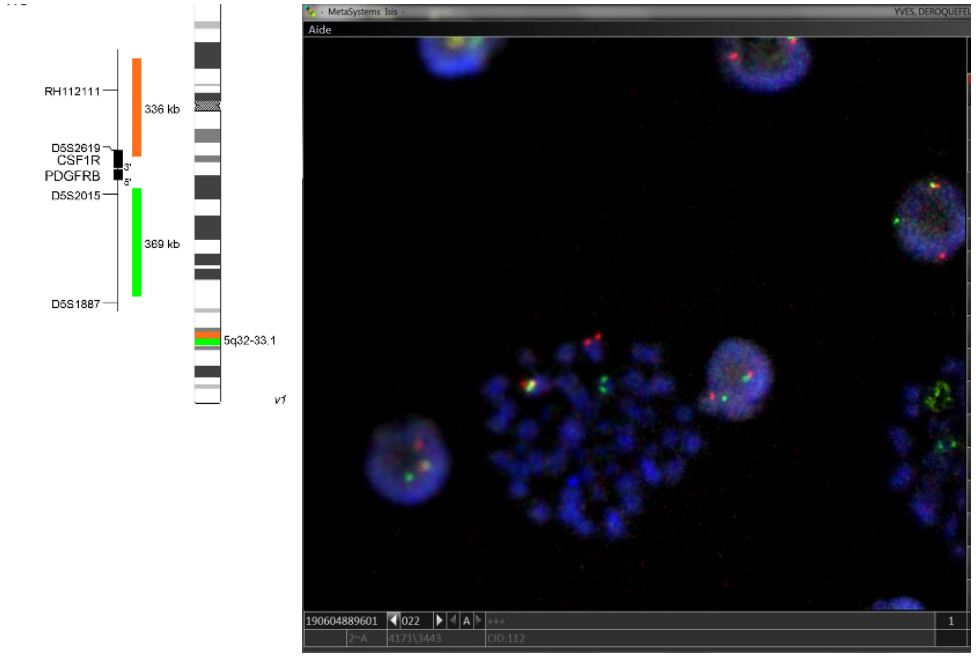
Caryotype : 46,XY,t(5;16)(q32;p13)



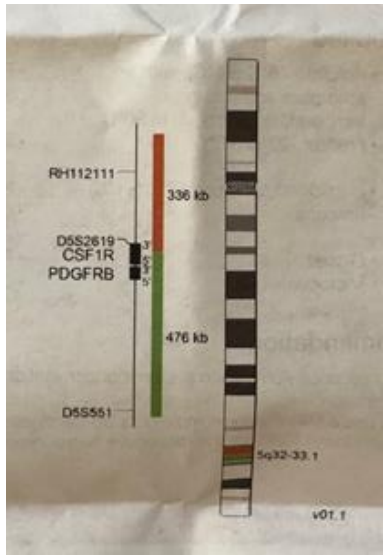
Hôpital Saint Louis Dr.Tueur, Dr.Chat, Dr.Cuccuini

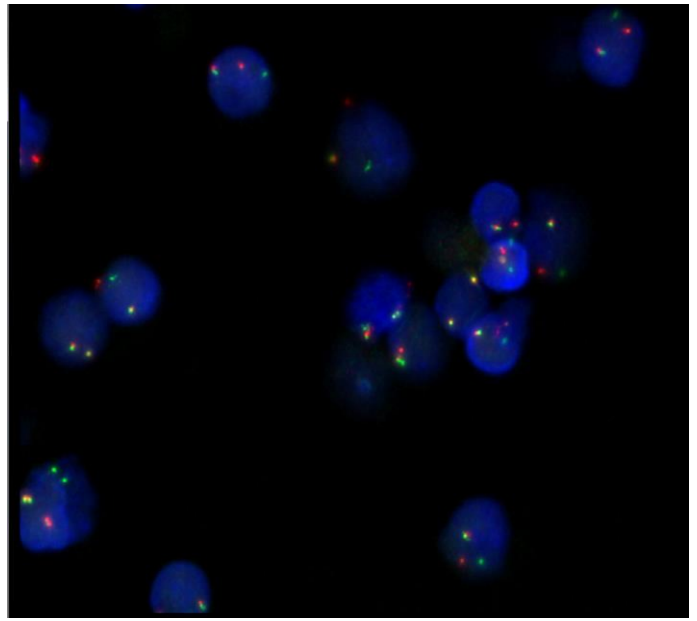
Tester un patient pos PDGFRB transcrit CRAMP1-PDGFRB en BM et caryo t(5;16)

FISH:



Sonde PDGFRB remaniée sur patient PDGFRB CRAMP1 pos avec t(5;16) au caryotype





Sonde CSFR1 remaniée sur patient PDGFRB-CRAMP1 MAIS différence dans remaniement indiquant que le dérvié der(5) garde la partie complète centromérique de CSFR1 rouge et petite partie verte (petite fusion), partenaire sur le 16 (partie verte télomérique qui couvre plus PDGFRB donc remaniement de PDGFRB par CSFR1!

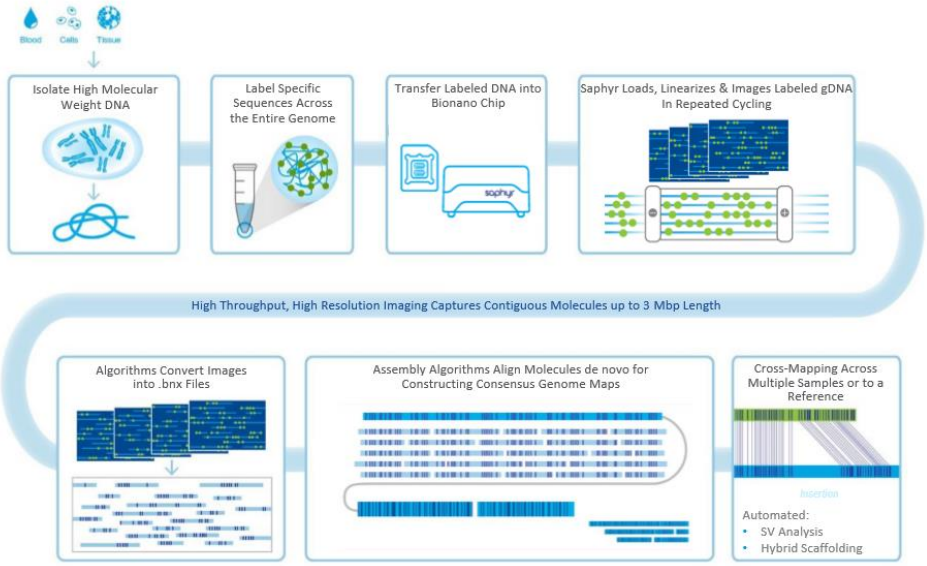
Conclusion : inutile dans protocole ALLtogether le RNAseq donnera le partenaire, on veut juste savoir si « ABL like » la réponse donnée par PDGFRB/CSF1R

Hôpital Saint Louis Dr.Tueur, Dr.Chat, Dr.Cuccuini

Optical genome mapping

- Required viable cells for high molecular weight DNA extraction *(>300 kb)*
- Fluorescent labelling of DNA molecules used for imaging and mapping to a human genome reference
- Allows genomic aberrations detection with a sensitivity of 5-10% (somatic pipeline) :
  - Structural variants : deletion, insertion, inversion, duplication, and translocations *(with a resolution of ~5-10kb)*
  - Copy number variations (CNVs)
  - Aneuploidies
- Previous studies showed a high concordance of OGM results compared to other cytogenetic methods in ALL
- ~10 center within GFCH* testing OGM in hematology

  (*GFCH Francophone Group of Cytogenetic in Hematology)
- Prospective frontline workflow under current evaluation :
  - Detection of translocations and copy number aberrations required for risk stratification
  - Results expected within 10 days
- If OGM validated : Switch to OGM for cytogenetic results for ALL cases

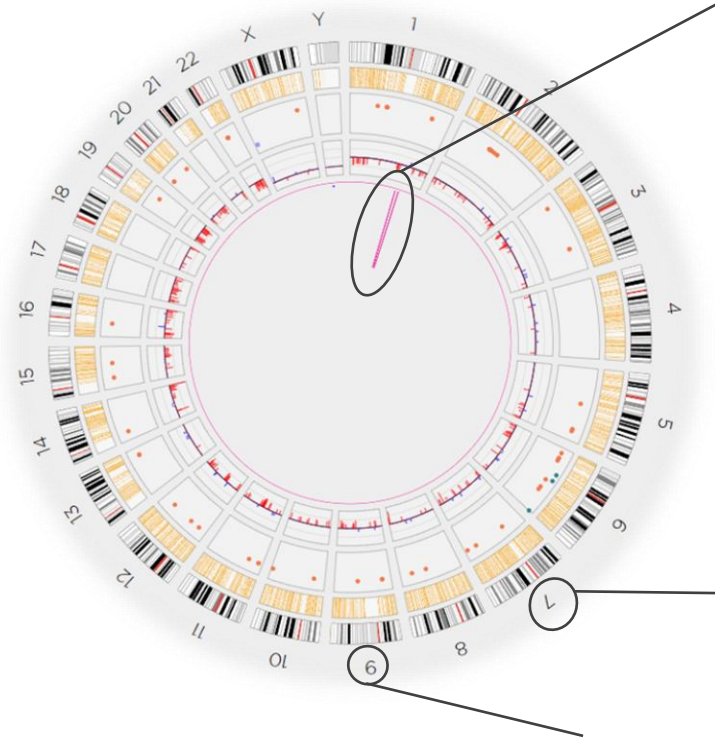


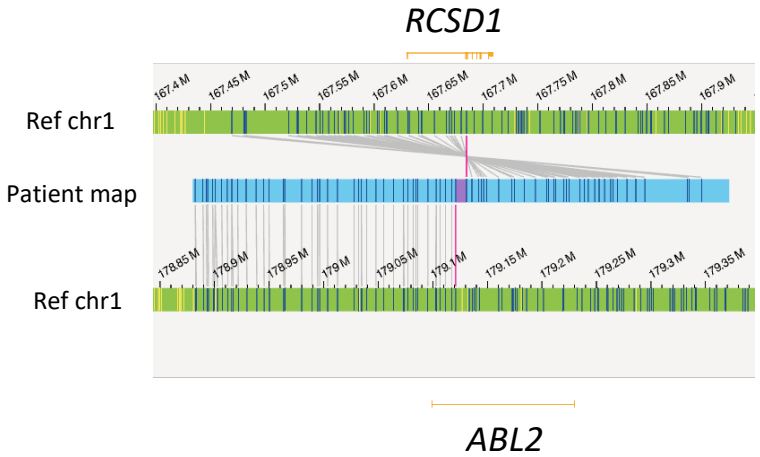
K Rack, J De Bie, G Ameye, O Gielen, S Demeyer, J Cools, K De Keersmaecker, et al. **« Optimizing the Diagnostic Workflow for Acute Lymphoblastic Leukemia by Optical Genome Mapping»**. *American Journal of Hematology* 97, nº 5 (mai 2022): 548-61. https://doi.org/10.1002/ajh.26487

V Lestringant, N Duployez , D Penther , I Luquet, C Derrieux, A Lutun, et al. **« Optical genome mapping, a promising alternative to gold standard cytogenetic approaches in a series of acute lymphoblastic leukemias ».** Genes Chromosomes Cancer. oct 2021;60(10):657-67

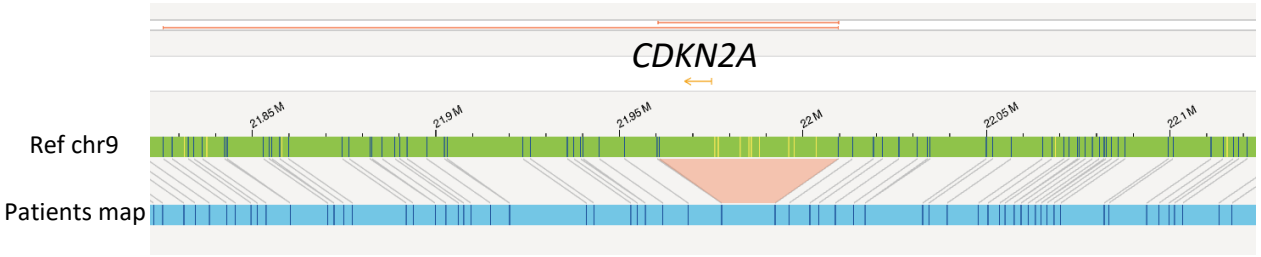
ACCORD ALLtogether
Suite demande du Pr HELENE CAVE 28/02/22 :
OK OGM si méthode VALIDEE/ACCREDITEE(EEQ)

OGM analysis of an illustrative B-ALL case

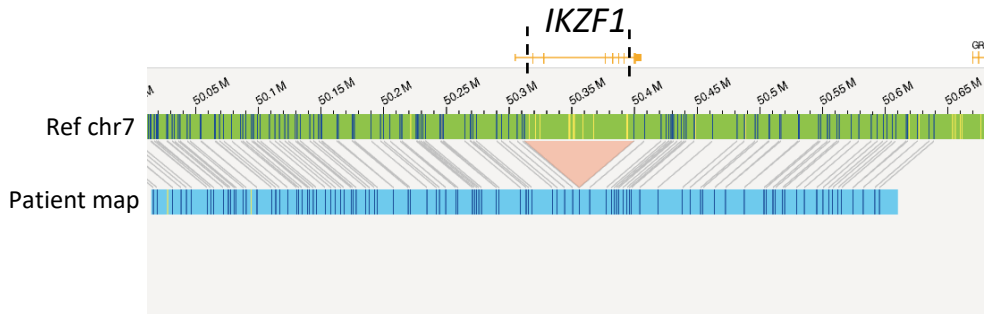




RCSD1-ABL2 transcript



CDKN2A focal biallelic deletion



IKZF1 del(2-7) *confirmed by PCR*

Saint Louis hospital, Dr.Kim, Dr.Chat,Dr.Cuccuini, Pr.Clappier.