ALLTogether
Consortium (ATG) protocol

CYTOGENETIC STRATIFICATION

UPDATES GUIDELINES octobre 2022

GFCH 1 févier 2023

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GEN-GR

CYTO-GR

or CNA group A w/o CYTO-HR

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*

Inf ou égal à 16ans

GEN-PR

CYTO-HR

or CNA group B w/o CYTO-GR

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*

+ *Ig::MYC* Update Guidelines

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

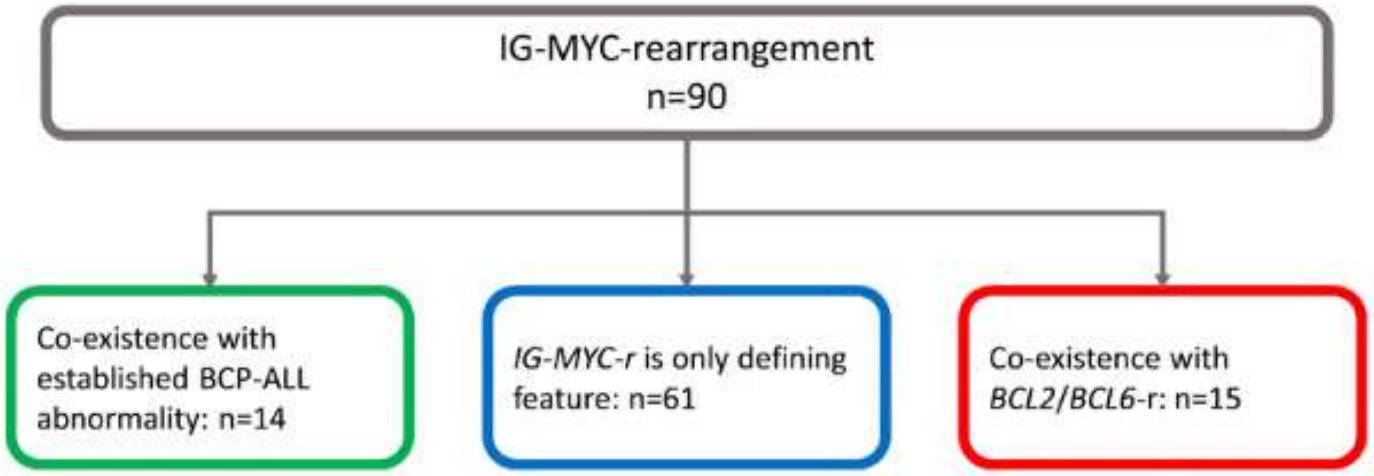
Molecular characterisation and clinical outcome of B-cell precirsor ALL with Ig:: MYC rearrangement

Bomken, et al,Heamatologica 2022

- Approximately 0.5% patients who present with surface immunoglobulin negative BCP-ALL are characterise by a translocation between an immunoglobulin (IG) locus and MYC (being any of t(8;14)(q24;q32), t(2;8)(p12;q24) or t(8;22)(q24;q11))
- Defined as **having IG::MYC in the absence of other subtype** defining genetic abnormalities and without translocation of BCL2 or BCL6, this group has a poor event free survival.
- Patients with BCP-ALL and IG::MYC are **eligible for inclusion on the trial unless a concomitant BCL2/6 translocation is present**.
- **Some IG::MYC rearrangements are sub-clonal to other subtype defining genetic events** (e.g. KMT2A-r, hig hyperdiploidy) → In this situation, **stratification will be based on the primary genetic abnormality**.

Molecular characterisation and clinical outcome of B-ALL with IG::MYC rearrangement

Bomken et al,Heamatologica, 2022



*NB : IG::MYC may co-occur as a **sub clonal abnormality** to HeH, KMT2A-r, iAMP21, BCR:: ABL1,IGH::DUX4*

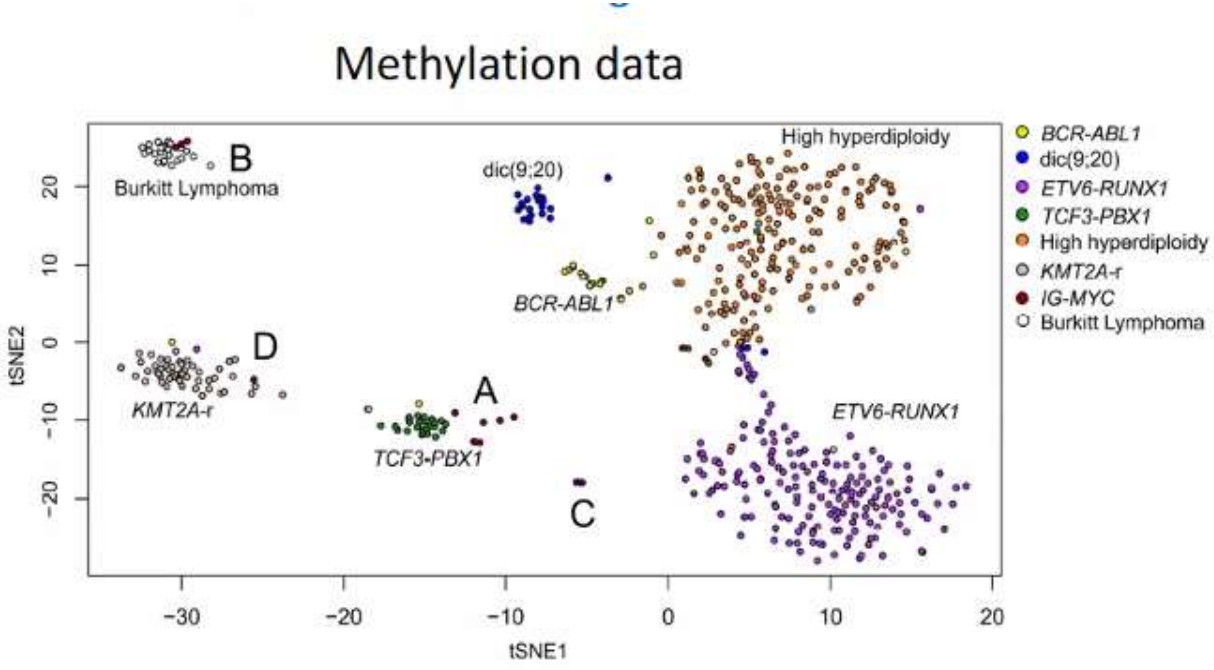
NB : Si 8q24 anormal au caryotype du diagnostic toujours penser à MYC

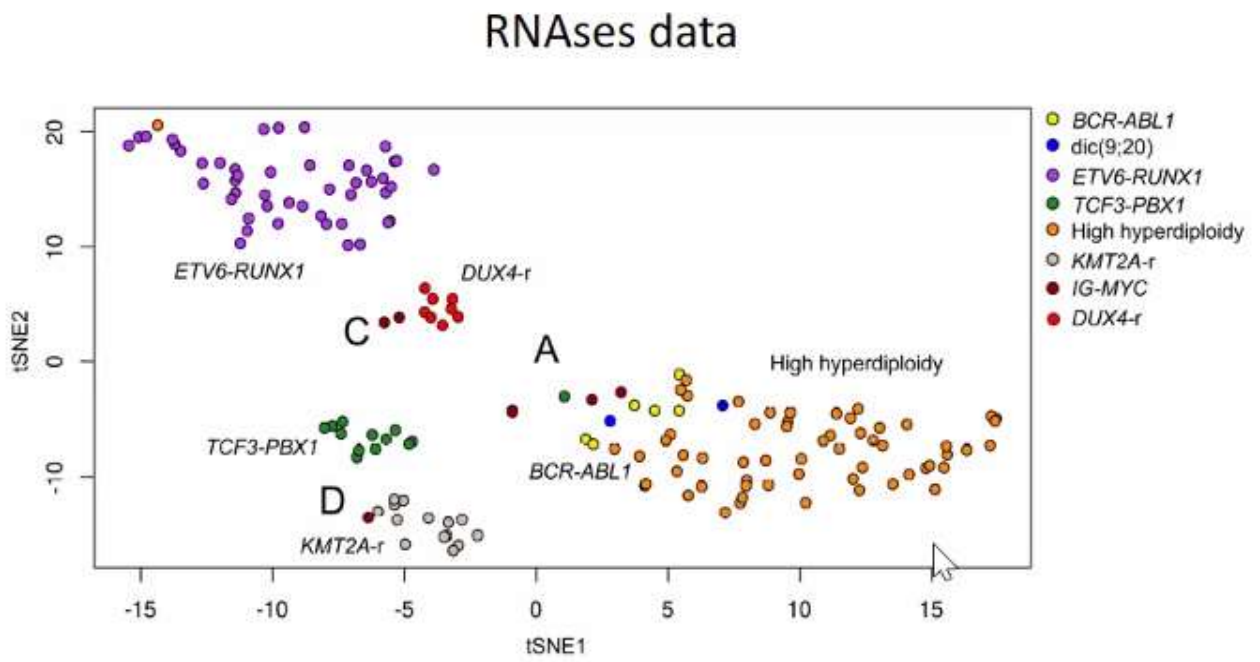
Molecular characterisation and clinical outcome of B-ALL with IG::MYC rearrangement

Bomken et al,Heamatologica, 2022

Coexistence with an ALL-specific CA (n=14)

Methylome (5/14) and Transcriptome (6/14) analyses





NB : 3 cases co-cluster with Burkitt AL/LL

Update of the genetic guidelines – Incorporate *IG*::*MYC* screening

Patients with HR genetics (KMT2A-r, NH, HoL, iAMP21 or IG::MYC) are not eligible for treatment de-intensification and should hence be allocated to IR-high group unless their TP1 MRD is >5% in which case they should be allocated to the HR group.

-> *IG:: MYC* fusions must be confirmed by FISH.
We strongly recommended using MYC break apart probes such as Cytocell Break apart (LPS027).
On reflection we have decided **not to recommended a specifIc *MYC* probe.** *It is out of sync with rest of the guidelines.*
However, we have to kept in a genetic statment about ensuring the chosen porbe spans the whole region to detect variable breakpoints.

-> It is **mandatory to exclude rearrangement of BCL2 and/or BCL6 using FISH in all *MYC-r***

-> **Identification of MYC- r should be followed by confirmation of the IG partner locus (IGH,IGK,IGL)**

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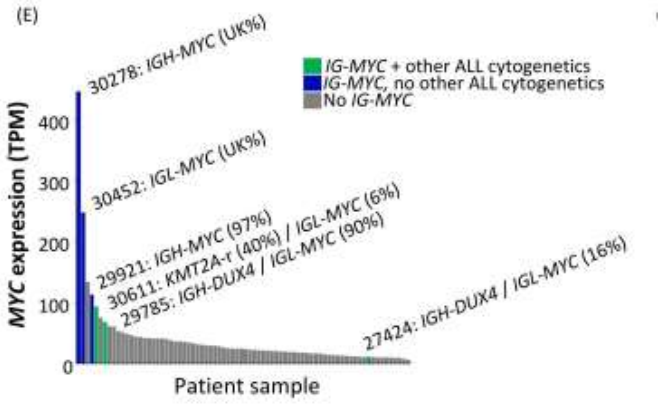
-> **Identification of MYC- r should be followed by confirmation of the IG partner locus (IGH,IGK,IGL)**

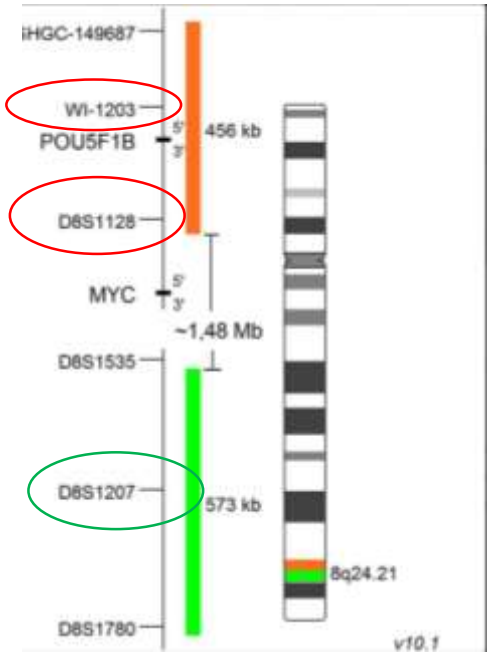
NB :
Ideally, all BCP ALL patients should be screened for *IG::MYC* but a **hierarchical strategy is acceptable** because the stratification is based on the primary CA.
Howewer, the identification of *IG::MYC* **must not prevent screening for other abnormalites.**



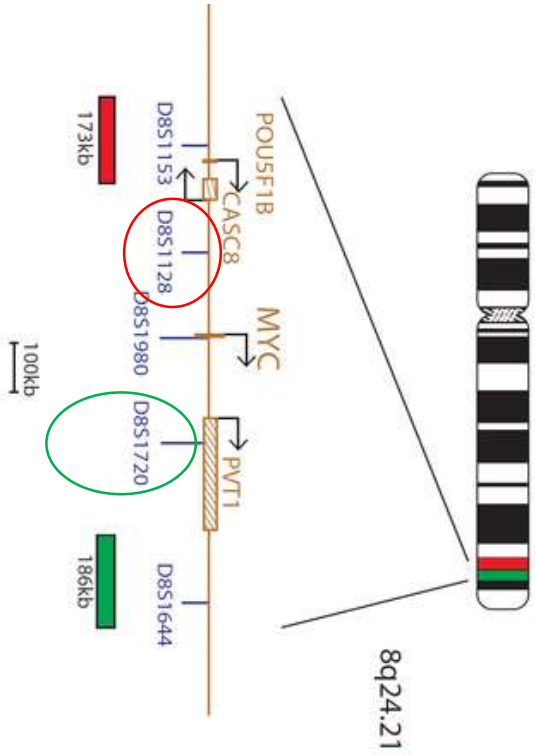
MYC break apart probes

Sonde MYC qui couvre tous les points de cassures (Metasystem, Cytocell(sysmex), Vysis)

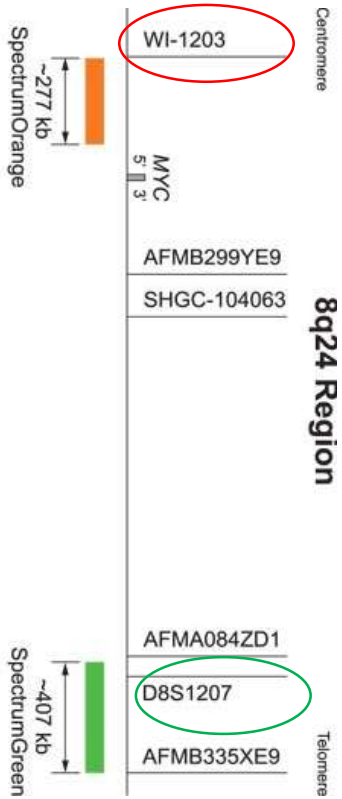
Metasystems



Cytocell



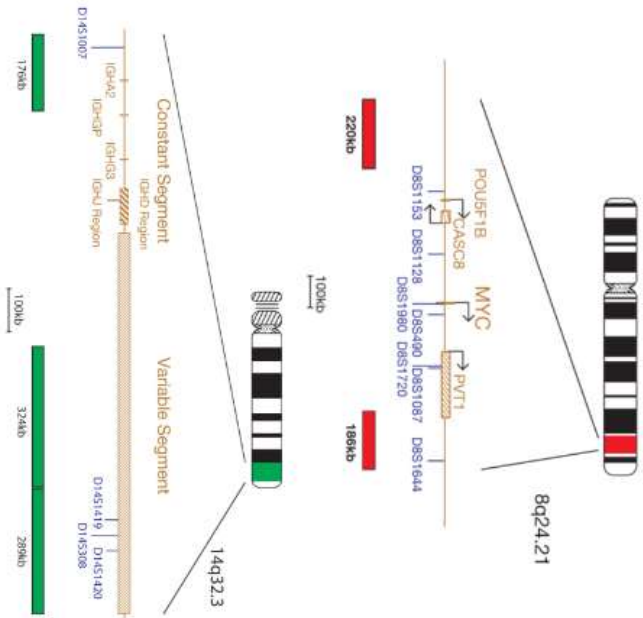
Vysis Abbott



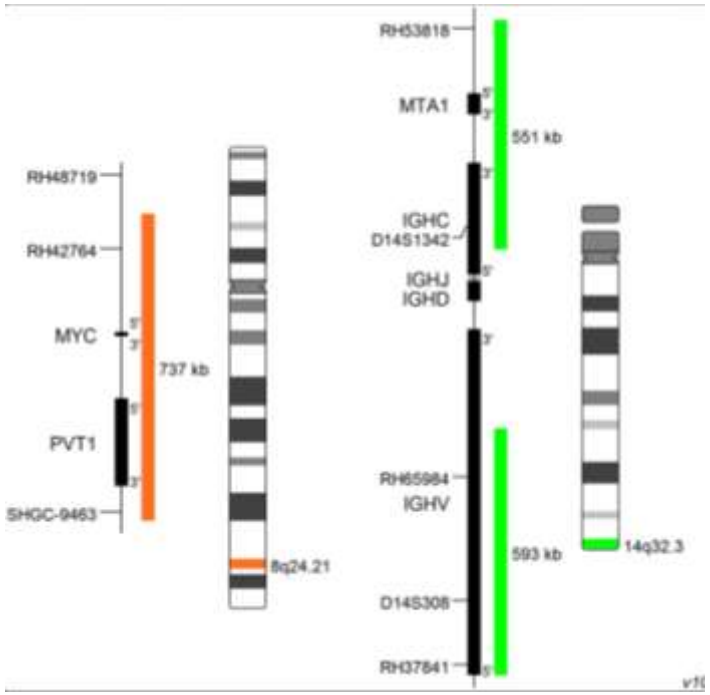
IGH::MYC Dual fusion probes

(Cytocell, Metasystem, Vysis,)

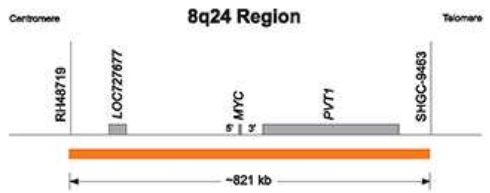
Cytocell



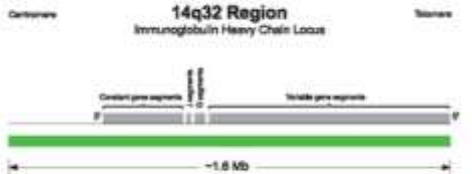
Metasystem



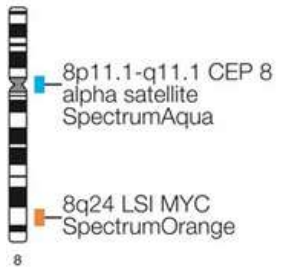
Vysis Abbott



LSI MYC SpectrumOrange Probe

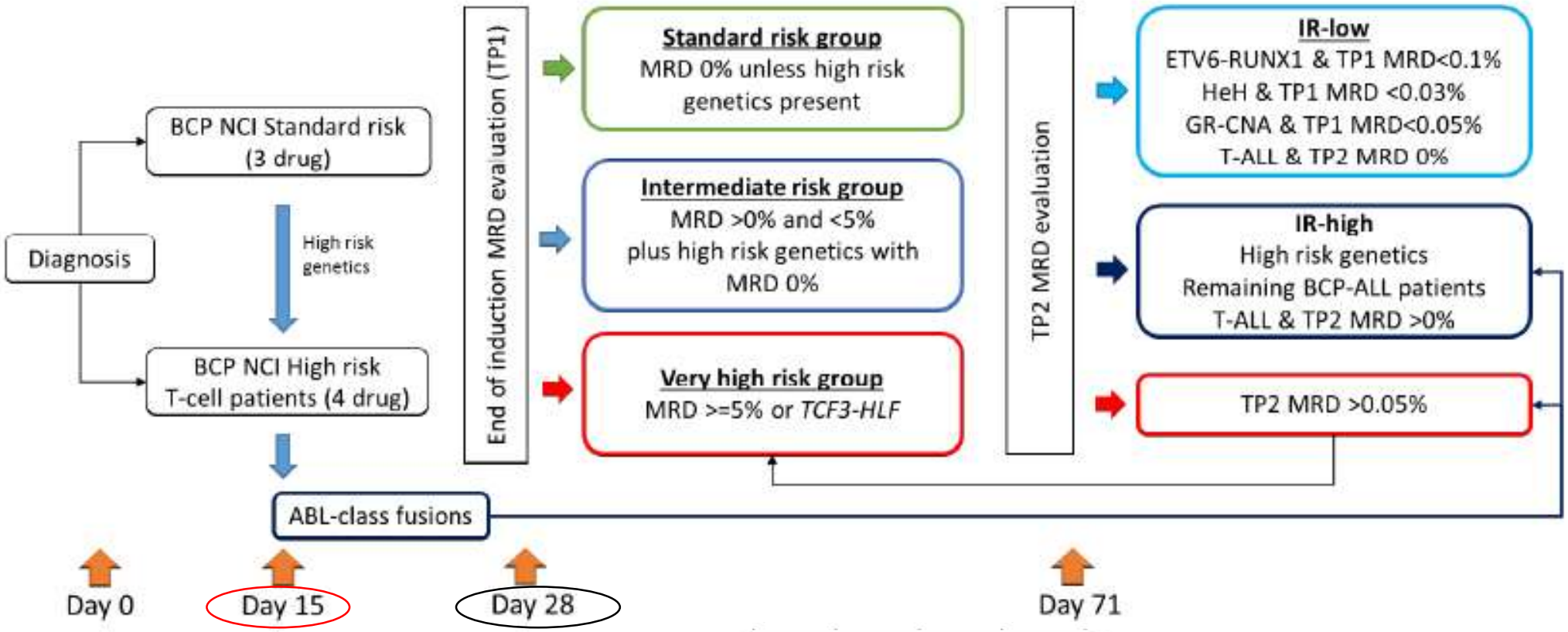


LSI IGH SpectrumGreen Probe



Update of the genetic guidelines – Incorporate *IG::MYC* screening

Patients with HR genetics (KMT2A-r, NH, HoL, iAMP21 or IG::MYC) are not eligible for treatment de-intensification and should hence be allocated to IR-high group unless their TP1 MRD is >5% in which case they should be allocated to the HR group.



ALLTogether B-ALL: La FISH en pratique

Panel 1 : type LAL-B «classique» (résultat avant J15)

- ***ETV6::RUNX1***
- ***BCR::ABL1***
- ***KMT2A***
- ***TCF3***

Panel 2, si panel 1 non informatif (résultat pour J15)

- ***ABL*-class**
  - ***ABL1 (si ABL1 anormal avec BCR::ABL1)***
  - ***PDGFRB/CSFR1***
  - ***ABL2***
- ***IG::MYC***
  - ***MYC ou IGH::MYC***

Panel 3 , si *MYC* pos (résultat pour J28) :

- ***BCL2 et BCL6* (si positif : cas exclus du protocole)**
- **Si *IGH* negatif : *IGK et IGL***

ALLTogether B-ALL : La FISH en pratique
Screening des ABL-class

Panel 1 : type LAL-B «classique» (résultat avant J15)

- *ETV6::RUNX1*
- *BCR::ABL1*
- *KMT2A*
- *TCF3*

Panel 2, si panel 1 non informatif (résultat pour J15)

- ***ABL*-class ***
  - ***ABL1 (si ABL1 anormal avec BCR::ABL1)***
  - ***PDGFRB/CSFR1***
  - ***ABL2***
- *IG::MYC*
  - *MYC*
  - *IGH::MYC*

Panel 3 , si *MYC* pos (résultat pour J28) :

- ***BCL2 et BCL6* (si positif : cas exclus du protocole)**
- **Si *IGH* negatif : *IGK et IGL***

- *Sauf pour les hypodiploidies mais y compris les Hyperdiploidies élevées*

- *Voir à l'usage, MAIS l'idée est de tenter de demander un amendement pour réduire les « Hyper » à des cas Atypiques +++ :*
  - *nombre modal 51-53*
  - *chromosomes surnuméraires atypiques :*
    pas de trisomie 17 ou 18 ***et*** *presence de trisomie 5 ou 20*
    (Ensheai A, Lancet Haem, 2021)
  - *delIKZF1*

ALLTogether T-ALL: La FISH en pratique

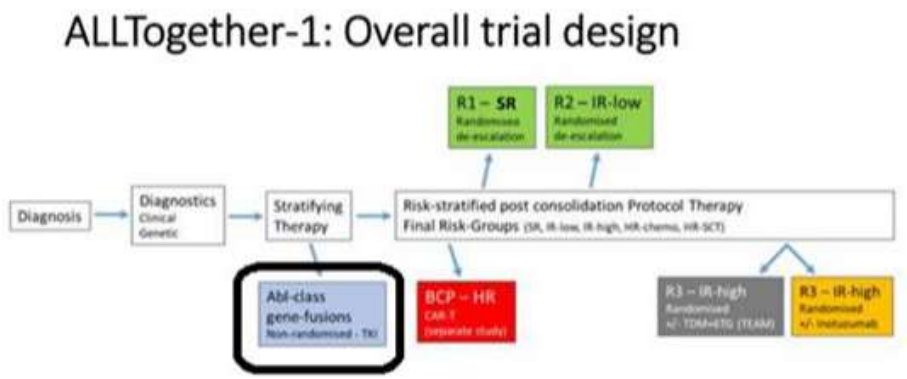
Patients with T-ALL must be screened for BCR::ABL1, ABL-class and KMT2A fusions (1).

Panel 1 :

- ***BCR::ABL1***
- ***KMT2A***

Panel 2 : *ABL*-class (resultats pour J15)

- ***ABL1 (si ABL1 anormal avec BCR::ABL1)***
- ***PDGFRB/CSFR1***
- ***ABL2***



At the time of writing these guidelines there is uncertainty as to whether T-ALL with ABL-class fusion should be treated with imatinib.
Hence, we recommend **that all patients with T-ALL are screened for ABL-class fusions** but that treatment with imatinib should be discussed on an individual basis with the national principal investigator. (1)

(1) Lab guidelines Summary of the recommendations from the ALLTogether Genetics group for the genetic screening of patients treated on the ALLTogether1 Protocol Version 4.0 DDMMM2022

Table 1: Definition of actionable chromosomal and genomic abnormalities in the ALLTogether paediatric ALL trial

Full name	Abbreviation	Definition	Notes
ETV6::RUNX1	*ETV6::RUNX1*	t(12;21)(p13;q22)/*ETV6::RUNX1*	
High hyperdiploidy	HeH	51-67 chromosomes	
KMT2A gene fusions	*KMT2A*-r	Any genetic rearrangement that results in the fusion of the 5' portion of *KMT2A* with the 3' portion of a partner gene. The most prevalent partner genes are: *AFF1/AF4, MLLT3/AF9, MLLT1/ENL, MLLT10/AF10* and *MLLT4/AF6/AFDN*. Collectively they account for >90% *KMT2A*-r cases in paediatric ALL.	1) *KMT2A* was previously named *MLL*. 2) All *KMT2A*-r patients will be classified in the same manner; so the identification of the partner gene is not required.
Near-haploidy	NH	Clones with fewer than 30 chromosomes	Doubled-up sub-clones which have 48-58 chromosomes will be included in the subgroup.
Low hypodiploidy	HoL	Clones with 30-39 chromosomes	Doubled-up sub-clones whose karyotypes have 60-78 chromosomes (i.e. near-triploidy) will be included in the subgroup.
Intrachromosomal amplification of chromosome 21	iAMP21	Three or more extra copies of a FISH probe to the *RUNX1* gene on a single abnormal chromosome 21	iAMP21 can be defined using a DNA array
Primary *IG::MYC*	*IG::MYC*	Surface immunoglobulin negative BCP ALL with any of t(8;14)(q24;q32)/*IGH::MYC*, t(2;8)(p12;q24)/*IGK::MYC* or t(8;22)(q24;q11)/*IGL::MYC* demonstrated by FISH	Patients with another actionable chromosomal/genetic abnormality will be stratified according to that other abnormality. Patients with additional rearrangements of *BCL2* and/or *BCL6* are excluded from the protocol
t(17;19)(q22;p13)/ *TCF3::HLF*	t(17;19)	t(17;19)(q22;p13)/*TCF3::HLF*	
Gene fusions involving *ABL1, ABL2, PDGFRB* and *CSF1R*	ABL-class fusions	Any genetic rearrangement that results in the fusion of a variable 5' partner gene with the 3' portion of *ABL1, ABL2, PDGFRB* and *CSF1R*. Numerous partner genes have been described. The most frequent are *EBF1* and *ETV6*.	Patients with *BCR::ABL1* fusion are excluded from this subgroup and from the protocol. Identification of the partner gene is not required for classification.
Good risk UKALL CNA profile*	CNA-GR	No deletions affecting *BTG1, CDKN2A/B, EBF1, ETV6, IKZF1, PAX5*‡, *RB1*, and PAR1 Isolated deletion of *BTG1* or *ETV6* or *PAX5*‡ deletion Only two deletions - *ETV6* and *BTG1*, *ETV6* and *CDKN2A/B*, *ETV6* and a *PAX5*‡ deletion	See Table 4 for details of calling UKALL-CNA profile
Poor risk UKALL CNA profile*	CNA-PR	Any other deletion profile including all deletions of *IKZF1, EBF1, RB1 and* PAR1	See Table 4 for details of calling UKALL-CNA profile

* MLPA was used to develop the CNA profile and is published in Moorman et al (2014) Blood;124(9):1434-44.
‡ For PAX5, intragenic amplifications are coded with the deletions as they are predicted to be functionally equivalent.