FISH interphasique sur tissus dans les lymphomes B diffus à grandes cellules

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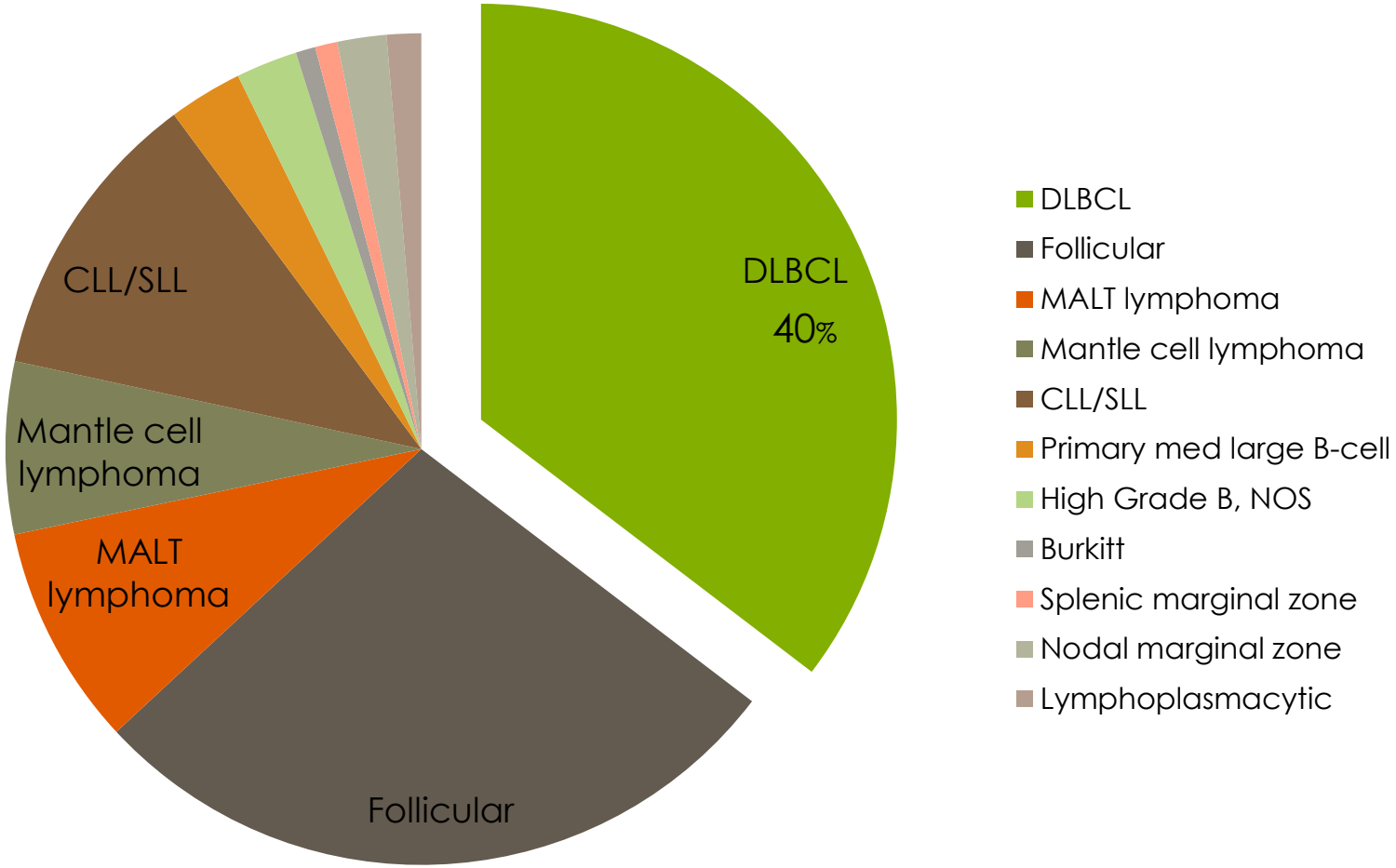
Les lymphomes B diffus à grandes cellules (DLBCL)

- Les lymphomes B diffus à grandes cellules font partis des lymphomes non hodgkiniens(LNH)

- Ce sont les plus fréquents , puisqu'ils représentent environ 40 % des LNH

Les DLBCL dans les Lymphomes non Hodgkiniens



Classification des LNH de haut grade

Diffuse large B-cell lymphoma (DLBCL), not otherwise specified (NOS)

Germinal-centre B-cell-like (GCB)

Activated B-cell-like (ABC)

DLBCL subtypes

T-cell/histiocyte-rich large B-cell lymphoma

Primary DLBCL of the CNS

Primary cutaneous DLBCL, leg type

Epstein-Barr virus–positive DLBCL, NOS ~~of the elderly~~

EBV+ mucocutaneous ulcer

Primary mediastinal (thymic) large B-cell lymphoma

Intravascular large B-cell lymphoma

DLBCL associated with chronic inflammation

Lymphomatoid granulomatosis

ALK-positive DLBCL

Plasmablastic lymphoma

Primary effusion lymphoma

HHV8-positive, DLBCL, NOS

B-cell lymphoma, with features intermediate between DLBCL and classical Hodgkin lymphoma

~~B-cell lymphoma, with features intermediate between DLBCL and Burkitt lymphoma~~

High grade B-cell lymphoma,

With MYC and BCL2 and/or BCL6 rearrangements

NOS

DLBCL,NOS

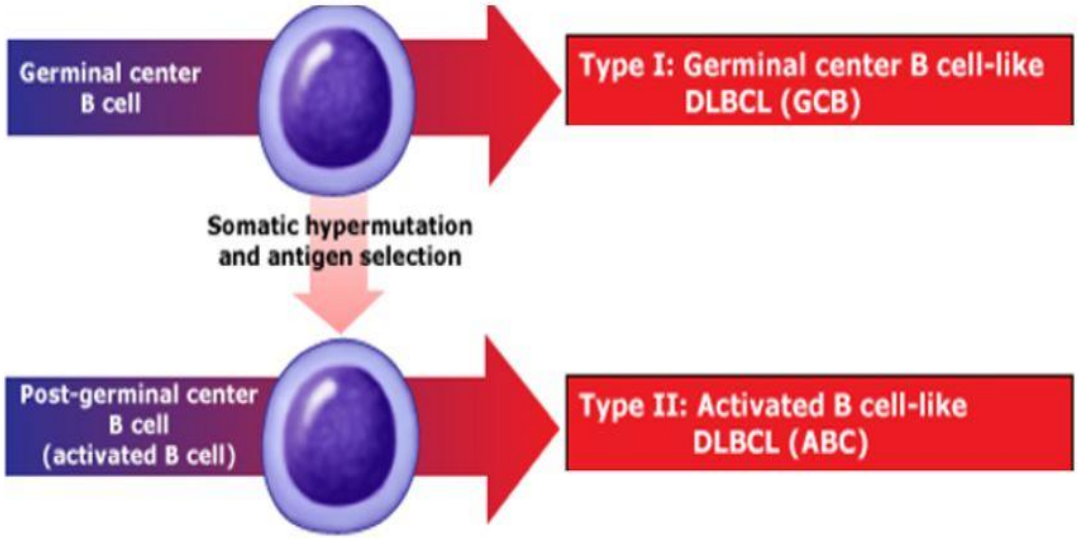
- Germinal center B-Cell (GC-B)
- Actived B cell-like (ABC),Non-GC

- Renseigne sur l'agressivité

Treatment

- Standard Chemotherapy regimen → R-CHOP
  - Rituximab, Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone

- Success depends on Cell origin and Genetic Factors!



5-Year Overall Survival

Double Hit DLBCL

- BCL6 and/or BCL2 PLUS MYC translocation
- High rates of relapse and poor survival with standard R-CHOP
- Pts are candidates for more aggressive da-EPOCH-R

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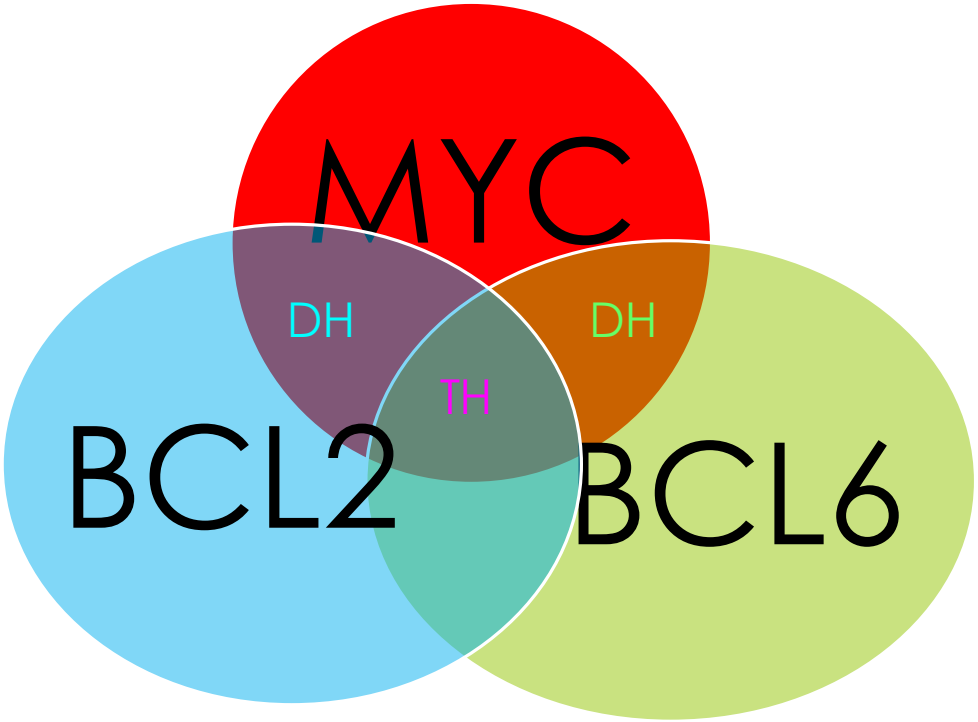
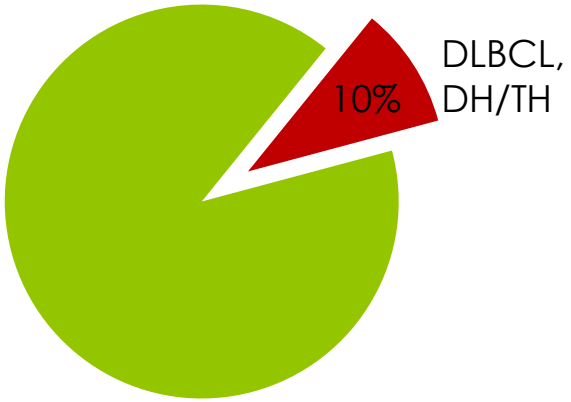
NOS

High grade B-Cell lymphoma, with MYC and BCL2 and/or BCL6 Rearrangements



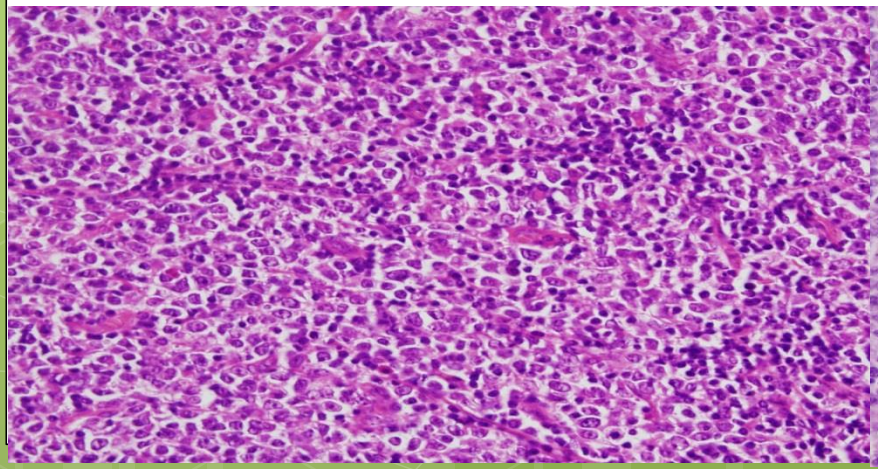
Les DH et TH dans les DLBCL

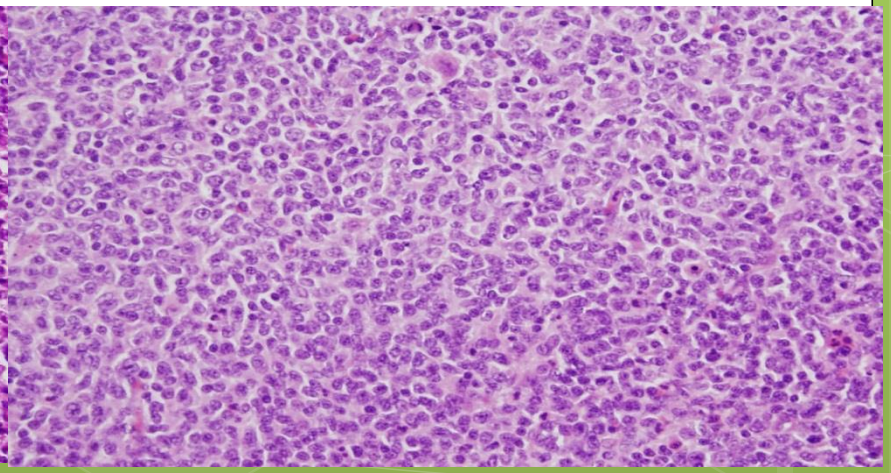


La victoire se conquiert collectivement

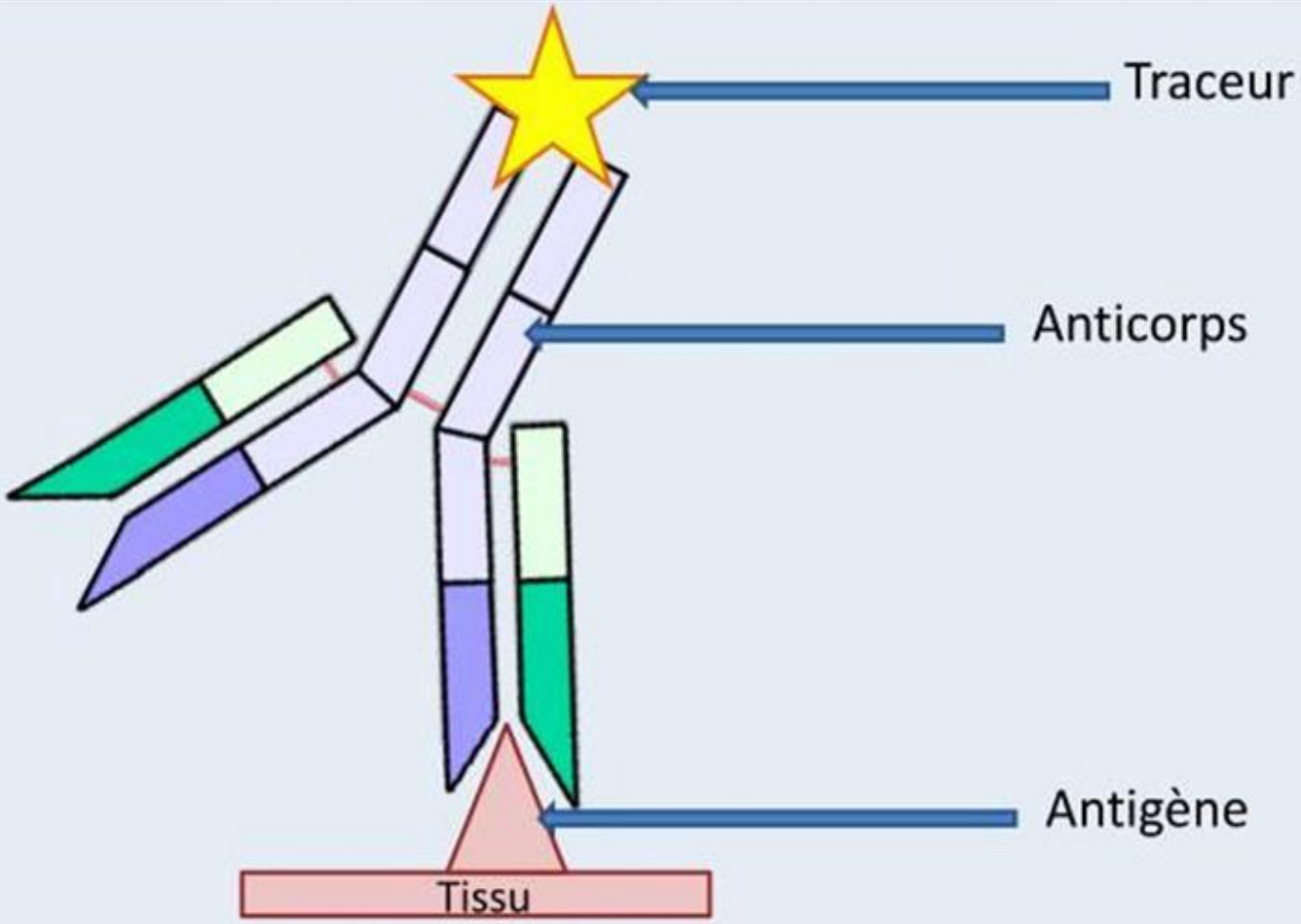
ANATOMO-PATHOLOGIE

Histologie : *grandes cellules avec un agencement diffus, effacement en général complet , parfois partiel de l'architecture du ganglion*

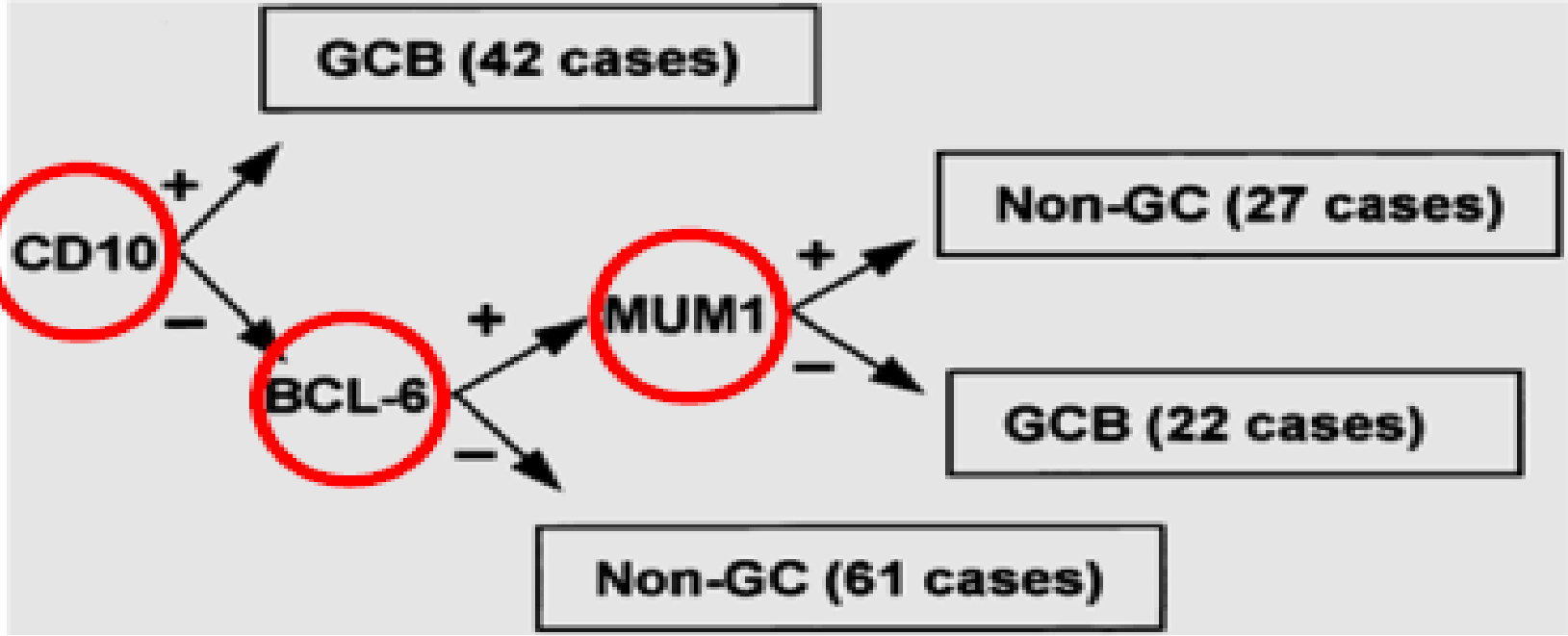




PRINCIPE IHC

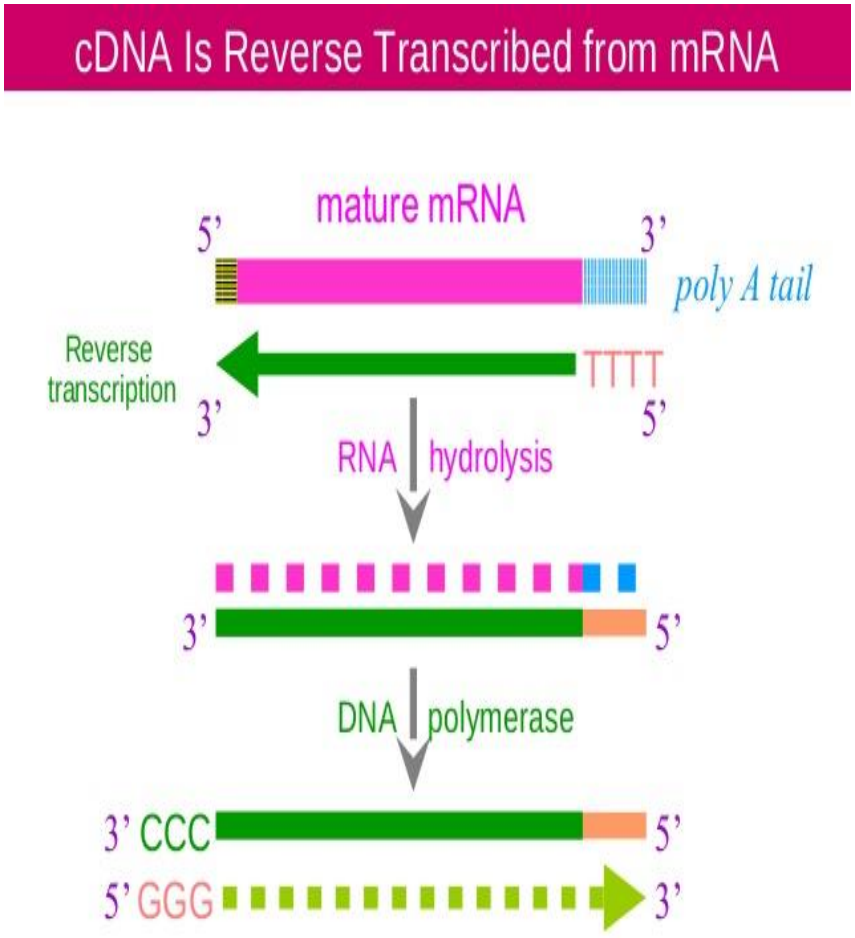


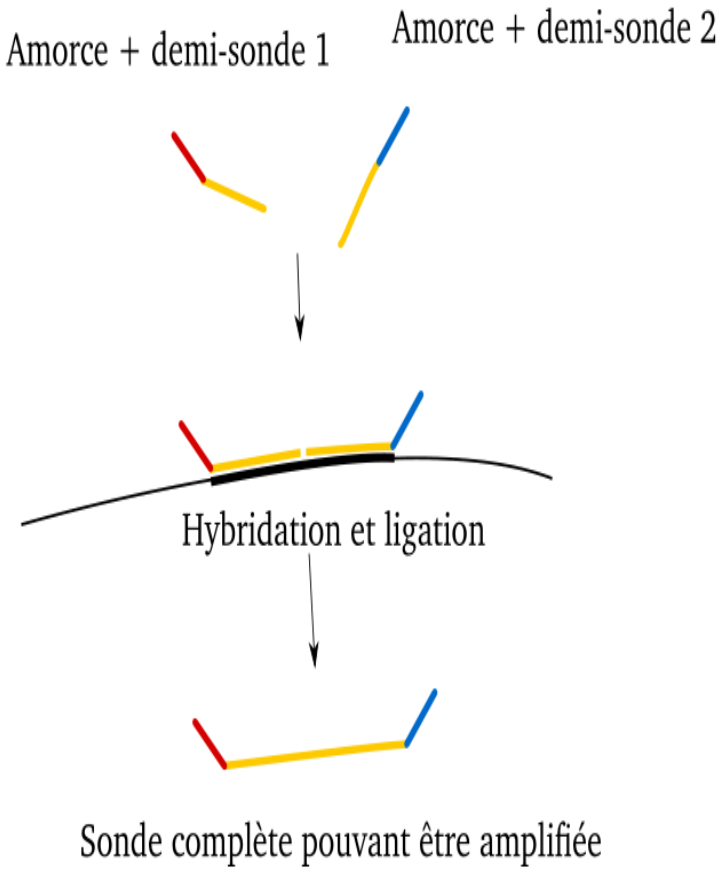
Identification en IHC des sous-types du DLBCL



Classification en pratique diagnostique quotidienne par IHC d'après l'algorithme de Hans *(Hans et al. Blood 2004)* défini à partir de l'expression différentielle de **CD10, BCL6 et MUM1**

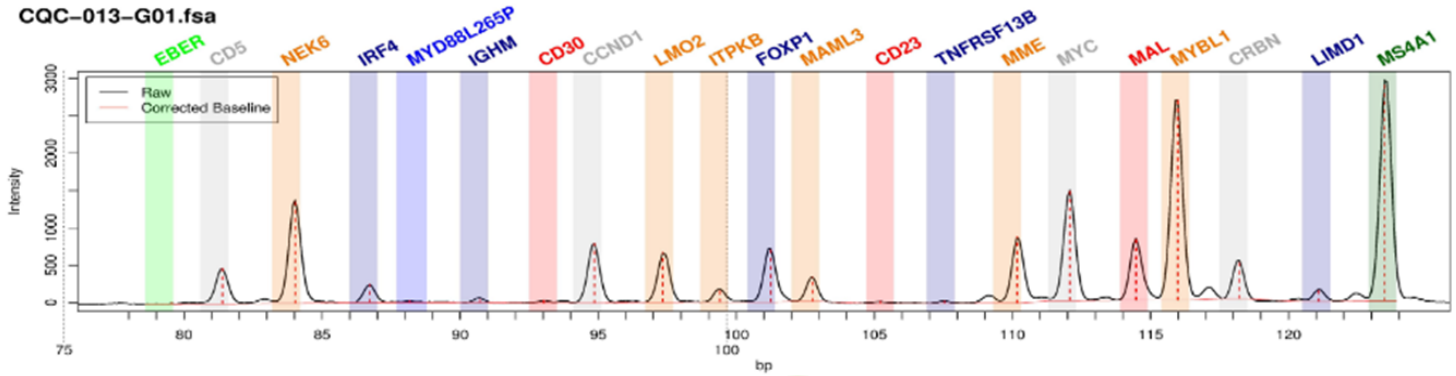
RT-MLPA DLBCL

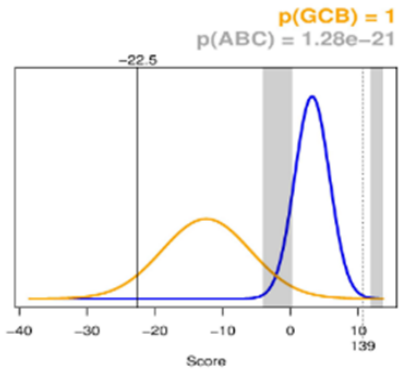




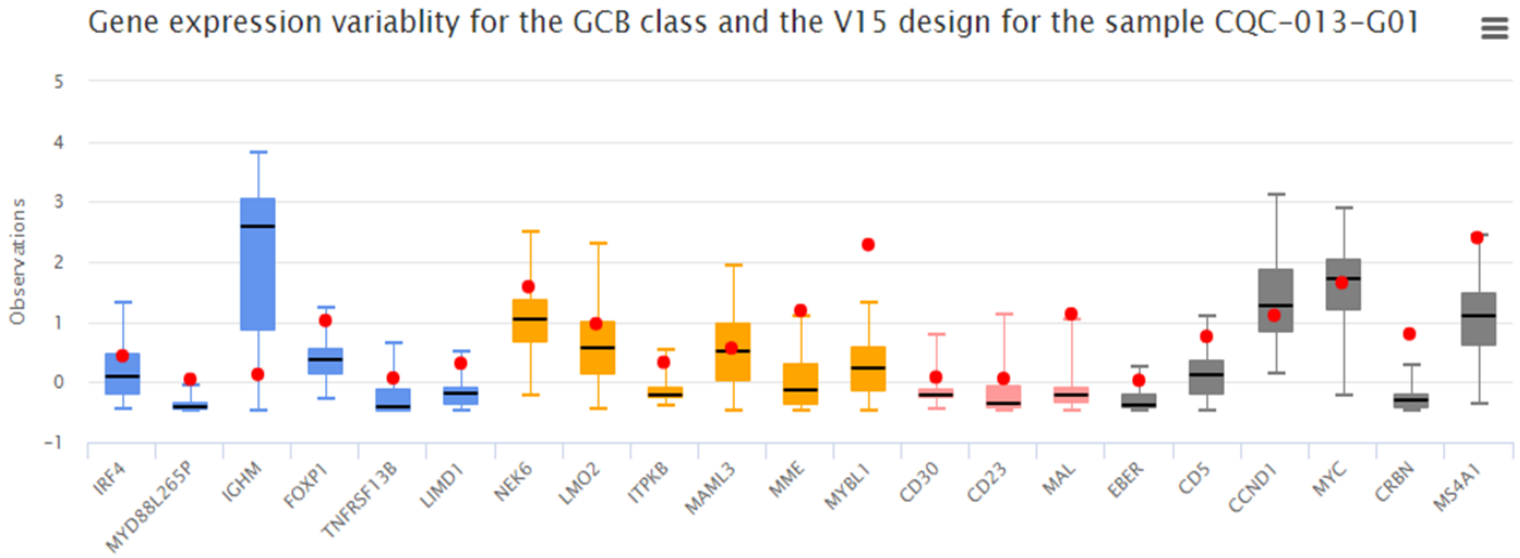
Sample CQC-013-G01 predicted as GCB

PROFILE & CLASSIFICATION | ALERTS 0 | EXPRESSION INFORMATIONS | PEAKS INFORMATIONS | EXTRA PD



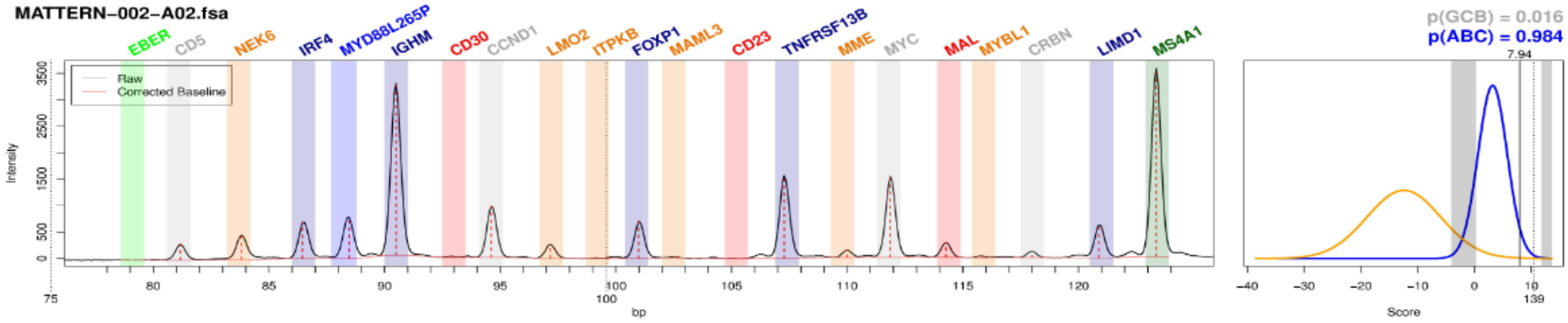


Genes associated with ABC | Genes associated with GCB | Genes associated with PMBL | Others

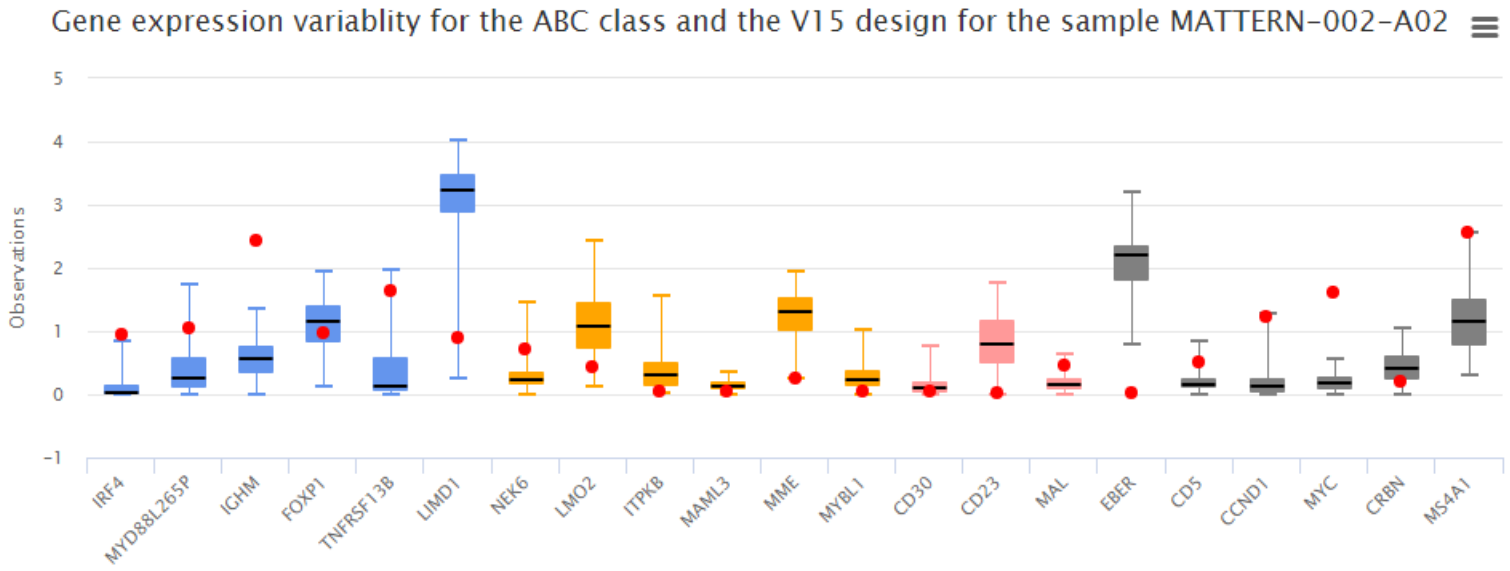


Sample MATTERN-002-A02 predicted as ABC

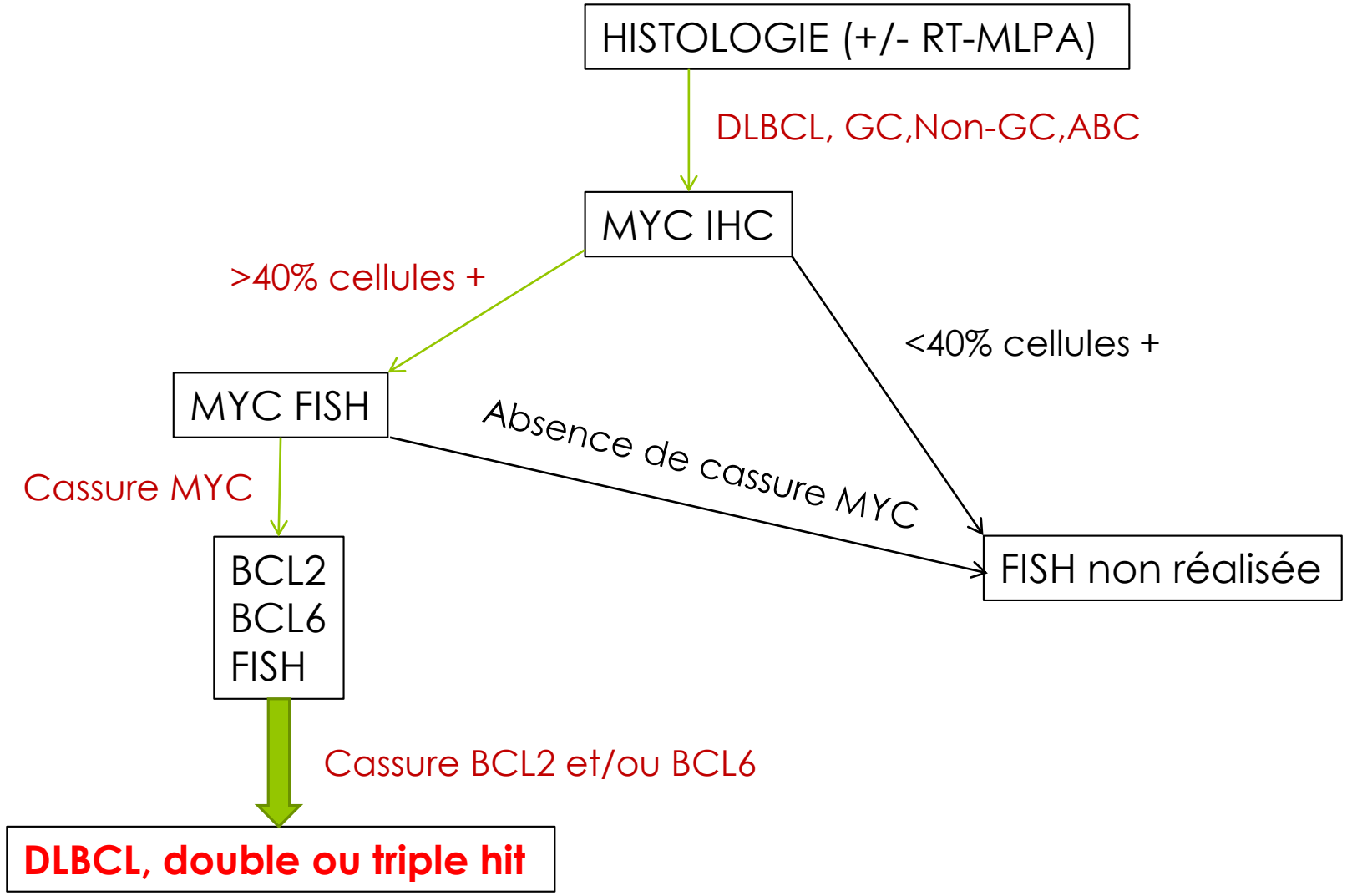
PROFILE & CLASSIFICATION | ALERTS 1 | EXPRESSION INFORMATION | PEAKS INFORMATION | EXTRA PI



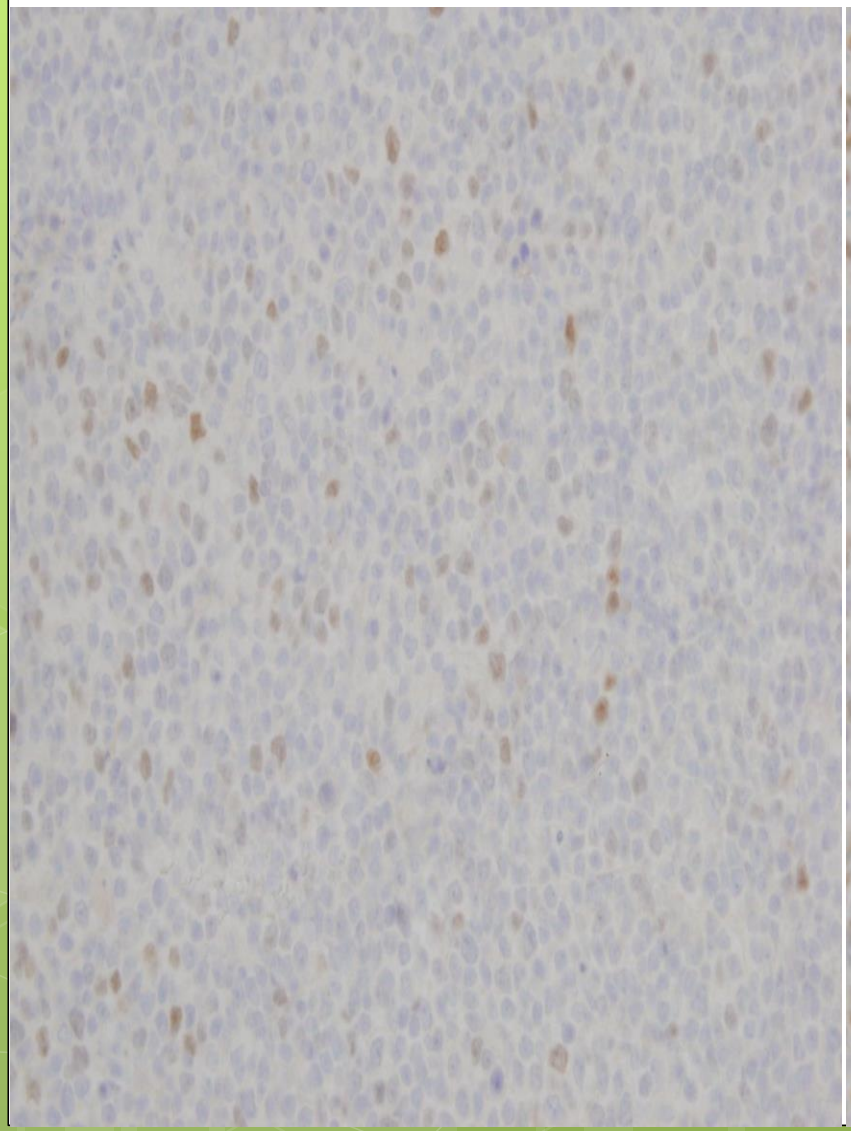
Genes associated with ABC | Genes associated with GCB | Genes associated with PMBL | Others

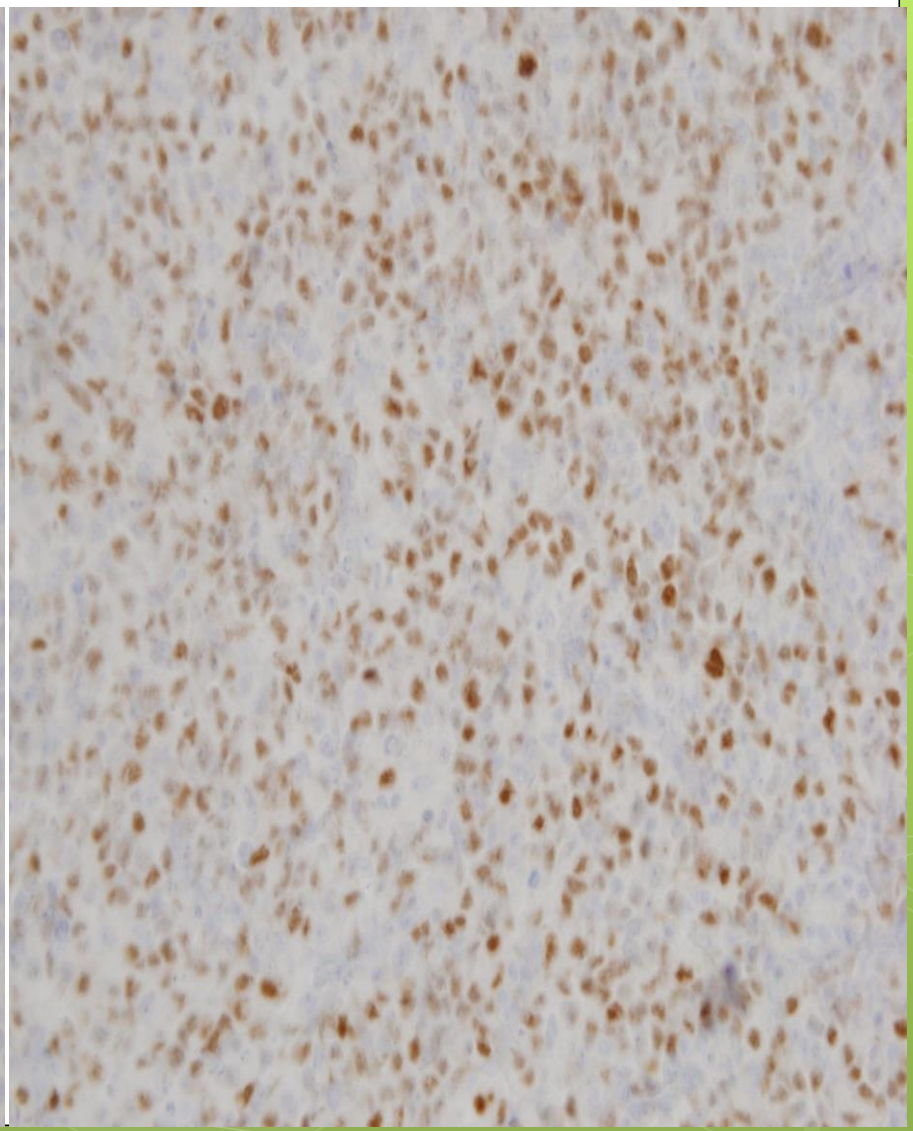


Diagnostic du DLBCL avec DH/TH

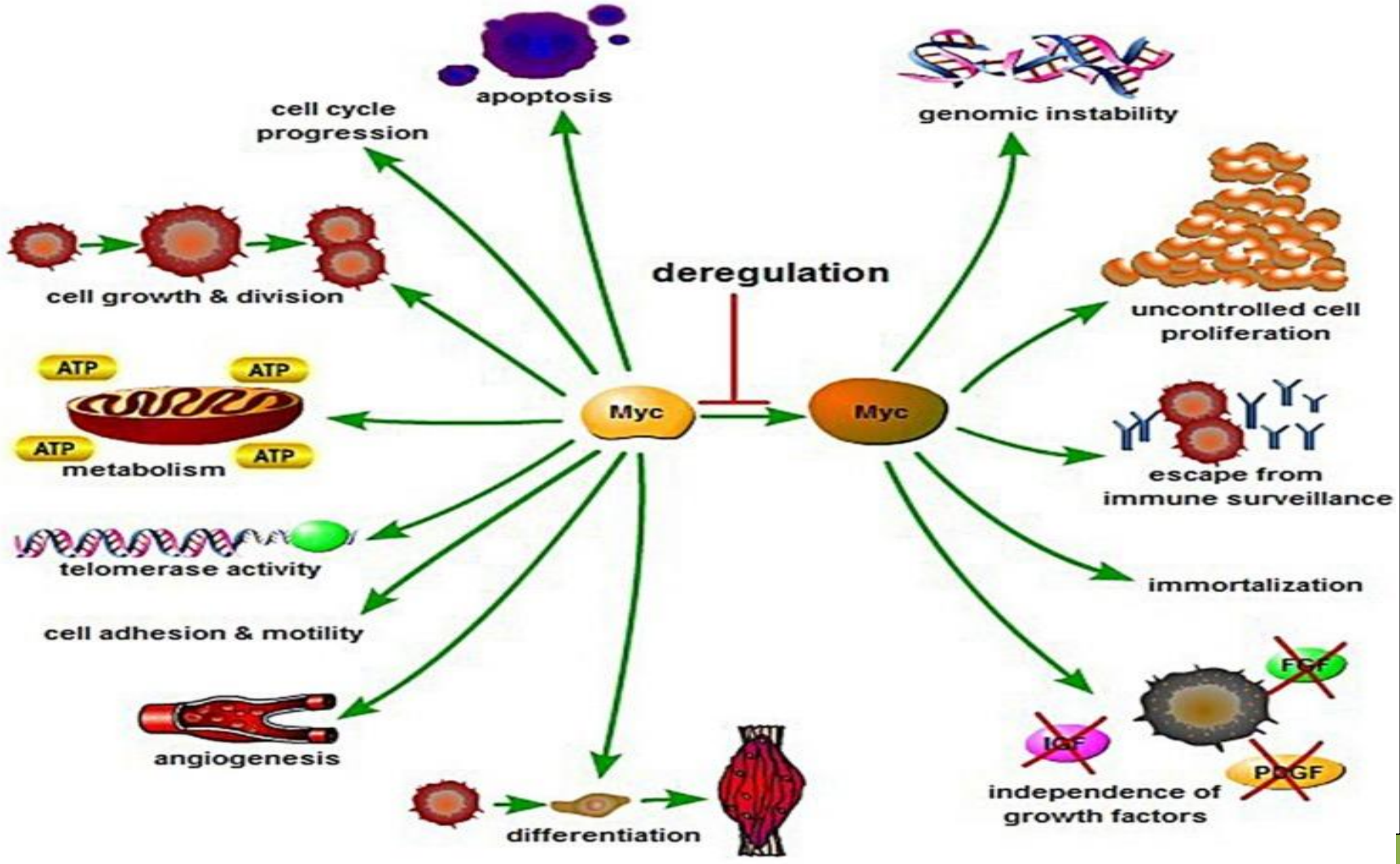


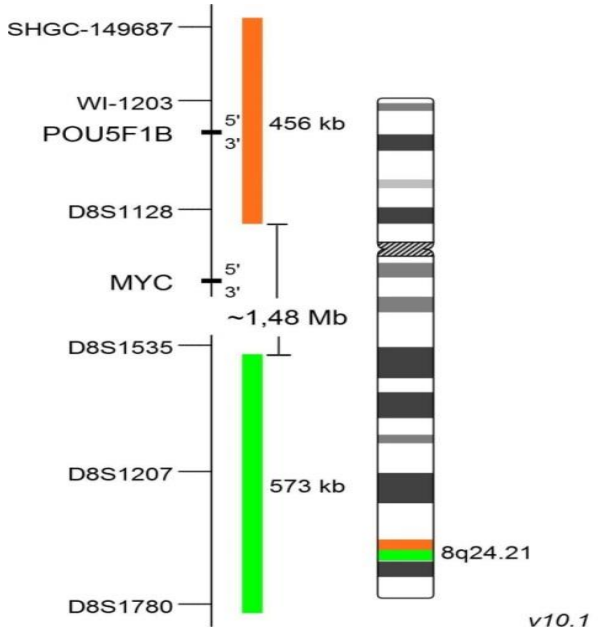
Lecture de MYC en IHC





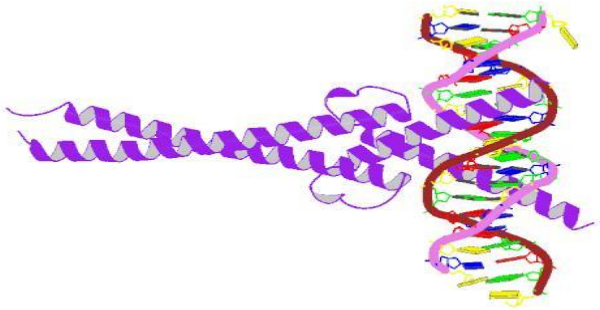
Biology and regulation of Myc in cellular processes control





MYC

8q24



- La régulation de la croissance cellulaire
- La différenciation cellulaire
- L'apoptose

MYC

proto-oncogène

Amplification

Translocation

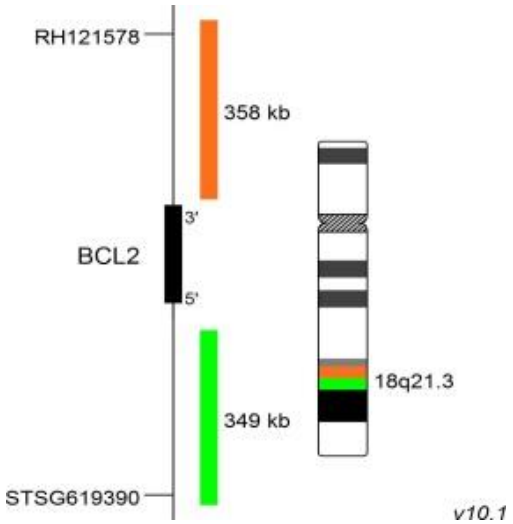
Mutation

oncogène

INSTABILITE GENOMIQUE

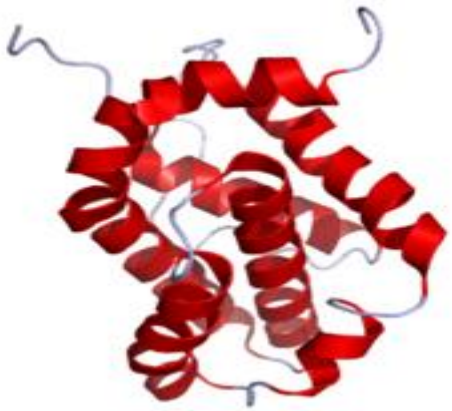
PROLIFERATION DESORDONNEE DES CELLULES

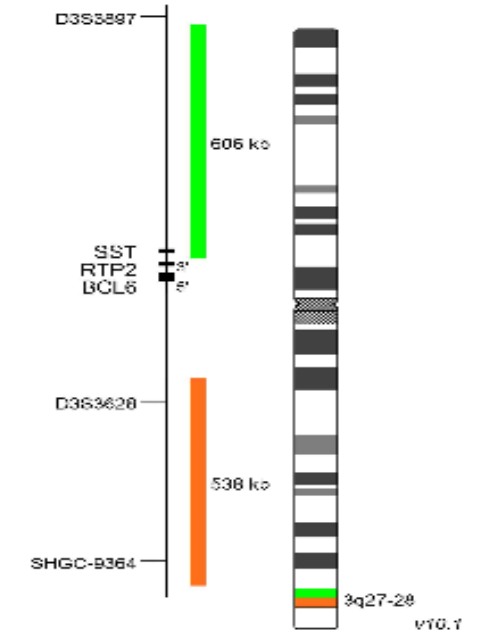
INHIBITION DE L'APOPTOSE



BCL2

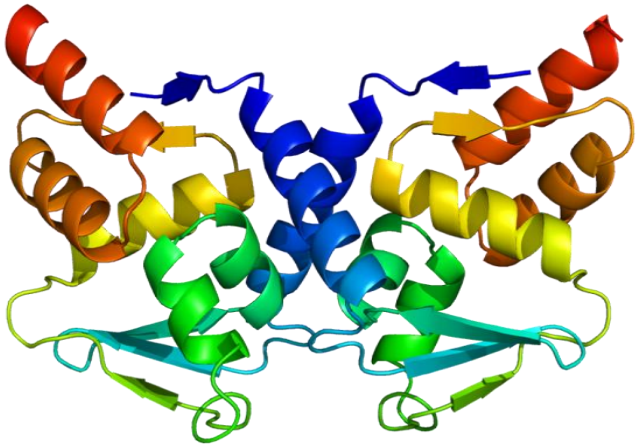
18q21

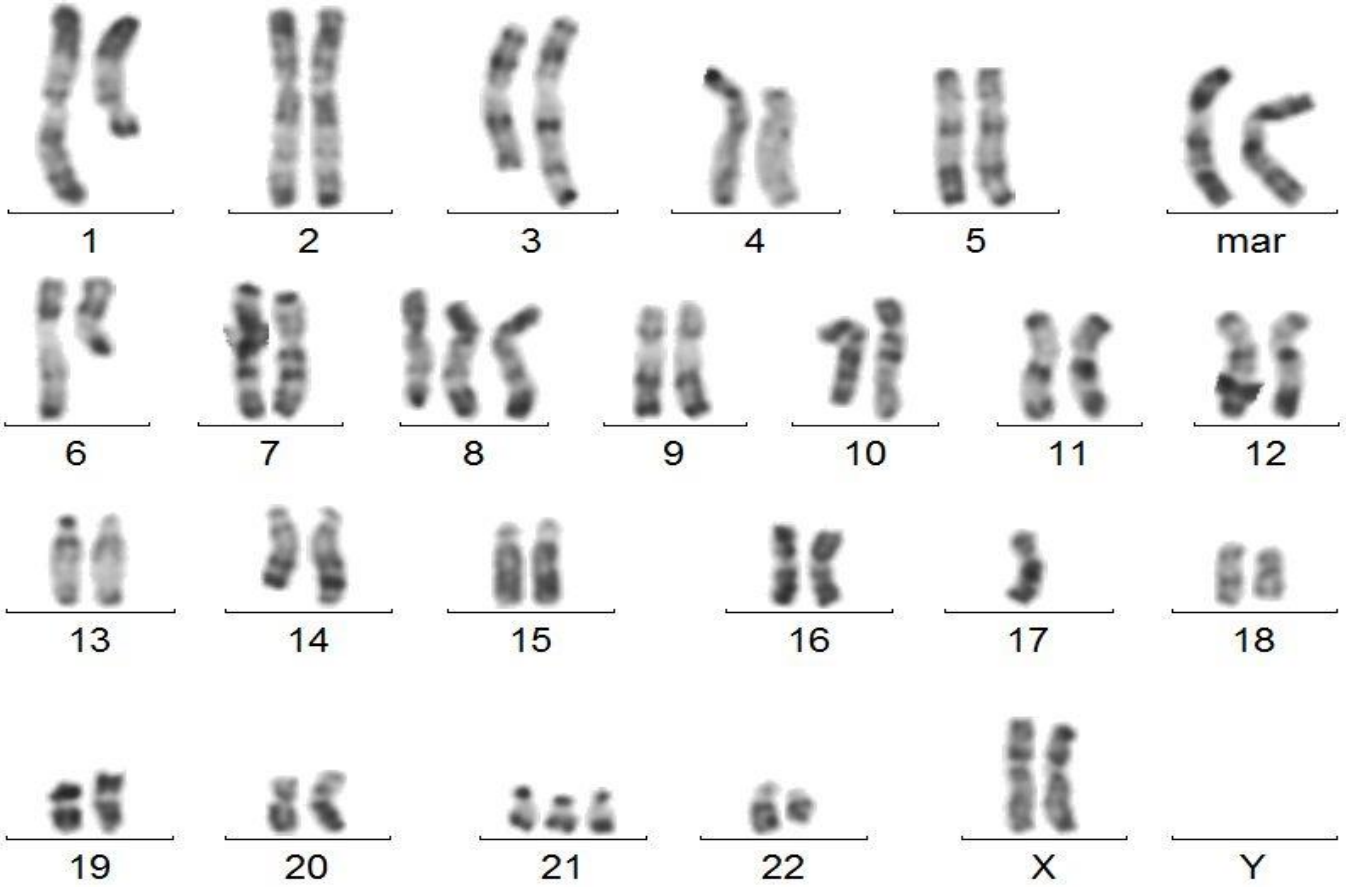




BCL6

3q27

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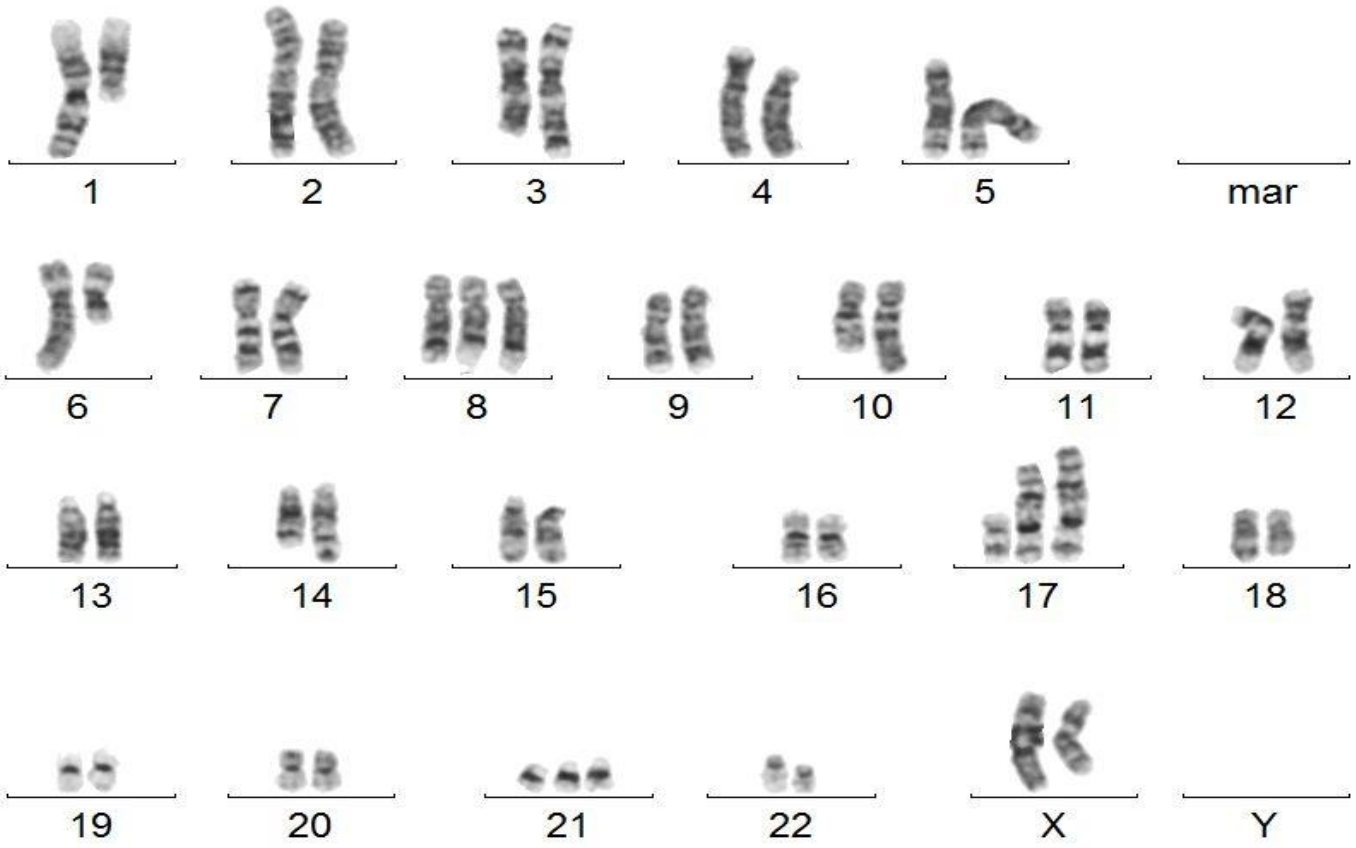
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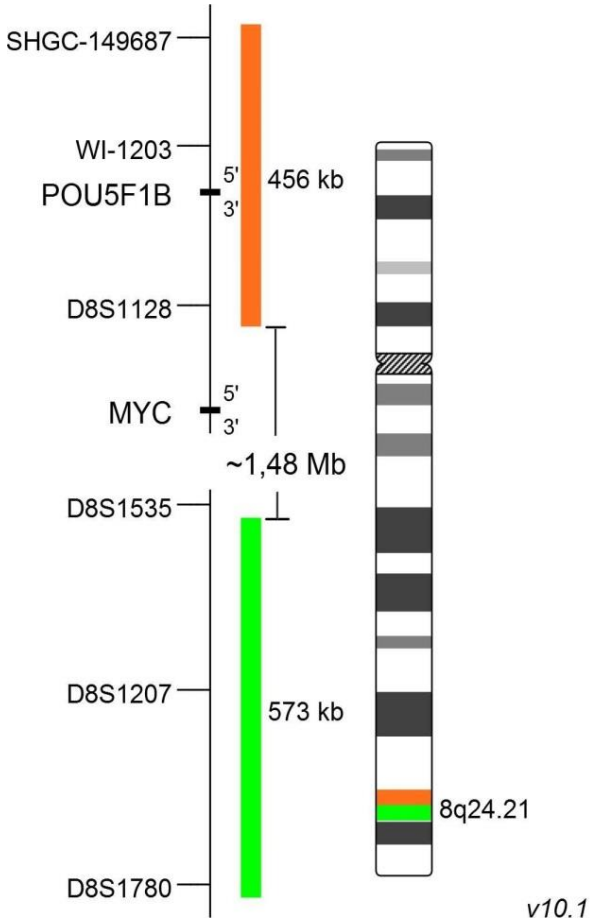
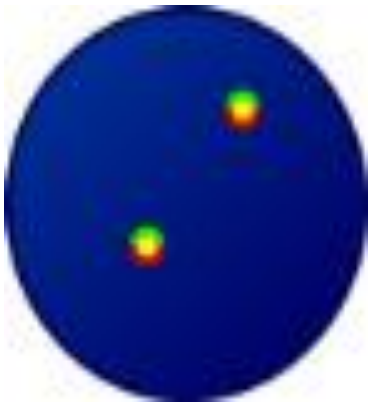
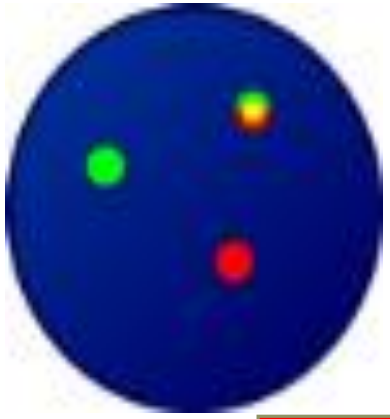
LECTURE SONDE XL MYC
Break Apart Metasystems



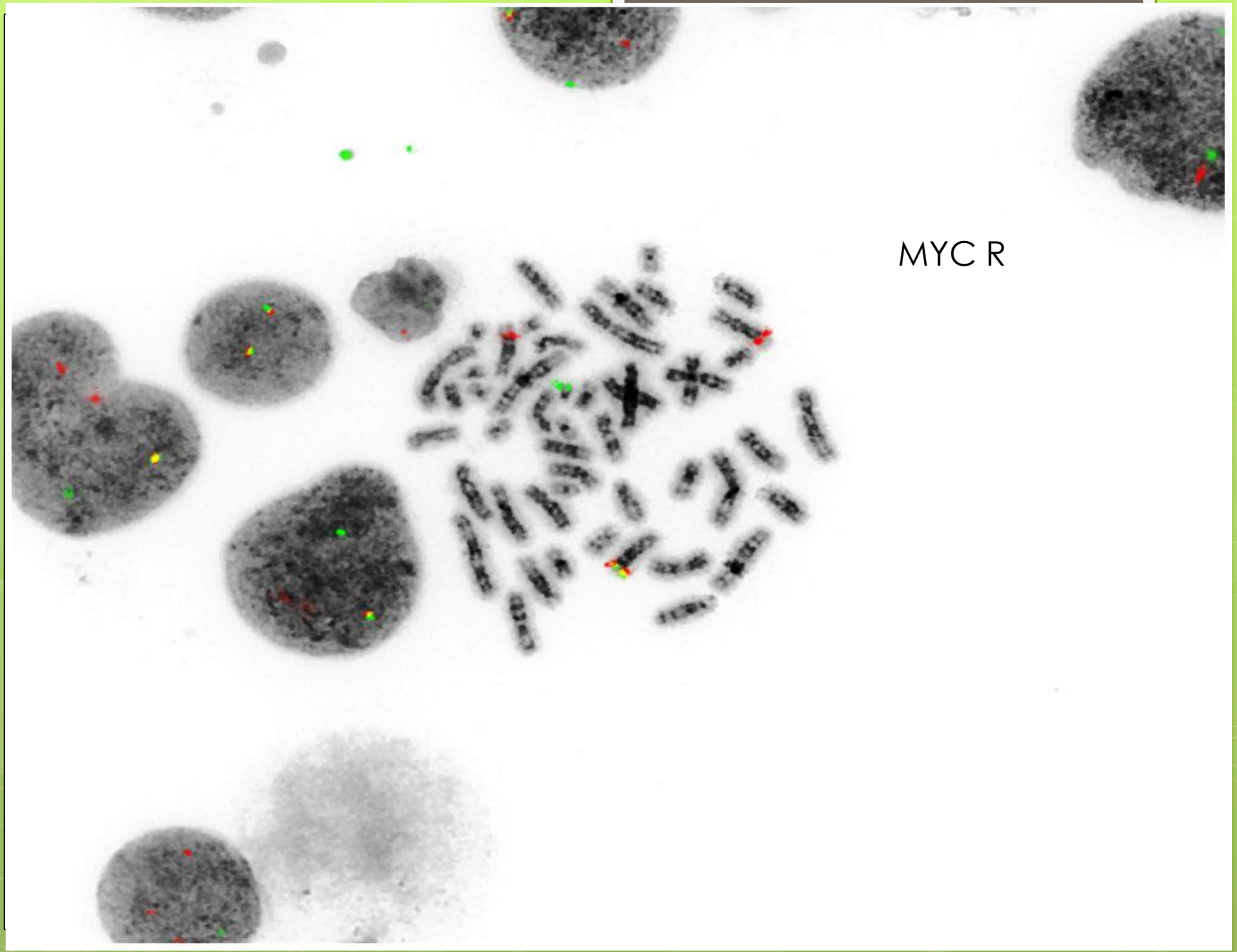


Noyau normal :
2 signaux de fusion



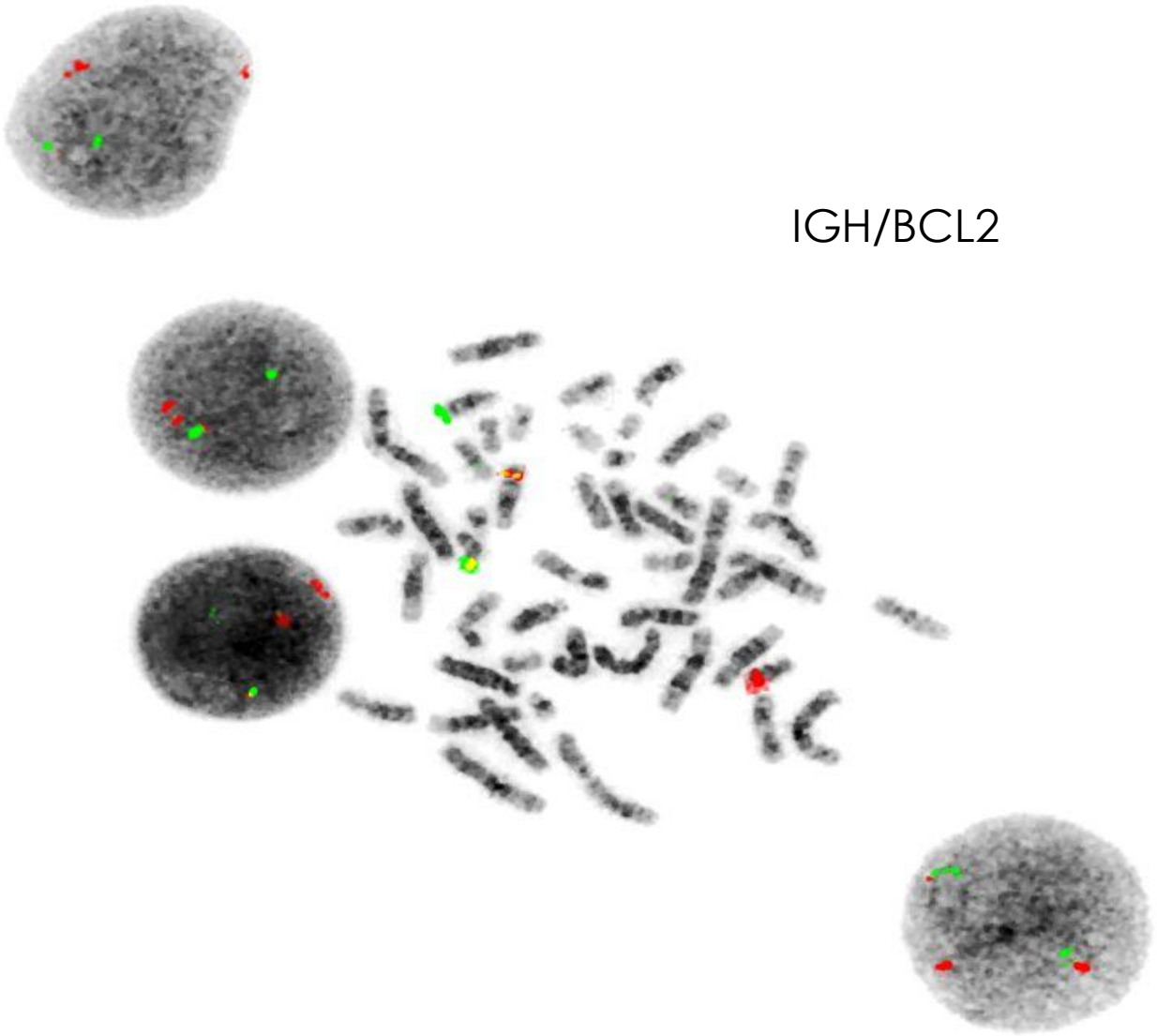
Noyau anormal:
1 signal Vert
1 signal Rouge
1 signal de fusion

Cassure du gène

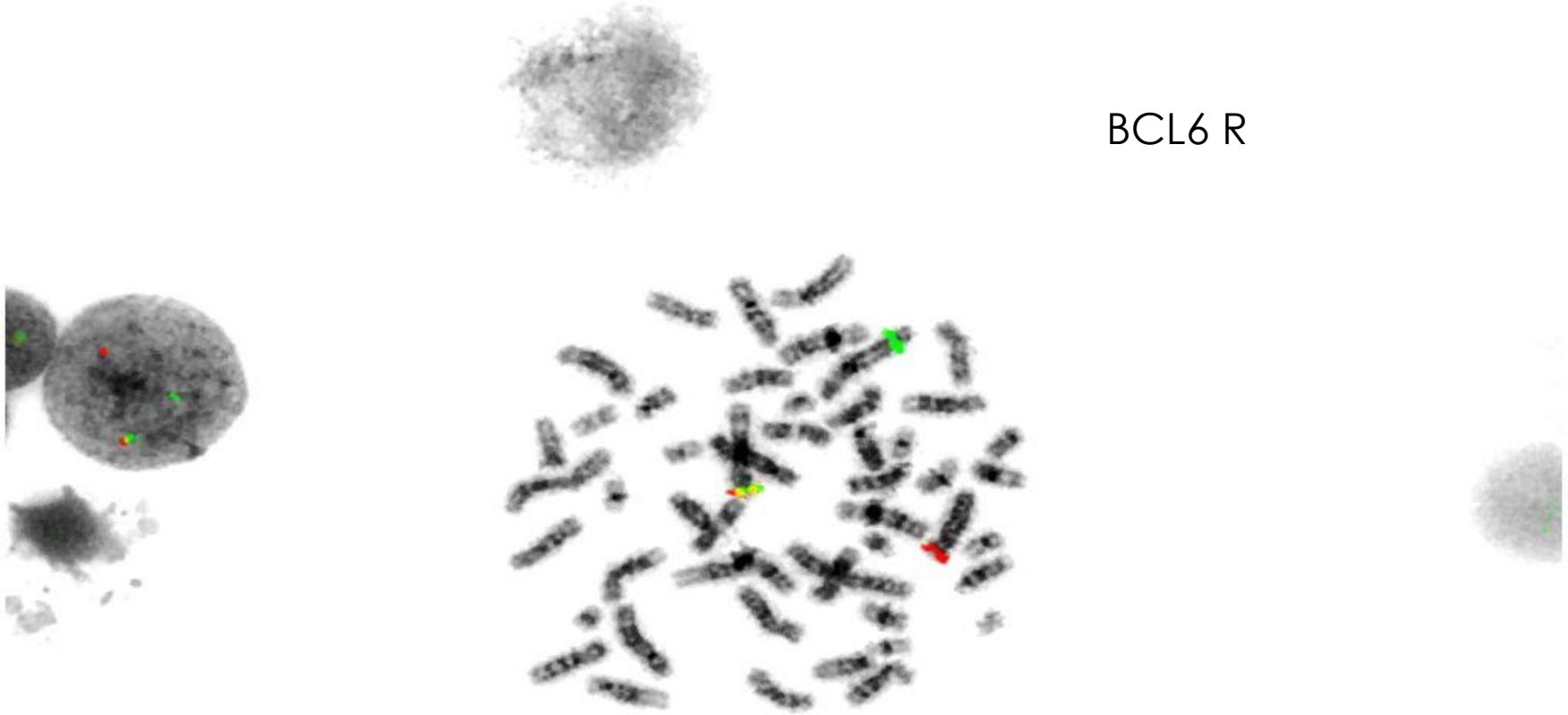


MYC R

IGH/BCL2



BCL6 R



Envoi des lames

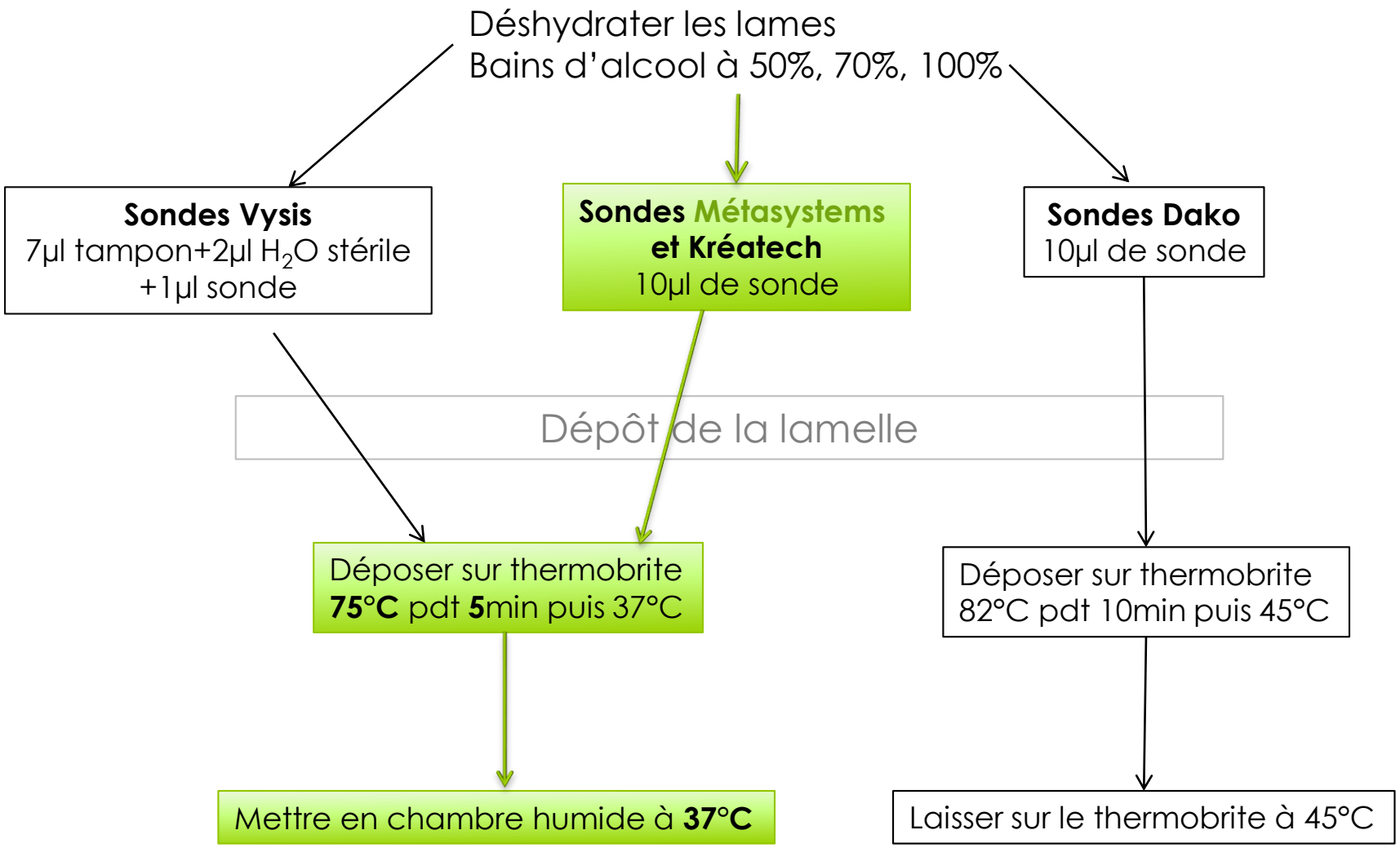
Référence IPM	Envoyé le	Réceptionné le	Référence Hémato/cytogénétique	Remarques
18M00214	01/02/2018	01/02/2018	1802L010868	RT-MLPA
18M00401	23/02/2018	23/02/2018	1802L260462	FISH C-Myc
18M00435	28/02/2018	28/02/2018	0803L010615	FISH C-Myc
18M00522	14/03/2018	14/03/2018	1803L141029	RT-MLPA
18M00831	09/05/2018	09/05/2018	1805L110345	FISH C-Myc
18M00889	17/05/2018	22/05/18	1805L220730(RTMLPA) 1805L220733(Fish)	FISH C-Myc + RTMLPA

Les lames sont transmises, par coursier interne, déparaffinées (45 minutes à 56° puis 3 bains de Xylène et 3 bains d'alcool pour éliminer le xylène (non soluble à l'eau)

FISH sur coupes déparaffinées

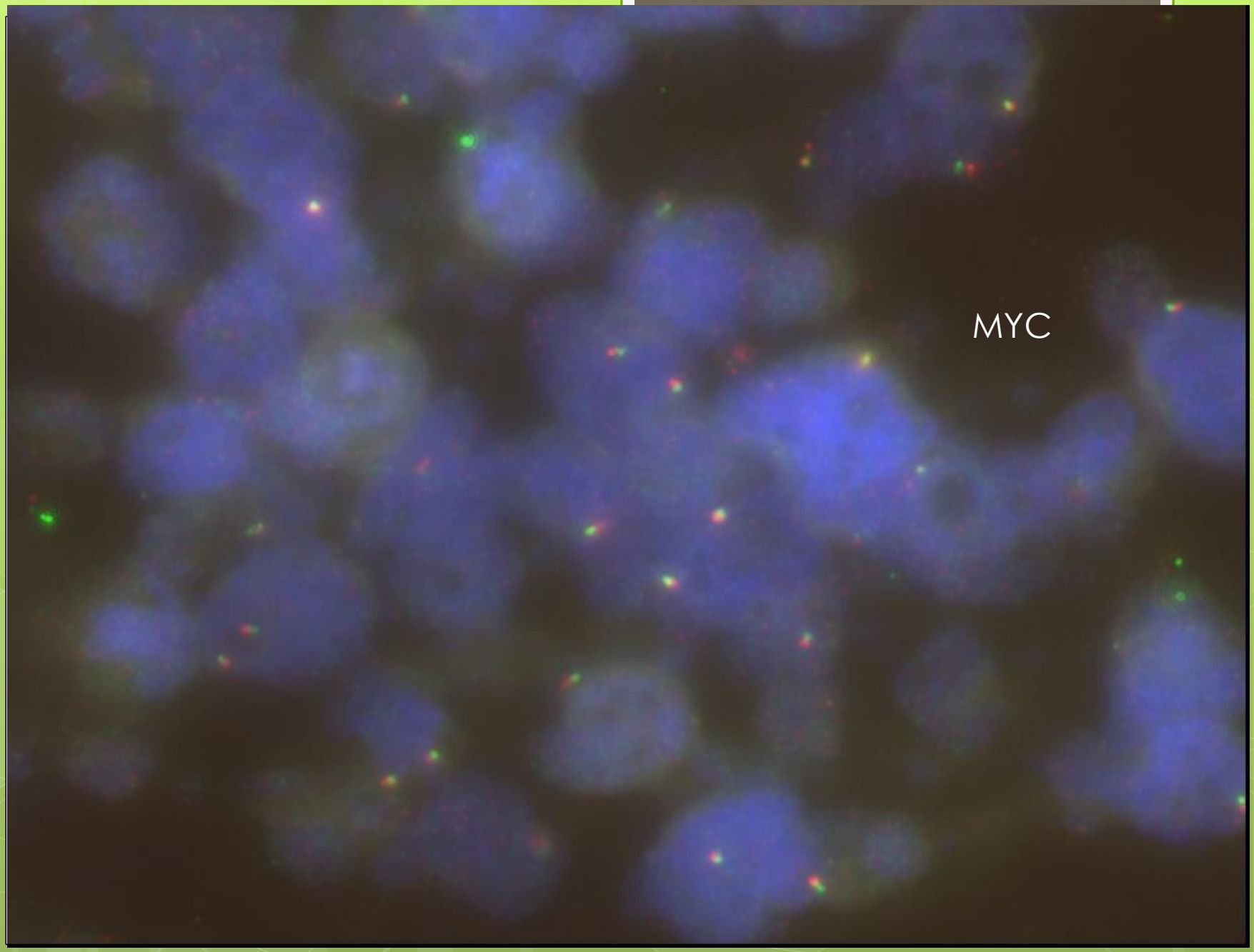
- Immerger la lame dans la solution de prétraitement(sodium thiocyanate = NASCN) à 80°C pendant 10 minutes
- La transférer dans une jarre d'eau distillée pendant 3 minutes
- Plonger la lame dans la solution de protéase (protéase Buffer II + 250mg de protéase) chauffée à 37°C pendant 15 minutes.
- La transférer dans une jarre d'eau distillée pendant 3 minutes
- Sécher à l'air libre pendant 5 minutes.
- Effectuer l'hybridation par codénaturation

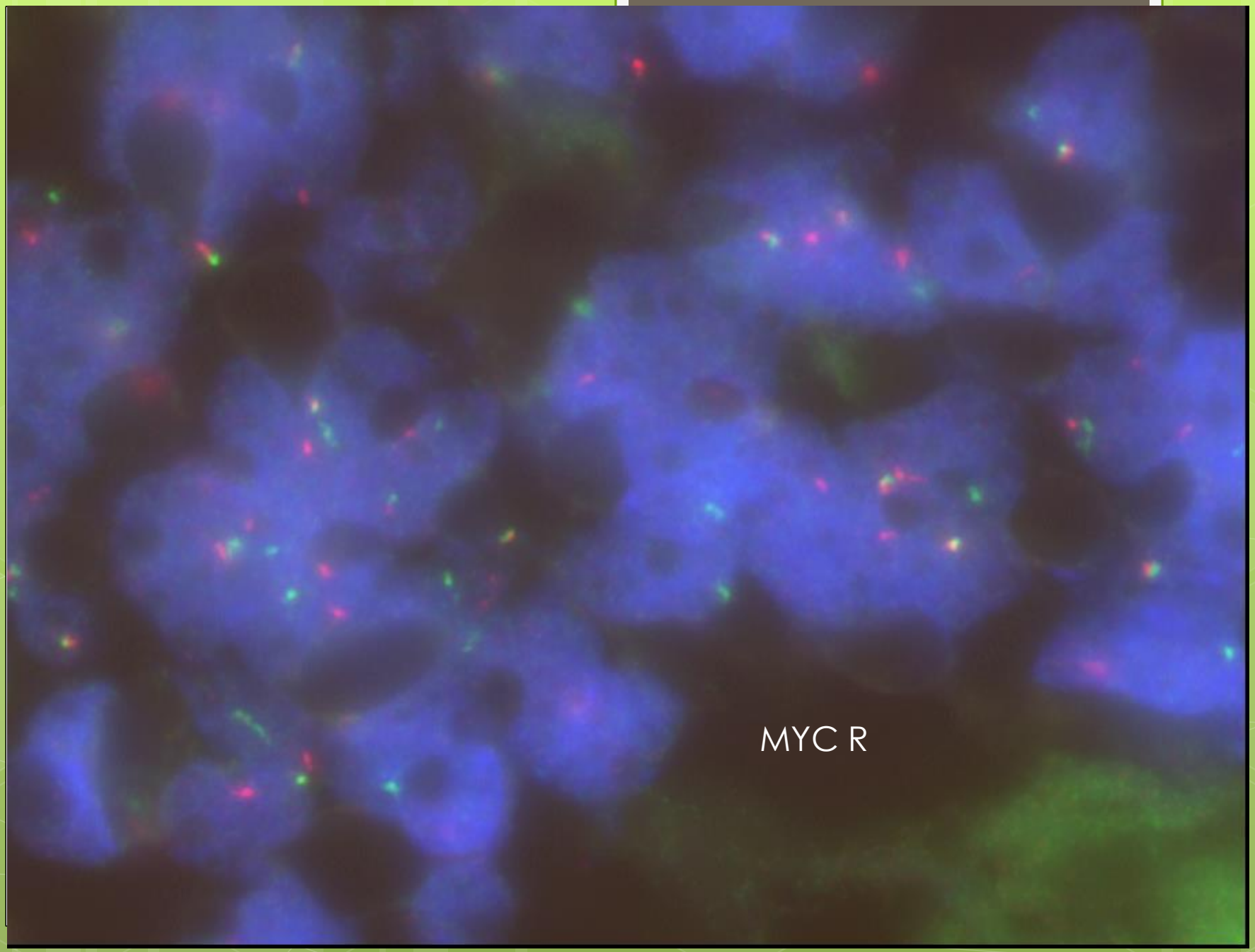
Hybridation des lames par codénaturation

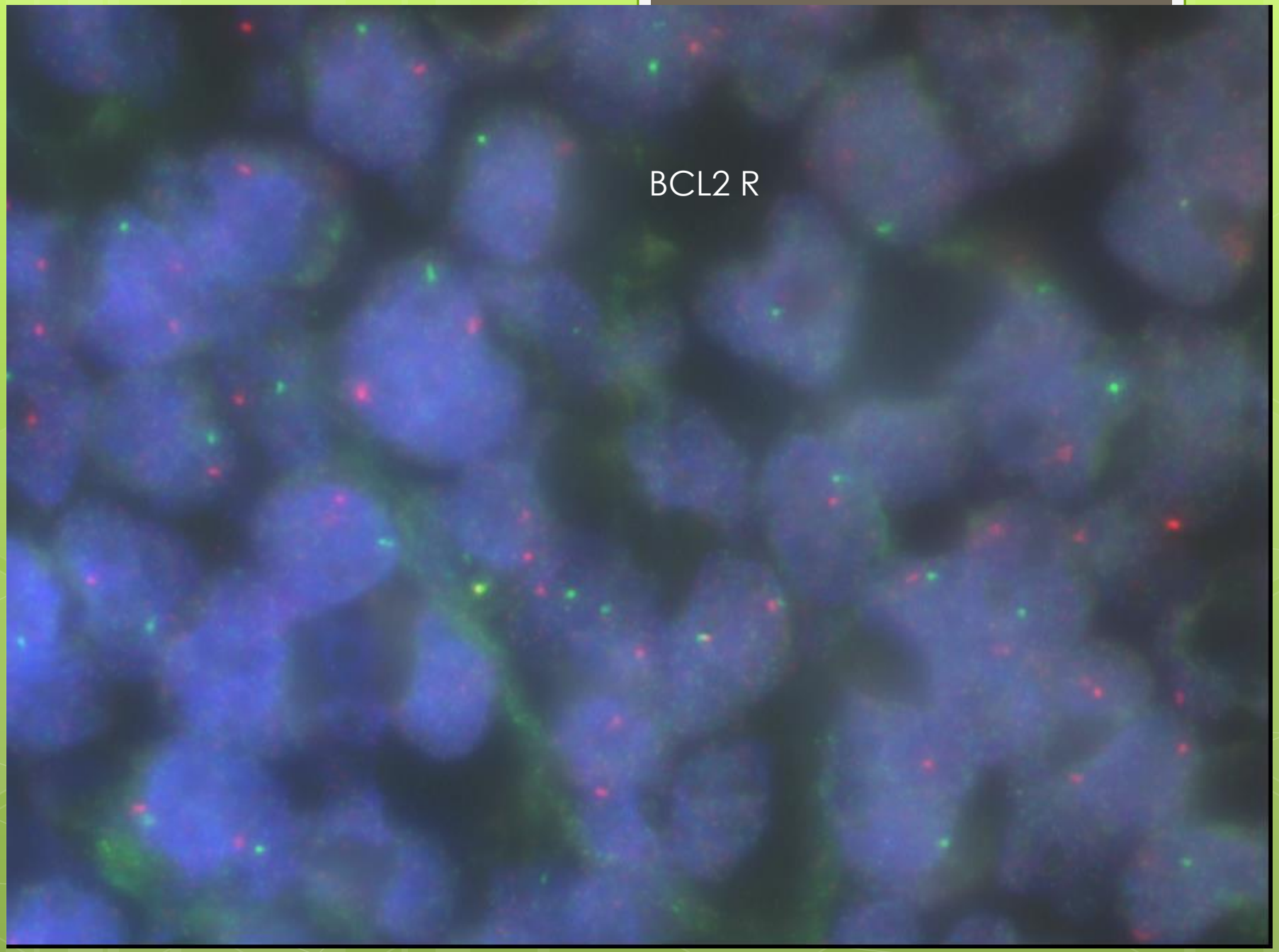


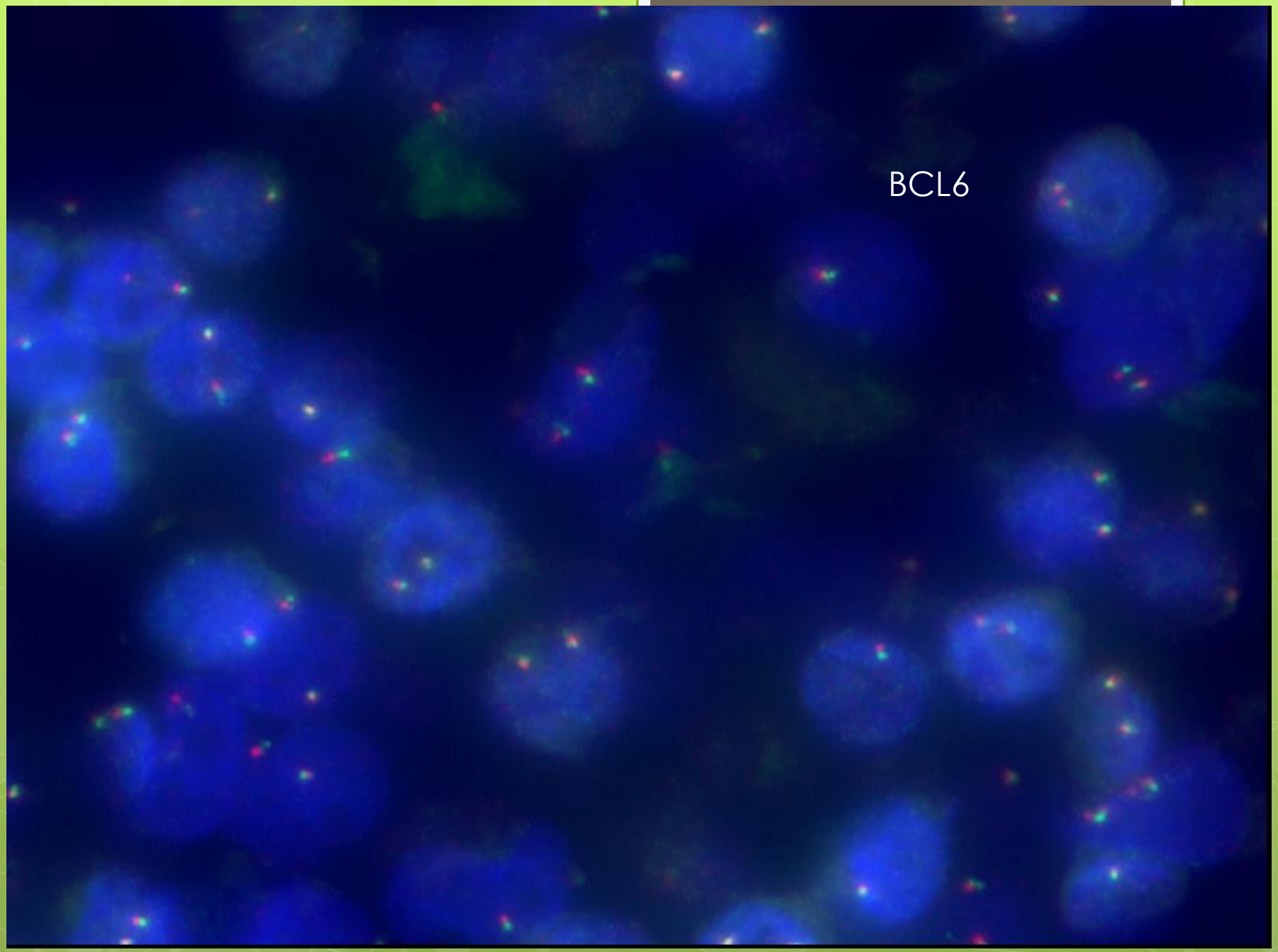
Lavages des lames

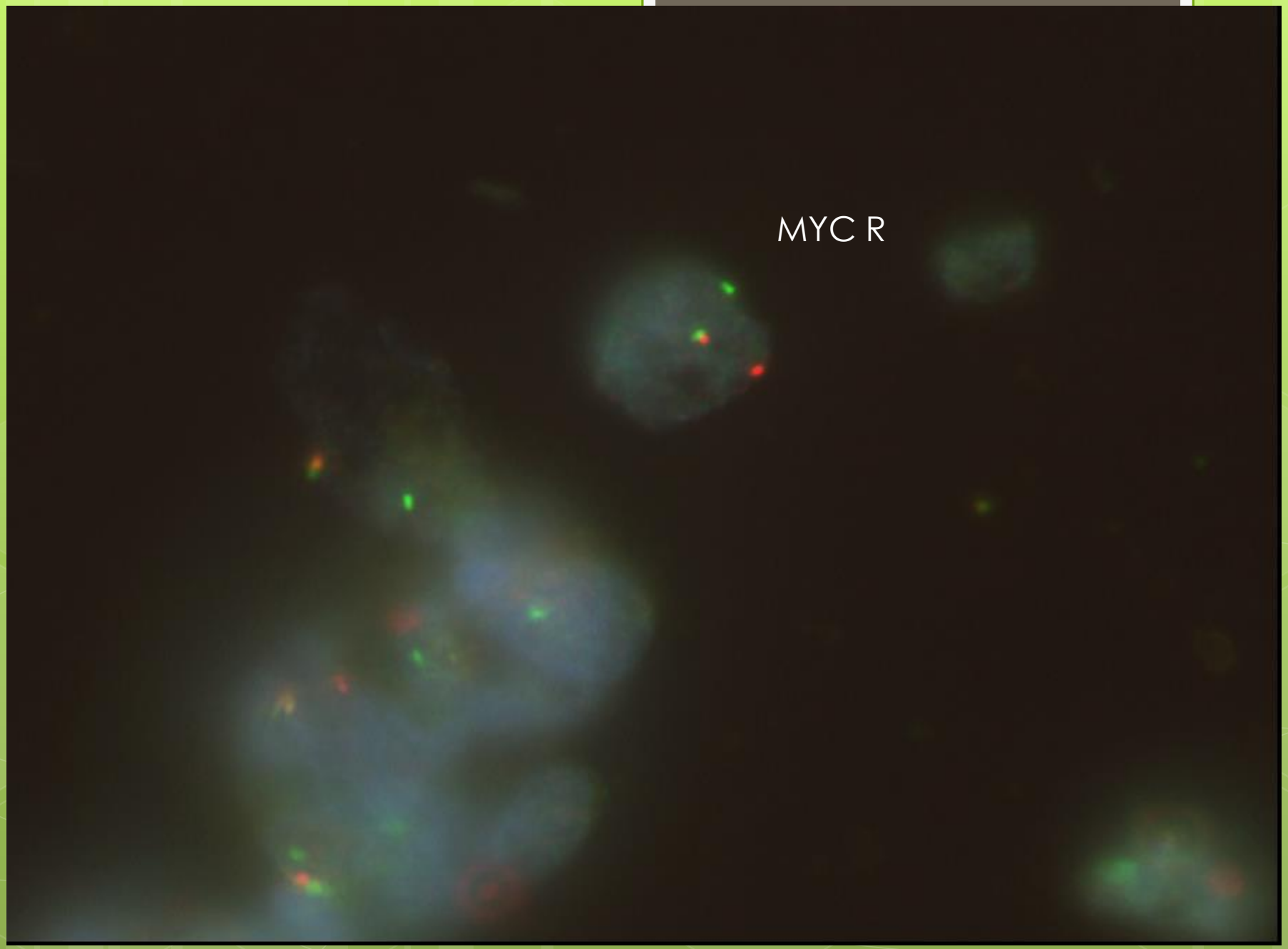
- Enlever délicatement la lamelle
- Plonger les lames dans une solution de 0,4xSSC/0,3% NP40 à 73°C pendant 2 min
- Puis dans du 2xSSC/0,1% NP40 pendant 30 secondes à 1 minute
- Déposer une goutte de DAPI à 0,20 µg/ml (environ 25 µl) sur la lame et ajouter une lamelle 24x60
- Stocker le lames au réfrigérateur et à l'obscurité jusqu'à la lecture.

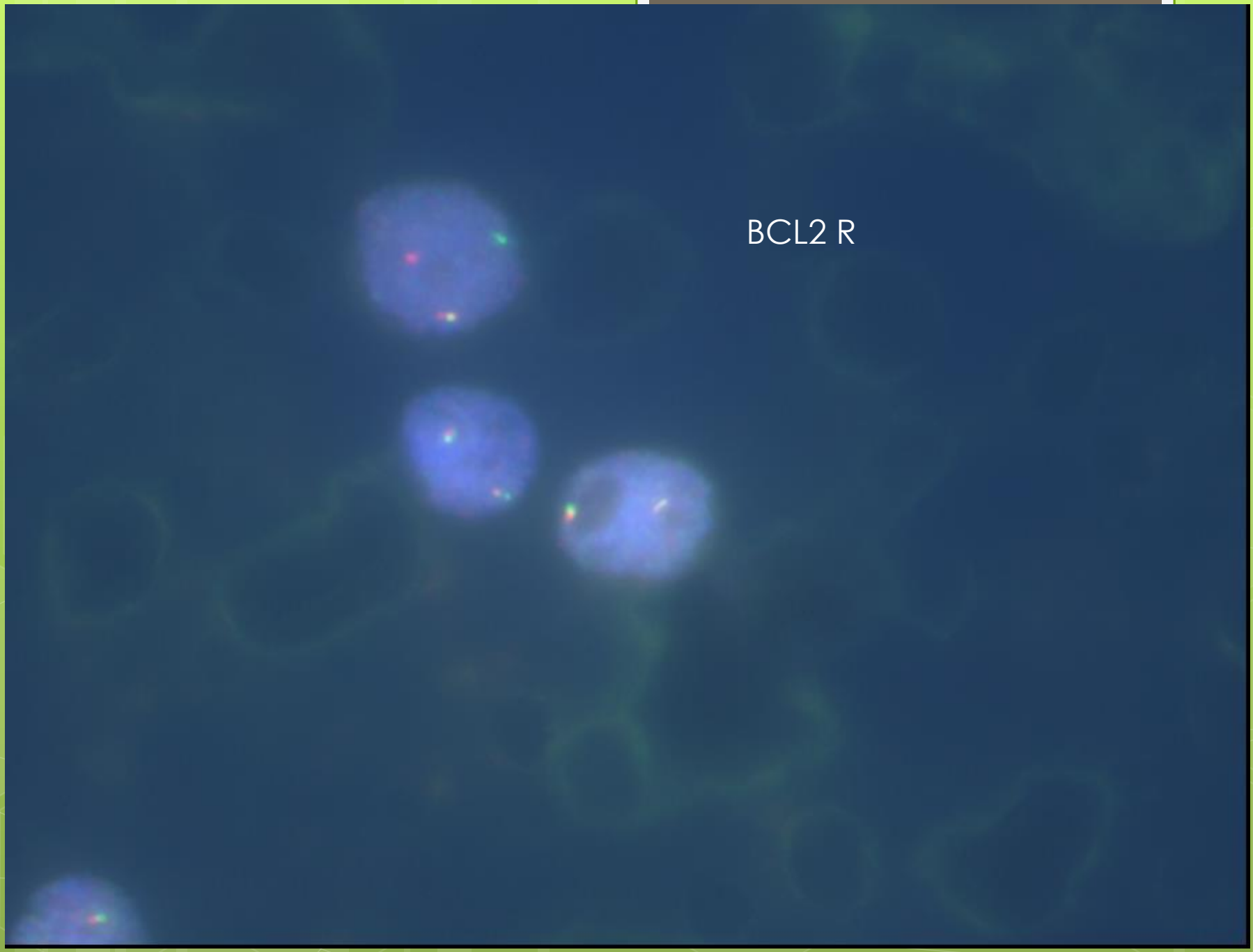

MYC

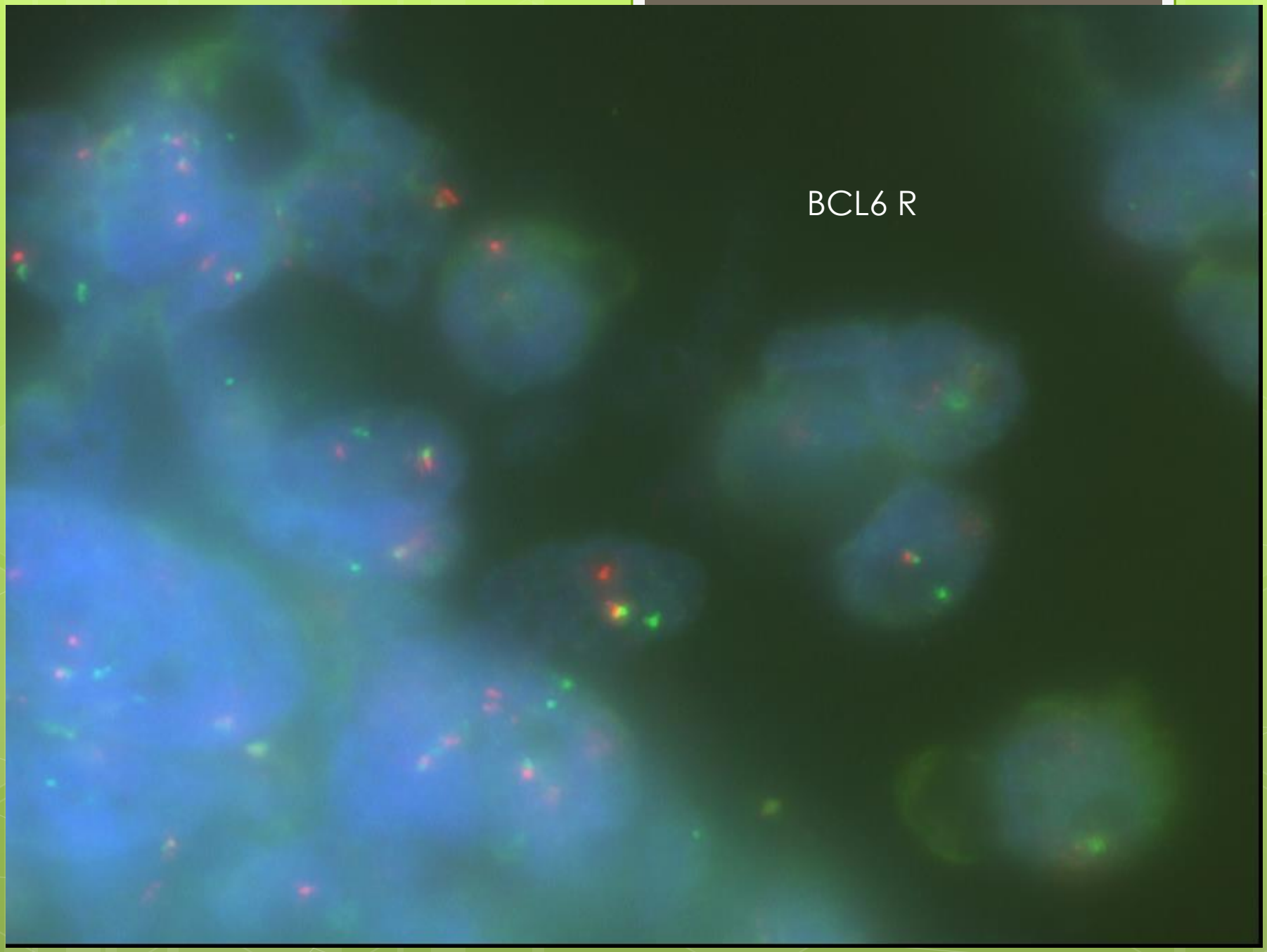

MYC R


BCL2 R

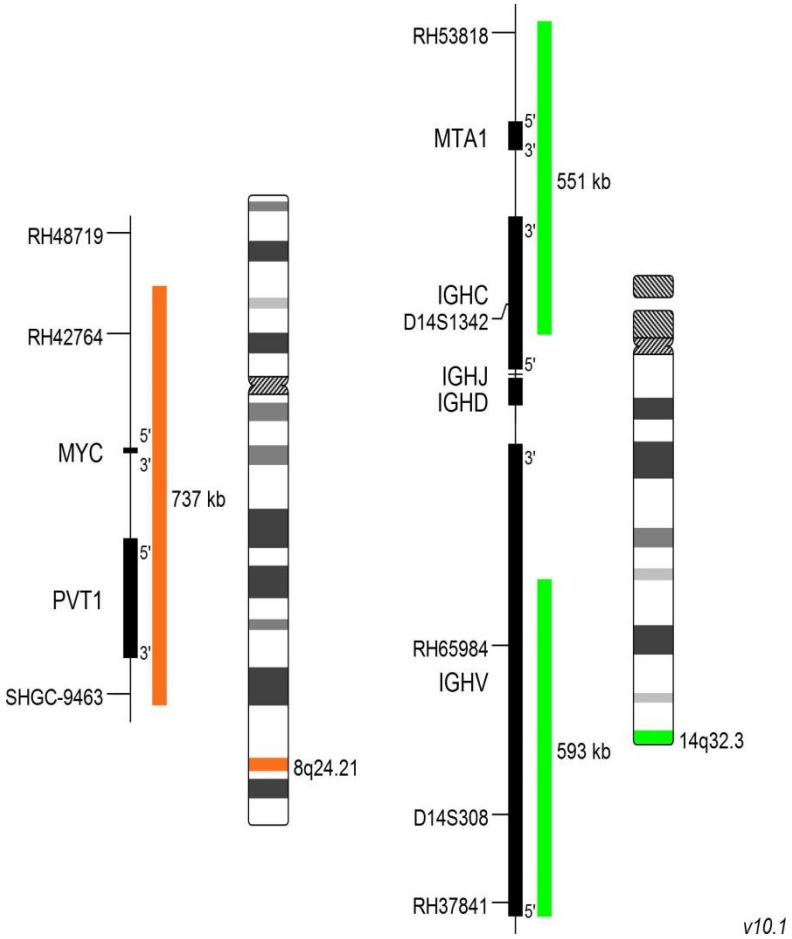

BCL6

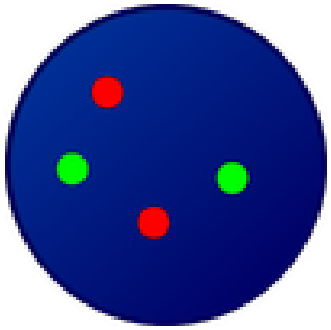

MYC R


BCL2 R

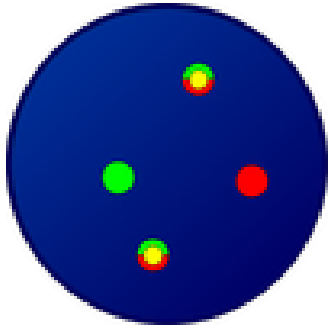

BCL6 R

LECTURE SONDE XL IGH/MYC
Double fusion Metasystems



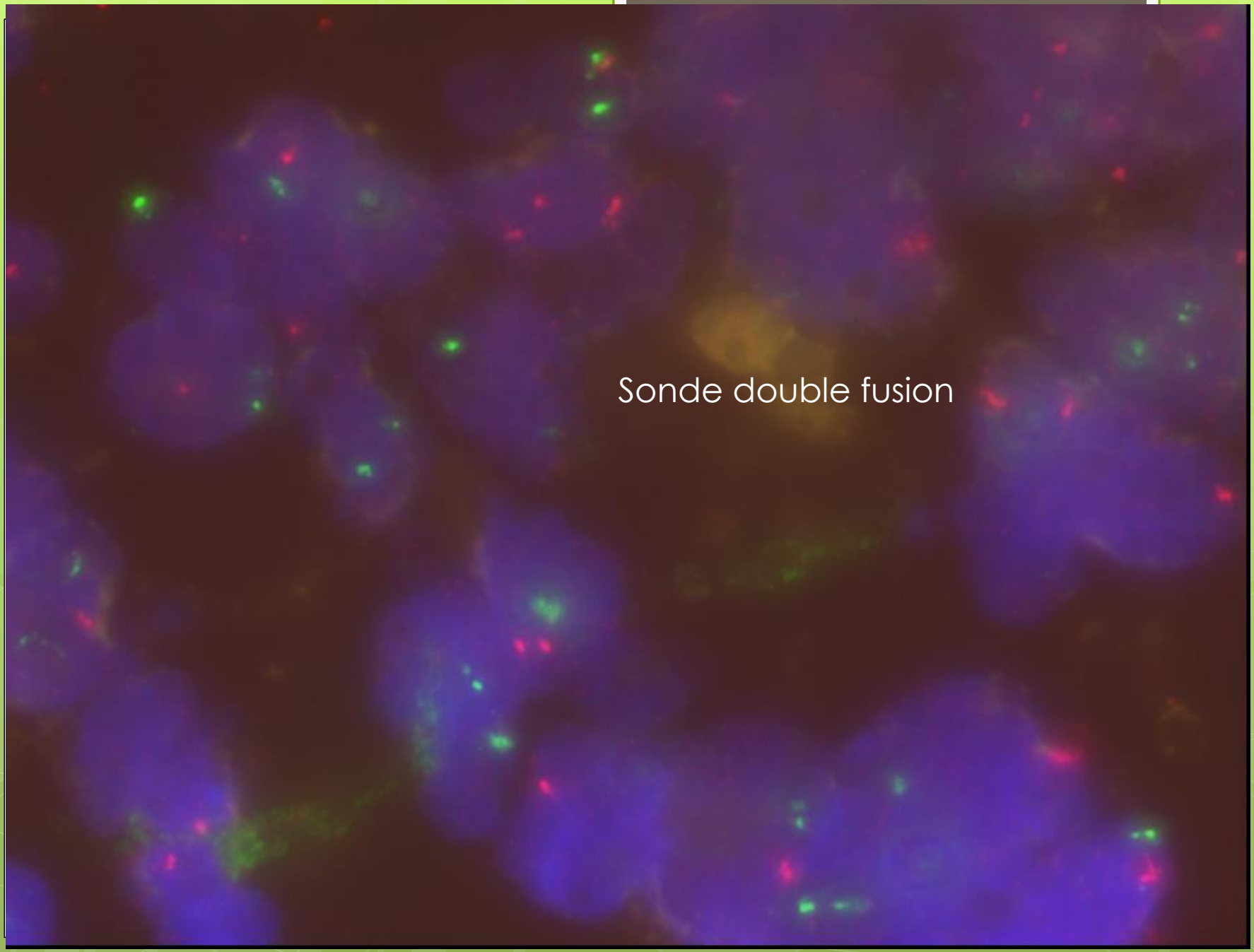


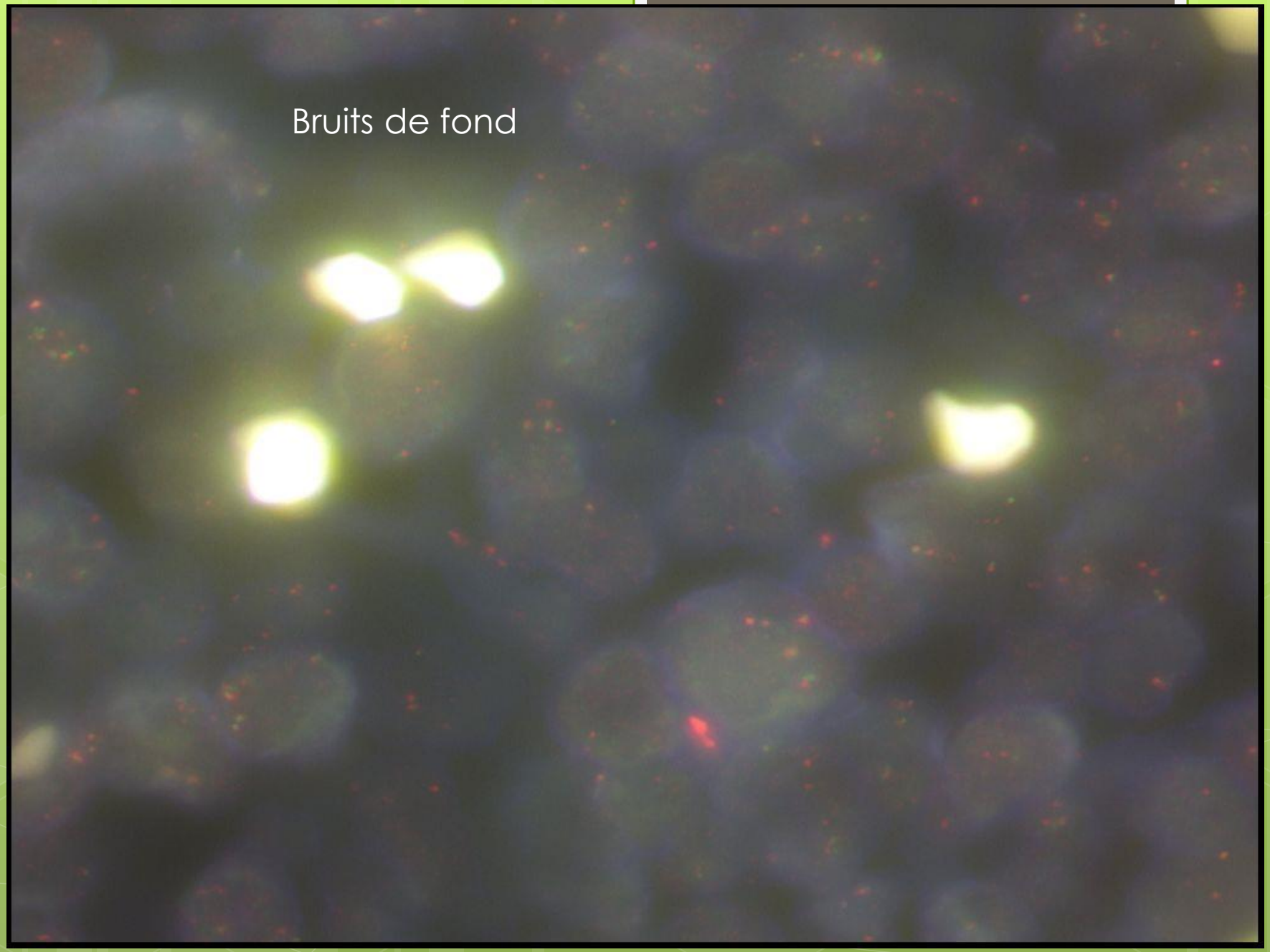
Noyau normal
2 signaux verts
2 signaux rouges
Pas de fusion

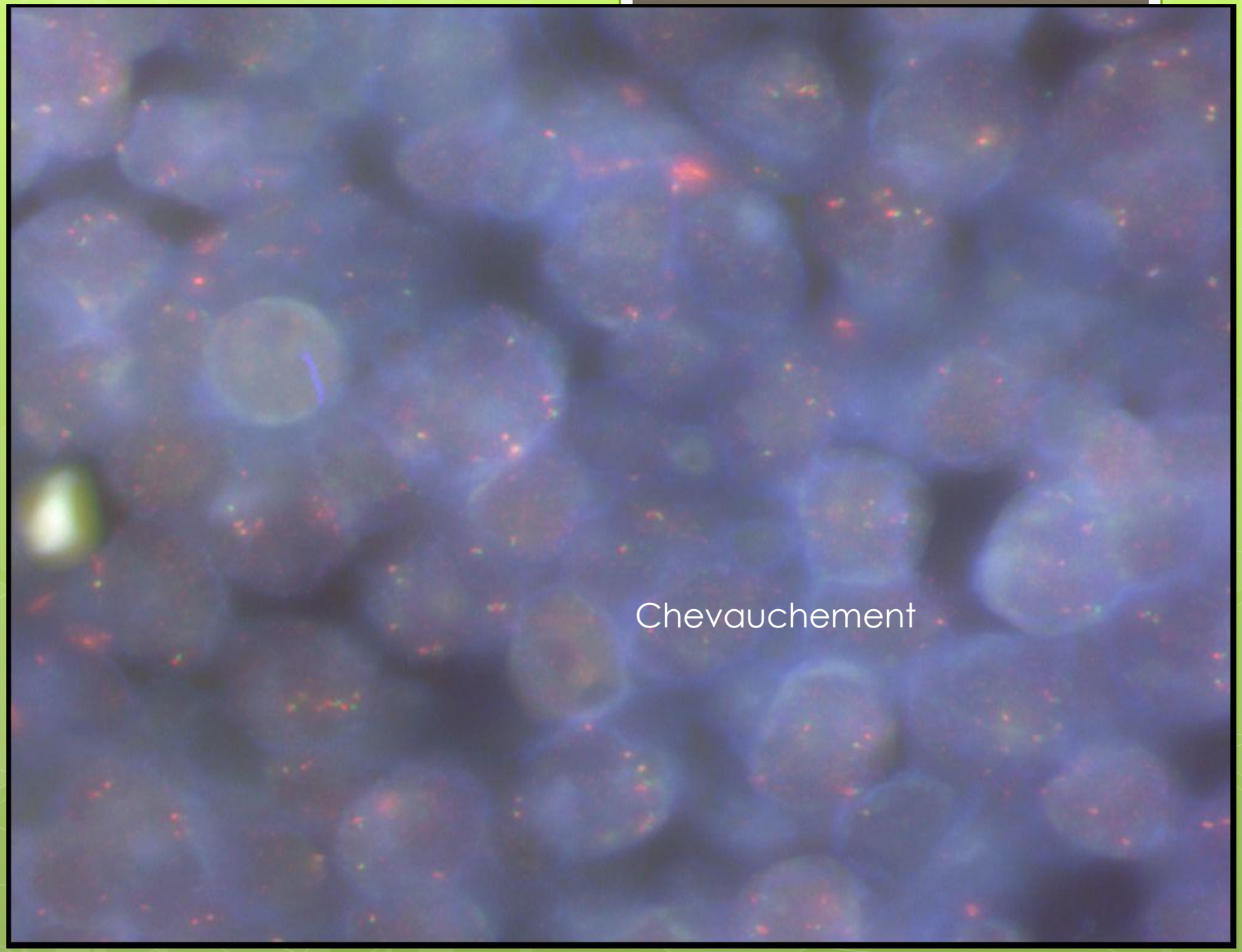


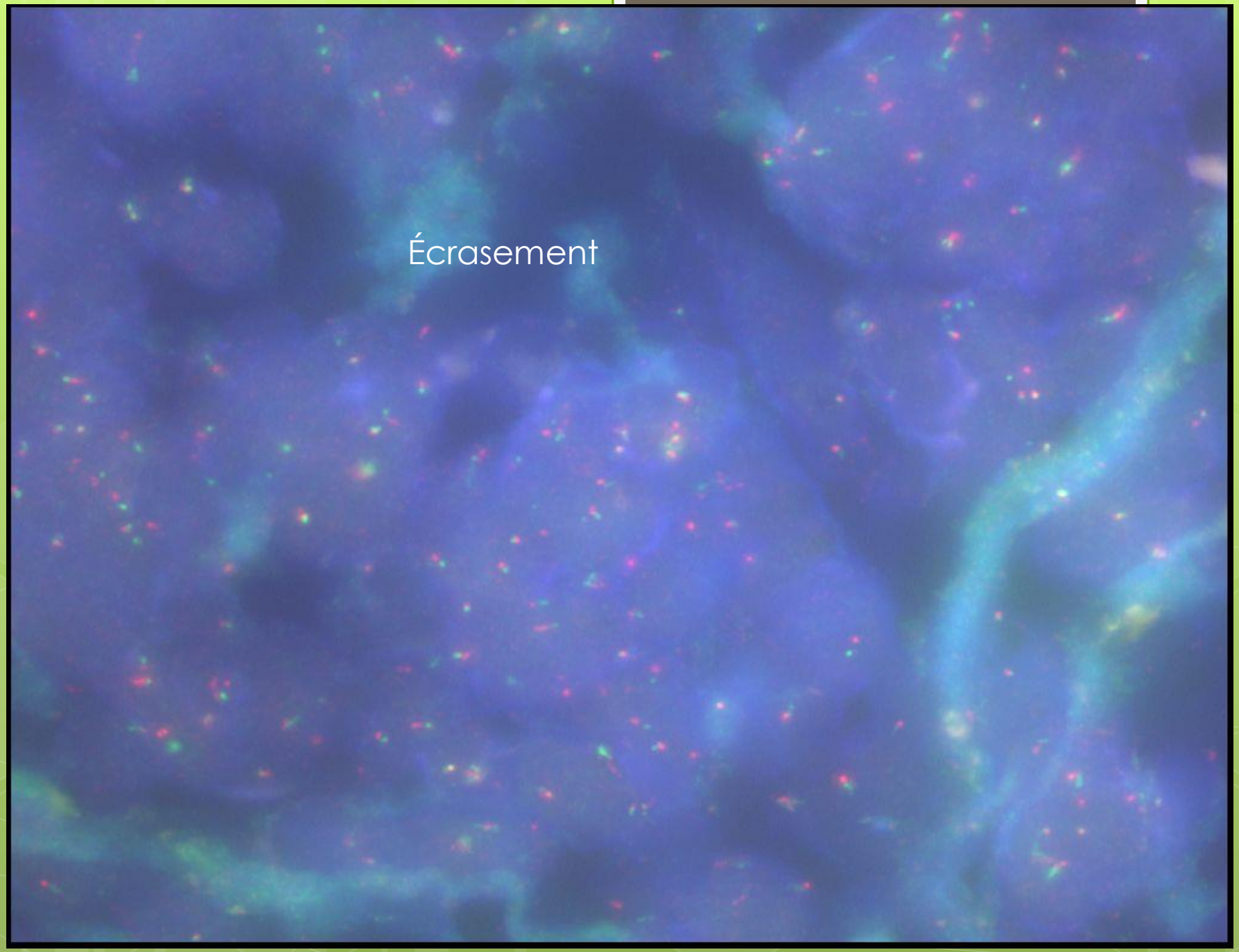
Noyau anormal
1 signal vert
1 signal rouge
2 signaux fusionnés

T(8;14), avec fusion IGH/MYC


Sonde double fusion


Bruits de fond

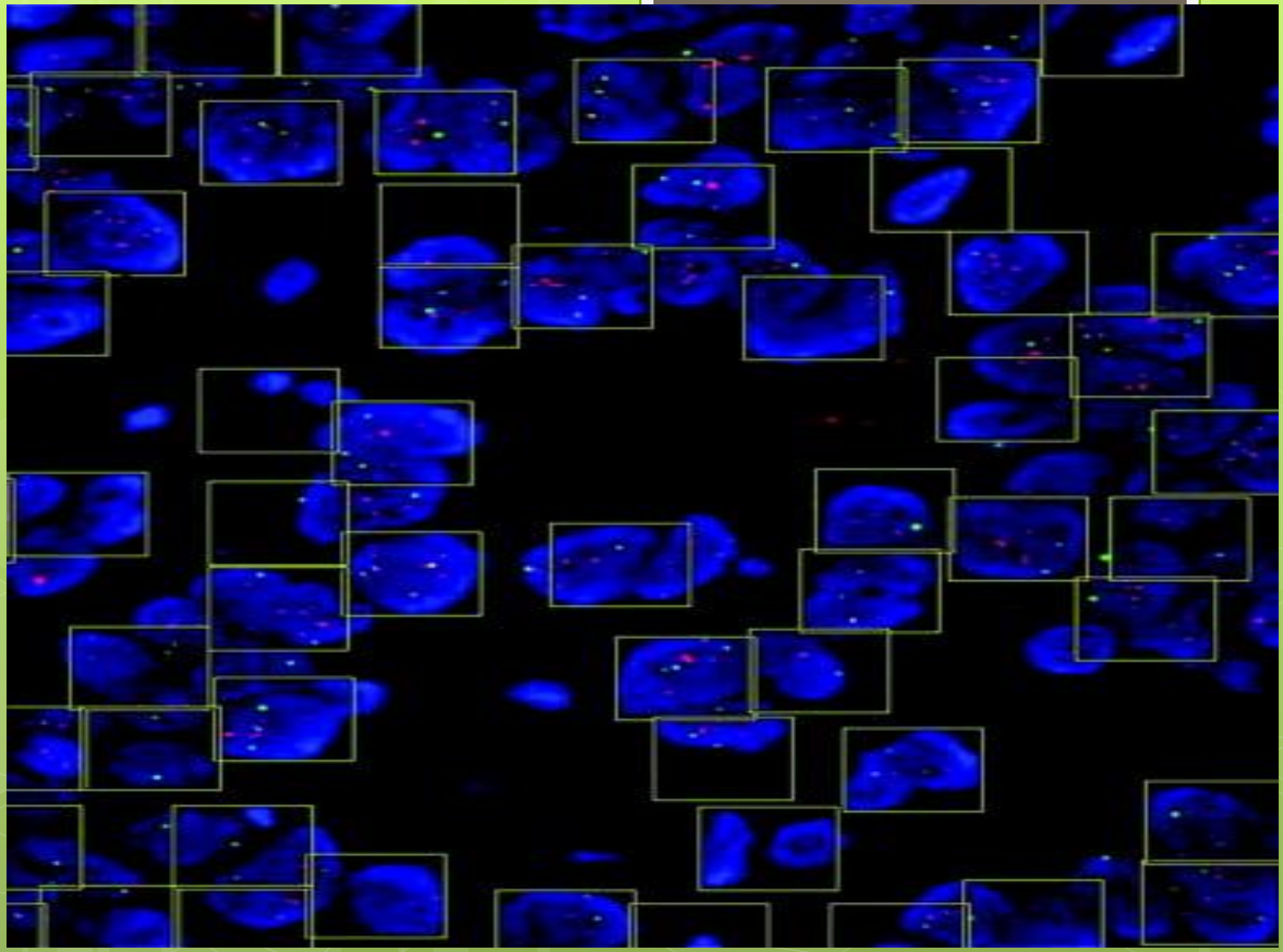

Chevauchement


Écrasement

LECTURE

- Épaisseur de la coupe (Différents plans)
- Bruits de fond
- Aspect des noyaux
  - Chevauchement
  - Écrasement
    - Mal délimité
- Utilisation de sondes BA

Module TissuFISH pour MetaCyte



EN PRATIQUE CLINIQUE (OMS 2016)

Comment poser le diagnostic de Lymphome B de Haut Grade avec DH ou TH ?
Comment sélectionner les cas pour la FISH (DLBCL ?)

- Pas de réel consensus
- Recherche de l'expression de MYC-BCL2 par IHC
- FISH MYC :
  - Se concentrer sur les tumeurs qui ont un profil GC,
  - Le réarrangement de MYC (MYC R) étant rare pour les profils ABC
  - Particulièrement chez les patients « jeunes » (<70 ans)
  - Utilisation de l'IHC pour le dépistage, FISH uniquement sur ces tumeurs qui ont une expression de la protéine MYC >40 %
  - Faire la FISH BCL2 (BCL6 ?) lorsque MYC est réarrangé
  - TT : R-CHOP(CHOP – cyclophosphamide, doxorubicine, vincristine et prednisone ,R-CHOP – CHOP avec rituximab (Rituxan))

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