un evento promosso da

SERVIZIO SANITARIO REGIONALE
EMILIA-ROMAGNA

ISTITUTO ROMAGNOLO PER LO STUDIO DEI TUMORI DINO AMADORI

SCHOOL OF NGS

Nuove frontiere del Next Generation Sequencing nella diagnostica oncologica ed ematologica

04 Novembre 2022

Centro Congressi FEDERICO II Napoli

ANTONIO PINTO - STEFANIA CRISCI

UOSC EMATOLOGIA ONCOLOGICA – INT NAPOLI– IRCCS – G. Pascale

L'NGS nei linfomi: il laboratorio incontra il clinico

NGS IN LYMPHOMAS MAY ENABLE

- diagnostic refinement through multiple genomic biomarkers identified simultaneously (potentially challenging differential diagnoses)
- risk stratification (prognosis and therapy prediction)

…. AND DISCLOSE

- genomic alterations (therapeutically targetable alterations and vulnerabilities)
- biomarkers of drug resistance and real-time monitoring, with early detection of relapse (opening the way for personalized medicine)
- a mutation database (a source for new drugs in lymphoma)

DETECTION CAPACITY OF GENOMIC ABERRATIONS WITH DIFFERENT TECHNOLOGIES

		Single Nucleotide Variants/ InDels	Copy Number Alterations[3]	Structural Variants[4]	IG/TR Clonality	Cell of Origin	Tumor Purity
Targeted	Fluorescence *in situ* Hybridization		✓	✓			
	Single gene analyses[1]	✓			✓		
	Amplicon-based gene panel sequencing	✓			✓		
	Capture-based gene panel sequencing	✓	▽	✓	✓		▽
Digital/ Arrays	Genomic arrays		✓				✓
	Methylation arrays		✓			✓	✓
	Gene expression[2]					✓	
Genome Wide	Whole transcriptome sequencing	▽		▽	✓	✓	
	Whole exome sequencing	✓	▽	▽	✓		✓
	Whole genome sequencing	✓	✓	✓	✓		✓

de Leval L, et al. Blood 2022

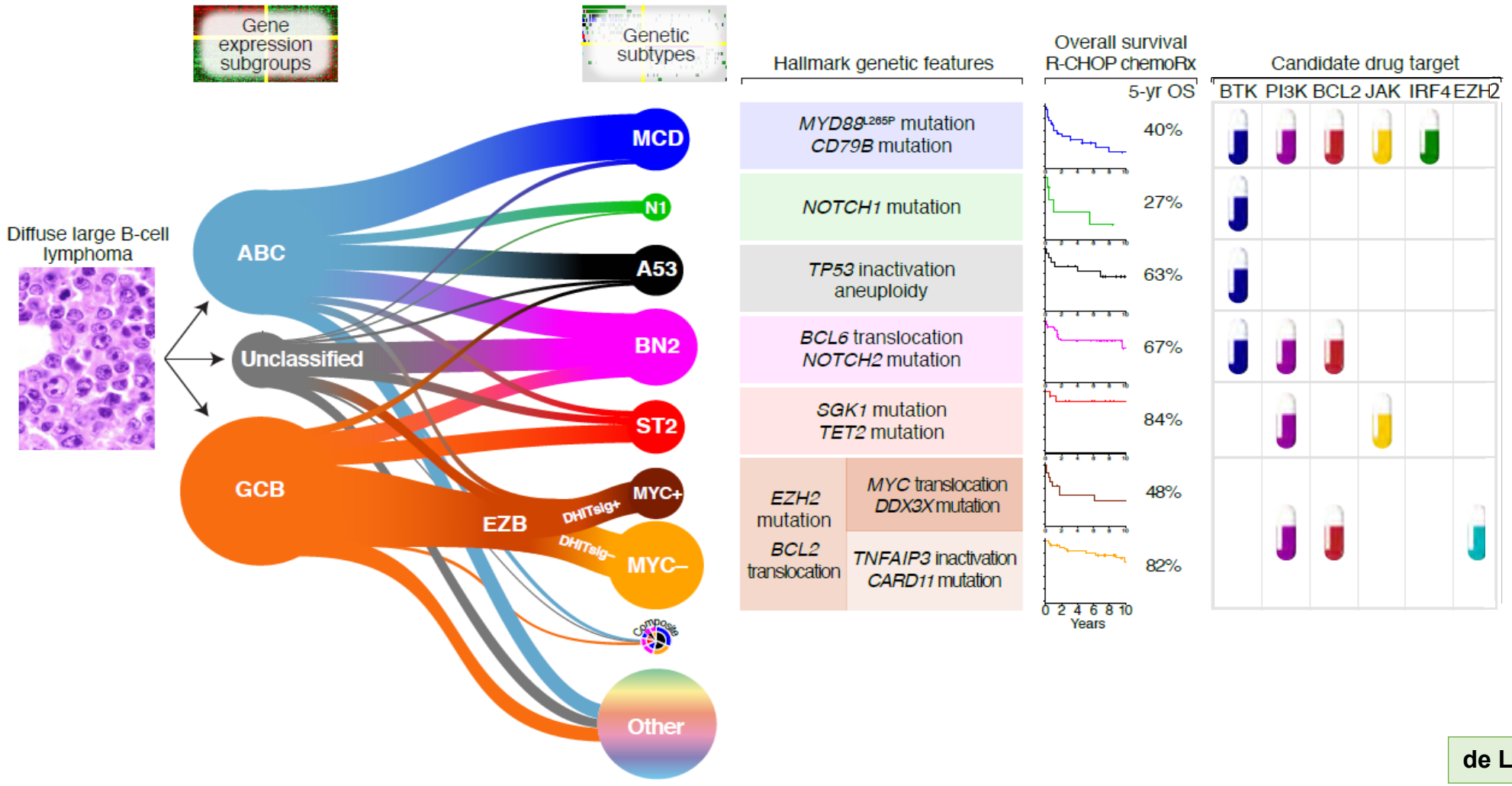
DNA METHYLATION AND CHROMATIN PROFILING

Epigenetic mechanisms play critical roles in lymphomagenesis and have significant clinical diagnostic and outcome implications. A DNA methylation imprint of the cellular origin is useful for diagnostic and patient stratification purposes.

1. **Aberrant histone modifications are critically relevant to lymphomagenesis. Extensive changes in the activity of regulatory elements are targets of drugs such as BET inhibitors. For example**
   - I. **EZH2, after gain-of-function mutations, causes profound spreading of the H3K27me3 promoter repressive mark, which is reversed by EZH2 inhibitors**
   - II. **KMT2D loss-of-function mutations cause loss of enhancer-activating H3K4me1 and may be reverted through inhibition of histone demethylases**
2. **Recurrent hypermethylation of specific genes is harboured by Lymphoid neoplasms, including**
   - I. **CDKN2A, a canonical tumour suppressor gene, is related to disease progression**
   - II. **SMAD1 is a biomarker for chemotherapy resistance**
   - III. **TET2 may present epigenetic modifier mutations due to a hypermethylation effect that drives an aberrant cytosine methylation patterning, a universal finding in lymphoid neoplasm.**

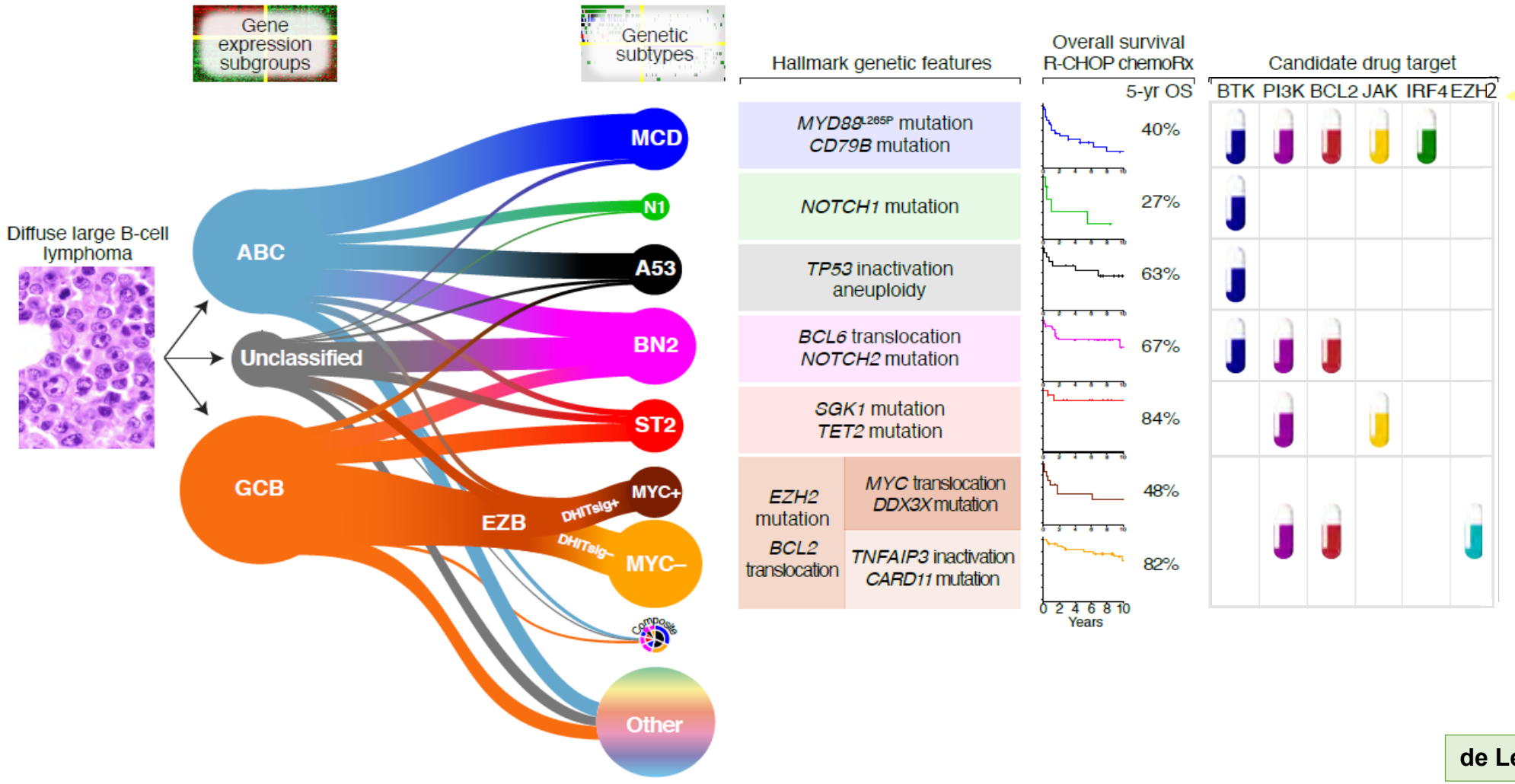
de Leval L, et al. Blood 2022

APPROACH TO DIAGNOSING HIGH-GRADE B-CELL LYMPHOMAS

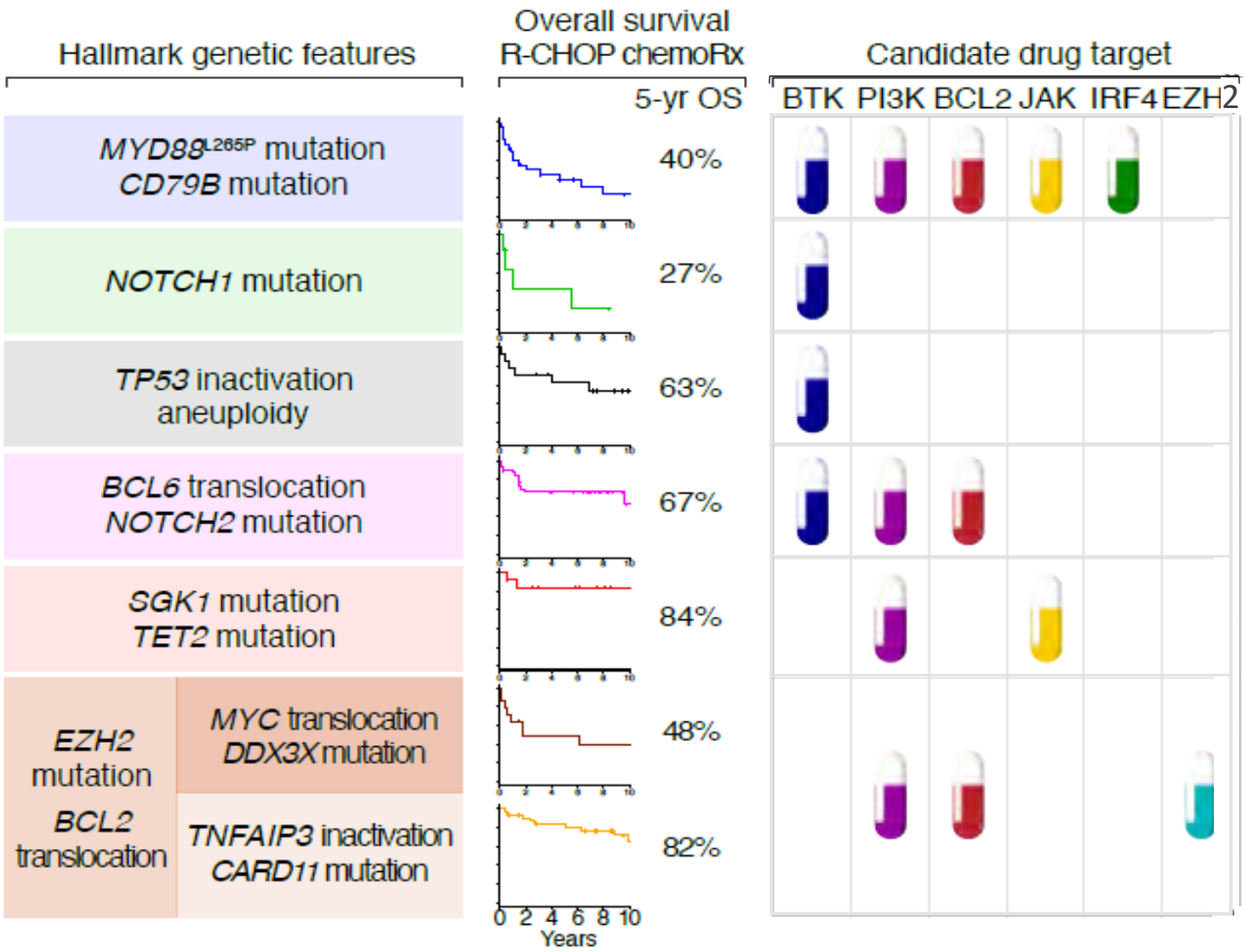


de Leval L, et al. Blood 2022

APPROACH TO DIAGNOSING HIGH-GRADE B-CELL LYMPHOMAS



de Leval L, et al. Blood 2022



de Leval L, et al. Blood 2022

REAL LIFE

NGS in Lymphoma Diagnosis

PRACTICAL CONSTRAINTS:

1. The optimal source is nucleic acids extracted from fresh surgical biopsies or liquid samples.
   ⇒ Yet, clinical assays still need to be optimally fit for formalin-fixed paraffin-embedded (FFPE) tissues, the common diagnostic material. Also, fresh needle biopsies are suboptimal samples.

2. Large and complex panels by capture-based NGS are required to analyze chromosomal aberrations, CNA, SNV and INDEL simultaneously and to provide uniform coverage for sensitive detection of subclonal somatic abnormalities.
   ⇒ Still, amplicon target enrichment is applied as well.

3. Tumour cell content, gene selection, sequencing platform, sequence coverage/depth, background artefacts, unique molecular identifiers, variant interpretation and turnaround time are critical parameters for NGS-based assays.
   ⇒ Still, the procedures and the quality of data differ among laboratories.

4. A comparison of sequential biopsies may be necessary depending on the clinical question.
   ⇒ Still, only the most recent sample is available for analysis in case of disease recurrence.

Navrkalova V, et al. The Journal of Molecular Diagnostics 2021

NGS in Lymphomas - Next steps

Validation & Standardization

There has yet to be a standard approach. Features such as gene selection, sequencing platform, read depth, and variant analysis can differ among laboratories

SOP

A standard operating procedure (SOP) for the classification of the oncogenicity of somatic variants should be devised

Liquid biopsies

The use of NGS on liquid biopsies will be a breakthrough not only towards tailor-made therapies at diagnosis but also towards a real-time and dynamic monitoring of tumour responses to treatment

NGS DIAGNOSTIC ALGORITHM

PRE-ANALYTICAL (1)

A. Clinical History
B. Sampling method
C. Sample stratified diagnostic vs. monitoring

ANALYTICAL (2)

A. Sample preparation
B. Library preparation, pooling

→ **NGS SEQUENCING and Generation of FastQ file**

INTERPRETATION (3)

A. Reads Alignment and Variant Calling
B. Annotation
C. Filtering
D. Validation/Interpretation (SNV, INDELs…)

REPORT (4)

A. Report generation:
- Mutation hotspot
- Actionable variant
- Prognostic value

B. Clinical decision making

Clinical Case

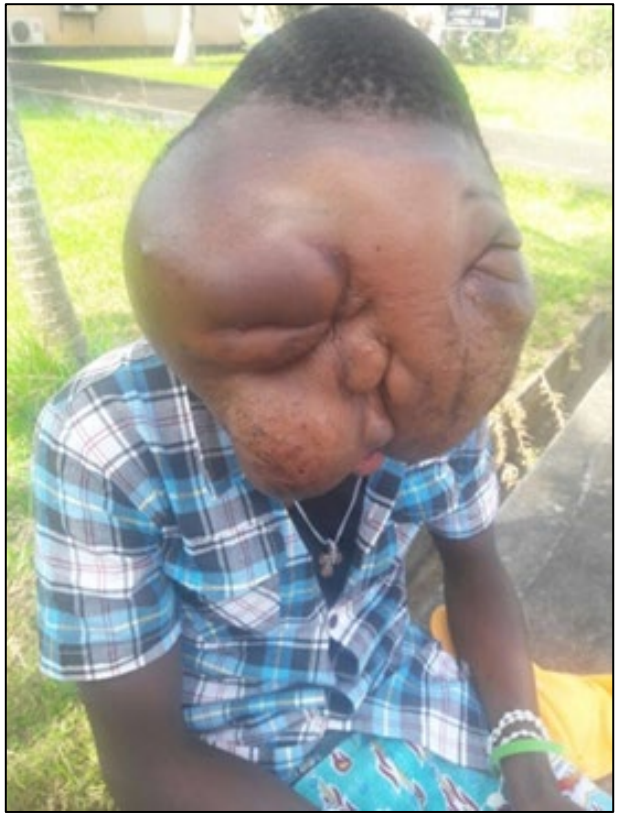
A STRANGE KIND OF NHL

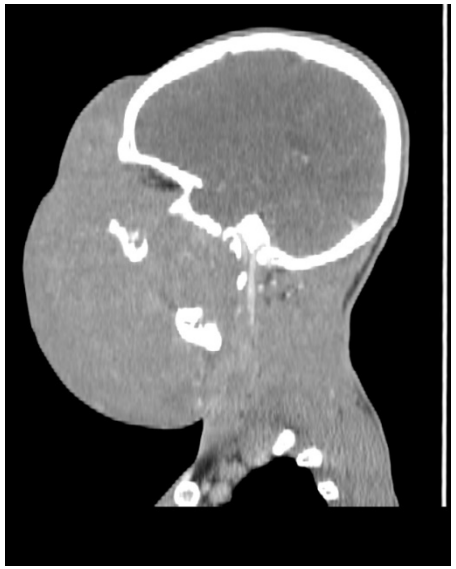
«…between the stables and the 'starry sky'!»

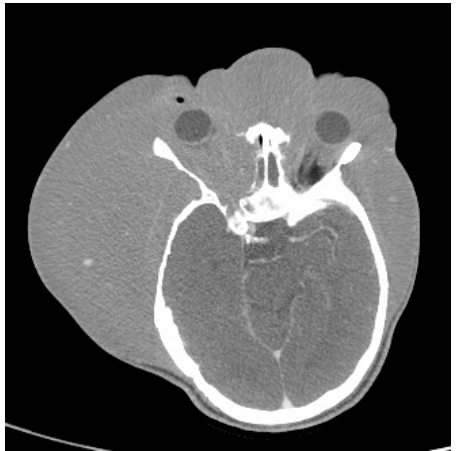
Topics covered

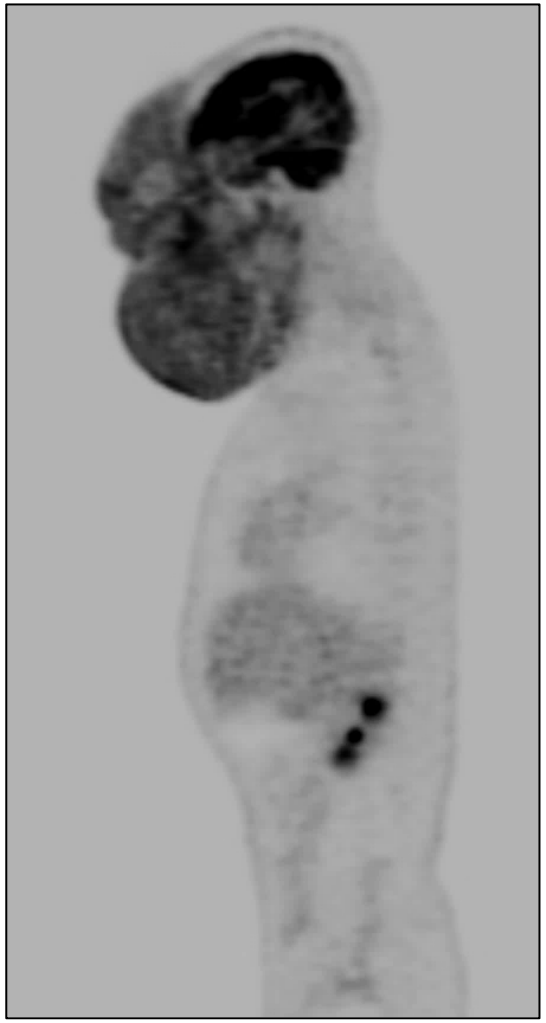
- Troubled diagnosis at onset (2016) and relapse (2022)
- Contradictory clinical picture
- Response to induction treatment?
- Late progression yet contradictory
- Contribution of NGS and new therapy

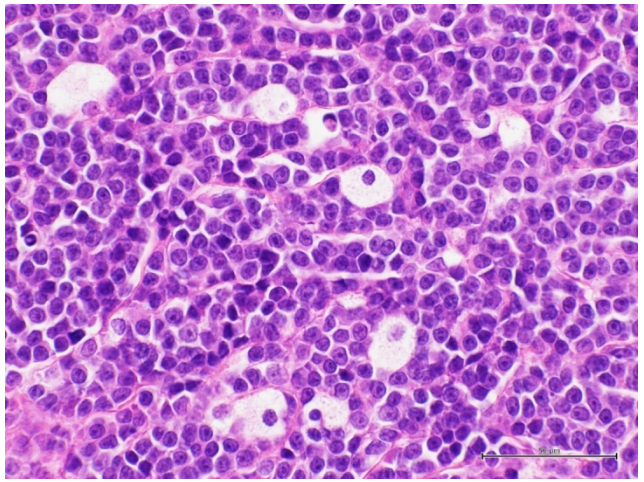
OCTOBER 2016, Multiple biopsies: Indolent NHL (Ivory Coast-France) vs. high suspicious for Burkitt lymphoma with t(8;14) and 'STARRY SKY' (Naples)

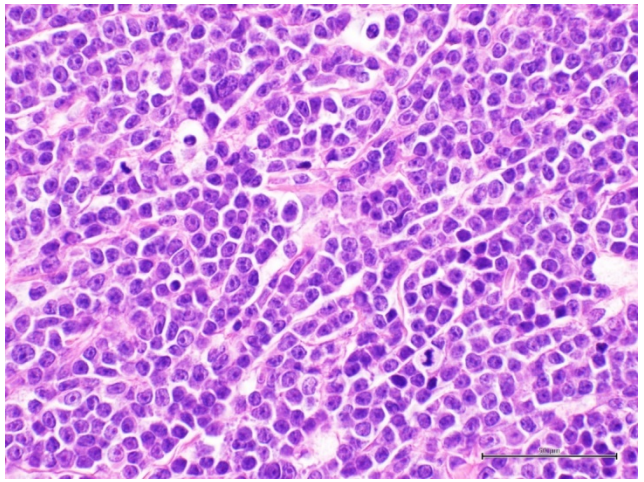






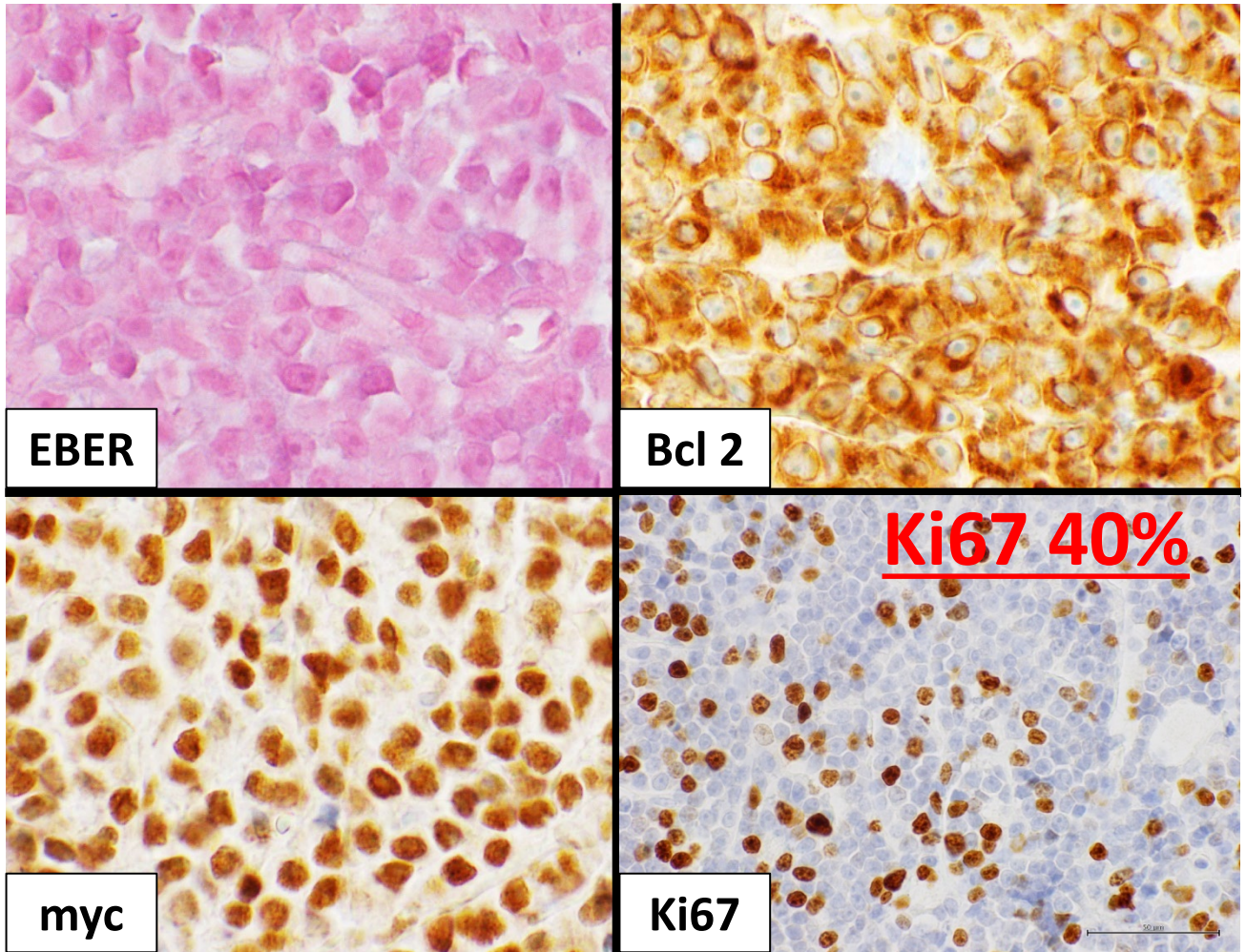


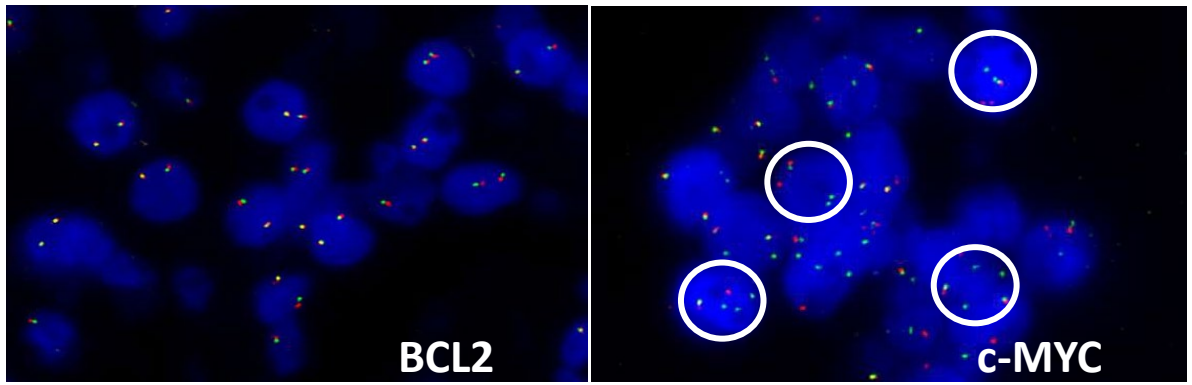




- Age: 17, Male
- African ethnic origin

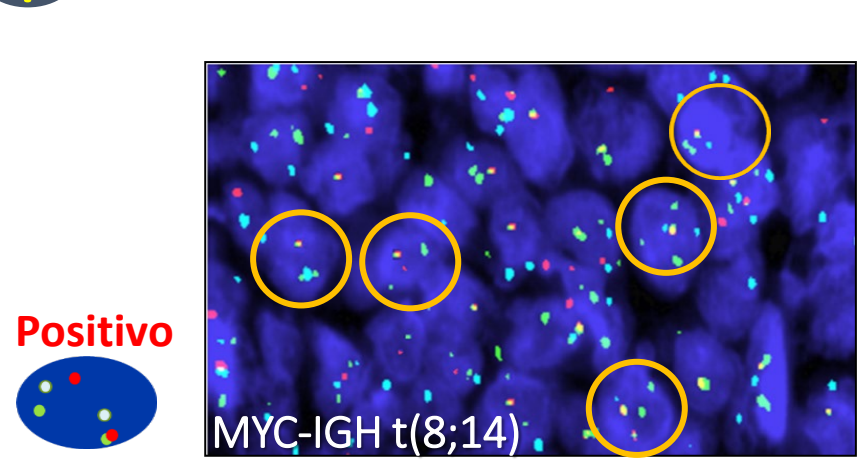
IHC: CD20+CD10-,BCL6-, BCL2+(100%), MUM1+,c-myc+(100%),EBER-,Tdt-,Ki67 40%





Negativo

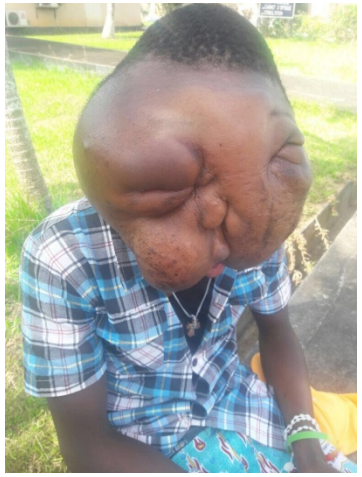
Positivo



Positivo

FISH: MYC-IGH t(8;14): POSITIVE

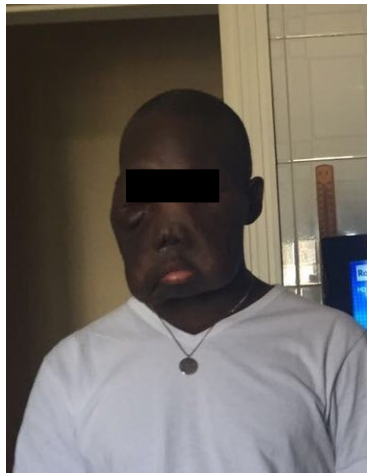
PCR: IGH/BCL2 & IGH/CCND1 & API2/MALT1: all NEG.,
IGH rearrangement monoclonal



Induction Treatment

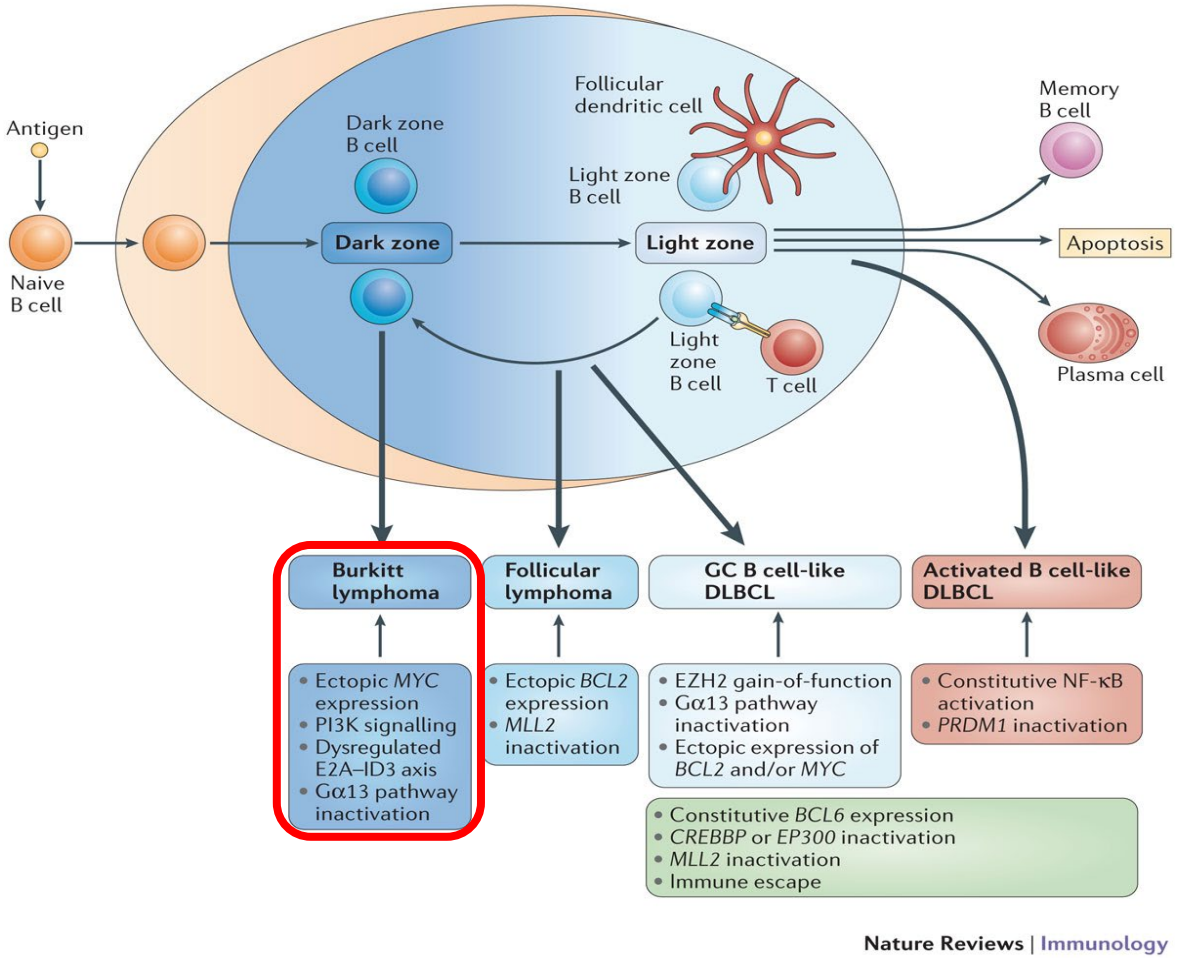
R-CODOX-M/R-IVAC x 4 cycles, w/ SNC prophylaxes → Partial response (only!)

JAN 2017: 2nd line treatment w/ GDP (2 cycles) +
IFRT followed by ASCT consolidation

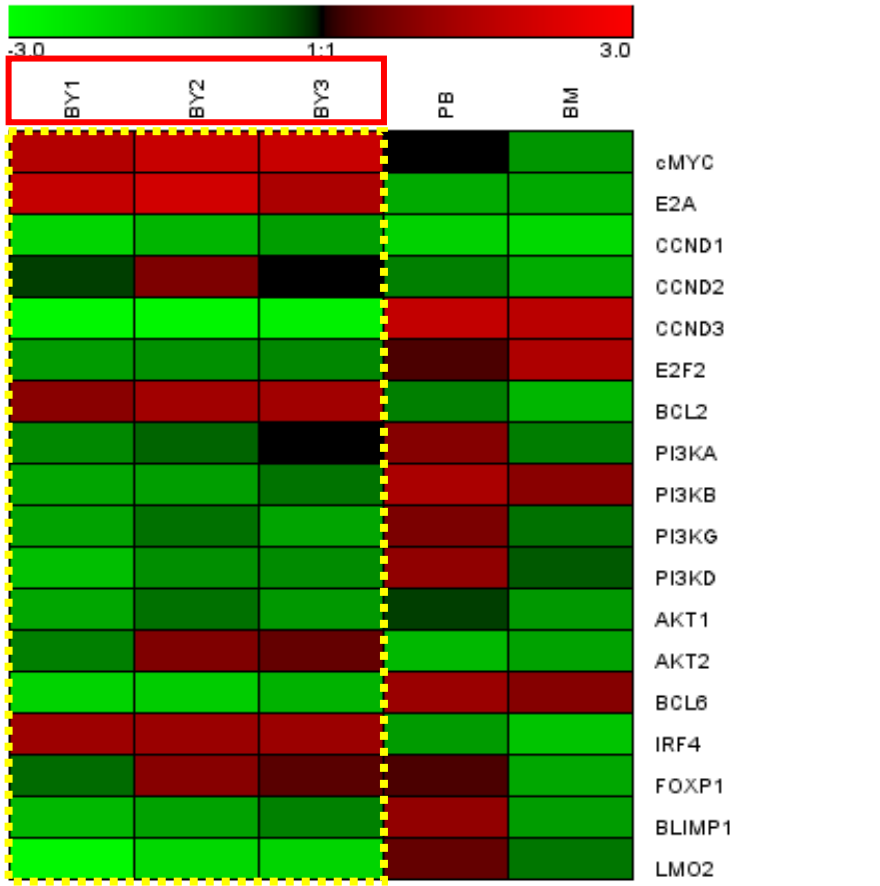


Follow-up

GENE EXPRESSION PROFILING



1 Step



NON-CONCORDANCE WITH BL GENE SIGNATURE

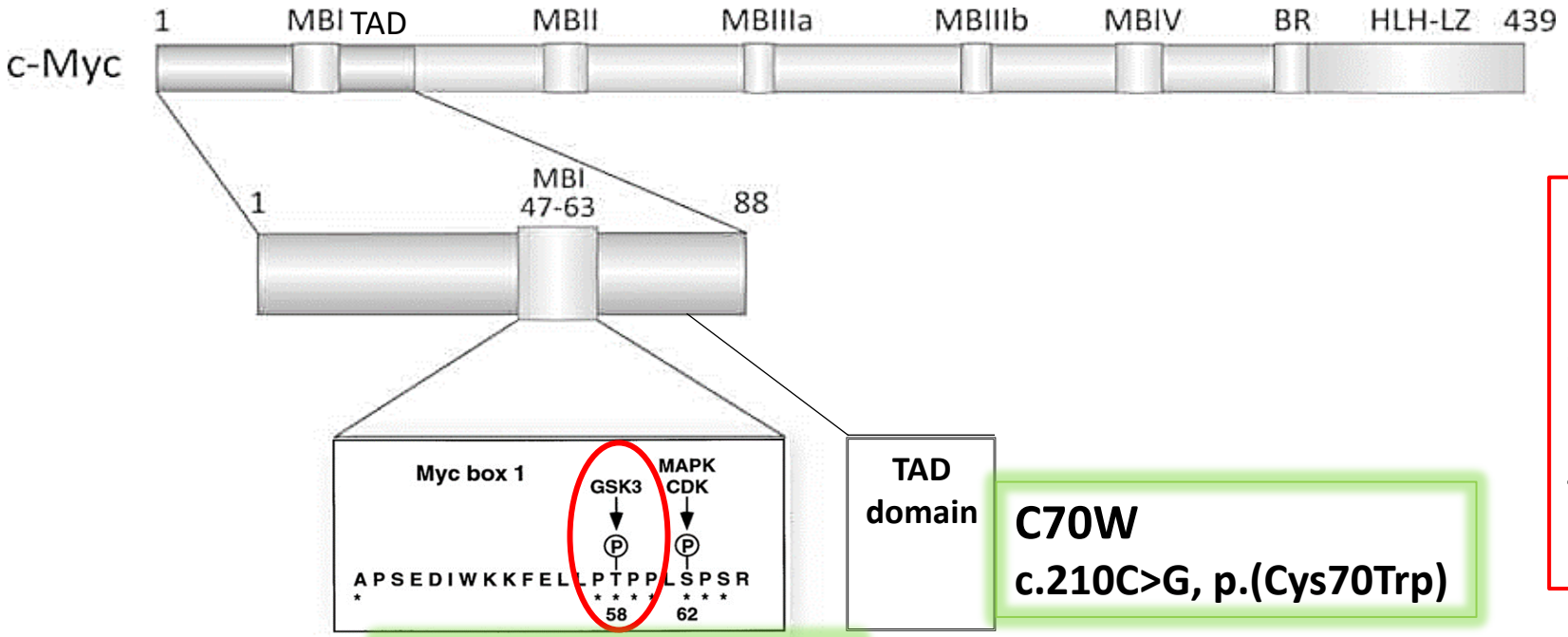
2 Step

Sanger sequencing of c-MYC gene

Basso, K., Dalla-Favera, R. Nat Rev Immunol 2015

SEQUENCING OF c-MYC ONCOGENE

Paired-end Sanger sequencing with 34 primer pairs



T58N
c.173C>A, p.(Thr58Asn)

C70W
c.210C>G, p.(Cys70Trp)

Most of c-MYC mutations target functional domains that enhance the oncogenic potential of MYC by different mechanisms, including increased protein stability and transcriptional function, or by impairing the induction of the proapoptotic element BIM

c-MYC: NM_002467.4

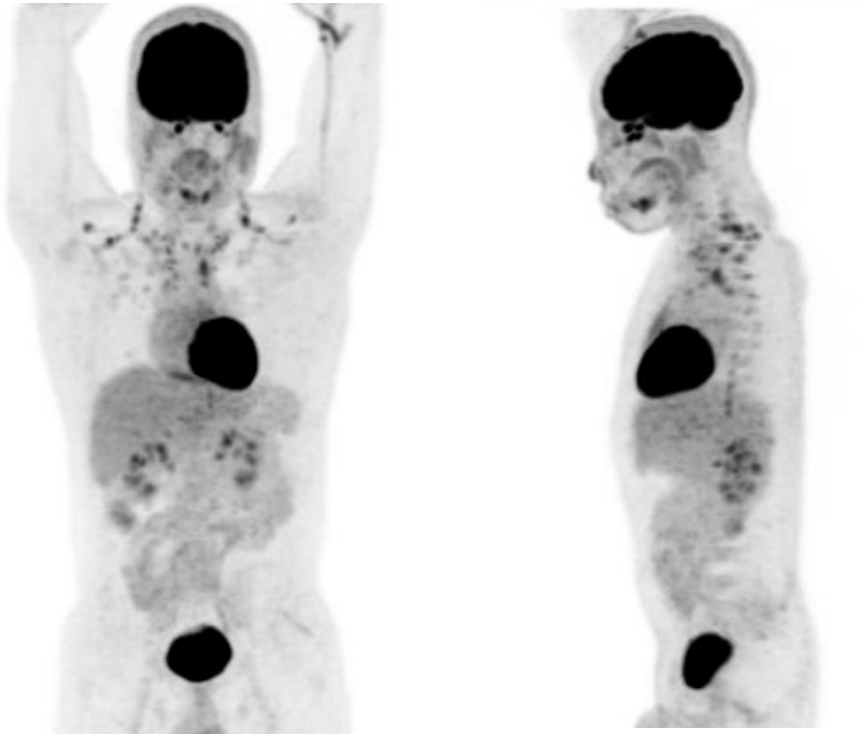
Andresen C, et al. Nucleic Acids Research 2012 - Modified

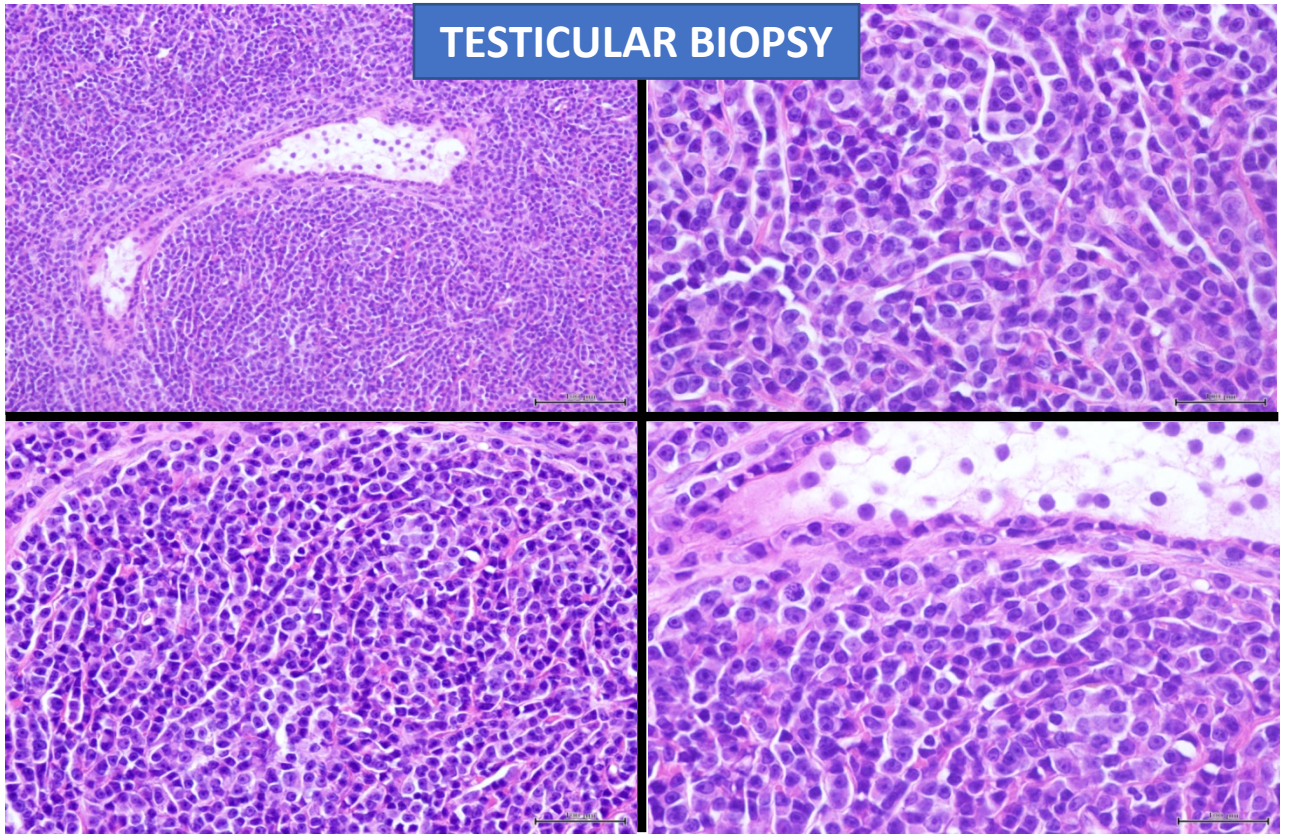
Recurrence: September 2022 – Histology: *«comparable to 2016 … HGL-NOS/WHO 2017, Ki67→30 to 40% (transformation from a low-grade NHL?).»*

Normal LDH

Site of Recurrence:

- PET NEGATIVE: mandibular, paravertebral (T5-T6, at RMN), testicular (PET-, ECO+, Hystology)
- PET POSITIVE: orbital (SUV 3.2)(Histology)





2022 MOLECULAR PROFILING

PCR: IGH/BCL2 & IGH/CCND1 & API2/MALT1: ALL NEGATIVE,
IgH GENE REARRANGEMENT: MONOCLONAL

NanoString GEP

(Lymph2Cx assay)

COO-ABC

2016 BIOPSIES

NanoString GEP

(Lymph2Cx assay)

COO-Unclassified

TARGETED SEQUENCING: CAPTURE-BASED NGS-LYMPHOMA SOLUTION PANEL

2022

Gene Transcript, CDNA, Protein	Type Consequence	Pathogenicity Classification	Orbital biopsy VF	Testicular biopsy VF	BMA VF	PB VF
CHD2 NM_001271, c.2636C>T, p.(Ala879Val)	SNP missense	Likely Pathogenic	44,8%	-	-	-
CHD2 NM_001042572, c.725C>A, p.(Ser242*)	SNP nonsense	VUS	-	47%	-	-
CHD2 NM_001271, c.4173dupA, p.(Gln1392Thrfs*17)	INDEL frameshift	VUS	5,1%	5,3%	-	-
KRAS NM_004985, c.37G>T, p.(Gly13Cys)	SNP missense	Pathogenic	48,8%	47,8%	-	-
MYC NM_002467, c.255C>G, p.(Cys85Trp)	SNP missense	Pathogenic	48%	51,6%	-	-
MYC NM_002467, c.218C>A, p.(Thr73Asn)	SNP missense	Pathogenic	47,8%	51,2%	-	-
IGH clonality-NGS			IGHV4-34*02- D3-10*01-J5*02	IGHV4-34*02- D3-10*01-J5*02	-	-

TARGETED SEQUENCING: CAPTURE-BASED NGS-LYMPHOMA SOLUTION PANEL

2016

Gene Transcript, CDNA, Protein	Type Consequence	Pathogenicity Classification	Perimandibular biopsy VF	Cheek biopsy VF	BMA VF	PB VF
CHD2 NM_001271, c.4173dupA, p.(Gln1392Thrfs*17)	INDEL frameshift	VUS	-	5,2%	-	-
KRAS NM_004985, c.37G>T, p.(Gly13Cys)	SNP missense	Pathogenic	20,1%	20,9%	-	-
MYC NM_002467, c.255C>G, p.(Cys85Trp)	SNP missense	Pathogenic	40,4%	38,3%	-	-
MYC NM_002467, c.218C>A, p.(Thr73Asn)	SNP missense	Pathogenic	39,8%	38,7%	-	-
IGH clonality-NGS			IGHV4-34*02/ D3-10*01/J5*02	IGHV4-34*02/ D3-10*01-J5*02	-	-

COSMIC Catalogue Of Somatic Mutations In Cancer GRCh37

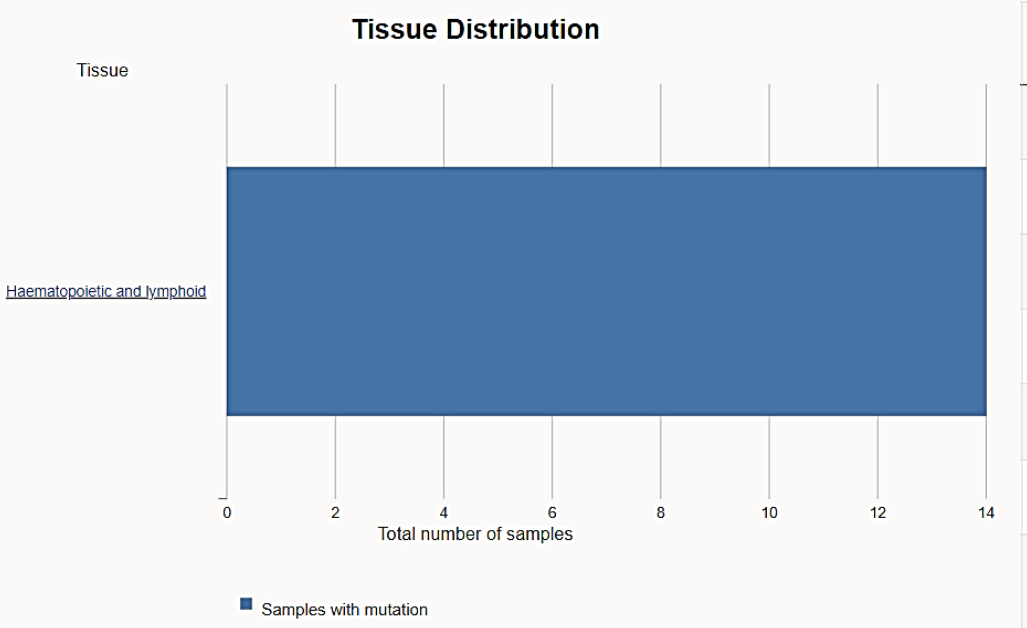
c-MYC Variant c.218C>A (p.T73N)

This variant is in protein domain (exon 2):

- **Transcription regulator Myc, N-terminal**

SIFT (v6.2.0): DELETERIOUS (1.0)

Genomic Mutation ID	COSV52373588
Legacy Identifier	COSM3316881
Gene name	MYC
AA mutation	p.T73N (Substitution - Missense, position 73, T→N)
CDS mutation	c.218C>A (Substitution, position 218, C→A)
SNP	No
Nucleotides inserted	n/a
Genomic coordinates	GRCh37, 8:128750681..128750681, view Ensembl contig
CDD	NP_002458.2
HomoloGene	31092, view the multiple sequence alignment
Ever confirmed somatic?	Yes
FATHMM prediction	Pathogenic (score 0.99)
Remark	n/a
Recurrent	n/a
Drug resistance	n/a
Alternative Ids	22698486{MYC_ENST00000259523}, 75415112{MYC_ENST00000524013}



Primary Histology	Histology Subtype 1
Lymphoid neoplasm	Burkitt lymphoma
Lymphoid neoplasm	Burkitt lymphoma
Lymphoid neoplasm	Burkitt lymphoma
Lymphoid neoplasm	Burkitt lymphoma
Lymphoid neoplasm	Burkitt lymphoma
Lymphoid neoplasm	Burkitt lymphoma
Lymphoid neoplasm	Diffuse large B cell lymphoma
Lymphoid neoplasm	Diffuse large B cell lymphoma

COSMIC Catalogue Of Somatic Mutations In Cancer GRCh37

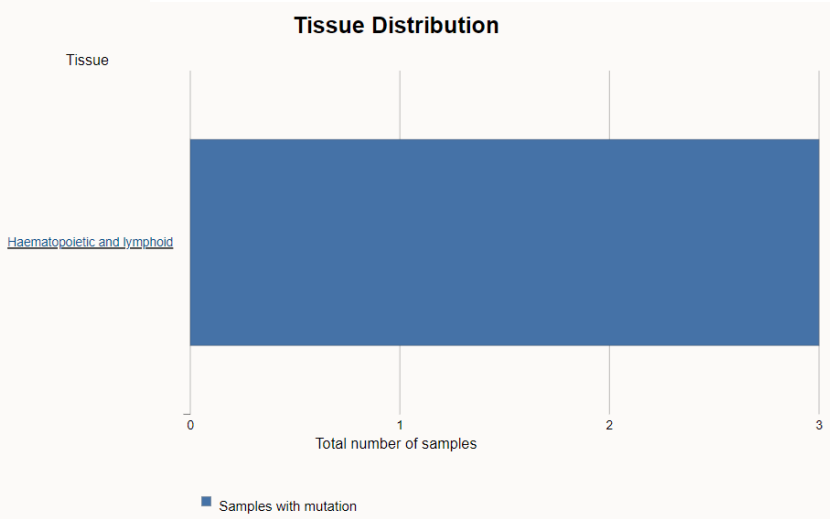
c-MYC Variant c.255C>G(p.C85W)

Genomic Mutation ID	COSV104388511
Legacy Identifier	COSM9213571
Gene name	MYC
AA mutation	p.C85W (Substitution - Missense, position 85, C→W)
CDS mutation	c.255C>G (Substitution, position 255, C→G)
SNP	No
Nucleotides inserted	n/a
Genomic coordinates	GRCh37, 8:128750718..128750718, view Ensembl contig
CDD	NP_002458.2
HomoloGene	31092, view the multiple sequence alignment
Ever confirmed somatic?	No
FATHMM prediction	Pathogenic (score 0.94)
Remark	n/a
Recurrent	n/a
Drug resistance	n/a
Alternative Ids	22706204{MYC_ENST00000259523}, 75424564{MYC_ENST00000524013}

This variant is in protein domain (exon 2):

- **Transcription regulator Myc, N-terminal**

SIFT (v6.2.0): DELETERIOUS (0.99)



Tissue Subtype 1	Primary Histology	Histology Subtype 1
Lymph node	Lymphoid neoplasm	Diffuse large B cell lymphoma
NS	Lymphoid neoplasm	Diffuse large B cell lymphoma
NS	Lymphoid neoplasm	Burkitt lymphoma

COSMIC Catalogue Of Somatic Mutations In Cancer GRCh37

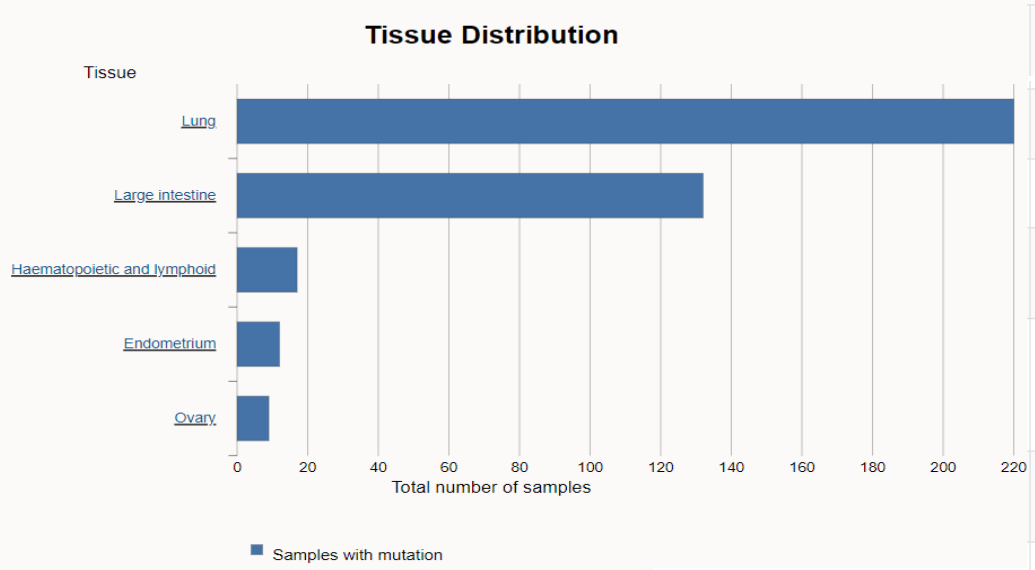
KRAS Variant c.37G>T(p.G13C)

This variant is in protein domain (exon2):

- **Small GTPase superfamily**
- **Small GTP-binding protein domain**
- **Mitochondrial Rho-like**
- **P-loop containing nucleoside triphosphate hydrolase**

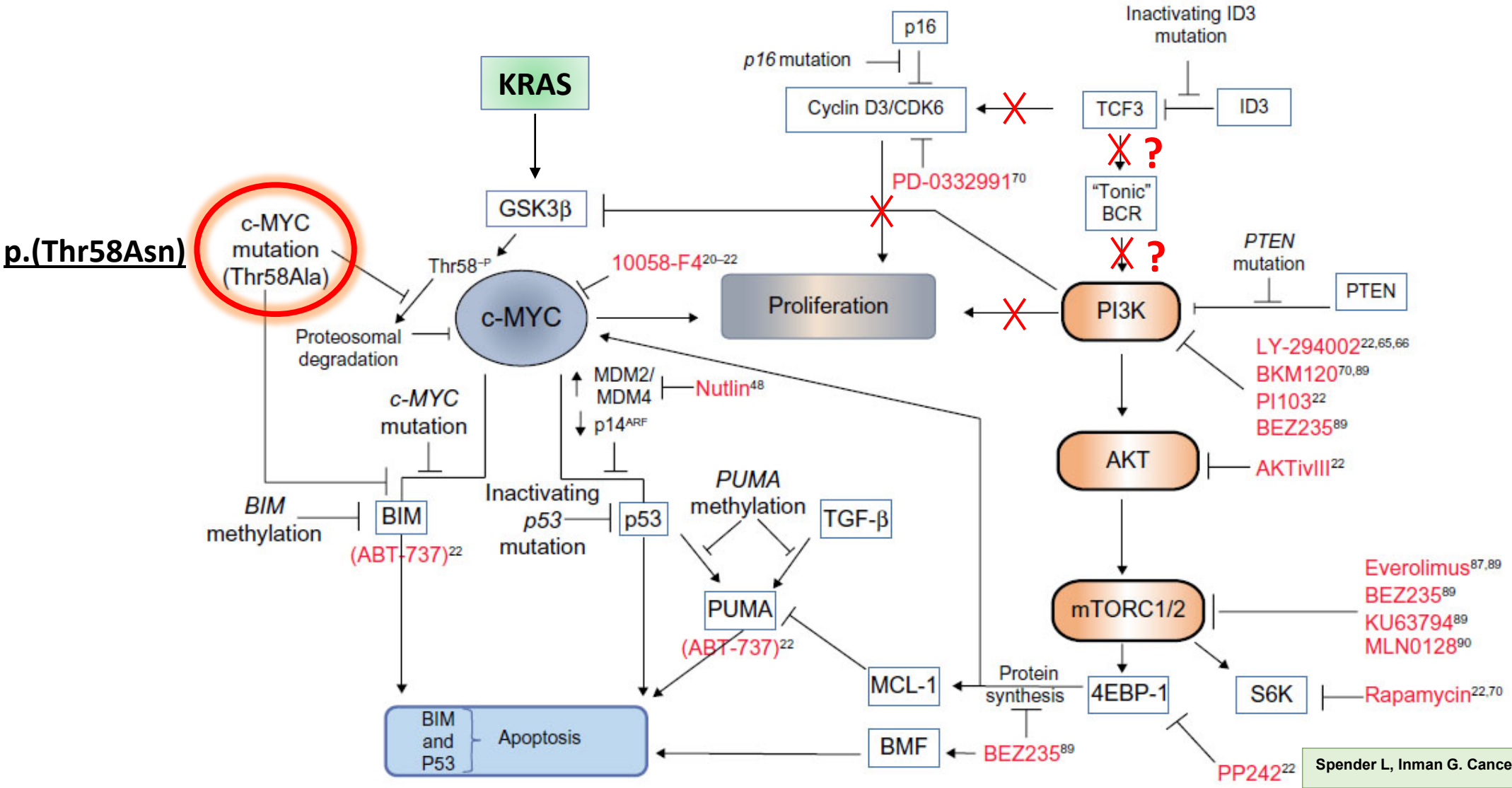
SIFT (v6.2.0): DELETERIOUS (1.0)

Genomic Mutation ID	COSV55497378
Legacy Identifier	COSM527
Gene name	KRAS
AA mutation	p.G13C (Substitution - Missense, position 13, G→C)
CDS mutation	c.37G>T (Substitution, position 37, G→T)
SNP	No
Nucleotides inserted	n/a
Genomic coordinates	GRCh37, 12:25398282..25398282, view Ensembl contig
CDD	NP_203524.1
HomoloGene	n/a
Ever confirmed somatic?	Yes
FATHMM prediction	Pathogenic (score 0.98)
Remark	n/a
Recurrent	n/a
Drug resistance	n/a
Alternative Ids	29423429{KRAS_ENST00000311936}, 88011751{KRAS_ENST00000556131}, 87542567{KRAS_ENST00000557334}



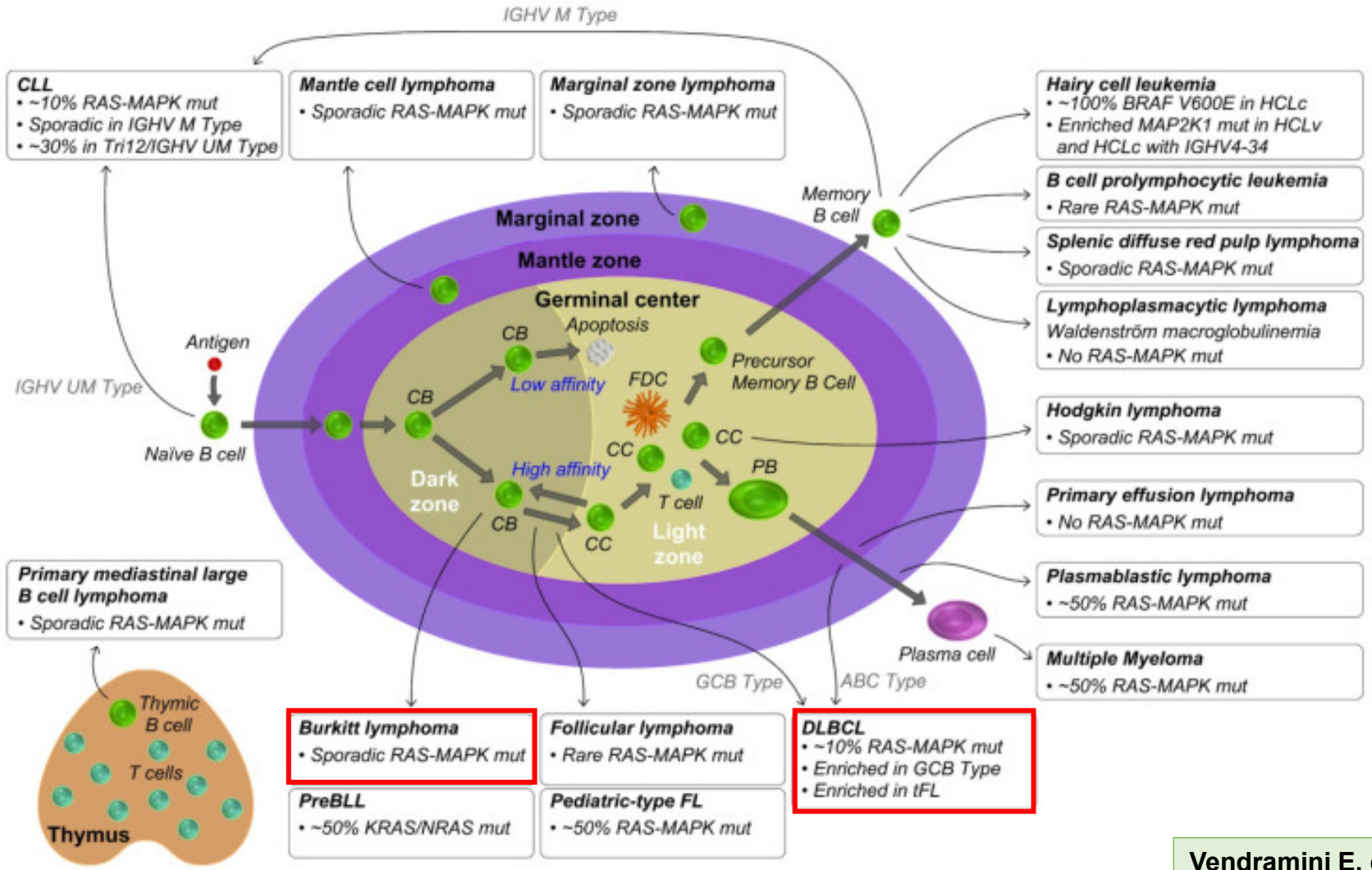
Primary Histology	Histology Subtype 1
Lymphoid neoplasm	Plasma cell myeloma
Lymphoid neoplasm	Plasma cell myeloma
Lymphoid neoplasm	Peripheral T cell lymphoma unspecified
Lymphoid neoplasm	Chronic lymphocytic leukaemia-small lymphocytic lymphoma
Haematopoietic neoplasm	Chronic myelomonocytic leukaemia
Haematopoietic neoplasm	Acute myeloid leukaemia

COOPERATING MUTATIONAL EVENTS IN MYC-DRIVEN LYMPHOMAGENESIS



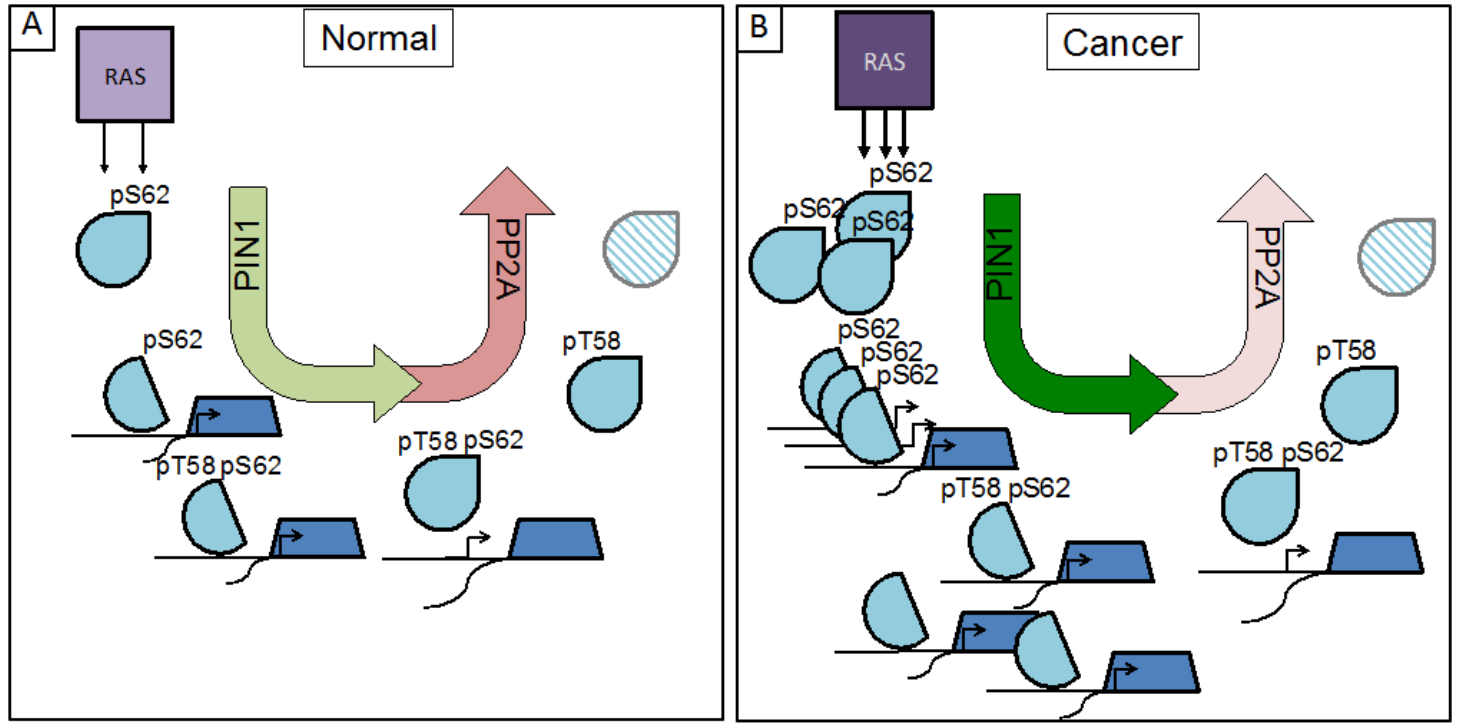
Spender L, Inman G. Cancer Manag Res 2014

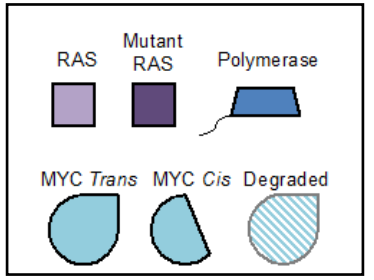
MATURE B CELL LYMPHOPROLIFERATIVE DISORDERS AND RAS-MAPK PATHWAY DEREGULATION



Vendramini E, et al. Cancers (Basel) 2022

RAS AND MYC: CO-CONSPIRATORS IN CANCER





A. In normal cells, RAS signaling leads to MYC phosphorylation at S62, which supports PIN1 isomerization of P63 to cis, recruitment of MYC to target gene promoters, and activation of the basal transcription machinery. Subsequent phosphorylation of MYC at T58 results in a second PIN1 isomerization of P63 to trans that is associated with the release of MYC from DNA, PP2A mediated pS62 dephosphorylation and MYC degradation.

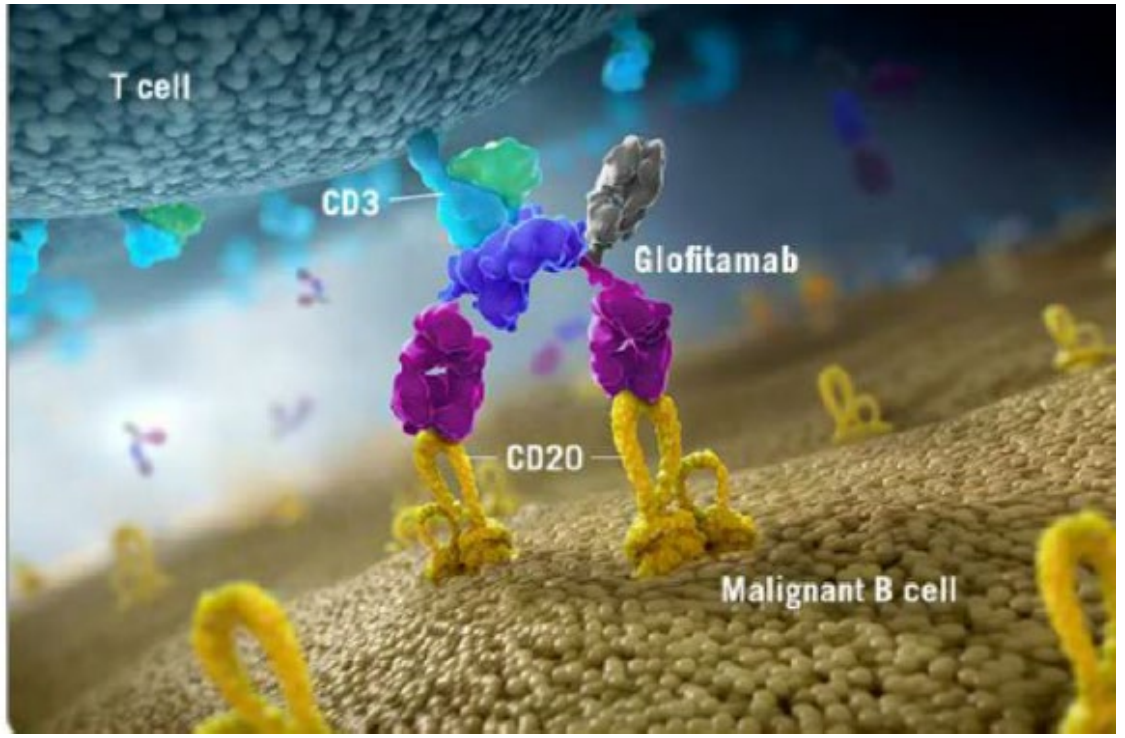
B. In RAS-driven cancer, increased signaling from mutant RAS along with active PIN1 and suppressed PP2A leads to an accumulation of active MYC that can drive pro-tumor transcriptional programs.

LNH- MUTATIONAL SPECTRUM	CLINICAL CASE (MYC, KRAS, CHD2)
T-HGL of indolent B-cell LNH (TNFAIP3, GPR34, CD274, TNFRSF14, TET2, EZH2, NOTCH2, NOTCH1, CREBBP, KMT2D, MYC, TP53)	✓
DLBCL NOS GCB (EZH2, GNA13, MEF2B, KMT2D, B2M, TNFRSF14, CREBBP)	✓
DLBCL NOS ABC (MYD88 p.L265P, CD79B, PIM1, PRDM1/BLIMP1, MYC)	✓
HGL NOS (MYD88, CD79B, TBL1XR1, MYC, KMT2D, TP53, BCL2, EZH2 CREBBP, TNFRSF14)	✓
BL SPORADIC (MYC, TCF3, ID3)	✓

«… the aggressive subtype documented through NGS prompted us to enrol the patient in an appropriate clinical trial…»

Phase 1 study NP39488

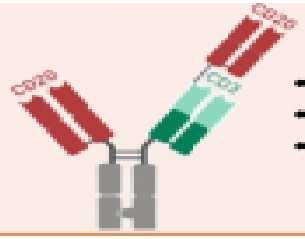
Polatuzumab (ADC, anti-CD79b) + **Glofitamab** (Bispecific Antibody, antiCD20xCD3)



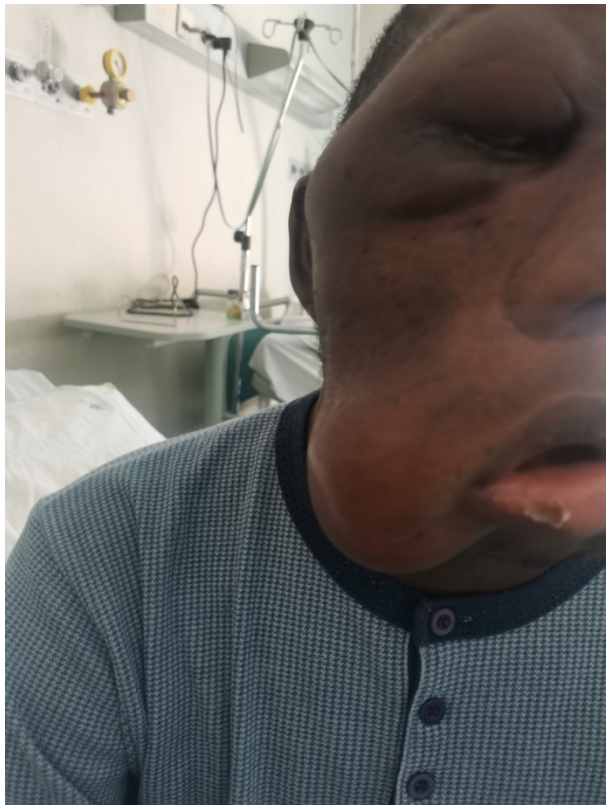
NCT03533283

Espansion Part for relapsed/refractory

DLBCL and High-grade B-cell lymphoma

glofitamab	**$(CD20)_2$ x CD3** 	• humanized mouse IgG1-based antibody • bivalent CD20 and monovalent CD3ε binding • modified Fc devoid of FcγR and complement binding

Flares occurring 36 hours after 1° step-up dose of Glofitamab



Clinical case – The contribution of NGS

Diagnosis

Despite renewed efforts on the histological classification of high-grade lymphomas, still exists a grey zone with clinical and pathological conundrums

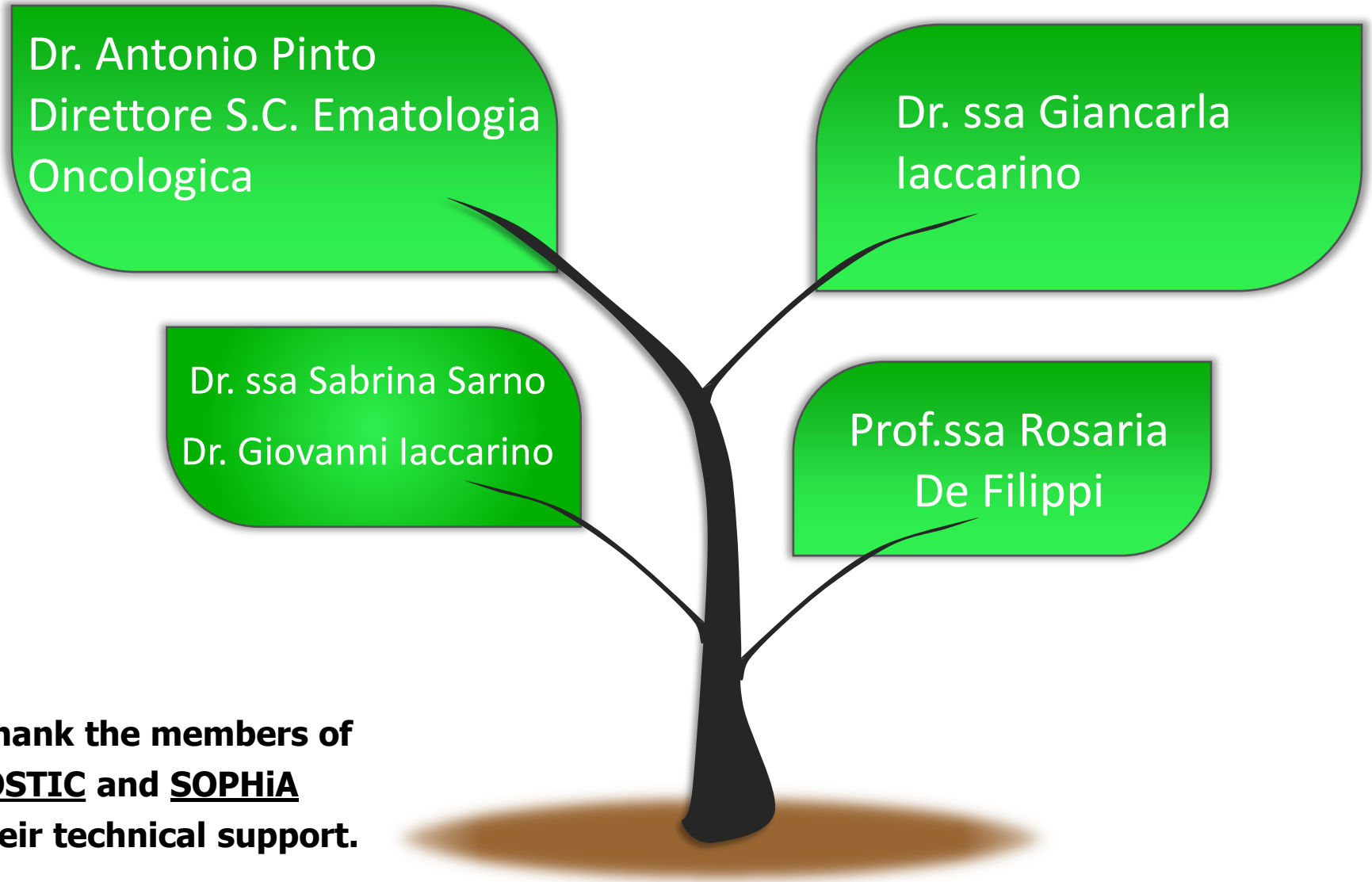
Prognosis

Clinico-pathological prognostication tools do not adequately encompass the heterogeneities of high-grade lymphomas, while histological classification is far from remedying.

Therapy

NGS may retain diagnostic and prognostic value in high-grade NHL.
Moreover, genomic alterations detectable through NGS may represent an additional source for therapeutically targetable alterations and vulnerabilities, well beyond immunotherapies such as glofitamab and polatuzumab adopted in this clinical case.

Thankful Tree



I would like to thank the members of ARROW DIAGNOSTIC and SOPHiA GENETICS for their technical support.