The young side of
LYMPHOMA

gli under 40 a confronto

Verona, Centro Congressi Camera di Commercio
26-27 settembre 2025

Novità nella definizione del rischio nel MCL

Giulio Eugenio Mandelli

S.C. Anatomia Patologica – ASST degli Spedali Civili di Brescia

Disclosures of Name Surname

Company name	Research support	Employee	Consultant	Stockholder	Speakers bureau	Advisory board	Other
Johnson & Johnson	X		X				

Prognostic factors: old and new (?)

Current clinical use	Potential future use
Age	MCL35 RNA expression analysis
Performance status	Measurable residual disease testing
CNS involvement at diagnosis	Somatic mutations (e.g. in *NOTCH1* and others)
Stage of disease (I and II vs III and IV)	FDG PET
Serum level of B2M	
Serum level of LDH	
Morphology (classic vs blastoid)	
MCL International Prognostic Index (MIPI)	
Ki-67 (< 30% vs ≥ 30%) and inclusion into combined-MIPI	
p53 expression by immunohistochemistry *TP53* mutations/deletions by sequencing analysis	

WHO Classification of Tumours, 5th Edition, Volume 1: Hematolymphoid Tumours, IARC (International Agency for Research on Cancer), 2022
Silkenstedt E, Dreyling M. Mantle cell lymphoma-Advances in molecular biology, prognostication and treatment approaches. Hematol Oncol. 2021 Jun;39

In your center, the pathologist's report includes

Prognostic factors: state of the art

Features	Newly diagnosed MCL	R/R MCL*
Accepted ultra-high-risk features	De novo blastoid or pleomorphic histology with high-risk mutations[40] Ki-67% ≥50%† in involved tissue biopsy with blastoid or pleomorphic histology[12,41] ‡*TP53* mutation (R273) with other high-risk gene mutations (*KMT2D, NSD2, CCND1, NOTCH1, CDKN2A, NOTCH2,* or *SMARCA4* mutations) and extensive disease burden[42] CNS involvement with systemic disease[43]	Transformed blastoid or pleomorphic histology (transformed from classic histology; ie, disease resistant to BTKi, venetoclax, and anti-CD19 CART)[40] Primary BTKi-refractory disease [12] Refractory to ≥3 previous lines of standard therapy (including BTKi)[12] Triple-resistant MCL (disease resistant to BTKi, venetoclax, and anti-CD19 CART)[12,33]
Accepted high-risk features	Blastoid or pleomorphic histology[40] Ki-67 ≥50%† in involved tissues with classic histology[12,41] *TP53* mutation[14] and/or del(17p) by FISH, *TP53* overexpression by IHC, and/or non-*TP53* mutations (*NOTCH1/NOTCH2, KMT2D, NSD2,* and *SMARCA4* mutations)[44] CK[45] *MYC* rearrangement and/or amplification[46-50] *TP53* expression in >50% of cells or a high combined MIPI score Simplified MIPI score ≥6.2[51] Bulky disease‖	High-risk features for patients with newly diagnosed disease[12] ≤2 previous lines of standard therapy Progression within 24 months of first-line therapy[52] MRD$^+$ status after therapy§

Jain P, Wang M. High-risk MCL: recognition and treatment. Blood. 2025 Feb 13;145(7):683-695

Prognostic factors: state of the art

Features	Newly diagnosed MCL	R/R MCL*
Accepted ultra-high-risk features	De novo blastoid or pleomorphic histology with high-risk mutations[40] Ki-67% ≥50%† in involved tissue biopsy with blastoid or pleomorphic histology[12,41] ‡*TP53* mutation (R273) with other high-risk gene mutations (*KMT2D, NSD2, CCND1, NOTCH1, CDKN2A, NOTCH2,* or *SMARCA4* mutations) and extensive disease burden[42] CNS involvement with systemic disease[43]	Transformed blastoid or pleomorphic histology (transformed from classic histology; ie, disease resistant to BTKi, venetoclax, and anti-CD19 CART)[40] Primary BTKi-refractory disease[12] Refractory to ≥3 previous lines of standard therapy (including BTKi)[12] Triple-resistant MCL (disease resistant to BTKi, venetoclax, a
Accepted high-risk features	Blastoid or pleomorphic histology[40] Ki-67 ≥50%† in involved tissues with classic histology[12,41] *TP53* mutation[14] and/or del(17p) by FISH, *TP53* overexpression by IHC, and/or non-*TP53* mutations (*NOTCH1/NOTCH2, KMT2D, NSD2,* and *SMARCA4* mutations)[44] CK[45] *MYC* rearrangement and/or amplification[46-50] *TP53* expression in >50% of cells or a high combined MIPI score Simplified MIPI score ≥6.2[51] Bulky disease‖	Hig disease[12] ≤2 previous lines of standard therapy Progression within 24 months of first-line therapy[52] MRD+ status after therapy§

biology of MCL

Jain P, Wang M. High-risk MCL: recognition and treatment. Blood. 2025 Feb 13;145(7):683-695

Prognostic factors: state of the art

pattern of resistance to treatments

R/R MCL*
Transformed blastoid or pleomorphic histology (transformed from classic histology; ie, disease resistant to BTKi, venetoclax, and anti-CD19 CART)[40] Primary BTKi-refractory disease [12] Refractory to ≥3 previous lines of standard therapy (including BTKi)[12] Triple-resistant MCL (disease resistant to BTKi, venetoclax, and anti-CD19 CART)[12,33]
High-risk features for patients with newly diagnosed disease[12] ≤2 previous lines of standard therapy Progression within 24 months of first-line therapy[52] MRD$^+$ status after therapy§

Jain P, Wang M. High-risk MCL: recognition and treatment. Blood. 2025 Feb 13;145(7):683-695

Recognizing high-risk MCL: new tips

➢ Recommended Ki67 cut-offs of 50%

- The Ki-67% **combined with the simplified MIPI** score, is an **independent prognostic** indicator in MCL
- high Ki-67% (≥50%) had **significantly shorter OS** than those with a low Ki-67% (20 vs 118 months).

Jain P, Wang M. High-risk MCL: recognition and treatment. Blood. 2025 Feb 13;145(7):683-695
Jain Pet al. Genomic profiles and clinical outcomes of de novo blastoid/pleomorphic MCL are distin ct from those of transformed MCL. Blood Adv. 2020;4(6):1038-1050.

Recognizing high-risk MCL: new tips

➢ Cytogenetic assessment for complex karyotype (CK)

- A CK and/or complex chromosomal rearrangements, breakage, fusion, and genomic instability are associated with an aggressive clinical course
- A CK at initial diagnosis is linked to a **significantly shorter OS after chemotherapy** → median of 4.5 vs 11.6 years
- Patients with a **secondary acquired** CK had worse survival than those with a de novo CK

Jain P, Wang M. High-risk MCL: recognition and treatment. Blood. 2025 Feb 13;145(7):683-695
Greenwell IB et al.Complex karyotype in patients with mantle cell lymphoma predicts inferior survival and poor response to intensive induction therapy. Cancer. 2018;124(11):2306-2315

Recognizing high-risk MCL: new tips

➢ Next-generation sequencing is recommended to detect somatic mutations

- **Best solution for multigene testing**
- Mutations in *TP53, KMT2D, NSD2, CCND1, NOTCH1, NOTCH2, CDKN2A, NOTCH2* and other genes correlated with **aggressive MCL**
- Somatic mutations in *TP53, KMT2D, NSD2, SMARCA4, CCND1, TRAF2, NFKBIE* genes are reported with **ibrutinib resistance**
- **change in mutational status from baseline** samples to samples at disease progression → re-testing (?)

Jain P, Wang M. High-risk MCL: recognition and treatment. Blood. 2025 Feb 13;145(7):683-695
Khouja M e al. Comprehensive genetic analysis by targeted sequencing identifies risk factors and predicts patient outcome in Mantle Cell Lymphoma: results from the EU-MCL network trials. Leukemia. 2024 Dec;38(12):2675-2684
Hill HA et al. Genetic mutations and features of mantle cell lymphoma: a systematic review and metanalysis. Blood Adv. 2020;4(13):2927-2938.

SOX-11 oncogene

- +CD70⁺CD27⁺ Tregs
- +Angiogenesis PDGFA
- +Cell adhesion FAK kinase
- +Musashi-2

Cyclin D dysregulation

- Truncation 3' UTR
- Higher expression of cyclin D signature
- CDKN2A deletion (9p21.3)

Unstable genome

- High aneuploidy
- Recurrent breakage fusion bridge cycles
- Chromothripsis
- High CNA, SV's

BCR and NFκB

- *NFKBIE* mutations
- CARD11/BCL10/MALT1 pathway
- PI3K/AKT/mTOR pathway
- Alternate NFκB pathway mutations - *BIRC3, TRAF2*

Lymphoid microenvironment

- "Immune depleted" TME
- Exhausted T cells
- High CD163⁺ macrophages and high Tregs
- CSF-1R axis and tumor - associated macrophages
- SOX-11+CD70⁺CD27⁺ Tregs
- High CCL3, CCL4, CXCR4

Epigenetic aberrations

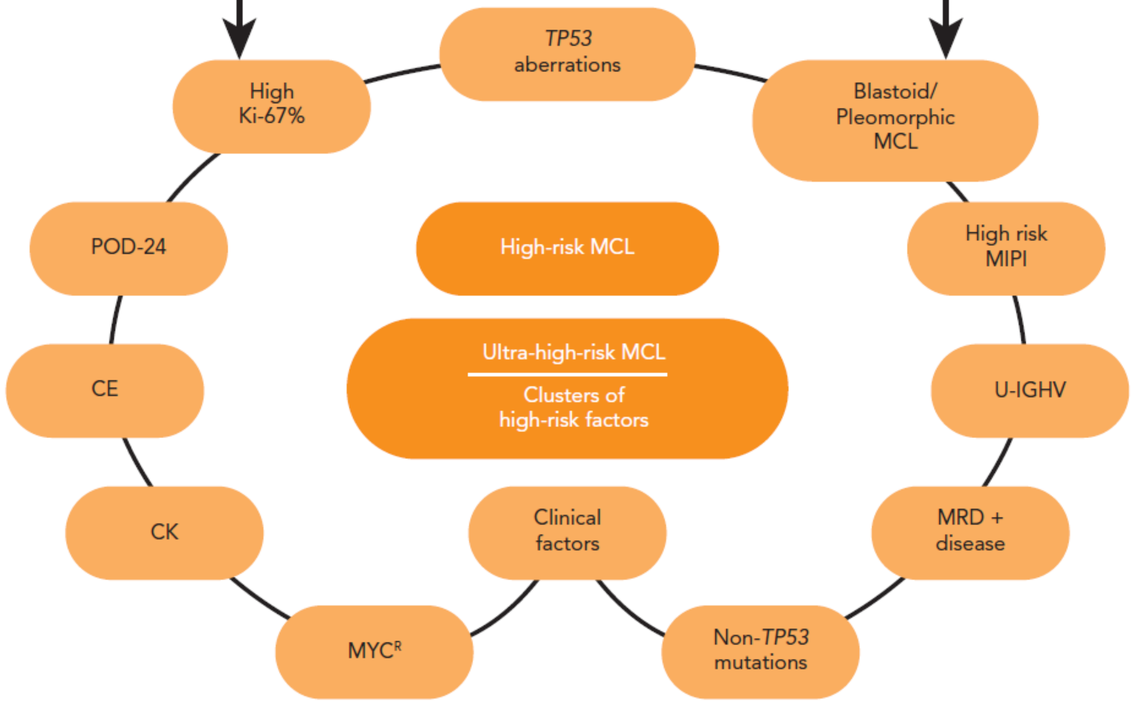
- PRMT5 upregulation
- DNMT3A-MEF2B and MYC-OXPHOS axis
- + EZH2
- Higher EpiCMIT score
- *NSD2* GOF mutations
- *KMT2D* LOF mutations
- *SMARA4/SWI* mutations

Metabolic reprogramming

- + OXPHOS pathway overactivation in ibrutinib-resistant MCL
- + EGR1
- DNMT3A-MEF2B and MYC-OXPHOS axis

Miscellaneous

- *NOTCH1, NOTCH2* GOF mutations
- + CTPS1
- HNRNPH non-coding mutations
- Aberrant mRNA processing
- High IL-2R
- + TIGIT and + MDSCs
- Transcription regulation -*UBR5* mutations
- BACH2 downregulation
- HSP90-MYC-CDK9 axis



Relapsed disease

- ≥ 3 prior lines (with BTKi resistance)
- Disease transformation
- Triple-resistant MCL (BTKi, BCL2i and anti-CD19 CART)

Which new prognostic test/marker is used at your center?

Brescia NGS experience in MCL

73 genes targeted NGS panel SNV – INDEL – CNV

SOPHiA GENETICS™

ARID1A	*CREBBP*	*KRAS*	*STAT3*
ATM	*CXCR4*	*MAP2K1*	*STAT5B*
B2M	*DDX3X*	*MEF2B*	*STAT6*
BCL2	*DNMT3A*	*MFHAS1*	*TBL1XR1*
BCL6	*EP300*	*MYC*	*TCF3*
BCOR	*ETV6*	*MYD88*	*TET2*
BIRC3	*EZH2*	*NFKBIE*	*TNFAIP3*
BRAF	*FAT1*	*NOTCH1*	*TNFRSF14*
BTG1	*FBXW7*	*NOTCH2*	*TP53*
BTK	*FOXO1*	*NRAS*	*TP63*
CARD11	*GNA13*	*PIM1*	*TRAF2*
CCND1	*HIST1H1E*	*PLCG1*	*TRAF3*
CCND3	*ID3*	*PLCG2*	*XPO1*
CD28	*IDH2*	*PRDM1*	
CD58	*INPP5D*	*PTEN*	
CD79A	*IRF4*	*PTPRD*	
CD79B	*ITPKB*	*RHOA*	
CDKN2A	*JAK3*	*SETD2*	
CDKN2B	*KLF2*	*SF3B1*	
CIITA	*KMT2D*	*SOCS1*	

Brescia NGS experience in MCL

Cohort and Samples

N patients		11
Age		47-81 (median 66)
Sex	male	8
	female	3
Histology*	classic	8
	blastoid	2
	pleomorphic	1
Ki67 index	≥30%	7
	<30%	4
P53 IHC	>50%	3
	≤ 50%	5
MIPI (classic)	LR	5
	IR	1
	HR	4
NGS timing	onset^	6
	relapse°	5

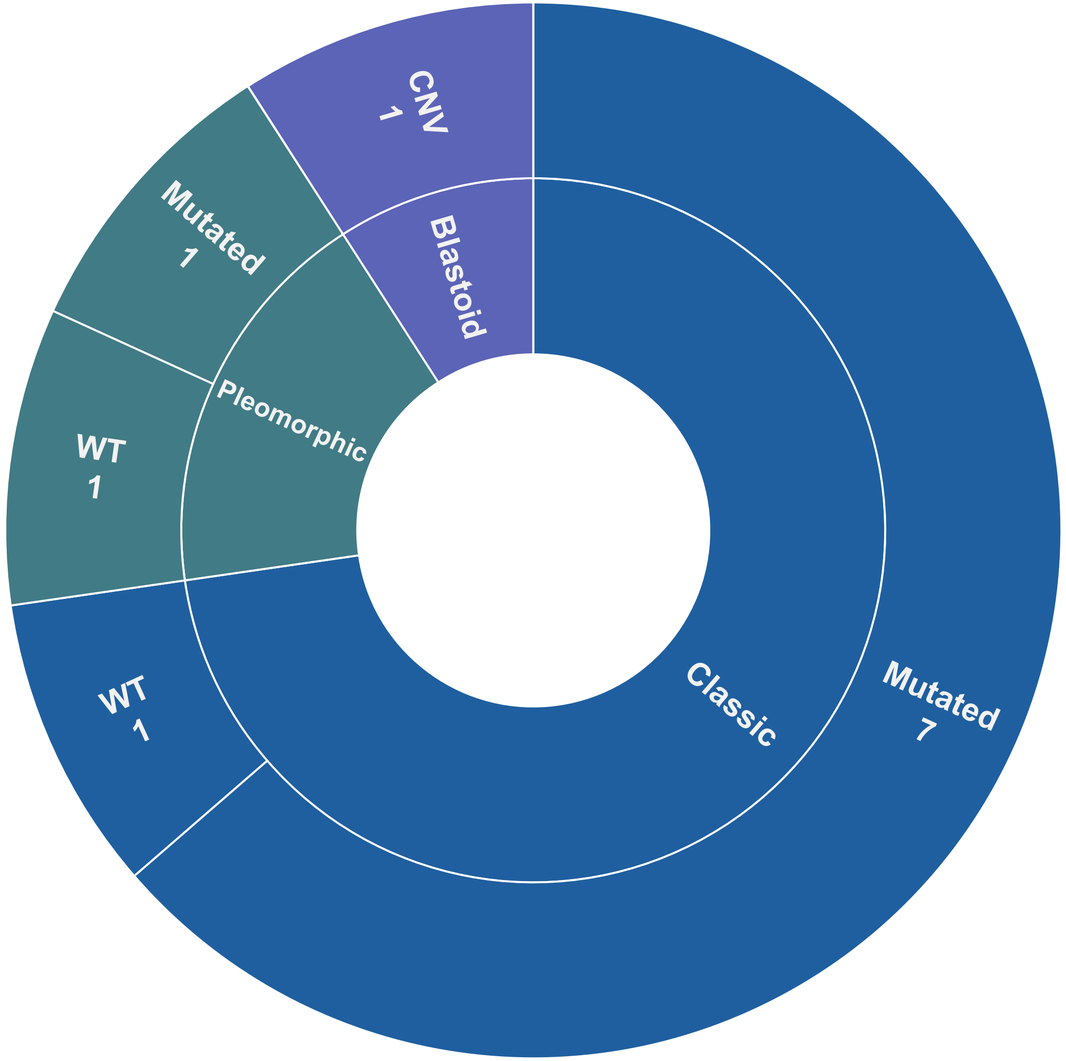
* All cases Ciclin D1+
^ All cases presented at Stage 4a
° NGS testing **only** at relapsed disease and corresponding tissue/BM

- **7 Lymph Node FFPE**
- **1 Skin Biopsy FFPE**
- **1 Salivary Gland FFPE**
- **3 Bone Marrow EDTA**

Brescia NGS experience in MCL

Mutational landscape

	GENE	MUTATION	VAF
#1	BIRC3	c.1704_1727del p.(Phe569_Val576)del	50,5%
#2	ATM	c.9023G>A p.(Arg3008His)	1,20%
#3	Wild Type		
#4	ATM	c.9022C>T p.(Arg3008Cys)	56,80%
#5	BIRC3	c.1540_1541del p.(Leu514Alafs*4)	14,40%
	TP53	c.764_766del p.(Ile255del)	32,40%
#6	KMT2D	c.12466dup p.(Gln4156Profs*12)	30,30%
	TP53	c.814G>T p.(Val272Leu)	73,80%
#7	CCND1	c.142G>C p.(Val48Leu)	42,6%
#8	CREBBP	c.5837del p.(Pro1946Hisfs*30)	1,7%
	BIRC3	c.1580-1G>A	1,2%
#9	Wild Type		
#10	KMT2D	c.15566G>T p.(Gly5189Val)	25,40%
	TP53	c.722C>A p.(Ser241Tyr)	23,90%
	TP53	c.672+1G>T	25%
#11	CNV BCL2 and MYC		



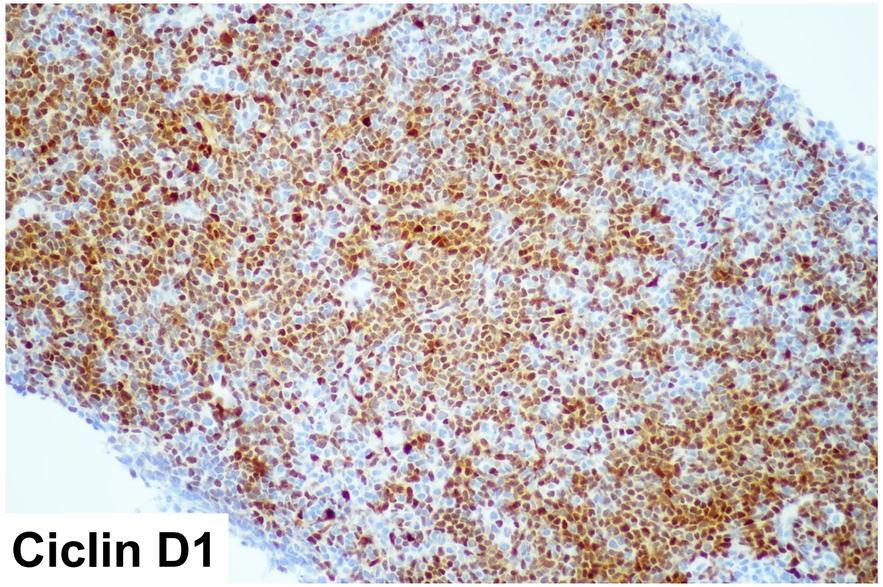
Brescia NGS experience in MCL

p53 IHC and TP53 mutation

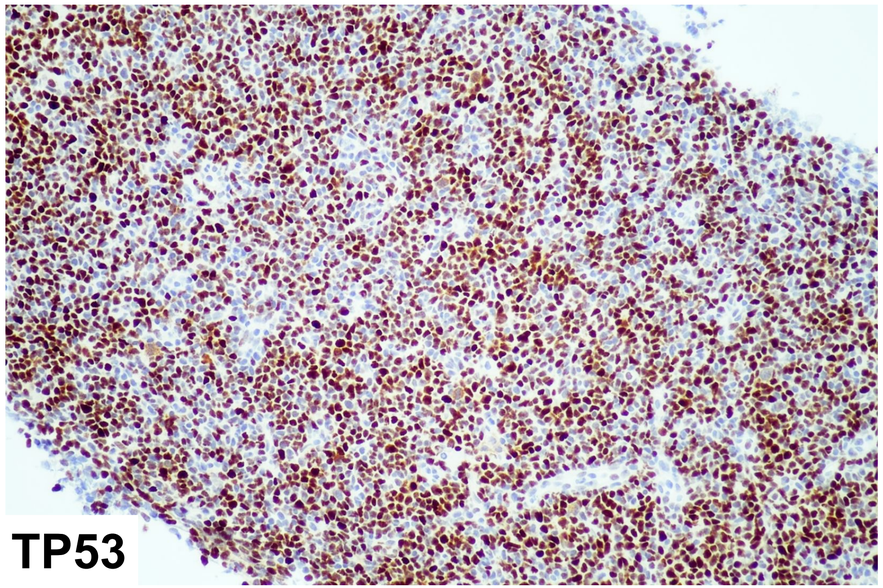
	GENE	MUTATION	VAF
#5	BIRC3	c.1540_1541del p.(Leu514Alafs*4)	14,40%
	TP53	c.764_766del p.(Ile255del)	32,40%
#6	KMT2D	c.12466dup p.(Gln4156Profs*12)	30,30%
	TP53	c.814G>T p.(Val272Leu)	73,80%
#10	KMT2D	c.15566G>T p.(Gly5189Val)	25,40%
	TP53	c.722C>A p.(Ser241Tyr)	23,90%
	TP53	c.672+1G>T	25%

- 3/3 **TP53 mutated** cases **had >50% p53** protein expression in IHC
- 5/8* TP53 wild type cases had ≤ 50% p53 protein expression in IHC

* IHC NA in 3 cases



Ciclin D1



TP53

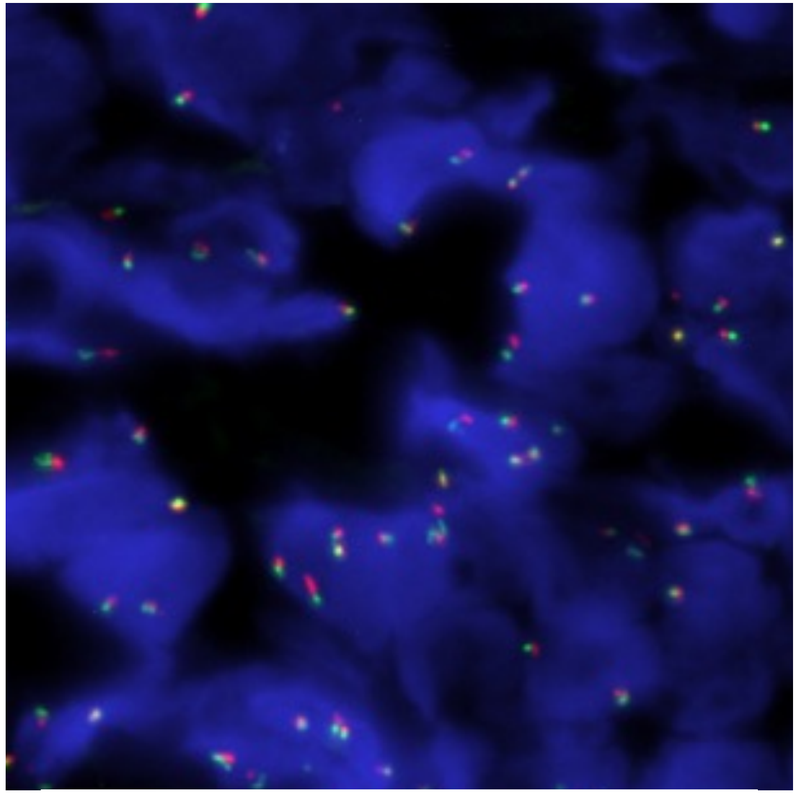
Brescia NGS experience in MCL

CNV confirmed in FISH as CN *gain*

	GENE	MUTATION	VAF
#11	CNV BCL2 and MYC		

Prognostic relevance:

- MYC rearrangement
- BCL2 amplification



MYC FISH – break apart probe

Wang L et. MYC rearrangement but not extra MYC copies is an independent prognostic factor in patients with mantle cell lymphoma. Haematologica. 2021 May 1;106(5):1381-1389
Khouja M e al. Comprehensive genetic analysis by targeted sequencing identifies risk factors and predicts patient outcome in Mantle Cell Lymphoma: results from the EU-MCL network trials. Leukemia. 2024 Dec;38(12):2675-2684

Brescia NGS experience in MCL

Onset-tested cohort

	Age	MIPI	Histo	Ki67	Mutation	Therapy I Line	Response	Notes
#1	70	IR	C	50%	ATM	RBACx6+RTT +RTX maintenance	PR	
#2	47	LR	C	15%	Wild Type	Watch and Wait	SD	Last FU 25/06/2025
#3	63	HR	C	5%	BIRC3 TP53	RCHOP-RDHAP	RC	ASCT suspended for complication; programmed RTX maintenance
#4	81	HR	C	5%	BIRC3 CREBBP	R-Bendamustin lower dose	Ongoing (responding)	IRC → dialysis at onset
#5	73	HR	P	55%	Wild Type	RBACx6	RC	
#6	59	LR	P	90%	TP53 KMT2D	RCHOP-RDHAP ASCT FEAM	RC	Aug2025-Nov2025 → Watch and Wait; programmed RTX maintenance

Brescia NGS experience in MCL

Onset-tested cohort

	Age	MIPI	Histo	Ki67	Mutation	Therapy I Line	Response	Notes
#1	70	IR	C	50%	ATM	RBACx6+RTT +RTX maintenance	PR	
#2	47	LR	C	15%	Wild Type	Watch and Wait	SD	Last FU 25/06/2025
#3	63	HR	C	5%	BIRC3 TP53	RCHOP-RDHAP	RC	ASCT suspended for complication; programmed RTX maintenance
#4	81	HR	C	5%	BIRC3 CREBBP	R-Bendamustin lower dose	Ongoing (responding)	IRC → dialysis at onset
#5	73	HR	P	55%	Wild Type	RBACx6	RC	
#6	59	LR	P	90%	TP53 KMT2D	RCHOP-RDHAP ASCT FEAM	RC	Aug2025-Nov2025 → Watch and Wait; programmed RTX maintenance

Brescia NGS experience in MCL

Onset-tested cohort

	Age	MIPI	Histo	Ki67	Mutation	Therapy I Line	Response	Notes
#1	70	IR	C	50%	ATM	RBACx6+RTT +RTX maintenance	PR	
#2	47	LR	C	15%	Wild Type	Watch and Wait	SD	Last FU 25/06/2025
#3	63	HR	C	5%	BIRC3 TP53	RCHOP-RDHAP	RC	ASCT suspended for complication; programmed RTX maintenance
#4	81	HR	C	5%	BIRC3 CREBBP	R-Bendamustin lower dose	Ongoing (responding)	IRC → dialysis at onset
#5	73	HR	P	55%	Wild Type	RBACx6	RC	
#6	59	LR	P	90%	TP53 KMT2D	RCHOP-RDHAP ASCT FEAM	RC	Aug2025-Nov2025 → Watch and Wait; programmed RTX maintenance

Brescia NGS experience in MCL

Relapsed-tested cohort

	Age	MIPI	Histo	Ki67	Mutation	DFS* (m)	Therapy I Line	Therapy II Line	Res I	Res 2	Notes
#1	76	LR	C	70%	ATM	185	prot. R-HDS con 2° Mel	Ibrutinib	RC	RP	
#2	66	LR	C	30%	KMTD2 TP53	43	RCHOP-RDHAP +ASCT FEAM +RTX maintenance *	Ibrutinib	RC	Ongoing	*suspendend cause ICU for COVID
#3	61	LR	C	50%	BIRC3	95	RCHOP-RDHAP +ASCT FEAM	Ibrutinib	RC	RP	ASCT suspended for complication; programmed RTX maintenance
#4	74	HR	B	70%	CNV BLC2 MYC	28	RBACx6	Ibrutinib → Pirtoprutinib*	RC	RC	*switch cause of toxicity

*DFS calculated from the end of I line therapy to the time of relapse

NOTE: one patient tested in NGS (relapsed) is being followed by another hematology center

Take home messages

- Talk to your pathologist/biologist
- Trust p53 immunohistochemistry
- NGS for multigene testing

Open questions

- Should we switch to 50% cut-off for Ki-67?
- On which specimen should karyotyping be performed?
- Should we test *MYC* and *TP53* by FISH always?

UNIVERSITÀ DEGLI STUDI DI BRESCIA

Ringraziamenti

Sistema Socio Sanitario
Regione Lombardia
ASST Spedali Civili

S.C. Anatomia Patologica
A.S.S.T. Spedali Civili di Brescia
Università degli Studi di Brescia
Prof.ssa Luisa Lorenzi
Dott.ssa Flavia Melotti
Dr. Mattia Facchetti
Dr. Iacopo Ghini

Laboratorio Patologia Molecolare
Dr.ssa Medicina Daniela
Dr.ssa Galvagni Anna
Prof William Vermi
Tecnici di Laboratorio

Reparto di Ematologia
A.S.S.T. Spedali Civili di Brescia
Dr.ssa Alessandra Tucci
Dr Alessandro Re
Dr.ssa Chiara Pagani

Laboratorio Ricerca in Ematopatologia
Dr. Gallucci Daniele
Dr.ssa Silvia Lonardi
Dr.ssa Emma Albertini
Dr.ssa Francesca Filippini