VII CORSO del GRUPPO ITALIANO DI EMATOPATOLOGIA

La DIAGNOSTICA EMATOPATOLOGICA nell'ERA della MEDICINA di PRECISIONE

Post–CAR-T Grey-Zone Relapse in B-Cell Lymphoma: Diagnostic Challenges and Mechanisms of Tumor Plasticity

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Clinical Case: Male, 47 years old

1

December 2019

Clinical Presentation

Multiple laterocervical lymphadenopathies, present for 2–3 months
Maximum diameter: 3 cm
Excisional biopsy performed - lymph node entirely submitted for histological evaluation

Diagnosis : Follicular lymphoma, Grade 2 (WHO classification), predominantly follicular growth pattern
t(14;18)(IgH/BCL2) detected

No evidence of bone marrow involvemen

1L: Obinutuzumab + CHOP for six cycles (December 2019 – April 2020)

2

April 2020

Post immunochemotherapy: PET-positive with high SUV - **Biopsy of mesenteric lymph node.**

Clinical question: suspected transformation to DLBCL.

Histological diagnosis: involvement by peripheral B-cell lymphoma, predominantly small- to medium-sized cells, with scattered large cells.

No evidence of transformation to diffuse large B-cell lymphoma

2L: 2 cycles of R-DHAP (April–June 2020) → Stable Disease (SD)

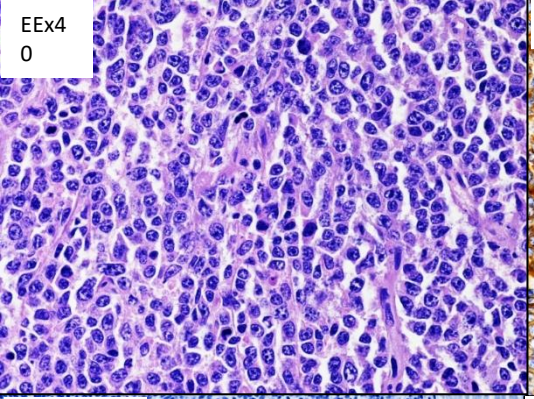
3

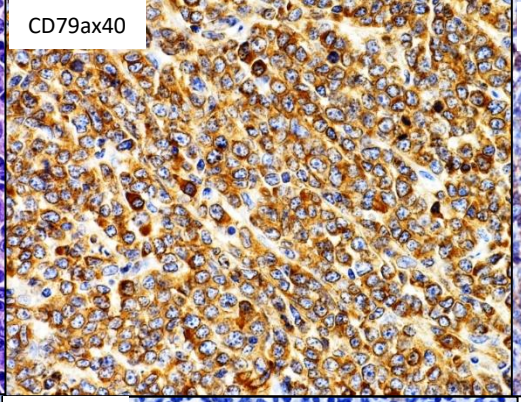
December 2020

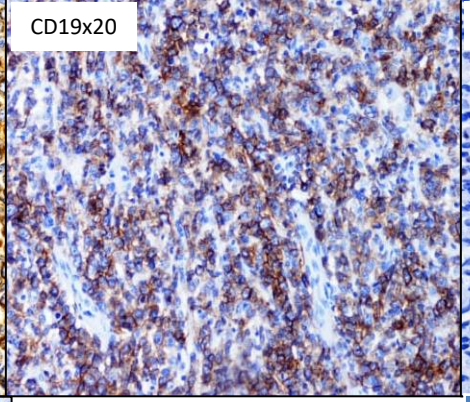
Clinical Presentation:
Large mesenterici lymphadenopathy with appearance of mediastinal involvement

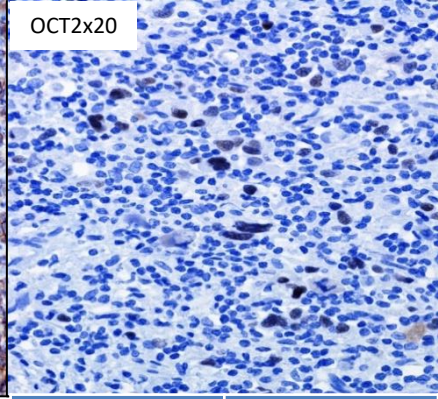
Macroscopic Findings:
Nodular lesion measuring 2 × 1 × 1.5 cm

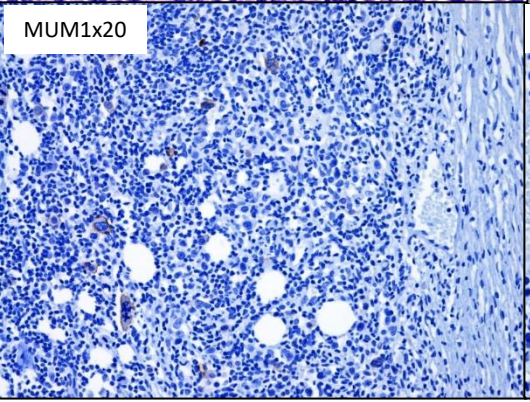
Histological Diagnosis:
Polymorphic Medium – Large cells proliferation with extension to fat tissue around.
Necrosis.

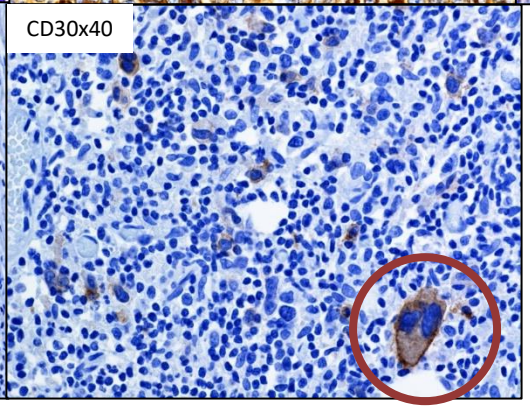












CD20 Negative

FISH MYC: negative

Ab	Result
CD20	-
CD79 alfa	+
CD19	+
OCT2	Isolated cells
CD30	Isolated cells
CD10	+
BCL6-/+	
MUM/IRF4	-
MYC	<5%
Ki67	50-80%

DLBCL , germinal center type sec. HANS

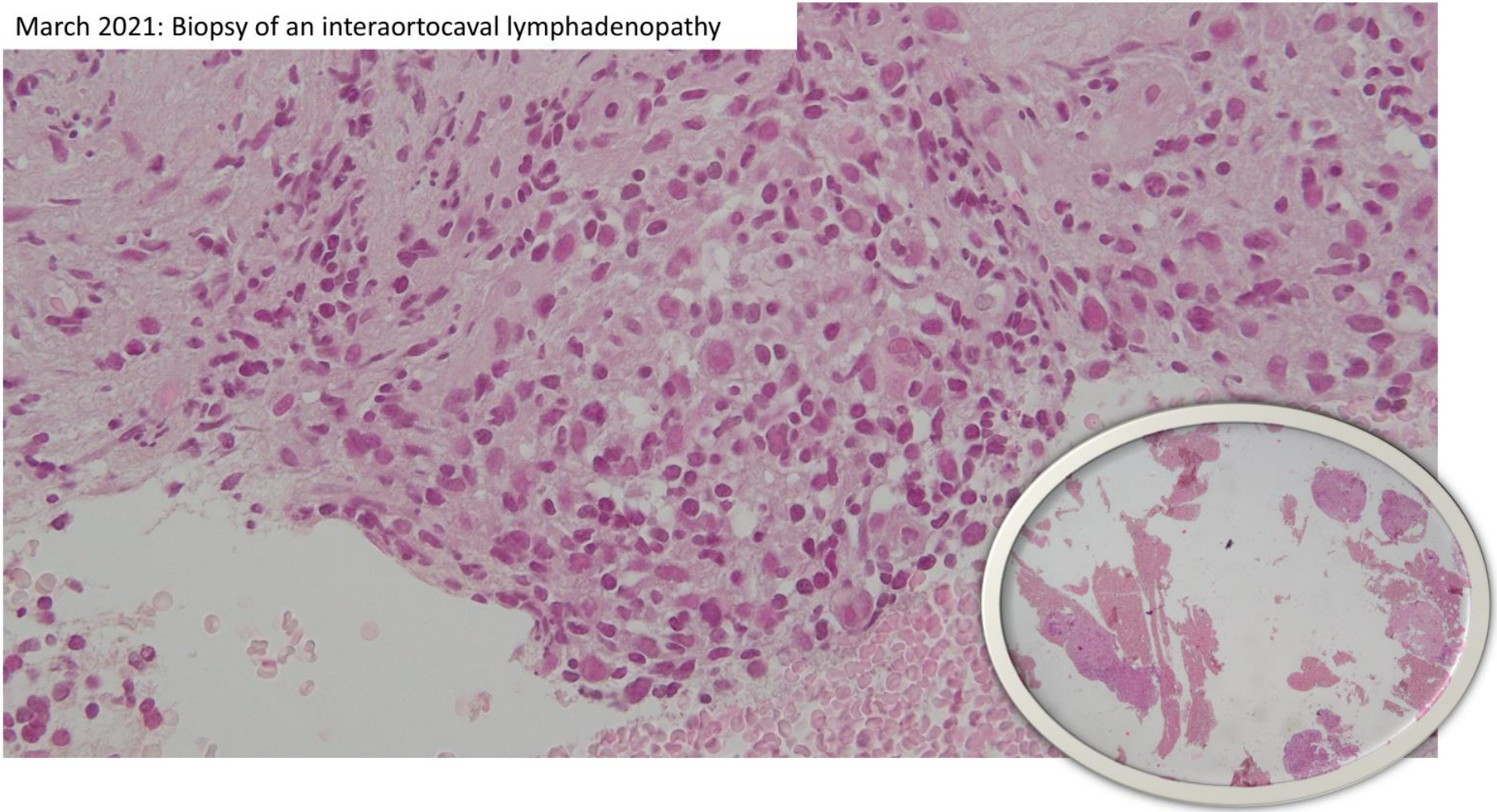
3L: Clinical trial in RE with odronextamab (CD20xCD3 bispecific antibody) from July to November 2020 → Progressive Disease (PD)

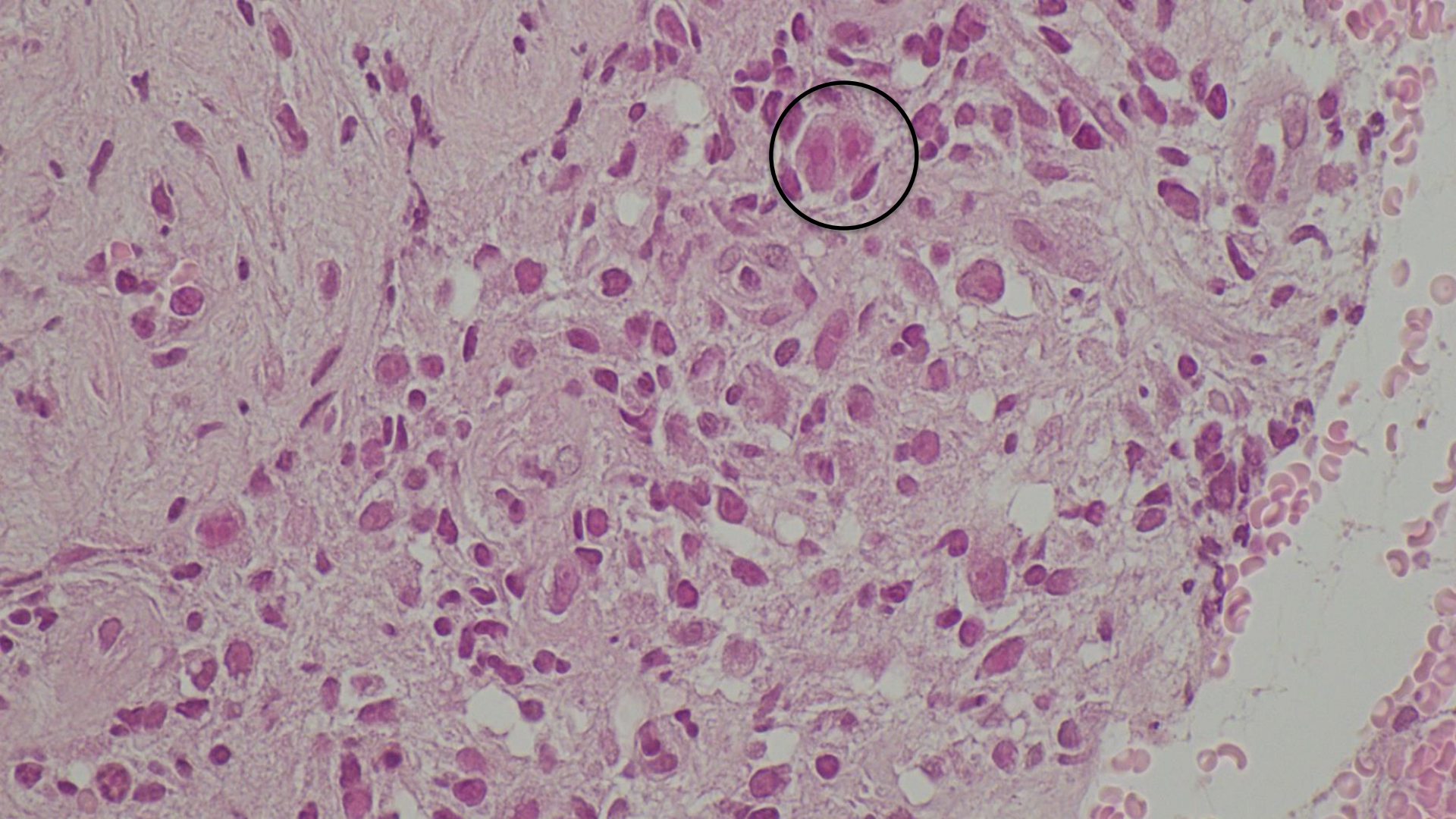
4 **The patient was referred to Policlinico Sant'Orsola for CAR-T therapy.**

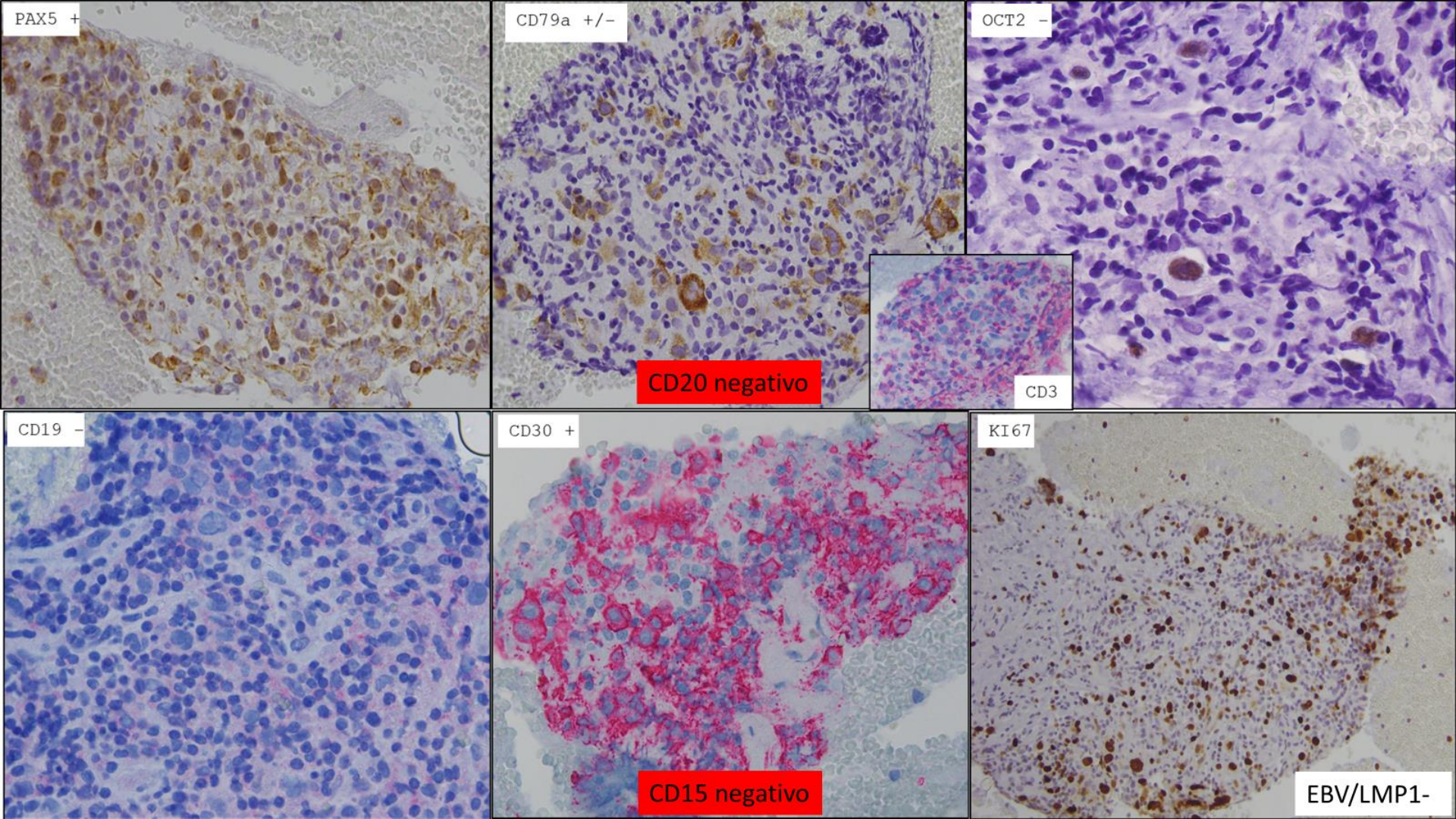
28/10: lymphocytapheresis was performed.
Bridging therapy (BT): 1 cycle of ICE chemotherapy.
Outcome: skeletal progressive disease (PD), but resolution of B symptoms.
05/02/2021: reinfusion of axicabtagene ciloleucel (axi-cel).
Complicated by grade 1 CRS (cytokine release syndrome).
PET scan at 1 month:
Progressive disease due to increased nodal SUV uptake.
Resolution of skeletal uptakes.
Given the patient's good clinical condition and suspected pseudoprogression, close follow-up was continued.
PET scan at 2 months:
Deauville score 4 (DS4), meaning stability of some uptakes and resolution of others.
Overall: initial mixed response after CAR-T therapy, with suspected pseudoprogression followed by partial metabolic stabilization

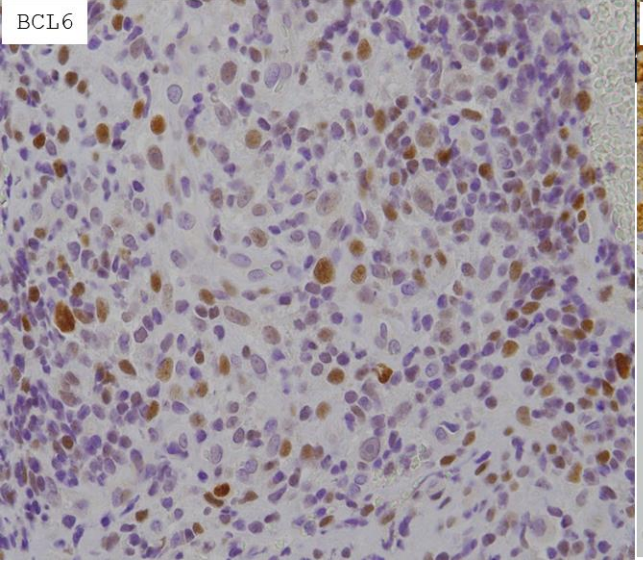
March 2021: A biopsy was performed due to the recurrence of B symptoms

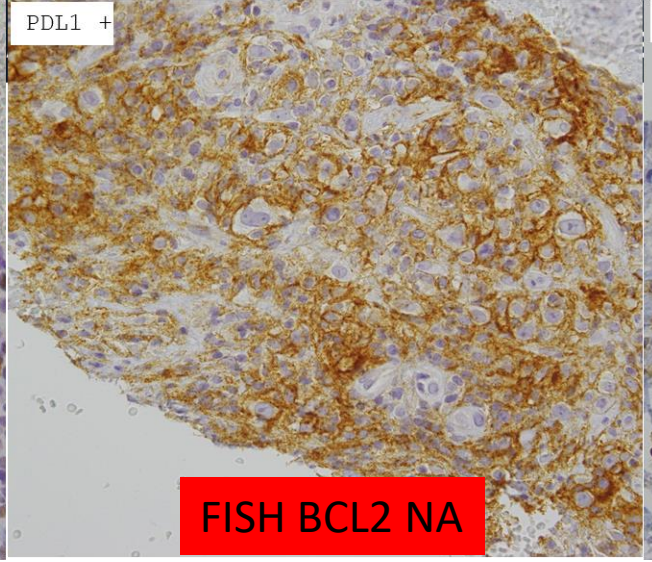
March 2021: Biopsy of an interaortocaval lymphadenopathy



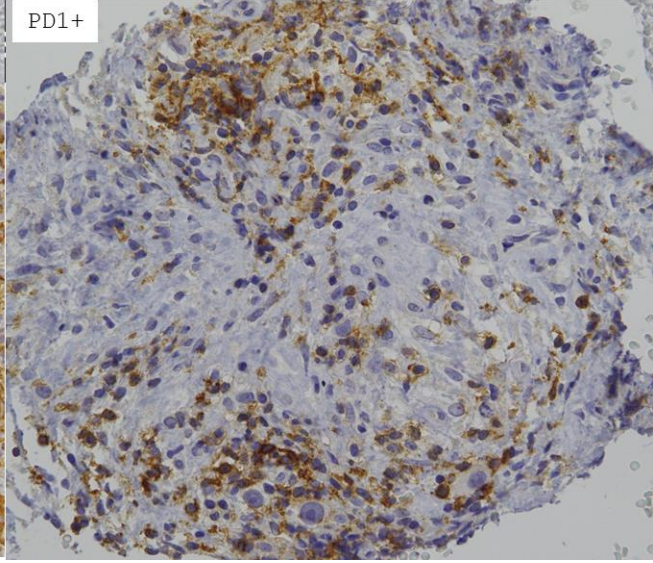



PAX5 +
CD79a +/-
CD20 negativo
CD3
OCT2 -
CD19 -
CD30 +
CD15 negativo
KI67
EBV/LMP1-





FISH BCL2 NA



CD20-	CD79a +/-	CD19-	OCT2-	PAX5+	CD30+	CD15-	BCL6+	PDL1+	PD1+	EBV-

B-cell lymphoma with aberrant B-cell phenotype and Hodgkin-like features

Unclassifiable form related to the previous disease, with intermediate features between Hodgkin lymphoma and extramediastinal B-cell lymphoma.

5

5° - line Pembrolizumab __> Progressive Disease

6

6th -line treatment with Brentuximab Vedotin: complete response (CR), consolidated with allogeneic transplant from a related donor

The patient is currently alive and has been in complete remission for 4 years

		CD20	CD79a	CD19	OCT2	PAX5	CD30	BCL2	BCL6	CD10	MUM1	ki67	MYC
Novembre 2019	tFL	+	+					IHC + t(14; 18) +	+	+		20/25 %	
Aprile 2020	Peripheral B cell lymphoma NO DLBCL	+	+					+	+	-		40%	
Dicembre 2020	DLBCL, CG sec. HANS	-	+	+	-	+	Rare cellule	+	+/-	+	-	50-80%	Isolated cells
2021 Post CART	GZL	-	+/- w	-	-	+	+	NA	+	NA	NA	50%	NA

Mechanisms of resistance and treatment of relapse after CAR T-cell therapy for large B-cell lymphoma and multiple myeloma

Transplant Cell Ther. 2023 July ; 29(7): 418–428. doi:10.1016/j.jtct.2023.04.007.

nature reviews clinical oncology

Spectrum, pathobiology, mechanistic insights and diagnostic challenges of post-CAR T cell therapy lymphoproliferative disorders

blood advances

Clonal hematopoiesis in patients receiving chimeric antigen receptor T-cell therapy

Peter G. Miller,[1,2,*] Adam S. Sperling,[1-3,*] Elliott J. Brea,[1] Mark B. Leick,[4] Geoffrey G. Fell,[5] Max Jan,[1,2,6] Satyen H. Gohil,[1,2,7] Yu-Tzu Tai,[1] Nikhil C. Munshi,[1] Catherine J. Wu,[1,2] Donna S. Neuberg,[5] Marcela V. Maus,[4] Caron Jacobson,[1] Christopher J. Gibson,[1,2,†] and Benjamin L. Ebert[1,2,8,†]

haematologica 2018; 103:e215

Sequential loss of tumor surface antigens following chimeric antigen receptor T-cell therapies in diffuse large B-cell lymphoma

CAR-T exerts a strong selective pressure capable of reshaping tumor biology and lineage differentiation

Resistance of B-Cell Lymphomas to CAR T-Cell Therapy Is Associated With Genomic Tumor Changes Which Can Result in Transdifferentiation

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TABLE 1. Clinical Characteristics of Patients

Patient #	Sex (Age)	Initial Diagnostic	Therapy Before CART	CART	Time to Relapse After First CART Infusion (mo)	Second CART (Y/N) (Interval Between First and Second CART) (mo)	Overall Survival (mo) and Status After Last CART Infusion
1	M (43)	tFL-DLBCL	R-CHOP followed by multiple lines of additional therapies*	Axicabtagene ciloleucel	11	N	8 (DOD)
2	F (60)	HGBCL_DH	DA-EPOCH-R followed by multiple lines of additional therapies†	Tisagenlecleucel	11	Y (10)	3 (DOD)
3	M (68)	DLBCL, NOS	R-CHOP followed by R-DHAC	Axicabtagene ciloleucel	4	N	14 (DOD)
4	M (71)	DLBCL, NOS	R-CHOP followed by R-DHAC	Axicabtagene ciloleucel	3	Y (8)	1 (DOD)
5	F (66)	DLBCL, NOS	R-CHOP+HD-MTX followed by R-DHAC	Axicabtagene ciloleucel	4	N	15 (complete remission)
6	M (5)	BL	R-CHOP/R-CHOP like followed by lines of additional therapies‡	Tisagenlecleucel	1.5	N	10 (complete remission)
7	M (69)	tFL-DLBCL	R-CHOP followed by R-ICE	Tisagenlecleucel	3	N	6 (stable)
8	F (55)	DLBCL, NOS	R-CHOP followed by R-DHAP-like	Axicabtagene ciloleucel	3	Y (3)	3 (progression)
9	M (13)	BL	R-CHOP/R-CHOP like followed by multiple lines of additional therapies§	Tisagenlecleucel	1.5	N	1.5 (DOD)

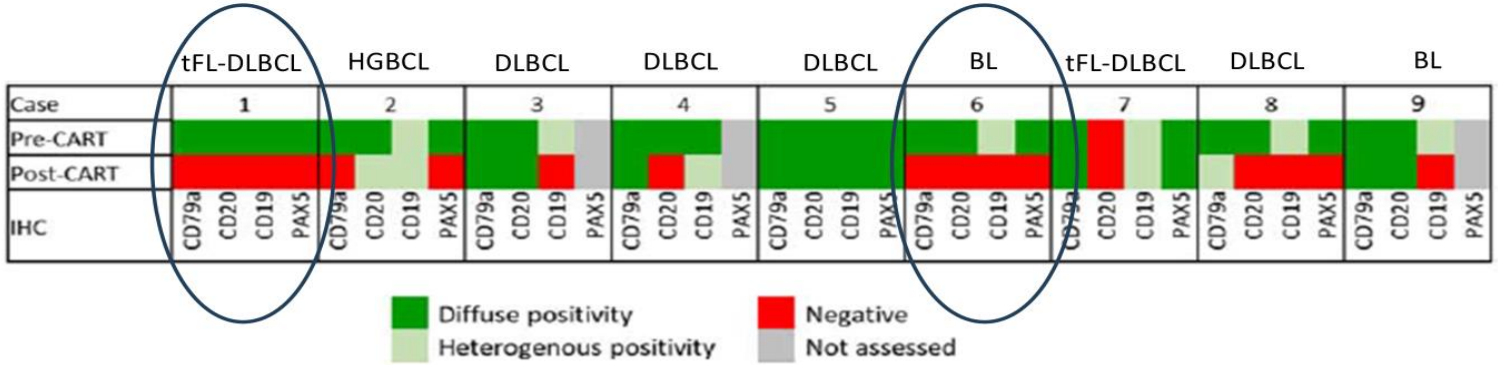


FIGURE 1. Evolution of B-cell markers in pre-CART versus post-CART lymphoma tissues. This representation highlights a global tendency to the loss of various B-cell markers including CD19 in most patients.

Histopathologic features were mostly retained at relapse in 7/9 patients, except the frequent loss of 1 or several B-cell markers.

The remaining 2 cases displayed a dramatic phenotypic shift in post-CART tumors, with the drastic downfall of B-cell markers and emergence of T-cell or histiocytic markers, **despite the persistence of identical clonal immunoglobulin gene rearrangements.**

Main Biological Mechanisms of Immunophenotypic Escape

1. Antigen loss / immune escape
•Downregulation or loss of:
CD20, CD19, CD79a, PAX5

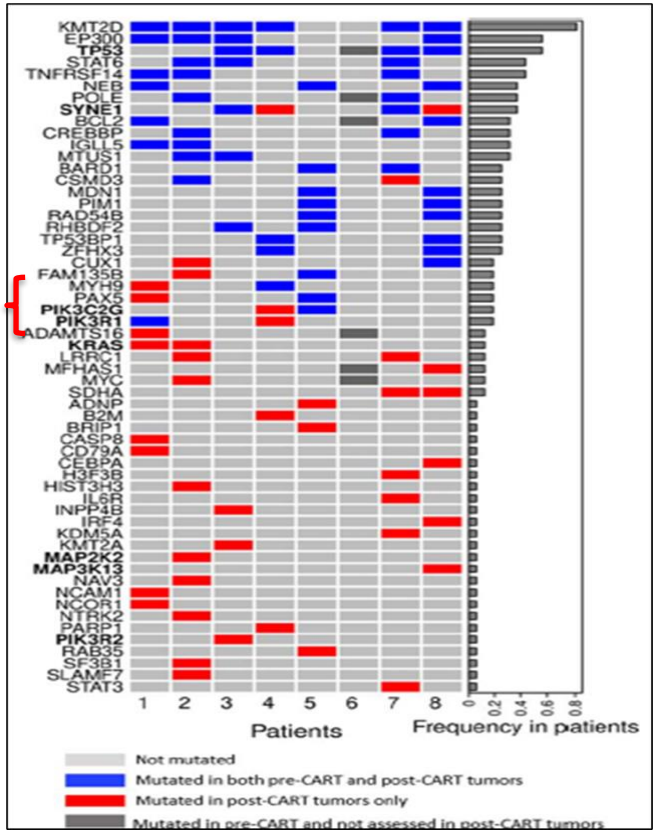
Mechanisms:
• Epigenetic silencing
•**Downregulation B cell program at RNA level**
•Prior anti-CD20 exposure

2. Tumor plasticity and transdifferentiation
Loss of B-cell identity

Emergence of:
•Hodgkin-like phenotype
•T-cell markers (CD2/CD3/CD7)
•Histiocytic phenotype

Persistence of identical IG rearrangements supports clonal relationship

3. Genomic remodeling under CAR-T pressure:
•**PI3K pathway** (PIK3R1/PIK3R2)
•**KRAS/MAPK** signaling
•TP53 alterations
•Epigenetic regulators (KMT2D, EP300)
• **Mutation of PAX5** : role of stable B lineage commitment



4. Microenvironment-mediated resistance:

CART activity can be affected by an immunosuppressive tumor microenvironment (TME).

- PD-1 / PD-L1 axis activation : PD1/PDL1 staining in stromal cells, in lymphoma cells and tumour infiltrating macrophages
- Immunosuppressive macrophage-rich microenvironment
- Functional CAR-T exhaustion

One can hypothesize that the interaction of PD1+ tumor cells with PD-L1+ macrophages could contribute to sustain their presence within the TME, which in turn could inhibit CAR T cells.

Strategies to Overcome Post–CAR-T Phenotypic Escape

Diagnostic strategies

1) Mandatory re-biopsy at relapse
2) Compare with all previous pathology specimens
3) Integrate morphology + IHC + molecular data

Expanded immunophenotypic panel
Mandatory B-cell markers
CD19
CD20
CD79a
PAX5
OCT2 / BOB1

Additional markers
CD30
PD-L1
CD15
CD3/CD2/CD7
CD68/CD163
EBER

Molecular confirmation

Perform when possible:
IG clonality studies
FISH comparison with original lymphoma
NGS panel
Evaluation of target antigen expression

Practical Take-Home Messages

Key messages for the pathologist

- Post–CAR-T relapses may show dramatic phenotypic shifts
- Loss of CD19/CD20 does NOT exclude clonal persistence
- Gray-zone" or Hodgkin-like morphology may represent immune escape rather than a new lymphoma

Diagnostic pearls

Never rely on a limited IHC panel
Always compare with previous biopsies
Clonality studies are critical in ambiguous cases
Interpret PD-L1 and CD30 expression in therapeutic context

Clinical relevance

Correct recognition of post–CAR-T phenotypic escape may:

Prevent diagnostic errors

Identify new therapeutic vulnerabilities

Direct patients toward salvage immunotherapy or transplant

Re-biopsy after CAR-T is not optional — it is biologically and therapeutically essential

Grazie per l'attenzione