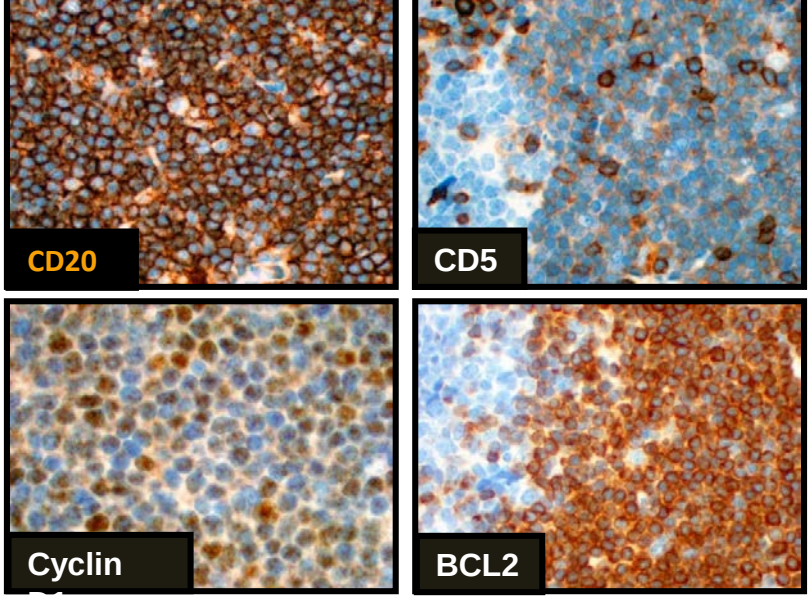
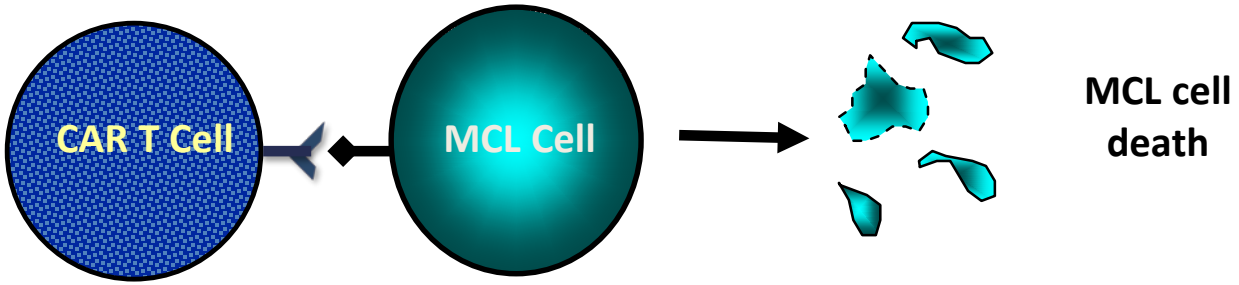
Clinical History

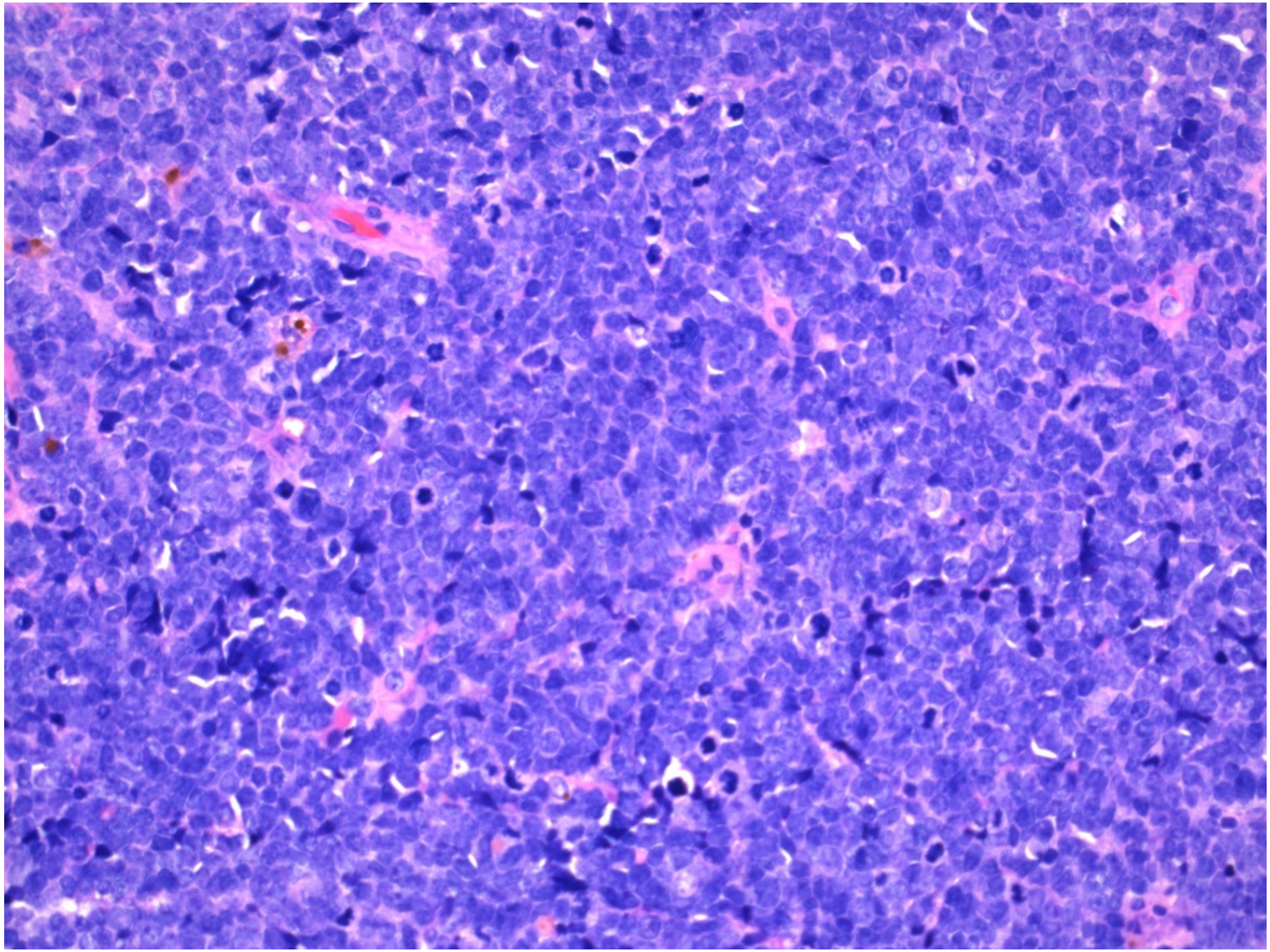


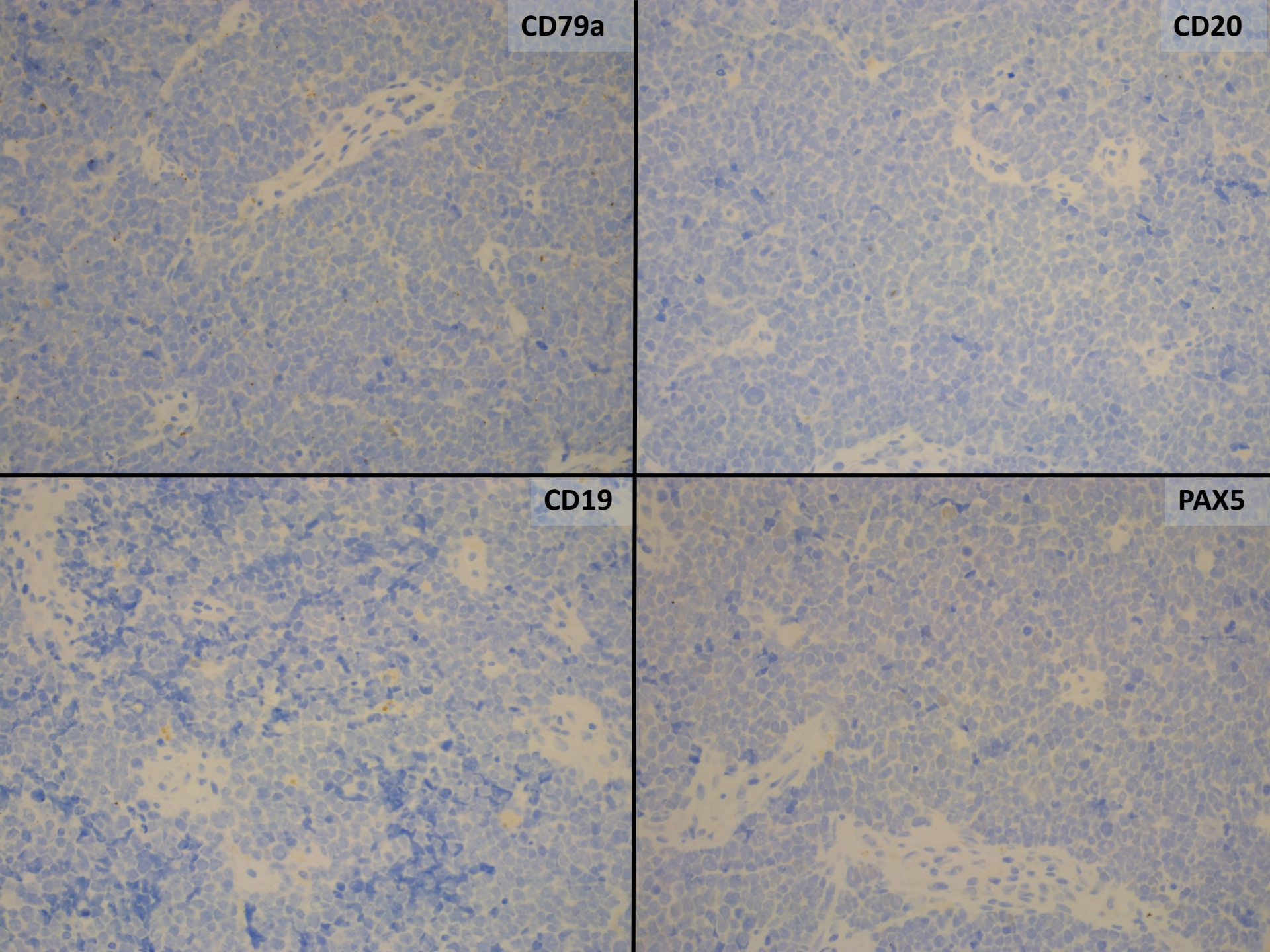
- H/o stage IV mantle cell lymphoma (MCL) initially diagnosed in the right groin and treated in **2002** with 8 cycles of R-HCVAD with CR
- Relapse of MCL in **2004** during the 4th cycle of R maintenance; pt achieved PR, consolidated by XRT
- Relapse of MCL in **2008** at the base of tongue; treated with 7 cycles of R/Velcade. Biopsy showed no disease but PET uptake suggested otherwise
- In 11/**2012** patient was diagnosed with relapsed MCL in leukemic phase and achieved CR, however in 11/**2013** her MCL relapsed and she was started on **BTK inhibitor** ibrutinib that she took until **2/2015** when the bone marrow showed 15% involvement by MCL
- Patient was treated with EPOCH+Velcade in 2/2015 and in **3/2015** by hyperfractionated Cytoxan followed by **CART19 cells**

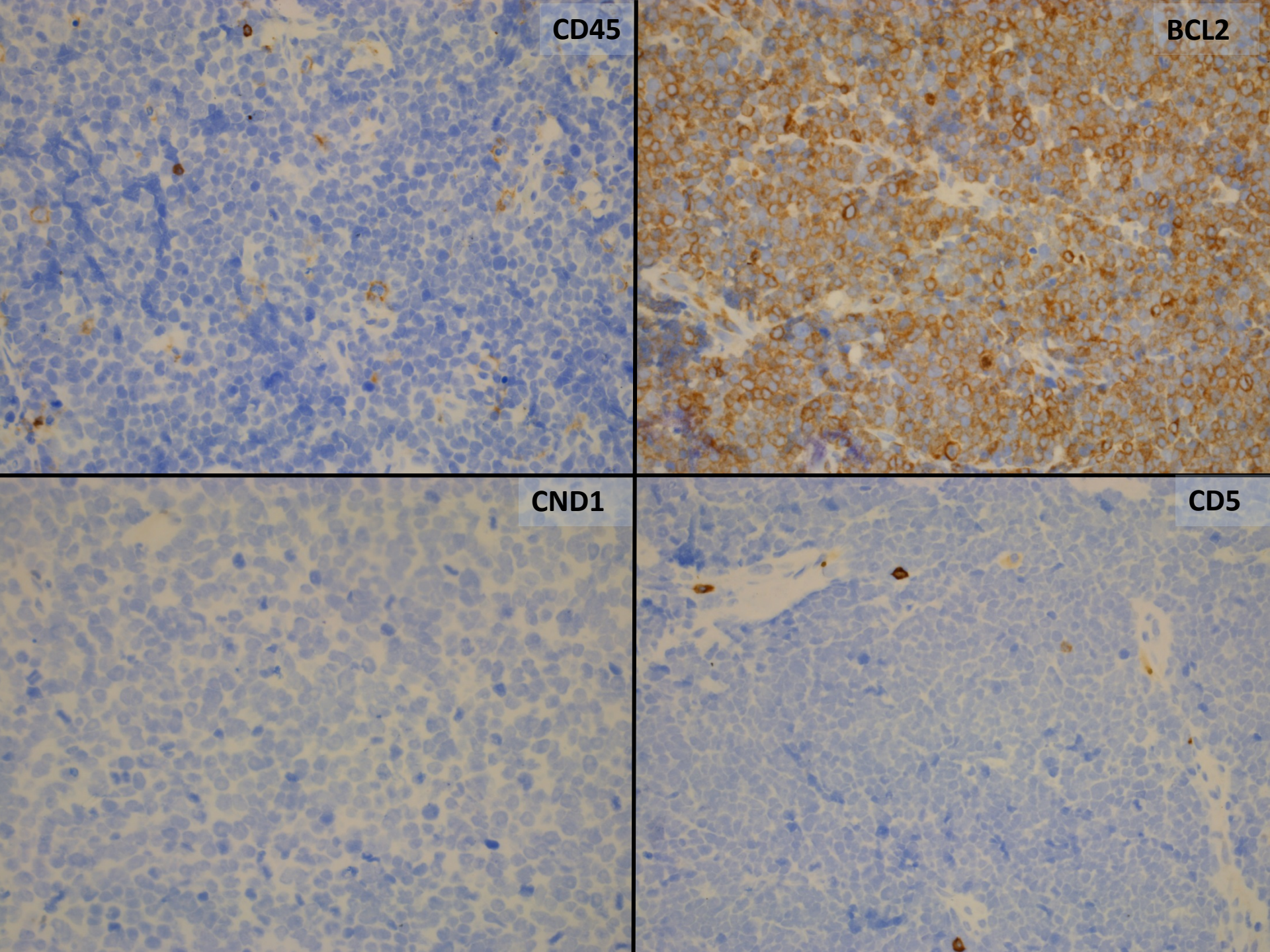


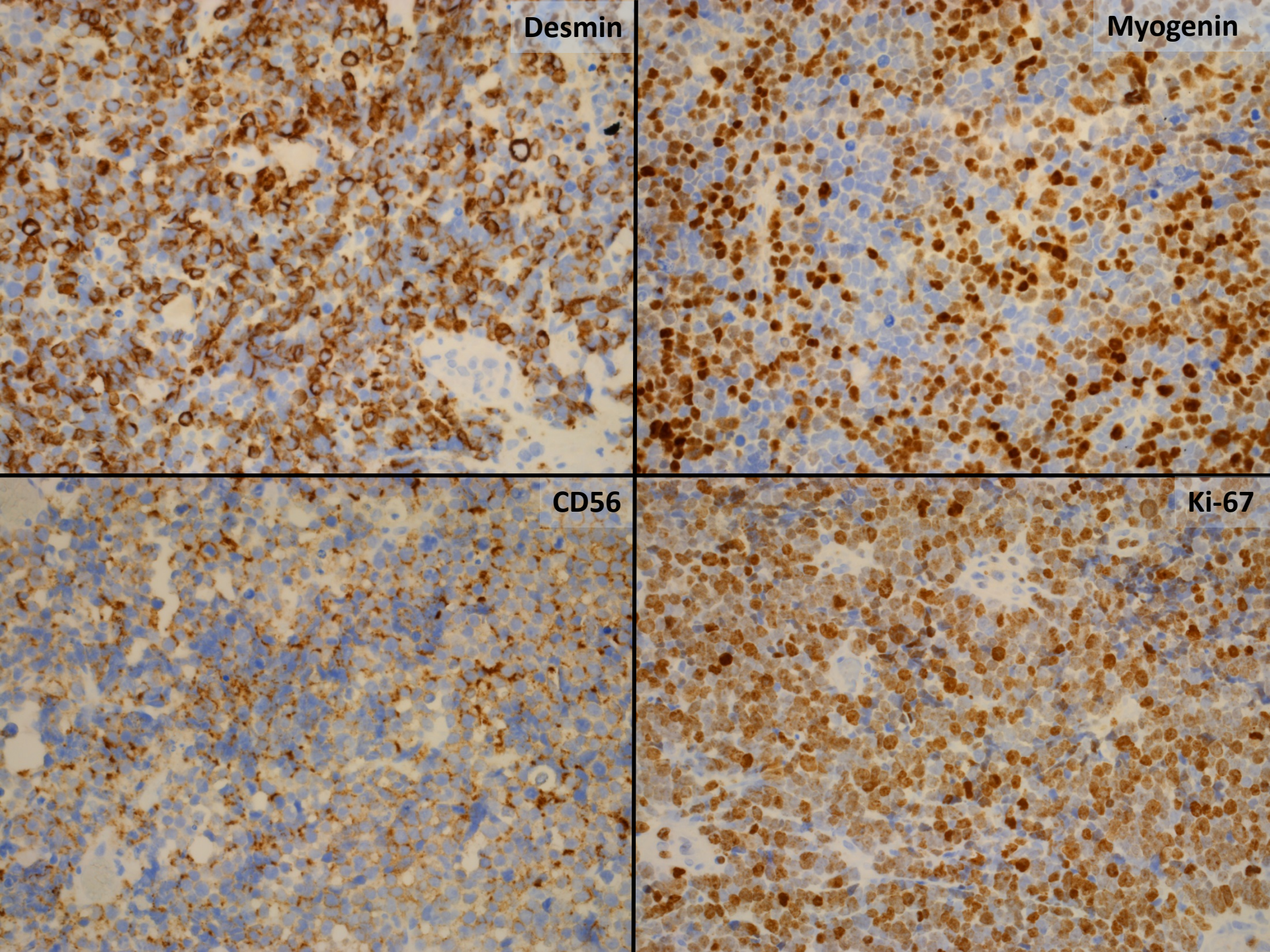
- In **5/2015**, the patient reported progressive enlargement of left supraclavicular lymph node

Tumor morphology (05/2015)




CD79a
CD20
CD19
PAX5


CD45
BCL2
CND1
CD5


Desmin
Myogenin
CD56
Ki-67

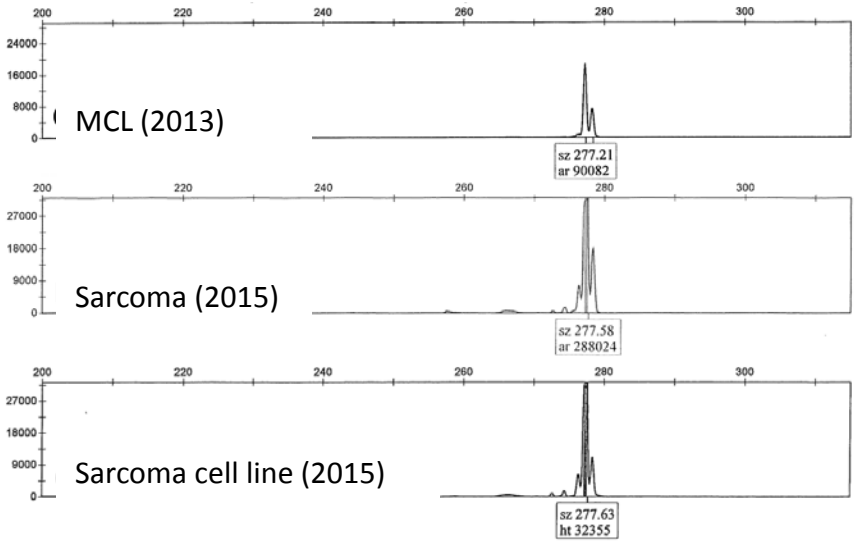
Diagnosis

Poorly differentiated tumor of mesodermal origin with evidence of rhabdomyoblastic and possible neuroblastic differentiation:

poorly differentiated sarcoma (PD-Sc) with striated muscle and (?) limited neural differentiation

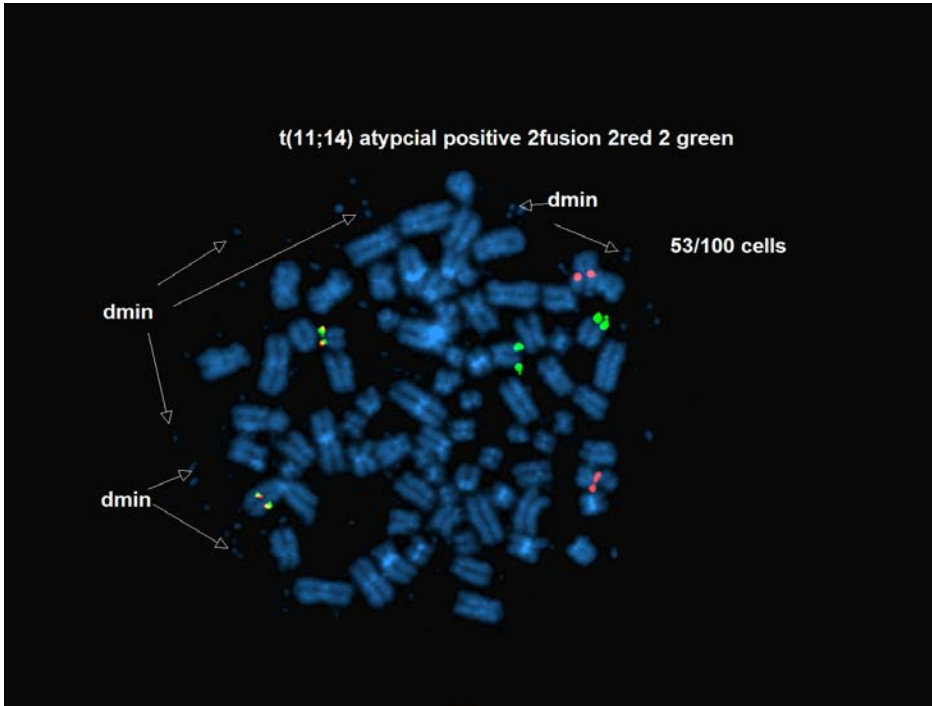
Clonal relationship of the PD-Sc and MCL

- Molecular studies performed on sarcoma tissue and cell line: both positive for the immunoglobulin (IgH) gene rearrangement matching the IgH rearrangement of the MCL



- FISH for IgH-Cyclin D1 (CND1) gene fusion: positive in sarcoma cells

  (as also seen in MCL; not shown)



The key questions:

1. What are the mechanisms of the trans-differentiation?

2. Can we provide any therapeutic guidance?

Trans-differentiation of lymphoma (including MCL) has been described in the past but:

- it has been limited to histiocytic/dendritic sarcoma, hence to malignancies of other immune cells (*M Hure et al. 2012*)
- causes, let alone mechanisms, remained unknown

Potential causes of trans-differentiation: spontaneous or therapy-induced (history of multiple therapies favors the latter).

In CART19-treated patients tumor conversions have been seen to:

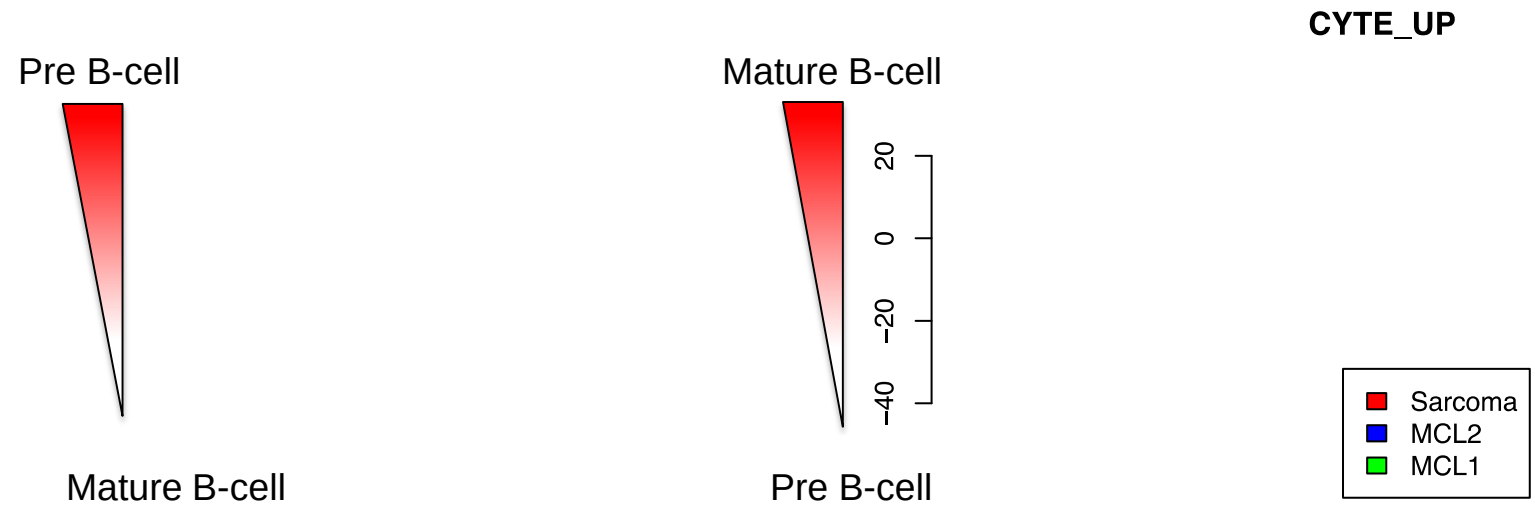
- CD19- plasmablastic lymphoma in CLL pt (*A. Evans et all. 2015*)
- AML in ALL pts (*E. Jacoby et al. 2016*)

both these “trans-differentiations” are also fairly limited: one is a form of large-cell transformation, the other “dedifferentiation” to a common progenitor cell

In-depth analysis of patient's MCL and PD-Sc

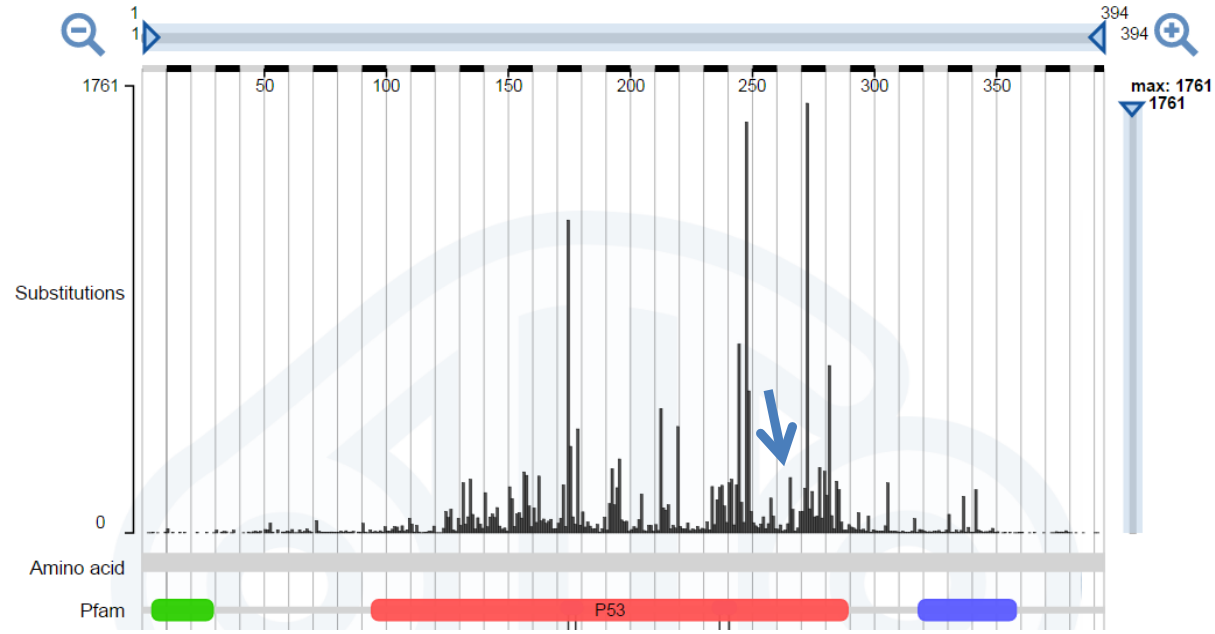
- Analysis (RNA-Seq) of MCL and primary and cultured PD-RMSc cells for gene expression
- Analysis (WES) of MCL and PD-Sc cells for gene mutations
- Analysis of MCL and PD-RMSc cells for genome-scale DNA methylation

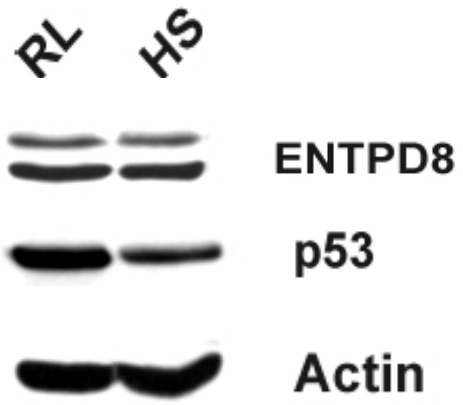
Comparative genome-scale gene expression analysis in MCL and PD-Sc cells



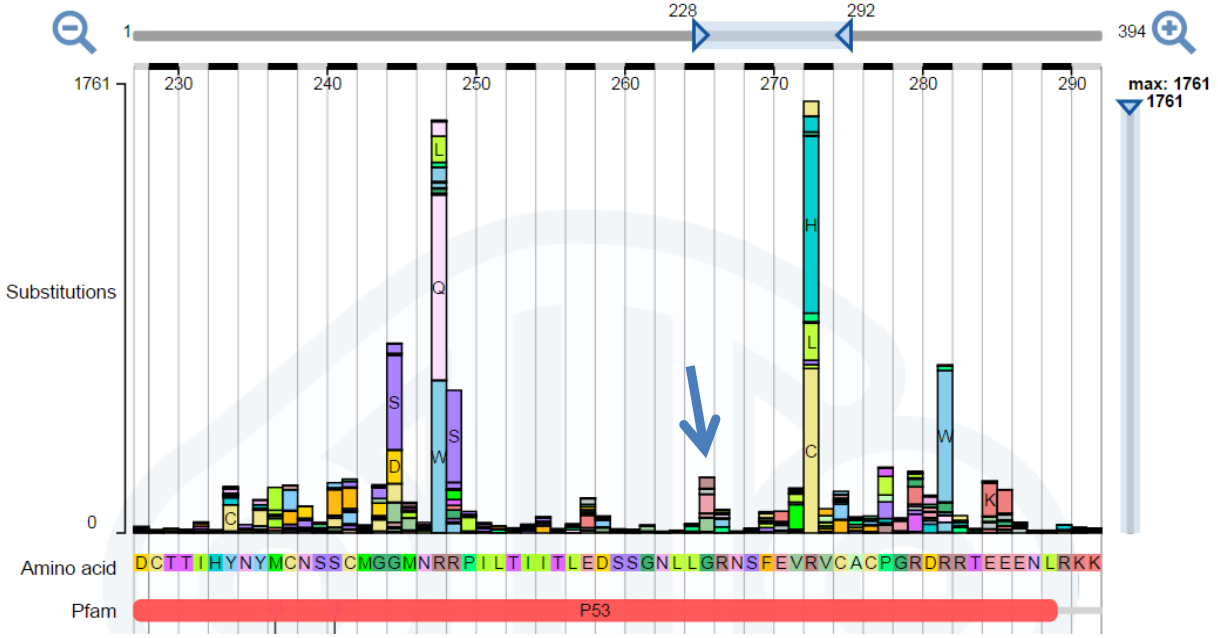
Similar results were obtained using Hoffman and Biocarta data sets as reference

ENTPD8 (G165R): novel mutation with unknown oncogenic potential
p53 (G266V): pathogenic, seen in carcinomas of lung, colon, pancreas, and liver





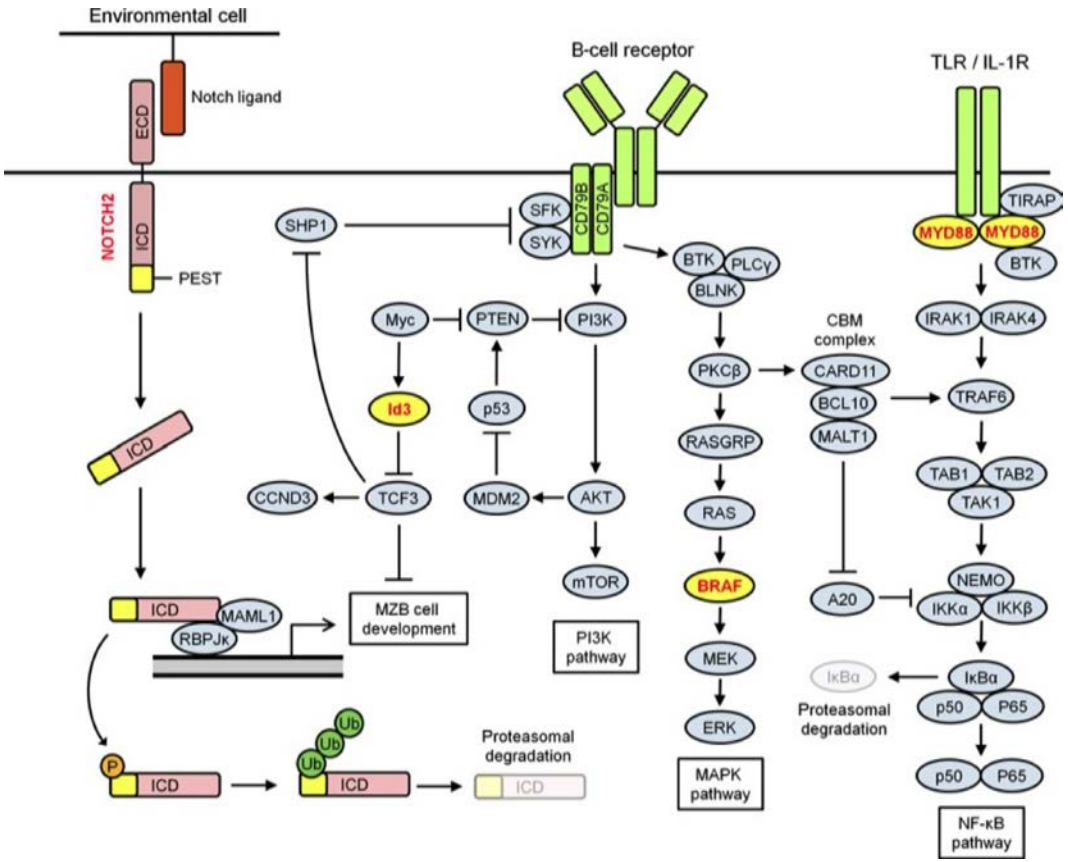
Mutation distribution in p53 gene



Gene promoter DNA methylation in MCL vs. PD-Sc

Number of gene promoters evaluated: 24770

Number of gene promoters differentially methylated between MCL and PD-Sc: **12054** including 547 promoters of miR genes



Promoter de-methylation in PD-Sc of genes associated with muscle and neuronal differentiation

	Gene #	
GO: contractile fiber	22	CAV3, OBSCN, ABLIM2, MYL4, TNNC2, MYH3, MYL1, ANKRD2, MYLPF, MYLK2, CSRP3, CACNA1S, TNNT3, MYO18B, TRIM54, ANK2, SORBS2, SVIL, ATP2A1, RYR1, MYOM1, PLEC
GO: muscle contraction	18	**FXYD1**, MYL4, TNNC2, **TRPV1, DRD2,** MYH3, MYL1, **DAG1**, **ANKRD2**, MYLK2, **CACNG1**, CACNA1S, TNNT3, RYR1, **SMPX, CHRND**, MYOM1, **MB**
GO: transmission of nerve impulse	22	PRX, KCNMB3, SCN2B, DRD2, GABRA6, SLC12A5, ASZ1, MYLK2, KCNIP1, DMPK, SLC17A7, PDE7B, MUSK, GABRR1, GRM2, P2RX1, RAPSN, NMUR2, SLC1A6, GHRL, CACNA1A, HTR2A
GO: neuro-transmitter binding	12	**HTR3E**, **SSTR5**, **MCHR1**, GABRR1, **GABRA1**, **SLC6A11**, NMUR2, **GABRA6**, **MC2R**, **SORCS1**, **BRS3**, **CHRND**
GO: neuron differentiation	23	**NRTN**, **NDN**, **DRD2**, **RXRA**, **BRSK2**, **CABP4**, **RPGRIP1**, **CDH4**, **TP73**, **LINGO1**, **HOXC8**, **DLX1**, **BDNF, LAMB2, GBX2, GHRL, BMPR1B, PITX3, LHX8, DCLK1**, CACNA1A, **NGF**, **CDH23**

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DNA mutational analysis
CHOP genomic core
H.Y. Wang

RNA expression analysis
E. Orlando
H. Bitter

Flow cytometry
Qun-bin Xiong

Cytokine expression analysis
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Panel Dx: Mantle cell lymphoma transdifferentiated to sarcoma