# Unmet challenges in high risk hematological malignancies: from benchside to clinical practice

Turin, September 13-14, 2018

## Histopathological and Biological characterization of high risk Hodgkin Lymphoma (CHL)

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**Histopathological factors and biomarkers which influence the prognosis of CHL**

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**ABVD, BEACOPP with escalations, brentuximab vedotin, PD-1 blockade or combinations**

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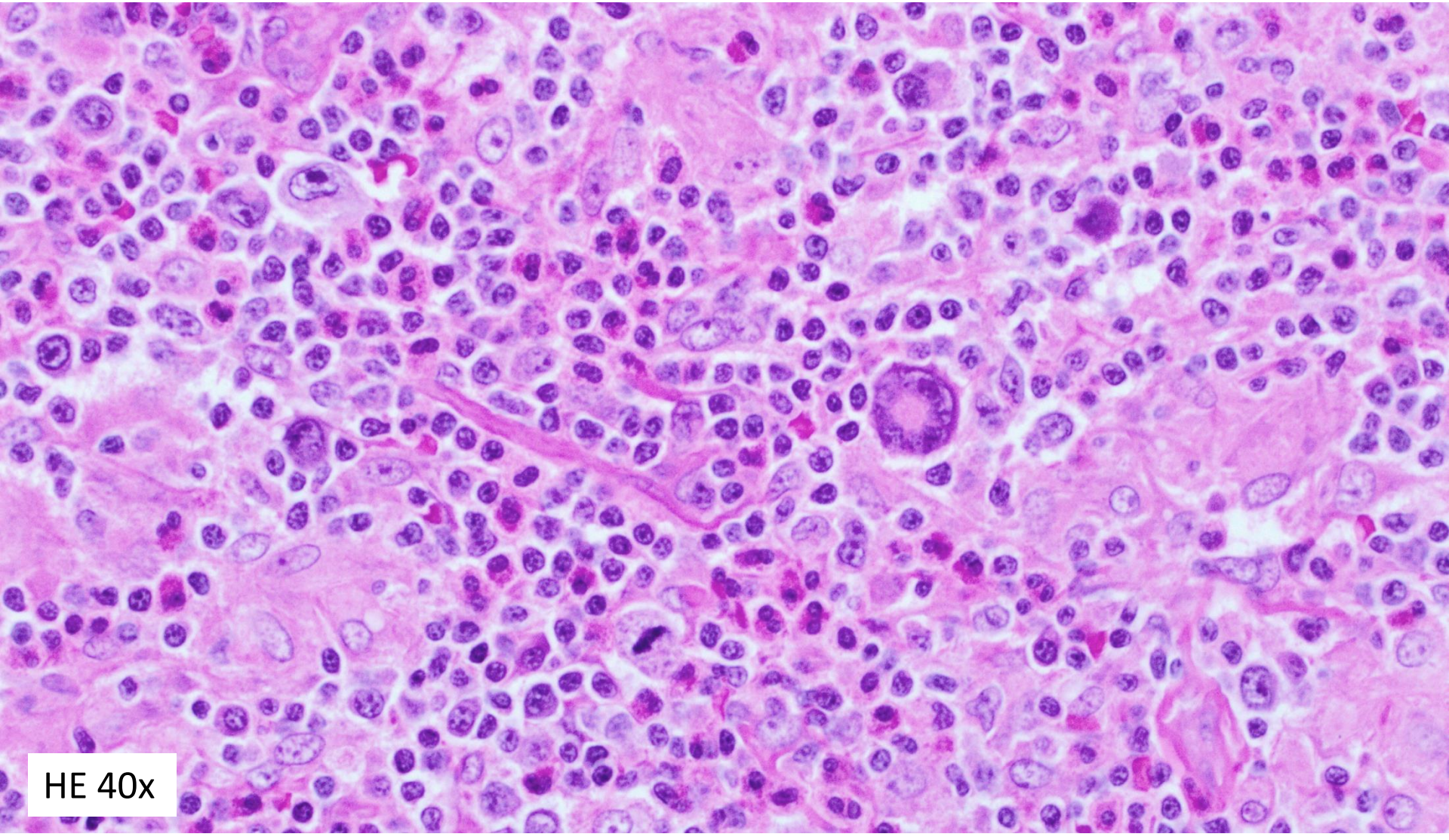
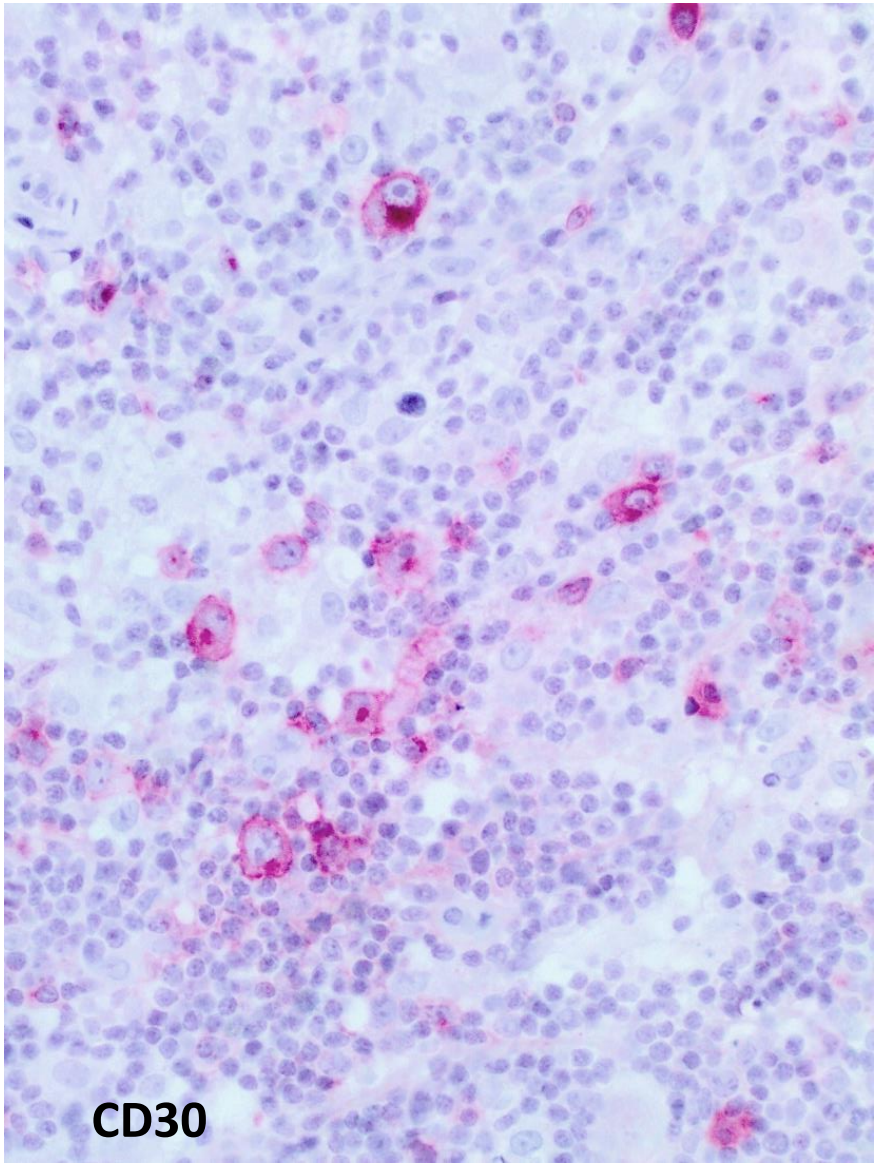
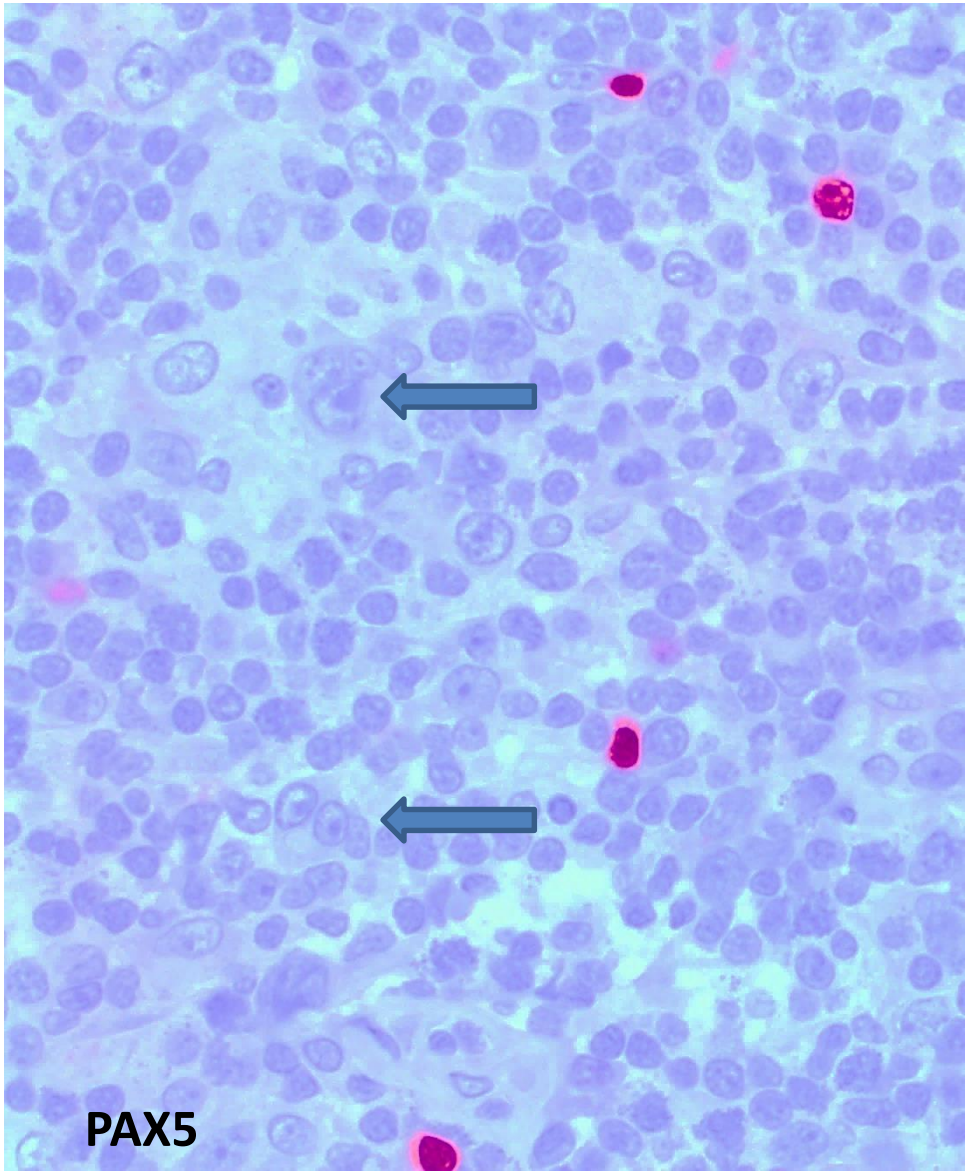
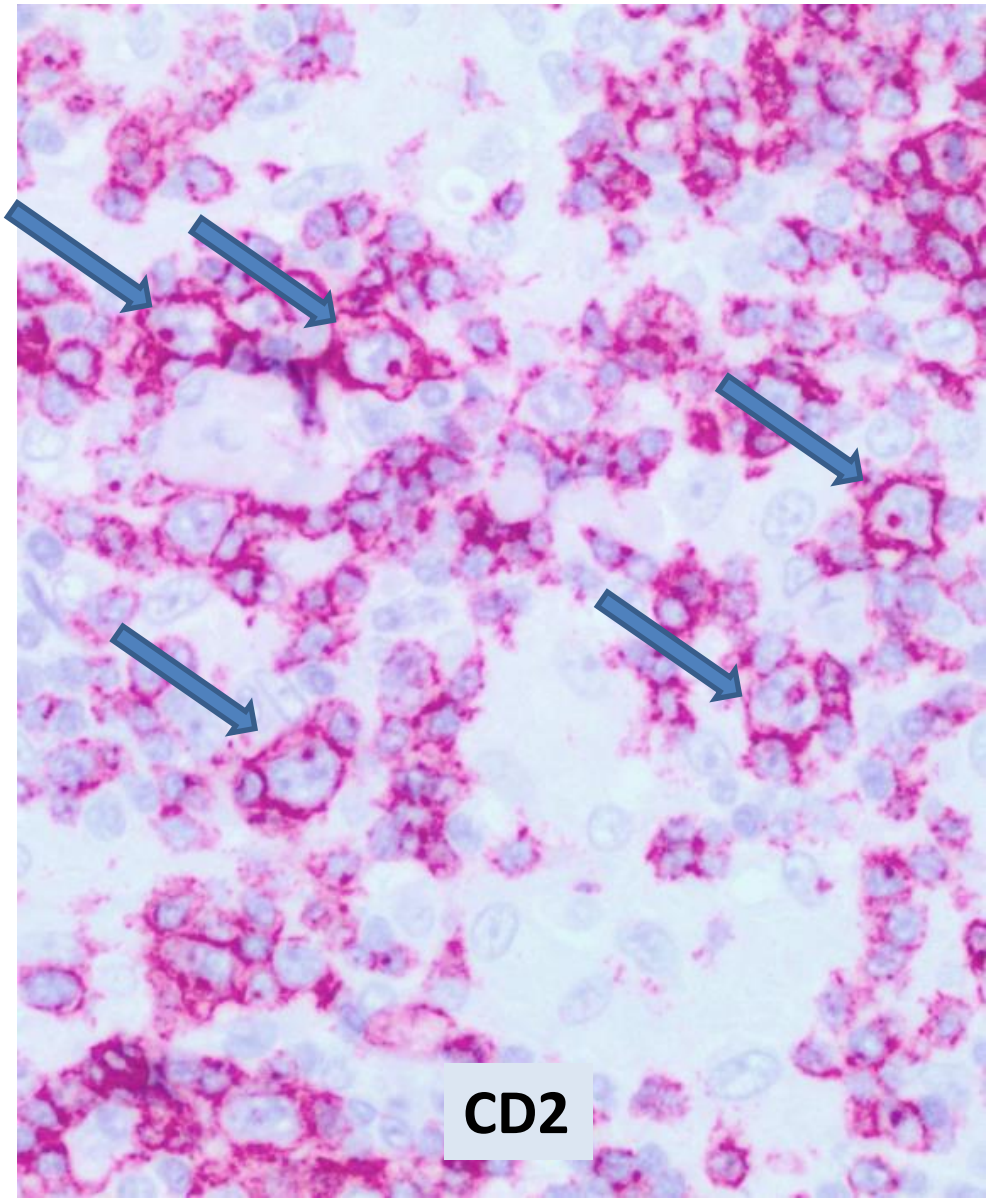
- **microenvironment:** CD68, perforin, FOXP3, PD-1, CD20: prognostic impact not significant

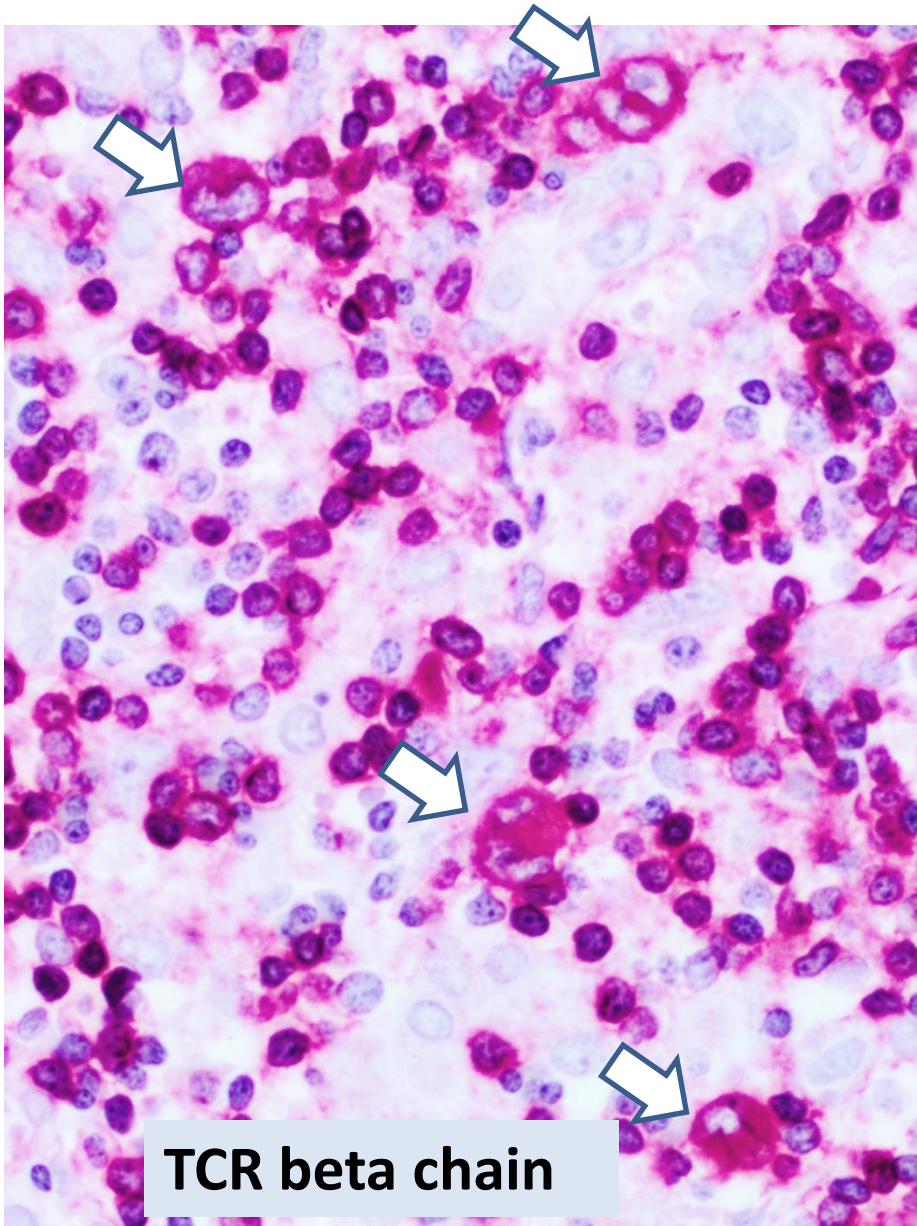
2017: Biopsy from  a 54 year old male patient diagnosed by the primary pathologists as a relape of the classical Hodgkin lymhoma first diagnosed in 2016



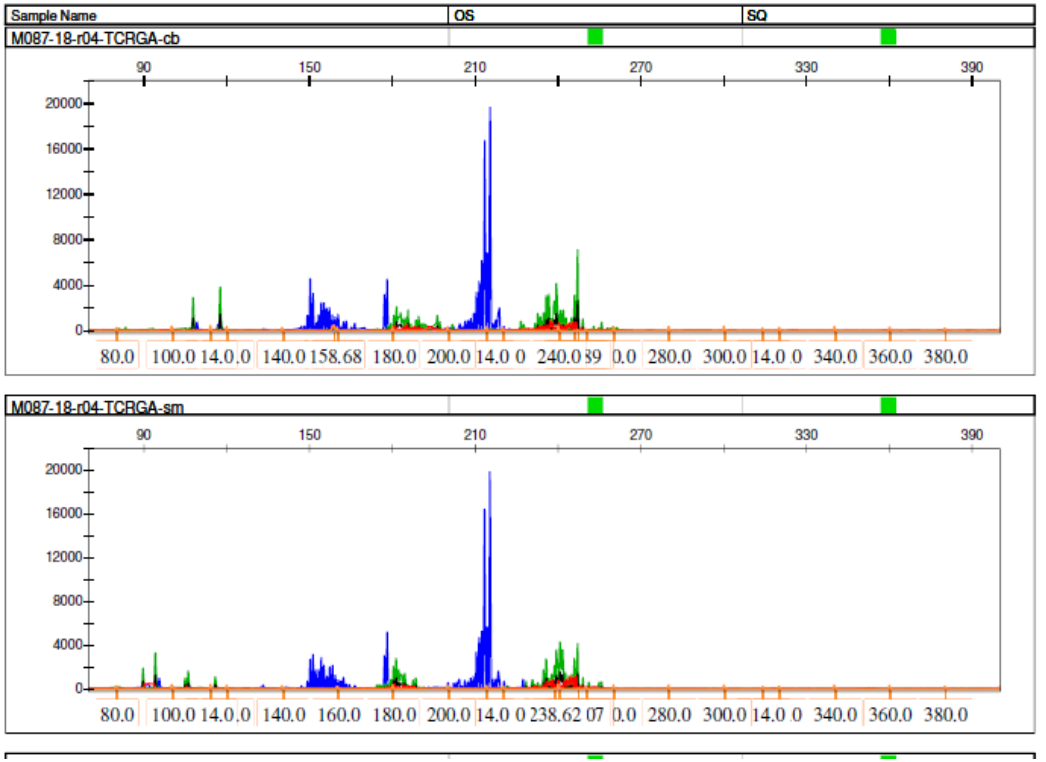

CD30


PAX5


CD2


TCR beta chain

| Biomarker | Expression* |
|---|---|
| CD30 | -/+ |
| PAX5 | - |
| MUM1/IRF4 | -/+ |
| CD3 | - |
| CD5 | - |
| CD4 | - |
| CD8 | - |
| **CD2** | **+** |
| **TCR beta chain** | **+** |



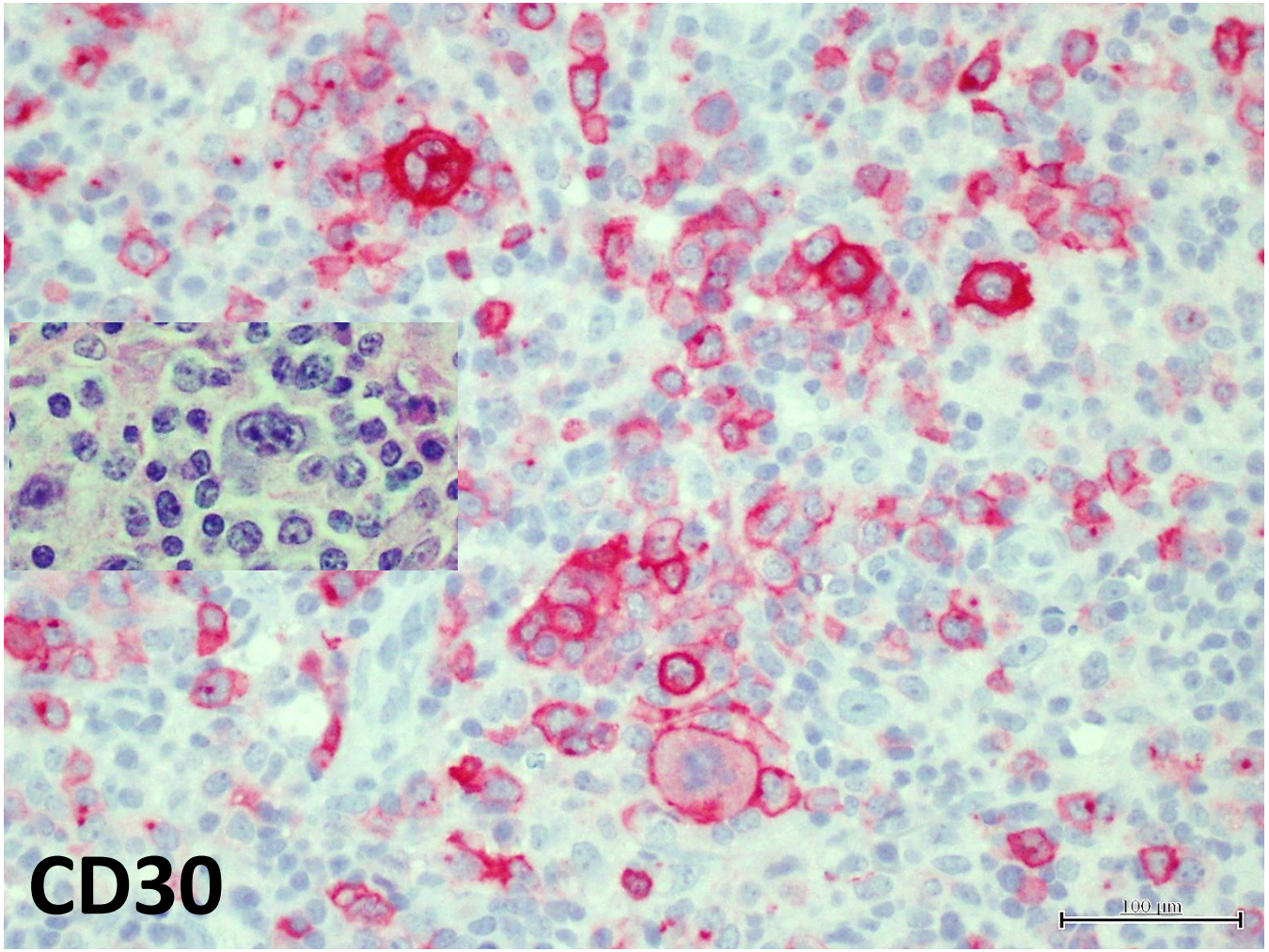

* in neoplastic cells

**Conclusion:**

This lymphoma fullfils all criteria of a peripheral T-cell lymphoma

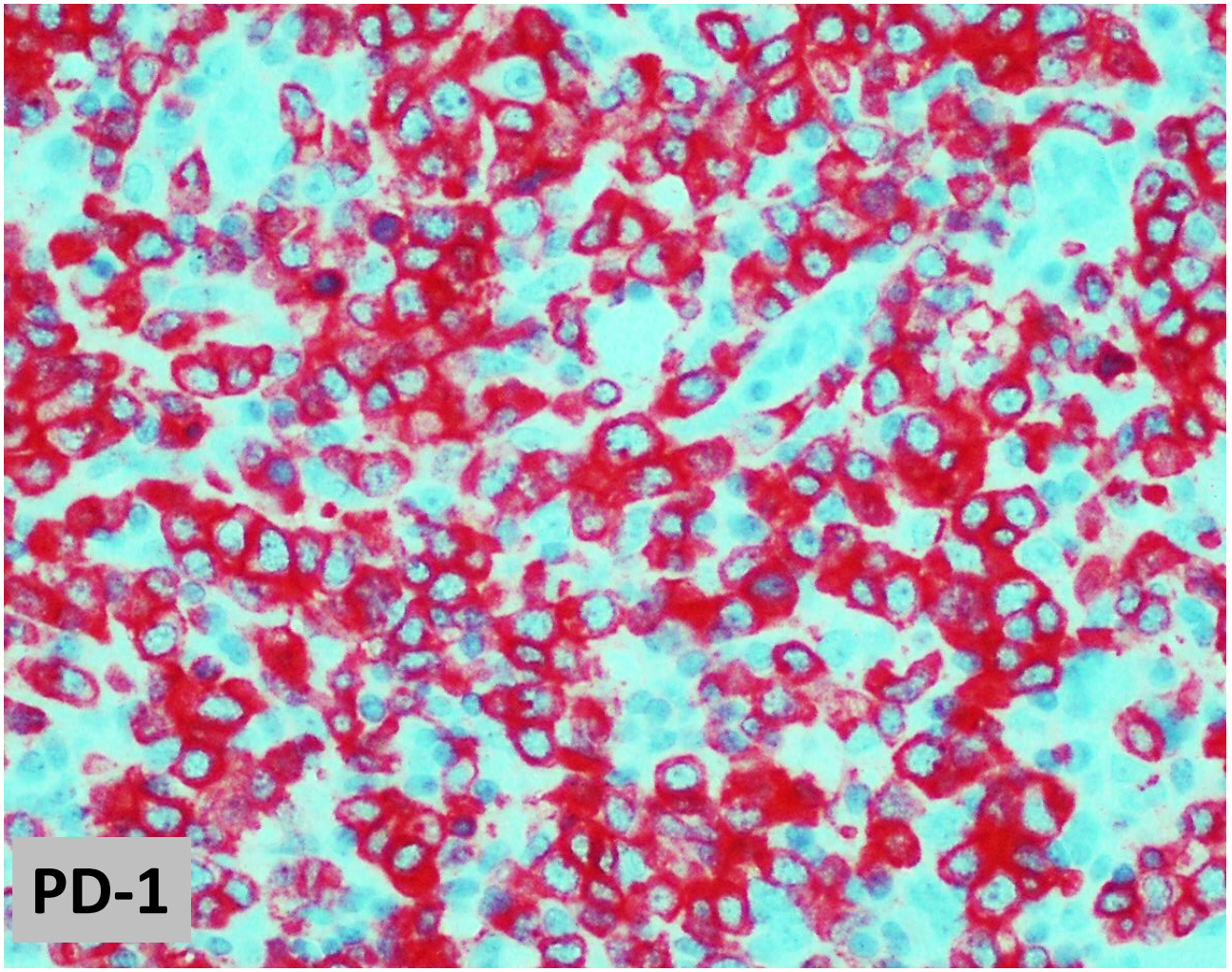
67 year old male patient with generalized lymph node swellings.

Biopsy sent in for reference pathology assessment of whether the diagnosis of **classical Hodgkin lymphoma (CHL)** can be confirmed

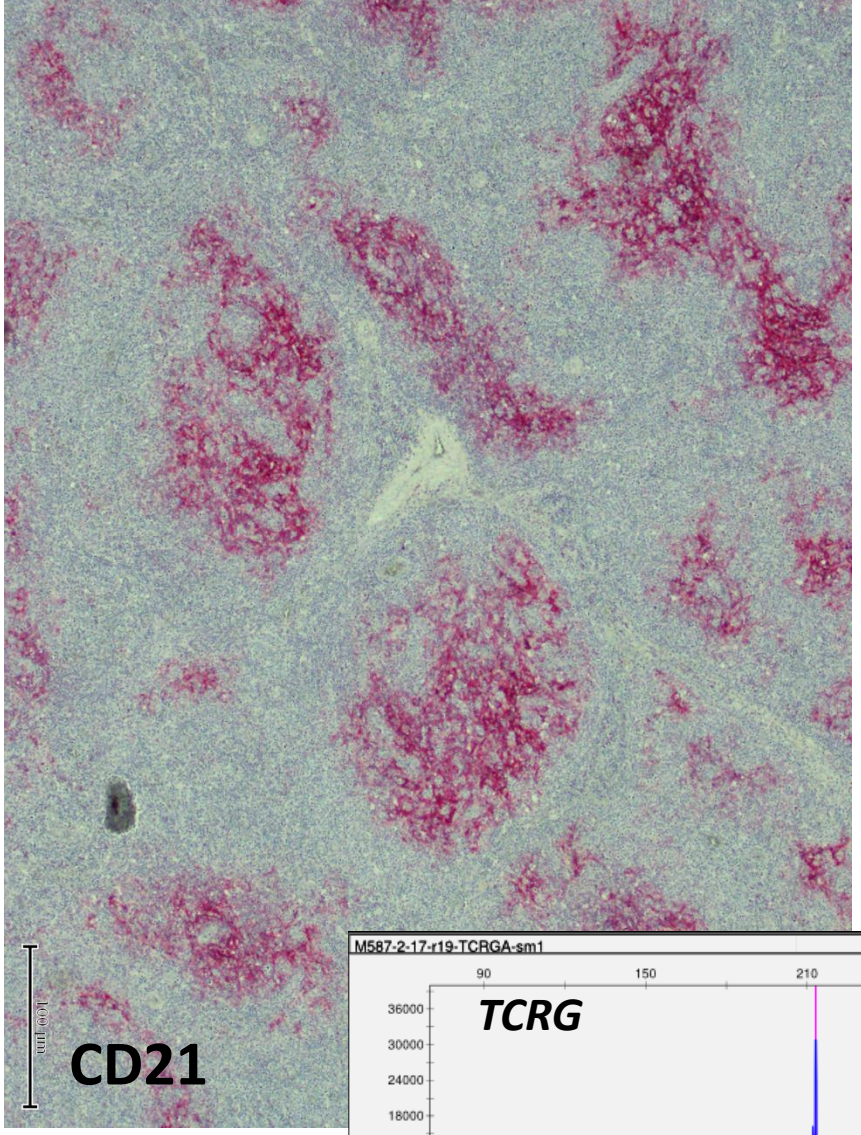


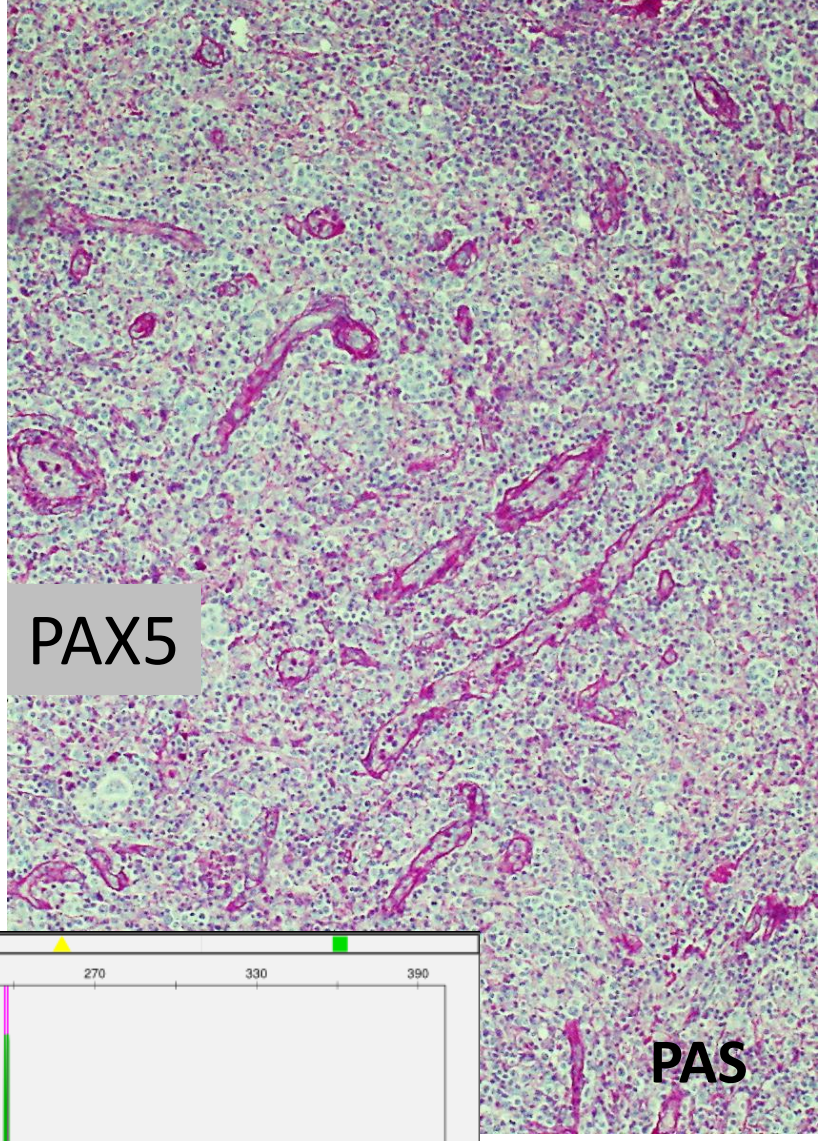

Koch E 8624/17 P9270 629 CHL vs AITL

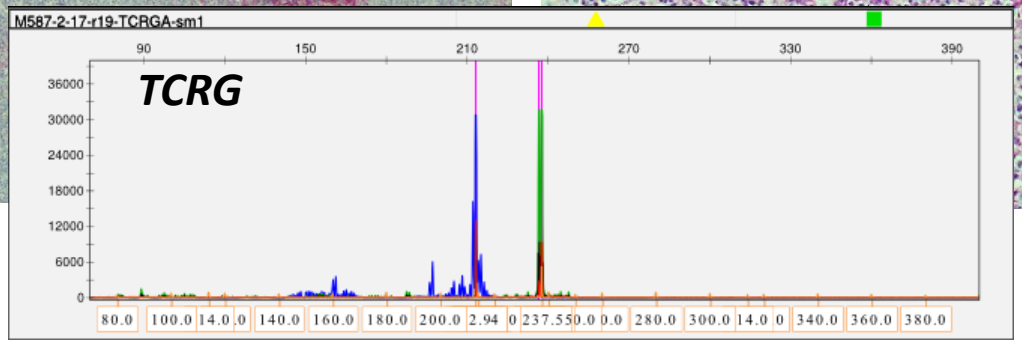
Same case as seen before




ICOS immunostaining was strongly positive as well




**Diagnosis: angioimmunoblastic T-cell lymphoma**

## Histopathological factors and biomarkers which influence the prognosis of CHL

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**Biomarkers of:**

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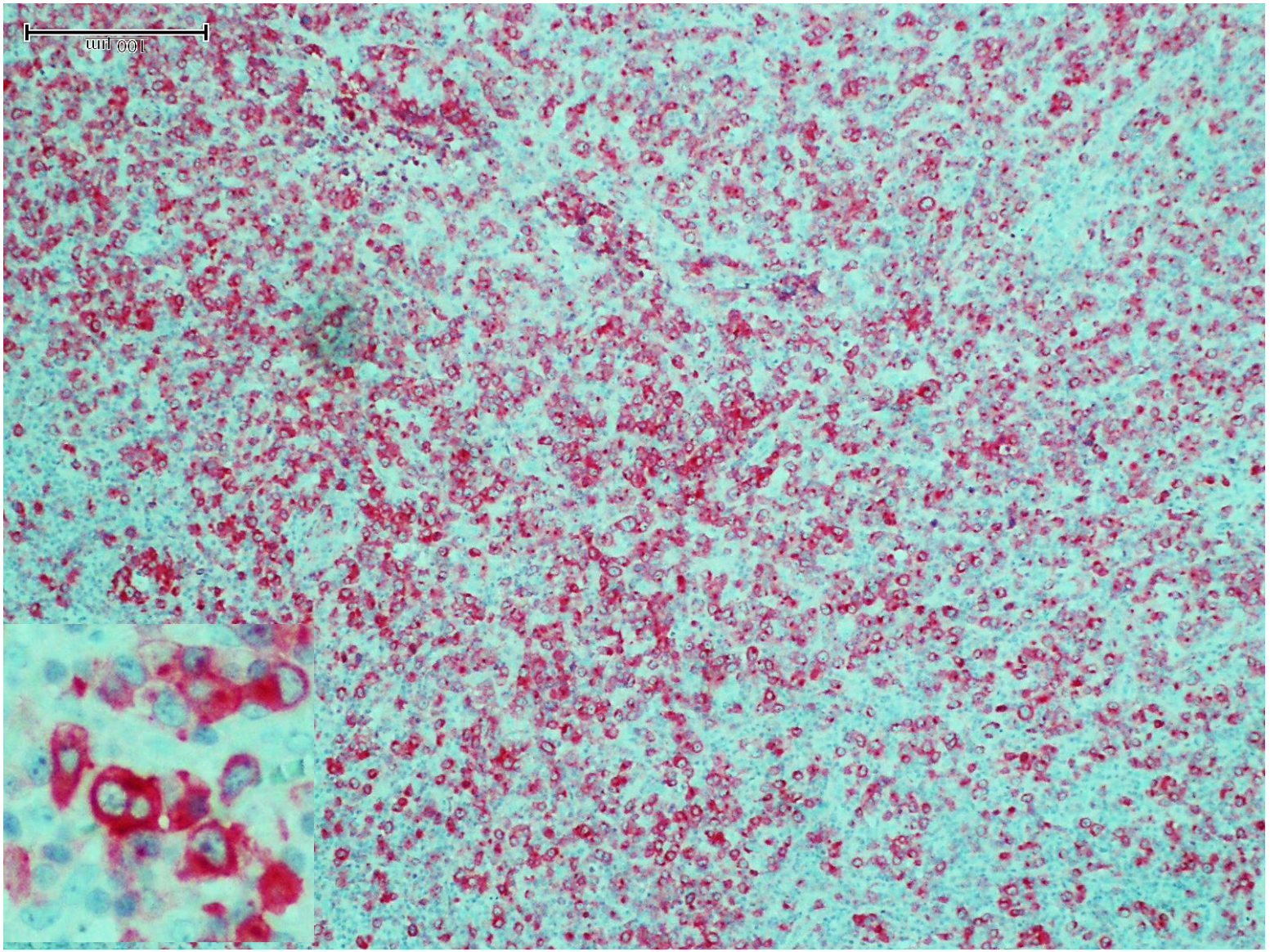


**Extremely tumor cell rich  classical Hodgkin lymphoma stage IV seems to be of high risk:**

CD30+, PAX5+. IRF4+, OCT2a+. BOB.1-, CD20-, T-cell marker-

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**Approach by the Gascoyne group: to develop a robust predictor of OS in advanced stage CHL not using single biomarkers but a combination of marker genes by gene expression**

## Gene Expression–Based Model Using Formalin-Fixed Paraffin-Embedded Biopsies Predicts Overall Survival in Advanced-Stage Classical Hodgkin Lymphoma

**Results**
A 23-gene outcome predictor was generated. The model identified a population at increased risk of death in the validation cohort. There was a 29% absolute difference in 5-year OS between the high- and low-risk groups (63% *v* 92%, respectively; log-rank $P < .001$; hazard ratio, 6.7; 95% CI, 2.6 to 17.4). The predictor was superior to the International Prognostic Score and CD68 immunohistochemistry in multivariate analyses.

**Conclusion**
A gene expression–based predictor, developed in and applicable to routinely available FFPET biopsies, identifies patients with advanced-stage cHL at increased risk of death when treated with standard-intensity up-front regimens.

*J Clin Oncol 31:692-700. © 2012 by American Society of Clinical Oncology* *

**Problem:** The 23 gene expression-based assay failed in combination with FDG-PET imaging to predict treatment response in advanced CHL in two studies *(CRUK/07/033 and US intergroup SO816 trial)* presented at a Lugano meeting

* Scott/Gascoyne et al 2012 JCO

**Chrisrtian Steidl and his group developed a new gene expression model** to capture the biology of CHL and discover noval and robust biomarkers that predict outcomes after autologous stem-cell transplantation.
*(Chan FC/Steidel C et al: J Clin Oncol 2017).*
The GE model was based on 18 outcome associated and 12 housekeeping genes-

**Current situation:** This new GE modell is not yet clinally applied since it still needs validation by independent studies

***The following authors followed a different approach by combining the predictive role of interim PET scan with biomarkers***
**in a huge Retrospective European Mulitcentre Cohort Study**.

*Claudio Agostinelli*, Andrea Gallamini*, Luisa Stracqualursi*, Patrizia Agati*, Claudio Tripodo, Fabio Fuligni, Maria Teresa Sista, Stefano Fanti, Alberto Biggi, Umberto Vitolo, Luigi Rigacci, Francesco Merli, Caterina Patti, Alessandra Romano, Alessandro Levis, Livio Trentin, Caterina Stelitano, Anna Borra, Pier Paolo Piccaluga, Stephen Hamilton-Dutoit, Peter Kamper, Jan Maciej Zaucha, Bogdan Małkowski,Waldemar Kulikowski, Joanna Tajer, Edyta Subocz, Justyna Rybka, Christian Steidl, Alessandro Broccoli, Lisa Argnani, Randy D Gascoyne, Francesco d'Amore, Pier Luigi Zinzani†,* ***Stefano A Pileri†***

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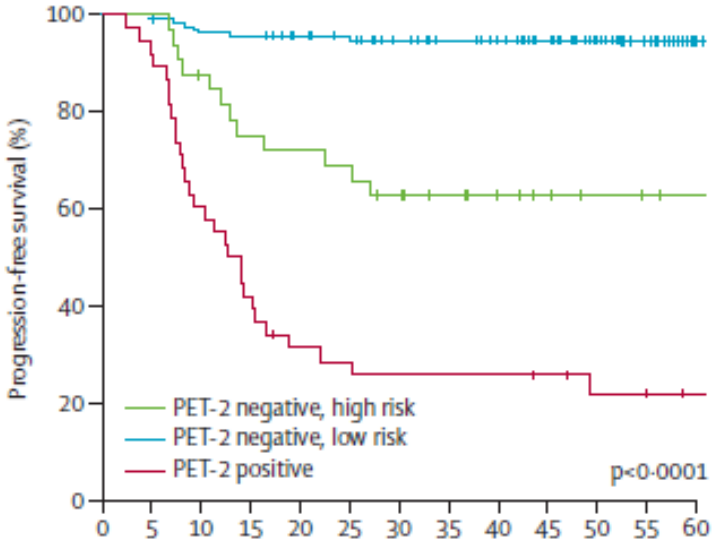
thelancet.com/haematology 2016

# Results:

**Minor finding**: In the Cox regression analysis FOXP3 and P53 remained the only biomarkers that are statistically associated with prognosis: better OS with FOXP3 and worse OS with p53.

**Major finding:** the application of CART (Cox multivariate analysis classification and regression) revealed:

- no other marker identified **a higher unfavourable risk group than a positive PET scan**.




| Number at risk | | | | | | | |
|---|---|---|---|---|---|---|---|
| PET-2 negative, low risk | 137 | 131 | 124 | 111 | 99 | 78 | 51 |
| PET-2 negative, high risk | 33 | 28 | 23 | 19 | 15 | 10 | 8 |
| PET-2 positive | 38 | 23 | 11 | 8 | 8 | 6 | 4 |

In consequence, the combination of biomarkers with PET was restricted to **PET-negative scans. This** resulted in the distinction of two risk groups:
a **low** and **a medium high risk** group.

**The PET negative medium high risk group is characterized by:**

- **> 25% or more CD68 positive cells,**
- a diffuse or rosetting PD-1 pattern
- absence of STAT1 expression

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**Summary of the Retrospective European Mulitcentre Cohort Study**

1. Positive PET scan proved to be the strongest marker for a **high risk group** of CHL patients.

1. the combination of biomarkers with PET negative scans identified an important **mediumhigh risk group** which is not recognized by PET alone.

2. The PET negative scan identified low risk patients which can safely be treated with standard ABVD regimen.

3. the medium risk PET negative group warrants a more agressive treatment approach.

**These important findungs of this Retrospective European Mulitcentre Cohort Study need a prospective validation**

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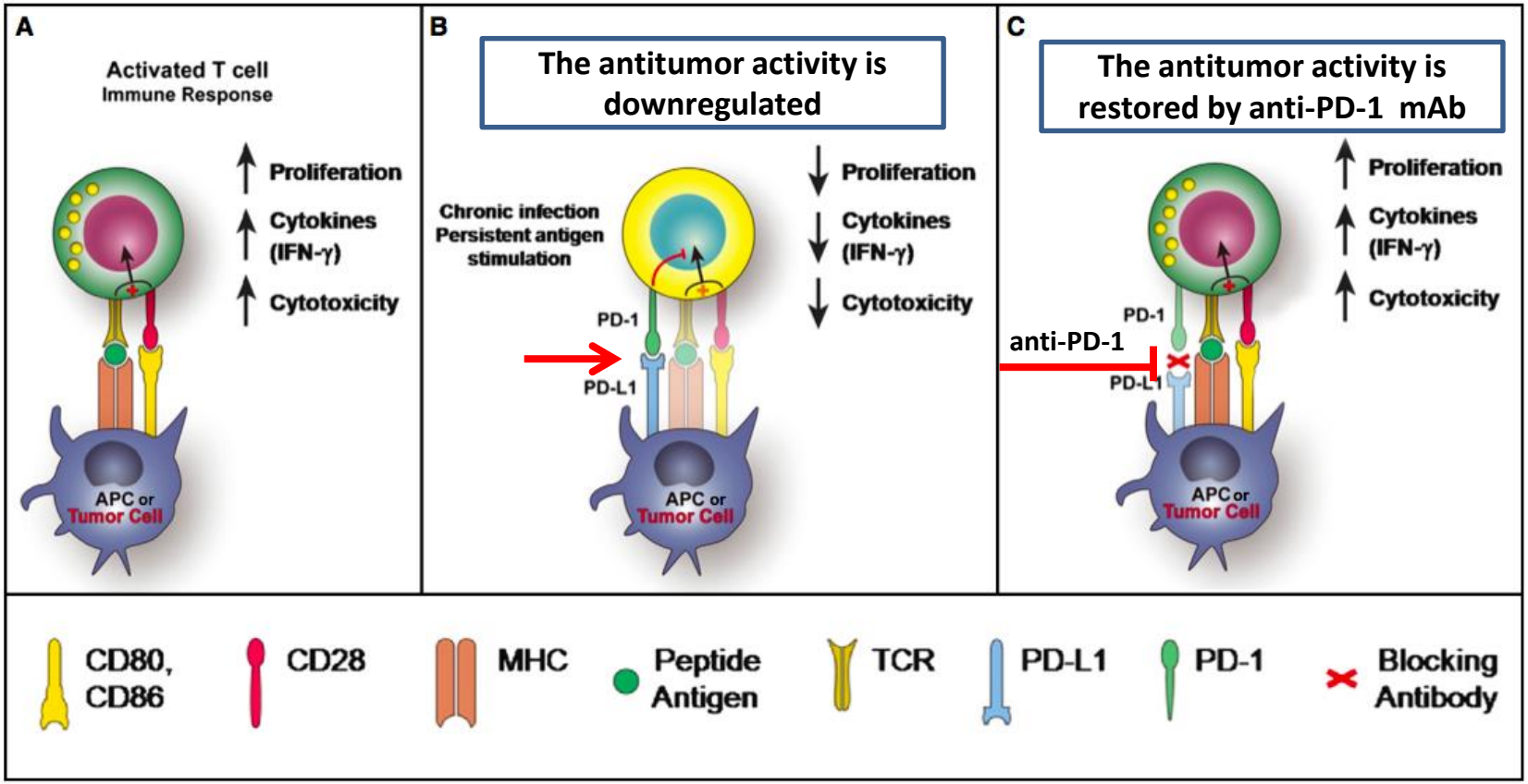
## Immune blockade of T cells by the PD-1/PD-L1 pathway is particulary efficient in many CHL

**Question:** can a success of PD-1 blockade treatment be predicted?




**The Shipp group reported** that in CHL the 9p24.1 copy gains and MHC class II positivity are potenital predictors of a favorable outcome after PD-1 blockade.

However, a possible pathogenic role of bystander histiocytes expressing large amounts of PD-L1 appears to be not included in the investigations.

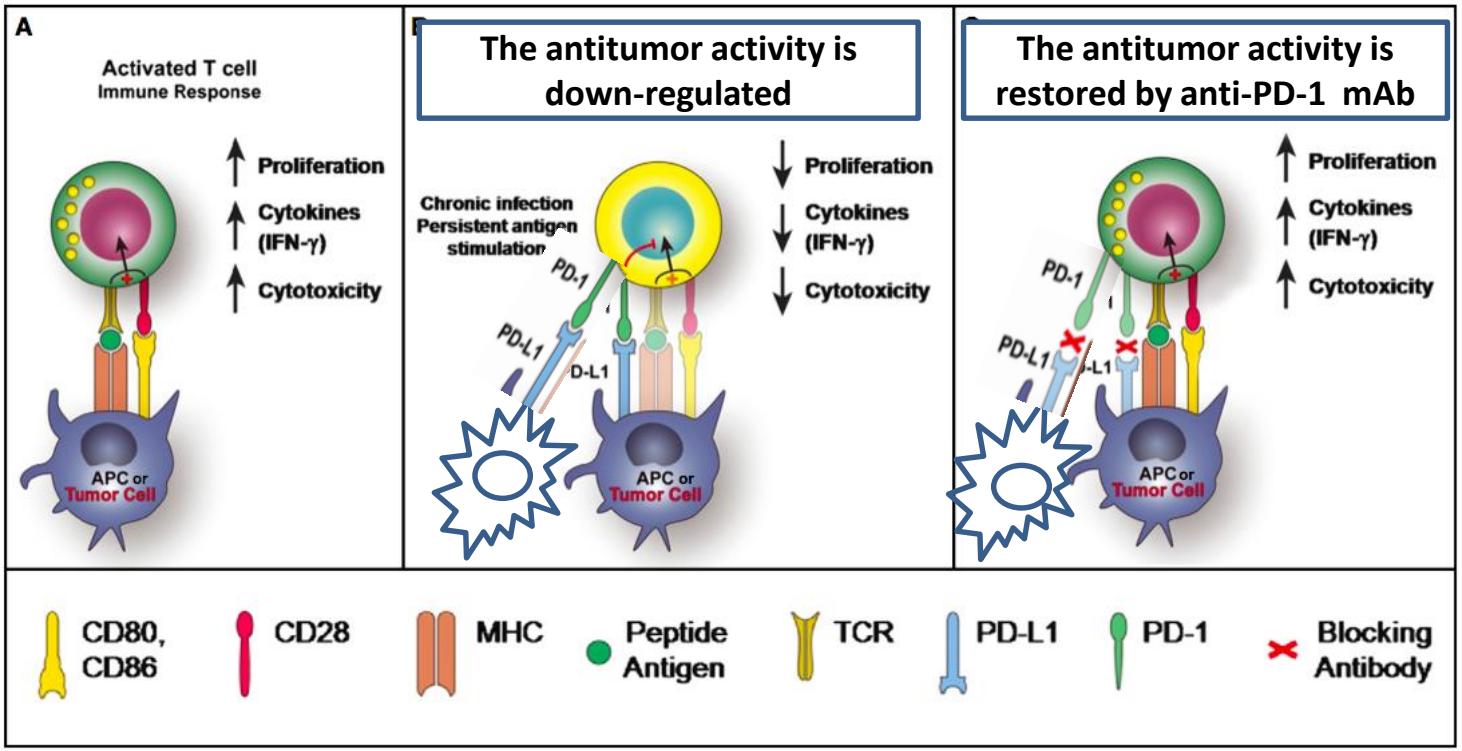
Roemer/Shipp et al JCO 2018

## *Immune blockade of T cells by the PD-1/PD-L1 pathway*

Herbst et al: Nature 2014 and others provided evidence, that most response to anti-PD-L1 blockade was observed in patients with tumours expressing high levels of PD-L1, especially when PD-L1 was expressed by tumour-infiltrating immune cells.

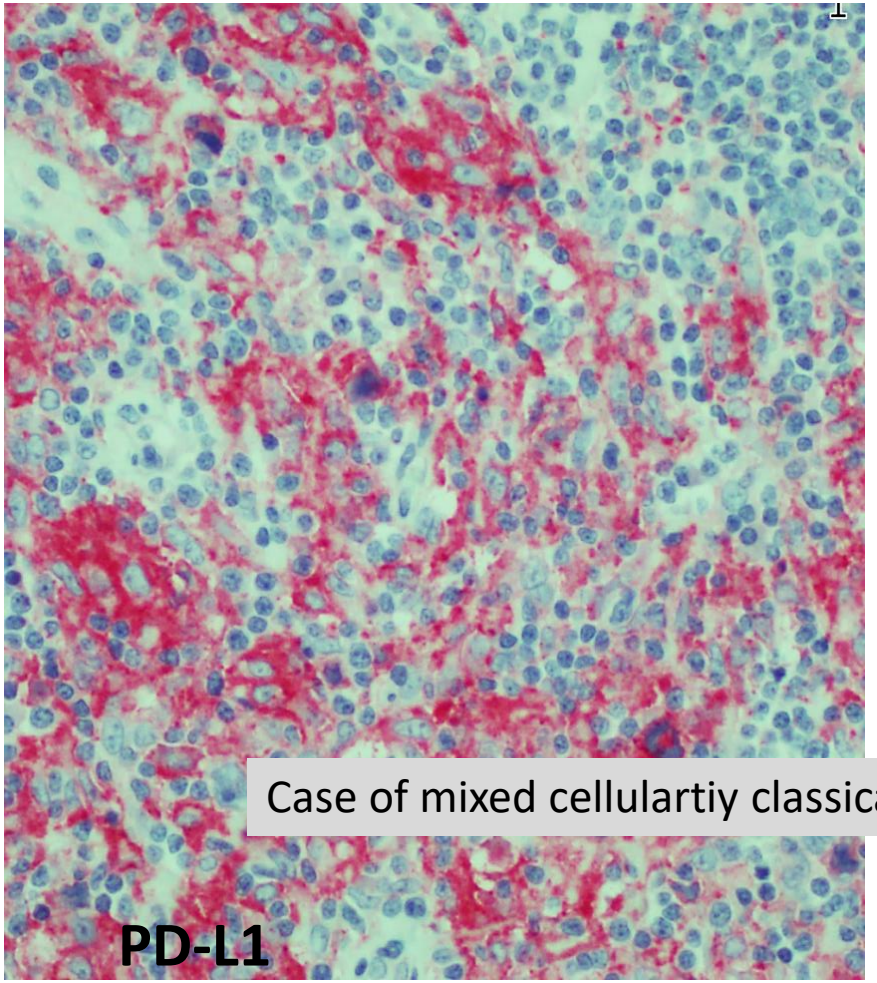
**Open questions**:

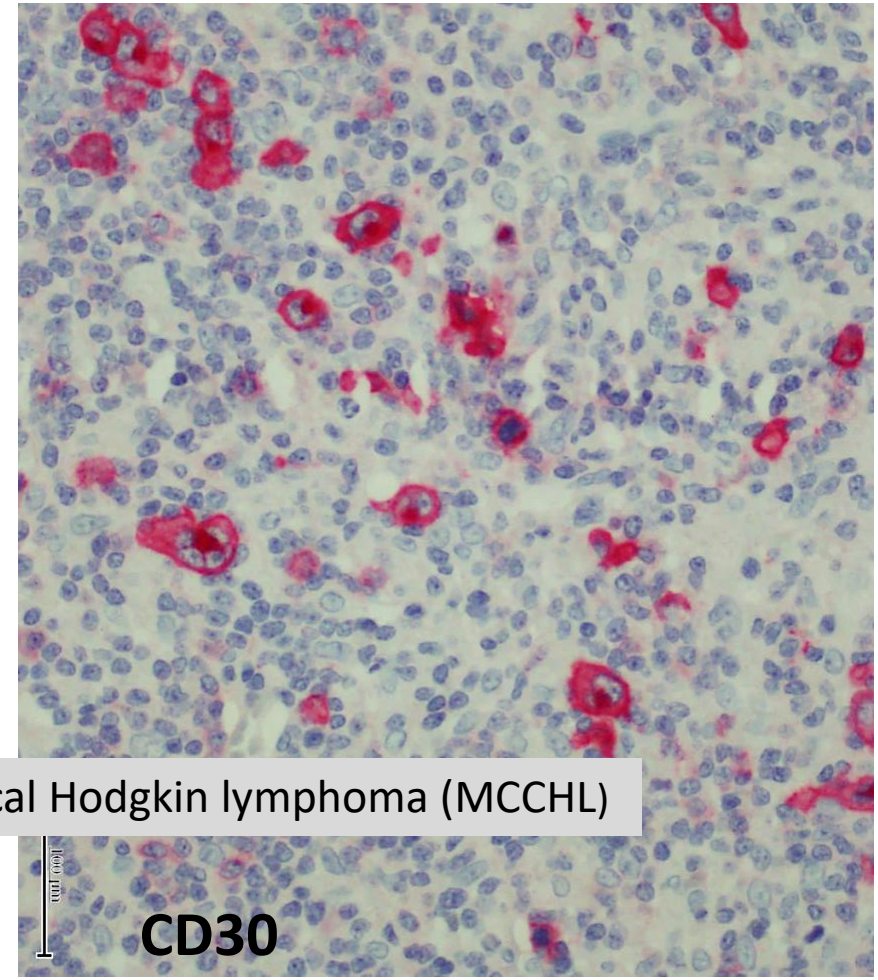
- can this be valid for classical Hodgkin Lymphoma?



***Immune blockade of T cells by the PD-1/PD-L1 pathway***

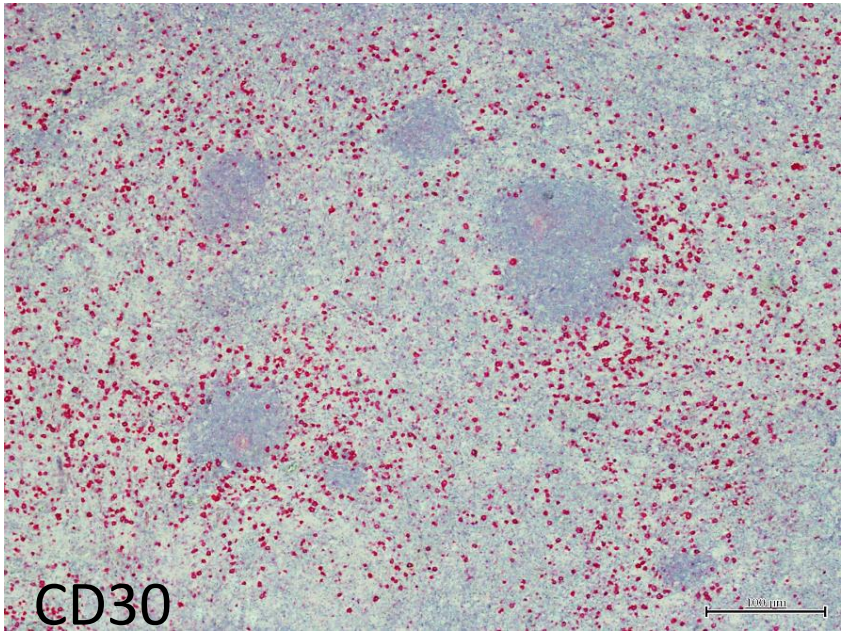
Because of the evidence provided by Herbst et al: Nature 2014 and others we investigated like Carey et al Blood 2017 the presence and the quantity of tumor associated bystander macrophages and their vicinity to HRS cells, first by single staining and then by double staining

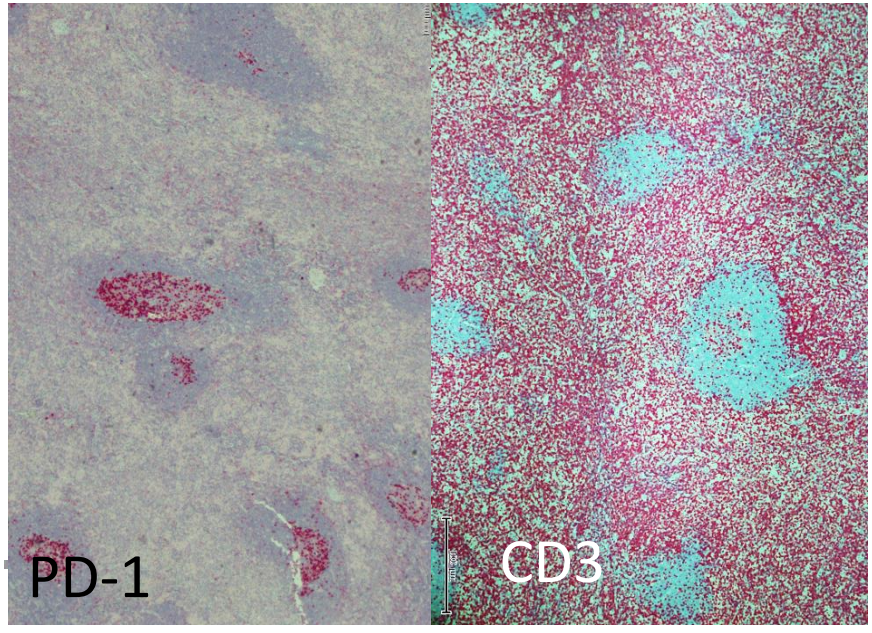


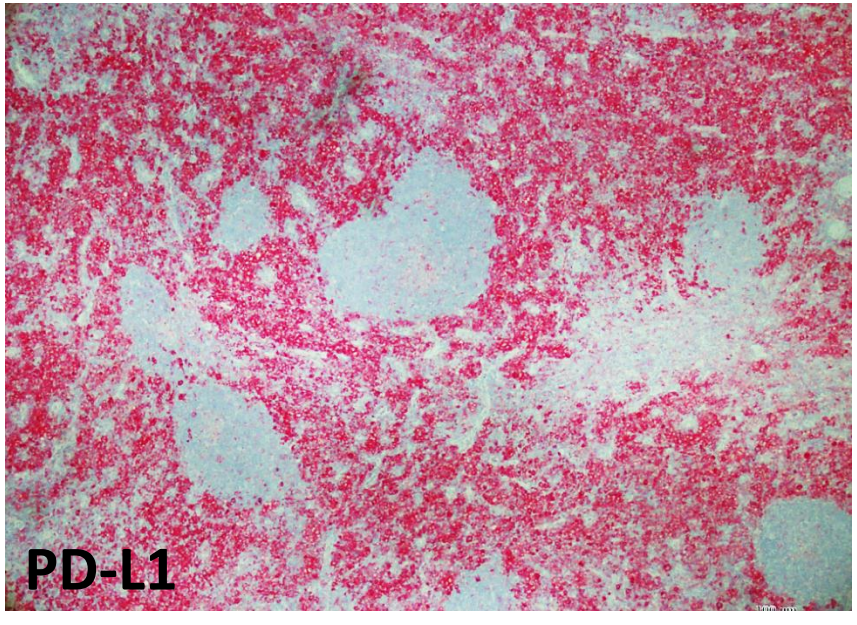



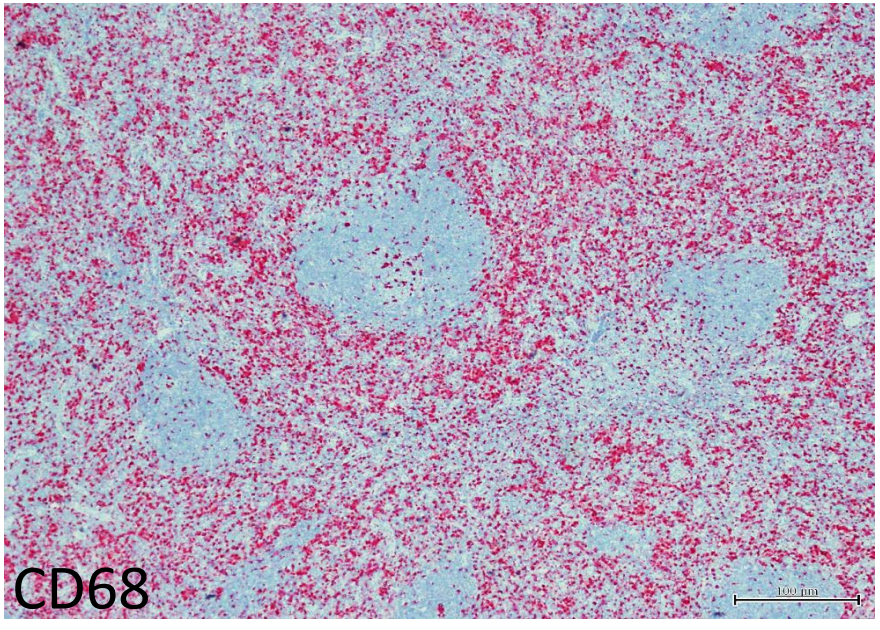

Case of mixed cellulartiy classical Hodgkin lymphoma (MCCHL)

# Mixed cellulartiy classical Hodgkin lymphoma (MCCHL)



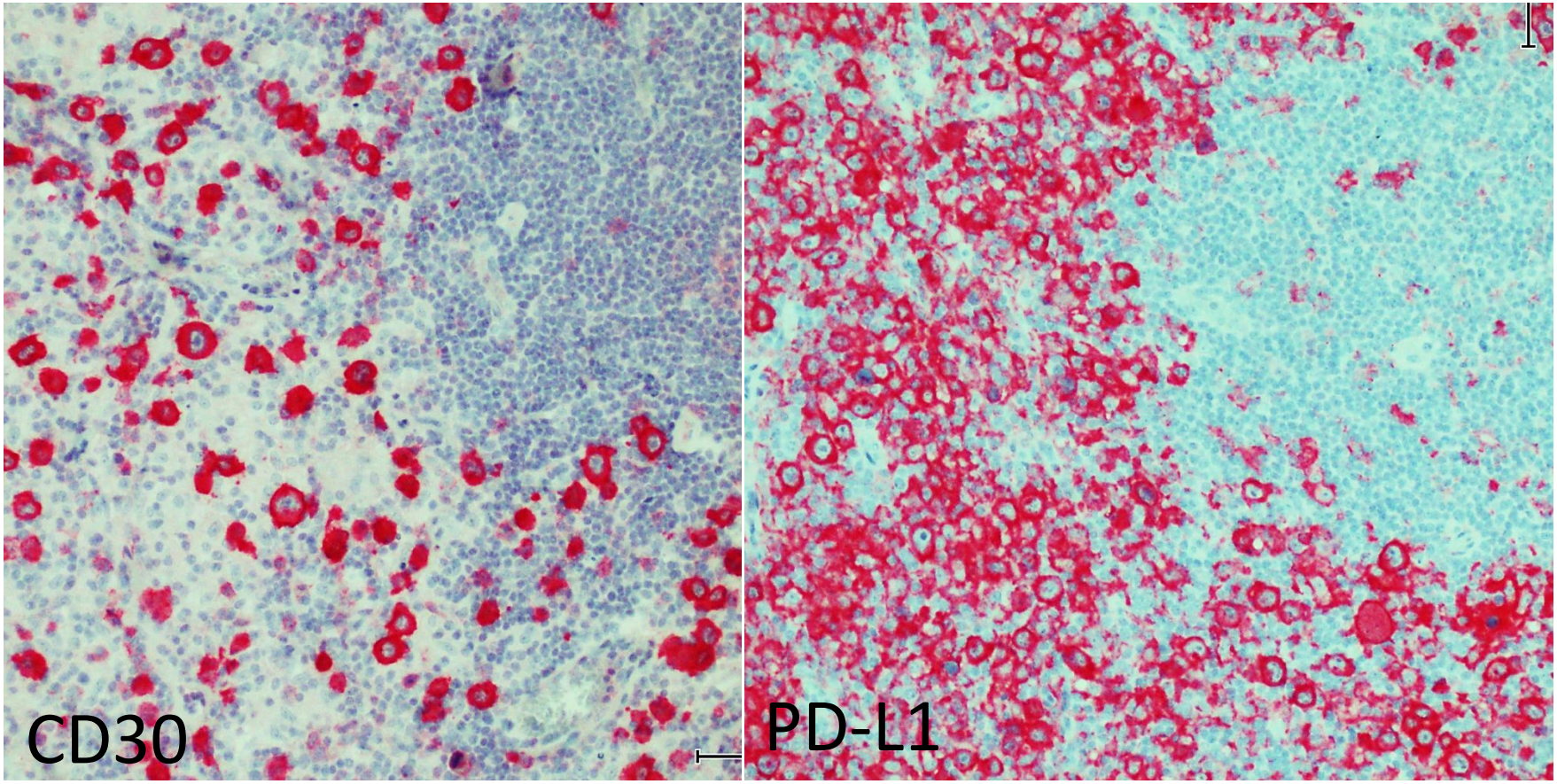



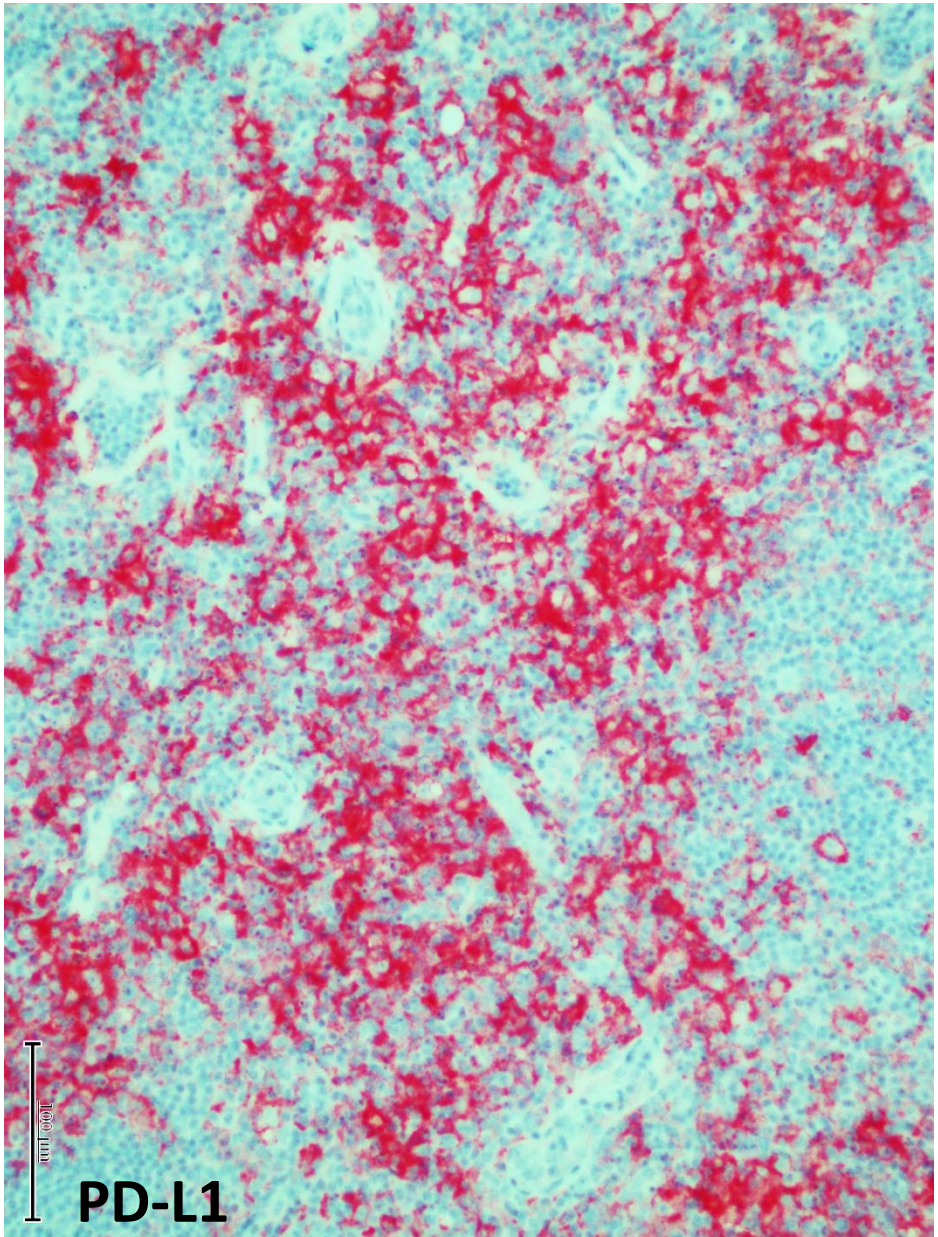



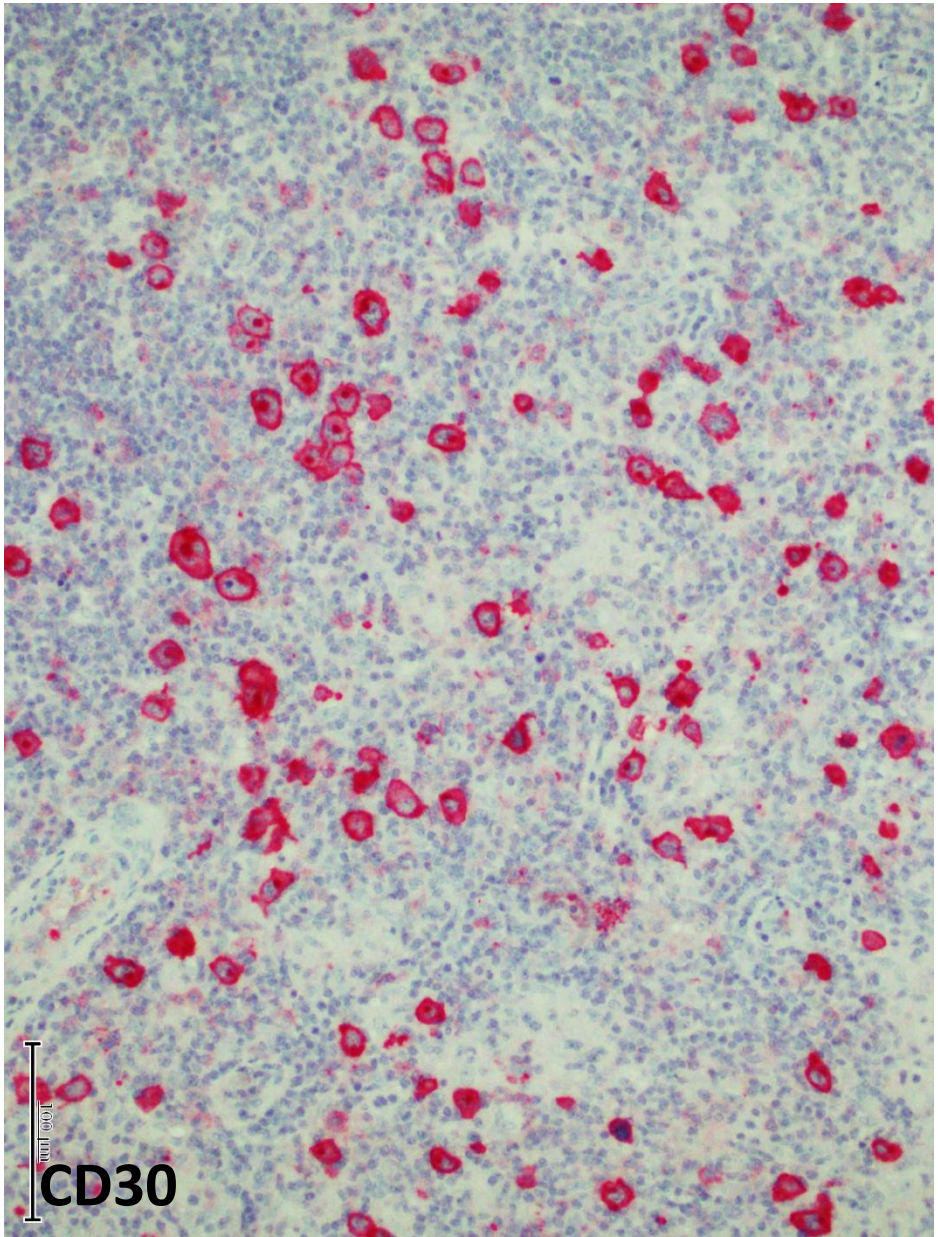
# Mixed cellulartiy classical Hodgkin lymphoma (MCCHL)

same case as seen before

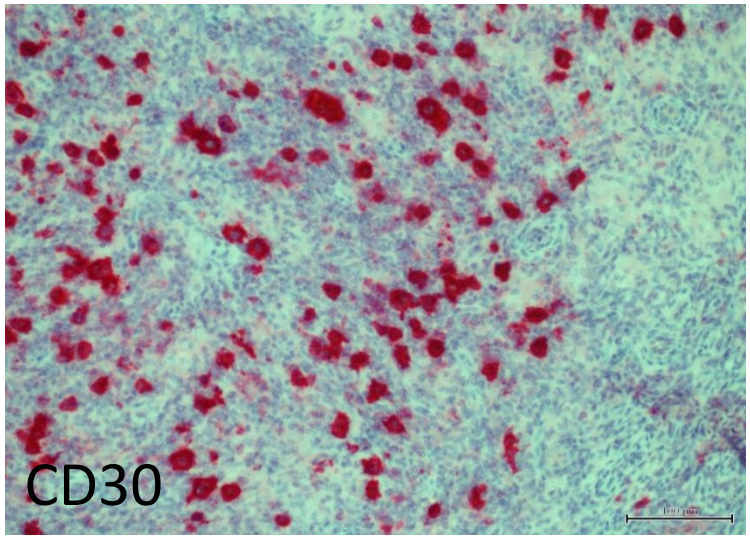


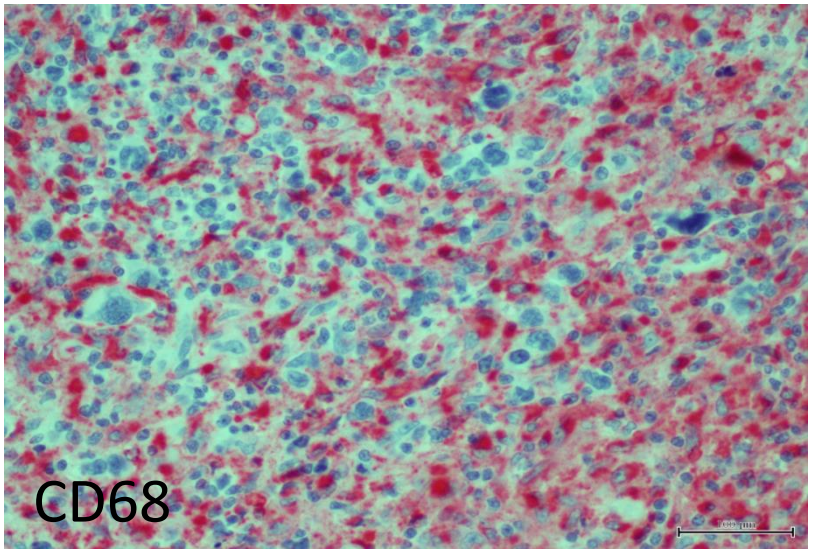
# NSCHL

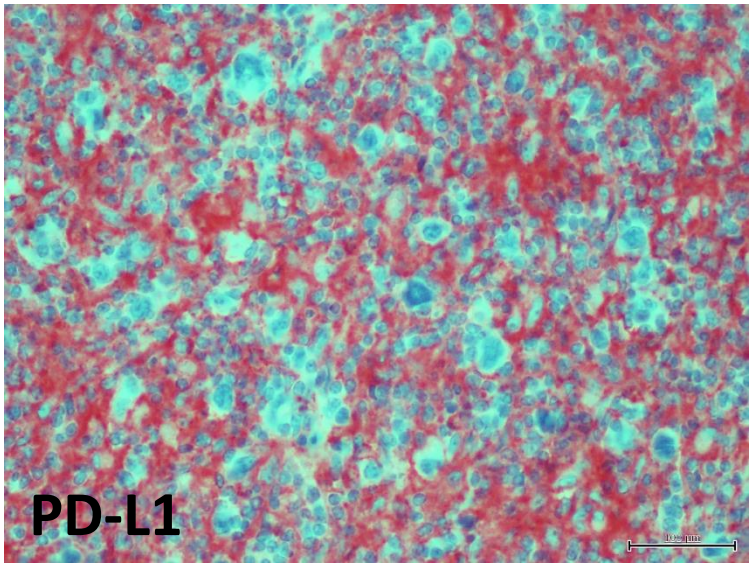


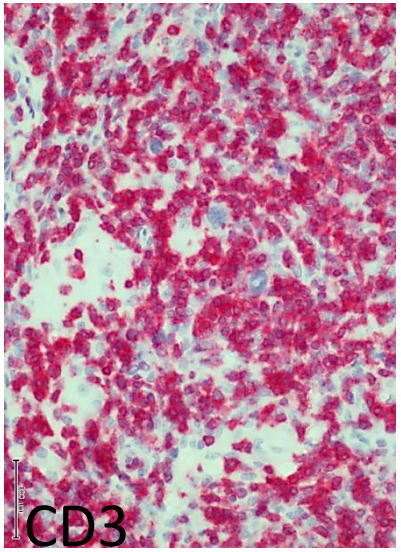



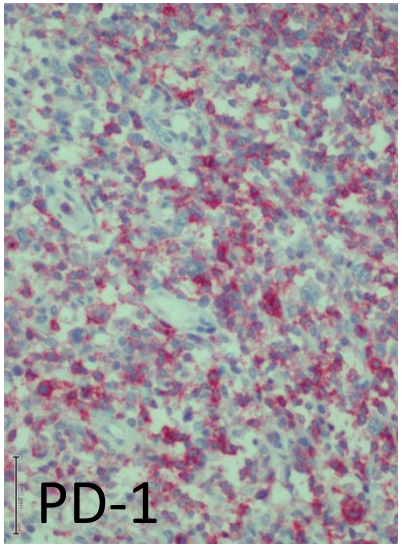
# Nodular sclerosing classical Hodgkin lymphoma (NSCHL)



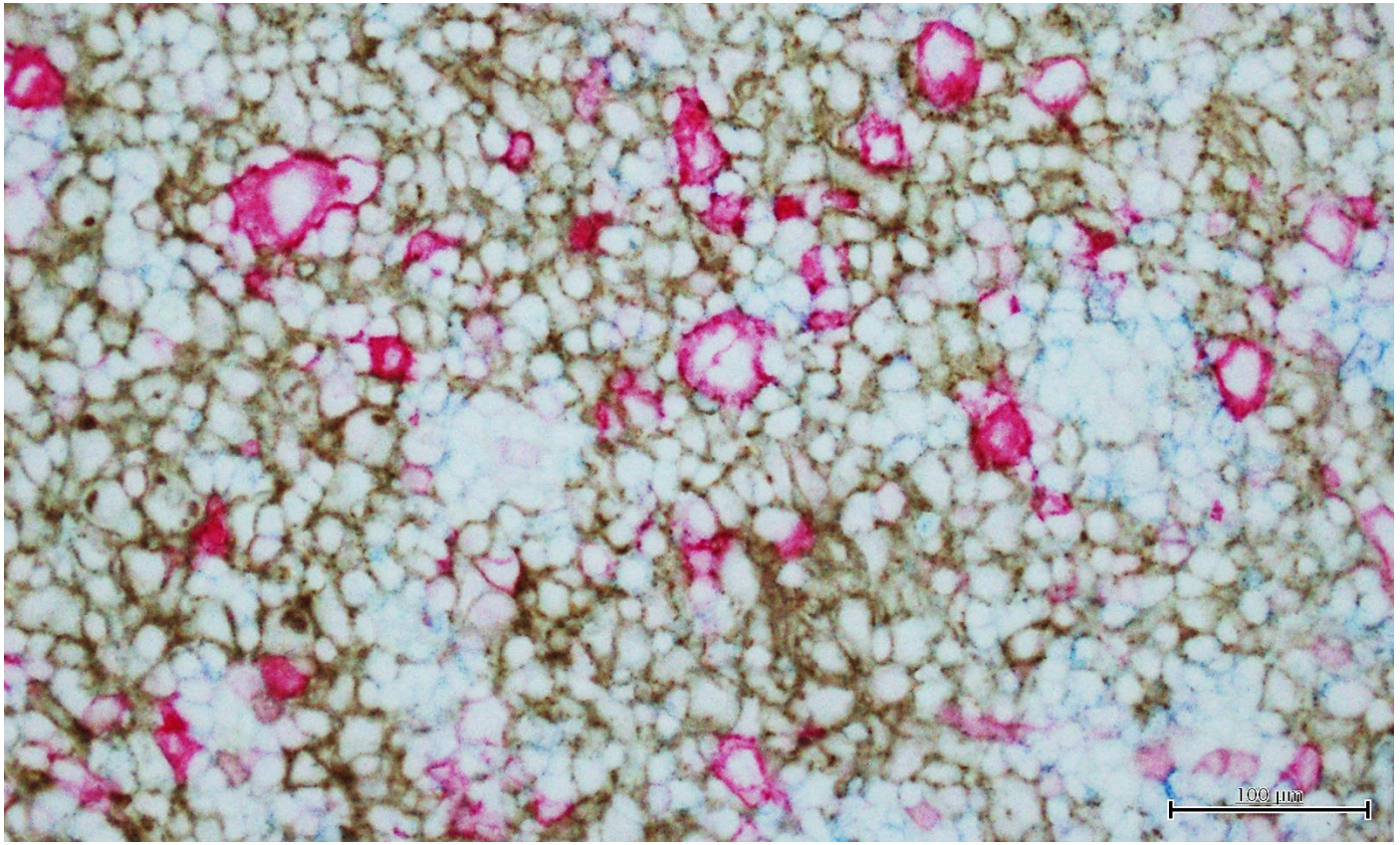




**Question:** can an expression of PD-L1 reliably identified on HRS cells by single colour staining?

## Double staining:
## Ratio and distance between HRS cells and associated histiocytes

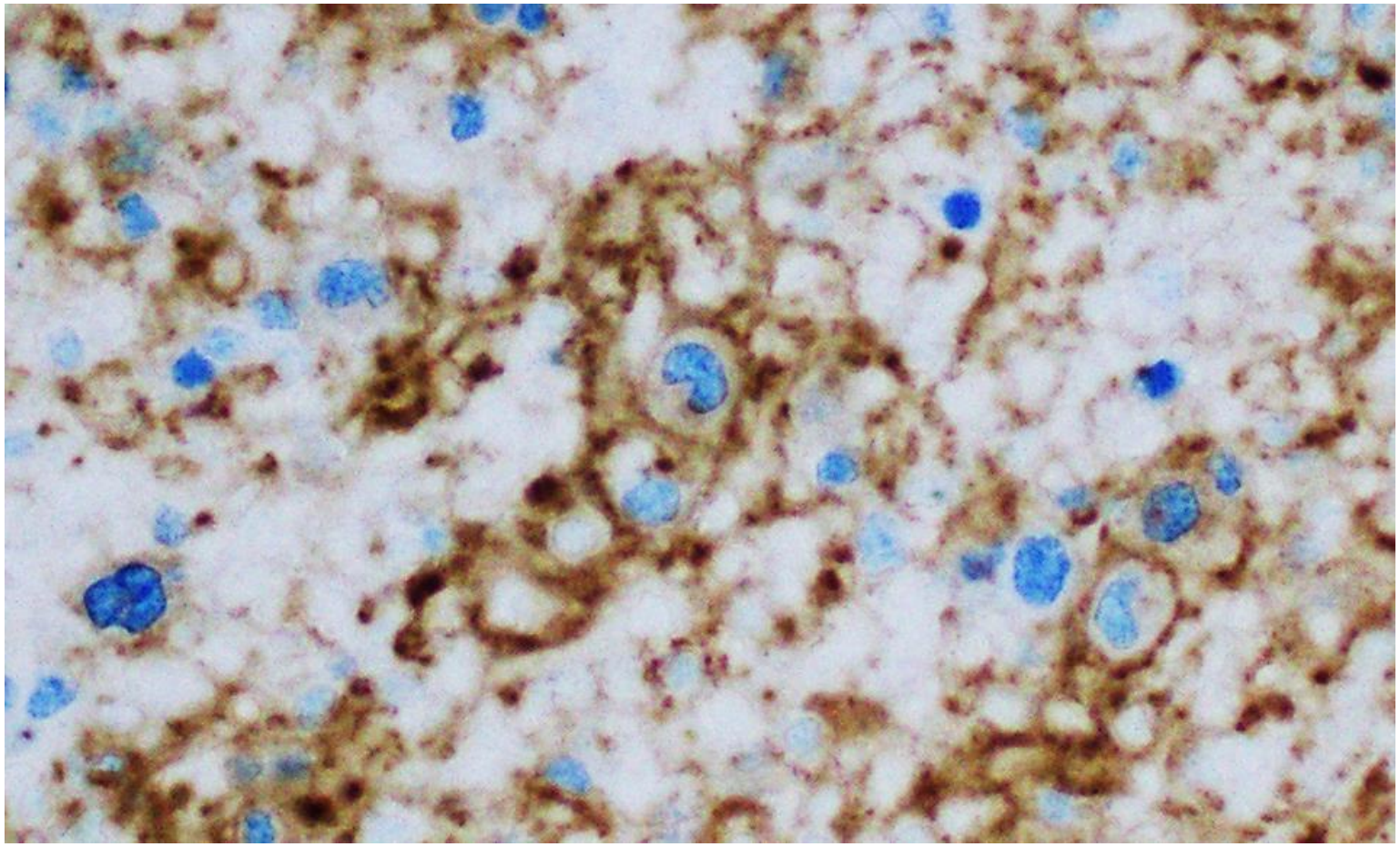



**MCCHL: CD30 red = HRS cells, PD-L1 brown = histiocytes, PD-1 blu = T cells**

**Conclsion:**

PD-L1+ histiocytes exceed HRS cells in number in the majority of CHL cases

# Double staining of the nuclei of HRS cells and the cellular projections of PD-L1+ histiocytes show the frequent close association between HRS cells  PD-L1+ histiocytes



NSCHL  MUM1 blue = large cells = HRS celles,  small cells = plasma cells;
PD-L1 + in  brown = histiocytes

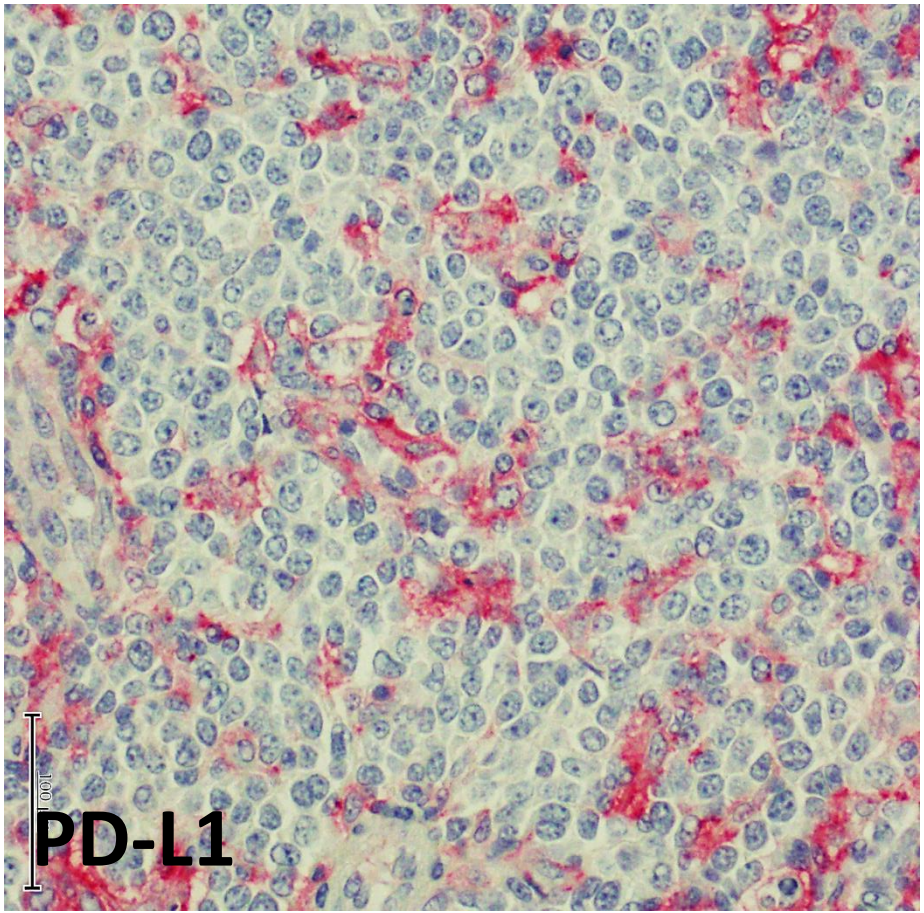
**In 70% of diffuse large B-cell lymhomas (DLBCLs) :**

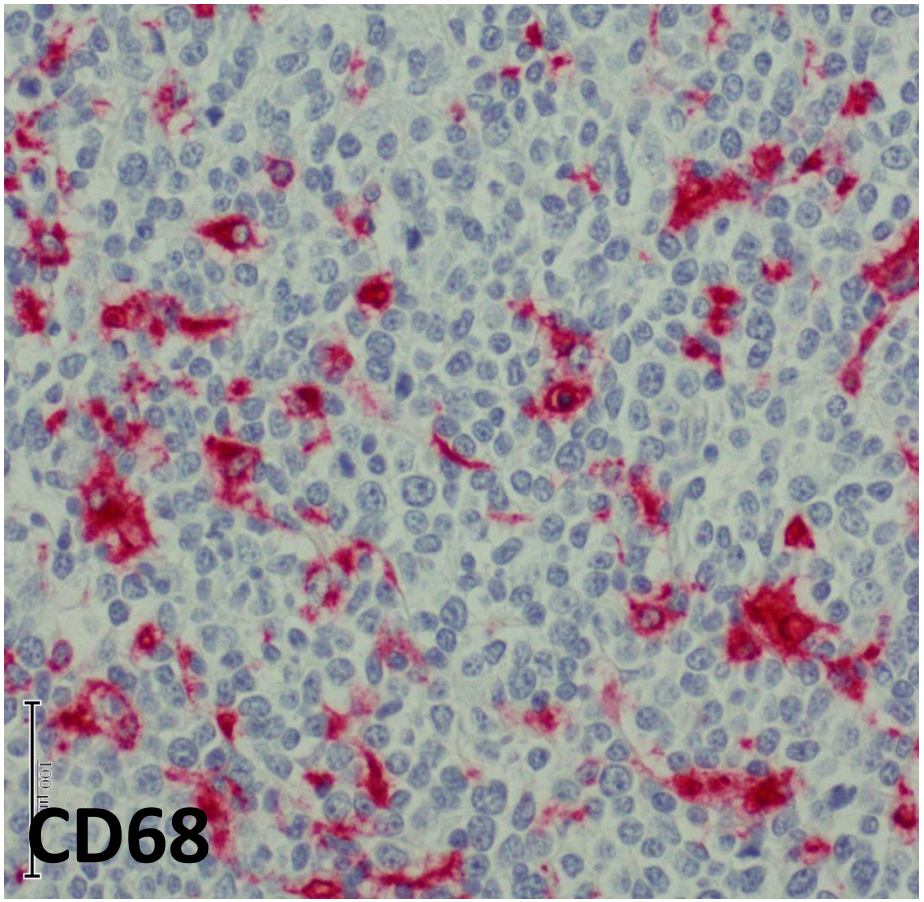
1.) the anti-PD-1 blockde is not successful

2. ) PD-L1+ histiocytes are much lower in number than the lymphoma cells,

2.) there is **no close association** beween PD-L1+ histiocytes and lymphoma cells

3.) the lymphoma cells do not express PD-L1



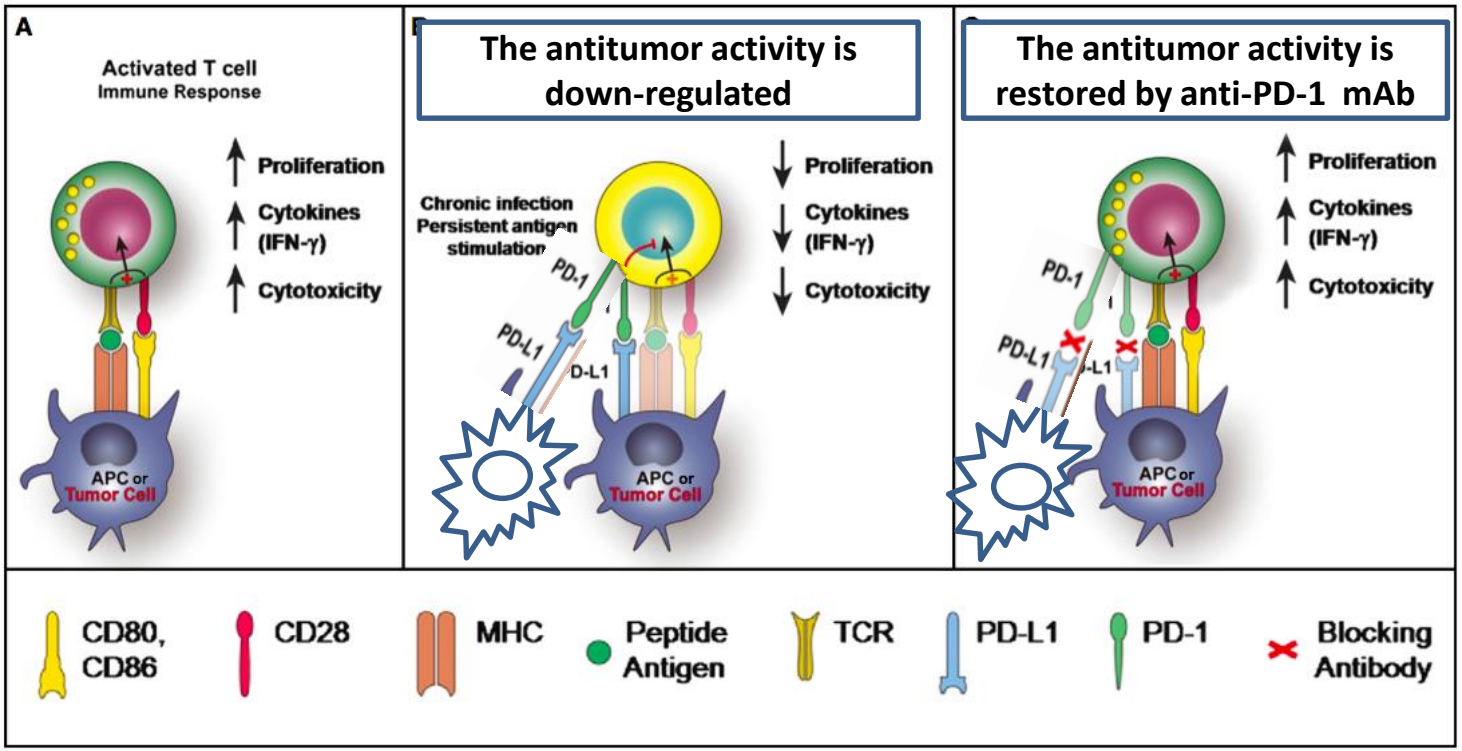



## *Immune blockade of T cells by the PD-1/PD-L1 pathway*

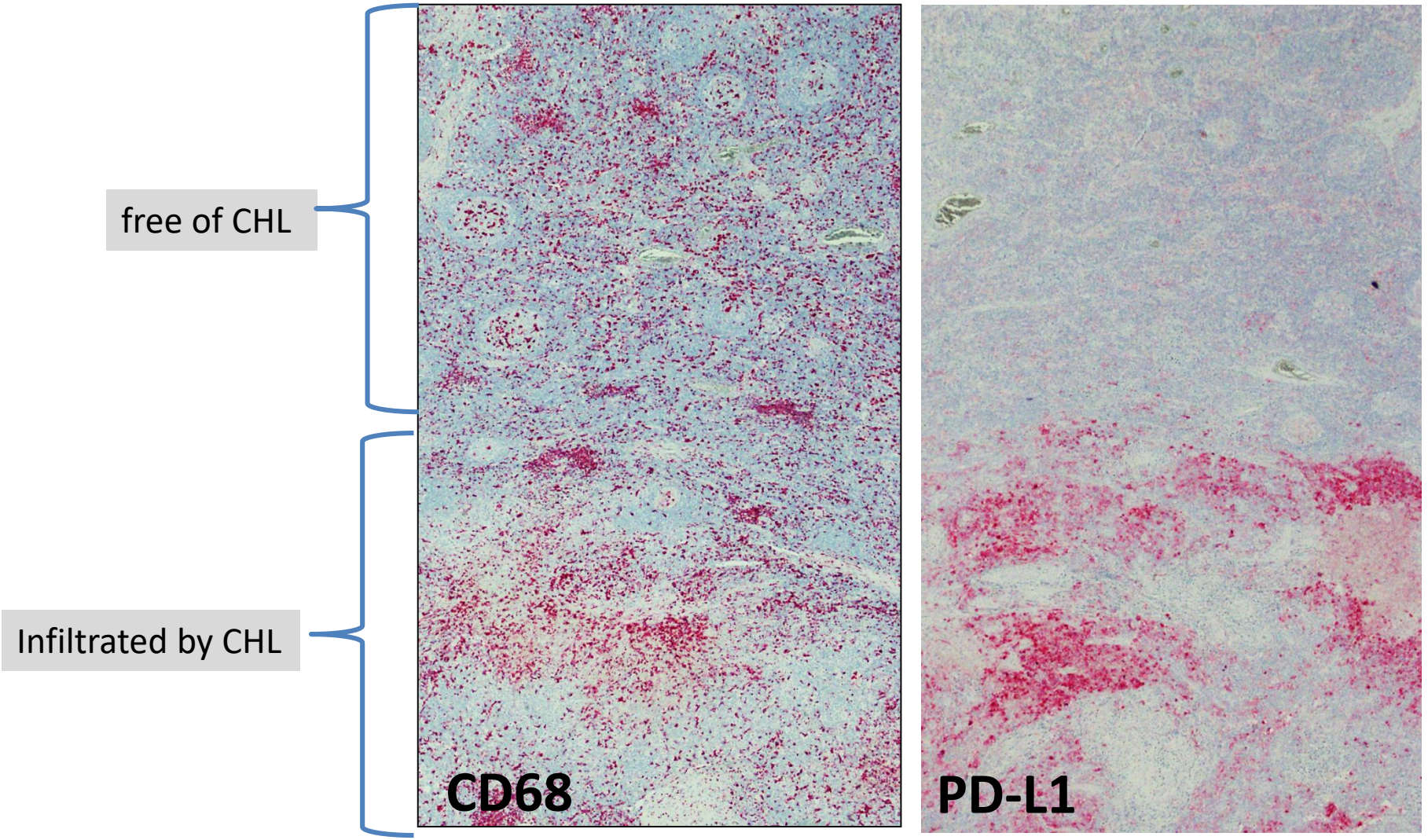
**Hypothetical conclusions:**

- The huge expression of PD-L1 by the histiocytes in CHL might significantly contribute to the downregulation of the anti-tumor activity of the many T-cells present in CHL.
- could therefore the PD-1 blockade therapy be so successful in many CHL cases?

**Questions:** is the up-regulation of PD-L1 by the histiocytes induced by HRS cells



## Observation on partially involved lymph nodes of 3 CHL cases




The expression of PD-L1 only in the infiltrated area suggests that PD-L1 expression by the histiocytes is induced by the HRS cells and seems to explain why PD-L1+ histiocytes are abundant in CHL .

# Summary and Implications

Cases which look like Hodgkin lymphoma but are not Hodgkin lymphoma need to be recognized for the correct treatment regimen.

The great prognostic histologic differences between the subtypes of cHL has disappeared by modern polychemo - and radiotherapy . High risk cases are usually not identified.

Single biomarkers with a strong prognostic/predictve power valid in all cases of cHL fail to idenfiy high risk cases.

The predictive prognostic power of two gene expression studies using a combination of genes claimed to identify high risk cases but this was not confirmed by other studies.

A model combining biomarkers and PET identified two high risk groups and revealed that

- PET positivity is the strongest adverse prognostic indicator.
- PET negative scan cases are composed of a low and a high risk group which cannot be recognized by PET alone and biomarkers alone.
- The low risk group can be treated with standard ABVD
- the high risk groups need a more aggressive treatment.

# Summary and Implications

So far a modell for predicting risk groups in which treatment with the anti-PD-1 blockade is not successful has not been reported.

PD-L1-positive histiocytes are abundant in many CHL cases and are in close vicinity to HRS cells

The pathogenic role of the abundant PD-L1 positive histiocytes warrants clarification.

The up-regulation of PD-L1 on histiocytes seen only in the vicinity to HRS cells suggests that HRS cells induce the up-regulation of PD-L1 on the bystander histiocytes

I am curious to hear your questions
and
opinions

## *Immune blockade of T cells by the PD-1/PD-L1 pathway*

**Herbst et al: Nature 2014 and others provided evidence:**
Most response to anti-PD-L1 blockade was observed in patients with tumours expressing high levels of PD-L1, especially when PD-L1 was expressed by tumour-infiltrating immune cells.

**Questions**:
- can this be valid also for classical Hodgkin Lymphoma;
- is the PD-L1 expression on tumor associated macrophages dependent on P24.1 copy gains