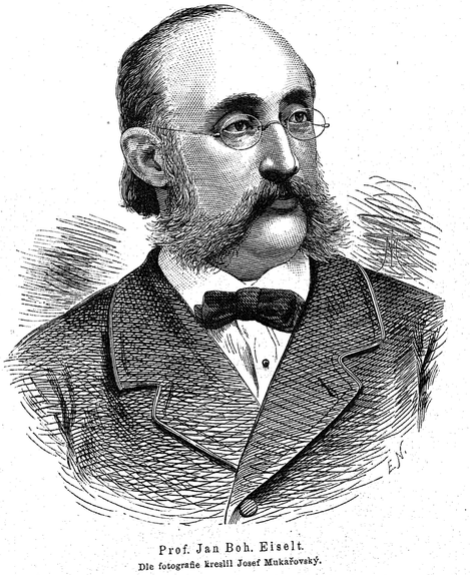


1831- 1908

MicroRNAs in lymphoproliferative disease with a focus on Hodgkin lymphoma

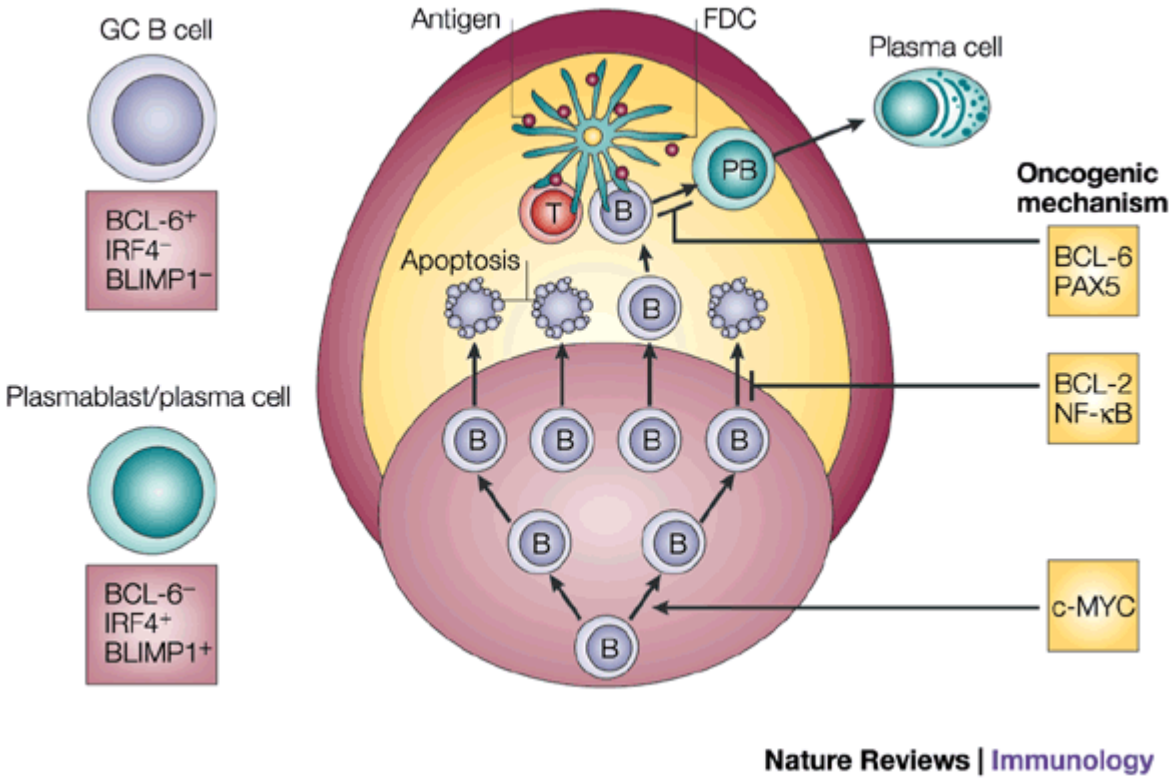
Dr. Tomáš Stopka

HL origin is GC-B

Malignancy	SHM	Ongoing SHM	GC B-cell expression profile[27, 71, 99, 130]	Putative cell of origin*
Mantle-cell lymphoma	No (except for a small percentage[131])	No	No	Pre-GC B cell
Chronic lymphocytic leukaemia (CLL)‡	Yes and no	No	No	Antigen-experienced B cell (pre- or post-GC)
Burkitt lymphoma	Yes	No	Yes	GC B cell
Follicular lymphoma	Yes	Yes	Yes	GC B cell
Marginal-zone lymphoma — nodal, extranodal (MALT) and splenic	Yes (except for some splenic variants[132])	Yes (prevalent in MALT lymphomas[133])	No§	GC B cell or post-GC B cell
GC B-cell-like DLBCL	Yes	Yes	Yes	GC B cell
Activated B-cell-like DLBCL	Yes	No	No	GC B-cell subset or extra-GC mutated B cell[23, 25]
Lymphoplasmacytic lymphoma (LPL)	Yes	Yes	No¶	Post-GC B cell
Multiple myeloma	Yes	No	No	Post-GC B cell
Hodgkin lymphoma (classical type)	Yes	No	No	GC or post-GC B cell
Hodgkin lymphoma (nodular lymphocyte pre-dominant type)	Yes	Yes	Yes	GC B cell

L Di Lisio et al. 2012

Germinal-centre B cells.



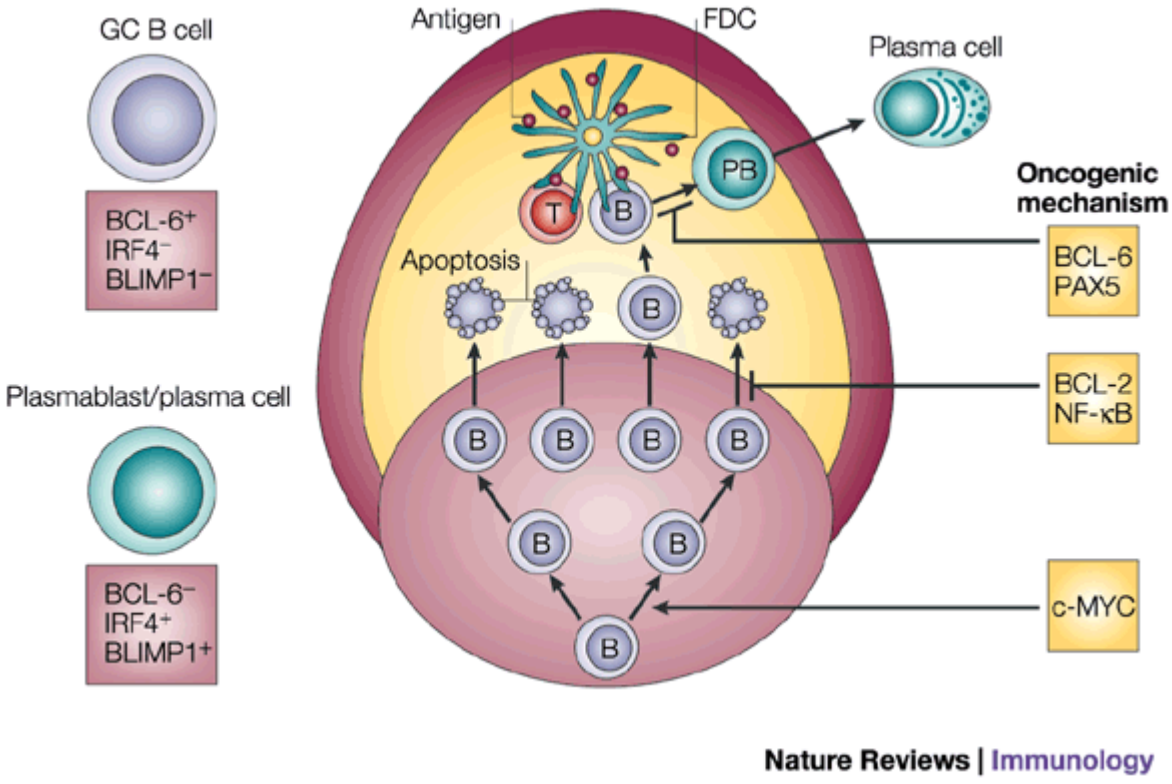
1) GC-B cells mutate their immunoglobulin genes (w/ AID)

2) GC-Bs acquire mutations to maintain/ improve the affinity of the B-cell receptor (BCR) for antigen

3) leading to a rescue from programmed cell death and differentiation.

Shaffer et al. 2002

Mechanisms of malignant transformation of GC-B cells.



1) **c-MYC overexpression** is a result of translocation by Burkitt lymphoma cells

2) **nuclear factor- B (NF- B) pathway** activation blocks apoptosis in diffuse large B-cell lymphomas (DLBCLs), marginal-zone lymphomas, Hodgkin lymphomas and Epstein–Barr virus-related lymphomas.

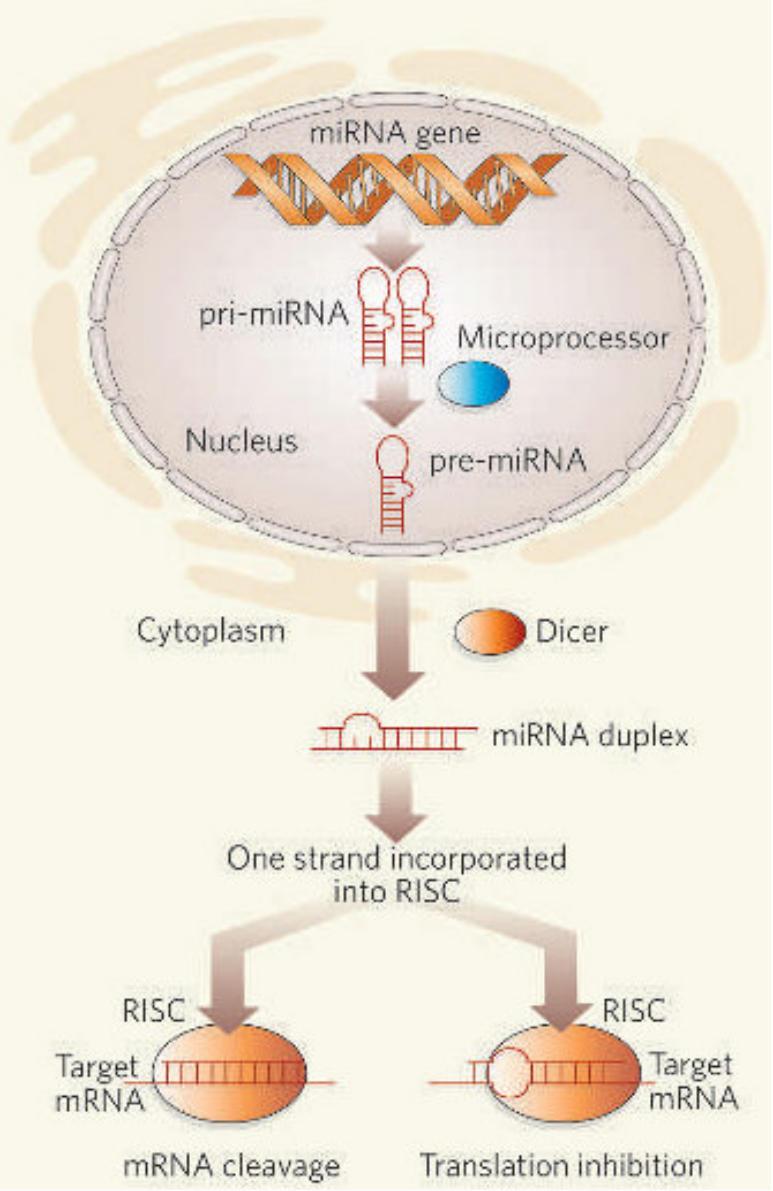
3) ***BCL2* gene** translocation, amplification or transcriptional activation blocks apoptosis and stimulates GC-B proliferation in various non-Hodgkin lymphomas.

4) **Translocation of *PAX5*** (paired box gene 5) in lymphoplasmacytic lymphomas is also likely to block plasmacytic differentiation..

Shaffer et al. 2002

microRNAs

- class of small RNA molecules (~ 22 nts)
- highly conserved
- regulate gene expression at posttranscriptional level
  - ➢ members of a RISC complex
  - ➢ binding to 3´UTR of target mRNA leads to translational repression or degradation of the mRNA
- implicated in number of cellular processes – metabolism, development, differentiation
- involved in solid tumors, leukemias, lymphomas *(rev. in Dalmay 2008, Croce 2008)*

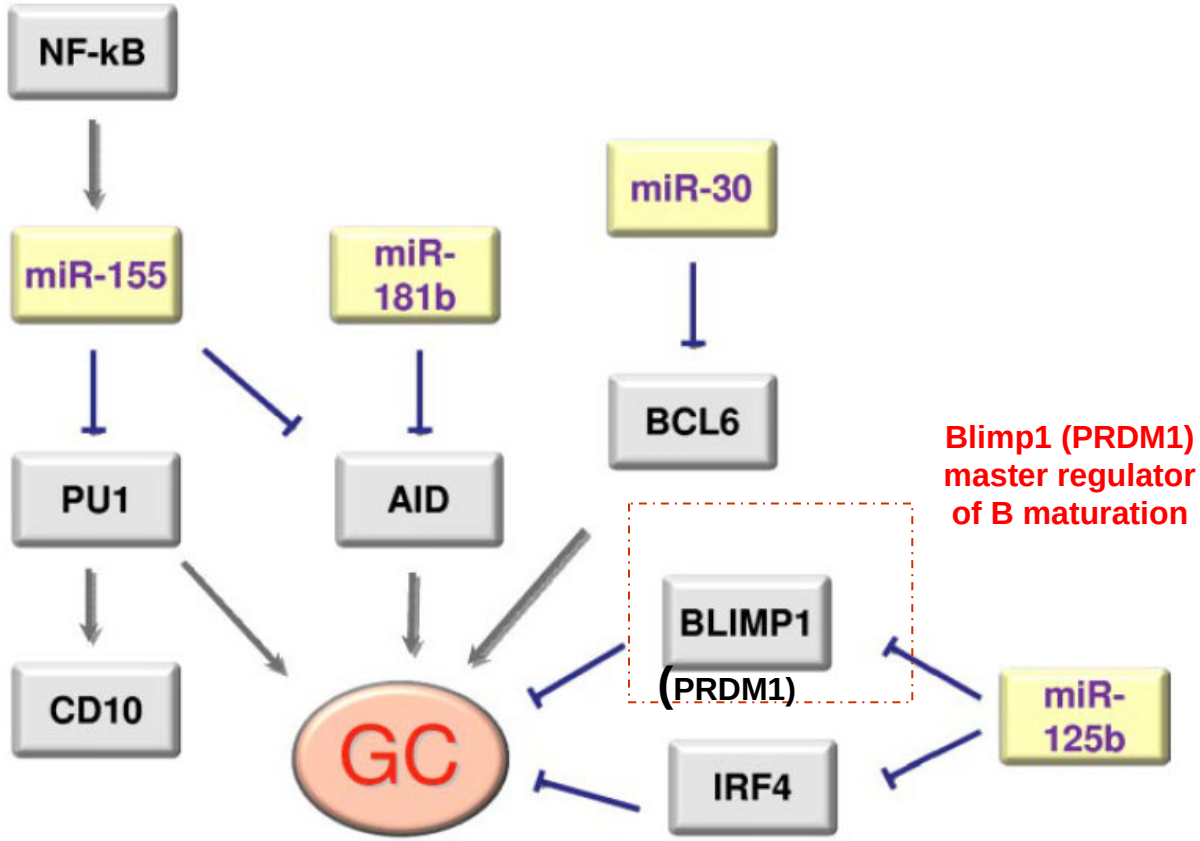


Stage-specific microRNAs involved in B-cell differentiation

	Naive B cells	Germinal center B cells	Memory B cells	Plasma cells
⇑	miR-181[31] mi-34a[24] miR-223[16,31,32]	miR-17-5p[16,32] miR-181b[25,32] miR-125b [31,33] miR-155 [26]	miR-223[16,32]	
⇓		miR-223[31-33] miR-150[22,31,33]	miR-181b[31-33] miR-17-5p[16,32]	miR-181b[16,32] miR-17-5p[32] miR-30[16,32]

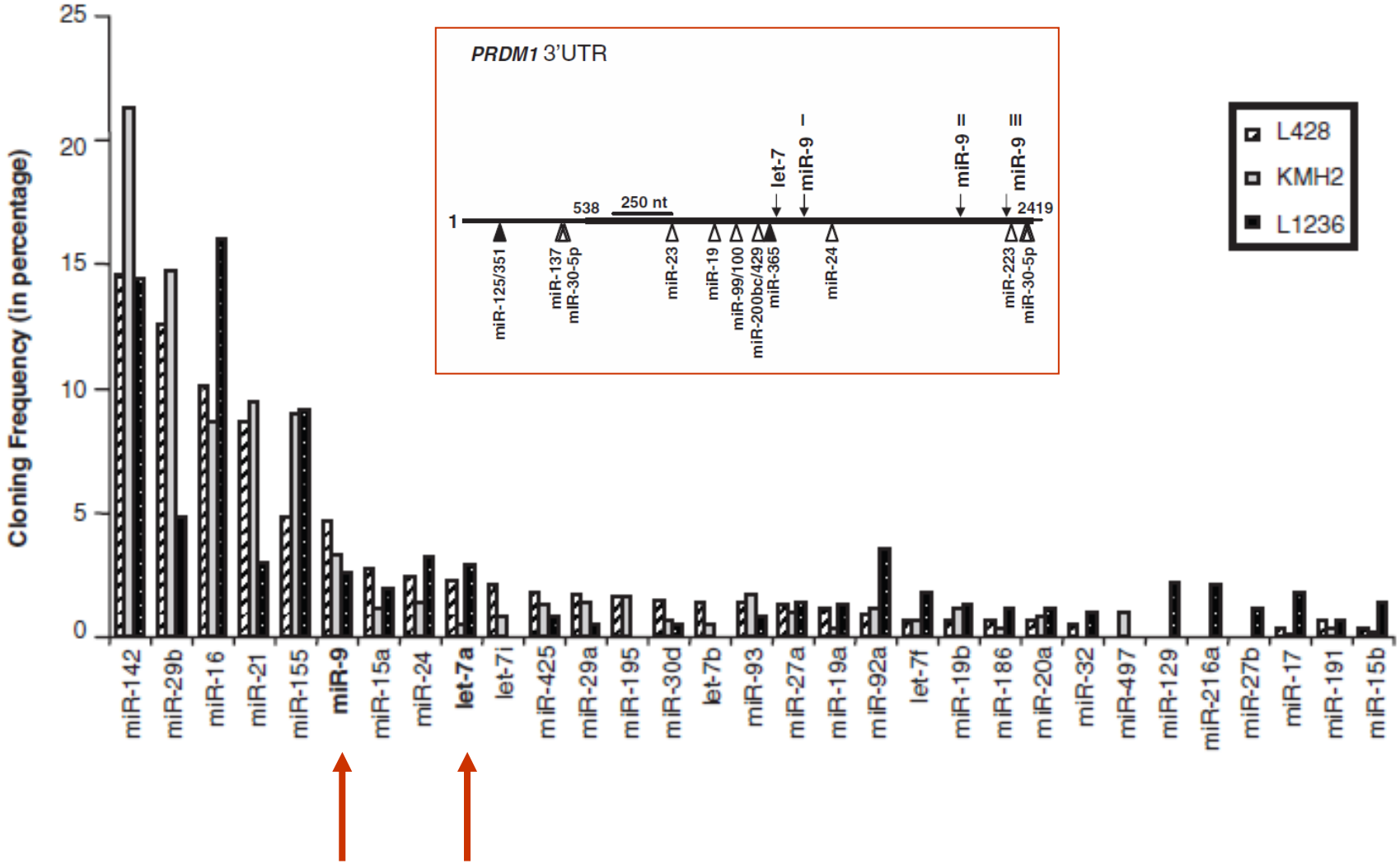
L Di Lisio et al. 2012

Interactions between genes and microRNAs in GC differentiation



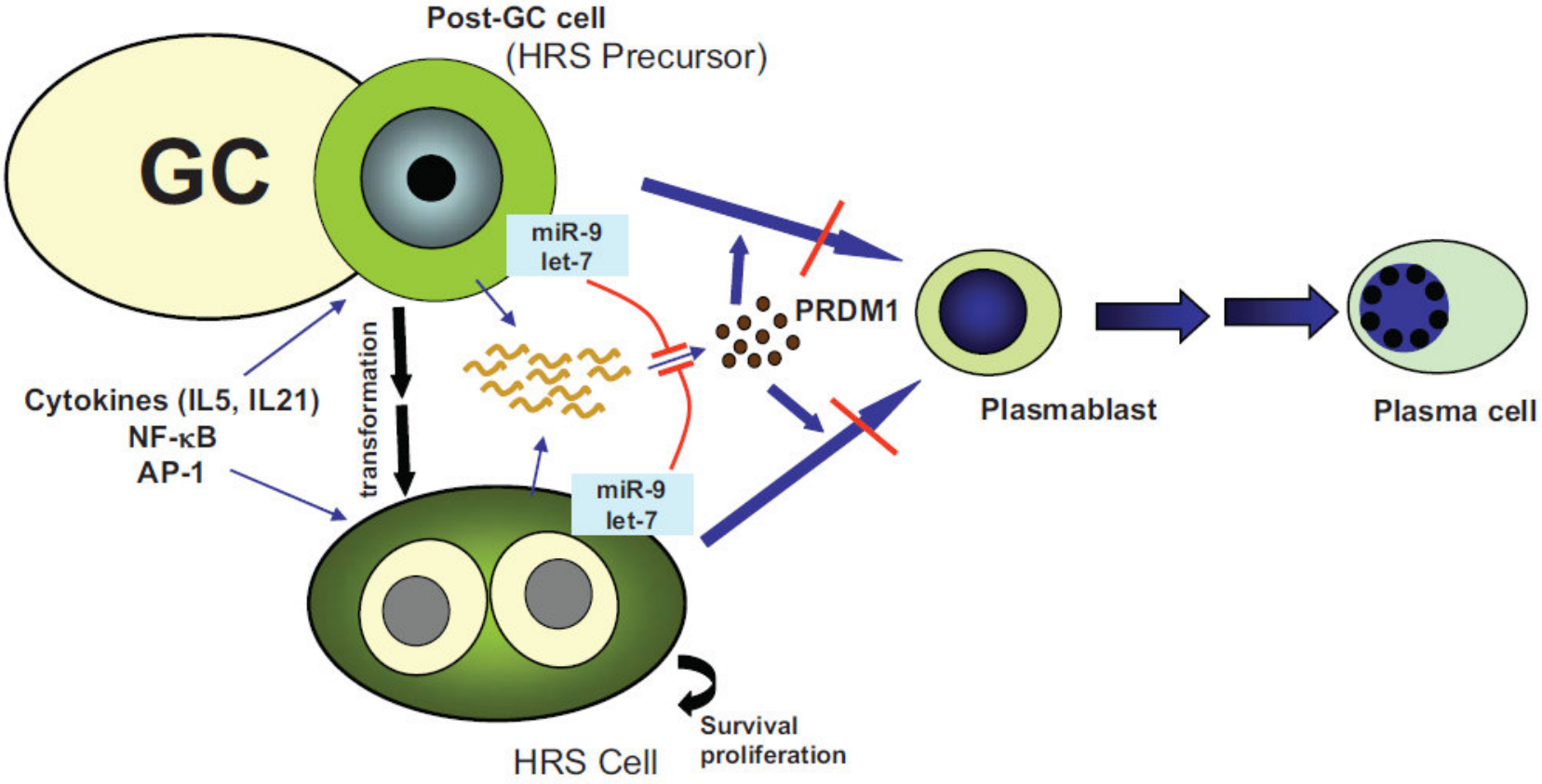
L Di Lisio et al. 2012

miR-9 inhibits Blimp1 (*PRDM1)* in Hodgkin/Reed-Sternberg cells



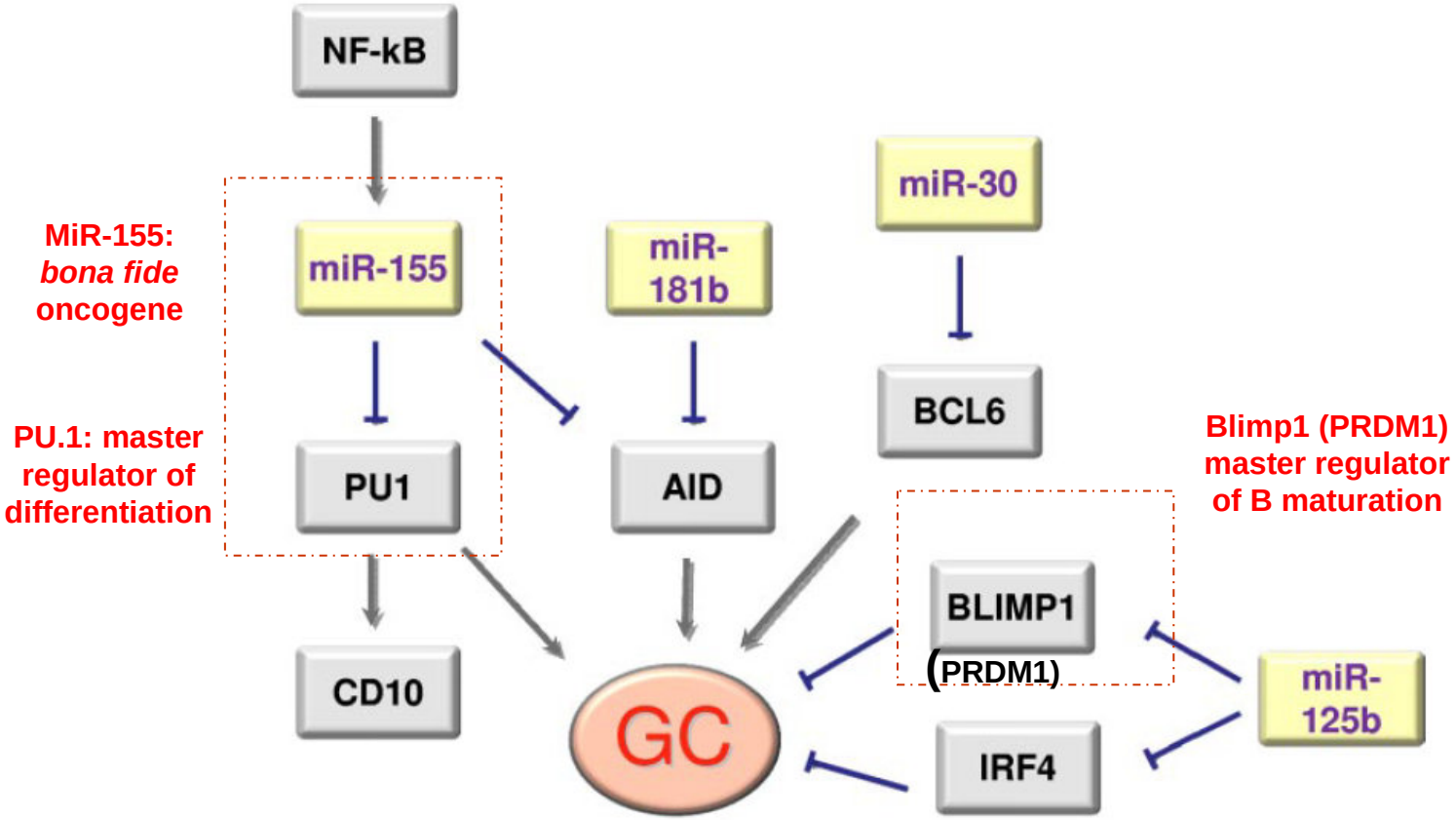
Nie et al. 2008

microRNA-mediated *PRDM1* inactivation by miR-9 in Hodgkin/Reed-Sternberg cells

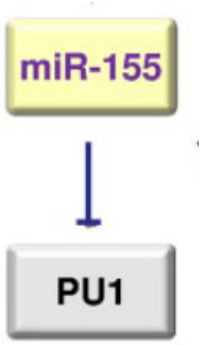


Nie et al. 2008

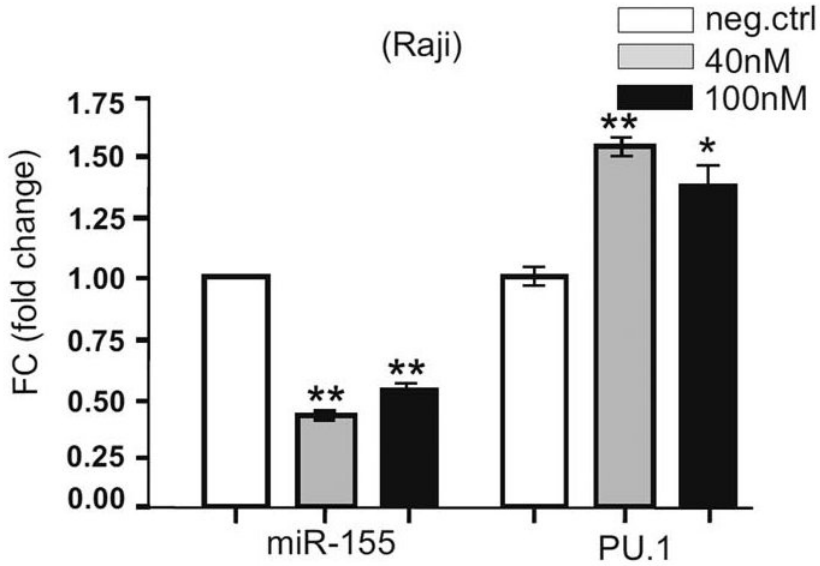
Interactions between genes and microRNAs in GC differentiation

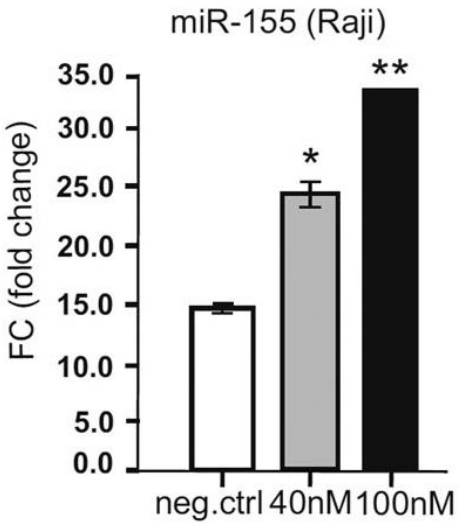


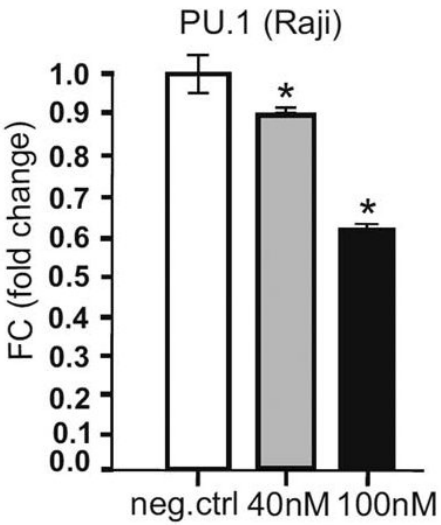
L Di Lisio et al. 2012



miR-155 targets and regulates PU.1 expression



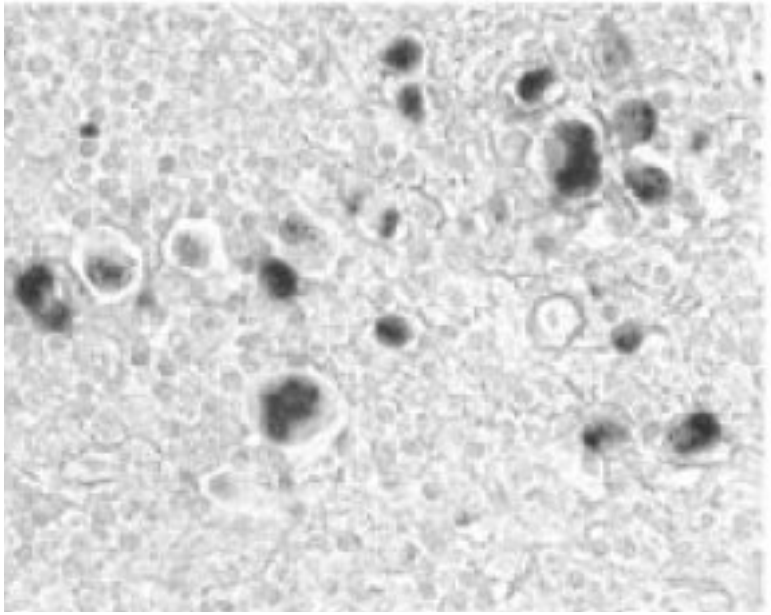




Vargova et al. 2011, Fig 5

BIC (miR-155) RNA-ISH in HL

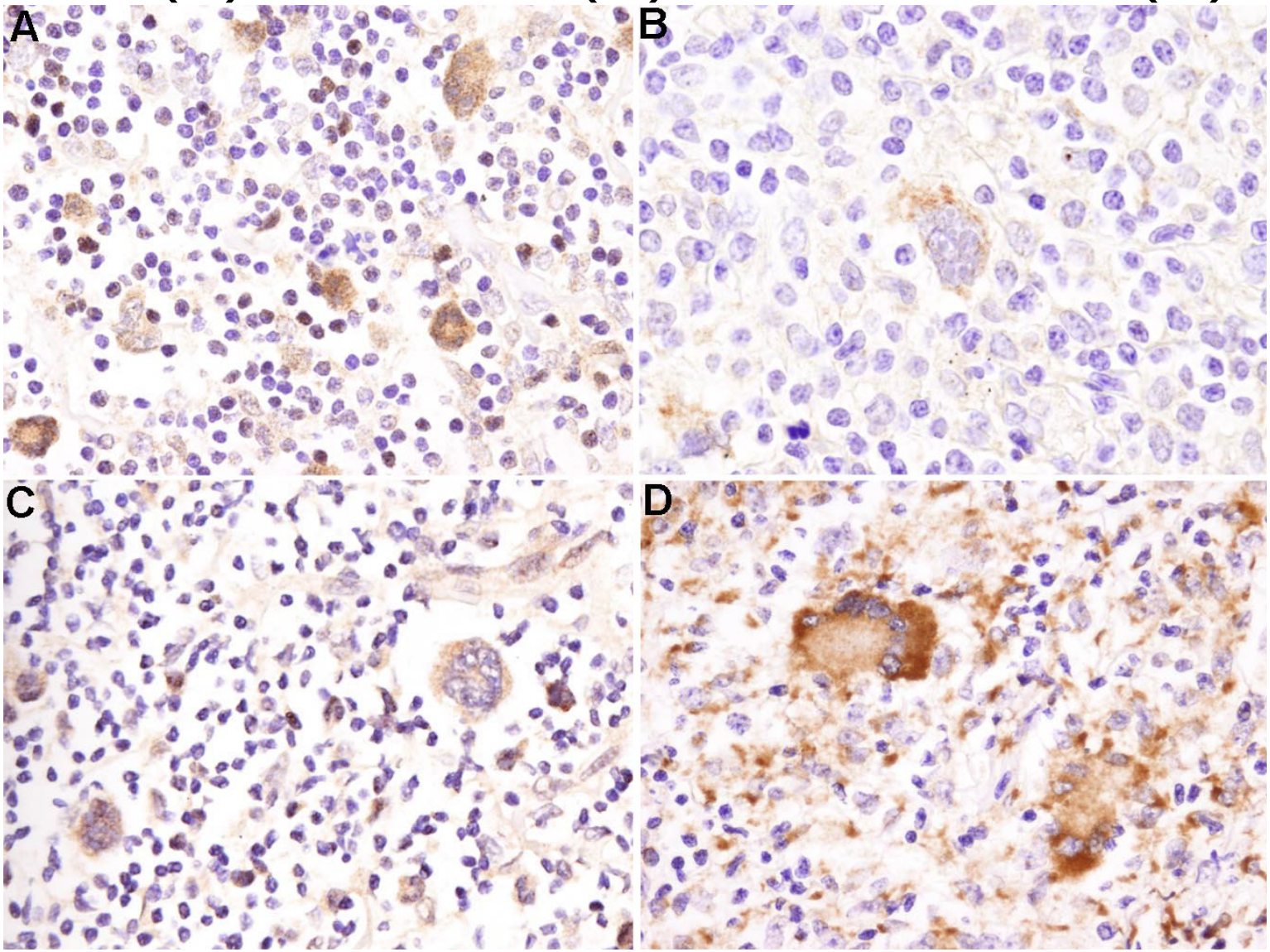
Tissue	BIC$^-$	BIC$^+$
FL*	15	0
BL*	8	1
MCL	6	0
MZL	0	2
DLBCL	6	12
PMBL	0	8
HL*	5	53
TCRBCL†	16	1
ALCL*	7	0
TCL	0	3



Hodgkin and Reed-Sternberg cells

Kluvier et al. 2005

Cytoplasmatic expression in Hodgkin and Reed-Sternberg cells of miR-21 (A), miR-134 (B), miR-138 (C), and miR-155 (D).



Navarro et al. 2007

Hypothesis

- Lymphomas originate from a block of differentiation.
- miR-155 dependent inhibition of PU.1 involves HL pathogenesis.
- *How can we use this in clinical questions (biomarkers)?*

Questions

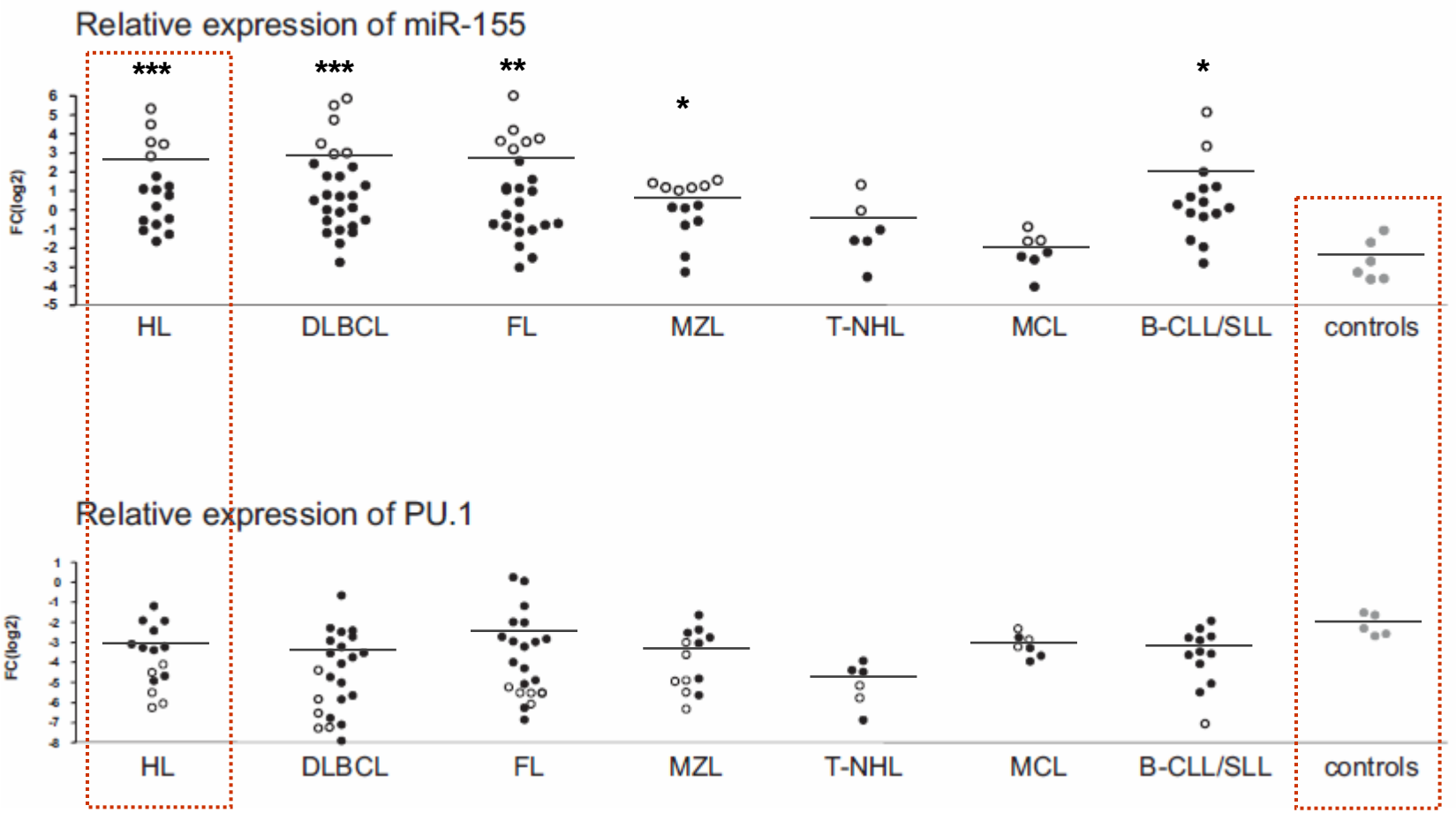
- To compare miR-155 levels across lymphomas?
- In what other lymphomas is miR-155 overexpressed?
- Is PU.1 repressed in that lymphomas?

- relationship btw miR-155 and PU.1 and clinical course of the patients?

miR-155/PU.1 in lymphoma

*** p < 0.0001
** p < 0.001
* p < 0.01
○ miR-155 above average
● miR-155 bellow average

- RNA isolation from the biopsies of lymph nodes (Trizol)
- RQ-PCR (TaqMan) – normalization: RNU44, GAPDH



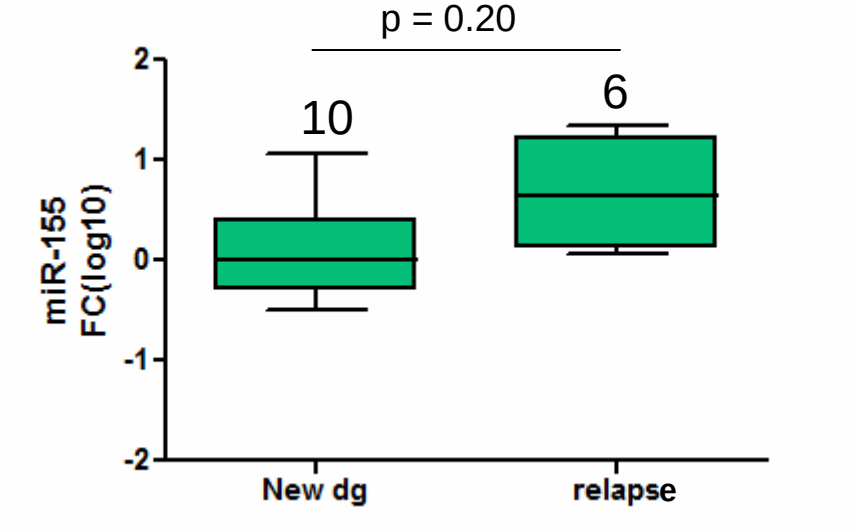
Hodgkin lymphoma

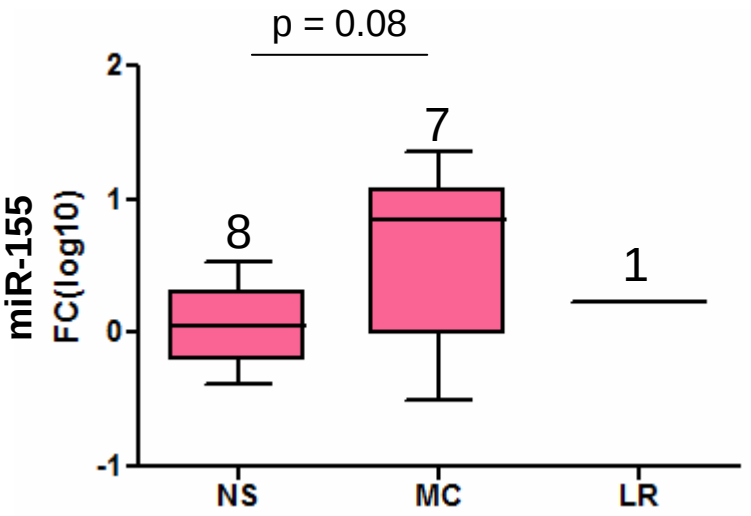
Description of patients

N = 16
Male = 8, Female = 8
Age
median = 28, min = 20, max = 67

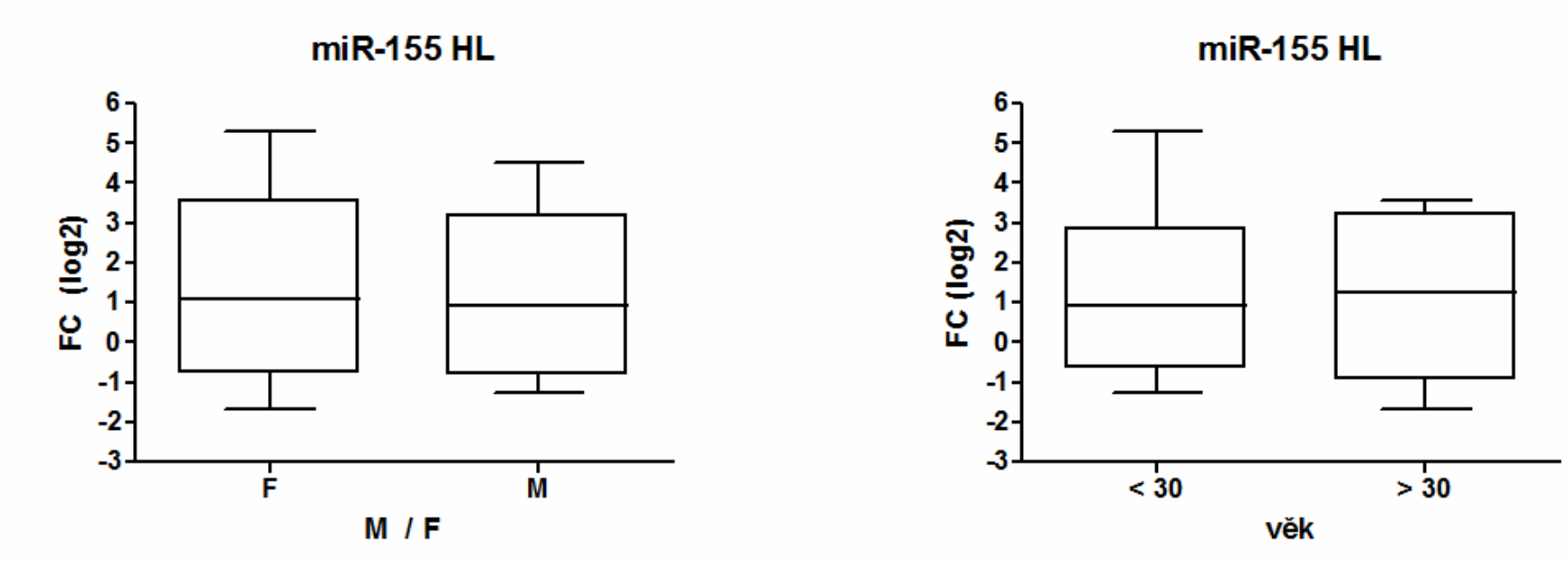
Nodular sclerosis = 8
Mixed cellularity = 7
Lymphocyte rich = 1

New diagnosis = 10
Relaps = 6



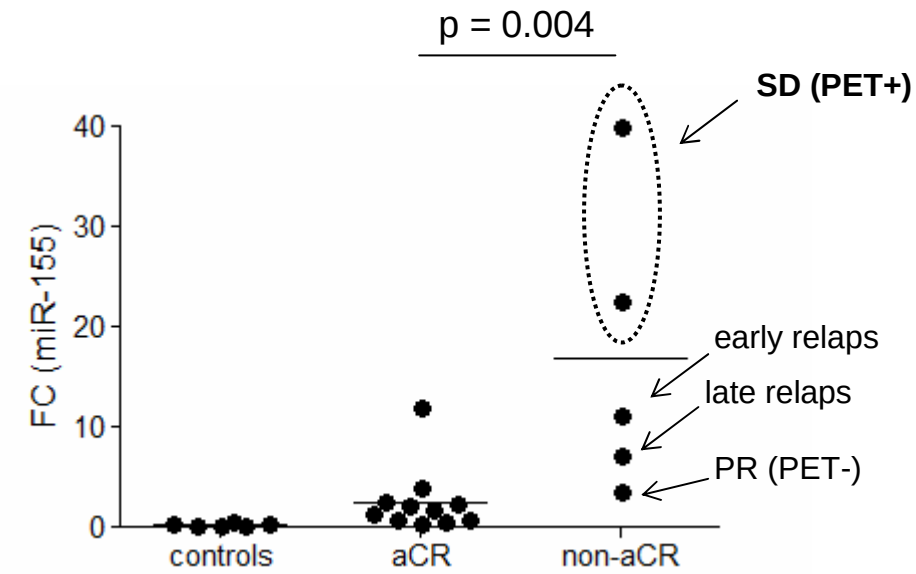


NS – nodular sclerosis, MC – mixed cellularity, LR – leukocyte rich


miR-155 HL
FC (log2)
6
5
4
3
2
1
0
-1
-2
-3
F
M
M / F
miR-155 HL
FC (log2)
6
5
4
3
2
1
0
-1
-2
-3
< 30
> 30
věk

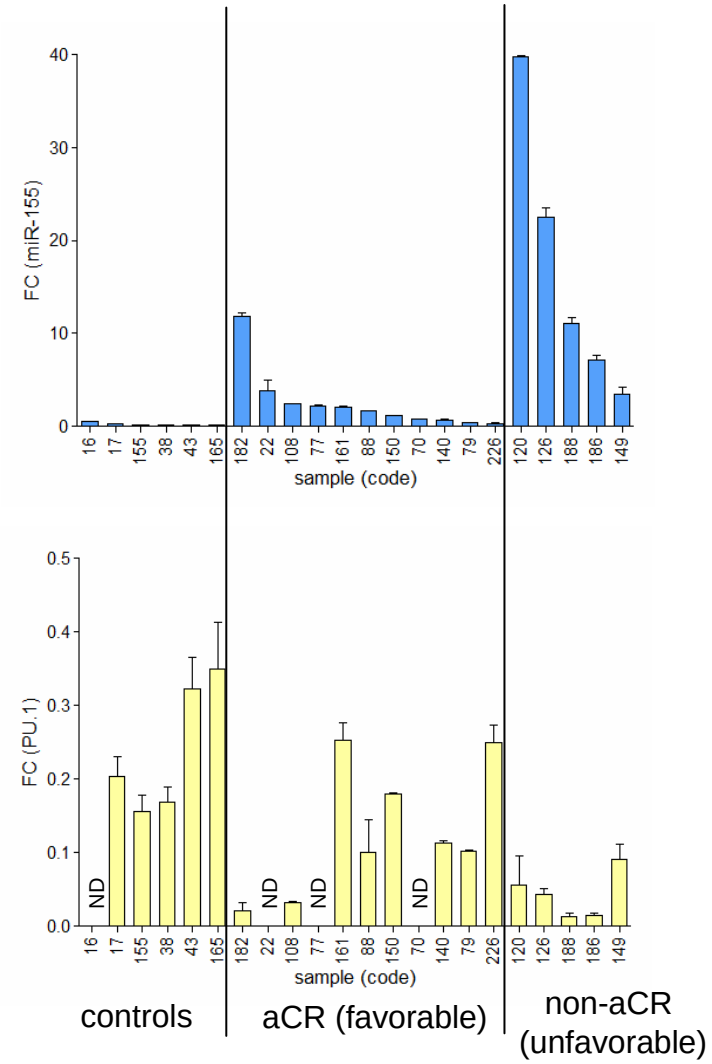
Hodgkin lymphoma

and response to first line of therapy



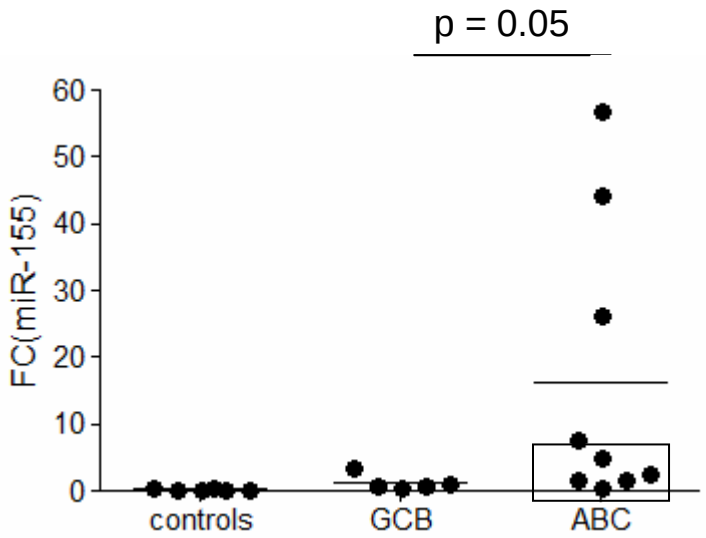
aCR – achieved remission, non-aCR – not achieved remission

- response to first line of therapy is a valuable prognostic marker in HL
- miR-155 is upregulated in lymph nodes of patients that did not achieve remission or relapsed very early

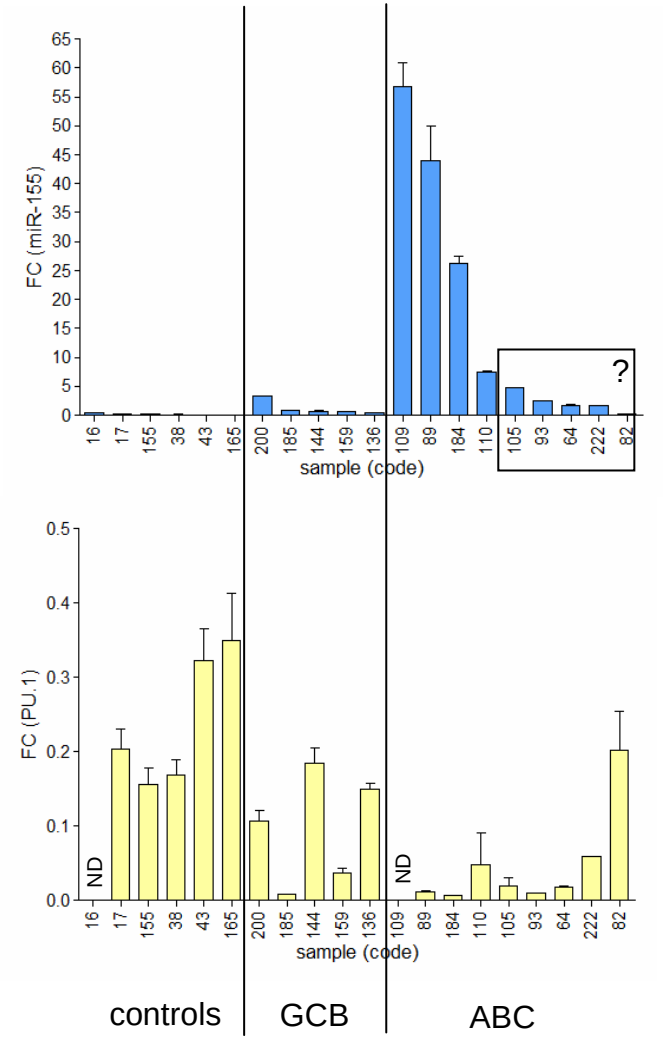


Diffuse large B-cell lymphoma

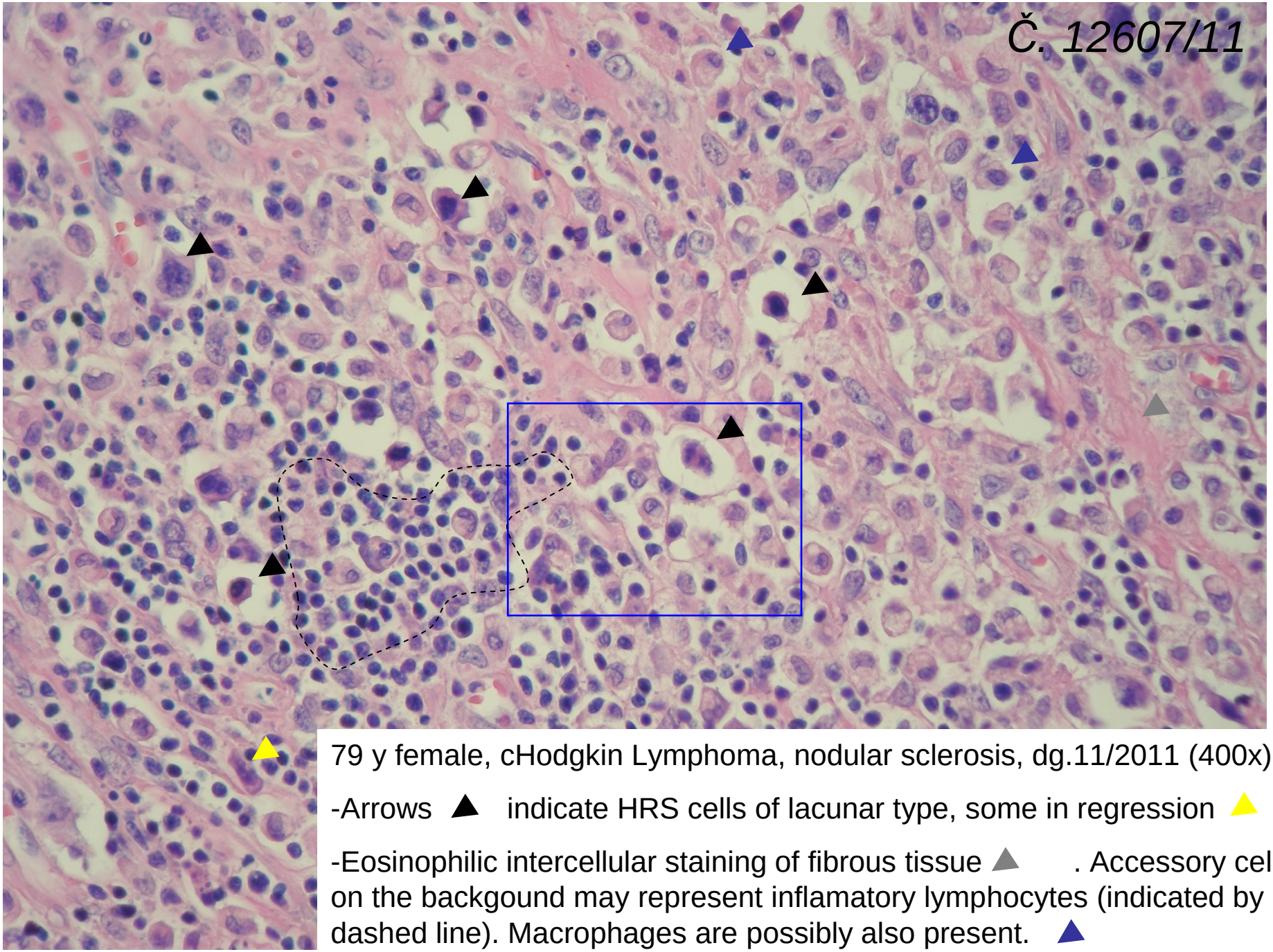
and its subtypes



GCB – germinal center B cell like, ABC – activated B cell like



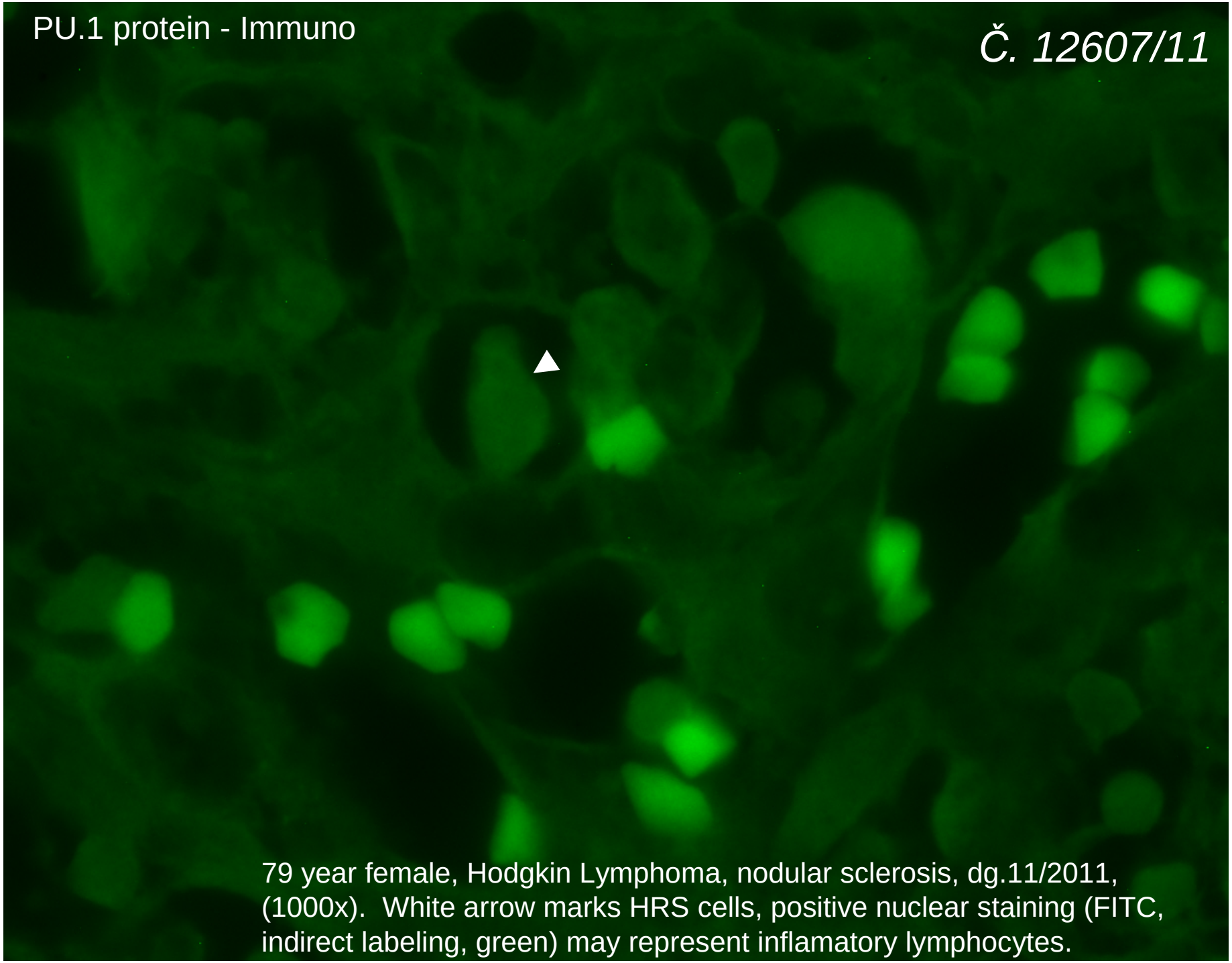
- ABC-DLBCL subtype is more aggressive than GCB-DLBCL
- miR-155 is upregulated in lymph nodes of patients with ABC-DLBCL compared to GCB-DLBCL *(Eis et al. 2005)*



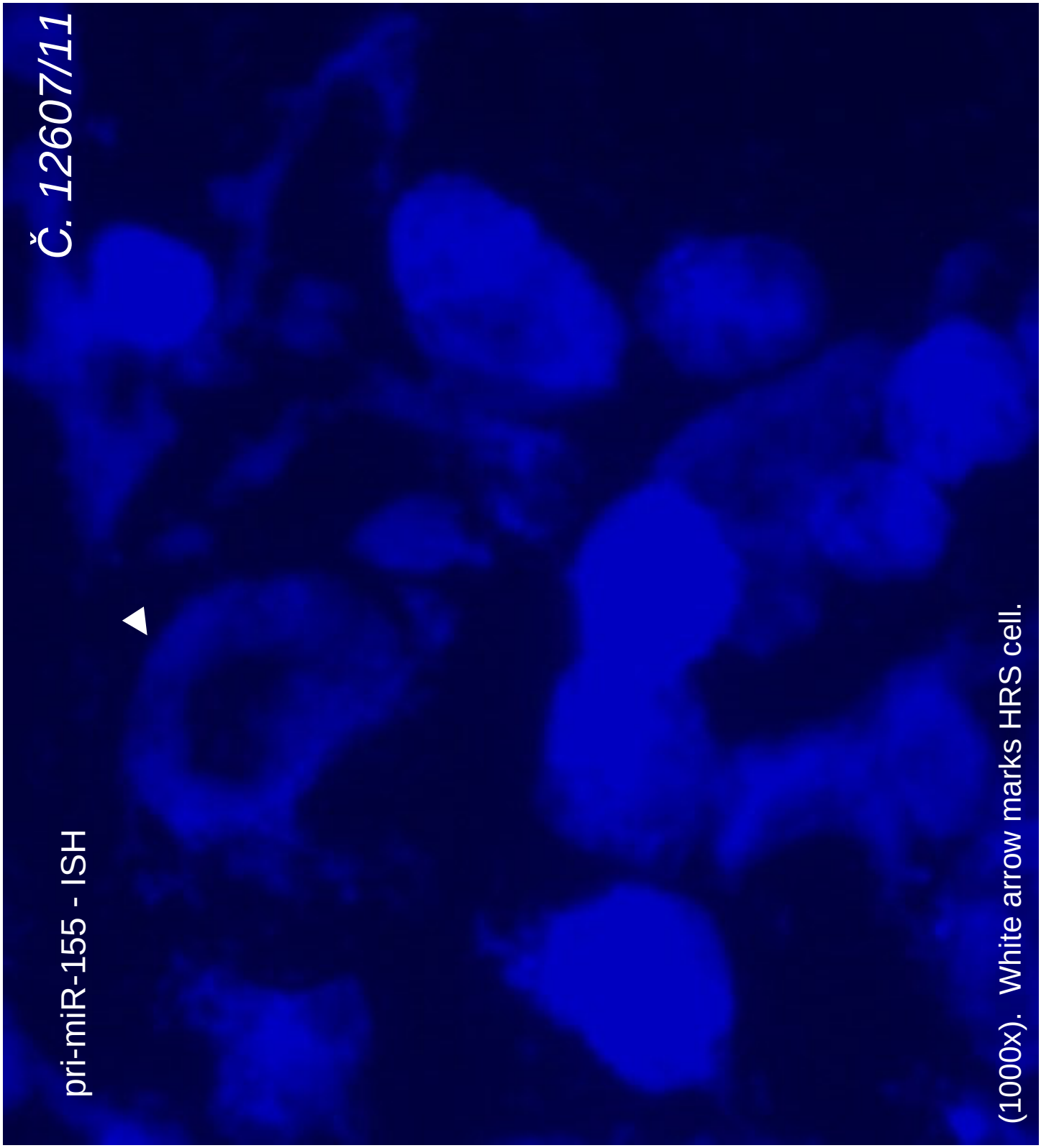
79 y female, cHodgkin Lymphoma, nodular sclerosis, dg.11/2011 (400x)

-Arrows ▲ indicate HRS cells of lacunar type, some in regression ▲

-Eosinophilic intercellular staining of fibrous tissue ▲ . Accessory cells on the backgound may represent inflamatory lymphocytes (indicated by dashed line). Macrophages are possibly also present. ▲


PU.1 protein - Immuno
Č. 12607/11
79 year female, Hodgkin Lymphoma, nodular sclerosis, dg.11/2011, (1000x). White arrow marks HRS cells, positive nuclear staining (FITC, indirect labeling, green) may represent inflamatory lymphocytes.

79 year female, Hodgkin Lymphoma, nodular sclerosis, dg.11/2011



Conclusions

- miR-155 is upregulated in HL and B cell malignancies from GCs incl. DLBCL, B-CLL/SLL, FL, MZL.

- miR-155 expression is reflected by downregulation of its target: major hematopoietic transcription factor PU.1

- in B cell malignancies (incl. HL), miR-155 upregulation may reflect aggressiveness exemplified by patients that did not achieve complete remission or relapsed early.

Acknowledgements

- **1st Faculty of Medicine**
- H. Hušková, K. Vargová, V. Kulvait (Inst. pathophysiology)
- Libor Staněk, Pavel Dundr, Radek Jakša (Inst. pathology)
- **General Faculty Hospital**
- M. Trněný, K. Siebertová-Korecká, A. Berková, J. Karban (1st Dept Medicine- hematology)

- **3rd Faculty of Medicine, University Hospital Kralovské Vinohrady**
- H.Mociková, J.Marková, Ľ.Gahérová, T.Kozák (Dept. of Clinical Hematology)
- Z.Vernerová (Inst. pathology)