Waldenström macroglobulinemia and miRNA

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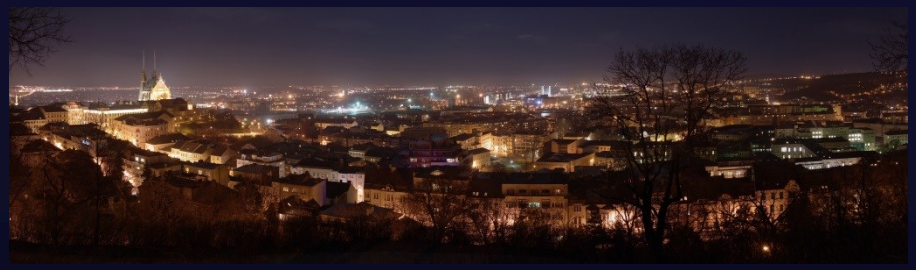
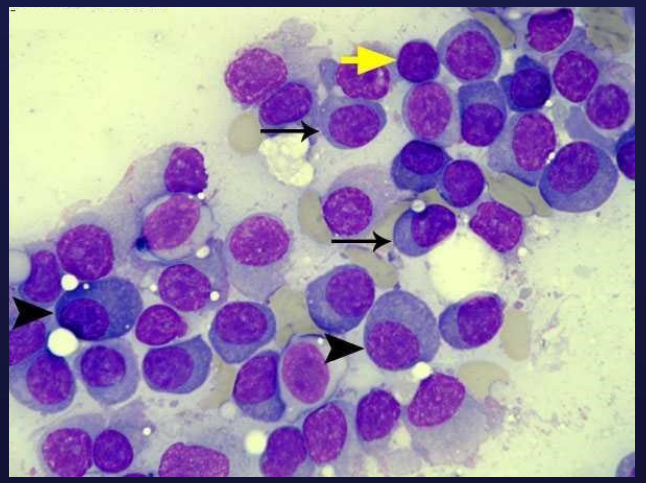


13th Workshop
Multiple Myeloma
April 10-11, 2015
Mikulov

Waldenström macroglobulinemia (WM)

- lymphoplasmacytic lymphoma with immunoglobulin M (IgM) monoclonal protein



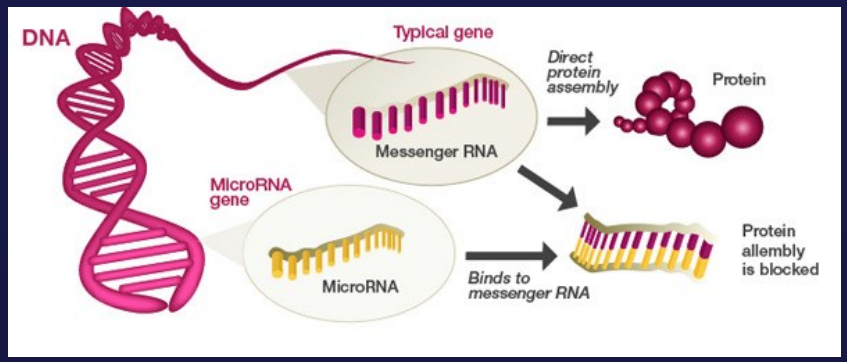
Diagnosis

- ≥10% clonal lymphoplasmacytic cells
- presence of monoclonal IgM
- L265P mutation in MYD88
  - detectable in more than 90% of patients
- difficult to differentiate from **IgM-MM** or **IgM-MGUS**
  - MYD88 mutation, flow cytometry

- microRNA/circulating microRNA?

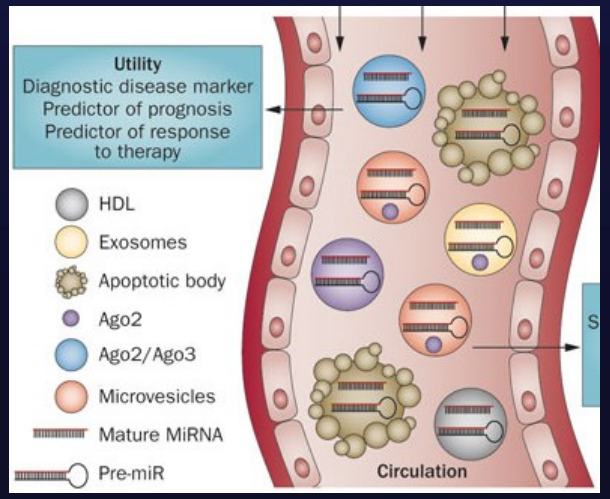
MicroRNA (miRNA)

- single stranded RNAs
- regulators of gene expression
- disease pathogenesis and cancer



Circulating miRNA

- in various body fluids
- stable in microvesicles
- easily accessible, easy detection
- connected to pathological condition



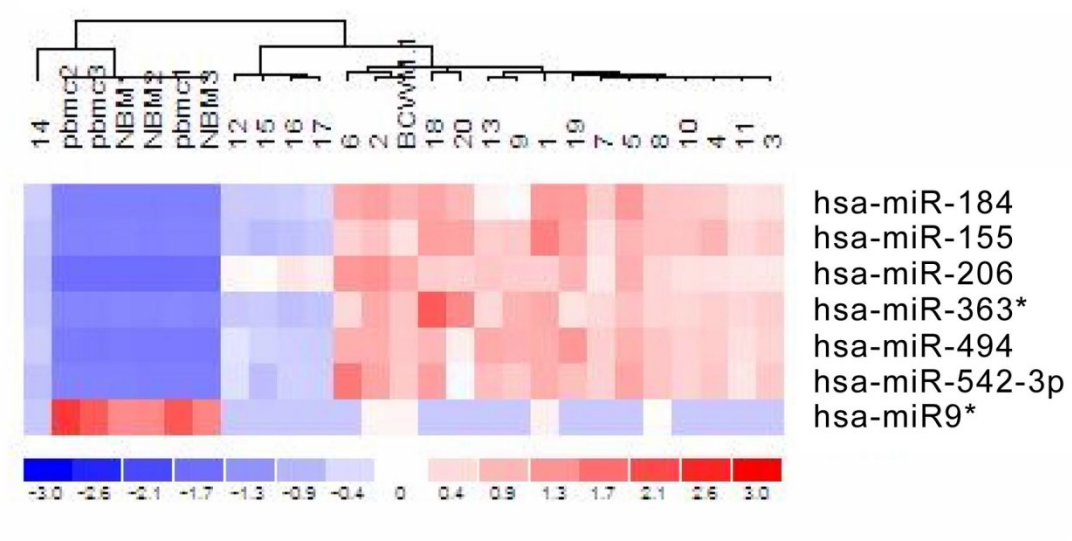
http://www.medscape.com/

Wittmann et al., 2010; Wang, 2009, Sanger miRBase

miRNA
in Waldenström macroglobulinemia

WM cells

- minimal changes in cytogenetic studies and gene expression analysis
- microRNA (miRNA) signature differentiates WM cells from their normal counterparts



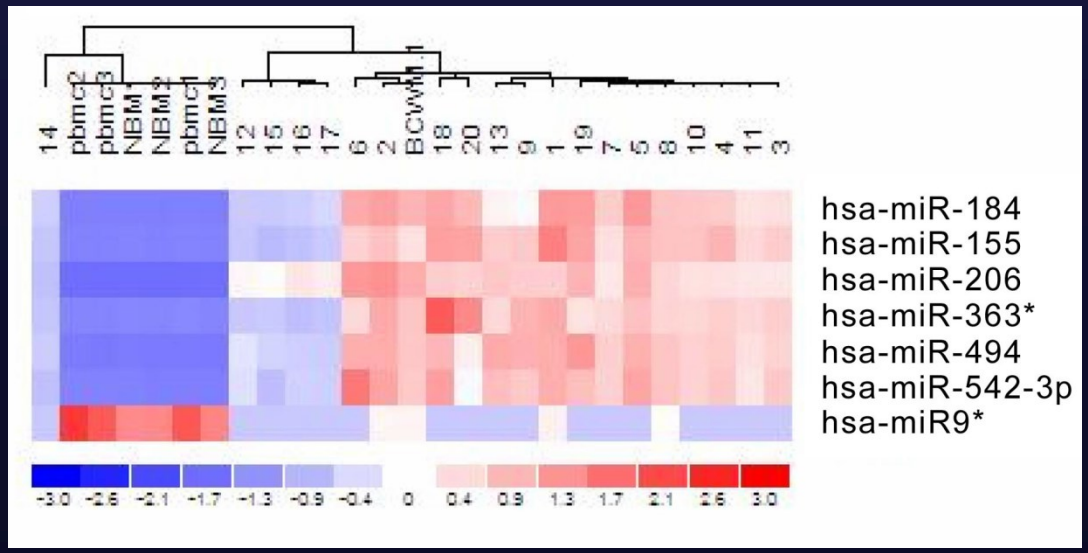
Roccaro et al., 2009; 2010; Zhang et al., 2012

miRNA
in Waldenström macroglobulinemia

WM cells

miR-155 – pivotal role in WM *in vitro* and *in vivo*

- pathogenesis, prognosis, microenvironment
- In plasma – potential biomarker of WM



Roccaro et al., 2009; 2010; Zhang et al., 2012

Our goal

study of circulating miRNA in WM

Aims of study

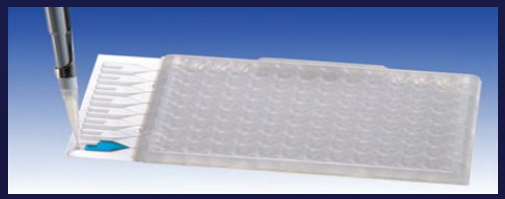
- to analyze profile of differentially expressed serum miRNA in WM patients in comparison to IgM-MGUS as well as IgM-MM and healthy donors

- to validate this profile on a larger cohort of WM and IgM-MGUS as well as IgM-MM and healthy donors using qPCR

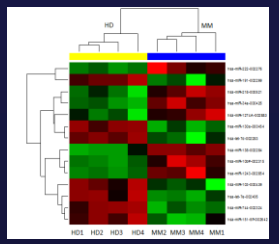
- to use circulating serum miRNA as marker of WM

Workflow

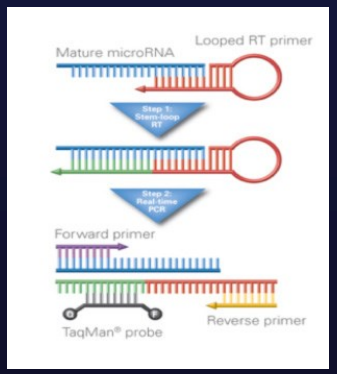
Serum miRNA isolation



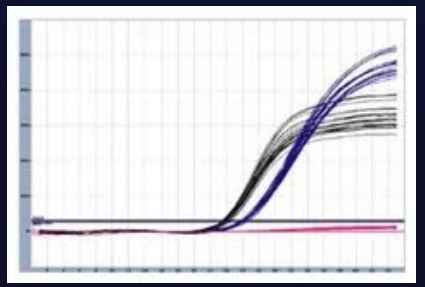
TaqMan Low Density Arrays (TLDA)



Differently expressed miRNA evaluation



qPCR Specific TaqMan miRNA assays

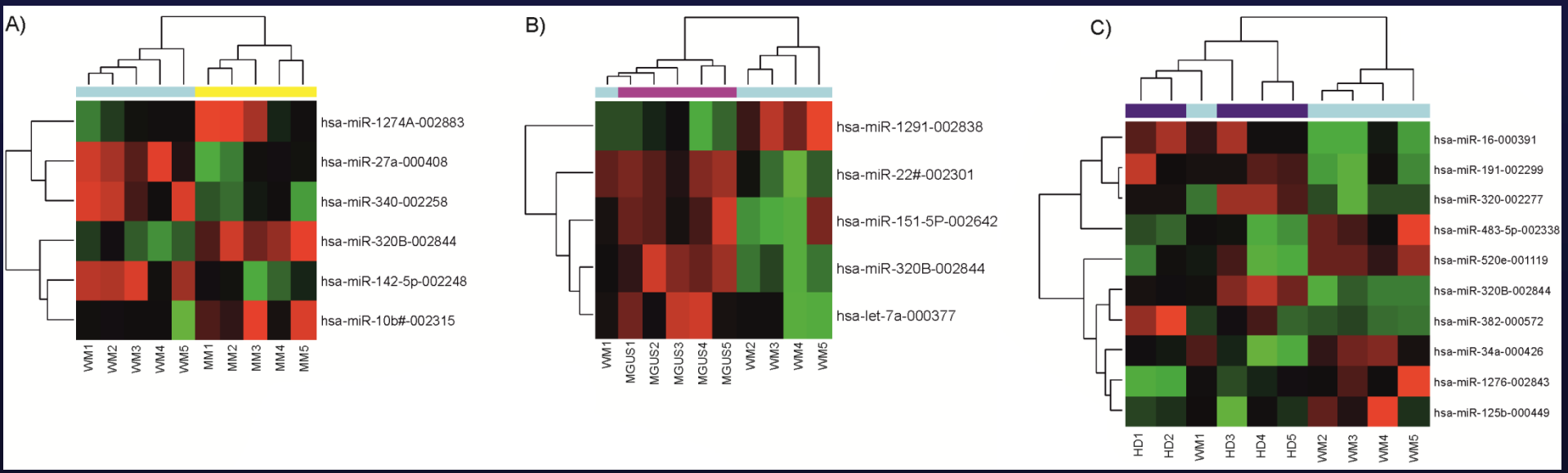


Data analysis

Correlation with
clinical parametres

Results – TLDA screening

- 6 differently expressed miRNA between WM and IgM-MM
- 5 differently expressed miRNA between WM and IgM-MGUS
- 10 differently expressed miRNA between WM and HD
- validation: miR-320a, miR-320b, let-7a, miR-151-5P



Kubiczková-Bešše *et al*. 2014, Am J Hematol

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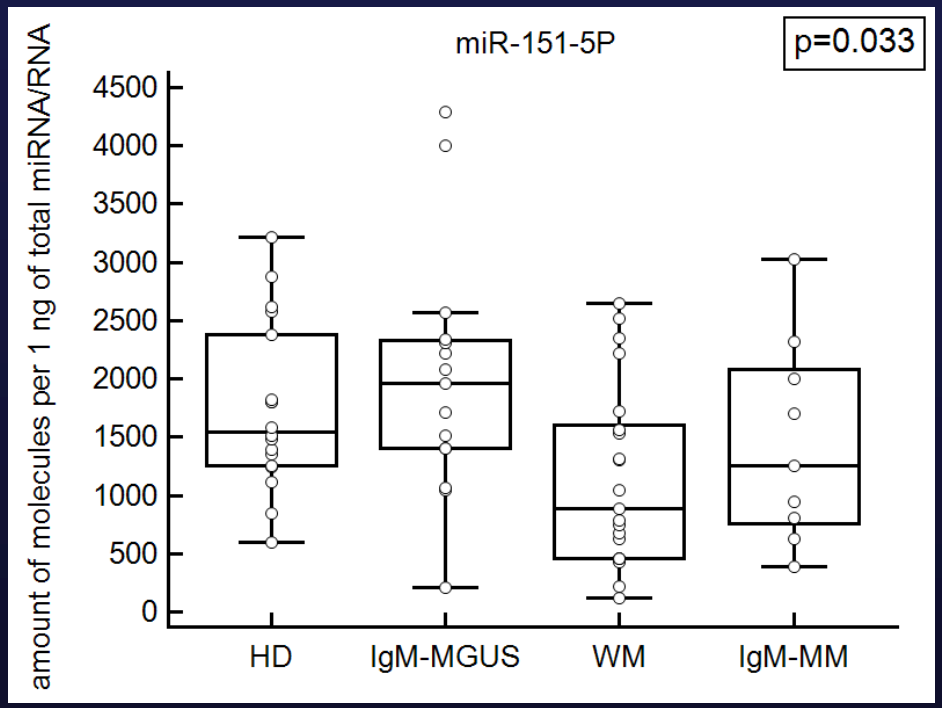
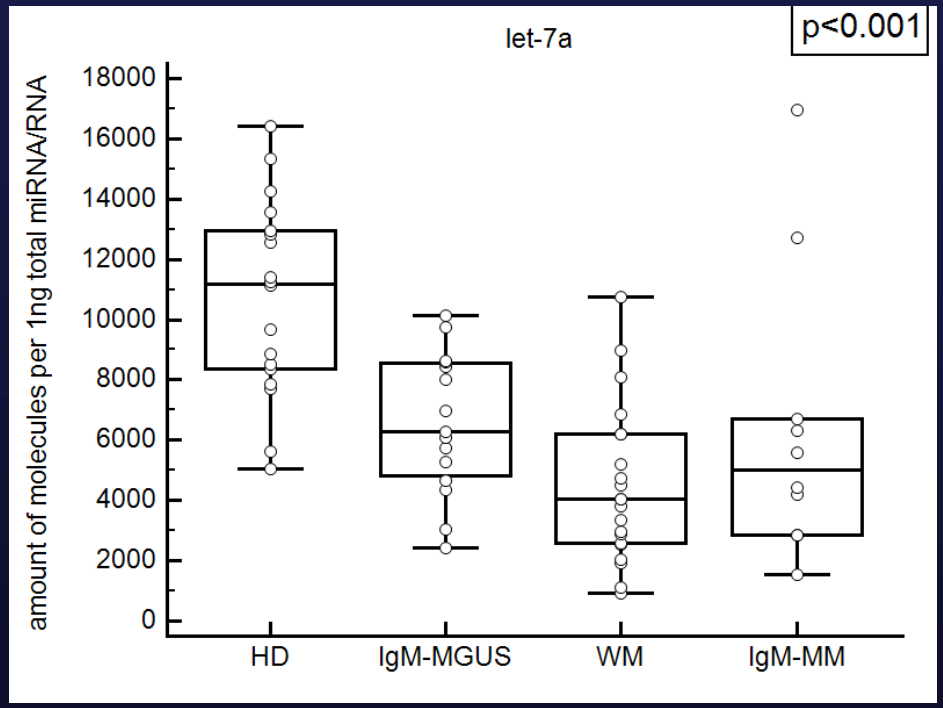
Patients´ characteristics

	HD	IgM-MGUS	WM	IgM-MM
Gender: males-females	50%-50%	40%-60%	66%-34%	50%-50%
ISS stage: I-II-III	ND	ND	ND	60%-30%-10%
D-S substage: A-B	ND	ND	ND	90%-10%
Light chains: kappa-lambda	ND	46.7%-53.3%	85.7%- 14.3%	50%-50%

Results - validation

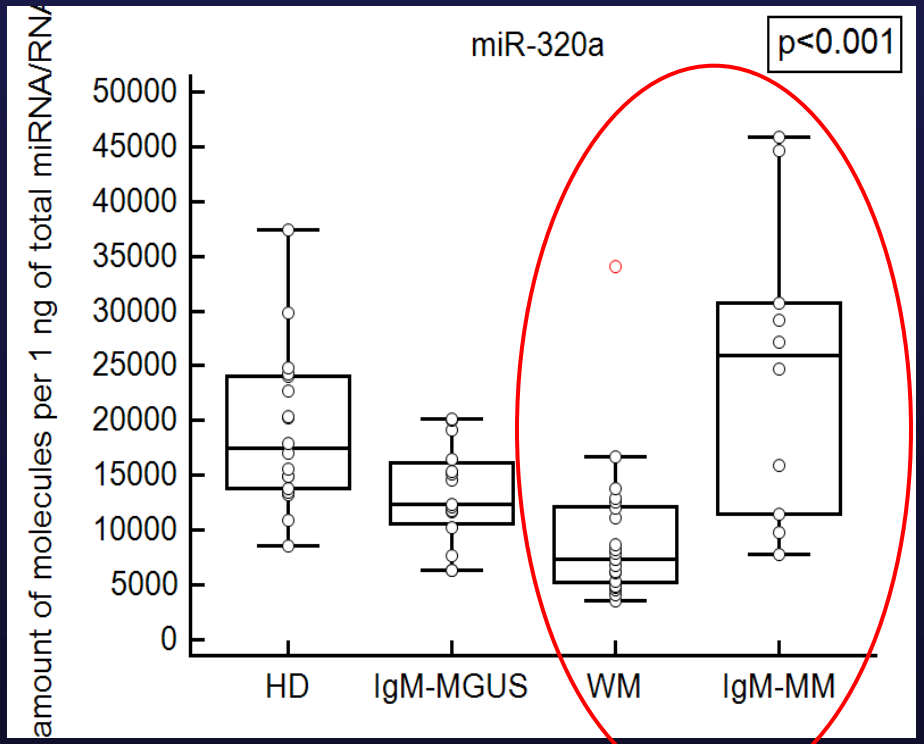
- miR-151-5P and let-7a:

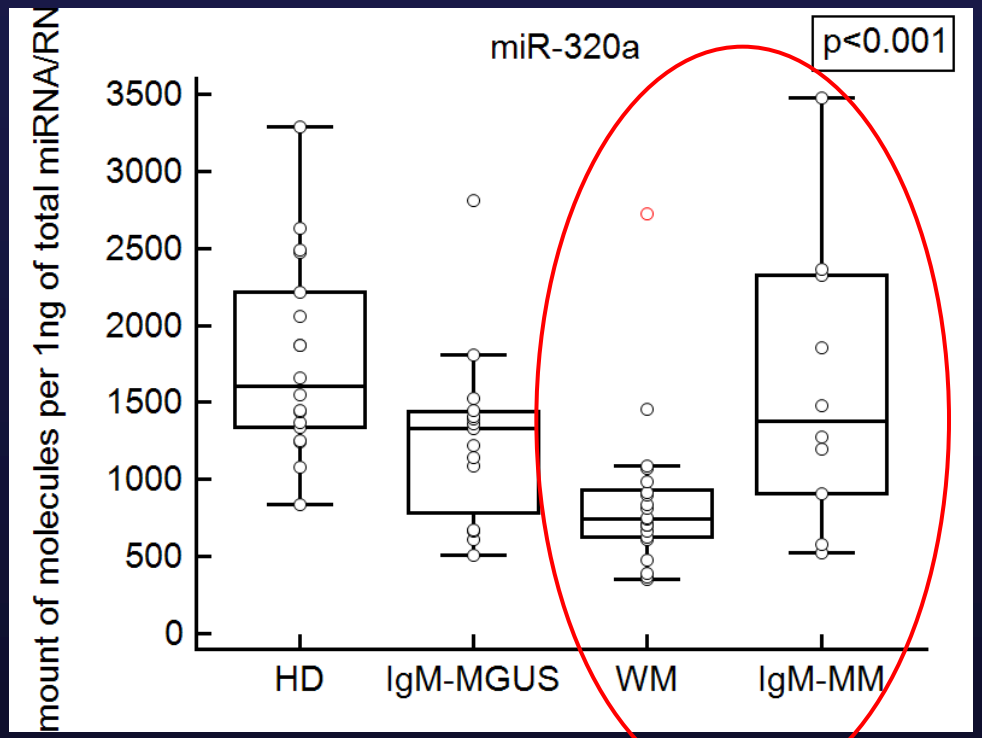




Results - validation

- miR-320a and miR-320b:





different expression between

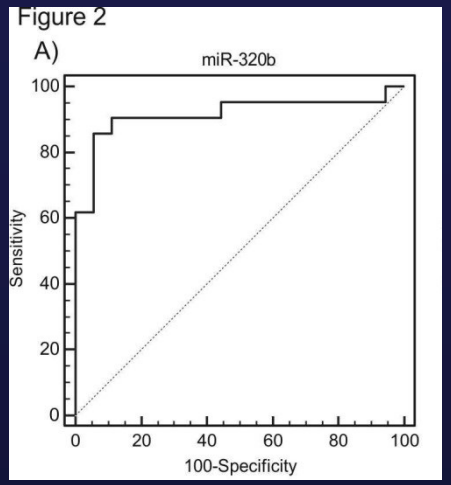
WM and all other groups of samples ($p<0.05$)

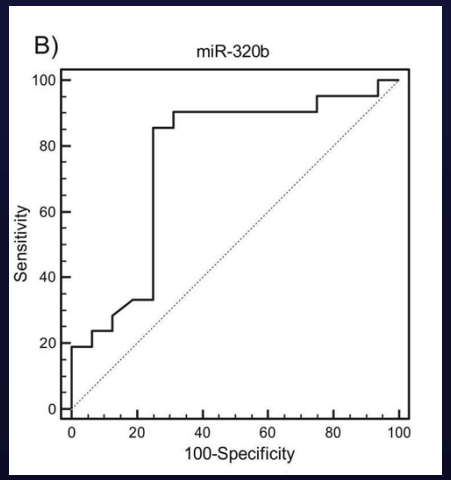
Results – ROC analysis

- serum miRNA-320b and miR-320a discriminate:

- WM from HD,
  sensitivity: 90.5%, specificity: 94.4%
  AUC: 0.921

- WM from IgM-MGUS
  sensitivity: 90.5%, specificity: 73.3%
  AUC: 0.743



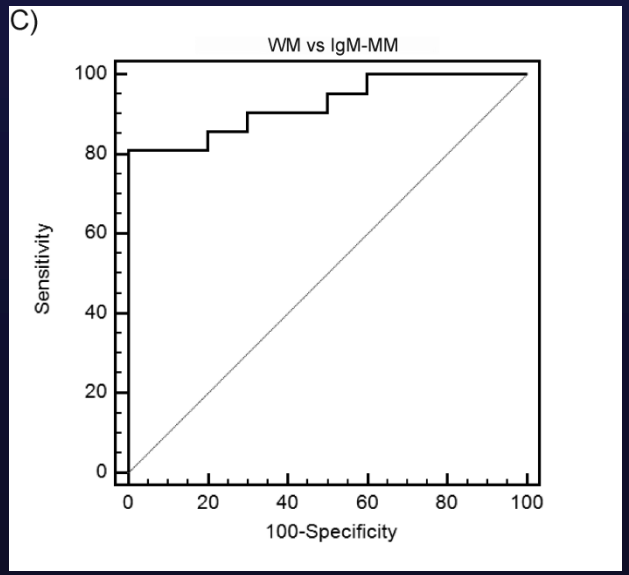


Kubiczková-Bešše *et al*. 2014, Am J Hematol

Results – ROC analysis

- serum miRNA-320b and miR-320a discriminate:

- WM from IgM-MM
  sensitivity: 81.0%, specificity: 100.0%
  AUC = 0.924



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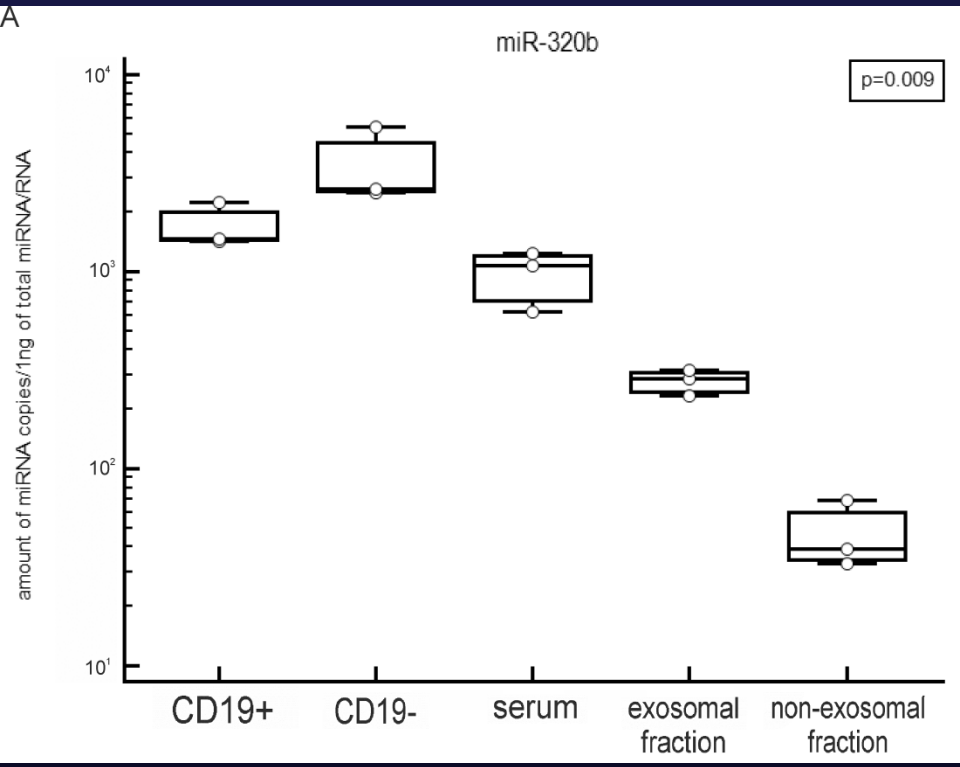
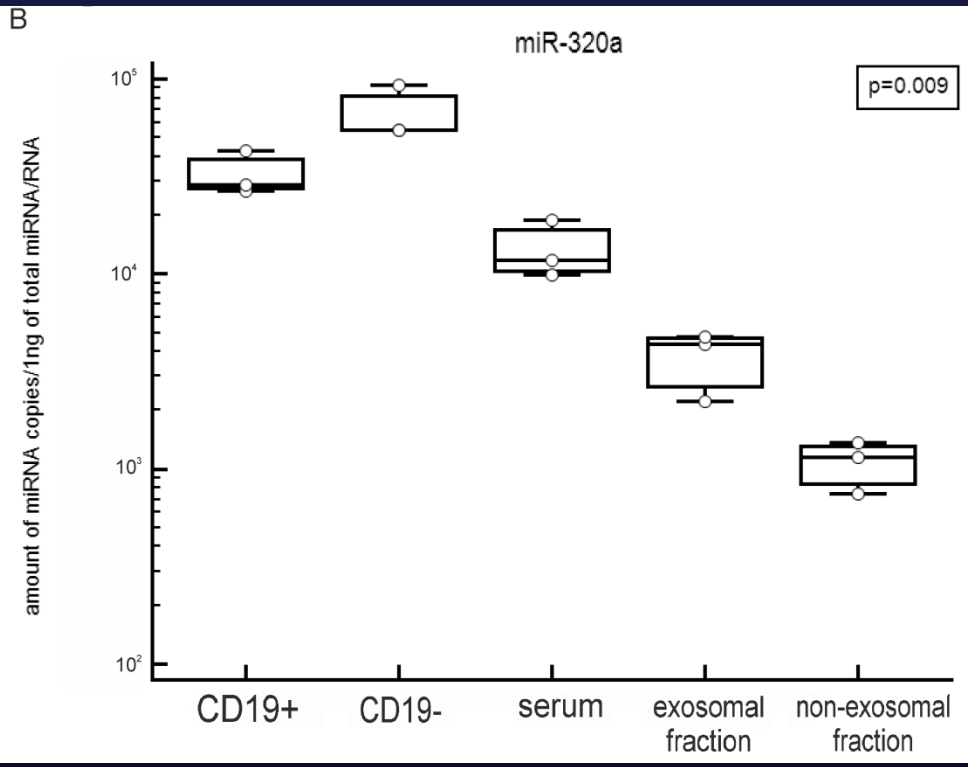
miRNAs in different fractions

- present in both CD19+ and CD19- cellular fractions
- higher levels in exosomal fractions





Kubiczková-Bešše *et al*. 2014, Am J Hematol

Summary

Circulating miR-320a and miR-320b:

- distinguish WM patients from IgM-MM, IgM-MGUS and HD
- potentially could serve as a minimally invasive biomarkers for WM diagnosis
- need a larger validation cohort of patients
- prediction of IgM-MGUS evolution?

ACKNOWLEDGMENTS

Babak Myeloma Group,
Dep. of Pathological Physiology, Faculty of Medicine, MU

Ševčíková Sabina

Bešše Lenka
Kubaczková Veronika
Sedlaříková Lenka
Gromesová Barbora
Stanková Monika
Kutálková Kateřina
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Bezděková Renata
Hanáková Božena
Říhová Lucie
Sáblíková Barbora
Suská Renata
Štouračová Marcela
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Kuglík Petr

Mikulášová Aneta
Smetana Jan
Wayhelová Markéta

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Adam Zdeněk
Krejčí Marta
Pour Luděk

Institute of biostatistics and analysis
Faculty of Medicine, MU

Jarkovský Jiři

Brožová Lucie
Budinská Eva
Ihnatová Ivana

Institute of Molecular and Translational Medicine,
Palacký University, Olomouc

Ščudla Vlastimil
Pika Tomáš
Minařík Jiří