Pathogenesis of multiple myeloma

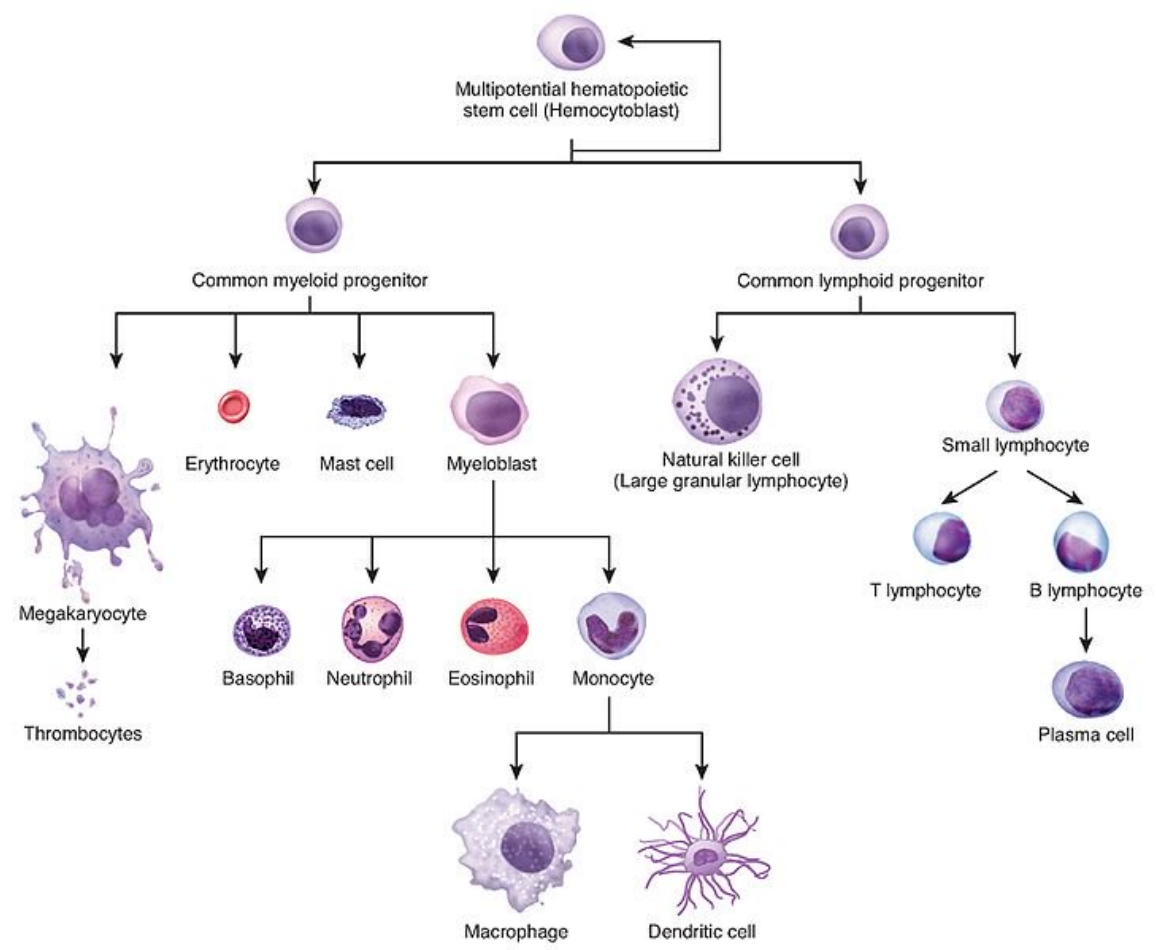
Doc. RNDr. Sabina Ševčíková, Ph.D.
Babak myeloma group
ÚPF LF MU

Monoclonal gammopathies

- Abnormal proteins in serum or urine
- These proteins produced by a clone of plasma or lymphoid cells
  - MGUS
  - Multiple myeloma
  - Plasma cell leukemia
  - Primary amyloidosis
  - Solitary plasmocytoma
  - Waldenström macroglobulinemia

World Health Organization
Adam, 2011

Hematopoesis



Plasma cells

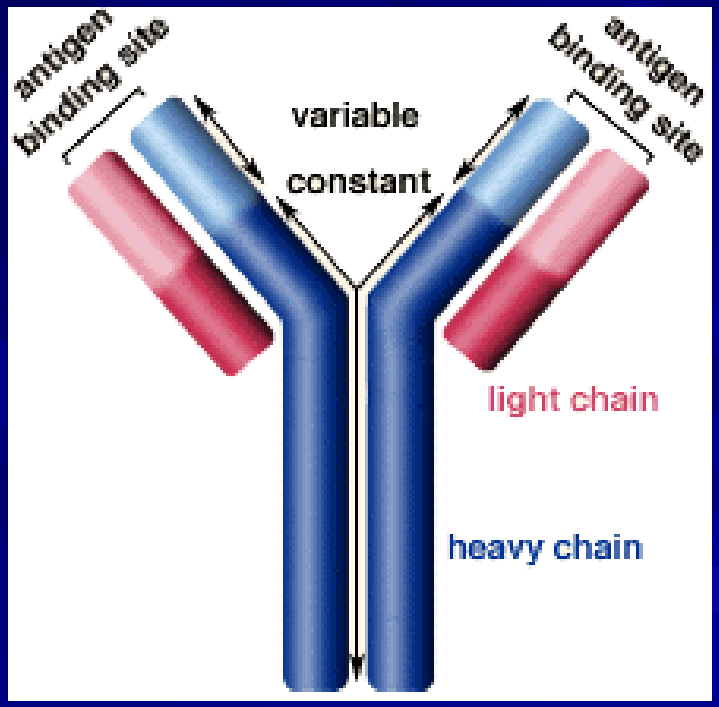
- Cells that produce antibodies
- Usually around 5% in the bone marrow
- In MM, they are malignant
- Produce monoclonal immunoglobulin= paraprotein

Antibodies

- To find and destroy foreign objects in the organism
- Eliminate pathogen
- IgG, IgA, IgM, IgE and IgD

- IgG – only one that enters placenta
- IgA – produced in mucous membranes, protects entry to body
- IgM – produced first after infection – body protection in the first few days
- IgE – parasites and allergic reactions
- IgD –unclear, co-expressed on surface of mature B cells

Basic structure of immunoglobulin



MGUS

- Precancerosis
- In people over 50 – 3-4%
- Risk of progression into MM > 1% every year

Multiple myeloma

- Second most common hematological malignancy
- 10% of hematological malignancies
- Median age at diagnosis 65
- Incidence 5.7/100 000
- More common in men
- Infiltration of bone marrow by malignant PC
- Osteolytic lesions
- Presence of M-Ig in serum and/or urine

Hájek, 2012
Anderson, 2011

PCL

- Most aggressive monoclonal gammopathy
- Incidence 0.04/100 000
- More than 20% of circulating PC and more than $2x10^9/L$
- Median age at diagnosis - 52
- Median survival 1.5 years or shorter

Primary amylodosis

- Amyloid deposits in organs
- Production of abnormal protein filaments
- Rare
- May be present together with MM
- Survival around 40 months

Solitary plasmocytoma

- Solitary lesion of abnormal PC
- Without bone marrow involvement
- Without osteolytic lesions
- Risk of progression into MM – 10% in 3 years

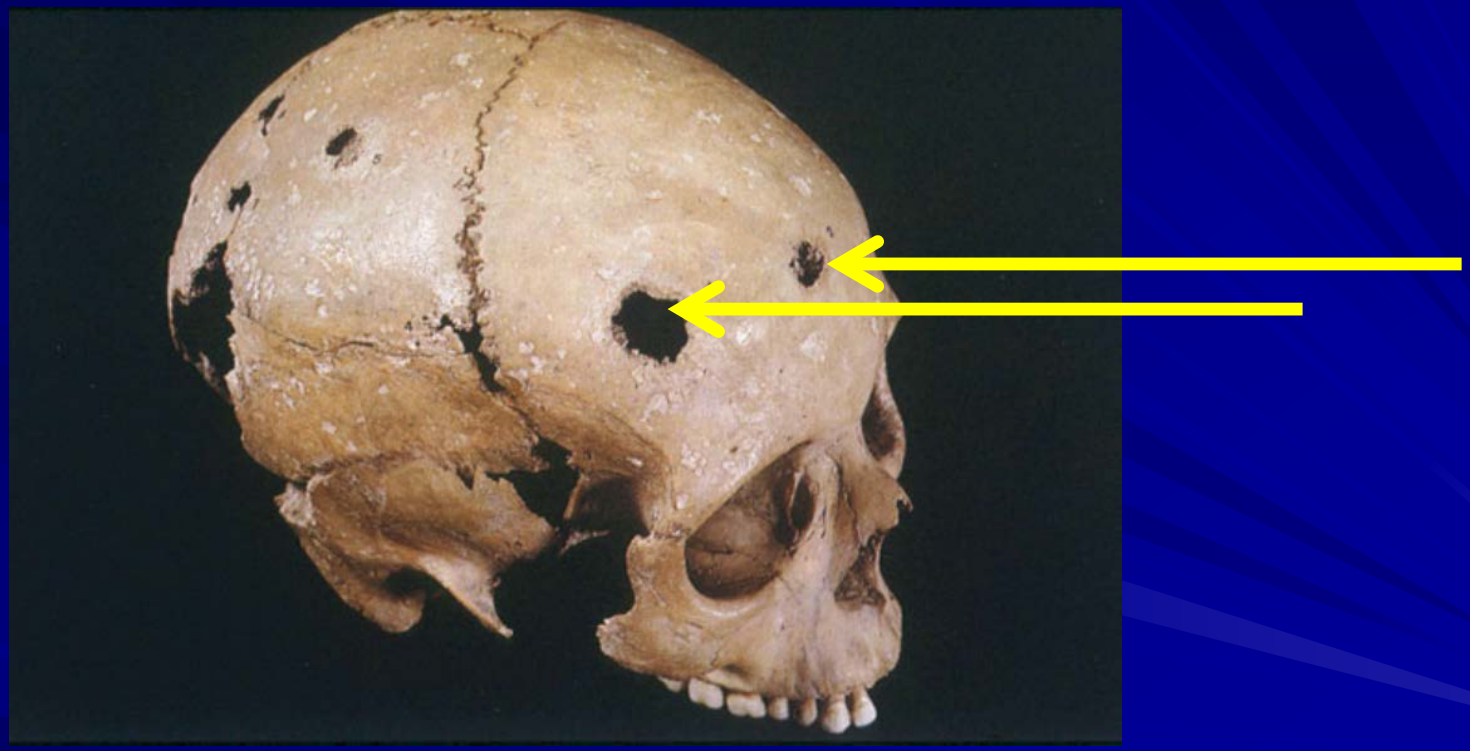
Waldenstrom macroglobulinemia

- Affects lymphocytoplasmoid cells (cells with characteristics of both PC and lymphoid cells)
- Production of large amount of IgM
- Very rare
- May progress from IgM MGUS

Multiple myeloma

History of MM

Male skull from the bronze age with MM characteristics



Capasso, 2005

History of MM

- 1844 – first documented case – Sarah Newbury (Dr. Solly)

destruction of sternum fractures of bones destruction of femur

Kyle et Rajkumar, 2008

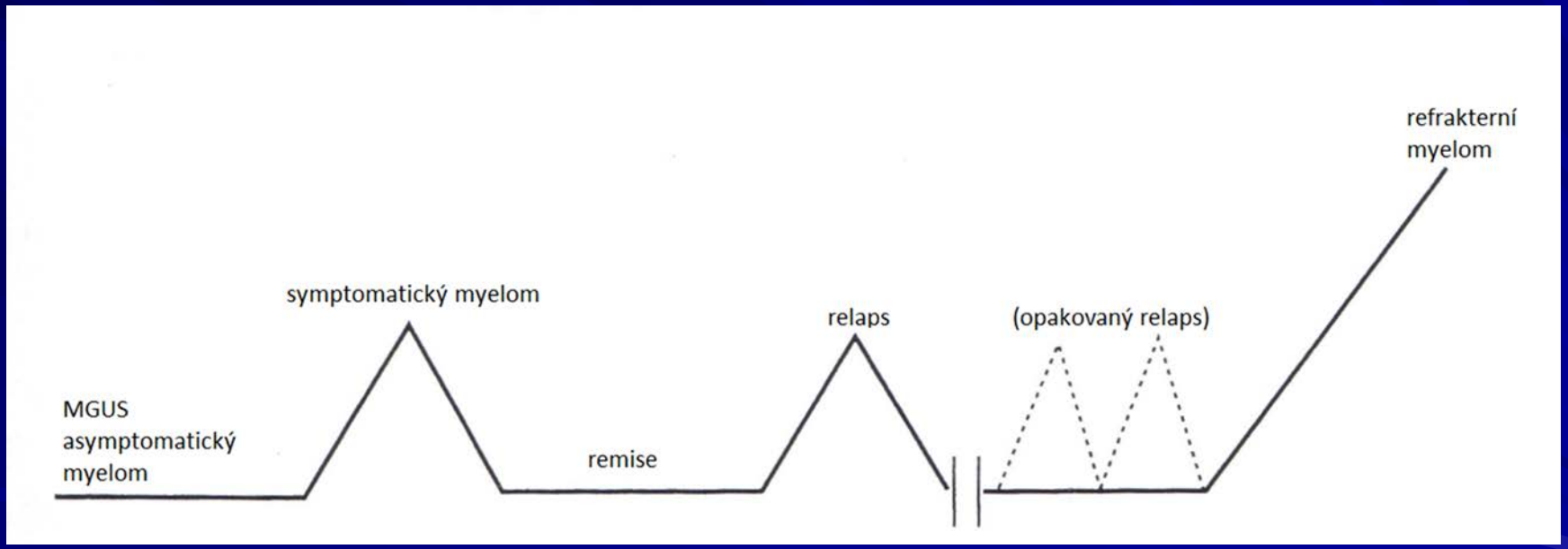
History of MM

- 1845 – presence of proteins in urine of patients (Bence Jones – Bence Jones protein)
- Kahler's disease – Prague physician Otto Kahler

Kyle et Rajkumar, 2008

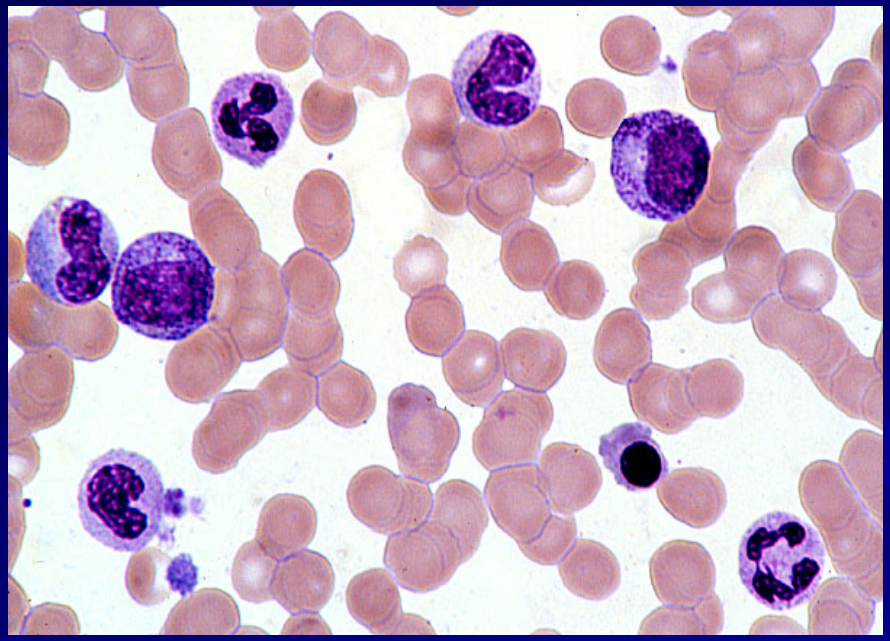
Pathogenesis of MM

- Multistep transformation

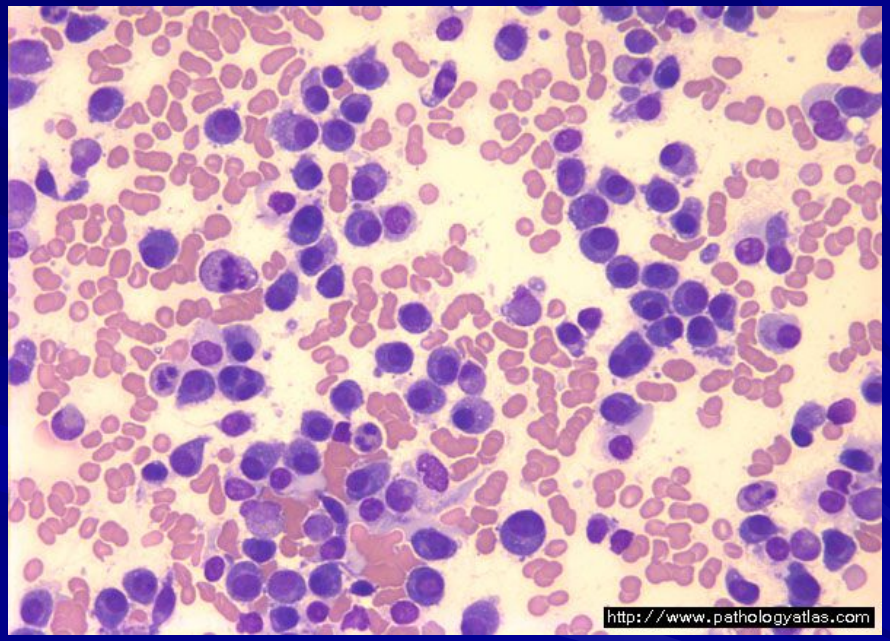


Špička, 2005

Healthy bone marrow



MM bone marrow



www.pathologyatlas.com

Multiple myeloma

the clinician's viewpoint

(doc. Minařík FNOl)

Causes of MM

- Unknown but
  - Decrease of immunity dependent on age
  - Hormonal changes
  - Chemicals
  - Radiation

- → changes leading to unstable genome of PC

Manifestation of MM

- 1) bone marrow:
  - ↓ ery → anemia
  - ↓ leukocytes → decrease of immunity
  - ↓ thrombocytes → bleeding

Manifestation of MM

- 2) osteolytic lesions:
  - Bone pain
  - Weakening of bone structures
  - Spontaneous fractures
  - Calcium increase in serum

Manifestation of MM

- 3) Defects in immunoglobulins - paraprotein:
  - Hyperviscosity
  - Accumulation in blood vessels
  - Decrease in proper function of immunity

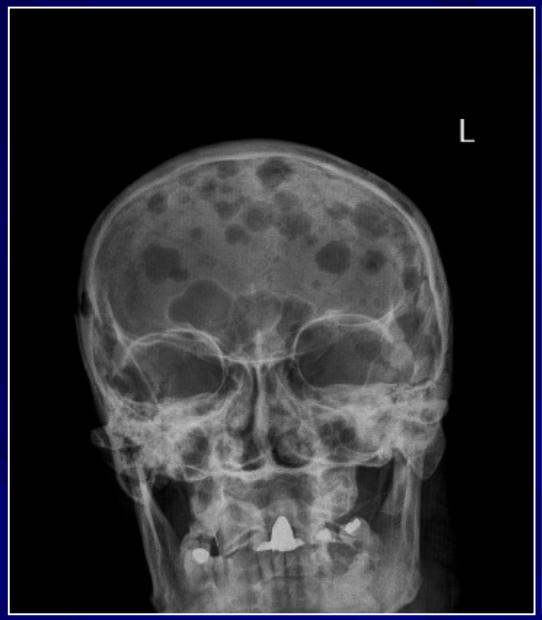
Diagnosis of MM

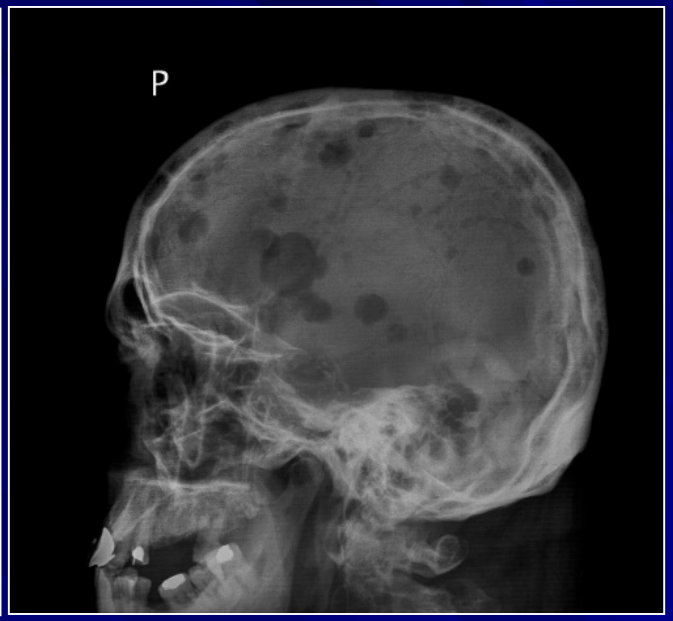
- Difficult, not specific (pain, weakness, repeated infections, tiredness – similar to other diseases)

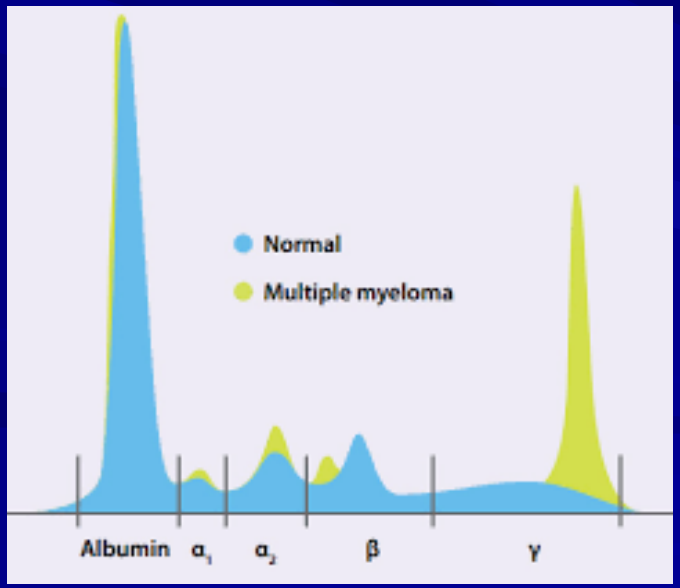
1) Number of MM cells in the BM
2) Presence of abnormal proteins in blood/urine
3) Typical bone changes

Diagnostic methods

- Blood and urine test – electrophoresis of proteins
- Sampling and analysis of bone marrow
- Imaging – X-rays, CT, MRI, PET-CT, bone density


L


P


Normal
Multiple myeloma
Albumin
α1
α2
β
γ

Diagnostic methods - general

- Various indirect parameters:
  - Blood count
  - Calcium levels
  - Kidney function
  - Levels of antibodies
  - Liver function
  - Bone metabolism

Diagnostic methods- special

- Beta-2-macroglobulin
- Cytogenetics
- FACS
- MM cell proliferation
- Antiangiogenic cytokines
- Free light chain ratio
- Albumin
- Staging

Staging and course of disease

- 3 stages of disease
- I-III – level of disease
- A/B kidney function

- Course – individual, varies
- From light form of disease to kidney failure, broken vertebrae, immune deficiency, bleeding….

MM treatment

- Orange peel and opioids
- Chemotherapy
- Transplants
- Immunomodulatory drugs
- Proteasome inhibitors

Hájek, 2012
Anderson, 2011

MM prognosis

- Untreated patients survive 14 months
- Standard therapy – 3-4 years
- Transplants – 6-7 years
- New drugs – 5-year survival for more than 80% of pts

Hájek, 2012

Chemotherapy and transplants

- Treatment protocol Junior – Senior
- Melphalan
  - Alkylating agent
- Prednisone
  - Glucocorticoid – apoptosis of heme cells

- Transplants since 1957
  - Autologous – up to 65, even tandem
  - Allogeneic – in clinical trials

Hájek, 2012
Anderson, 2011

Treatment options in MM

- IMIDs
- Proteasome inhibitors

Treatment options in MM

- IMIDs
- Proteasome inhibitors

Thalidomide

- 1953- Chemie Grünenthal
- 1957- distribution
- Sedative
- Morning sickness
- Teratogenic properties
- Only tested on rats

White House Archive

- About 10 000 children born – only about 40% survived

- FDA - Dr. Francis Kelsey

Sedlaříková, 2012

Thalidomide

- 1964 – Jason Sheskin – leprosy patient
- 1993- Judah Folkman – angiogenesis in hematology
- 1994 – Bart Barlogie - refractory MM, thalidomide
- Clinical study – 84 pts, 1/3 of pts response
- 2006 – FDA – approval
- Unpleasant side effects - neuropathy

Sedlaříková, 2012

IMIDs immunomodulatory drugs

- Analogues of thalidomide – lenalidomide a pomalidomide
- Pleiotropic effect on MM:
  - T-cell co-stimulation
  - Antiangiogenic properties
  - Anti-inflammatory properties
  - Apoptosis and cell cycle progression
  - Inhibition of MM and BM interactions

Sedlaříková, 2012

Treatment options in MM

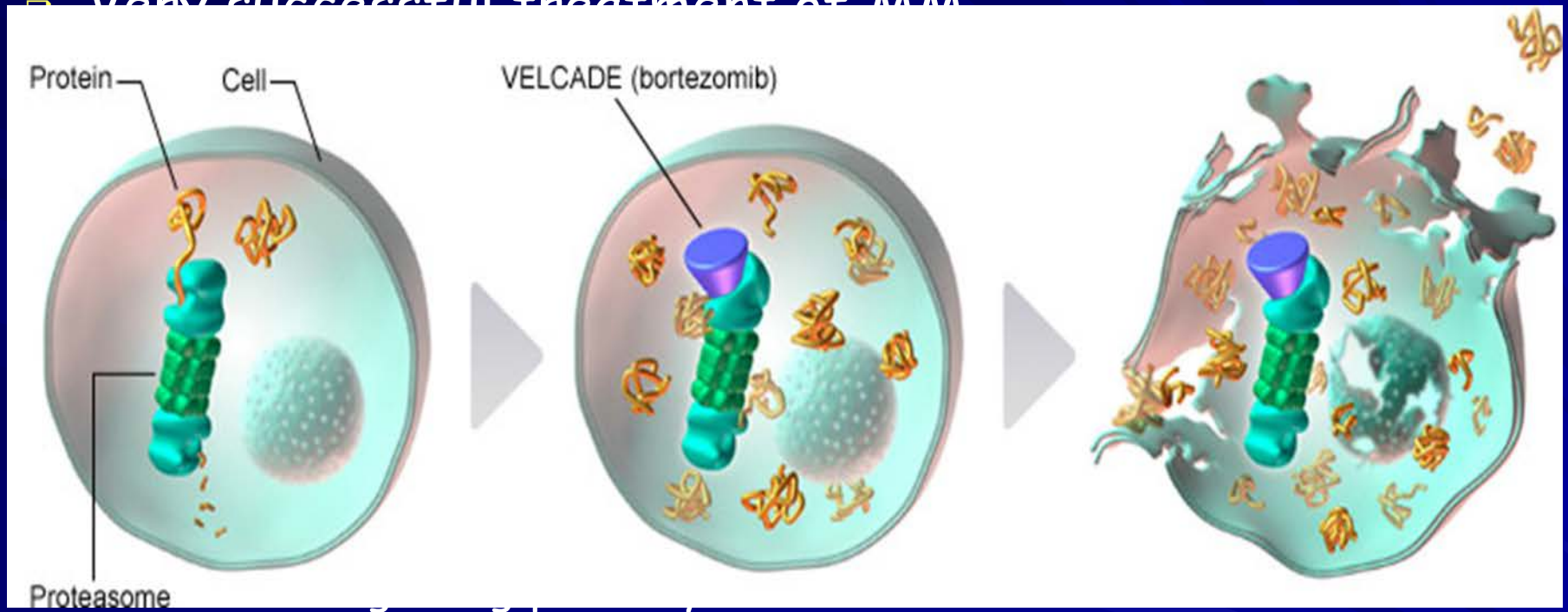
- IMIDs
- Proteasome inhibitors

Treatment options in MM

- IMIDs
- Proteasome inhibitors

Proteasome inhibitors

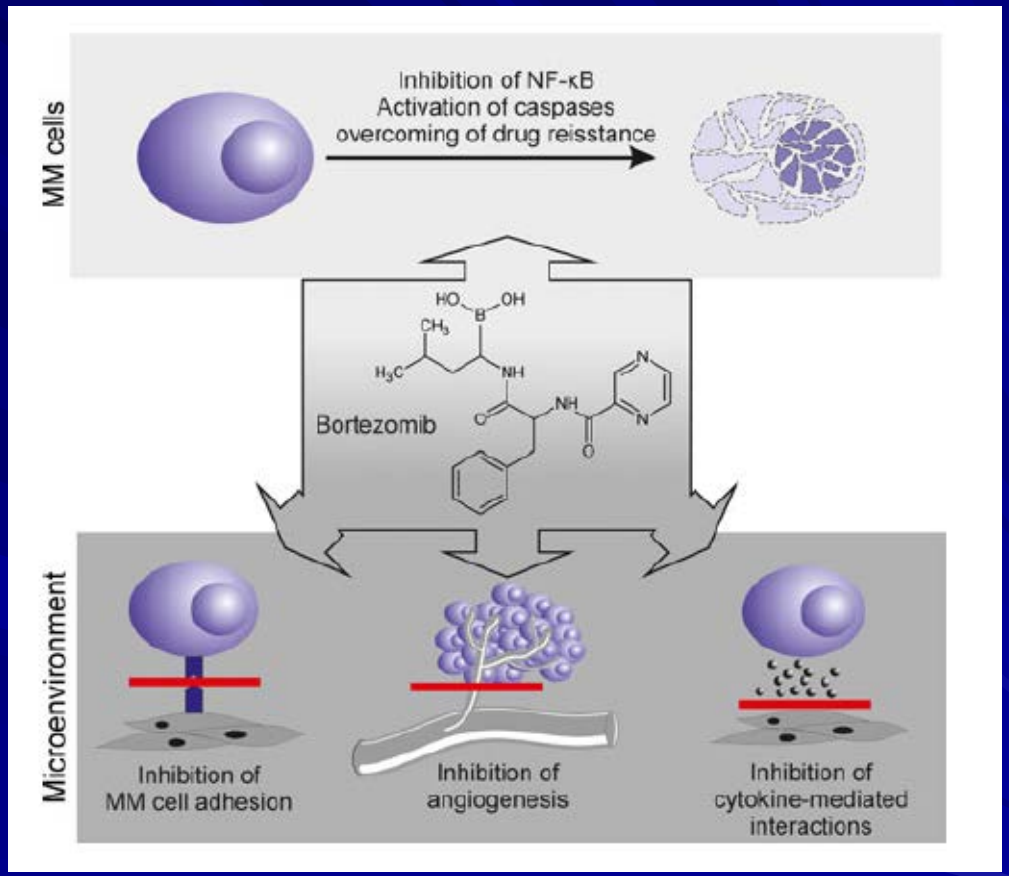
- Very successful treatment of MM



Kubiczková, 2014

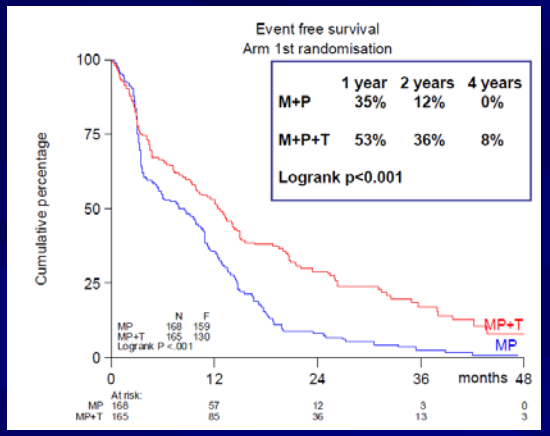
Effect of proteasome inhibitors on BM microenvironment

- Cell signaling – apoptosis
- Inhibit cell adhesion, angiogenesis, interactions

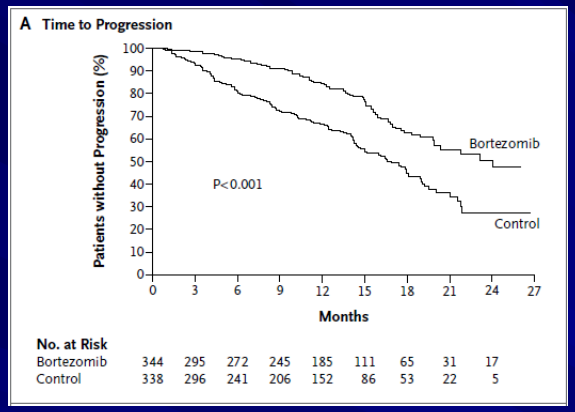


Kubiczková, 2014

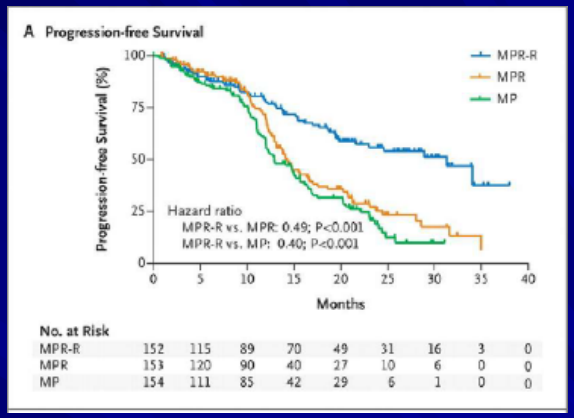
New drugs increase life but do not cure…..yet



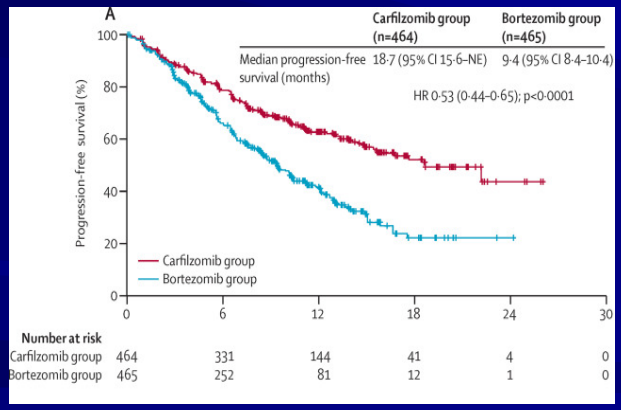
Thalidomid (Myrin)



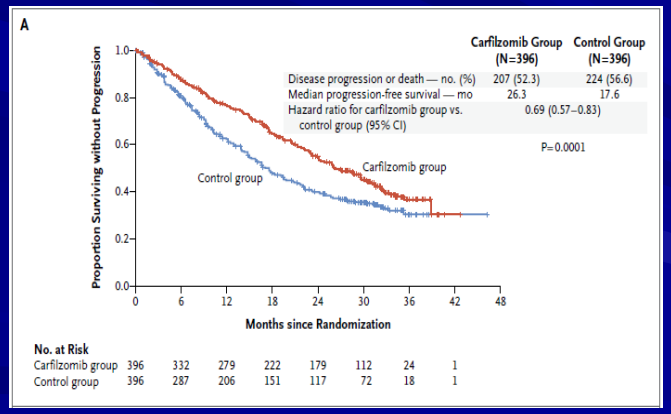
Bortezomib (Velcade)



Lenalidomid (Revlimid)



Carfilzomib > Bortezomib



Carfilzomib+Revlimid> Revlimid

New drugs- new hope

- New proteasome inhibitors:
  - Carfilzomib (Kyprolis)
  - Ixazomib (Ninlaro)
- Monoclonal antibodies:
  - Daratumumab (Darzalex)
  - Elotuzumab (Empliciti)
- Perspective for newly diagnosed:
  - Early diagnostics → early treatment (new criteria 2014)
  - New combinations of drugs

What you can buy for 1 year of treatment:

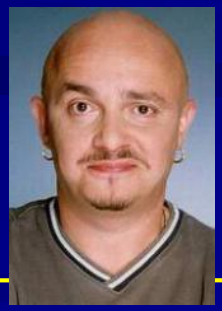
Myrin

Velcade/
Bortezomib

Revlimid

Imnovid

Differences in treatment

	others	MM
Before		
Treatment		
After		

Multiple myeloma

the molecular part

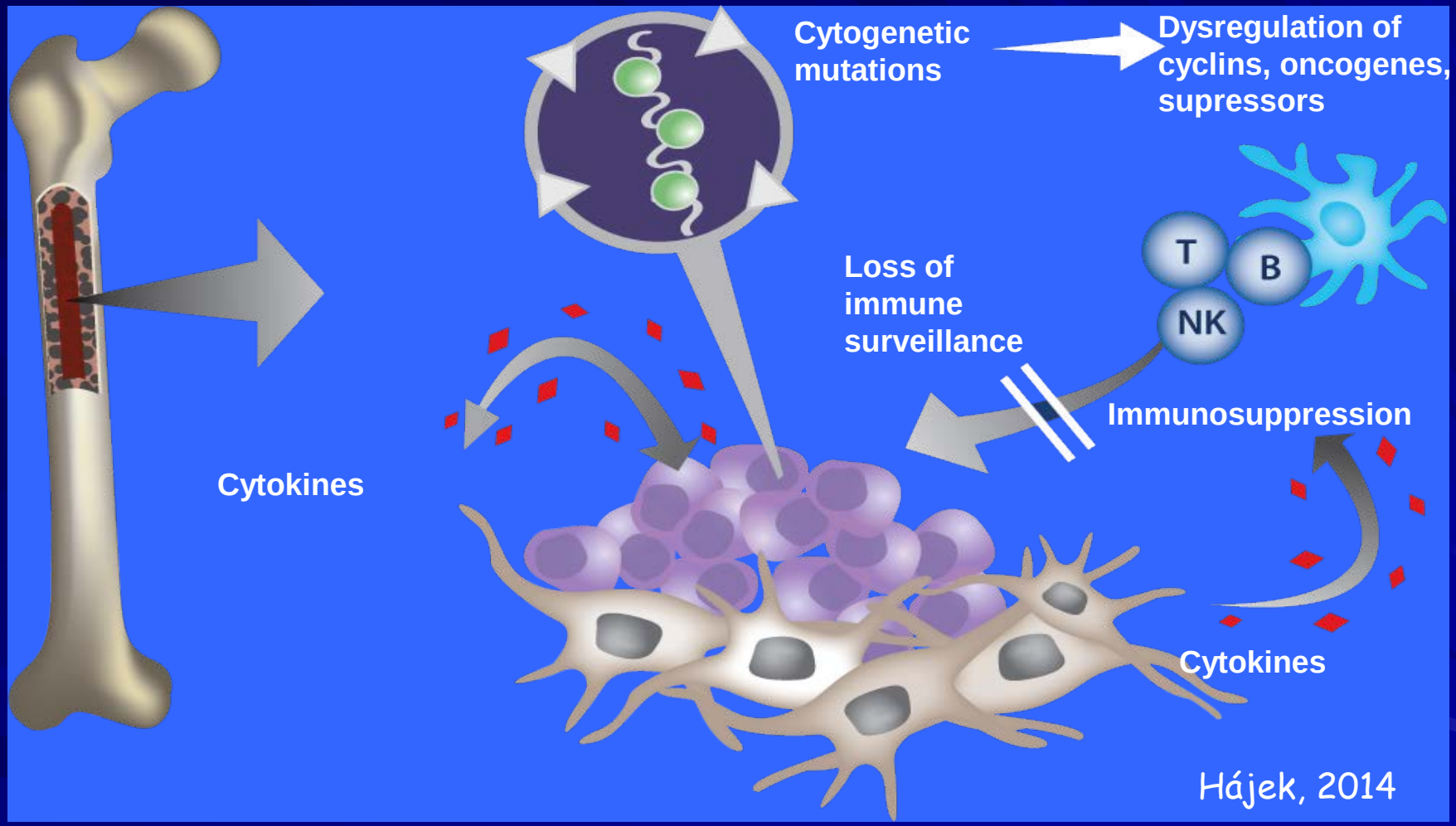
MM pathogenesis

- Unknown
- Genetic predisposition – close relatives - 6 times risk
- Pesticides, herbicides, radiation

- Mutations in PC
- Changes in BM microenvironment – PC growth

Hájek, 2012
Anderson, 2011

MM pathogenesis



Flowcytometry

- MM diagnosis - $CD19^{-}CD56^{+}CD38^{+}CD138^{+}$
- Risk of progression from MGUS to MM
- Detection of MRD – higher PFS and OS in MRD^{-} pts

Hájek, 2012
Anderson, 2011
Říhová, 2013

Cytogenetic aberrations in MM

- Unstable genome – deletions, amplifications translocations
- Changes accumulate in time
- Numerical and structural changes of chromosomes
- Aneuploidy of odd chromosomes and translocation of IgH locus

Palumbo, 2013
Anderson, 2011
Kuglík, 2012
Němec, 2012

Numerical aberrations in MM

- Non-hyperdiploid (48 < >74) x hyperdiploid (48-74)
- Hyperdiploid:
  - Trisomies of 3,5,7,9,11,15,19,21 – better prognosis
- Non-hyperdiploid:
  - Monosomies of 8,13,14,16,17,22

Palumbo, 2013
Anderson, 2011
Kuglík, 2012
Němec, 2012

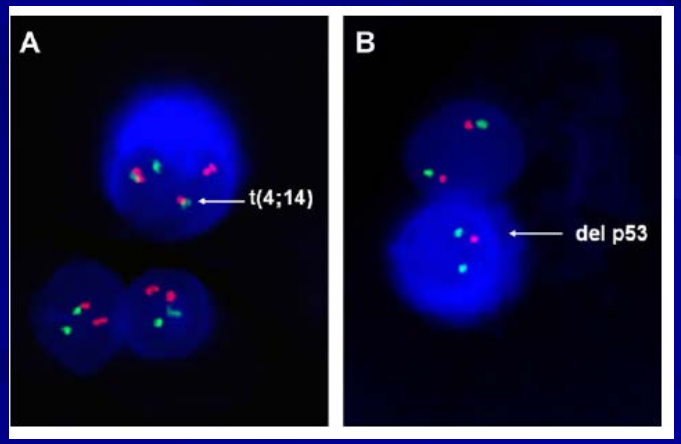
Structural aberrations

- Translocations of locus 14q32 (IgH)
- Primary changes:
  - t(11;14) 15-20% cyclin D1
  - t(4;14) 10-15% FGFR3/MMSET
  - t(14;16) 2-10% c-MAF
  - t(6;14) 5% cyclin D3
- Secondary changes:
  - Complex karyotypes - MYC
  - Deletion or duplication of 1q21
  - Deletion or monosomy of chr 13
  - Deletion of chr 17 – deletion of TP53

Palumbo, 2013
Anderson, 2011
Kuglík, 2012
Němec, 2012

FISH

- Most MM cells do not cycle – classical cytogenetics basically useless
- i-FISH – much better results
- Prognosis – t(4;14), t(14;16), t(14;20) and del (17p) – bad prognosis



Kuglík, 2012
Němec, 2012
Bešše, 2015

Diagnostics

- Bone marrow biopsies
- Painful, unethical to repeat too often
- New marker? Liquid biopsies?

Why liquid biopsies?

Limitations of classic biopsies

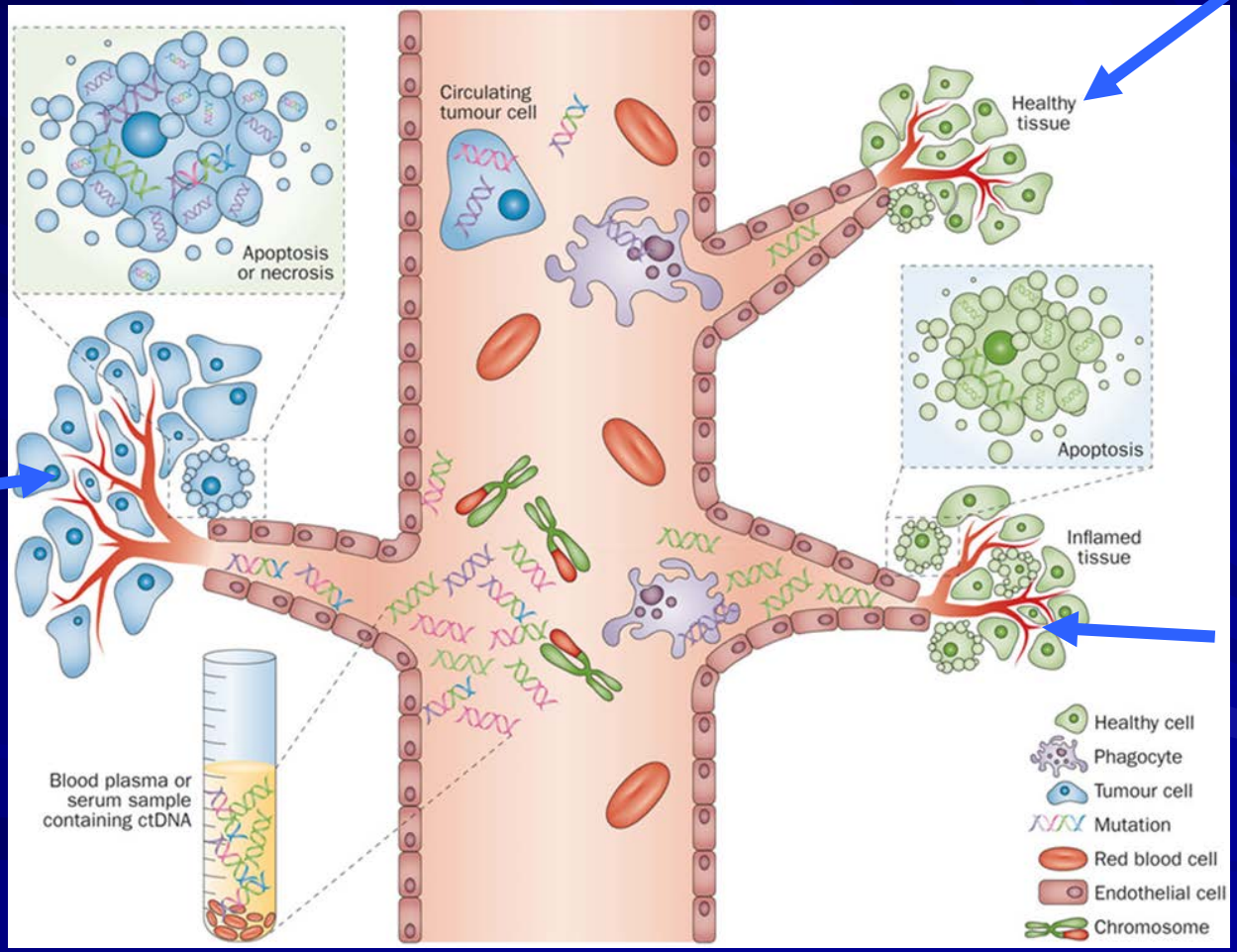
- Invasive
- Painful
- One site of tumor – heterogeneity not represented
- In MM – presence of subclones in focal lesions

Liquid biopsies

- Biopsies of PB
- Detection of circulating tumor cells
- Detection of circulating nucleic acids
- Easier sampling
- Entire heterogeneity of the tumor

Liquid biopsies

healthy tissue



tumor

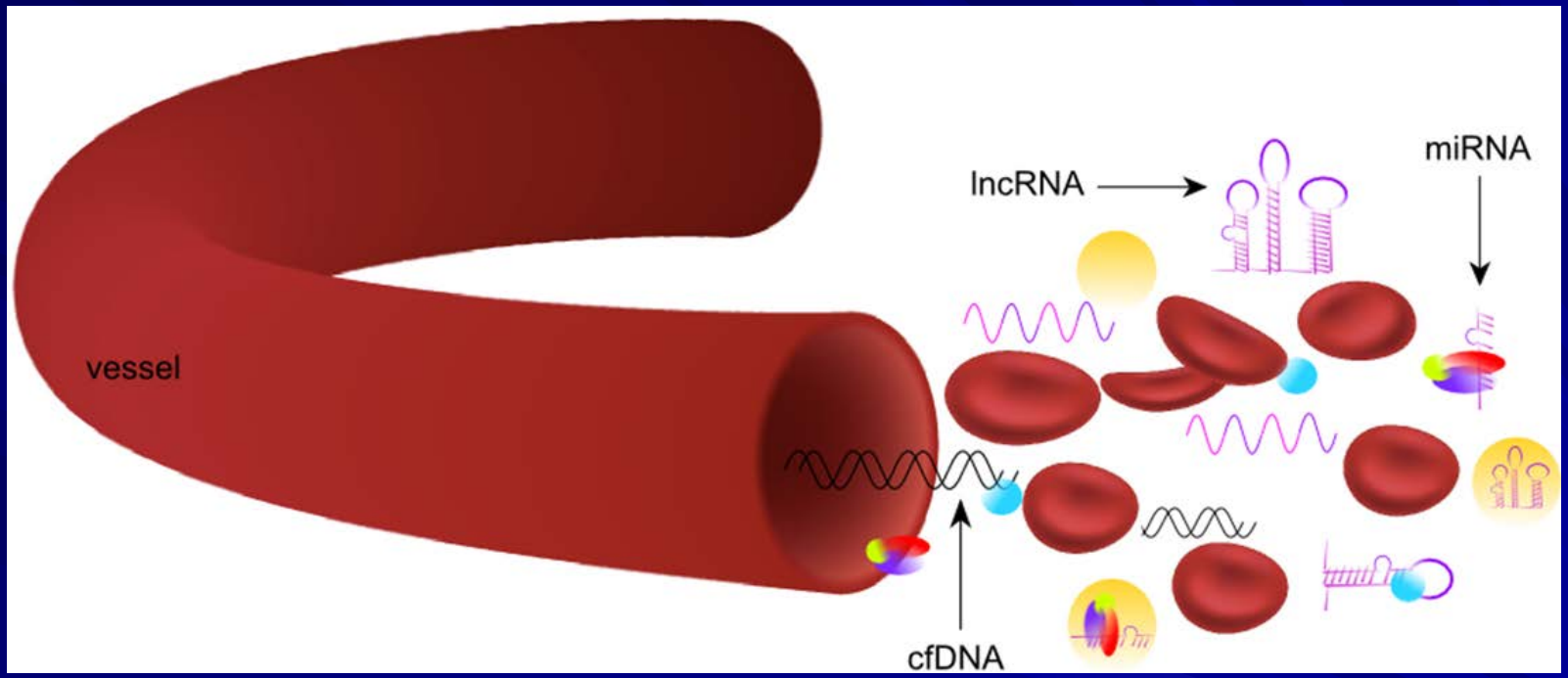
inflammation

Crowley et al 2013

Circulating PC (cPC)

- Prognostic marker
- Loss of dependence on BM microenvironment
- Changes of adhesion molecules, chemokines, aberrations
- Faster progression MGUS to MM
- Higher BM infiltration in MM
- Negative prognostic marker in refractory MM
- 5-20% cPC – worse survival regardless of age
- > 5% cPC – prognosis like PCL

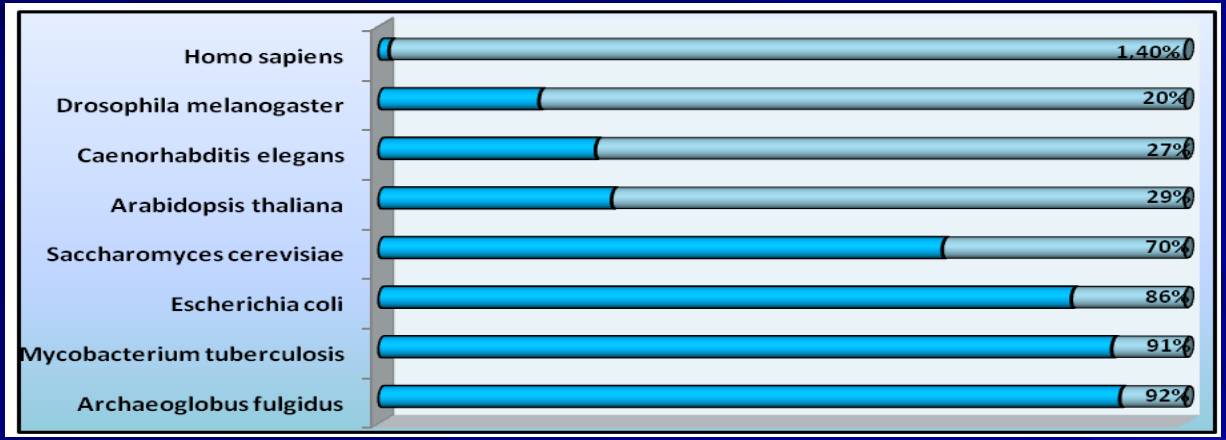
Which molecules?



KO suplementum 2017

Non-coding RNAs

- Less than 1.5% of human genome codes for proteins
- More than 90% is transcribed
- Most common non coding RNAs : rRNA, tRNA

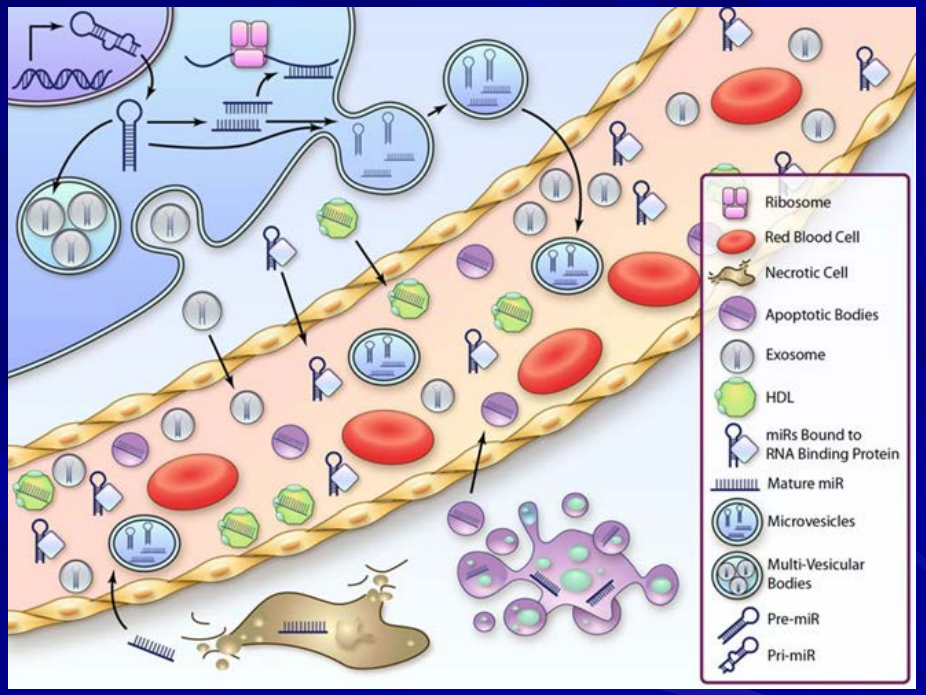


(Sana, 2012)

- Basic division:
  - Short ncRNA < **200 bp** < long ncRNA

Circulating ncRNA

- Body fluids
- Stable and resistant to RNAses
- Easily accessible
- Cell communications
- Diagnostics
- Relapse monitoring
- MRD monitoring



http://circresearch.com/gallery/tag/circulating-mirna/

New markers for MM

- MicroRNA
- Cell-free DNA

New markers for MM

- MicroRNA
- Cell-free DNA

microRNA

- Short noncoding RNAs
- 20-22 nt long
- Post-transcriptional regulation of gene expression
- Physiological processes (proliferation, differentiation)
- Tumorigenesis

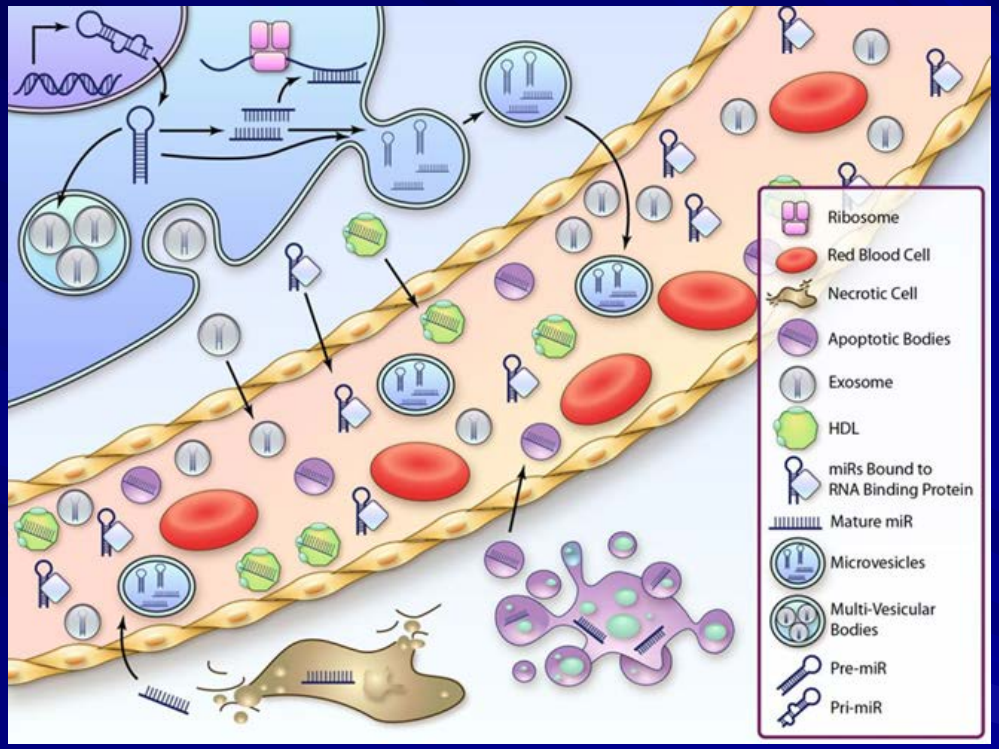
Lee, 1993
Calin, 2002

History of miRNA

- Identified in 1993 in *C. elegans*
- Lin-4 – larval stages of *C. elegans*
- About 1/3 of human genes regulated by miRNA
- About 2200 of human miRNA
- miR-15a and miR-16 identified in region 13q14 – potential oncogenes in CLL

Lee, 1993
Calin, 2002

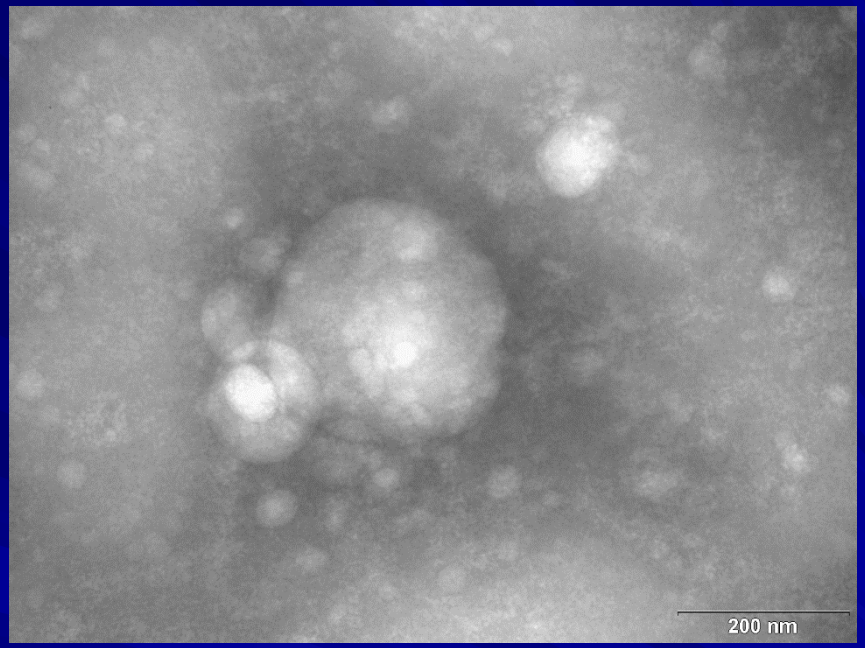
Circulating miRNA



http://circresearch.com/gallery/tag/circulating-mirna/

Exosomes

- 50-140 nm vesicles
- Proteins, NA
- Active secretion from cells
- Change gene expression, signaling in cells
- Remove chemo from cells actively
- Support tuorigenesis – miRNA transport



Our pilot study

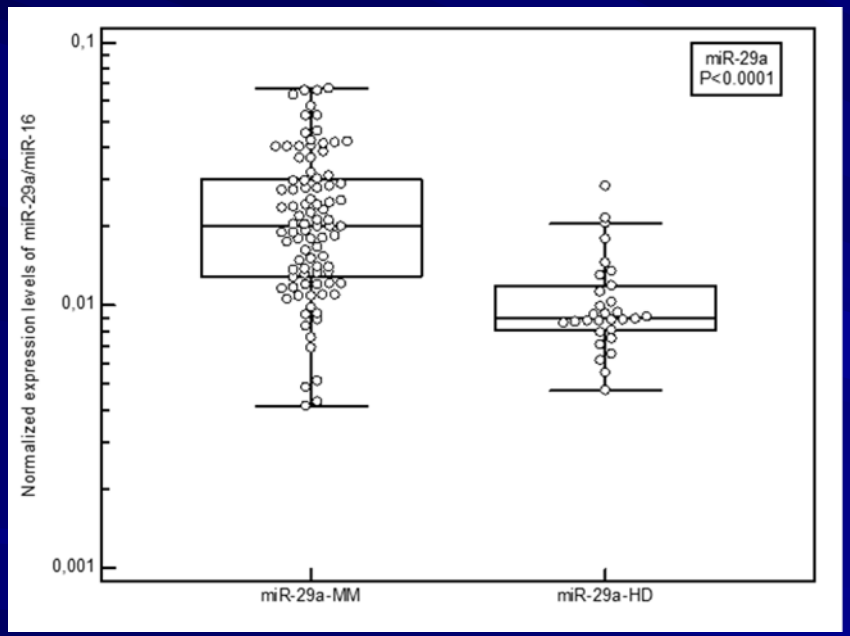
- 4 miRNA chosen based on MM pathogenesis
- Their presence analyzed in serum of PB

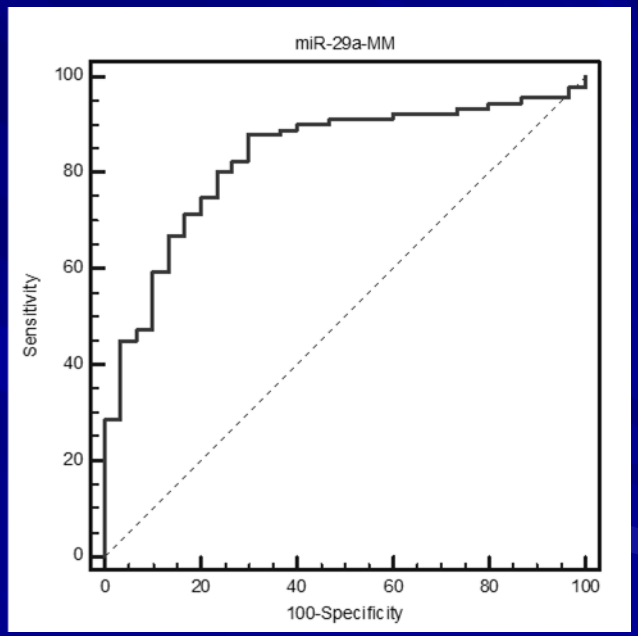
  - miR-410 – locus 14q32 – MM translocation
  - miR-660 – aberrant expression in MM
  - miR-142-5p – aberrant expression in MGUS and MM
  - miR-29a – increased expression in PC

Ševčíková, 2012

Results of pilot study

- miR-29a increased in MM
- Specificity 70%, sensitivity 88%, AUC=0.832





Ševčíková, 2012

Circulating miRNA as MG markers

- MM, MGUS and HD
- 103 MM at diagnosis
- 18 MM at relapse
- 57 MGUS
- 30 HD

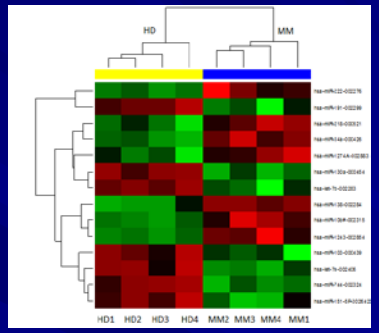
Kubiczková, 2014

Methods

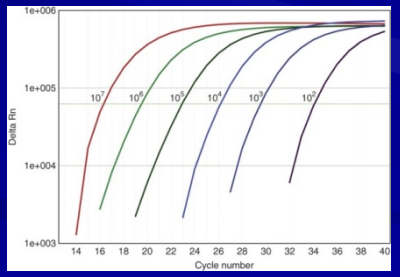
miRNA isolation

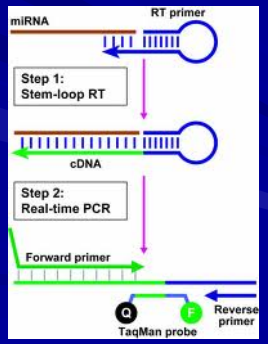
TaqMan Low Density Arrays

Differential expression analysis

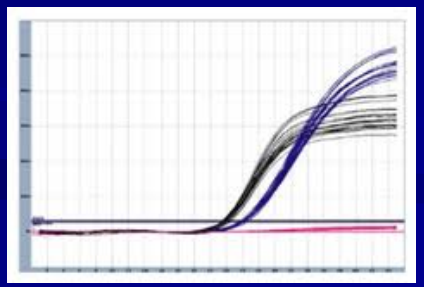


qPCR
Specific TaqMan miRNA assays





Data analysis



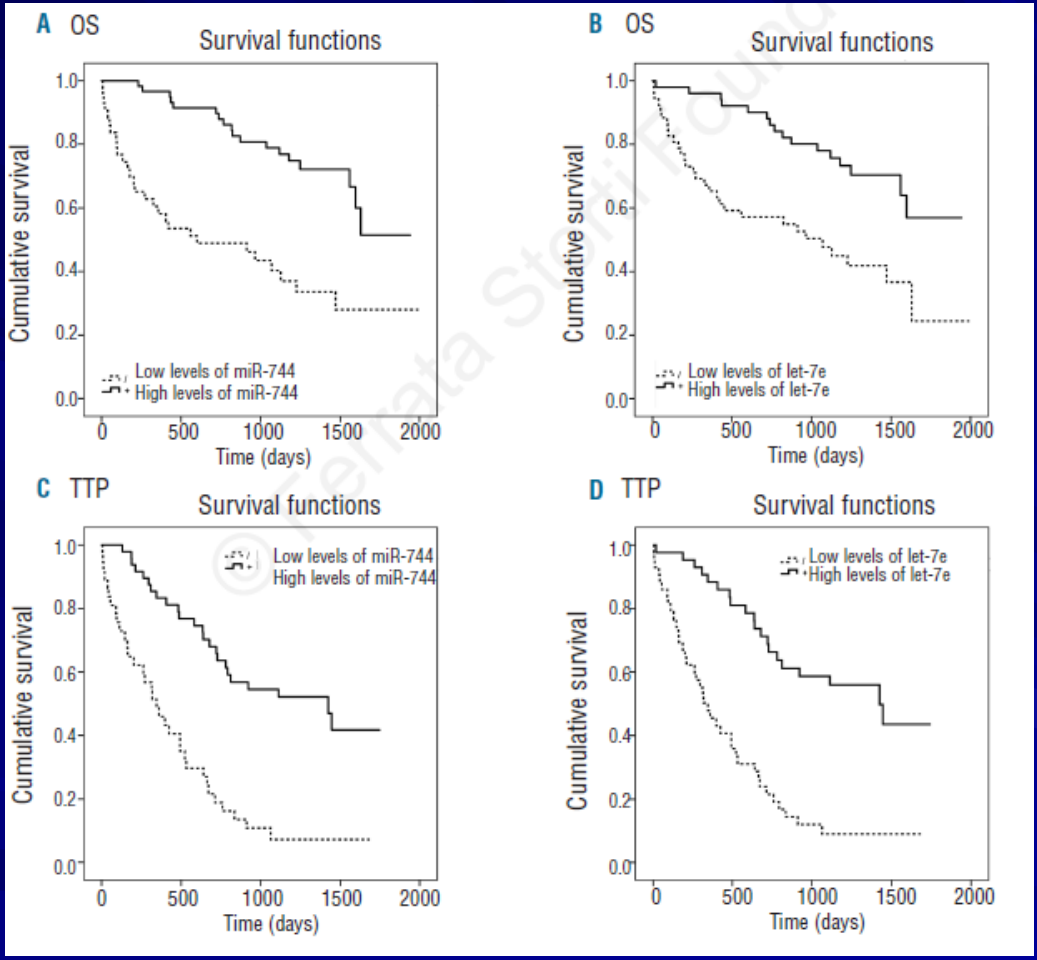
Correlation with clinically important parameters

Circulating miRNA as MG markers

- miR-744, miR-130a, miR-34a, let-7d, let-7e deregulated in MG vs HD
- Combination of miR-34a and let-7e: MM vs HD vs MGUS
- No correlation with PC in BM – other pathological changes in MM?

Kubiczková, 2014

Low levels of miR-744 and let-7e - shorter OS



Kubiczková, 2014

New markers for MM

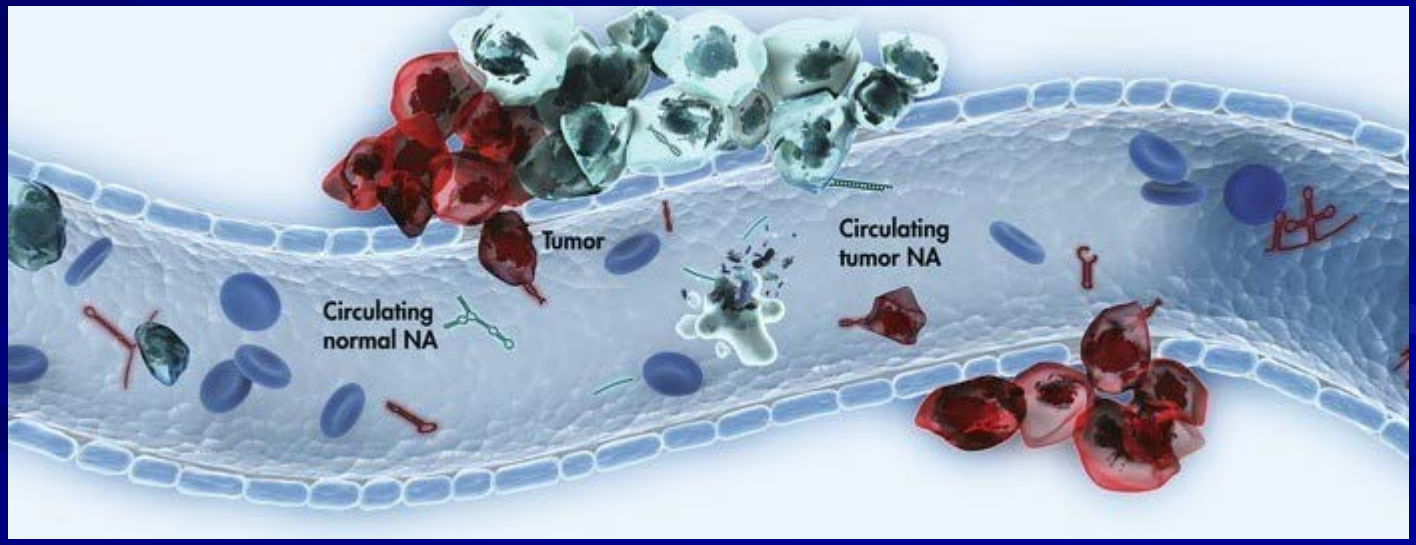
- MicroRNA
- Cell-free DNA

Cell-free DNA

- Short fragments of DNA (180 bp) in PB
- First described in 1949 (Mandel et Métais)
- 1977 – described in tumor patients (Leon et al)
  - Higher levels in pts than controls
  - Higher levels in metastases
  - Lower levels after radiotherapy
- 1994 – cfDNA carrying RAS mutation in MDS
- Used in prenatal diagnostics

cfDNA levels

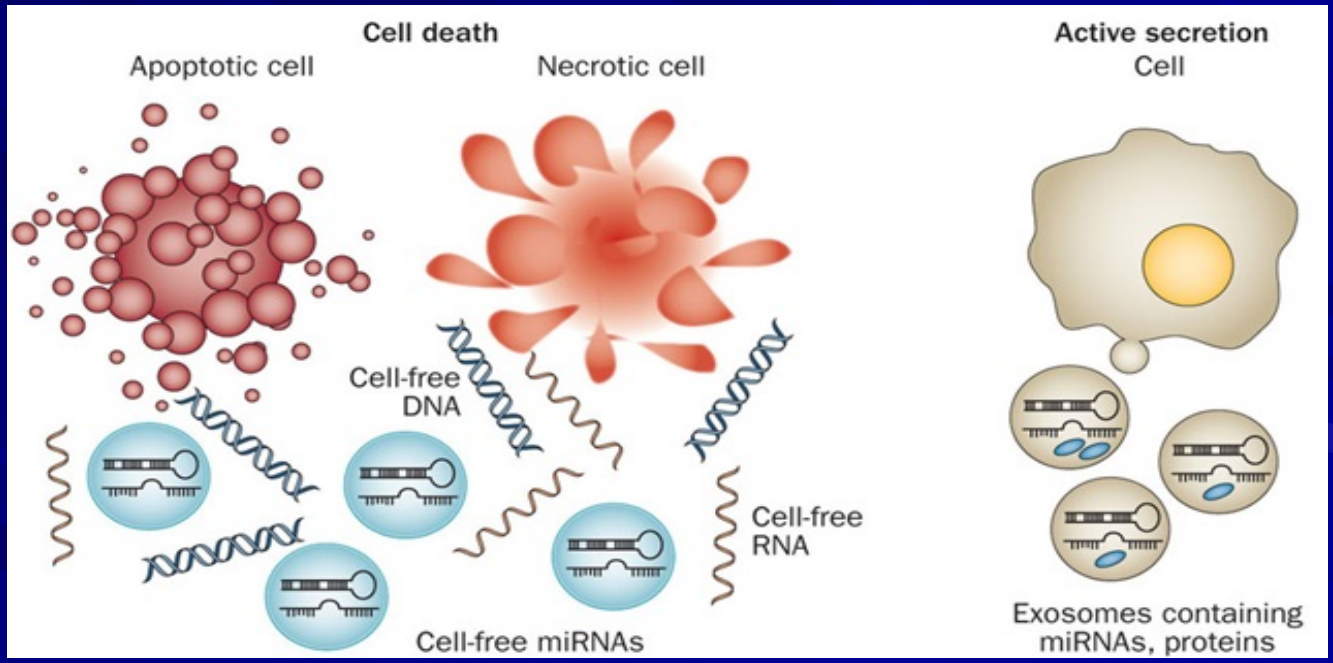
- Physiologically low (10-100 ng/ml)
- Change of quantity and quality in pathology
- Higher levels (1000 ng/ml) in tumors, inflammations – but not diagnostic



http://biomarkerinsights.qiagen.com/2016/08/17

How are cfDNA released from cells?

- Apoptosis
- Necrosis (tumor cells)
- Active release (cell signaling)



Schwarzenbach et al 2014

Our cfDNA project

- Detect a patient specific VDJ rearrangement of the IgH locus in BM
- Check the rearrangement in cfDNA
- Follow the dynamics of the molecules after treatment

- Analyzed 85 pts, follow up up to 2 years after start of treatment

Summary - cfDNA

- cfDNA carry various reaarangments
- Possible to follow MRD in MM?

Summary

- MM disease of older people
- New drugs dramatically improved survival
- Need more specific easily accessible markers
- Several possibilities – miRNA, cfDNA, lncRNA…
- Improvement in diagnostics and follow-up of patients

Liquid biopsies in the future?

- PB biopsies – all heterogeneity of the tumor
- Less painful
- Several molecules
- Great potential

Acknowledgments

Babákova myelomová skupina,
Ústav patologické fyziologie, Lékařská fakulta, MU

Sabina Ševčíková

Veronika Kubaczková
Lenka Sedlaříková
Božena Bollová
Martina Valachová
Jana Gregorová
Veronika Kelbichová
Kamila Novosadová

Oddělení klinické hematologie,
Fakultní nemocnice Brno

Miroslav Penka

Martina Almáši
Renata Bezděková
Božena Hanáková
Lucie Říhová
Barbora Sáblíková
Renata Suská
Pavla Všianská

Interní hematologická a onkologická klinika
Fakultní nemocnice Brno
Zdeněk Adam
Marta Krejčí
Luděk Pour
Viera Sandecká
Martin Štork

Institut biostatistiky a analýz
LF MU
Jiří Jarkovský
Lucie Brožová

Oddělení klinické hematologie
Fakultní nemocnice Ostrava
Roman Hájek
Tomáš Jelínek
Fedor Kryukov
Elena Kryukova
Zuzana Kufová

Laboratoř molekulární cytogenetiky
Ústav experimentální biologie, PřF MU
Petr Kuglík

Aneta Mikulášová
Jan Smetana
Markéta Wayhelová

Ústav patologické fyziologie
Lékařská fakulta, MU
Anna Vašků

Thanks for your attention