Role of genetic abnormalities in monoclonal gammopathy of undetermined significance pathogenesis

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Faculty of Medicine, Masaryk University

MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS)

Permanently higher risk of malignant disorder development

MGUS consistently precedes MM

Landgren, O. et al. 2009. Blood 113(22): 5412-5417.
Weiss, B. M. et al. 2009. Blood 113(22): 5418-5422.

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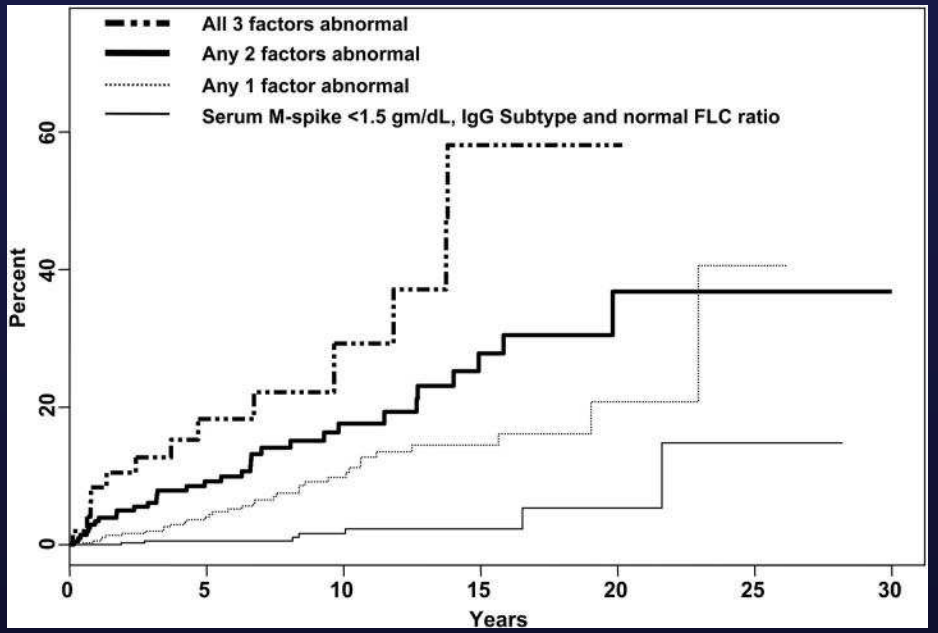
Prognostic factors progression from MGUS to MM

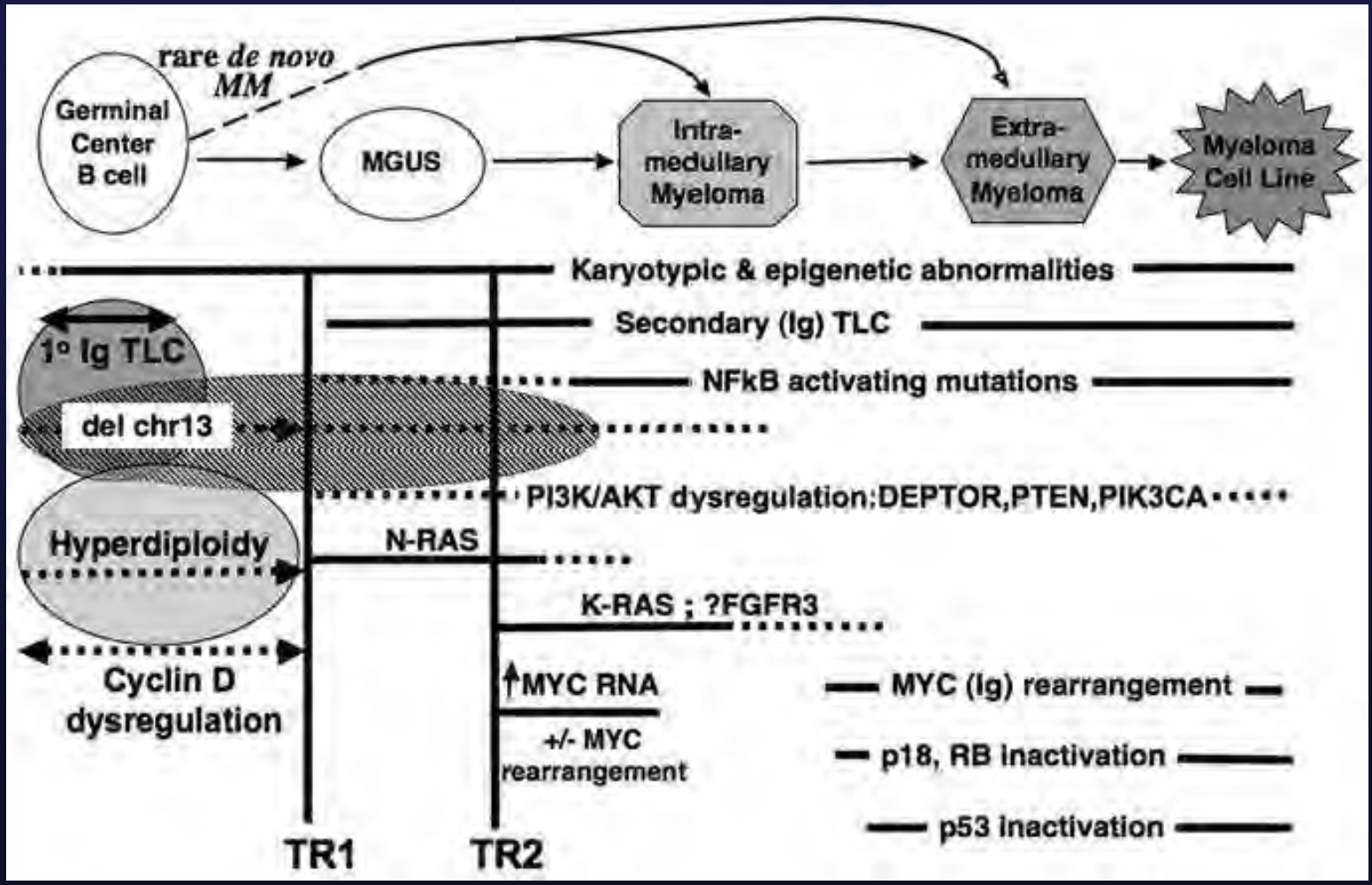
- **Serum M-Ig subtype**
  (IgG, IgA, IgM, IgD, IgE)
- **Serum M-Ig size**
  (<15g/l, >15g/l)
- **Serum free light chain ratio**
  (FLC ratio)
- **BMPCs infiltration**
  (<5%, >5%)
- **Flow cytometry analysis**
  (95%<aPCs/BMPCs<95%)



- **Chromosomal abnormalities**

Cesana, C. et al. 2002. J. Clin. Oncol. 20(6): 1625-1634.
Kyle, R. A. et al. 2004. Mayo Clin. Proc. 79(7): 859-866.
Pérez-Persona, E. et al. Blood 110(7): 2586-2592.
Rajkumar, S. V. et al. 2005. Blood 106(3): 812-817.

Molecular pathogenesis of MGUS and MM

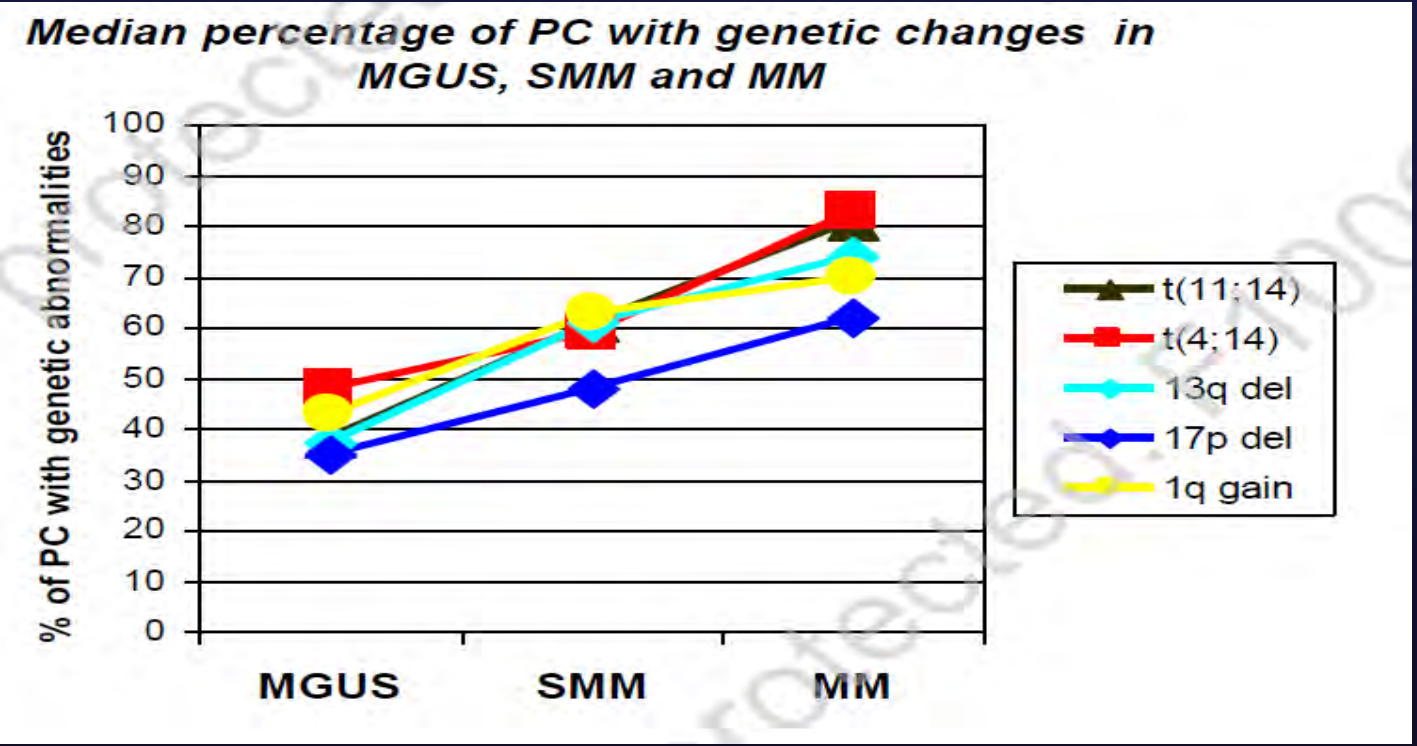


Zingone, A., Kuehl, W. M. 2011. Semin. Hematol. 48(1): 4-12.

Chromosomal abnormalities

- progression from MGUS to MM involves a clonal expansion of genetically abnormal PCs:

  median proportion of PCs with *IGH* translocations and del(13)(q14) is significantly lower in MGUS than in MM (34 % vs. 76 %; 37 % vs. 74 %)



López-Corral, L. et al. 2011. Clin. Cancer Res. 17(7): 1692-1700.

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Our results – iFISH analysis

60 MGUS, 36M/24F, age median 64 years, follow-up median 26 months

Chromosomal abnormality	Frequency	% cytogen. abnormal PCs
del(13)(q14) (*RB1*)	21 % (11/52)	71 % (26 % – 92 %)
del(17)(p13) (*TP53*)	2 % (1/52)	37 % (37 % – 37 %)
disruption 14q32 (*IGH*)	71 % (31/44)	55 % (22 % – 94 %)
t(11;14)(q13;q32) (*CCND1/IGH*)	50 % (2/4)	60 % (31 % – 88 %)
t(4;14)(p16.3;q32) (*FGFR3/IGH*)	10 % (4/39)	35 % (30 % – 96 %)
t(14;16)(q32;q23) (*IGH/MAF*)	6 % (1/16)	64 % (64 % – 64 %)
gain 1q21 (*CKS1B*)	13 % (6/48)	62 % (21 % – 93 %)
hyperdiploidy	15 % (7/47)	49 % (25 % – 86 %)

New project - IGA MZČR NT/13492

Project title:

Role of genetic abnormalities in development and progression of precancerosis monoclonal gammopathy of undetermined significance

Recipient:	Faculty of Science, Masaryk University
Co-Recipient:	University Hospital Brno
Project duration:	1. 4. 2012 – 31. 12. 2015

Aims

1. **Set up and optimize whole genome screening of genetic abnormalities of MGUS** patients using AgilentSurePrint G3 CGH+SNP platform.

2. **Analyze potential genetic markers of MGUS transformation** based on comparison of whole genome screening between groups of MGUS patients and MM patients.

3. **Confirm presence and clarify significance of known genetic abnormalities** in MGUS (deletion of *RB1* and *TP53*, *IGH* translocation, gain 1q21) and focus on study of significance of *C-MYC* and *RAS* genes (or other genes based on whole genome screening) by molecular cytogenetic (FISH, aCGH) as well as expression levels (RT-PCR).

4. **Analyze genome of phenotypically normal PCs population.**

Procedure

serum

bone marrow

clinical and biochemical tests

flow cytometry

FACS

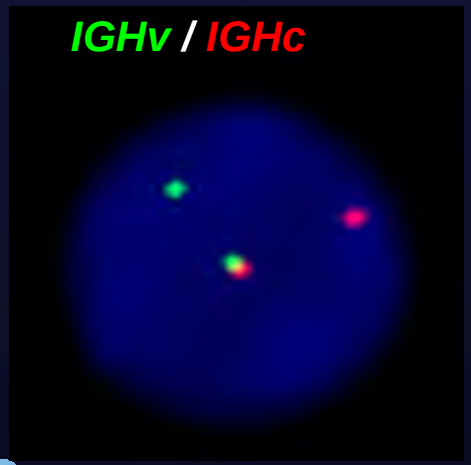
microscopic slides

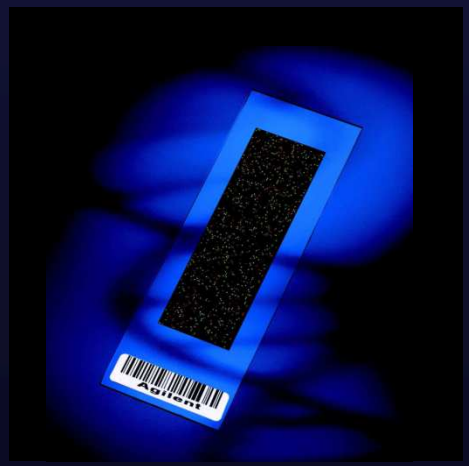
cell pellets

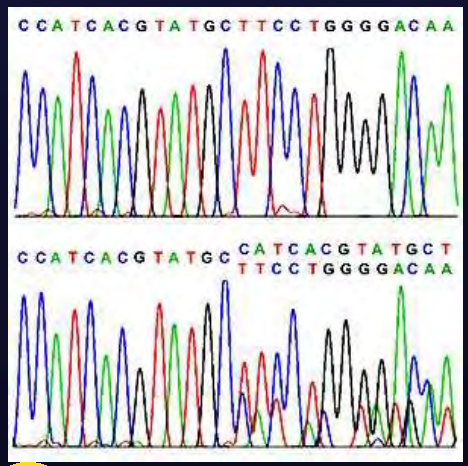
iFISH

WGA - aCGH

RT-PCR, sequencing







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Time schedule 1

Year	Number of individuals
2012	20 MGUS (+ 10-15 archived samples)
2013	25 – 30 MGUS
2014	25 – 30 MGUS
2015	10 – 20 MGUS
Total	90 – 110 MGUS

Time schedule 2

First year:

We plan to **optimize CGH+SNP microarray** method and perform gene expression experiments on chosen samples.

Second and third year

We plan to supplement results of aCGH by **I-FISH** of chosen ***IGH* translocations** and translocations involving ***C-MYC*** as well as expression data of chosen genes (C-MYC and ***RAS***). Whenever possible, we will analyze **genotype of normal PCs population** isolated from MGUS patients.

Fourth year:

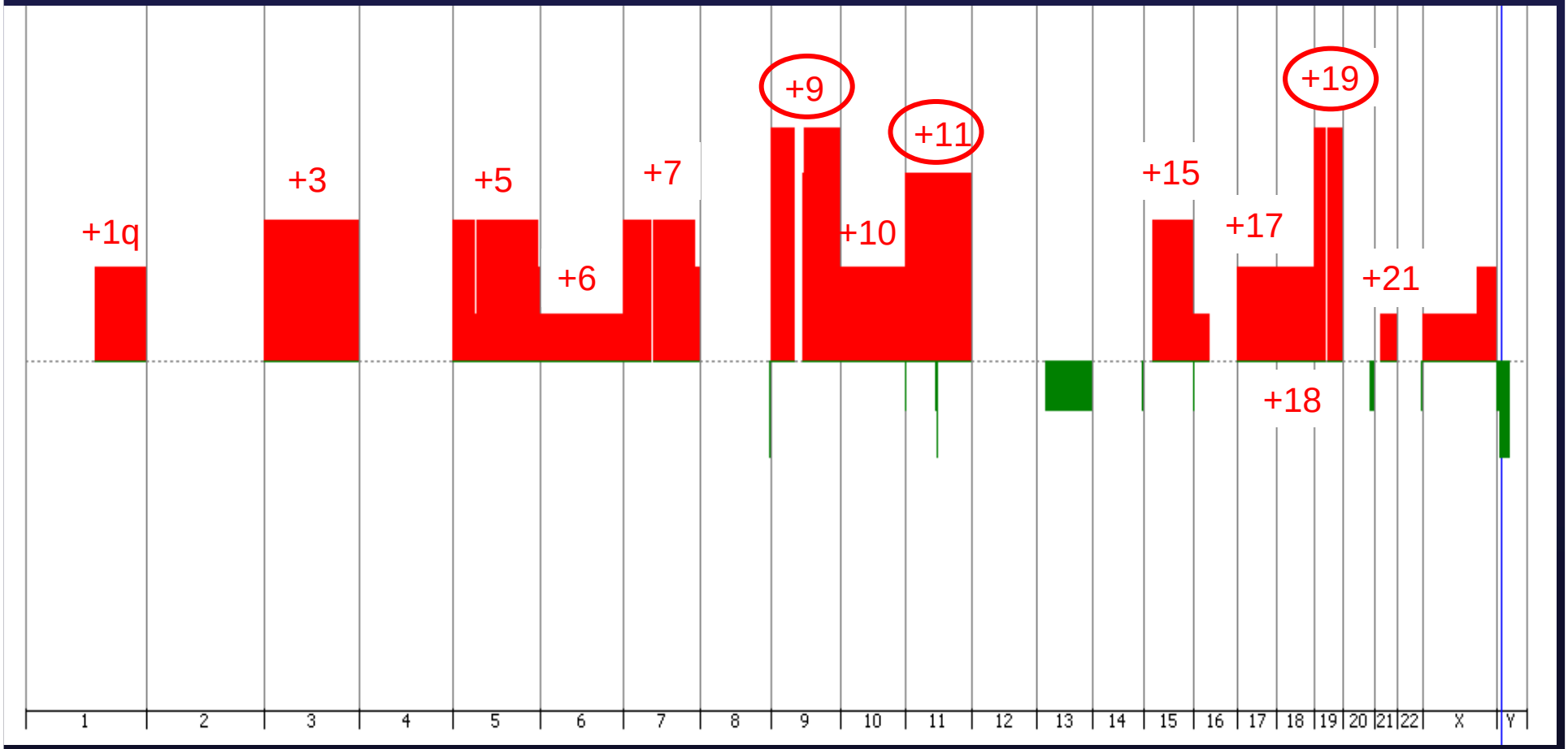
We will evaluate genetic background of this precancerosis and significance of individual abnormalities in abnormal and normal populations in connection to MGUS transformation. At the end of this project, we will analyze all results of our molecular cytogenetic experiments, we will concentrate on **correlations with clinical, biochemical, flow-cytometric and other characteristics of patients**, we will perform summary statistical analyses and we will suggest an **identification algorithm of high-risk MGUS patients** for transformation into MM.

First results – aCGH analysis

- 12× array-CGH (Agilent Human Genome CGH Microarray Kit, 4x44K)
- 4× MGUS – PCs (CD138+)
- 8× MGUS – aPCs (CD138+CD19-CD56+/-)

	Purity	Number of cells	Amount of DNA
PCs	97,5 % (93,2 % - 99,3 %)	$0{,}175\times10^6$ ($0{,}110\times10^6$ - $0{,}300\times10^6$)	739 ng (262 ng - 805 ng)
aPCs	96,8 % (93,3 % - 99,3 %)	$0{,}031\times10^6$ ($0{,}010\times10^6$ - $0{,}160\times10^6$)	59,5 ng (15,6 ng - 1150 ng)

Hyperdiploid MGUS – 6/12 (50 %)



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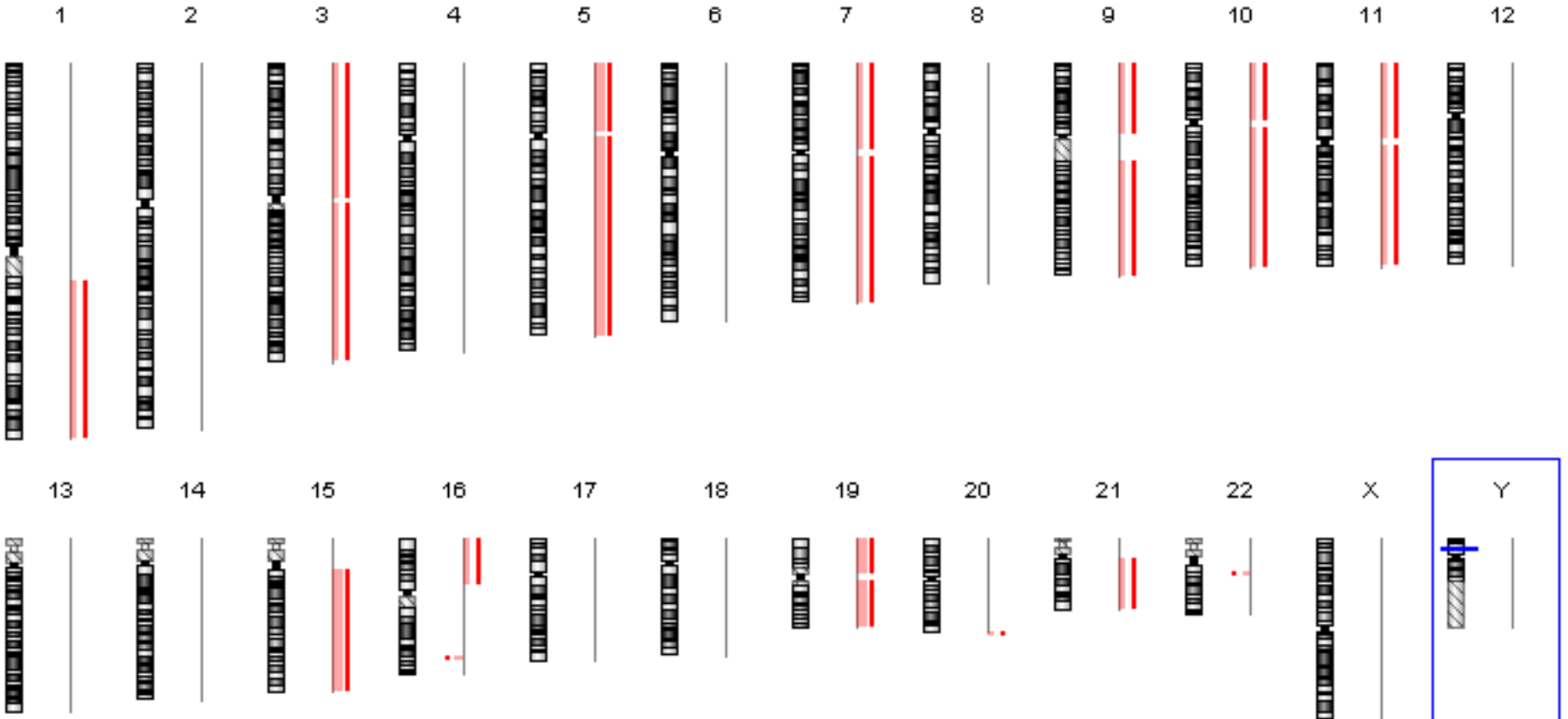
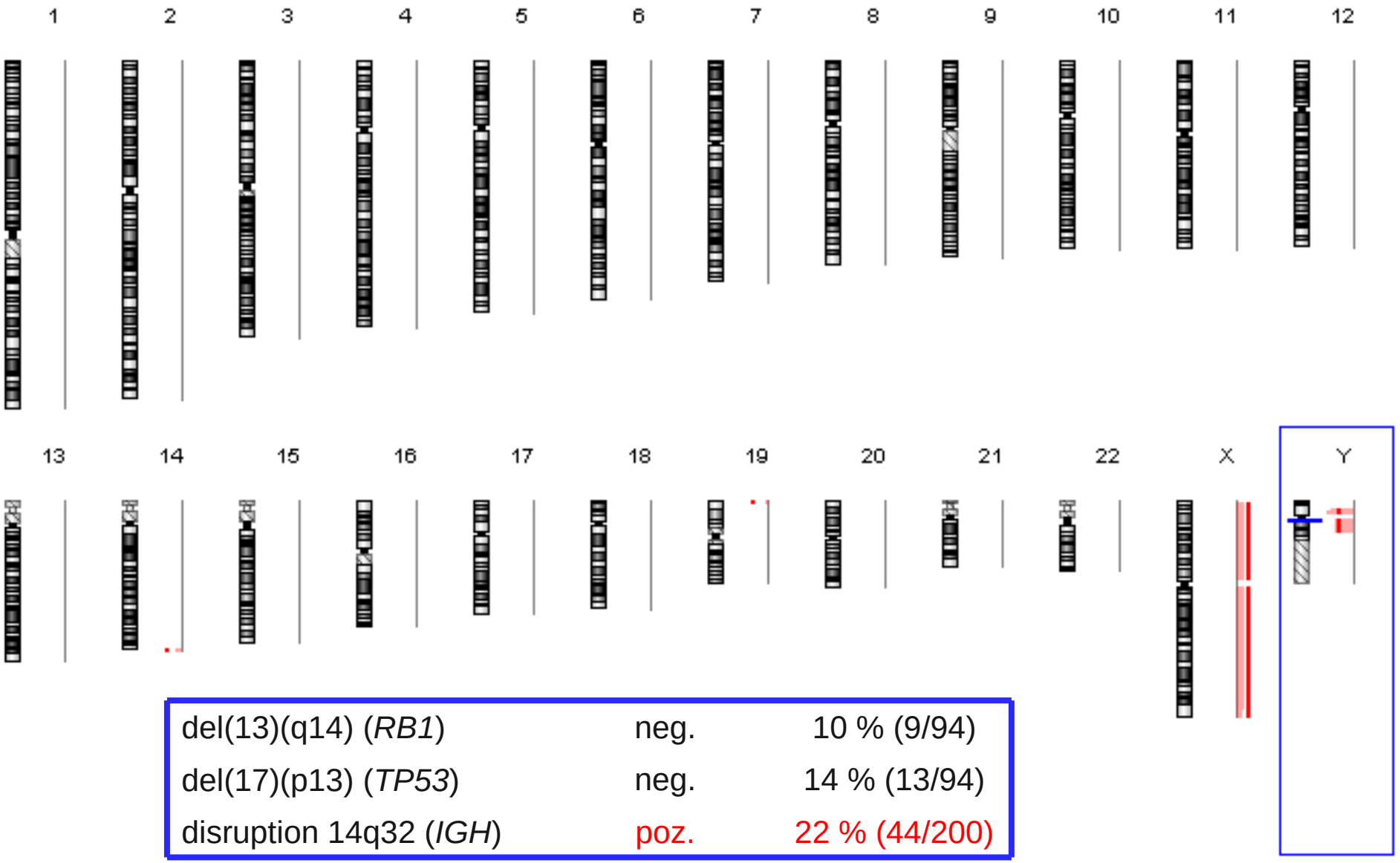
MGUS 1



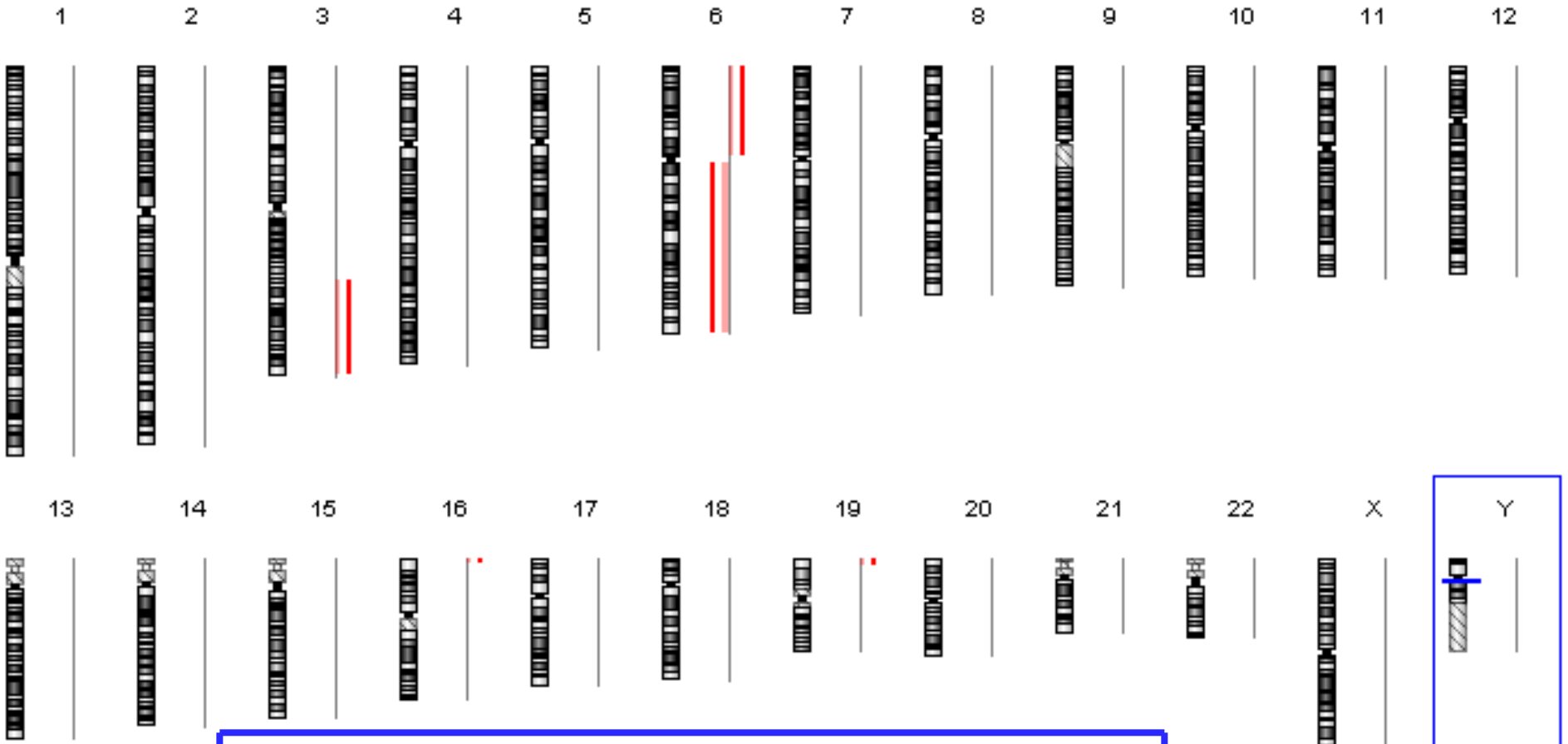
del(13)(q14) (*RB1*)	neg.	4 % (4/100)
del(17)(p13) (*TP53*)	neg.	9 % (9/100)
disruption 14q32 (*IGH*)	neg.	11 % (11/100)
gain 1q21 (*CKS1B*)	poz.	91 % (91/100)
hyperdiploidy	H (+5, +9, +15)	86 % (86/100)

MGUS 15



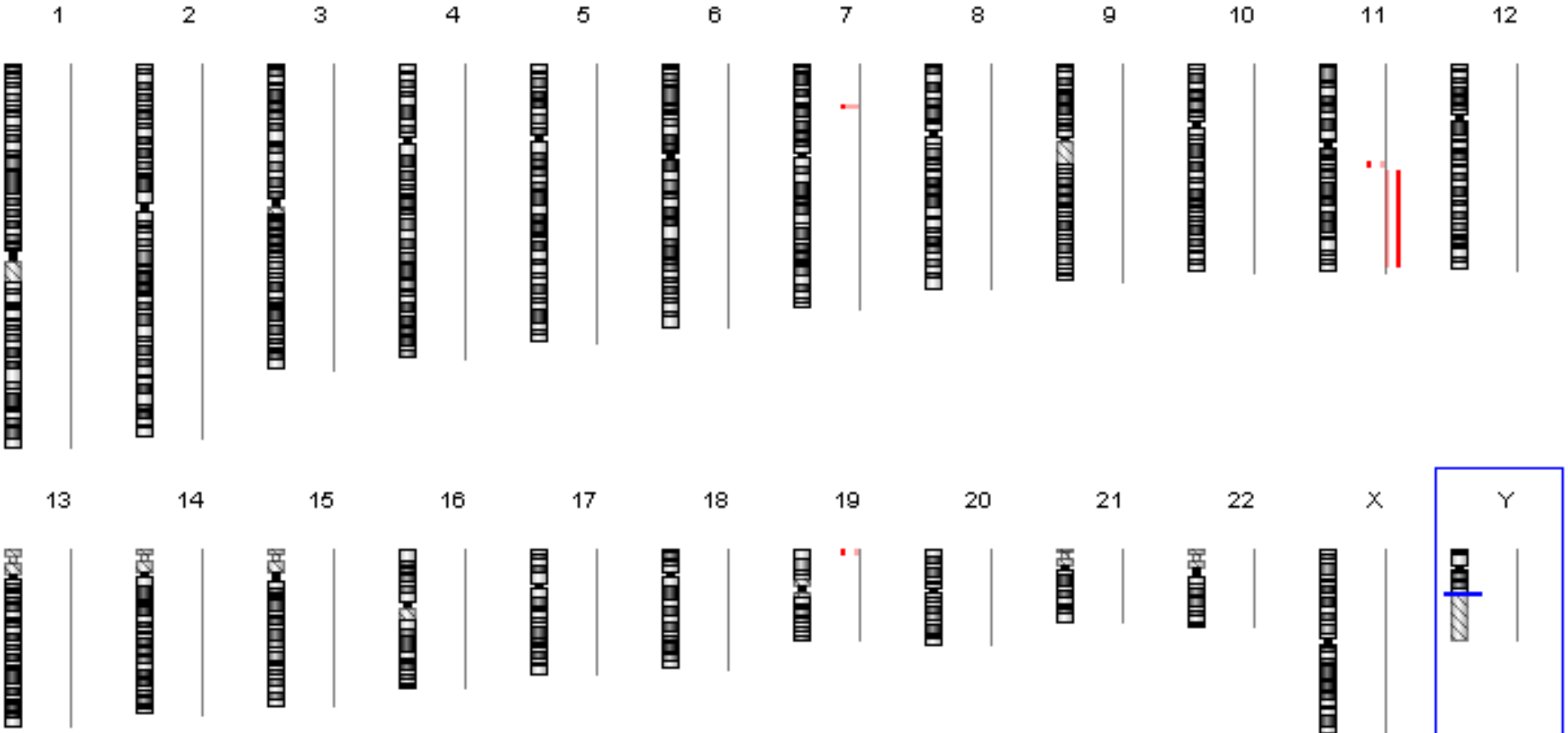
del(13)(q14) (*RB1*)	neg.	10 % (9/94)
del(17)(p13) (*TP53*)	neg.	14 % (13/94)
disruption 14q32 (*IGH*)	poz.	22 % (44/200)

MGUS 4



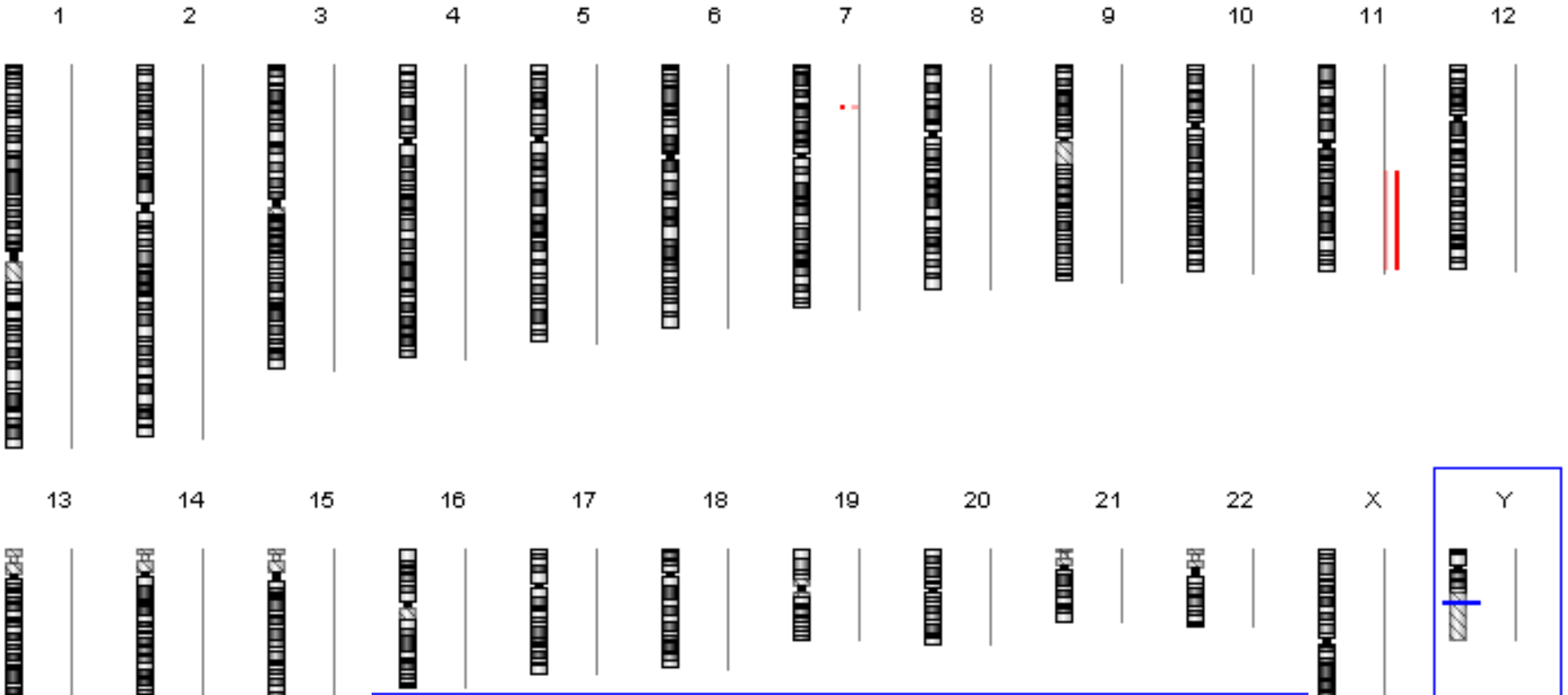
del(13)(q14) (*RB1*)	neg.	2 % (2/100)
del(17)(p13) (*TP53*)	neg.	3 % (3/100)
disruption 14q32 (*IGH*)	neg.	5 % (5/100)
gain 1q21 (*CKS1B*)	neg.	5 % (5/100)
hyperdiploidy	NH	2 % (2/100)

MGUS 8



disruption 14q32 (*IGH*)	poz.	62 % (62/100)
t(4;14)	neg.	2 % (2/100)
t(14;16)	neg.	5 % (5/100)

MGUS 9



del(13)(q14) (*RB1*)	neg.	8 % (8/100)
del(17)(p13) (*TP53*)	neg.	2 % (2/100)
disruption 14q32 (*IGH*)	poz.	94 % (94/100)
t(11;14)	poz.	88 % (88/100)
gain 1q21 (*CKS1B*)	neg.	0 % (0/85)
hyperdiploidy	NH	(100 bb)

Conclusions

- MGUS research is complicated ~ small amount of PCs for analyses
- We have optimized whole genome amplification and following array-CGH in MGUS, thus array-CGH from small amount of DNA
- Array-CGH profile is different between MGUS individuals, but MGUS cohort is too small for making any conclusions.
- We plan to:
- Investigate a large group of MGUS individuals by array-CGH
- Focus on specific genes that could potentially be related to the MGUS transformation
- Correlate cytogenetic data with clinical, biochemical, flow-cytometric and other characteristics

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