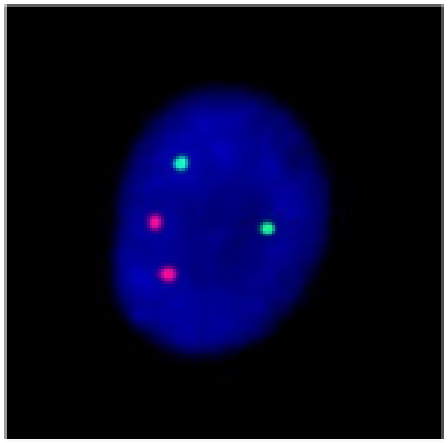
다발성 골수종(Multiple Myeloma)의 세포유전 검사

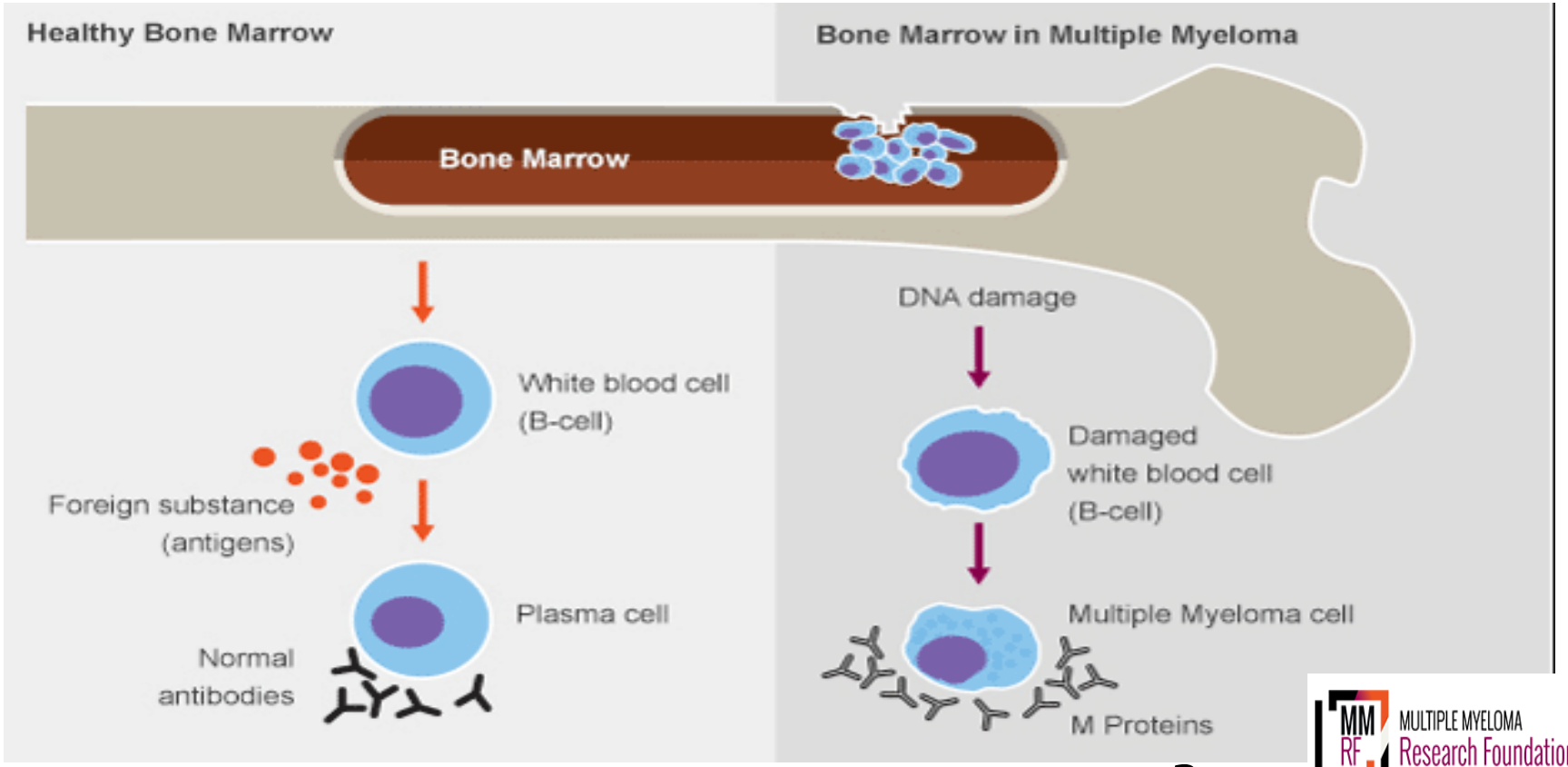


2017.6.14

세포유전검사실
함 명 희

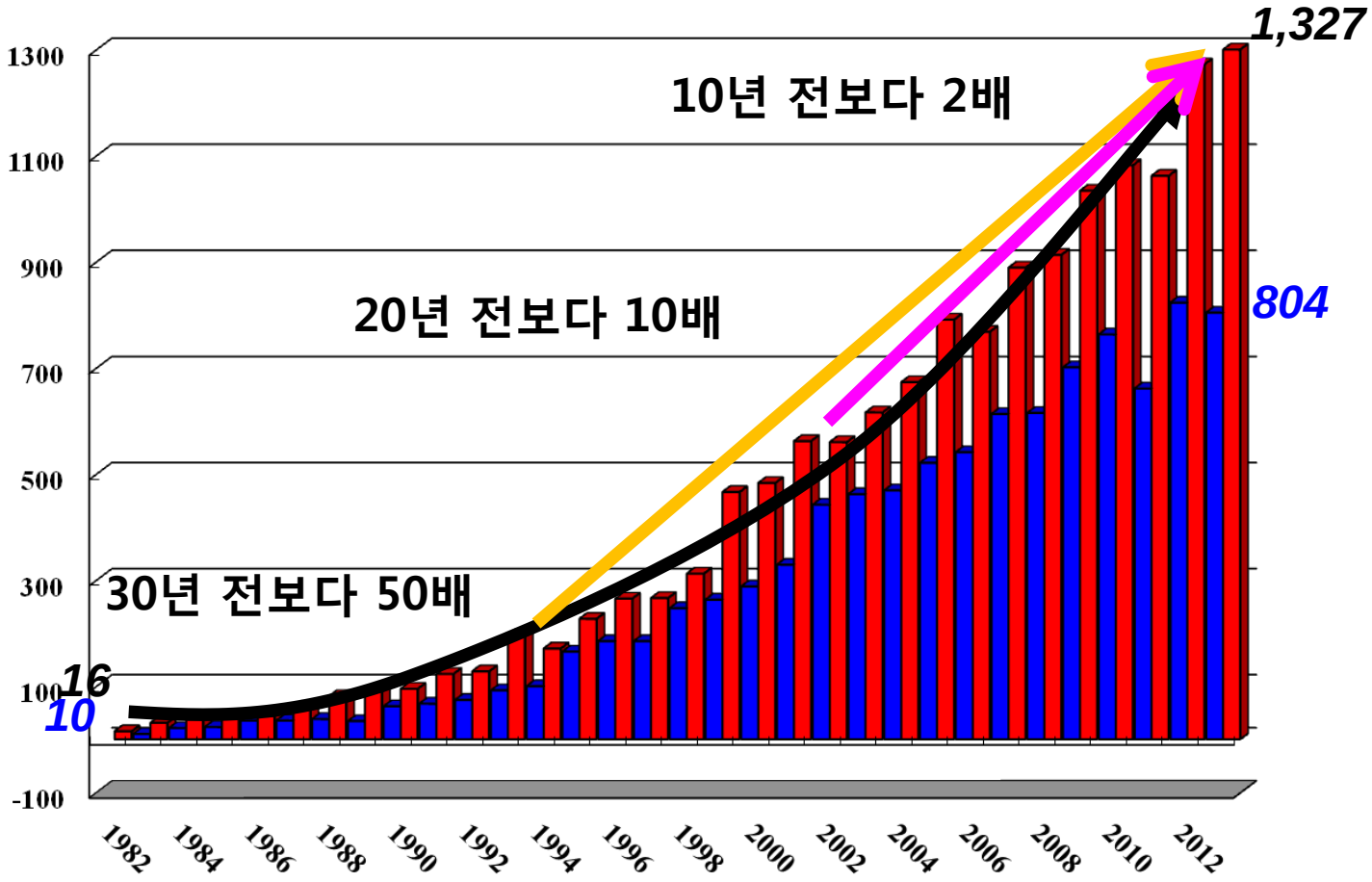
Multiple Myeloma?

Multiple Myeloma is a malignant neoplasm of plasma cells that accumulate in bone marrow, leading to bone destruction and marrow failure.

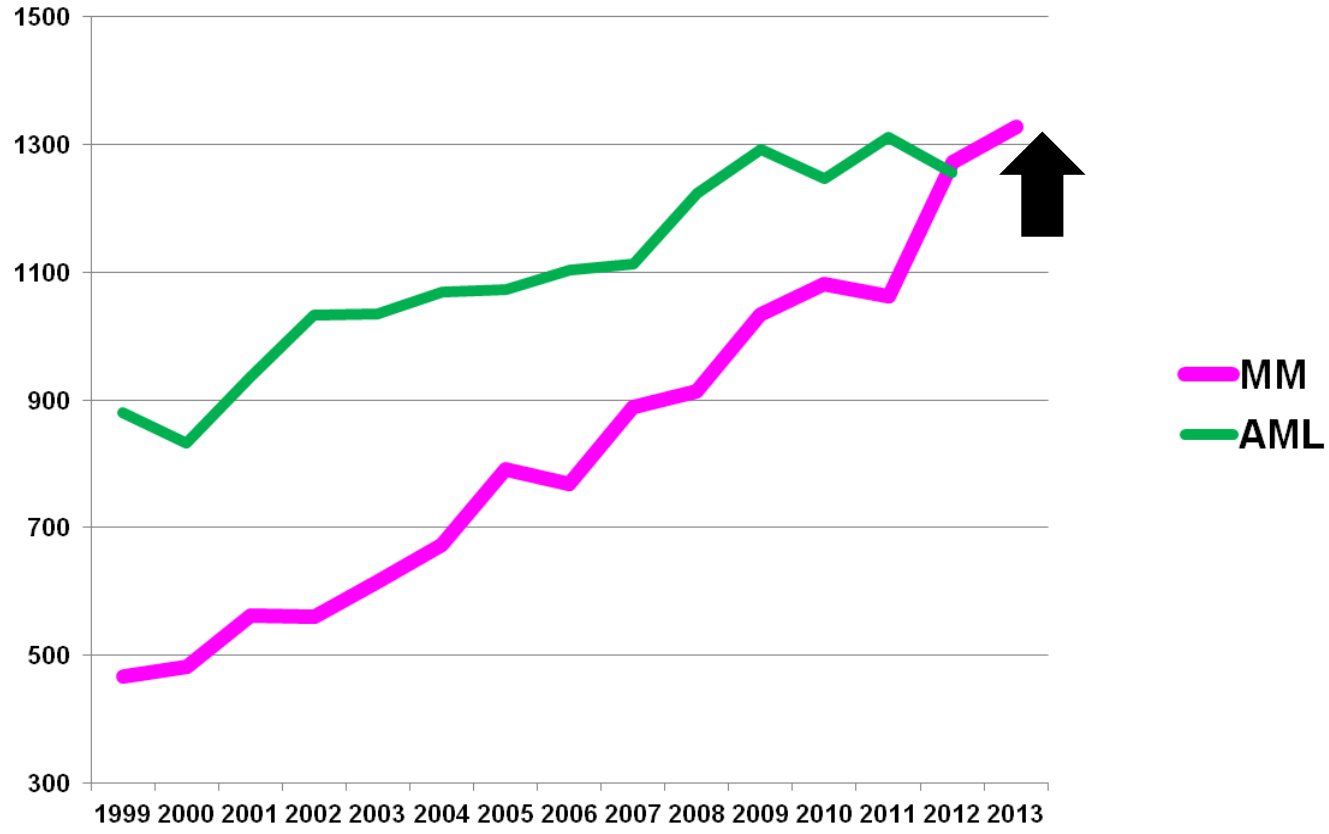


MULTIPLE MYELOMA Research Foundation

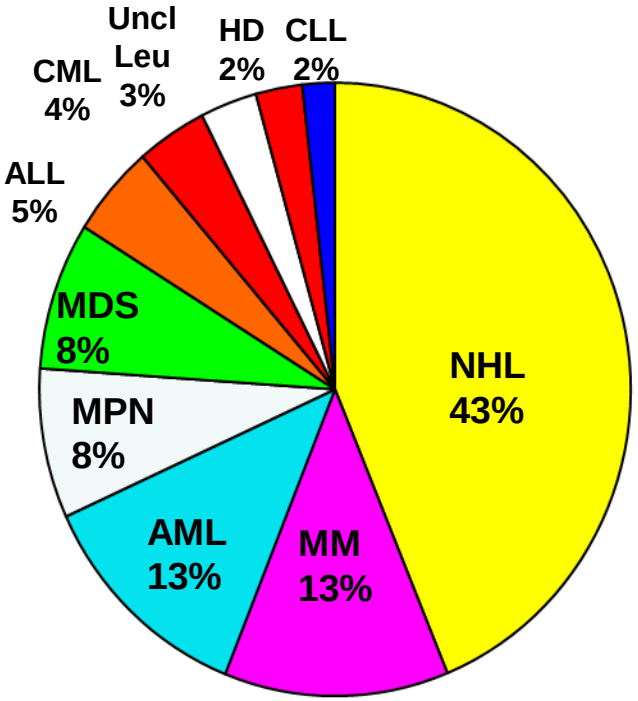
한국의 다발골수종 발생률/사망률



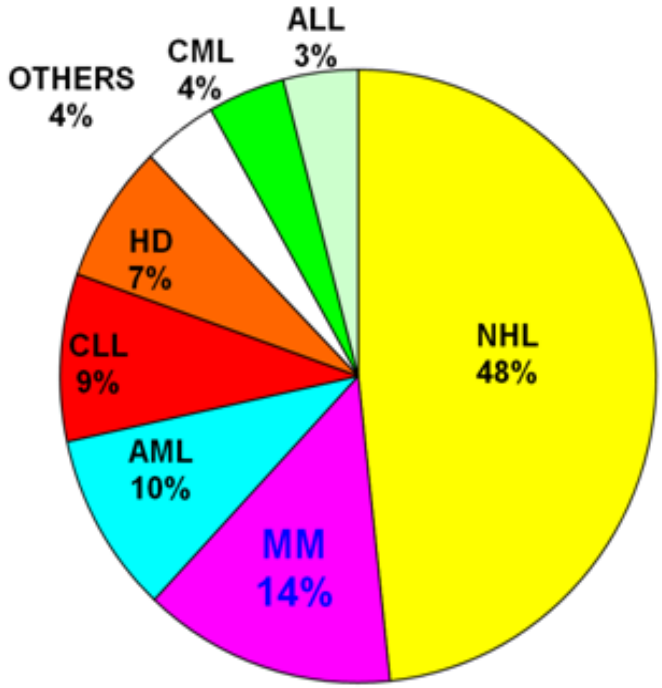
Crude Incidence of MM and AML recent 10 years in SK



Relative Incidence of Hematologic Malignancy in SK and US

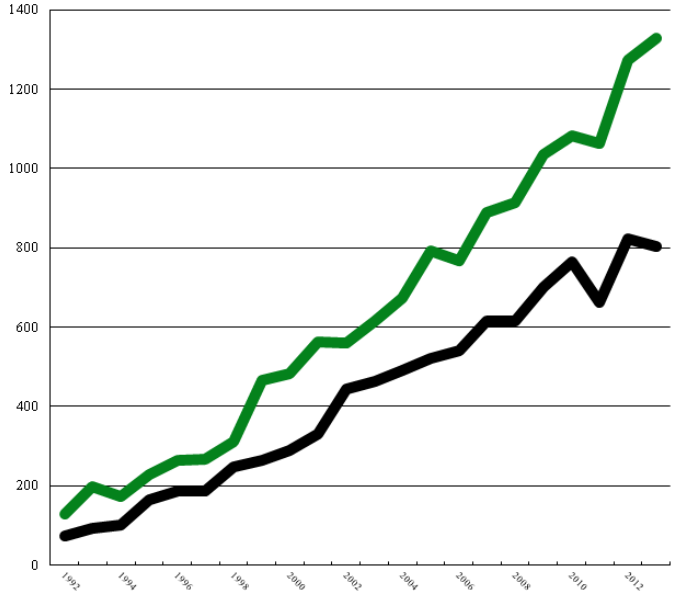


SK 2012

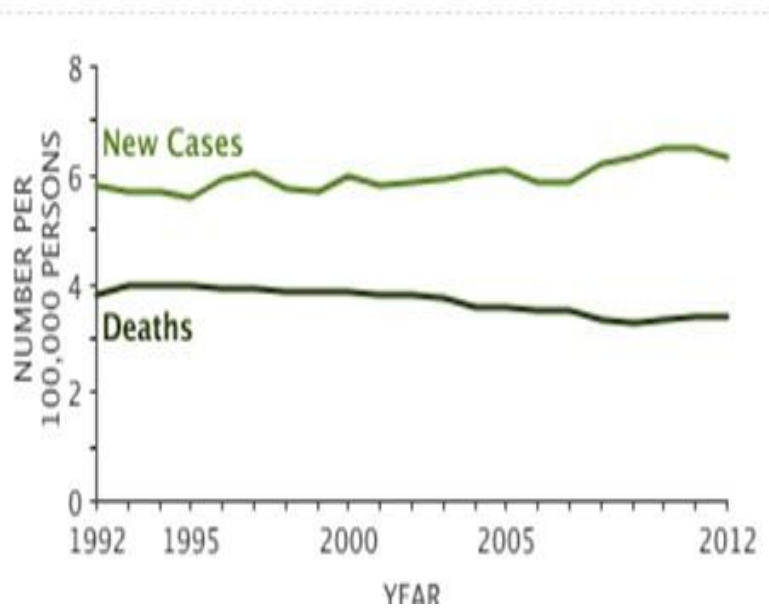


US SEER 2010

한국의 다발골수종 발생율/사망률 1992-2013



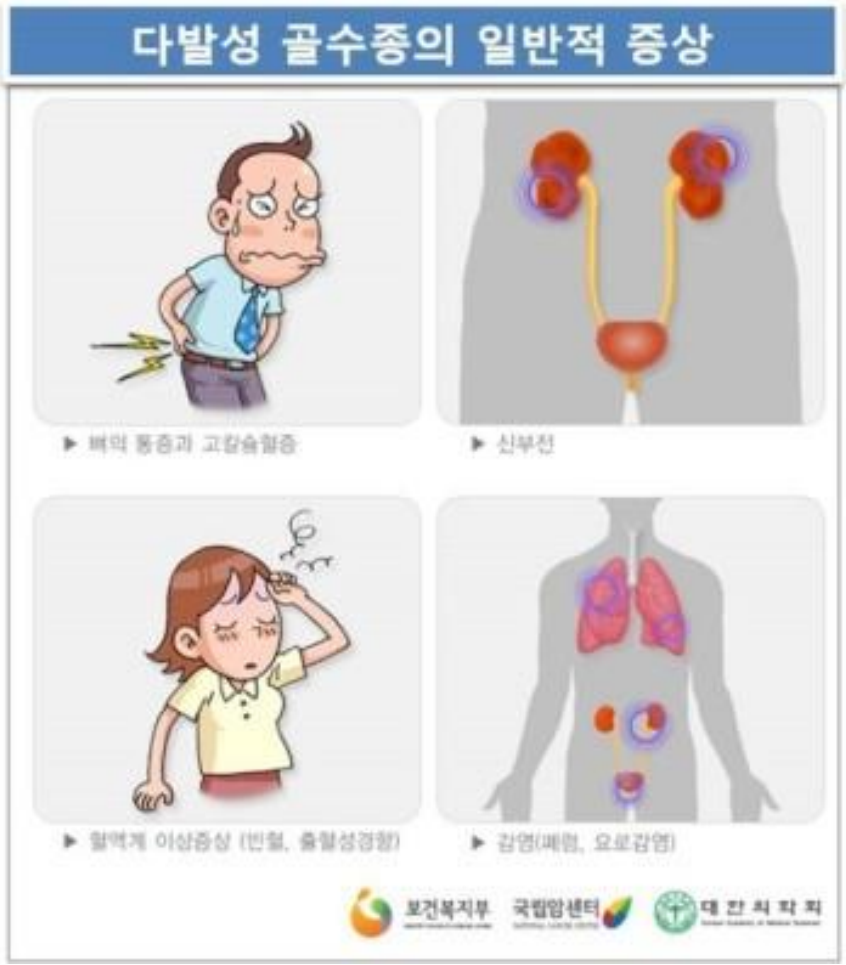
한국



미국

Signs and symptoms

- **C**alcium elevated
- **R**enal failure
- **A**nemia
- **B**one pain
- Infection



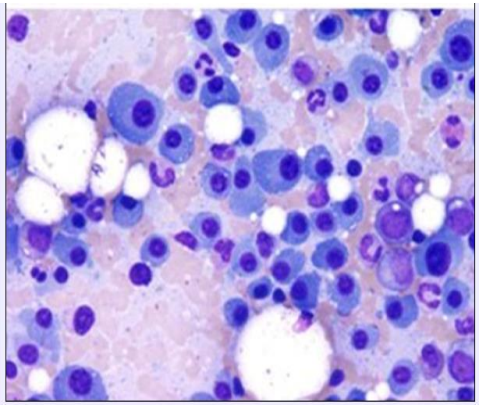
Diagnostic criteria

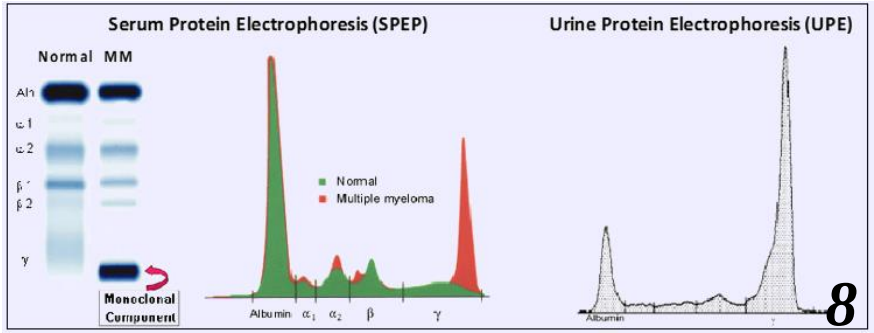
NAME	DEFINITION
Monoclonal Gammopathy of Undetermined Significance (MGUS)	• Monoclonal protein present but usually ‹ 3.0 g/dL • No CRAB features or other indicators of active myeloma • Bone marrow monoclonal plasma cells ‹ 10%
Asymptomatic or Smoldering Multiple Myeloma (SMM)	• Higher level of disease than MGUS: serum M-component can be › 3.0 g/dL and/or bone marrow plasma cells › 10%, but • No CRAB features or other indicators of active myeloma
Active or Symptomatic Myeloma	• Monoclonal protein present, and • One or more " CRAB " features and/or indicators of organ damage*

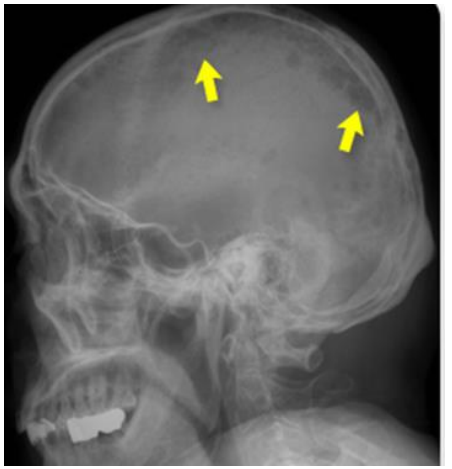
**Organ damage classified as "CRAB" or any other significant clinical problem linked to myeloma progression such as recurrent infections or neoropathy unrelated to treatment*

C - Calcium elevation (>10mg/dL)
R - renal dysfunction (creatinine >2mg/dl or creatinine clearance (<40ml/min)
A - anemia (hemoglobin <10g/dL or >2g/dL decrease from patient's normal)
B - bone disease (one or more osteolytic lesions detected on skeletal radiography, WBLC CT or PET/CT)

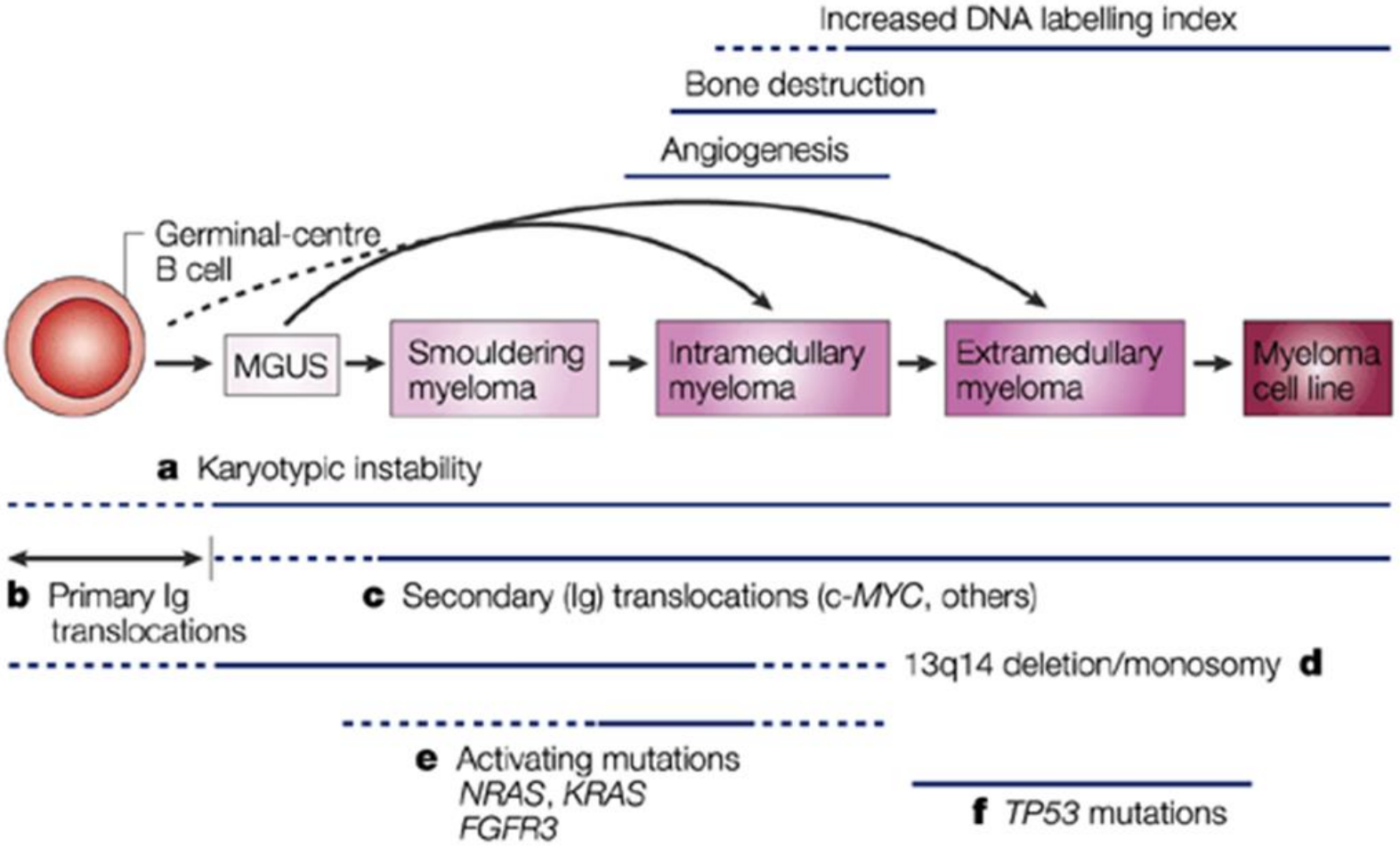
One or more "CRAB" features or other significant problem required for diagnosis of **Symptomatic Myeloma**







Pathogenesis

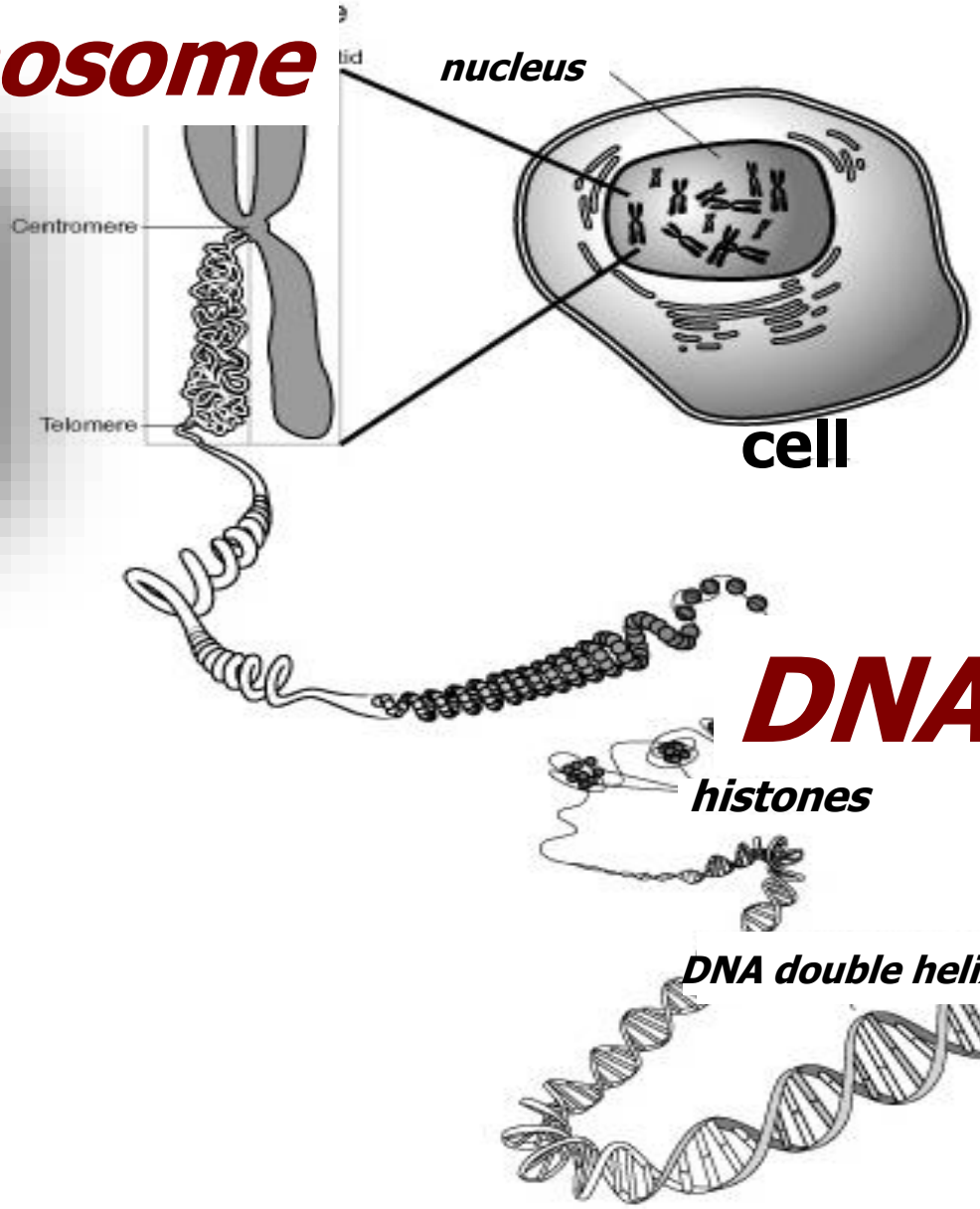


Nature Reviews | Cancer

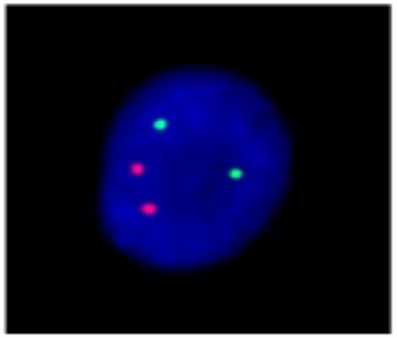
Diagnostic workup

CBC, diff-count/ blood cell morphology, BUN/creatinine / albumin/ electolyte with calcium, Serum light chain assay/Serum quantitative Ig, Serum LDH/ β2-microglobulin	
Bone marrow aspirate and biopsy	
Immunohistochemistry(BM bx.)	CD138, kappa/lambda
Immunophenotyping	CD19,CD38,CD45,CD56,CD138
Cytogenetic analysis	
FISH	1q gain, CDKN2A deletion, RB1 deletion, p53, IGH, t(4;14), t(14;16)

Chromosome

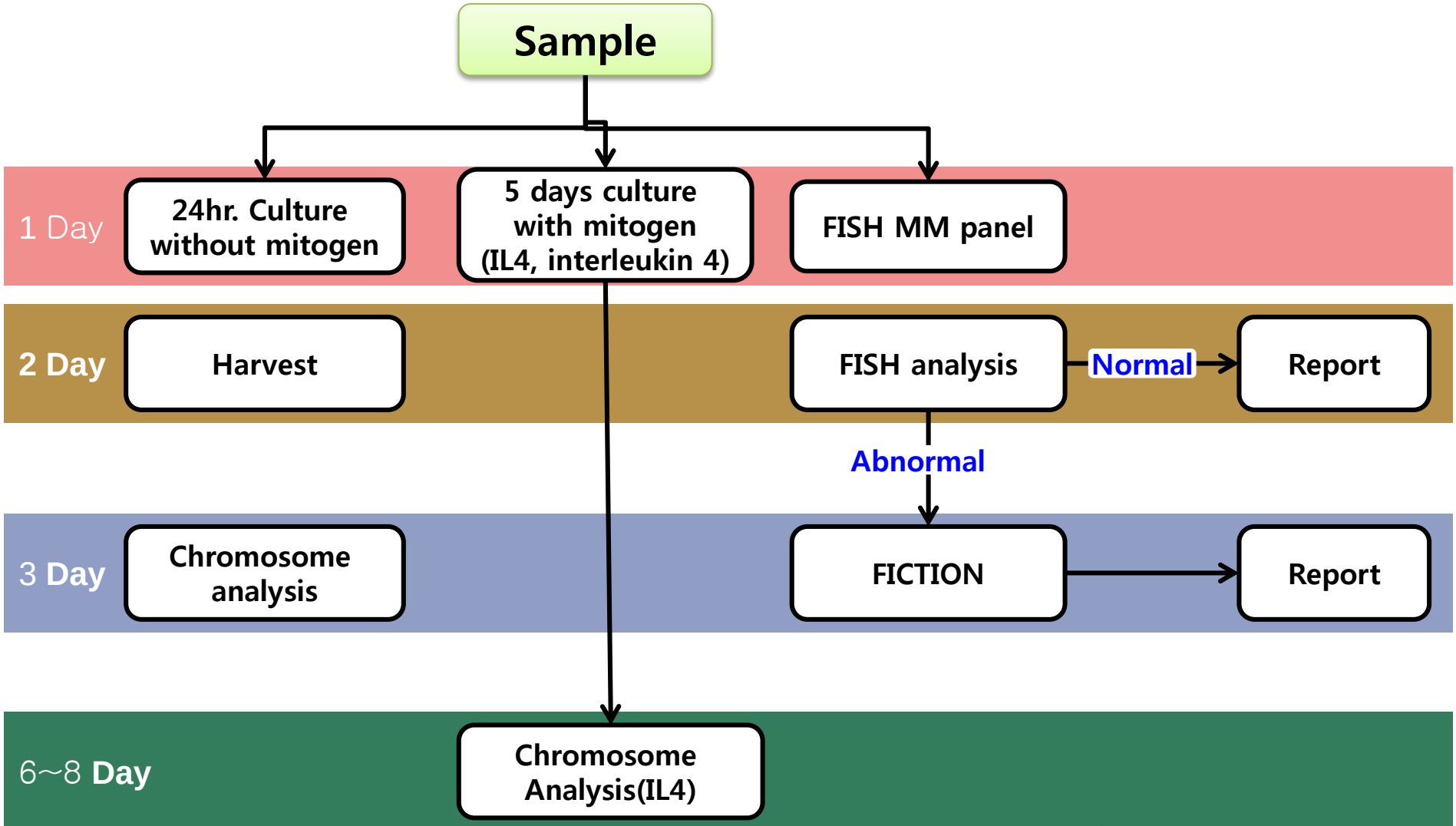


FISH



DNA study

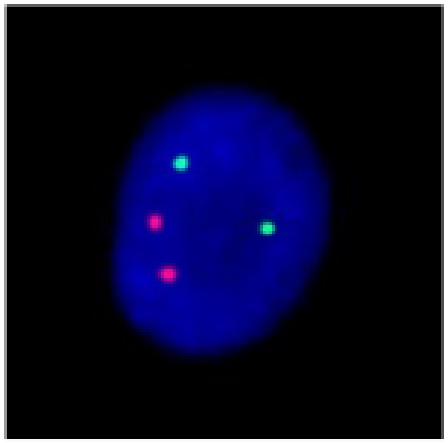
Work flow


Sample
1 Day
24hr. Culture without mitogen
5 days culture with mitogen (IL4, interleukin 4)
FISH MM panel
2 Day
Harvest
FISH analysis
Normal
Report
Abnormal
3 Day
Chromosome analysis
FICTION
Report
6~8 Day
Chromosome Analysis(IL4)


# of cytogenetics & FISH

Chromosome analysis	Long culture period(2~7 day)
	Low proliferation rate of plasma cells in vitro
	Abnormal clone is not detected until disease has reached an advanced stage
	Poor chromosome banding quality
	Sensitivity N/20 metaphase
FISH	Circumvents the need for cell division(1~2 day)
	Abnormalities by artifact
	Available for the detection of submicroscopic alterations
	for the clarification of complex alterations and also for MRD purposes
	Sensitivity N/200~300 interphase

Complementary G-banding vs. FISH



100 %

iFISH

G-banding

Chromosome aberrations in MM

- Marked karyotypic instability(significant molecular heterogeneity)

Nonhyperdiploidy	~half	
Recurrent translocations with IGH gene(14q32)	11q13(CCND1)	16%
	4p16(FGFR3)	15%
	16q23(MAF)	5%
	6p21(CCND3)	3%
	20q12(MAFB)	2%

Hyperdiploidy	~remaining half
Multiple trisomies usually do not have coexistent 14q32 translocations.	Odd no. chromosome 3, 5, 7, 9, 11, 15, 19, 21

Chromosome aberrations in MM

Hypodiploidy (<46)

- Loss of 13, specifically 13q14.3 and/or
- Loss of 17, specifically 17p13.1(*TP53*)
- Includes structural abnormalities chr. 1,4,6,14,16,20
- Loss of 8, 17, Y, X
- 1p loss, 1q gain, 4q loss, 6q loss, 20 loss
- Rearrangement 14q32, 16q
- 70~90 chromosome, double content
- Adverse prognosis

Chromosome aberrations in MM

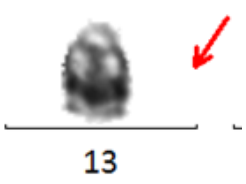
Hyperdiploidy

- Gain of 3, 5, 7, 9, 11, 15, 19, 21
- standard risk category as long as no deletion 13q or 17p
- the most common translocation: t(11;14)

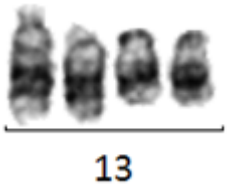
Chromosome aberrations in MM

Deletion of 13q/loss of 13

- Most frequent(~50% of abnormal karyotype)
- Interstitial deletion of 13q involving 13q14.2(RB1) or 13q14.3(D13S319);
  cryptic, only FISH
- Deletion with conventional cytogenetics(10~20%)
- Poor outcome, high relapse rate



-13



del(13)(q14q22)

ELSEVIER

Cancer Genetics and Cytogenetics 168 (2006) 124–132

CANCER GENETICS AND CYTOGENETICS

Identification of 13q deletion, trisomy 1q, and IgH rearrangement as the most frequent chromosomal changes found in Korean patients with multiple myeloma

Soo-Mee Bang[a,1,2], Young Ree Kim[b,1], Han Ik Cho[c], Hyun Sook Chi[d], Eul-Ju Seo[d], Chan Jeoung Park[d], Soo Jin Yoo[d], Hee Chan Kim[e], Hong Gu Chun[e], Hyun Chung Min[f,g], Bo Ra Oh[f,g], Tae Young Kim[f,g], Jae Hoon Lee[a], Dong Soon Lee[c,f,g,*]

[a]*Department of Internal Medicine, Gachon Medical School, Gil Medical Center, 1198 Guwol-dong, Namdong-gu, Incheon 405-760, Korea*
[b]*Department of Laboratory Medicine, Jeju National University College of Medicine, 66 Jeju-daehakro, Jeju 690-756, Korea*
[c]*Department of Laboratory Medicine, Seoul National University College of Medicine, 28 Yeongeon-dong, Jongno-gu, Seoul 110-744, Korea*
[d]*Department of Laboratory Medicine, College of Medicine, University of Ulsan and Asan Medical Center, 388-1 Pungnab2-dong, Songpa-gu, Seoul 138-736, Korea*
[e]*Department of Biomedical Engineering,* [f]*Cancer Research Institute, Seoul National University College of Medicine, Seoul, 28 Yeongeon-dong, Jongno-gu, Seoul 110-744, Korea*
[g]*National Research Laboratory for Molecular Cell Imaging, Seoul National University College of Medicine, 28 Yeongeon-dong, Jongno-gu, Seoul 110-744, Korea*

Received 19 October 2005; received in revised form 6 February 2006; accepted 15 February 2006

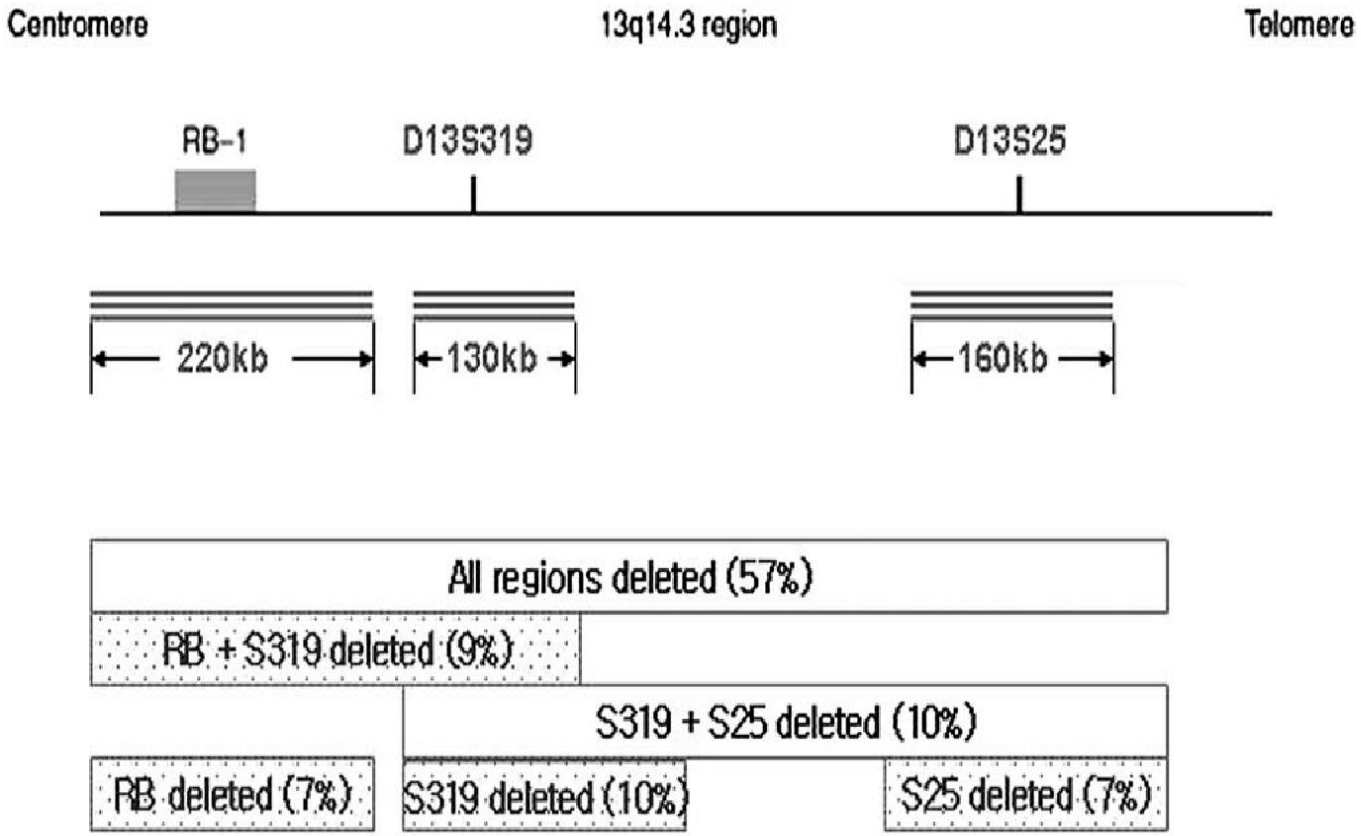
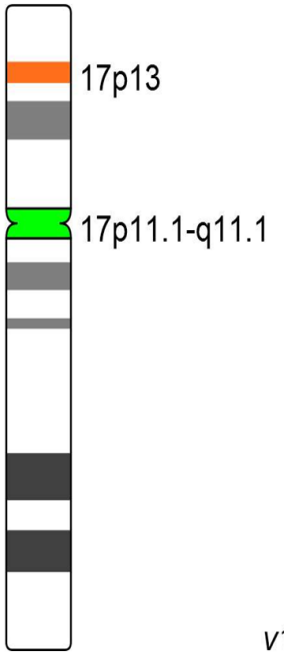


Fig. 2. The sensitivity of three different probes for 13q deletion. A total of 58 patients had at least one locus deleted on 13q. Among them, 33 patients (57%) proved to be deleted at all three loci. Six patients lost D13S25 plus D13S319 (10%), and five patients lost *RB* plus D13S319 (9%). Six patients lost only D13S319 (10%), and four patients each lost *RB* (7%) or D13S25 (7%).

Chromosome aberrations in MM

Deletion of 17p

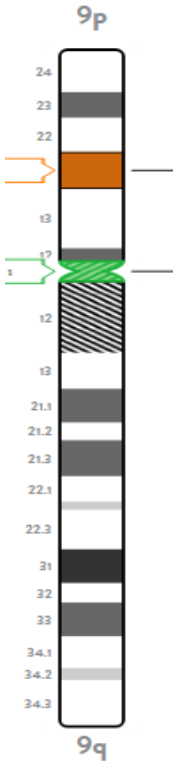
- Deletion of TP53 (~10%)
- Tumor suppressor gene
- Adverse prognostic outcome
- Occur as secondary events during disease progression.



Chromosome aberrations in MM

Deletion of CDKN2A

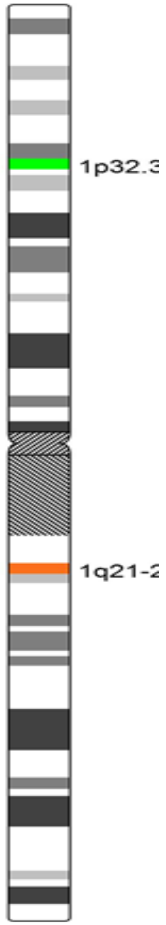
- Deletions of 9p13 or methylation
- If cryptic deletion, only FISH
- Tumor suppressor gene



Chromosome aberrations in MM

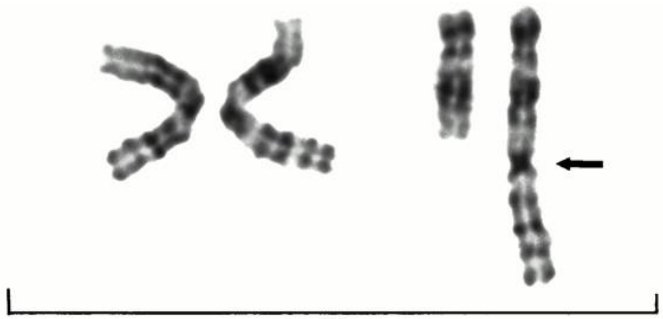
Chromosome 1 Abnormalities

- Deletions of 1p: 1p12~1p31
- Gains of 1q: 1q21,
  the second most frequent(40%~70%)
- Translocations involving either arm:
  t(1;15)(q10;q10),der(1;16)(q10;p10),
  der(1;19)(q10;p10), i(1)(q10)
- Unfavorable prognosis

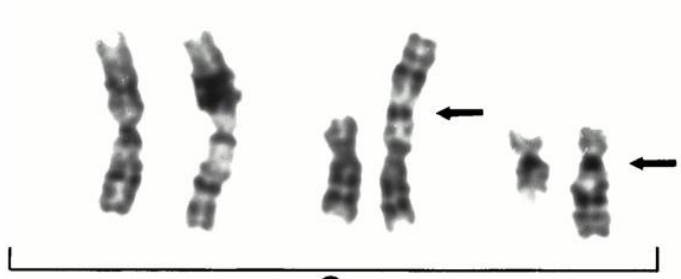


Chromosome 1 Abnormalities

A Der(1;15)(q10;q10)



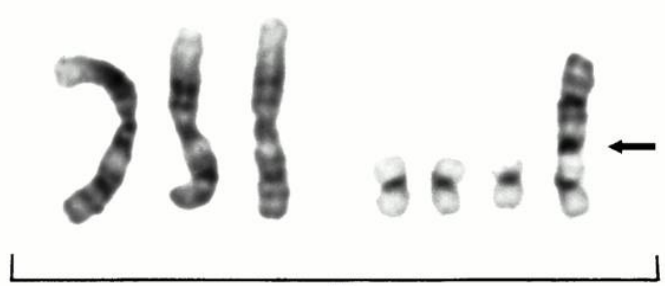
B der(12)t(1;12)(q10;q24)



der(8)t(1;8)(q10;p23) C der(16)t(1;16)(q10;p10)

der(5)t(1;5)(q10;q15) D der(16)t(1;16)(q10;p13)

der(5)t(1;5)(q21;q31) E der(17)t(1;17)(q10;q25)

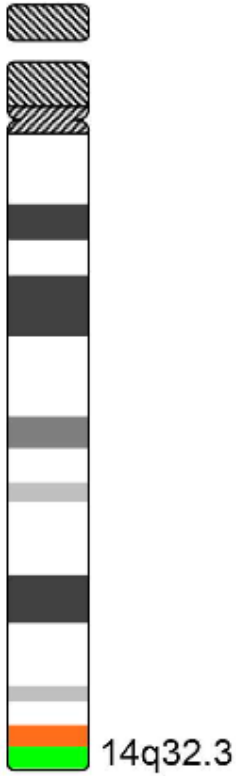


F der(19)t(1;19)(q10;p13)

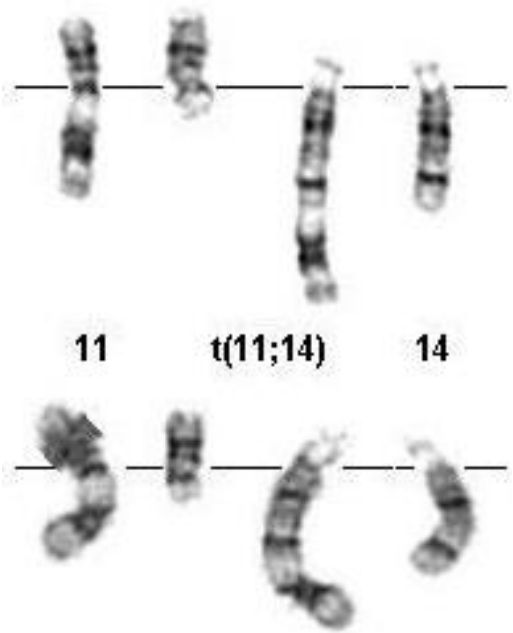
Chromosome aberrations in MM

IGH @ Rearrangement

- ~40% of patients with MM.
- Translocation of oncogenes into this region may lead to their increased expression, contributing to disease initiatior disease progression and therapeutic resistance.
- t(11;14): 20~25%, transforms to an aggressive phenotype after acquiring a secondary genetic "hit," CDKN2A inactivation by promoter methylation.
- t(4;14): 15%, cryptic, tend to be very frequent in hypodiploid karyotype, high-risk prognostic category
- t(14;16): 5~7%, cryptic, tend to be very frequent in hypodiploid karyotype

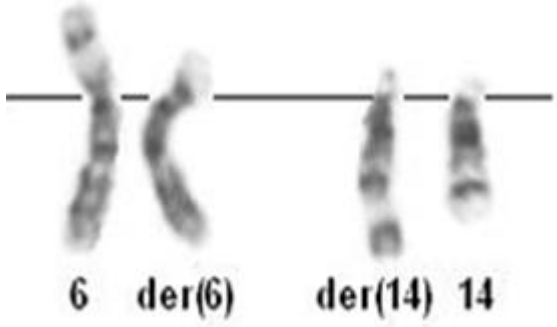


IGH @ Rearrangement



t(11;14)

t(14;16)

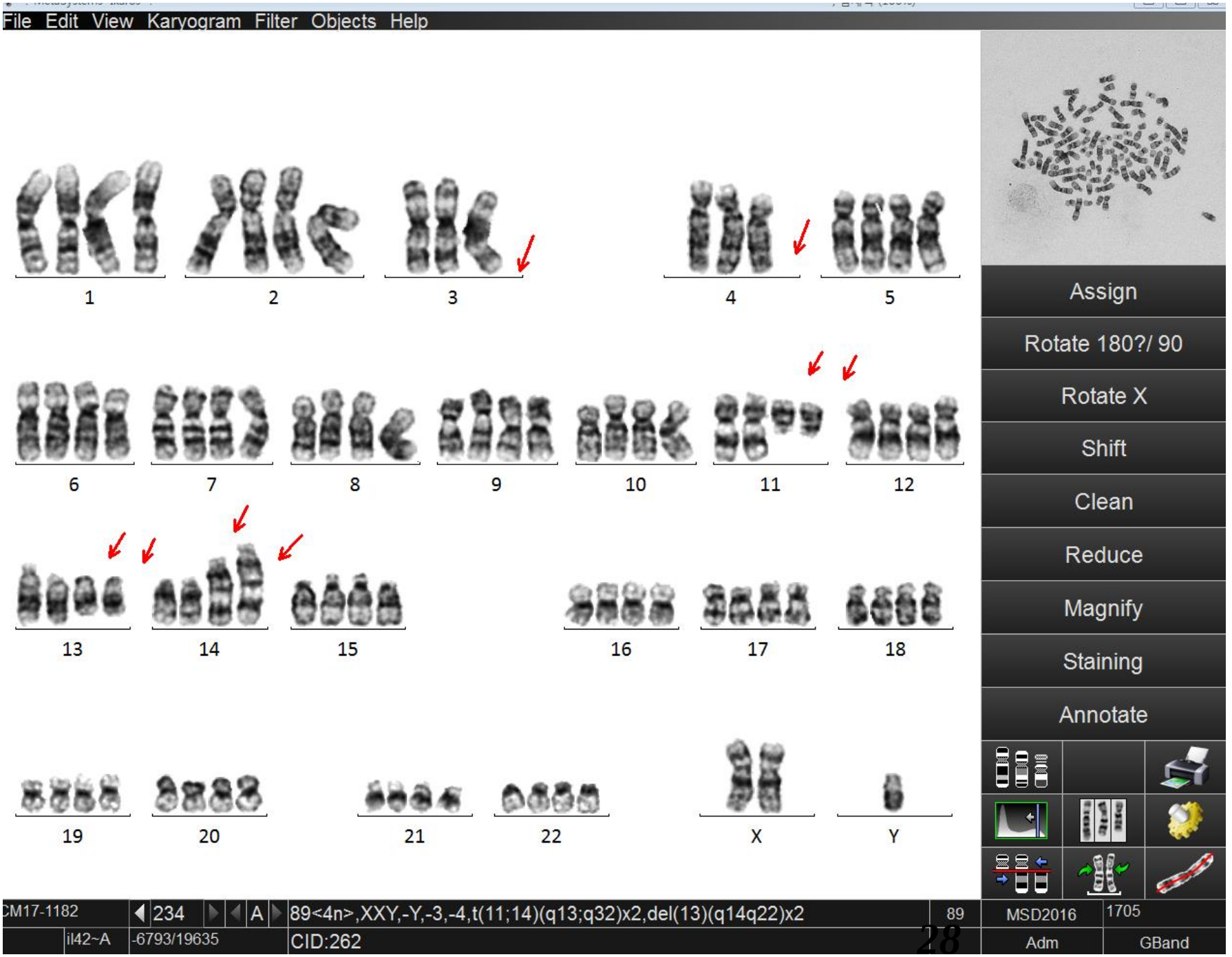


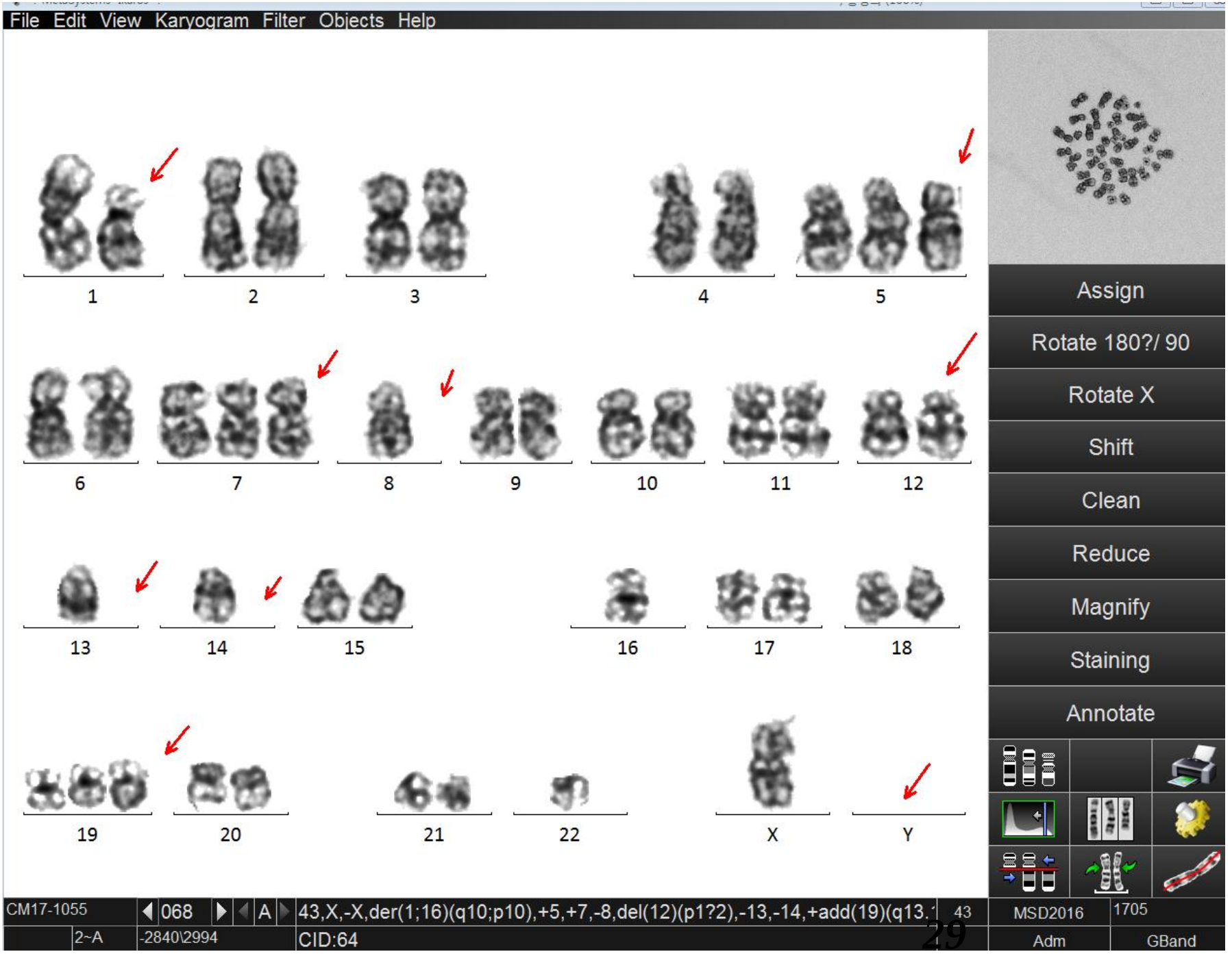
t(6;14)

Cytogenetic prognostic group in M.M

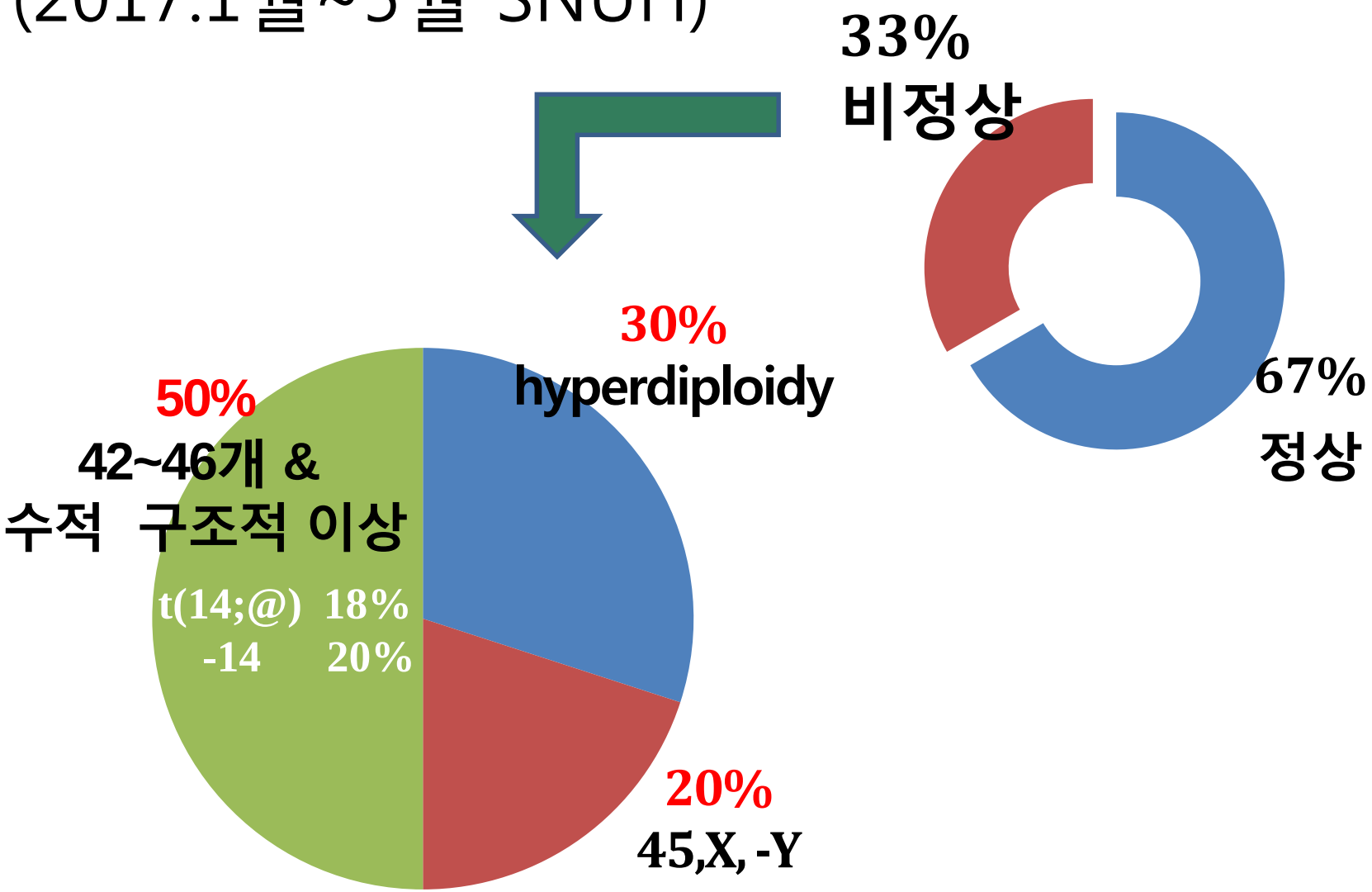
Unfavorable risk
t(4;14) or t(14;16)
Deletion 17p13 (p53)
13q deletion or aneuploidy
Hypodiploidy
complex abnormalities

Favorable risk
t(11;14)
Absence of unfavorable risk genetics
Hyperdiploidy

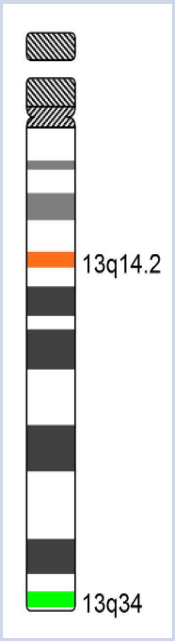
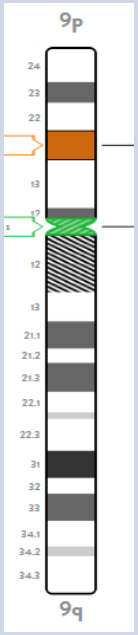
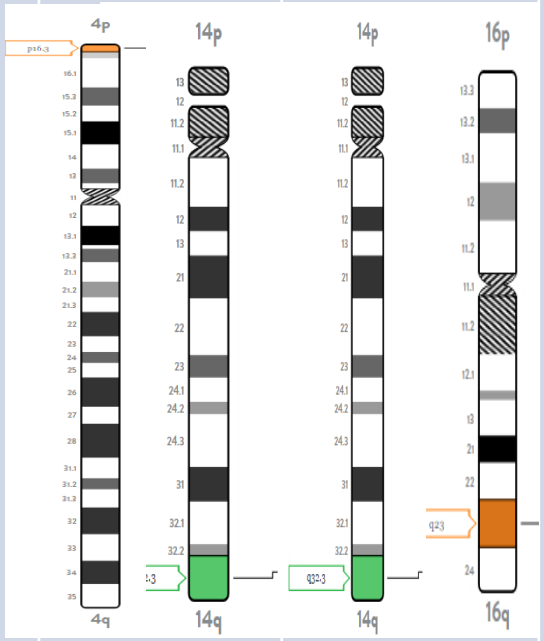
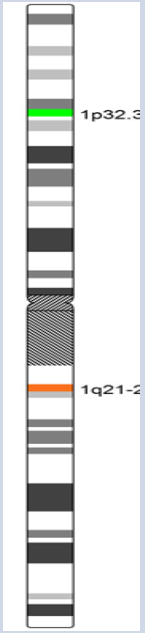
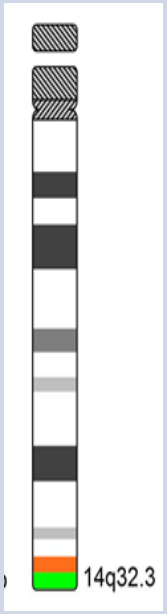
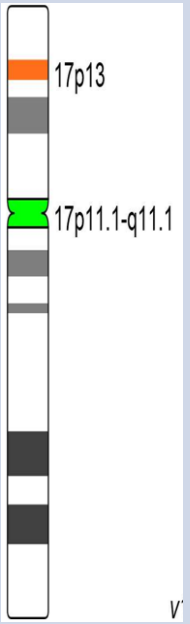

File Edit View Karyogram Filter Objects Help
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
Assign
Rotate 180?/ 90
Rotate X
Shift
Clean
Reduce
Magnify
Staining
Annotate
CM17-1182
234
A
89<4n>,XXY,-Y,-3,-4,t(11;14)(q13;q32)x2,del(13)(q14q22)x2
89
MSD2016
1705
il42~A
-6793/19635
CID:262
Adm
GBand


File Edit View Karyogram Filter Objects Help
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
X
Y
Assign
Rotate 180?/ 90
Rotate X
Shift
Clean
Reduce
Magnify
Staining
Annotate
CM17-1055
068
A
43,X,-X,der(1;16)(q10;p10),+5,+7,-8,del(12)(p1?2),-13,-14,+add(19)(q13.
43
MSD2016
1705
2~A
-2840\2994
CID:64
Adm
GBand

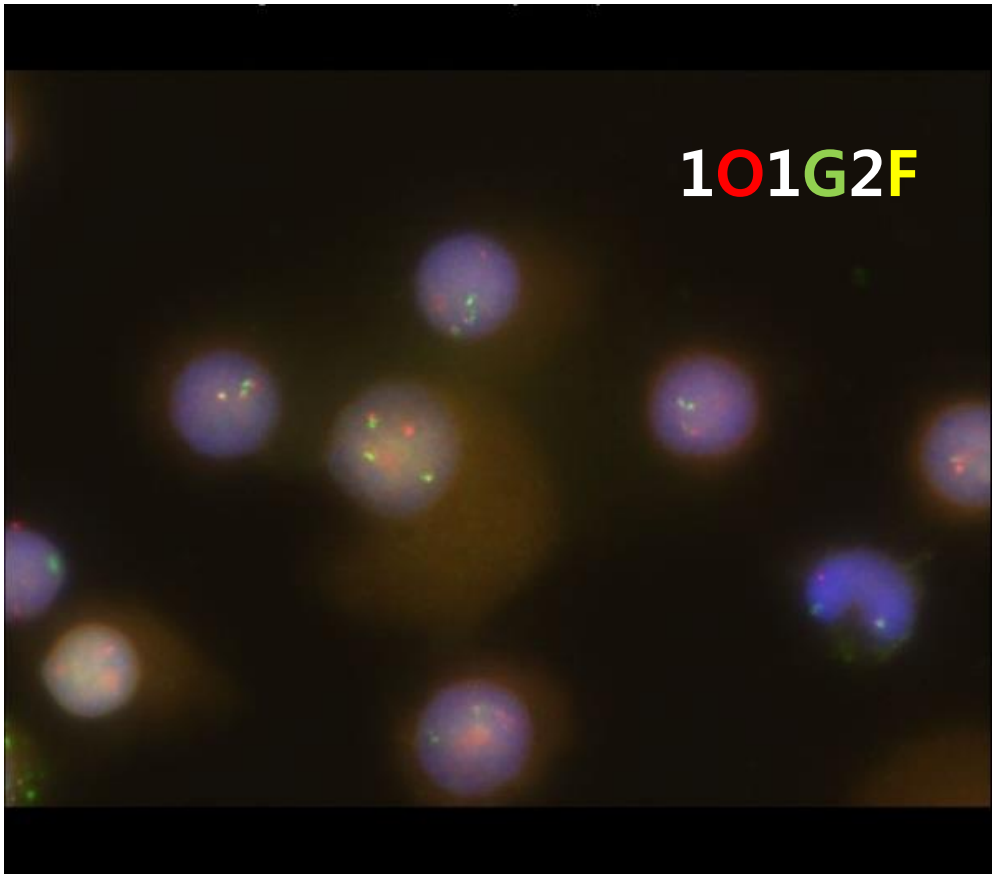
M.M 염색체검사 양성률 (2017.1월~5월 SNUH)



M.M panel-A(Diagnosis); 7 item
M.M panel-B(IMWG risk); 4 item

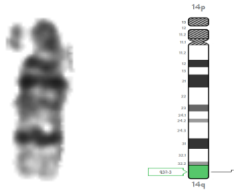
PROBE	D13S319 A B	CDKN2A A	T(4;14) A B	T(14;16) A B	1P/1Q A	IGH A	P53 A B
Locus & color							
Vendor	Meta systems	Abbott	Abbott	Abbott	Meta systems	Meta systems	Meta systems

Cryptic translocation



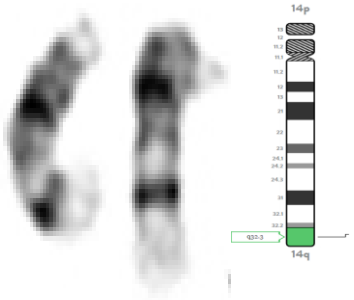
t(4;14)(p16;q32)

4

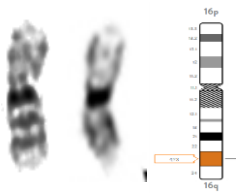


14

t(14;16)(q32;q23)



14

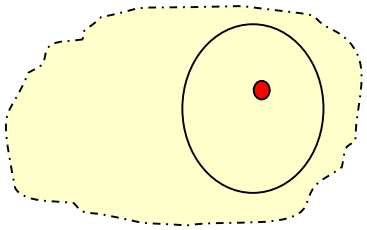


16

Secondary immunohistochemical stain (rabit to human kappa & lambda antibody-FITC)

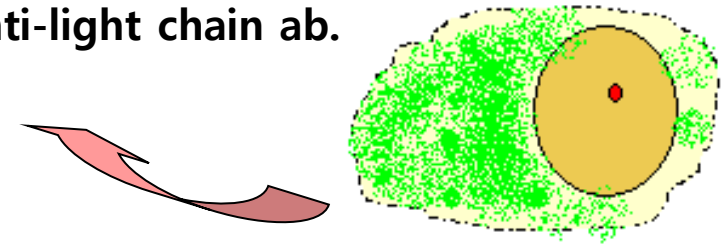
overnight

FISH staining



Reading

200 BM nucleated cells
100 plasma cells stained
with anti-light chain ab.



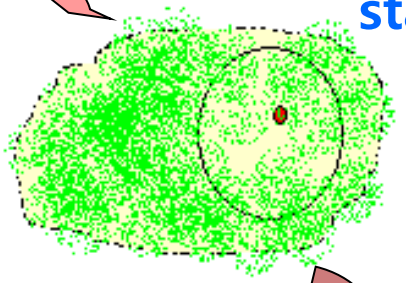
Counter stain & Mounting

DAPI

Washing & drying.

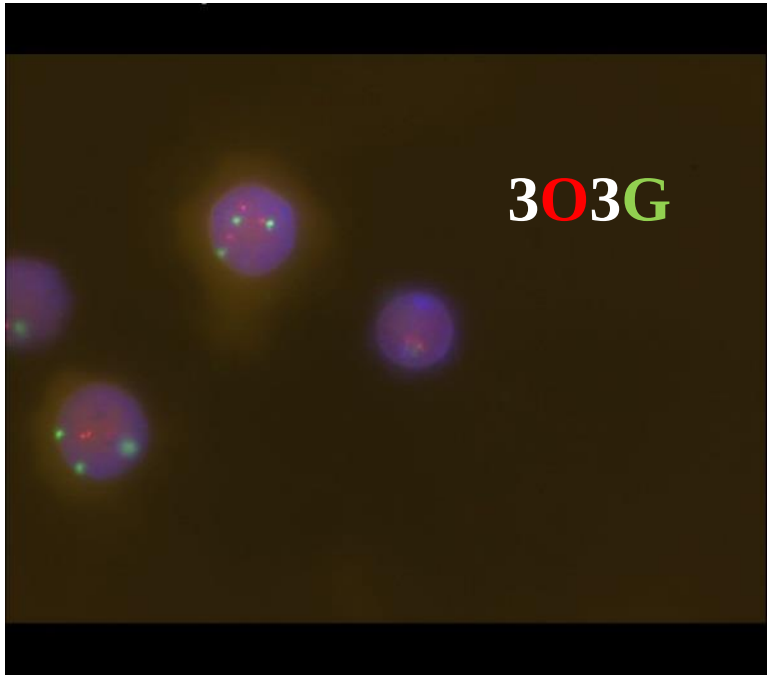
2 x SSC / PBS

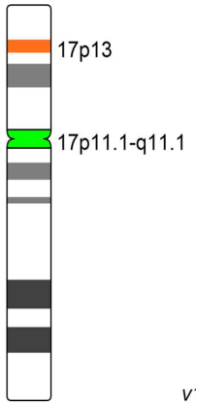
Anti light chain staining

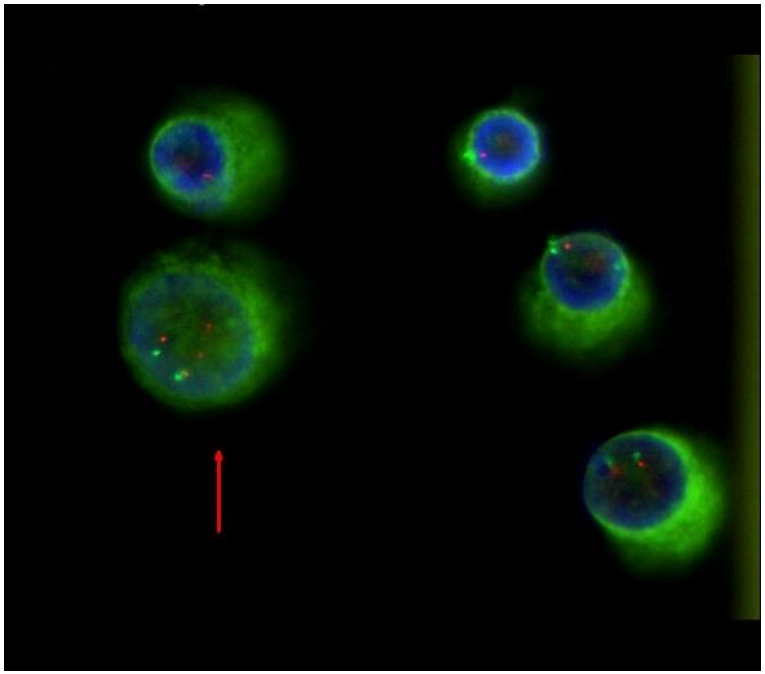


for 1 hours
at 37 ℃
in moisture

Washing & drying.







Plasma cell sorting;
Immunomagnetic bead or
FICTION (Fluorescence Immunophenotyping and interphase Cytogenetics as a Tool for the Investigation Of Neoplasms)

Treatment

- Chemotheraphy--기본
- 자가조혈모세포이식--65세 이하
- 동종조혈모세포이식—노령 ,전신상태불량
- 방사선치료--증상완화목적
- 뼈질환치료제—골절방지
- 수혈
- 투석--신기능 악화

Prognosis

- 치료저항성이 생겨 예후는 좋지 못함
- 항암화학요법이 도입되기 전에는 6개월 정도의 평균
- 현재는 항암치료 만으로도 2~3년의 생존기간을 보이고
- 자가조혈모세포이식을 한 경우는 5년
- 최근 도입된 신약들이 점점 광범위하게 도입됨에 따라 더 긴 생존기간을 보일 것으로 생각되며
- 국내에서는 보험 보험급여 문제로 효과 좋은 신약을 쓰기 힘들다.