“Chromosome analysis is currently the most important tool to assess risk and predict outcome in MM. With the development of new drugs in combinations with alternative transplantation modalities or in new combinations without transplantation – chromosome analyses will probably be even more important for selection of the best treatment for individual patients.”

H. Nahi, 2010, Hematology Centre, Karolinska University Hospital, Stockholm, Sweden
A chromosome is the visible state of genetic material. During cell division, mitosis, the chromosomes become highly condensed and are then visible as dark distinct bodies within the nuclei of cells.

A chromosome is made of two identical molecules of DNA efficiently packed into sister chromatids, which are held together at their centromere.

Each human chromosome has a short arm ("p" for "petit") and long arm ("q" for "queue") separated by a centromere.

The centromere is the structure where the mitotic spindle attaches prior to segregation. The ends of the chromosome are called telomeres.
The band width and the order of bands are characteristics of a particular chromosome.

Standard **G band staining** techniques allow between 400 and 600 bands to be seen on metaphase chromosomes.
Preparation of mitotic chromosomes

**Blood/BM**

1. Separate off red cells
2. Add culture medium to white cell suspension
3. Incubate at 37°C
4. Add colchicine
5. Separate off white cells
6. Hypotonic saline added
7. Cells spread onto slide by dropping
8. Stained
9. Photographed

**Timeframes:**
- **Min:** 5 days
- **Max:** 3 months
Structural Chromosomal rearrangements

**Ploidy level:**
The diploid number \( n = 46 \) is the number of chromosomes of a normal cell.

- **Hyperdiploid** – 47-57; **Hypodiploid** – 35-45 chromosomes
- **Triploid** = 3n = 72; near triploid – 58 – 80 chromosomes;
- **Tetraploid** = 4n = 96; near tetraploid – 81 – 103 chromosomes

**Numerical abnormalities:**
the loss of chromosome(s) and the gain of chromosome(s)

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**Translocations**
\( t(6;9) \)

**Duplication**

**Inversions**

**Deletions**
Chromosome rearrangements in hematological cancer diseases
No. of rearrangements ~ 540 / No. of cloned breakpoints ~ 350 / No. of genes involved 90

Clinical applications:

Diagnosis

CML = BCR/ABL fusion =
\( t(9;22)(q34;q11) \)

APL = RARA rearrangements =
\( t(15;17)(q24;q21) \) and other translocations with 17q21

Prognostic factor

Independent predictor of outcome in MDS, AML, MF and ALL

Marker

for the detection of the minimal residual disease (MRD)

Low rate of proliferation of the plasma cells (PCs) and a low percentage of PCs in bone marrow samples

Conventional karyotype analysis is informative in only 30% of MM cases
Chromosomal abnormalities (translocations, gains and losses) are very frequent in myeloma: most MMs show an average of 11 abnormalities per karyotype.
The complexity of structural and numerical chromosomal aberrations typical for MM has made it difficult to determine the individual prognostic contribution of specific chromosomal alterations.

1. 46,XY,der(1)t(1;4)(p12;p12)dup(1)(q25q32),+dup(5)(q13q31),+der(1;7)(p10;q10),der(8)t(4;8)(q31;p23),+del(9)(q22),der(11)t(11;13)(p15;q14),+14,+15,16,der(20)t(1;20)(p13;p11),der(21;22)(q10;q10)[cp17]/46,XY[29]

2. 47–52,X,der(X)t(X;1)(q28;q25),der(1)t(1;21)(q44;q11),+del(1)(p13),+3,+5,+7,+11,+15,der(16)t(16;17)(q24;q21),+19,–20[cp5]/46,XX[38]

3. 45,X,–X,der(2)t(2;2)(p21;q21),del(3)(q13),del(3)(q25),add(4)(p12),del(5)(p12),74psu dic(5;1)(q35;q10),del(6)(q21),del(11)(q23),t(11;14)(q23;q32),del(12)(q12q14),+12,–13[cp12]/46,XX[18]

4. 44–45,XX,del(6)(q21q23),der(10)t(10;10)(p15;q11),–9,–11,–12,der(14)t(2;14)16.4(p13;q32),+del(21)(q22),+r[cp6]/46,XX[7]

5. 41–43,X,–Y, der(1;15)(q10;p11)ins(1)(q32;q12),+del(1)(p13),t(4;20)(p15.2;p13),55.9del(5)(p13),–7,–8,del(10)(p12.2),–13,–14,t(14;16)(q32;q23),der(16)t(16;17)(q12;q21),+21,del(22)(q11.2),+mar[cp34]/46,XY[3]
The most common chromosomal abnormalities in MM detected by conventional cytogenetics

Structural abnormalities:

Rearrangements involving 14q32 (IGH gene) with various partner genes - translocations t(4;14); t(11;14); t(14;16), t(14;20) and other (70%)

Chromosome 13 deletions and monosomy (>50%)

Deletion of chromosome 17p (p53 gene)

Chromosome 8q24 rearrangements (c-myc gene)

Ploidy alterations:

Hypodiploidy and Hyperdiploidy > 50%
In clinical practice, the optimal method to detect chromosomal aberrations and ploidy in MM is interphase fluorescence in situ hybridization (I-FISH).

FISH is the technique in which a DNA probe is labeled with a fluorescent dye (that can be visualized under a fluorescent microscope) and then hybridized with target DNA on a microscopic slide.

When DNA is heated, DNA strands break apart, or denature, and the probes are able to hybridize to their complementary sequence in target DNA.

I-FISH is used to enumerate chromosomes and to detect chromosomal deletions, translocations, or gene amplifications in cancer cells.
FISH detection of 13q and 17p deletions

- Normal status
- Deletion
- Monosomy

Control probe

13q or 17p probes
Detection of the 14q32 alterations by the IGH gene Break Apart Rearrangement Probe

3’ to centromere

5’ Telomere

#14

der14

der??
Detection of the translocations t(4;14), t(6;14), t(11;14), t(14;16) and t(14;20)

Normal

Translocation

Green labeled IGH segment
Red labeled segments of chromosomes 4/6/11/16/20
Fusion between IGH and chromosomes 4/11/16
A high heterogeneity of translocation t(11;14) that was overlooked in previous investigations.

<table>
<thead>
<tr>
<th>IGH/CCND1</th>
<th>IGH</th>
</tr>
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<tbody>
<tr>
<td>2Y/O/G</td>
<td>Y/O/G</td>
</tr>
<tr>
<td>der 11</td>
<td>der 11</td>
</tr>
<tr>
<td>#11</td>
<td>#14</td>
</tr>
<tr>
<td>t(11;14)/11q A</td>
<td></td>
</tr>
</tbody>
</table>

| Y/O/2G    | 2Y/O/G |
| der 11    | der 11 |
| #11       | #14   |
| t(11;14)/11q A |

| 2Y/O/G    | 2Y/O/G |
| der 11    | der 11 |
| #14       | #14   |
| t(11;14); +der 14/11q A |

| 2Y/O/G    | O/G   |
| der 11    | der 11 |
| #11       | #14   |
| t(11;14); - #14/11q A |

| Y/O/2G    | 2O/G  |
| der 11    | der 11 |
| #11       | #14   |
| t(11;14),del Var IgH on #14/11q A |

| 2Y/O/G    | 3O/G  |
| der 11    | der 11 |
| #11       | #14   |
| t(11;14); 2x del IgH Var on #14/11q A |

| 3Y/O/G    | Y/2O/G |
| der 11    | der 11 |
| #11       | #14   |
| t(11;14),+der 14/11q A |

| Y/2O/G    | Y/3O/G |
| der 11    | der 11 |
| #11       | #14   |
| t(11;14),++der14/11q A |

| Y/O/G    | Y/O   |
| der 11    | der 11 |
| #11       | #14   |
| t(11;14); - der 11 |

| Y/O/G    | Y/O   |
| der 11    | der 11 |
| #11       | #14   |
| t(11;14); ++#11,der11 |

Typical t(11;14);++#11,der11
There are major problems with the quality of the bone marrow aspirates received for FISH studies; these frequently contain drastically fewer PCs than the corresponding smear used for morphological assessment. It is difficult for clinicians to accept that a normal FISH result from a patient that had 80% PC on the morphology slide could be meaningless, but that is the reality. Clinicians should be encouraged to send part of the first draw of the aspirate for FISH studies! [Ross F. et al., 2012]
The median proportion of plasma cells, is commonly low, ranging from 1-20%, within the bone marrow aspirates. The FISH technique cannot be performed directly as in other hematologic malignancies, PCs need to be selected, either by flow cytometry or immunomagnetic-bead based PC sorting (CD-138+ purified PCs), by the concomitant labeling of the cytoplasmic immunoglobulin light chain, by image analysis systems allowing morphological assessment of PCs and FISH scoring only in those designated as PC.

All of these methods give good results, the choice should be left to individual laboratories.

A significant fraction of samples will have too few PCs to allow such analyses to be made and will censor some patients in subsequent clinical evaluation.
The system is capable of performing high resolution, full color, automated or interactive morphological and fluorescent scanning.

It saves the coordinates and images of all cells found on the slides for future reference during the next phases of analysis.

It classifies the cells according to their morphology after MGG staining into six classifications: lymphocytes, PMN, normoblasts, myelocytes, blasts and PC.
Detection of the chromosome 13q status in the BM cells of patients with multiple myeloma (MM)

FISH False positive rates = 8-12%

Concentration of plasma cells in bone marrow = 1-30%

The use of the combined morphological and FISH analysis enables the most precise determination of chromosome 13 status in new diagnosed and treated MM patients [Hardan et al., Exp. Hematology 32 (2004)]

2011-2013 – combined morphological and FISH analysis of 25-40 cases /month
Chromosome 13q deletion and IgH abnormalities may be both masked by near-tetraploidy in a high proportion of multiple myeloma patients: A combined morphology and I-FISH analysis.
Chromosomal abnormalities are associated with prognosis in MM:

The detection of $t(4;14)$, $t(14;16)$, deletions of chromosome 13 and $p53$ will define **high-risk prognostic groups** that are not generally controlled with high-dose melphalan and autologous stem cell transplantation (ASCT), and should therefore be treated with more investigational therapies.

**Alternatively**, eligible patients who **do not have these poor risk factors** are more likely to benefit from a high-dose, melphalan-based, regimen followed by ASCT. Patients with $t(11;14)$ were initially believed to have an **unfavorable** category of MM, but in recent works their **improved** outcome was confirmed.

Non-hyperdiploidy is also proposed as an adverse prognostic factor

<table>
<thead>
<tr>
<th>Neutral prognosis</th>
<th>t(11;14), t(6;14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hyperdiploidy</td>
<td></td>
</tr>
</tbody>
</table>

| Poor prognosis | t(4;14); IGH@-FGFR3 |
|               | t(14;16); IGH@-MAF  |
|               | t(14;20); IGH@-MAFB  |
|               | P53 deletion         |
|               | Gain of 1q /Deletion of 1p |
|               | 16q deletion         |
Prognostic significance of del13q detected by FISH
Conflicting results!

In early investigations it was recognize as prognostic marker independent on the mode of treatment [Fonseca R. et al., 2004; Leibish P, 2006].

However, was shown that isolated del(13q) (it means, without t(4;14) or del17p), is not associated with a poor prognosis, thus prognostic value of del13q may be related to prognostic impact of associated other poor-risk markers [Chiecchio L. et al., 2009, Fonseca R. et al., 2009].

However, in the study of patients with relapsed or refractory MM who received treatment with lenalidomide plus dexamethasone, del(13g) was associated with a lower overall response rate (ORR) and a shorter progression-free survival (PFS).

Was suggested that prognostic value of del(13q) may be different at diagnostic and later stages of the disease. [Avet-Loiseau H. et al., 2010]
Prognostic significance of 1q+/1p- detected by FISH
Conflicting results!

Chromosome 1 abnormalities, particularly 1q gain(s) and 1p loss are a frequent finding in MM that are associated with disease progression (transition from MGUS/SMM to MM), shorter progression-free survival (PFS) and overall survival (OS). 1q2+ was identified as an independent factor predicting OS in the context of treatment with lenalidomide and dexamethasone in patients with recurrent MM [Chang H. et al., 2010, 2011; Klein U. et al., 2011]

However, later was shown that 1q21 gains with additional genetic abnormalities but not isolated 1q21 gain is a negative prognostic factor in newly diagnosed patients with multiple myeloma treated with thalidomide-based regimens [Grzasko N et al., 2012]

Combination of translocations with numerical aberrations
The presence of trisomies in patients with t(4;14), t(14;16), t(14;20) or p53 deletion ameliorates the usual adverse impact associated with these prognostic markers.
Mayo Clinic. Rochester, MN; Mayo Clinic in Arizona, Scottsdale, AZ, USA, 2012
The proportion of IGH abnormalities in the del13q group is three time higher than in the 13q norm group (~ 1000 cases)
Two major genetic categories of MM according to ploidy status:
[Smadja N, et al., 1998; Fonseca R, et al., 2003]

The **nonhyperdiploid MM** (< 48 chromosomes or more than 74 chromosomes): associated with primary translocations such as t(11;14), t(4;14), and t(14;16) = Abnormal IGH

The **hyperdiploid MM** (48 to 74 chromosomes, median 53 chromosomes): associated with trisomies especially of chromosomes 3, 7, 9, 11, 15, and 19.

Using the criteria of 2 or more trisomies from a 3-chromosome combination (+9, +11, +15), hyperdiploid myeloma can be detected with high specificity

**Normal IGH**

**FISH with t(11;14) probe:**
Detection of rare types of t(11;14) (~3% of all the t(11;14))
Detection of +11 = the possibility of hyperdiploidy
Proportion of cases with p53- and 1q+/1p- significantly higher in del 13q group

<table>
<thead>
<tr>
<th></th>
<th>13q norm</th>
<th>del 13q</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53-</td>
<td>0.6%</td>
<td>27.6%</td>
</tr>
<tr>
<td>1q+/1p-</td>
<td>13.4%</td>
<td>49.0%</td>
</tr>
</tbody>
</table>

Hyperdiploidy

13q norm IGH norm

del 13q IGH norm

del p53 1q+/1p-
Abnormal IGH

Proportion of cases with high-risk translocations \( t(4;14), t(14;16) \) and \( t(14;20) \)
is significantly higher in the 13q-group

13q norm

\[
\begin{align*}
\text{No IGH rearr} & \quad t(4;14) \\
\text{t(11;14)} & \quad t(6;14) \quad \text{Other IGH rearr}
\end{align*}
\]

del 13q

\[
\begin{align*}
\text{No IGH rearr} & \quad t(4;14) \\
\text{t(14;16)} & \quad t(14;20) \quad \text{Other IGH rearr} \\
\text{t(11;14)} & \quad \text{Other IGH rearr}
\end{align*}
\]
In \( t(14;16) \) and \( t(4;14) \) groups: proportion of \( 13q^- \) and \( 1q+/1p^- \) is significantly higher than in \( t(11;14) \); proportion of \( p53^- \) and \( 16q^- \) is almost the same.

Aberrations of \( P53 \) gene are the most predictive molecular markers for resistance to therapy and short overall survival (OS).

The prognosis for the group of MM patients with \( P53^- \) is clearly worse than the prognosis for patients with any of the other established unfavorable chromosomal abnormalities, regardless of therapy including allogeneic transplantation.
Unknown IGH rearrangements
Proportion of cases with p53- and 1q+/1p- is high in both groups – 13q norm and del13q-

<table>
<thead>
<tr>
<th></th>
<th>13q norm</th>
<th>del 13q</th>
</tr>
</thead>
<tbody>
<tr>
<td>p53-</td>
<td>7%</td>
<td>13%</td>
</tr>
<tr>
<td>1q+/1p-</td>
<td>30%</td>
<td>58%</td>
</tr>
</tbody>
</table>

13q norm
Unknown IGH rearr

Del 13q
Unknown IGH rearr

del p53
1q+/1p- 16q- ?
Translocation t(11;14)

All of the chromosome aberrations are associated

\[ \text{complete linkage, matching measure - 11}_14 \]

\[ \begin{align*}
1q & \text{ gain} \\
del(16q) \\
1q & \text{ gains} \\
del 1p \\
del p53 \\
del(IGH) \\
del IGH \\
del(13q) \\
del 13q
\end{align*} \]

\[ \text{matching similarity measure} \]

\[ \begin{align*}
&.6 \\
&.7 \\
&.8 \\
&.9 \\
&1 \\
&.6
\end{align*} \]

\[ \text{del 1p, del p53 and 1q gains are very mutually similar (Y \geq 0.9) and has a strong association with del(16q) (Y = 0.80) and 1q gain(Y \sim 0.73).} \\
\text{del (IGH) and del(13q) are very mutually similar (Y \sim 0.87);} \\
\text{Also this couple has also association with others (Y\sim 0.60).} \]
Report from the European Myeloma Network on interphase FISH in multiple myeloma and related disorders
Fiona M. Ross, Hervé Avet-Loiseau, et al, 26 European Medical Centers

“Wherever possible, testing should be carried out for t(4;14)(p16;q32), t(14;16)(q32;q23), p53 deletions, 1q gains (and 1p deletions in patients suitable for autograft).

An extended panel may include testing for t(11;14)(q13;q32), t(14;20)(q32;q12), ploidy status, and chromosomes 12 and 13 abnormalities.”

Galina Ishoev and Bella Weisman
THANK YOU VERY MUCH!!