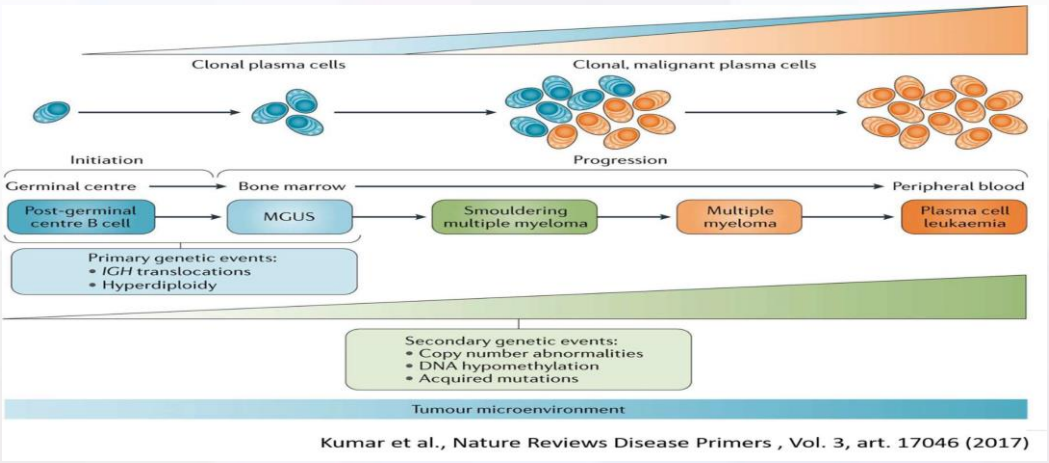
# Multiple Myeloma: Genetic Study

Multiple myeloma (MM) is a type of neoplasm that involves the clonal expansion of plasma cells, a type of cell that originates from the B lymphocyte and is responsible for the production of antibodies. These cells, when they develop abnormally and uncontrolled in the bone marrow, cause a set of symptoms that include bone pain, fatigue, recurrent infections, and high levels of calcium in the blood.

It is the third most common hematological malignancy, with around 3000-3500 new cases diagnosed in Spain each year. It accounts for 2% of cancer deaths and 20% of deaths from hematological malignancies[1].

There are four main categories of myeloma:

- Asymptomatic Myeloma: Patients with nonspecific symptoms that can be attributed to other diseases.
- Silent myeloma: characterized by presenting only a stable monoclonal protein peak.
- Smouldering myeloma: Slow accumulation of aberrant plasma cells in the bone marrow but without immediate or serious damage.
- Multiple Myeloma: The diagnosis is based on the detection of aberrant plasma cells (≥10% bone marrow) that interfere with the normal formation of blood cells in the bone marrow, leading to the appearance of anemia and leukopenia; the presence of paraproteins and associated complications such as bone lesions, renal failure and hypercalcemia .



Kumar et al., Nature Reviews Disease Primers , Vol. 3, art. 17046 (2017)

Myeloma is usually preceded by Monoclonal Gammopathy of Uncertain Significance (MGUS). During progression, genetic alterations accumulate.

## Clinical importance of chromosomal alterations in Multiple Myeloma

Chromosomal alterations are of great clinical importance, as they influence diagnosis, prognosis, risk stratification and response to treatment[2]. The key points of their relevance are described below:

### Diagnosis and pathogenesis :

Chromosomal alterations are fundamental in the development of MM. They are divided into primary and secondary events. Primary events contribute to the immortalization of plasma cells, and secondary events to the progression of the disease.

**Primary genetic abnormalities**:

In the primary phase of the disease, genetic abnormalities can be divided into two large groups:

- Hyperdiploid myeloma: involves rearrangements of the *IGH* gene (14q34) with several oncogenes, including *FGFR3, CCND3, CCND1, MAF* and *MAFB* on chromosomes 4, 6, 11, 16 and 20, respectively. These primary rearrangements juxtapose these oncogenes to the IGH enhancer region.
- Hyperdiploid myeloma: involves trisomies of chromosomes 3, 5, 7, 9, 11, 15, 19 and 21, along with a low prevalence of rearrangements *IGH* .

A small proportion of patients, IGH rearrangements and multiple trisomies are found .

**Secondary genetic abnormalities:**

Aberrant plasma cells undergo different stages of evolution over time. The genetic abnormalities that reflect this progression are:

- Deletion *(17p13): It is the most important* cytogenetic factor for prognosis, with a very negative effect on survival, more aggressive disease, greater prevalence of extramedullary disease .
- Chromosome 1 abnormalities (Loss of 1p and/or gain of 1q). Abnormalities in both arms of chromosome 1 are associated with shorter survival.
- *MYC* gene alterations (8q24). Late or secondary alteration in the genesis of MM, associated with adverse prognosis.

### Risk stratification:

Cytogenetic risk stratification mSMART developed by the Mayo Clinic (www.msmart.org)[3], classifies the different chromosomal abnormalities by risk group.

Mayo Clinic Risk Stratification for Multiple Myeloma (mSMART)

| Risk Group | Percentage of newly diagnosed patients with the abnormality |
|---|---|
| **Standard Risk** | 60% |
| Trisomies | |
| t(11;14) | |
| t(6;14) | |
| **High Risk** | 40% |
| t(4;14) | |
| t(14;16) | |
| t(14;20) | |
| del(17p) | |
| gain(1q) | |
| **Double-Hit myeloma**: Any 2 high risk factors | |
| **Triple-Hit myeloma**: Any 3 or more high risk factors | |

## Prognosis:

Patients with high-risk alterations tend to have shorter survival and faster disease progression. Standard or low-risk alterations have a better response to standard treatments, such as proteasome inhibitors or immunomodulatory agents .

To calculate the prognosis, several factors are considered, such as the **patient's characteristics** (comorbidities), the **tumor burden** (stage), the **biology of the disease** (cytogenetic alterations, increased lactate dehydrogenase (LDH) and presence of plasma cells in the blood), and the **response to treatment** .

Tumor burden is assessed by staging Durie-Salmon (DSS) and the International Staging System (ISS), based on serum β2-microglobulin and albumin values.

The biology of the disease is evaluated according to the presence or absence of the cytogenetic abnormalities considered high risk: t(4;14), t(14;16) and del(17p), the increase in lactate dehydrogenase ( LDH) and the presence of plasma cells in the blood.

In 2015, the International Myeloma Working Group (IMWG) published a revision of the ISS (R-ISS)[4], combining tumor burden and disease biology to achieve a personalized prognostic value for patient.

**Table 1.** Standard Risk Factors for MM and the R-ISS

| Prognostic Factor | Criteria |
|---|---|
| ISS stage | |
| I | Serum $\beta_2$-microglobulin < 3.5 mg/L, serum albumin ≥ 3.5 g/dL |
| II | Not ISS stage I or III |
| III | Serum $\beta_2$-microglobulin ≥ 5.5 mg/L |
| CA by iFISH | |
| High risk | Presence of del(17p) and/or translocation t(4;14) and/or translocation t(14;16) |
| Standard risk | No high-risk CA |
| LDH | |
| Normal | Serum LDH < the upper limit of normal |
| High | Serum LDH > the upper limit of normal |
| A new model for risk stratification for MM | |
| R-ISS stage | |
| I | ISS stage I and standard-risk CA by iFISH and normal LDH |
| II | Not R-ISS stage I or III |
| III | ISS stage III and either high-risk CA by iFISH or high LDH |

Abbreviations: CA, chromosomal abnormalities; iFISH, interphase fluorescent in situ hybridization; ISS, International Staging System; LDH, lactate dehydrogenase; MM, multiple myeloma; R-ISS, revised International Staging System.

Palumbo et al.(2015)

In 2022, the European Myeloma Network (European Myeloma Network) conducted a second revision of the ISS (R2-ISS)[5] where a risk scoring system was created and validated based on the different stratification factors already established in the R-ISS and added as a high-risk anomaly the gain of 1q.

To ensure uniform harmonization of staging criteria, only 4 cytogenetic markers widely available and considered high-risk are used in the R2-ISS [t(4;14),t(14;16), del(17p), and 1q gain].

International guidelines, however, recommend the study of other cytogenetic abnormalities to contribute to a personalized prognosis and therapeutic strategy[1,2,6,7].

### Treatment planning:

Stratification based on chromosomal alterations helps select personalized treatments [7]:
*High-risk patients may benefit from more intensive or combination therapies.
*The use of proteasome inhibitors such as Bortezomib is particularly beneficial for patients with del(17p) or(4;14).
*Patients with t(11;14) respond best to Venetoclax , a BCL-2 inhibitor.

### Monitoring of minimal residual disease (MRD):

Monitoring chromosomal alterations with techniques such as FISH (fluorescent in situ hybridization) is crucial to assess the persistence of tumor cells after treatment, in combination with flow cytometry study.

### Impact on research and development of new therapies:

The study of chromosomal alterations leads to the development of new drugs targeted at specific molecular alterations.

## Fluorescent in situ hybridization (FISH) in the cytogenetic study of multiple myeloma

Conventional cytogenetic analysis (karyotype), due to both a variable degree of plasma cell infiltration in the bone marrow and the low rate of proliferation of aberrant plasma cells, greatly underestimates the potential chromosomal abnormalities that can be found in MM.

Under these conditions, international guidelines recommend a method of enriching the sample in plasma cells (CD138+), followed by the fluorescent in situ hybridization technique (FISH), for the analysis of chromosomal alterations in interphase nuclei.

At Catlab , following these recommendations, we carry out the following procedure:

### Samples and Purification:

- It is worked on bone marrow aspirate samples with cytology counting of more than 10% of plasma cells.
- Plasma cells (CD138+) are purified by **negative selection** using monoclonal antibodies that bind to unwanted cells.
- Untouched CD138+ cells are collected by density gradient centrifugation.

### Cultivation and extraction:

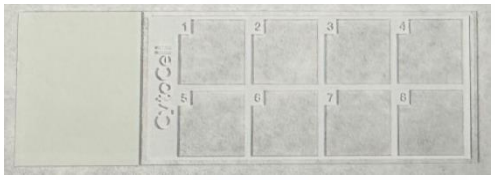
- The CPs are cultured for 72 hours and extraction is performed by cytogenetic procedure, obtaining cells in suspension.

### Hybridization:

- The suspended nuclei are spread on a slide and the FISH technique is performed: the material is labeled with specific fluorescent probes that emit light when they bind to the regions of the genome of interest.

- A **MultiFISH** panel is used , which allows the analysis, on a single slide, of the most relevant chromosomal alterations for the diagnosis and prognosis of MM:

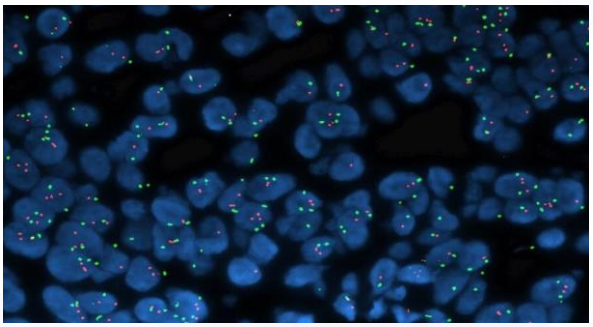
| |
|---|
| Hyperdiploidy ( chromosomes 5, 9 and 15) |
| *MYC* gene rearrangement |
| TP53 deletion . |
| t(14;20) rearrangement *IGH::MAFB* |
| t(4;14) rearrangement *IGH::FGFR3* |
| t(11;14) rearrangement *IGH::CCND1* |
| (14;16) rearrangement *IGH::MAF* |
| t(6;14) rearrangement *IGH::CCND3* |
| Gain 1q/ loss 1p . |



Portaobjectes MultiFISH (CytoCell)

### Analysis and interpretation:

With a fluorescence microscope, the fluorescence signals are visualized and the hybridization patterns obtained are interpreted.


Images of fluorescent signals in interphase nuclei

## Conclusion

In summary, multiple myeloma is a complex hematological disease with a wide variety of genetic alterations involved in its development. The FISH technique is a crucial tool to identify these alterations, and performing the MultiFISH technique routinely contributes to a more accurate diagnosis and a better therapeutic strategy. Thanks to the advancement in genetic techniques, such as FISH, clinicians can offer more personalized treatments and improve outcomes for patients with Multiple Myeloma.

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