INFORMATION FOR HEALTHCARE PROFESSIONALS ONLY

Cerba

Comprehensive biological monitoring of multiple Myeloma

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EXPERTISE AT LABORATOIRE CERBA

For 10 years, a number of national and international clinicians have entrusted the genetic and biological monitoring of MGUS (monoclonal gammopathy of unknown significance) for 20,000 of their patients to Laboratoire Cerba. Our laboratory also plays a major role in the evaluation of new treatments and in understanding the biology of these conditions, by treating more than 6,000 patients a year in international clinical trials. To do this, we strive to pool the expertise necessary for these special tests on a single platform, thus facilitating therapeutic management for clinicians, who continue to submit their biological and genetic tests to us on behalf of their patients. The main challenge is to define the best strategy for treating such diseases, symptomatic multiple myeloma especially.

At diagnosis, the clinical management of these conditions involves an increasing number of specialist tests, from biology to genetics, which thus determine the diagnostic and prognostic criteria and treatment strategy.

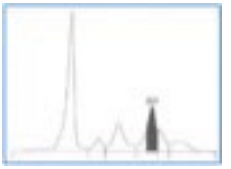
During therapeutic follow-up, these parameters are used to define the criteria for response to treatment according to the recommendations by the HAS and the IMWG, International Myeloma Working Group.

This document describes all of the tests carried out by Laboratoire Cerba for the treatment of multiple myeloma.

MONOCLONAL GAMMOPATHY EXPLORATION

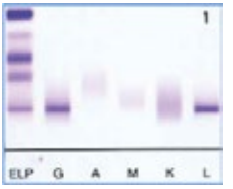
SERUM PROTEIN ANALYSIS BY CAPILLARY ELECTROPHORESIS

Analysis of the electrophoresis trace is used to look for a monoclonal immunoglobulin, characterised by the present of a straight peak. Quantification is carried out by orthogonal peak integration and it is necessary for the diagnosis, for evaluating treatment response and detecting potential relapse.



SERUM PROTEIN IMMUNOFIXATIONS

Carried out on agarose gel, immunofixation is used to confirm monoclonality and to determine the isotype (gamma, mu, alpha total kappa and total lambda anti-serums in first intention, and the more uncommon delta, epsilon, free kappa and free lambda anti-serums in second intention).
Pre-treatment with 2-mercaptoethanol is notably used to differentiate the various polymerisation states of monoclonal IgM.

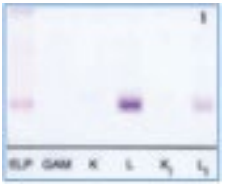


SERUM FREE KAPPA AND LAMBDA LIGHT CHAIN ASSAY (FREELITE®) WITH RATIO DETERMINATION

This test is recommended for the diagnosis and monitoring of treatment response in free light chain diseases and non- or low-secretory myeloma. Normalisation of the kappa/lambda ratio indicates a new stage of response to treatment.

URINE PROTEIN ELECTROPHORESIS

Urine protein electrophoresis by migration in HR (High Resolution) agarose gel can be prescribed to investigate pathological proteinuria, and can be used for the quantitative assessment of the Bence-Jones protein.



URINE PROTEIN IMMUNOFIXATION

On agarose gel, detection of Bence-Jones proteins is used to identify urine monoclonal free light chains.

G, A, M IMMUNOGLOBULIN WEIGHTED ASSAY

Turbidimetry is used to determine the status of other polyclonal immunoglobulins.

CRYOGLOBULIN TESTING

To demonstrate the potential cryoprecipitating effect of the monoclonal component.

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MONOCLONAL GAMMOPATHY EXPLORATION (CONTINUED)

HEVYLITE® TESTS

This test is used to assay immunoglobulin heavy and light chain pairs and to calculate the ratio between the monoclonal component and the residual immunoglobulins from the same class. This test is useful for quantifying the IgA which migrate into the electrophoretic zone of the beta-globulins, quantification of which is difficult by peak integration. An abnormal ratio is believed to predict shorter progression-free survival.

JOINT BETA-2 MICROGLOBULIN AND ALBUMIN ASSAY

For determination of the R-ISS (Revised International Staging System), prognostic evaluation tool recommended by the IMWG (International Myeloma Working Group) consensus committee.

STADE I

- Albuminaemia ≥ 3.5 g/dL
- Serum bêta-2 microglobulin< 3.5 mg/L
- Absence of unfavourable prognosis cytogenetic abnormality [t(4;14), t(14;16) or del (17p)]
- Normal serum Lactate Dehydrogenase (LDH) concentration

STAGE II

- All other situations that cannot be classified in Stage I or Stage III

STAGE III

2 criteria below:

- Serum bêta-2-microglobulin > 5.5 mg/L
- Presence of an unfavourable prognosis cytogenetic abnormality or abnormalities or elevated serum LDH concentration

CYTOLOGY AND IMMUNOPHENOTYPING

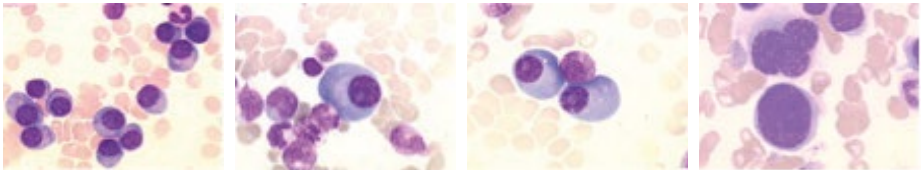
BLOOD COUNT

Detection of plasma cells on a blood smear. 15% of patients with myeloma have 1 to 3% circulating plasma cells. In 2% of cases, we detect plasmacytic leukaemia (plasma cells > 2 G/L or > 20%).

MYELOGRAPHY

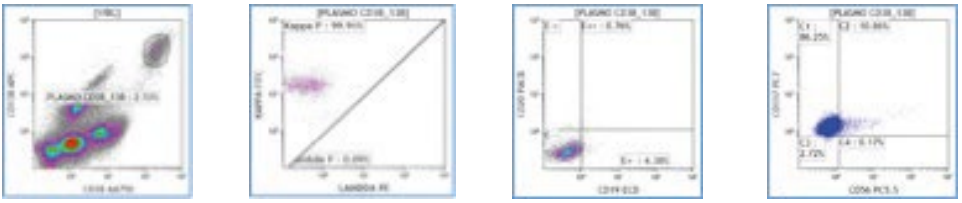
It shows bone marrow infiltration (>10% often dystrophic plasma cells).

Dystrophic plasma cells:
Appearance often heterogeneous, large plasma cells, gigantism, high nucleoplasmacytic ratio, nucleus abnormalities (uneven edges, loss of cluster structure: intermediate to fine chromatin, presence of nucleolus).



IMMUNOPHENOTYPING

CD38 and CD137 markers used to confirm the plasmacytic nature, to determine the monotypic character of the plasma cells studied and to study CD56 and CD117 prognostic markers.



MYELOMA CYTOGENETIC STUDIES

FISH ON CD138+ SORTED PLASMA CELLS

A first-intention cytogenetic test, selective plasma cell sorting by CD138 magnetic beads can be used to perform the interphase Fluorescence In Situ Hybridization (FISH) test on purified plasma cell preparations. A range of probes is available at the laboratory which is used to demonstrate stereotypical myeloma abnormalities for prognosis purposes. I.e. Probes P53 (17p13), LSI13 RB1 (13q14), IGH/FGFR3 t(4;14), IGH (14q32), IGH/CCND1t(11;14), IGH/MAF t(14;16), CKS1B/CDKN2C(P18) 1q amplification/1p deletion, study of chromosome 5,9 and 15 ploidy, 5q amplification detection.

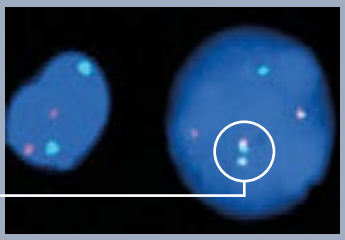
PLASMA CELL MAGNETIC SEPARATION

1 - Specific plasma cell marking: anti-CD138 Ab combined with a magnetic bead

2 - Washing

3 - Sample placed in a column with magnetic beads
Creation of a magnetic field
Collection of the negative fraction

4 - Creation of a magnetic field
Collection of the negative fraction



4p16: red fluorochrome - 14q32: green fluorochrome t(4:14)(p16:q32)t

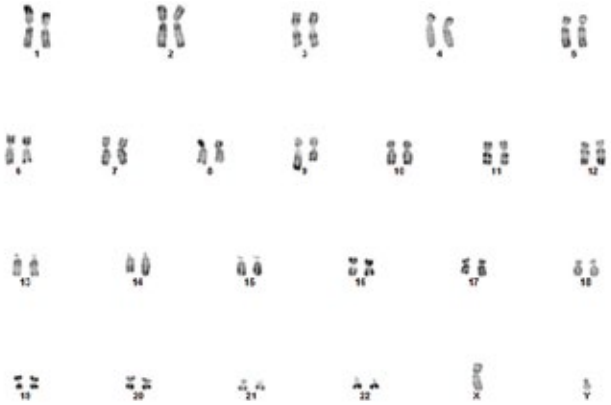
BONE MARROW KARYOTYPE

Reflecting plasma cell proliferation, the bone marrow karyotype is used to demonstrate clonal chromosome aberrations in 30% of myeloma cases, and especially the more advanced the disease. Chromosome aberrations may concern:

- the number: non-random gain in chromosomes 3, 5, 7, 9, 11, 15, 19 and 21 often seen in hyperdiploid karyotypes (50-55% of cases), monosomy of chromosomes 13, 14, 16 and 21 often observed in hypodiploid karyotypes, of favourable prognosis.

- the structure, often involving IGH loci in 14q32, P53 in 17p13, chromosomes 1 (1q amplification and 1p deletion) and 13.

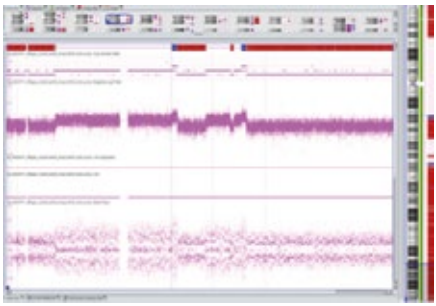
The potential increased plasma volume in bone marrow samples and the low mitotic index of plasma cells without effective mitogenic agents, are limiting factors to this technique, which requires lengthy culture (96h) in order to encourage plasma cell division and production of mitoses.



Example of a pseudodiploid and highly complex karyotype with chromosome aberrations, including chromosome 1 rearrangement and t(8;14) translocation, involving the MYC locus in 8q24 and IGH locus in 14q32.

SNP-ARRAY

New molecular cytogenetic techniques on DNA chip, Single Nucleotide Polymorphism array (SNP-array) or Cytoscan HD array performed on CD138+ sorted plasma cells, are used to detect abnormalities in the number of copies of a gene/chromosome region (deletion, gains, amplifications etc.) and to more accurately determine the multiple myeloma prognosis. In 2015, a meta-analysis with a genome-wide association study (GWAS) of more than 1,500 patients, demonstrated a significant association between the polymorphisms of the FOPNL locus on chromosome 16p13 and survival of patients with MM. This recent data will further improve the prognostic stratification of myeloma patients.



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