Myeloma MRD by deep sequencing from circulating tumor DNA does not correlate with results obtained in the bone marrow

Short title: ctDNA for evaluating MRD in myeloma

Céline Mazzotti[1], Laure Buisson[1], Sabrina Maheo [1], Aurore Perrot[2], Marie-Lorraine Chretien[3], Xavier Leleu[4], Cyrille Hulin[5], Salomon Manier[6], Benjamin Hébraud[1], Murielle Roussel[1], Laura Do Souto[1], Michel Attal[1], Hervé Avet-Loiseau[1] and Jill Corre[1]

[1]IUC-Oncopole and CRCT INSERM U1037, Toulouse, France, [2]Hematology Department, University Hospital, Nancy, France, [3]Hematology Department, University Hospital, Dijon, France, [4]Hematology Department, University Hospital, Poitiers, France, [5]Hematology Department, University Hospital, Bordeaux, France, [6]Hematology Department, University Hospital, Lille, France

Corresponding author: Jill Corre, Unit for Genomics in Myeloma, Institut Universitaire du Cancer – Oncopole, 1 avenue Irène Joliot-Curie 31100 Toulouse, France; phone: +33 531 156 173; fax: +33 531 156 227; e-mail: corre.jill@iuct-oncopole.fr

SUPPLEMENTAL DATA

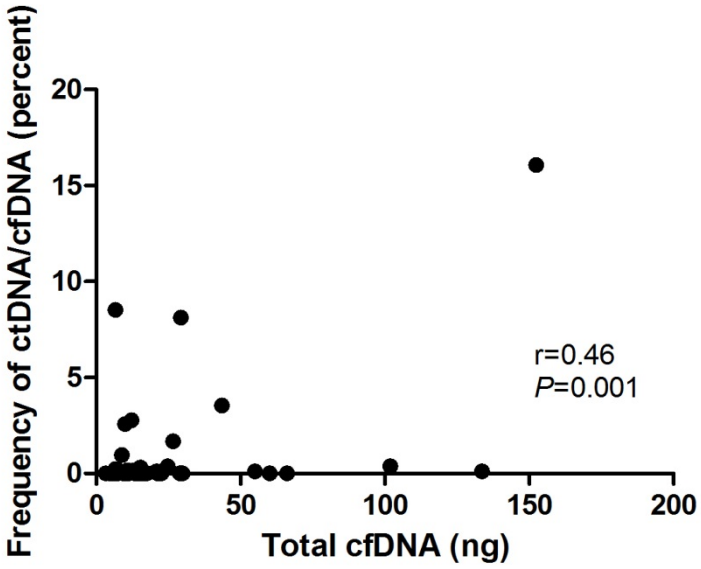
Supplemental methods

The study was approved by the Toulouse Ethics Committee and written informed consent was obtained for all the patients included. Paired bone marrow and blood samples were obtained from 10 patients at diagnosis and 37 patients during follow-up (Supplemental

Table). At diagnosis, plasma cells were isolated from bone marrow (collected in EDTA tubes) using CD138+ MAC-Sorting (Miltenyi Biotec, Paris, France). Post-sorting purity was systematically checked by May-Grünwald-Giemsa staining and cytological analysis of a spin from positive fraction. Only samples with more than 80% of plasma cells after sorting were kept for DNA extraction. DNA was then extracted with a Nucleospin tissue Kit (Macherey Nagel, Hoerdt, France). The clonal immunoglobulin genes rearrangements (*IGH*, *IGK*, *IGL*) were identified using Adaptive's NGS MRD Assay (Adaptive Biotechnologies, Seattle, WA, USA). For minimal residual disease quantification, DNA was directly extracted from the mononuclear layer of bone marrow samples and amplified by polymerase chain reaction using immunoglobulin gene-specific primers; the amplified products were then sequenced. A range of cells (~700,000 - $3x10^6$) were sequenced, allowing for 10^{-6} sensitivity when sufficient number of cells were present. Blood samples were directly collected in 2 ccfDNA Paxgene tubes (Qiagen, Hilden, Allemagne) and centrifuged as soon as received. Plasma obtained were used for cfDNA extraction, performed with the QIAamp Circulating Nucleic Acid Kit (Qiagen). cfDNA fragments were evaluated using the Tapestation 2200 (Agilent, Santa Clara, CA, USA), with High Sensitivity and genomic DNA reagents, and the obtained profiles are similar to what is known for cfDNA (a major proportion at 175bp and sometimes fragments at 350 and 500bp or more (but <1000bp), illustrating di- and tri-nucleosomes). A further fluorimetric quantitation was realized with a low range of standard provided in the Picogreen kit (Life Technologies, Carlsbad, CA, USA). The median cfDNA concentration was 10.5ng/mL (range 3.3-116.7) at diagnosis and 6.1 (range 4-48.2) during follow-up. Seven to 200ng of cfDNA was sent to Adaptive Biotechnologies, where same technique we performed in the bone marrow was applied. Clonotypes frequencies within bone marrow samples were determined by calculating the number of templates for each clonotype divided by the total

nucleated cells. In plasma, clonotype frequencies were determined by calculating the number of clonotype templates per mL of plasma.

Supplemental Figure 1. Relationship between myeloma ctDNA frequency and total cfDNA analyzed. r= Pearson's correlation coefficient.



Supplemental Table 1. Patient's population characteristics and detailed results obtained from bone marrow and plasma samples.