Am J Cancer Res 2021;11(9):4438-4454
www.ajcr.us /ISSN:2156-6976/ajcr0136158

Original Article

Predictive value of 1q21 gain in multiple myeloma is strongly dependent on concurrent cytogenetic abnormalities and first-line treatment

Alfredo Minguela[1], María A Vasco-Mogorrón[1], José A Campillo[1], Valentin Cabañas[2], María J Remigia[3], Mercedes Berenguer[4], María C García-Garay[2], Miguel Blanquer[2], Catalina Cava[5], José Antonio Galian[1], Lourdes Gimeno[1,6], María F Soto-Ramírez[1], María D Martínez-Hernández[1], Javier de la Rubia[3], Ana I Teruel[3], Manuel Muro[1], Adela Periago[5]

[1]Immunology Service, Clinic University Hospital Virgen de la Arrrixaca (HCUVA), Biomedical Research Institute of Murcia (IMIB), Murcia, Spain; [2]Hematology Service, Clinic University Hospital Virgen de la Arrrixaca (HCUVA), Biomedical Research Institute of Murcia (IMIB), Murcia, Spain; [3]Department of Hematology, University Hospital La Fe and School of Medicine and Dentistry, Catholic University of Valencia, Valencia, Spain; [4]Hematology Service, General University Hospital Santa Lucía, Biomedical Research Institute of Murcia (IMIB), Murcia, Spain; [5]Hematology Service, General University Hospital Rafael Méndez, Biomedical Research Institute of Murcia (IMIB), Murcia, Spain; [6]Human Anatomy Department, Medicine Faculty, Murcia University, Biomedical Research Institute of Murcia (IMIB), Murcia, Spain

Received June 8, 2021; Accepted July 28, 2021; Epub September 15, 2021; Published September 30, 2021

Abstract: Improved therapies in multiple myeloma (MM) have forced a constant risk stratification update, first Durie-Salmon, then international scoring systems (ISS), next revised-ISS (RISS) including high-risk cytogenetic abnormalities (HRCAs) such as del(17p) and t(4;14), and now R2-ISS including 1q21 gain has been proposed. Predictive value of 1q21 gain by itself or in concurrence with other cytogenetic abnormalities is evaluated in 737 real-world plasma cell neoplasm (PCN) patients under current therapies. Ten-year progression-free survival (10y-PFS) rates for patients with 2, 3 and >3 copies of 1q21 were 72.2%, 42.5% and 43.4% ($P<1.1\times10^{-17}$). Cox regression analysis confirmed that 1q21 gain was an independent prognostic factor for PFS (HR=1.804, P<0.0001, Harrell C-statistic =0.7779±0.01495) but not for OS (P=0.131). Gain of 1q21 was strongly associated with hypodiploidy (38.8% vs. 7.0%, $P=1.3\times10^{-22}$), hyperdiploidy (44.1% vs. 16.4%, $P=1.6\times10^{-13}$), HRCAs (12.6% vs. 3.5%, 1.8×10^{-5}), IGH breaks (12.3% vs. 2.1%, $P=2.1\times10^{-7}$) and del(13q) (8.0% vs. 4.0%, P=0.031). In our series, 1q21 gain by itself did not improve RISS predictive capacity in patients either eligible or ineligible for autologous stem cell transplantation (ASCT). However, compared with patients with other 1q21 gains: concurrence with hyperdiploidy improved the prognosis of ASCT-eligible patients from 62.5% to 96.0% 10-year overall-survival (10y-OS, P<0.002); concurrence with hypodiploidy improved the prognosis of ASCT-ineligible patients from 35.7% to 71.0% (P=0.013); and concurrence with del(13q) worsened the prognosis of ASCT-ineligible patients from 12.5% to 53.4% (P=0.035). Gain of 1q21 should be patient-wisely evaluated, irrespective of the RISS, considering its concurrence with other cytogenetic abnormalities and eligibility for ASCT.

Keywords: High-risk cytogenetic abnormalities, 1q21 gain, del(17p), t(4;14), plasma cell neoplasm, multiple myeloma, autologous stem cell transplantation, immunomodulatory and proteasome inhibitor treatments

Introduction

Novel first-line treatments in Multiple Myeloma (MM) combining immunomodulatory drugs (lenalidomide, thalidomide, or pomalidomide, -IMiDs-), proteasome inhibitors (bortezomib, carfilzomib, or ixazomib, -PIs-), monoclonal antibodies such as daratumumab and tandem autologous stem cell transplantation (ASCT) have increased the rate of complete response (CR) and have prolonged treatment-free and overall survival (OS) periods [1]. This has forced a constant update of the risk stratification criteria in these patients, from the Durie-Salmon staging system in 1978 [2], the international score system (ISS) in 2005 [3], to the revised

ISS (RISS) in 2015, which corrected the negative impact of high-risk cytogenetic abnormalities (HRCAs) such as the deletion of the 17p region -del(17p)- and t(4;14)-translocation IGH/FGFR3-detected by fluorescent *in situ* hybridization (FISH) [4]. Nonetheless, regardless of the type of treatment, MM shows substantial clinical heterogeneity in presentation and course within the same risk group, thus underlining the need for meaningful markers that allow us to adapt the therapy not only to the patient's biological and clinical conditions, but also to the real risk of the disease [5, 6]. For this reason, at the end of 2020, an update of the RISS (R2-ISS) was proposed [7] in order to correct the poor prognosis induced by chromosome 1 (chr1) abnormalities, specifically the gains of 1q21 region which increases the copy number of the cyclin kinase subunit 1B (CKS1B) gene.

Multiple myeloma (MM) is a genetically complex and heterogeneous neoplasm in which the concurrency of multiple genomic events leads to tumor development and progression. Genetic abnormalities in MM can be categorized into three types: chromosomal translocations, copy-number abnormalities (CNAs), and point mutations [8]. Immunoglobulin heavy chain gene (IGH, 14q32) translocations involving CCND1 (11q13), CCND3 (6p21), FGFR3/NSD2 (4p16), MAF (16q23) and MAFB (20q11) oncogenes are present in up to 50% of patients, and are considered key initiating events. CNAs most frequently occur due to gains (typically of odd chromosomes) and losses (most frequently chromosomes 13, 14, 16 and 22) of complete chromosomes, thus leading to hyperdiploidy in 50 to 60% of all MM cases or hypodiploidy in more than 50% of cases, respectively. Monosomy is more common in patients with non-hyperdiploid MM [9]. The long arm of chromosome 1 is gained (3 copies) or amplified (>3 copies) in nearly 50% of newly diagnosed MM patients and in up to 68% of relapsed/refractory cases. Deletion of 13q (del(13q)) is observed in up to 15% of cases. An intriguing opposite effect of monosomy 13 (favorable) and del(13q) (unfavorable) has been described in newly diagnosed MM patients treated with PIs and/or IMIDs [10]. Deletion of 17p13 (del(17p)) ranges from 5 to 12% in new diagnosed MM patients. This deletion entails the loss of the TP53 gene, which is a key tumor suppressor gene, and as a consequence is associated with very bad prognosis. Finally, whole-genome and whole-exome sequencing has led to the detection of 60 mutated genes which are considered driver genes [11].

Cytogenetic abnormalities involving the short and long arms of chr1 (gains, deletions, and balanced or jumping translocations) are regarded as major prognostic factors in MM and their presence is associated with unfavorable disease course, and shorter progression-free survival (PFS) and OS [12-17]. Chr1 abnormalities are also associated with higher frequency of immunoglobulin A (IgA) subtype, higher RISS stage, and concurrent HRCAs, especially monosomy 13 or del(13q) and del(17p) [13, 18, 19]. Clustering of such high-risk features among these patients may account for their unfavorable outcomes. Although the magnitude of the effects of chr1 abnormalities relative to other cytogenetic alterations is still uncertain, some results indicate that concurrent genetic abnormalities such as monosomy13/del(13q), del(17p) or t(4;14) significantly worsen the poor prognosis associated with 1q21 gain in MM patients [14, 19, 20]. Contradictory results about the impact of 1q21 CNAs on patient survival have also been reported. Some studies reported similar PFS and OS in MM patients with 3 or more than 3 copies [21-23], but others reported that PFS and OS were worse in patients with >3 copies of 1q21 [17, 24, 25].

Although it was suggested that chemotherapy regimens based on thalidomide, but not bortezomib, could overcome drug resistance and ameliorate the negative impact of 1q21 gain [21], subsequent studies reported that neither novel agent combinations nor ASCT can mitigate its adverse impact [13, 15, 16, 19, 26]. Over-expression of CKS1B gene due to 1q21 gains activates both MEK/ERK and JAK/STAT3 signaling pathways and promotes myeloma cell survival, drug-resistance and cancer progression [27]. The combination of specific inhibitors of STAT3 and MEK/ERK signaling pathways induces significant MM cell death and growth inhibition in CKS1B-overexpressing patients, suggesting that the outcome of these patients could improve if these drugs were implemented in the clinical practice. In the same line, it has been shown that NEDD8 inhibition overcomes both the toxicity associated with global protea-

somal inhibition and CKS1B-induced drug resistance, which provides a rationale for targeting the NEDD8 pathway in MM patients with 1q21 gain [28]. Besides, 1q21 gain promotes tolerance of genomic instability and drives resistance to DNA-damaging agents by inducing overexpression of the transcription factor interleukin enhancer binding factor 2 (ILF2). Optimizing the use of DNA-damaging agents in these MM patients could also contribute to improving their outcome [29].

Until these novel treatments can offer better opportunities to MM patients who overexpress CKS1B, it is necessary, on the one hand, to ascertain if regimens using novel agents can mitigate the adverse impact of Chr1 abnormalities, and on the other hand, to take into account all high-risk cytogenetic alterations [7, 30], in order to optimize risk stratification and treatment assignment for each patient. This study evaluated different controversial aspects regarding the impact of 1q21 gains on patient outcome on a large series of unselected real-world plasma cell neoplasm (PCN) patients. The predictive value of 1q21 gain was assessed in the outcome of asymptomatic and symptomatic PCNs. The copy number of 1q21 and its concurrence with other cytogenetic alterations were analyzed for current treatments, including IMiDs, PIs and ASCT, and confirmed in an independent series. Finally, the contribution that 1q21 gain can make to the RISS in MM under modern therapies was analyzed.

Materials and methods

Patients and samples

EDTA anti-coagulated bone marrow (BM) samples were obtained at diagnosis from 737 consecutive patients with PCNs in regular clinical practice from 7 hospitals in the Region of Murcia, Spain, as described previously [5, 6]. BM samples were also obtained for minimal residual disease (MRD) assessment 3 and 6 months after ASCT, and under the suspicion of loss of CR [5, 6]. Patients were enrolled between 2010 and 2017 and followed-up until March 2021. This study was approved by the Research Ethics Committee, Institutional Review Board (IRB-00005712). Written informed consent was obtained from all patients accordingly.

Following the international myeloma working group (IMWG) criteria [31], patients were classified in 352 monoclonal gammopathy of undetermined significance (MGUS), 64 smoldering multiple myeloma (SSM), and 321 MM. Mean follow-up for each stage were 55.2±27.3, 64.9±33.7 and 48.1±33.5, respectively. Standard risk stratification (sRisk) in MGUS (0 vs. 1-2 vs. 3 risk factors) [32], SMM (0 vs. 1 vs. >1 risk factors) [33] and MM (RISS-I vs. RISS-II vs. RISS-III) [31] was applied following updated criteria and patients were classified as low, intermediate, and high risk. In MGUS, disease progression was computed when it progressed to SMM or MM; in SMM, progression was computed when it progressed to symptomatic MM [5, 6]. MGUS and SMM patients progressing to symptomatic MM were treated as MM patients [5, 6]. In MM, progression, CR and relapse were estimated following the Uniform Response Criteria for Multiple Myeloma of the IMWG [34, 35]. Treatments and management were at the discretion of the hematologists based on patient condition and tumor risk. Briefly, conventional first-line therapy for patients who were not eligible for ASCT included bortezomib, melphalan and prednisone (VMP), bortezomib and dexamethasone (Vd) or more recently lenalidomide and dexamethasone (Rd). In ASCT-eligible patients, first-line therapy included bortezomib, cyclophosphamide and dexamethasone (VCD) or bortezomib, doxorubicin and dexamethasone (PAD) or more recently bortezomib, thalidomide and low-dose dexamethasone (VTd) or bortezomib, lenalidomide and low-dose dexamethasone (VRd), and ASCT conditioning with melphalan 200 mg/m^2 (dose ranging from 200 to 100-140 mg/m^2 if renal impairment) [5, 6].

A confirmatory series of 43 MM patients under VTd and ASCT regimen was included from the Group of Myeloma Studies of the Valencian Community (GREMI).

Immunophenotyping and minimal residual disease (MRD) monitoring

Immunophenotype of bone marrow plasma cells (BMPC) and MRD analyses were performed in a minimum of 2×10^6 white cells, with FACSCanto-II and DIVA-Software (Becton Dickinson; BD; San Jose, CA, USA) following consensus criteria [36-38], previously validat-

ed [5, 6, 39] and described in more detail in Supplementary Figure 1. Briefly, total PCs were identified as $CD38^{+++}CD138^{+/++}$ events and aberrant PC as $CD45^{low/negative}$ and/or $CD19^{low/negative}$ and/or $CD20^{+}$ and/or $CD27^{low/negative}$ and/or $CD56^{+}$ and/or monoclonal restriction for the heavy and/or light immunoglobulin chains [5, 6].

Fluorescent in situ hybridization (FISH)

Cytogenetic abnormalities were evaluated in interphase nucleus from BMPCs purified using RosetteSep® Human Multiple-Myeloma-Cell Enrichment Cocktail (Stemcell Technologies, Grenoble, France) and previously validated methods [5, 6]. The following FISH probes from Metasystems (Altlussheim, Germany) were used to evaluate the different abnormalities: translocations of the IGH region with break-apart IGH probe (cut-off: 3%) and with dual fusion probes to determine the most common IGH partners CCND1 (cut-off: 2%), FGFR3 (cut-off: 2%), MAF (cut-off: 2%) and MAFB (cut-off: 2%); copy number of chromosomes 5, 9 and 15 with 5p15/9q22/15q22 hyperdiploidy probes (cut-off: 10%); amplification/deletion of 17p13 (TP53) and 17q22 (LPO/MPO) with locus-specific probes (cut-off: 10%); amplification/deletion of 1q21-22 (CKS1B) and 1p32.3 (CDKN2C) with locus specific probes (cut-off: 10%), and monosomy-13/deletion 13q14.2 (DLEU1) and 13q34 (LAMP1) with locus specific probes (cut-off: 10%).

For each probe 300 plasma cells were analyzed with Metafer (Metasystems). Gain of 1q21 was defined when there were >2 CKS1B signals: 3 signals (3 copies, duplication) or >3 signals (>3 copies, amplification). Deletion of 13q was defined as one DLEU1 signal and two LAMP1 signals, and monosomy-13 as one signal of each gene [10]. Hyperdiploidy was defined with more than 3 signals of chromosomes 4, 5, 9, 11, 14, 13, 15, 16, 17 and/or 20. Hypodiploidy was defined with one signal of chromosome 1, 4, 5, 9, 11, 14, 13, 15, 16 and/or 20. One TP53 signal (either for deletion of 17p13 or monosomy-17) was computed as del(17p).

Statistical analysis

Statistical analyses were performed using the SPSS version 15.0 (SPSS Inc, Chicago, IL) and following previously described methods [5, 6]. Analysis of variance (ANOVA) and least significant difference (LSD) post hoc tests were used to analyze continuous variables. Progression-free survival (PFS) was estimated as months from the diagnosis date to disease progression or death. Survival curves were plotted according to the Kaplan-Meier method. The log-rank test was used to estimate significant differences. Multivariate analyses of prognostic factors for PFS and OS were performed using the Cox proportional hazards model (stepwise regression). Hazard ratio (HR) and 95% confidence interval were estimated. Harrell C-statistic was obtained using STATA-14 (Somersd package). $P<0.05$ was considered statistically significant.

Results

Patient characteristics

Table 1 summarizes the biological and clinical characteristics as well as treatments of symptomatic patients. MGUS, SMM and MM patients showed ten-year PFS rates (10y-PFS) of 86.4%, 50.0% and 40.6% ($P=2.2\times10^{-29}$) and 10y-OS rates of 90.8%, 75.0% and 54.5% ($P=1.2\times10^{-21}$), respectively (Supplementary Figure 2A). According to the standard risk stratification, low, intermediate and high risk MGUS patients showed 10y-PFS of 94.4%, 77.9% and 63.6% ($P=5.1\times10^{-6}$) and 10y-OS rates of 96.3%, 85.5% and 72.7% ($P=1.3\times10^{-4}$); SMM patients showed 10y-PFS rates of 58.3%, 50.0% and 20.0% ($P=0.05$) and 10y-OS of 87.5%, 75.0% and 40.0% ($P=0.01$); and MM patients showed 10y-PFS rates of 55.9%, 39.0% and 15.6% ($P=2.8\times10^{-8}$) and 10y-OS rates of 83.8%, 50.0% and 18.7% ($P=6.0\times10^{-17}$) (Supplementary Figure 2B). A total of 369 patients required treatment, including MM patients as well as MGUS and SMM that progressed to symptomatic MM, 238 ASCT-ineligible and 131 ASCT-eligible patients, 58 treated with PAD or VCD, and 73 with VTd or VRd.

Duplication and amplification of 1q21 showed similar adverse impact

First, we assessed the type (**Figure 1A**) and frequency (**Figure 1B**) of chr1 abnormalities in PCN stages. No abnormalities (2 copies), duplication (3 copies), amplification (more than 3 copies) of 1q21, and deletion of 1p32 (without 1q21 gain) were observed in 90.9%, 3.7%,

Table 1. Baseline characteristics of patients at the time of diagnosis

	MGUS (n=352)	SMM (n=64)	MM (n=321)
Demographic, biochemical and immunological characteristics			
Age, years, Mean ± SD	67.9±12.2	68.5±12.1	68.6±11.8
Female, n (%)	165 (46.9%)	40 (62.5%)	151 (47.0%)
Hemoglobin, g/dL, Mean ± SEM	14.3±5.7	12.9±2.51	10.8±1.7***
Serum calcium, g/dL, Mean ± SEM	9.44±0.04	9.47±0.11	9.71±0.09**
Serum creatinine, mg/dL, Mean ± SEM	1.19±0.07	1.14±0.07	1.74±0.13*
Serum albumin <3.5 g/dL, n (%)	34 (9.65%)	8 (12.5%)	103 (32.1%)***
Serum b2-microglobulin ≥3.5 mg/dL, n (%)	103 (29.3%)	20 (31.25%)	190 (59.1%)***
LDH ≥ upper limit of normal, n (%)	59 (16.8%)	10 (15.6%)	75 (23.4%)
Serum M-protein, g/dL, Mean ± SEM	1.05±0.08	1.69±0.13*	2.83±0.21***
Bence Jones protein, n (%)	116 (32.9%)	33 (51.5%)*	202 (62.9%)***
Free light chain ratio >20, n (%)	119 (33.8%)	35 (54.7%)**	212 (66.0%)***
IgG gammopathy, n (%)	248 (70.4%)	36 (56.2%)*	156 (48.6%)***
Immunoparesis, n (%)	121 (34.4%)	38 (59.3%)**	263 (91.9%)***
Bone marrow plasma cell (BMPC) counts			
Total BMPC histology, % (Mean ± SEM)	4.72±0.16	16.86±1.5***	34.52±1.6***
Total BMPC flow cytometry, % (Mean ± SEM)	1.09±0.09	3.55±0.54	13.11±1.5***
Fluorescent in situ hybridization (FISH) on purified BMPCs			
del(17p), n (%)	3 (0.85%)	3 (4.7%)*	23 (7.2%)***
1q21 gain, n (%)	32 (9.1%)	14 (21.9%)**	142 (44.2%)***
t(4;14), n (%)	1 (0.28%)	2 (3.1%)	13 (4.05%)***
t(14;16), n (%)	1 (0.28%)	0 (0.0%)	7 (2.2%)*
t(11;14), n (%)	5 (1.4%)	2 (3.1%)	26 (8.1%)***
Other IGH breaks	8 (2.3%)	1 (1.56%)	25 (10.2%)***
del(13q), n (%)	8 (2.3%)	3 (4.7%)	26 (8.1%)***
Monosomy 13	7 (2.0%)	5 (7.8%)*	74 (23.1%)***
Hypodiploidy, n (%)[1]	11 (3.13%)	6 (9.4%)*	94 (29.3%)***
Hyperdiploidy, n (%)[2]	29 (8.2%)	13 (20.3%)**	127 (39.6%)***
Clinical characteristics			
Osteolytic lesions, n (%)	9 (2.5%)	4 (6.25%)	160 (49.8%)***
Renal insufficiency, n (%)	93 (26.4%)	15 (23.4%)	106 (33.0%)*
Additional cardio-respiratory diseases, n (%)	89 (25.2%)	16 (25.0%)	87 (27.1%)
Additional endocrine diseases, n (%)	79 (22.4%)	17 (26.5%)	69 (21.5%)
Additional rheumatologic diseases, n (%)	37 (10.5%)	4 (6.25%)	13 (4.0%)
Additional oncological malignances, n (%)	25 (7.1%)	8 (12.5%)	27 (8.4%)
Additional hematological diseases, n (%)	15 (4.3%)	5 (7.8%)	29 (9.03%)
Risk stratification Low/Intermediate/High, n[3]	186/151/15	29/29/6	86/184/51
Treatments[4]			
No ASCT with VMP, Vd or Rd, n (%)	20 (5.6%)[5]	11 (17.2%)[5]	207 (64.5%)
ASCT with PAD or VCD, n (%)	0 (0.0%)	3 (4.6%)[5]	55 (17.1%)
ASCT with VTd or VRd, n (%)	2 (0.6%)[5]	12 (18.7%)[5]	59 (18.4%)

[1]Hypodiploidy: one chromosome 1, 4, 5, 9, 11, 14, 13, 15, 16 and/or 20. [2]Hyperdiploidy: more than two chromosomes for 4, 5, 9, 11, 14, 13, 15, 16, 17 and/or 20. [3]Risk stratification following standardized criteria for MGUS and SMM (score-0= low, 1= intermediate, and 2= high) [32, 33] and MM (RISS-I= low, II= intermediate and III= high) [31]. [4]ASCT: autologous stem cell transplantation; A: doxorubicin; C: cyclophosphamide; d: low-dose dexamethasone; M: melphalan, P: prednisone; V: bortezomib; R: lenalidomide; T: thalidomide. [5]MGUS and SMM patients who progressed to symptomatic PCNs and required treatment after first diagnosis. *P<0.05; **P<0.01 and ***P<0.001 MGUS vs. SMM or MGUS vs. MM (ANOVA and LSD tests or Chi square test).

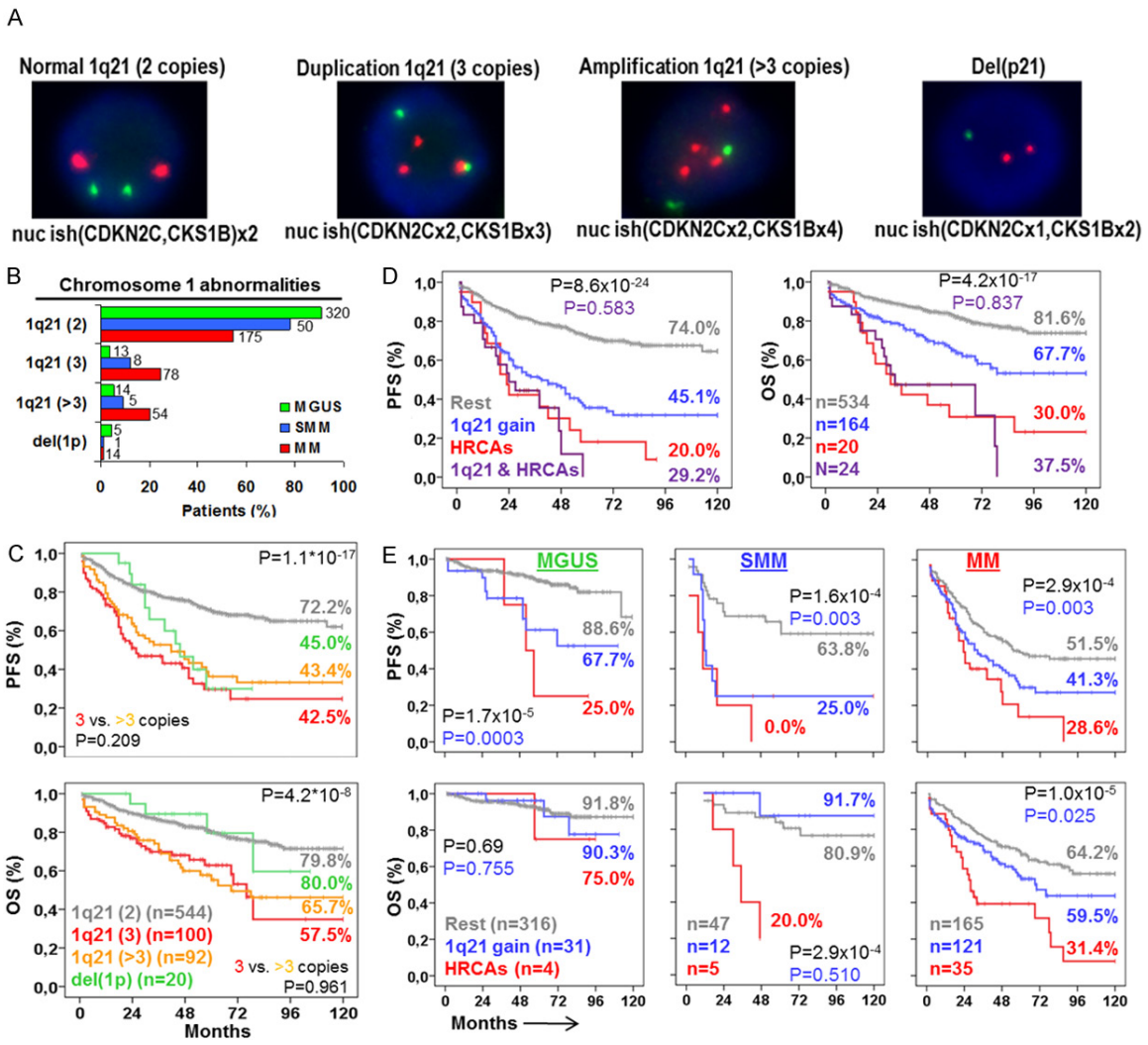


Figure 1. Predictive value of chromosome 1 abnormalities in asymptomatic and symptomatic PCNs. (A) Representative FISH images (left to right) of patients with normal status for 1q21-22 (CKS1B) and 1p32.3 (CDKN2C) regions (2 signals each), duplication of 1q21-22 (3 copies of CKS1B), amplification of 1q21-22 (>3 copies of CKS1B) and isolated deletion of 1p32.3 (1 copy of CDKN2C). Updated International System for Human Cytogenomic Nomenclature (ISCN) is indicated for each FISH image. (B) Frequency and number of patients with chromosome-1 abnormalities: no abnormalities -1q21 (2)-, duplication of 1q21 -1q21 (3)-, amplification of 1q21 -1q21 (>3)-, and deletion of 1p32 -del(1p)-. (C) Kaplan-Meier for progression-free survival (PFS) and overall survival (OS) according to chromosome-1 abnormalities in total PCN patients. *P* values in the Log-rank test for total patients or patients with 3 or >3 copies of 1q21 are shown. (D) Kaplan-Meier test for PFS and OS according to the concurrence of 1q21 gain and HRCAs -del(17p) and t(4;14)-. *P* values in the Log-rank test for total patients (black) or patients with 1q21 gain vs. 1q21 & HRCAs (violet) are shown. (E) Kaplan-Meier test for PFS and OS according to the type of PCN, the gain of 1q21 and the presence of HRCAs. *P* values in the Log-rank test for total patients (black) or patients with vs. without 1q21 gain (blue) are shown. Ten-year PFS and OS rates are shown for each group in (B-D).

5.4% and 4.4% of MGUS patients, in 78.1%, 12.5%, 9.4% and 1.6% of SMM patients, and in 54.5%, 24.6%, 20.0% and 1.4% of MM patients, respectively.

For patients without chr1 abnormalities, with 1q21 duplication, 1q21 amplification or del(1p), 10y-PFS rates were 72.2%, 42.5%, 43.4% and 45.0% ($P=1.1\times10^{-17}$) and 10y-OS rates were 79.8%, 57.5%, 65.7% and 80.0% ($P=4.2\times10^{-8}$). No significant differences were found between patients with duplication or amplification of 1q21 in 10y-PFS (P=0.209) or 10y-OS (P=0.961) rates. Patients with del(1p) alone (with-

out 1q21 gain) showed similar 10y-OS rate to patients without chr1 abnormalities (80.0% vs. 79.8%) (**Figure 1C**).

Next we analyzed the impact of the concurrence of HRCAs and 1q21 gain ("double-hit") on the outcome of total patients. Patients with HRCAs alone or in concurrence with 1q21 gain showed comparable 10y-PFS (20% vs. 29.2%, P=0.583) and OS (30.0% vs. 37.5%, P=0.837) rates (**Figure 1D**).

Finally, we evaluated the impact of 1q21 gain (more than 2 copies) in the outcome of PCN stages (**Figure 1E**). Patients without either HRCAs or 1q21 gain, compared with those with 1q21 gain or HRCAs, showed 10y-PFS rates of 88.6%, 67.7% and 25.0% ($P=1.7\times10^{-5}$) for MGUS; 63.8%, 25.0% and 0.0% ($P=1.6\times10^{-4}$) for SMM; and 51.5%, 41.3% and 28.6% ($P=2.9\times10^{-4}$) for MM patients. Therefore both 1q21 gain and HRCAs negatively impacted the PFS rates of the three PCN stages. However, in a similar study evaluating OS, only HRCAs showed an adverse effect in the three PCN stages, with 10y-OS rates for patients without either HRCAs or 1q21 gain, compared with those with 1q21 gain or HRCAs, of 91.8%, 90.3% and 75.0% (P=0.69) for MGUS; 91.7%, 80.9% and 20.0% ($P=2.9\times10^{-4}$) for SMM; and 64.2%, 59.5% and 31.4% ($P=1.0\times10^{-5}$) for MM patients. MM patients with 1q21 gain, but not MGUS or SMM patients, showed significantly lower 10y-OS rate than patients without these chr1 abnormalities (59.5% vs. 31.4%, P=0.025).

Association of 1q21 gain with other cytogenetic abnormalities

To discriminate the prognostic value of 1q21 gain, we first evaluated its association with other cytogenetic abnormalities in patients from all PCN stages. Comparing patients with and without 1q21 gain, significant association was found with hypodiploidy (38.8% vs. 7.0%, $P=1.3\times10^{-22}$), hyperdiploidy (44.1% vs. 16.4%, $P=1.6\times10^{-13}$), IGH breaks other than t(4;14), t(11;14) and t(14;16) (12.3% vs. 2.1%, $P=2.1\times10^{-7}$), t(4;14) (7.1% vs. 0.6%, $P=4.1\times10^{-6}$), del(13q) (8.0% vs. 4.0%, P=0.031) and del(17p) (6.6% vs. 3.3%, P=0.044). No association was found with t(11;14) or t(14;16) (**Figure 2A**). Cox regression analysis including all cytogenetic abnormalities confirmed that 1q21 gain (HR=1.804, P<0.0001, Harrell C-statistic =0.7779±0.01495) and del(17p) (HR=2.610, P<0.0001) were independent prognostic factors for PFS of patients from all PCN stages. However, del(17p) (HR=3.646, P<0.0001, Harrell C-statistic =0.8191±0.0164) and t(4;14) (HR=1.437, P=0.038), but not 1q21 gain (P=0.131), were independent prognostic factors for OS (**Figure 2B**).

To assess the influence of concurrent chromosomal abnormalities with 1q21 gain on patient outcome, patients were hierarchically classified in seven groups by prioritizing less frequent abnormalities (which were excluded from the successive groups): 1st, no 1q21 gain; 2nd, 1q21 gain alone (without other abnormalities); 3rd, 1q21gain+HRCAs; 4th, 1q21gain+del(13q); 5th, 1q21gain+IGHs-breaks other than t(4;14); 6th, 1q21gain+Hypodiploidy; and 7th, 1q21gain+Hyperdiploidy (**Figure 2C**, upper table). Patients with HRCAs, del(13q) and other IGH breaks in concurrence with 1q21 gain, compared with the other groups, showed higher numbers of BMPC in the immunophenotype study (21.6%±4.1, 16.5%±4.7 and 16.9%±2.7 vs. 8.35%±1.4, $P=2.7\times10^{-6}$), lower rates of stringent CR (sCR) with negative MRD (46.2%, 28.6% and 46.2% vs. 59.5%, P=0.055), higher relapse rates (80.0%, 100% and 87.5% vs. 42.7%, P=0.014), and lower 10y-PFS (33.3%, 6.1% and 25.0% vs. 51.2%, P=0.008) and 10y-OS (51.9%, 36.4% and 32.5% vs. 67.25%, P=0.004) rates. It should be noted that patients with 1q21 gain associated with hypodiploidy reached 100% negative MRD and showed the lowest rate of relapse (20%).

The Kapplan-Meier analysis for OS showed that no-1q21 gain, 1q21 gain alone, or 1q21 gain with hypodiploidy or hyperdiploidy had similar 10y-OS curves, but this curves were lower in patients with 1q21 gain concurrent with HRCAs, del(13q) and other IGH breaks (**Figure 2C**, lower graph).

Predictive value of 1q21 gain depends on first-line therapy

Next, we explored the impact of 1q21 gain, compared to HRCAs, on ASCT ineligible and eligible patients by evaluating the depth of response (sCR), the relapse of the disease after sCR, and the PFS and OS of patients (**Figure 3**).

A

Association of 1q21 gain with other cytogenetic abnormalities

	N	No 1q21	1q21 gain	P
Hypodiploidy[1]	111	7.0%	38.8%	**$1{,}3\times10^{-22}$**
Hyperdiploidy	169	16.4%	44.1%	**$1{,}6\times10^{-13}$**
Other IGH breaks	34	2.1%	12.3%	**2.1×10^{-7}**
t(4;14)	16	0.6%	7.1%	**4.1×10^{-6}**
del(13q)	37	4%	8%	**0,031**
del(17p)	29	3.3%	6.6%	**0,044**
t(11;14)	33	4.6%	4.9%	0.502
t(14;16)	8	0.9%	1.6%	0,34

[1] 87 out of 111 (77.7%) hipodiploid patients were monosomy 13

B

	Cox regression for PFS			Cox regression for OS		
	HR	95%CI	P	HR	95%CI	P
Sex	0.660	0.509-0.856	0.002	0.626	0.457-0.860	0.004
Age	1.051	1.037-1.064	0.001	1.092	1.072-1.112	0.001
NCP stage	1.986	1.675-2.353	0.001	2.336	1.883-2.899	0.001
1q21 gain	1.804	1,340-2.428	**0.001**	1.320	0.920-1.894	0.131
Hypodiploidy	0.744	0.517-1.069	0.110	0,867	0.564-1.334	0.517
Hyperdiploidy	1.144	0.862-1.517	0.351	0.936	0.699-1.392	0.936
Other-IGH	0.986	0.571-1.703	0.959	1.303	0.682-2.489	0.422
t(4;14)	1.437	0.734-2.812	0.290	2.233	1.043-4.799	**0.038**
del(13q)	1.279	0.810-2.021	0.291	1.400	0.825-2.373	0.212
del(17p)	2.610	1.638-4.1157	**0.001**	3.646	2.221-5.986	**0.001**

HR: hazard ratio; 95%CI: 95% confidence interval

C

Patients requiring treatment

	N	BMPC	sCR	Relapse	PFS	OS
	369	(%)	(%)	(%)	(%)	(%)
No 1q21 gain	195	8.1±0.9	55.2	45.2	47.0	63.6
1q21 alone	18	4.6±1.3	42.9	50.0	55.6	66.7
1q21 & Hypodip	29	10.6±1.9	100	20.0	51.7	72.4
1q21 & Hyperdip	49	10.1±1.7	40.0	55.6	50.6	66.3
1q21 & OtherIGH[1]	27	21.6±4.1	46.2	80.0	33.3	51.9
1q21 & del(13q)	11	16.5±4.7	28.6	100.0	9.1	36.4
HRCAs	40	16.9±2.7	46.2	87.5	25.0	32.5

[1] IGH breaks different to t(4;14)
BMPC: bone marrow plasma cells (%±SE)
sCR: stringent complete response

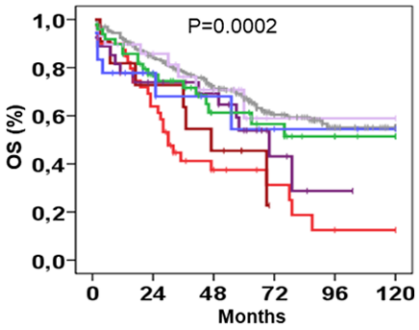


Figure 2. Association of 1q21 gain with other cytogenetic abnormalities and their impact on patient outcome. A. Association of 1q21 gain with other cytogenetic alterations. P estimated with the chi-squared test in patients from all PCN stages. B. Cox regression analysis of progression-free survival (PFS) and overall survival (OS) for sex, age, type of PCN and cytogenetic abnormalities. C. Hierarchical classification of patients that required treatment (n=369) according to the concurrence of chromosomal abnormalities with 1q21 gain. Upper table shows percentages of bone marrow plasma cells (BMPC) in the immunophenotype analysis, rate of complete response with negative minimal residual disease (nMRD), as well as relapse, 10y-PFS and 10y-OS rates of patient in these groups. Lower graph shows Kaplan-Meier and Log-rank tests for groups of patients with different cytogenetic abnormalities.

Higher rates of sCR were achieved in ASCT-eligible patients treated with PAD/VCD or VTd/VRd than in ASCT-ineligible patients, whether with HRCAs (75.0%, 50.0% and 28.6%), 1q21 gain (57.9%, 77.8% and 37.5%) or without these abnormalities (68.8%, 68.2% and 33.3%). In patients with HRCAs, only ASCT associated with VTd/VRd treatment sowed a lower relapse rate (50%) than the other 2 treatments (100%). Increasing rates of relapse were observed for ASCT-eligible patients treated with either VTd/VRd or PAD/VCD or for ASCT-ineligible patients whether with 1q21 gain (42.9%, 55.6% and 73.3%, respectively) or without this cytogenetic abnormality (24.0%, 52.6% and 66.7%, respectively) (**Figure 3A**).

Depending on the treatment but regardless of the cytogenetic alterations, 10y-PFS of ASCT-ineligible or ASCT-eligible patients treated with PAD/VCD or VTd/VRd were 32.4%, 38.5% and 63.6% ($P=9.1\times10^{-5}$) and 10y-OS were 46.0%, 67.7% and 87.2% ($P=4.7\times10^{-10}$) (**Figure 3B**).

For ASCT-ineligible patients, 10y-PFS of patients with HRCAs, 1q21 gain or neither HRCAs nor 1q21 gain (Rest) were 16.7%, 30.9% and 38.0% (P=0.01; P=0.014 between 1q21 gain and Rest) and 10y-OS were 25.0%, 37.0% and 48.8% (P=0.013, P=0.045 between 1q21 gain and Rest). Therefore, with these treatments, PFS and OS of patients with 1q21 gain were similar to those of patients with HRCAs (**Figure 3C**).

For ASCT-eligible patients treated with PAD or VCD, 10y-PFS of patients with HRCAs, 1q21 gain or Rest were 22.2%, 44.1% and 50.0% (P=0.113, P=0.616 between 1q21 gain and Rest) and 10y-OS were 33.3%, 79.4% and 81.3% (P=0.001, P=0.929 between 1q21 gain and Rest). Therefore, with this treatment, PFS and OS of patients with 1q21 gain were similar to those of patients without HRCAs (**Figure 3C**).

For ASCT eligible patients treated with VTd or VRd, 10y-PFS of patients with HRCAs, 1q21

A

	Stringen CR with negative EMR (%)				Relapse (%)			
	Total	HRCAs	1q21 gain	Rest	Total	HRCAs	1q21 gain	Rest
ASCT (VTd or VRd)	65.5	75.0	57.9	68.8	34.9	50.0	42.9	24.0
ASCT (PAD or VCD)	68.4	50.0	77.8	68.2	57.1	100	55.6	52.6
No-ASCT (VMP, Vd or Rd)	35.8	28.6	37.5	33.3	71.1	100	73.3	66.7

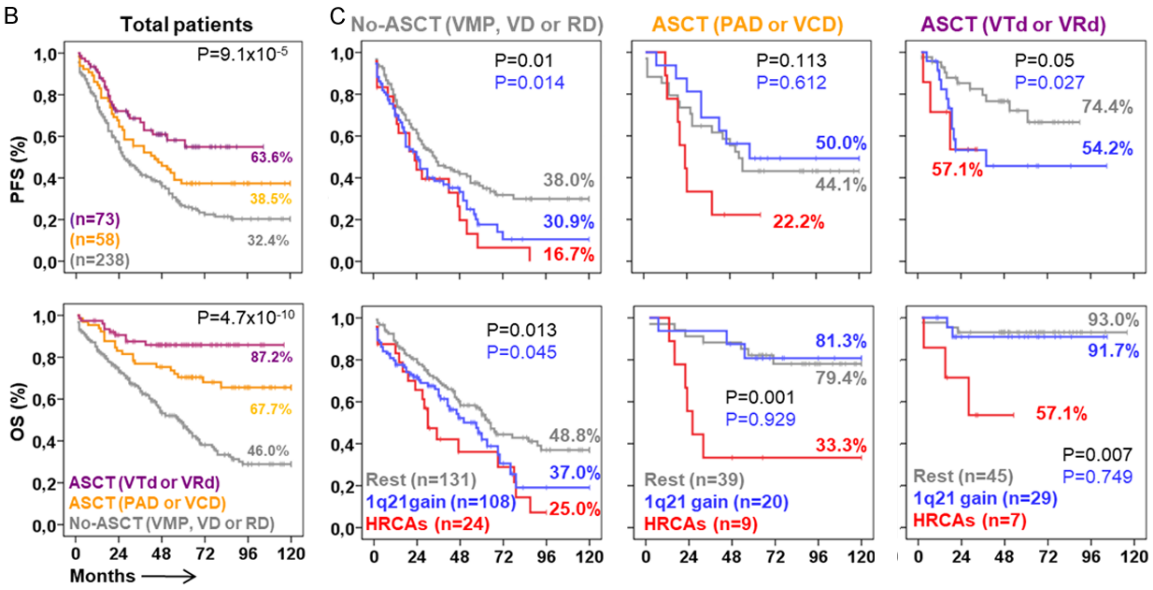


Figure 3. Predictive value of 1q21 gain depends on first-line therapy. A. Rate of stringent complete response (sCR) with negative minimal residual disease (MRD) and relapse after sCR according to the eligibility for autologous stem cell transplantation (ASCT) and the type of first-line therapy (No-ASCT with VMP, Vd or Rd; ASCT with PAD or VCD; or ASCT with VTd or VRd). B. Kaplan-Meier and Log-rank tests for progression-free survival (PFS) and overall survival (OS) according to the type of treatment. Ten-year PFS and OS rates are shown for each group. C. Kaplan-Meier test for PFS and OS according to the type of first-line treatment and the presence of HRCAs -del(17p) or t(4;14)-, 1q21 gain and the rest of patients (Rest). Ten-year PFS and OS rates are shown for each group. *P* values in the Log-rank test for total patients (black) or patients with 1q21 gain compared to the Rest of patients (blue) are shown.

gain or Rest were 57.1%, 54.2% and 74.4% (P=0.05, P=0.027 between 1q21 gain and Rest) and 10y-OS were 57.1%, 91.7% and 93.0% (P=0.007, P=0.749 between 1q21 gain and Rest). Therefore, with this treatment, PFS of patients with 1q21 gain was similar to that of patients with HRCAs, but the OS was similar to that of patients without HRCAs (**Figure 3C**).

Prognosis of 1q21 gain depends on concurrent cytogenetic abnormalities and first-line treatment

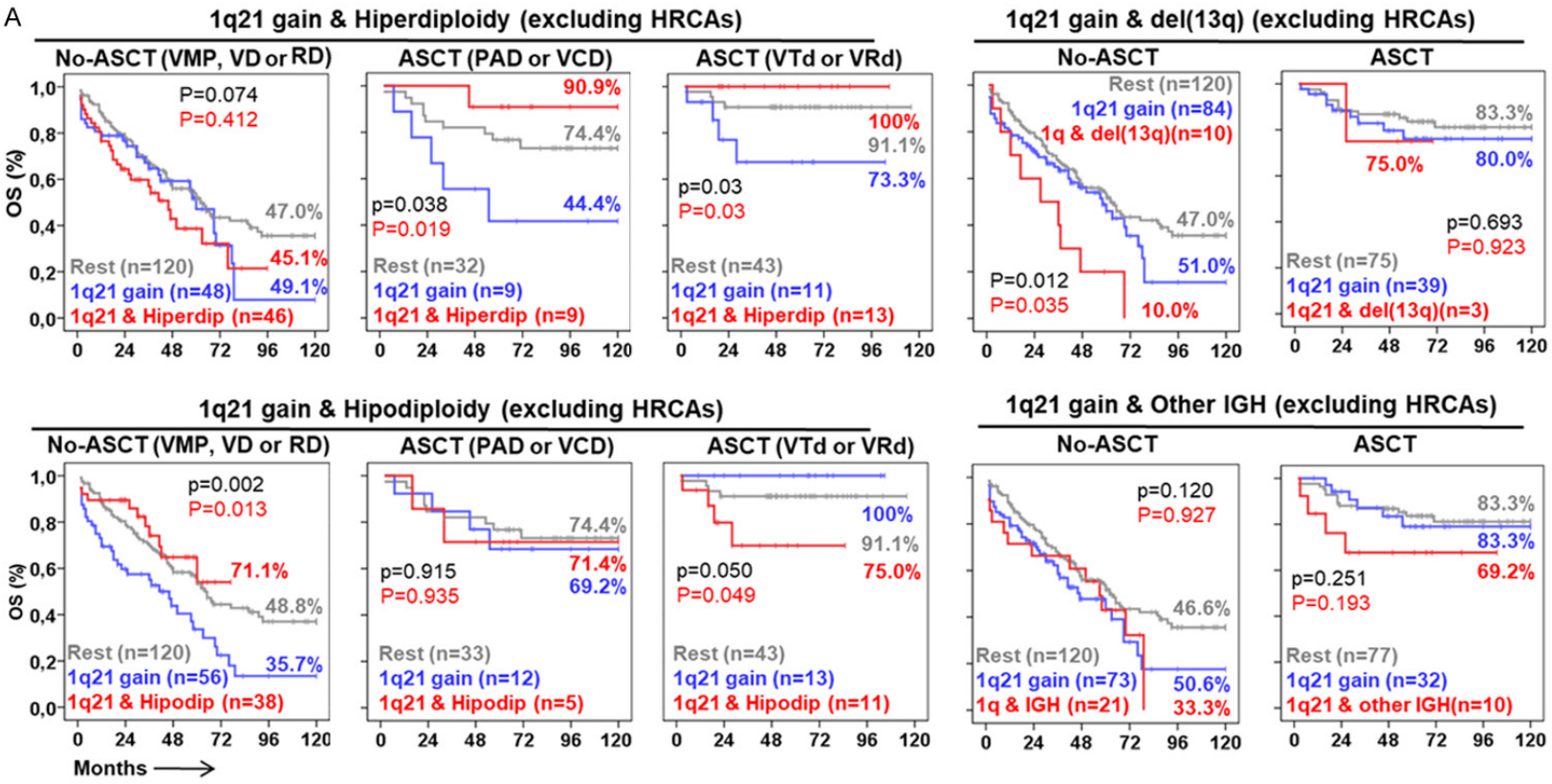
Next, we explored the differential impact of concurrent cytogenetic abnormalities associated with 1q21 gain on patients under different treatments. To avoid the negative impact of HRCAs, patients with HRCAs were excluded from these analyses (**Figure 4A**).

Hyperdiploidy associated with 1q21 gain showed no significant difference in the 10y-OS rate of ASCT-ineligible patients compared with other 1q21 gains (45.1% vs. 49.1%, P=0.412). However, patients with concurrent 1q21 gain and hyperdiploidy showed much favorable 10y-OS rates than patients with other 1q21 gains for both types of treatment associated with ASCT, PAD/VCD (90.9% vs. 44.4%, P=0.019) and VTd/VRd (100% vs. 73.3%, P=0.03).

Hypodiploidy associated with 1q21 gain showed better 10y-OS rate for ASCT-ineligible patients compared with other 1q21 gains (71.1% vs. 35.7%, P=0.013). However, while no impact was observed for ASCT-eligible patients treated with PAD/VCD (71.4% vs. 69.2%, P=0.935), VTd/VRd treatment associated with ASCT showed lower 10y-OS rates (75.0% vs. 100%, P=0.03) in patients with 1q21gain and hypodiploidy.

Del(13q) associated with 1q21 gain showed lower 10y-OS rate for ASCT-ineligible patients

A



B

HRCAs		Predictive value of 1q21 gain associated to other cytogenetic alterations (excluding HRCAs)								
		Hiperdiploidy		Hipodiploidy			del(13q)		Other IGH, no t(4;14)	
No-ASCT	ASCT	No-ASCT	ASCT	No-ASCT	ASCT (PAD/VCD)	ASCT (VTd/VRd)	No-ASCT	ASCT	No-ASCT	ASCT
Unfavorable	Unfavorable	No impact	Favorable	Favorable	No impact	Unfavorable	Unfavorable	No impact	No impact	Unfavorable[1]
0.05	$2.9x10^{-6}$	P=0.412	P=0.002	P=0.013	P=0.935	P=0.049	P=0.035	P=0.923	P=0.927	P=0.193

[1] this results need confirmation in larger series

Figure 4. Prognosis of 1q21 gain depends on concurrent cytogenetic abnormalities and first-line treatment. (A) Kaplan-Meier test for overall survival (OS) according to the type of treatment and the concurrence of 1q21 gain with hyperdiploidy, hypodiploidy, del(13q) or IGH breaks other than t(4;14). Ten-year OS rates are shown for each group. *P* values in the Log-rank test for total patients (black) or patients with 1q21 gain vs. 1q21 gain in concurrence with other abnormalities (red) are shown. (B) Summary of results from (A), including results for patients with HRCAs.

A

Confirmatory series

Patient information		FISH results	
Total patients (n)	43	FISH available	38 (88.4%)
Age, mean±SD	58.6±6.9	HRCA	4 (9.3%)
Female, n (%)	17 (39.5%)	1q21 gain	18 (41.8%)
Treatment		IGH breaks, no t(4;14)	13 (30.2%)
ASCT with VTd , n (%)	43 (100)	Hyperdiploidy	9 (20.9%)
		Hypodiploidy	6 (13.9%)

HRCA, high-risk cytogenetic abnormalities; ASCT, autologous stem cell transplantation

B

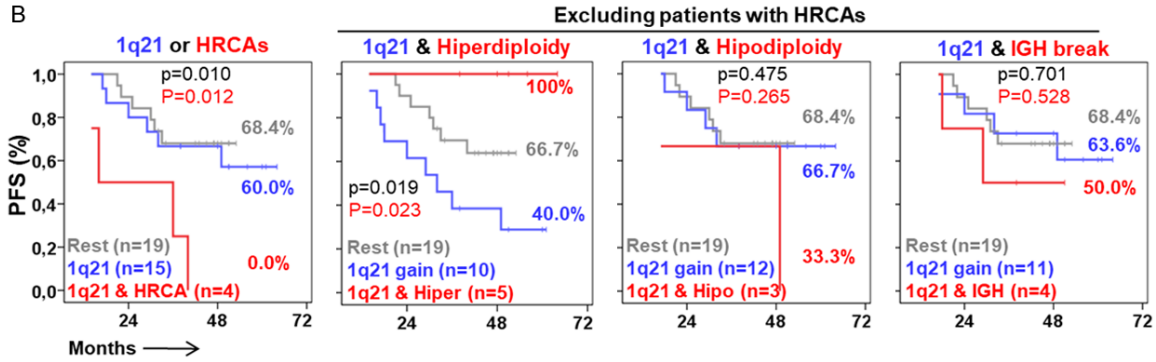


Figure 5. Confirmatory series. A. Table summarizing the biological and FISH characteristics as well as treatment of MM patients in the confirmatory series. B. Kaplan-Meier test for progression-free survival (PFS) for patients with HRCAs, 1q21 gain or other patients (Rest), as well as according to the concurrence of 1q21 gain with hyperdiploidy, hypodiploidy and IGH breaks other than t(4;14) and excluding patients with HRCAs. Five-year PFS rates are shown for each group. *P* values in the Log-rank test for total patients (black) or patients with 1q21 gain vs. 1q21 gain in concurrence with other abnormalities (red) are shown.

compared with other 1q21 gains (10.0% vs. 51.0%, P=0.035), but it was not significantly different in patients who were eligible for ASCT (75.0% vs. 80.0%, P=0.923). Nonetheless, this result should be confirmed in larger series.

IGH breaks other than t(4;14) associated with 1q21 gain showed no significant difference in the 10y-OS rate of ASCT-ineligible patients compared with other 1q21 gains (33.3% vs. 50.6%, P=0.927).

However, ASCT-eligible patients with these IGH breaks showed unfavorable 10y-OS (69.2% vs. 83.3%, P=0.193). Nonetheless, this result should be confirmed in larger series.

A summary of these results is shown in **Figure 4B**.

An independent confirmatory series of 43 ASCT-eligible MM patients treated with VTd was included to validate the results from our series (see **Figure 5A** for details). Mean follow-up for this series was 48.8±10.2 months. Patients with HRCAs showed lower 5y-PFS compared with patients with 1q21 gain (0.0% vs. 60.0%, P=0.012) or with the rest of patients (68.4%, P=0.01). Excluding patients with HRCAs, those with 1q21 gain and hyperdiploidy showed higher 5y-PFS than patients with other 1q21 gains (100% vs. 40.0%, P=0.023) and than the rest of patients (66.7, P=0.019); patients with hypodiploidy and IGH breaks other than t(4;14) concurrent with 1q21 gain showed lower 5y-PFS (33.3% and 50.0%, respectively) than patients with other 1q21 gains (66.7% and 63.6%, respectively) or the rest of patients (68.4%), but these results did not reach statistical significance (**Figure 5B**).

Contribution of 1q21 gain to current RISS stratification system

Given the complexity of the aforementioned interactions between the concurrence of other cytogenetic abnormalities with 1q21 gain and the type of treatment, and based on the proposition to modify the current RISS stratification system in order to include the 1q21 gain, our next analysis was aimed at ascertaining the contribution of 1q21 gain to the RISS.

Results of our series of patients clearly show that the inclusion of 1q21 gain in the current RISS stratification system does not improve its

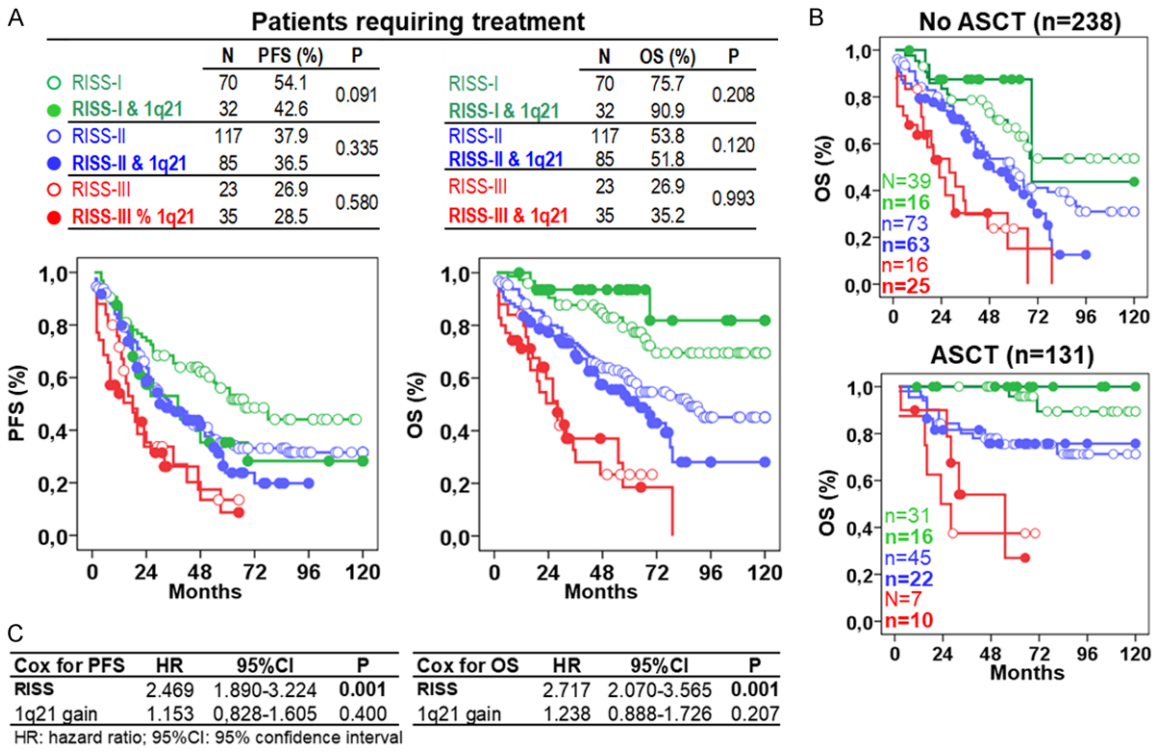


Figure 6. Gain of 1q21 did not complement standard risk stratification (RISS) of patients that required treatment. A. Kaplan-Meier test for progression-free survival (PFS) and overall survival (OS) according to the RISS and 1q21 gain. Ten-year PFS and OS rates and *P* values from the Log-rank test comparing patients with or without 1q21 gain for each RISS stage are described in the table above. B. Kaplan-Meier test for OS according to RISS and 1q21 gain in ASCT ineligible or eligible patients. C. Cox regression analysis of PFS and OS for RISS and 1q21 gain.

predictive capacity either for ASCT-eligible or for ineligible patients (see **Figure 6A**, **6B** for details). Cox regression analysis showed that 1q21 gain by itself was not an independent prognostic factor for PFS (P=0.400) or OS (P=0.207) that could complement the independent prognostic capacity of the RISS (HR=2.469, P=0.001 for PFS and HR=2.717, P=0.001 for OS) (**Figure 6C**).

Discussion

Improvement in MM patients' outcome has been and should be sustained in personalized therapies adapted to the risk of the disease and the biological condition of patients. However, due to underlying molecular variations of myelomatous cells, the clinical course of the disease is very heterogeneous, with patients experiencing long remission periods, or functional cures, and others relapsing or becoming refractory to therapy. Molecular abnormalities driving these differences should be incorporated into clinical care to further progress towards this objective. Incorporation of HRCAs, del(17p) and t(4;14), into the RISS has decisively contributed to improving risk stratification, but unfortunately only to moderate advances in the PFS or OS of these patients, as it is clearly shown in our results and many others [40-43]. Recently, there is a claim to incorporate chr1 abnormalities into the RISS [7]. However, although the negative impact of 1q21 gain has been widely demonstrated [12-17], numerous questions need answering in this particular case. Results in this manuscript decisively contribute to shed some light on some of these issues.

First, our results clearly support that both duplication and amplification of 1q21 are independent risk factors for the progression of the disease in MGUS, SMM and MM patients, and therefore, 1q21 gain analysis could be helpful in the clinical management of patients at all PCN stages. However, 1q21 gain negatively impacted the OS only in MM patients. There is controversy about the differential role of dupli-

cation/amplification of 1q21 gain [17, 21-25] and about the "double hit" effect of 1q21 gain in concurrence with HRCAs [19, 20]. Results from our series show that a similar negative impact on patient outcome was associated with duplication and amplification of 1q21, with no additional unfavorable effects when associated with the HRCAs del(17p) or t(4;14). In our series, HRCAs showed the most unfavorable clinical course at all PCN stages irrespective of the type and intensity of treatments; therefore, patients with HRCAs should be candidates for alternative (pomalidomide plus low-dose dexamethasone [42, 43]) or experimental therapies (CAR-T cells [44]) if we want to obtain better results in these patients.

Likewise, there is controversy as to whether 1q21 gain by itself or in concurrence with other factors is associated with such unfavorable prognosis [13, 18, 19]. Indeed, 1q21 gain was strongly associated with many other cytogenetic abnormalities, as plainly shown by ours and others' results [19, 20]. In fact, patients with 1q21 gain alone, in absence of other cytogenetic abnormalities, showed similar outcomes to patients without HRCAs or 1q21 gain after treatment. Therefore, its association with other cytogenetic abnormalities is what prints a negative effect on 1q21 gain. Remarkably, not all cytogenetic abnormalities associated with 1q21 gain showed similar results; therefore, two groups could be distinguished: abnormalities that increased the risk of 1q21 gain (HRCAs, IGH breaks other than t(4;14) and del(13q)) and abnormalities that reduced its risk (hyperdiploidy and hypodiploidy). But it is more important to take into account that these differential results were mostly conditioned by the type and intensity of first-line treatment received by patients.

For a better appreciation of the impact that the concurrence of chromosomal abnormalities and 1q21 gain had on patient outcome, patients with HRCAs were excluded from successive analysis. Thus, while hyperdiploidy in concurrence with 1q21 gain did not modify results in ASCT-ineligible patients as described previously [45], it was associated with close to 100% OS of ASCT-eligible patients treated either with PAD/VCD or VTd/VRd. In contrast, hypodiploidy in concurrence with 1q21 gain was associated with a favorable outcome in ASCT-ineligible patients, but with unfavorable prognosis in ASCT-eligible patients treated with VTd/VRd. The inverse effect of hyperdiploidy and hypodiploidy in patients' outcome is supported by the inverse relationship that exists in the appearance of these two cytogenetic abnormalities [9]. Besides, del(13q) in concurrence with 1q21 gain was associated with a worst outcome in ASCT-ineligible patients, but had no impact on ASCT-eligible ones. Finally, IGH breaks, excluding t(4;14), in concurrence with 1q21 gain had no impact on ASCT-ineligible patients, but increased the risk in ASCT-eligible patients; however, the latter result should be confirmed in new and larger series. Although most of these results were validated in an independent confirmatory series, they should be definitely validated in larger series.

Not many studies in the literature have analyzed the impact of the concurrence of other cytogenetic abnormalities with 1q21 gain, and in general they did not evaluate their impact depending on specific treatments, so their results were mainly influenced by dominant treatments, which in general did not include ASCT. Two consecutive studies with 104 and 411 MM patients mostly treated with front-line thalidomide (CTD/VMP, with ASCT in one third of patients) described that del(17p) and del(13q) worsen the poor prognosis associated with 1q21 [14, 19]. Results describing that del(13q) increases the risk of 1q21 gain are in line with the lower OS observed in patients from our series who were ineligible for ASCT and had this genotype. The inverse impact of monosomy 13 (unfavorable) and del(13q) (favorable) on OS was described in a series of 1181 MM patients receiving IMIDs and PIs, but not ASCT [10]. However, when these alterations appeared in concurrence with 1q21 gain in ASCT-ineligible patients, this effect seemed to be reversed, with hypodiploidy (77.7% due to monosomy 13) improving and del(13q) worsening the outcome of patients with 1q21 gain. As mentioned before, the latter was in concordance with previous results [19].

There is also controversy as to whether the new treatments are able to counteract the adverse effect of 1q21 gain [13, 15, 16, 19, 21, 26]. In view of our results, the answer is not dichotomous, since treatments including ASCT completely counteracted or even improved the neg-

ative effect of 1q21 gain in patients with concurrent hyperdiploidy or del(13q), but not in patients with concurrent HRCAs or hypodiploidy. In contrast, if ASCT is not an option, treatments will only be able to counteract the negative impact of 1q21 gain when in concurrence with hypodiploidy.

With these results in mind, our final objective was to assess the convenience of modifying the current risk stratification system (RISS). In contrast to some claims [7], our results clearly show that the inclusion of 1q21 gain (by itself) in the current RISS does not improve its predictive capacity at all, neither for ASCT-eligible nor ineligible patients.

In conclusion, 1q21 gain should be patient-wisely evaluated, irrespective of the RISS, also considering its concurrence with other cytogenetic abnormalities and the patient's eligibility for ASCT. Finally, we strongly recommend that, at least in patients with 1q21 gain, FISH probes for evaluating del/monosomy 13 and copy number of chromosomes 5, 9 and 15 should be assayed for a better evaluation of their hyperdiploidy or hypodiploidy status.

Acknowledgements

This work was funded by MINECO-Health Institute Carlos III (PI20/00161); Co-funded by European Regional Development Fund (ERDF), "A way to make Europe". We would like to acknowledge hematologists collaborating in the enrolment of patients: Drs. Catalina Cava Almohalla, Antonio Martínez Frances, Begoña Muiña Juarez, Horacio Cano Gracia, Victoriano Beltran Agullo and laboratory technicians María Carmen Martínez Solano, María Dolores García Arnao, María Elena Bernal Moreno, and the Group of Myeloma Studies of the Valencian Community (GREMI).

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Alfredo Minguela, Hospital Clínico Universitario Virgen de la Arrixaca, El Palmar, Murcia, Spain. Tel: +34-695344172; E-mail: alfredo.minguela@carm.es

References

[1] San-Miguel JF and Mateos MV. Can multiple myeloma become a curable disease? Haematologica 2011; 96: 1246-1248.

[2] Alberts DS, Durie BG and Salmon SE. Doxorubicin/B.C.N.U. chemotherapy for multiple myeloma in relapse. Lancet 1976; 1: 926-928.

[3] Greipp PR, Miguel JS, Dune BGM, Crowley JJ, Barlogie B, Bladé J, Boccadoro M, Child JA, Avet-Loiseau H, Kyle RA, Lahuerta JJ, Ludwig H, Morgan G, Powles R, Shimizu K, Shustik C, Sonneveld P, Tosi P, Turesson I and Westin J. International staging system for multiple myeloma. J Clin Oncol 2005; 23: 3412-3420.

[4] Palumbo A, Avet-Loiseau H, Oliva S, Lokhorst HM, Goldschmidt H, Rosinol L, Richardson P, Caltagirone S, Lahuerta JJ, Facon T, Bringhen S, Gay F, Attal M, Passera R, Spencer A, Offidani M, Kumar S, Musto P, Lonial S, Petrucci MT, Orlowski RZ, Zamagni E, Morgan G, Dimopoulos MA, Durie BG, Anderson KC, Sonneveld P, San Miguel J, Cavo M, Rajkumar SV and Moreau P. Revised international staging system for multiple myeloma: a report from international myeloma working group. J Clin Oncol 2015; 33: 2863-2869.

[5] Vasco-Mogorrón MA, Campillo JA, Periago A, Cabañas V, Berenguer M, García-Garay MC, Gimeno L, Soto-Ramírez MF, Martínez-Hernández MD, Muro M and Minguela A. Proliferation to apoptosis tumor cell ratio as a biomarker to improve clinical management of pre-malignant and symptomatic plasma cell neoplasms. Int J Mol Sci 2021; 22: 3895.

[6] Vasco-Mogorrón MA, Campillo JA, Periago A, Cabañas V, Berenguer M, García-Garay MC, Gimeno L, María F Soto-Ramírez MF, Martínez-Hernández MD, Muro M and Minguela A. Blood-based risk stratification for pre-malignant and symptomatic plasma cell neoplasms to improve patient management. Am J Cancer Res 2021; 11: 2736-2753. eCollection 2021.

[7] D'Agostino M, Lahuerta JJ, Wester R, Waage A, Bertsch U, Zamagni E, Mateos MV, Larocca A, Dall'Olio D, Van De Donk NWCJ, Cairns D, Rocchi S, Salwender H, Creixenti JB, Holt BVD, Gastone C, Ciccone G, Capra A, Dürig J, Bringhen S, Zweegman S, Cavo M, Goldschmidt H, Cook, Hernández-Rivas JM, San-Miguel J, Boccadoro M and Sonneveld P. A new risk stratification model (R2-ISS) in newly diagnosed multiple myeloma: analysis of mature data from 7077 patients collected by european myeloma network within harmony big data platform. Blood 2020; 136: 34-37.

[8] Cardona-Benavides IJ, de Ramón C and Gutiérrez NC. Genetic abnormalities in multiple myeloma: prognostic and therapeutic implications. Cells 2021; 10: 336-362.

[9] Debes-Marun CS, Dewald GW, Bryant S, Picken E, Santana-Dávila R, González-Paz N, Winkler JM, Kyle RA, Gertz MA, Witzig TE, Dispenzieri A, Lacy MQ, Rajkumar SV, Lust JA, Greipp PR and

Fonseca R. Chromosome abnormalities clustering and its implications for pathogenesis and prognosis in myeloma. Leukemia 2003; 17: 427-436.

[10] Binder M, Rajkumar S V, Ketterling RP, Greipp PT, Dispenzieri A, Lacy MQ, Gertz MA, Buadi FK, Hayman SR, Hwa YL, Zeldenrust SR, Lust JA, Russell SJ, Leung N, Kapoor P, Go RS, Gonsalves WI, Kyle RA and Kumar SK. Prognostic implications of abnormalities of chromosome 13 and the presence of multiple cytogenetic high-risk abnormalities in newly diagnosed multiple myeloma. Blood Cancer J 2017; 7: e600.

[11] Maura F, Bolli N, Angelopoulos N, Dawson KJ, Leongamornlert D, Martincorena I, Mitchell TJ, Fullam A, Gonzalez S, Szalat R, Abascal F, Rodriguez-Martin B, Samur MK, Glodzik D, Roncador M, Fulciniti M, Tai YT, Minvielle S, Magrangeas F, Moreau P, Corradini P, Anderson KC, Tubio JMC, Wedge DC, Gerstung M, Avet-Loiseau H, Munshi N and Campbell PJ. Genomic landscape and chronological reconstruction of driver events in multiple myeloma. Nat Commun 2019; 10: 3835.

[12] Marzin Y, Jamet D, Douet-Guilbert N, Morel F, Le Bris MJ, Morice P, Abgrall JF, Berthou C and Braekeleer MD. Chromosome 1 abnormalities in multiple myeloma. Anticancer Res 26: 953-959.

[13] Shah GL, Landau H, Londono D, Devlin SM, Kosuri S, Lesokhin AM, Lendvai N, Hassoun Chung HDJ, Koehne G, Jhanwar SC, Landgren O, Levine R and Giralt SA. Gain of chromosome 1q portends worse prognosis in multiple myeloma despite novel agent-based induction regimens and autologous transplantation. Leuk Lymphoma 2017; 58: 1823-1831.

[14] Grzasko N, Hajek R, Hus M, Chocholska S, Morawska M, Giannopoulos K, Czarnocki K, Druzd-Sitek A, Pienkowska-Grela B, Rygier J, Usnarska-Zubkiewicz L, Dytfeld D, Kubicki T, Jurczyszyn A, Korpysz M and Dmoszynska A. Chromosome 1 amplification has similar prognostic value to del(17p13) and t(4;14)(p16;q32) in multiple myeloma patients: analysis of real-life data from the Polish Myeloma Study Group. Leuk Lymphoma 2017; 58: 1-15.

[15] Giri S, Huntington SF, Wang R, Zeidan AM, Podoltsev N, Gore SD, Ma X, Gross CP, Davidoff AJ and Neparidze N. Chromosome 1 abnormalities and survival of patients with multiple myeloma in the era of novel agents. Blood Adv 2020; 4: 2245-2253.

[16] Biran N, Malhotra J, Bagiella E, Cho HJ, Jagannath S and Chari A. Patients with newly diagnosed multiple myeloma and chromosome 1 amplification have poor outcomes despite the use of novel triplet regimens. Am J Hematol 2014; 89: 616-620.

[17] Shah V, Sherborne AL, Walker BA, Johnson DC, Boyle EM, Ellis S, Begum DB, Proszek PZ, Jones JR, Pawlyn C, Savola S, Jenner MW, Drayson MT, Owen RG, Houlston RS, Cairns DA, Gregory WM, Cook G, Davies FE, Jackson GH, Morgan GJ and Kaiser MF. Prediction of outcome in newly diagnosed myeloma: a meta-analysis of the molecular profiles of 1905 trial patients. Leukemia 2018; 32: 102-110.

[18] Bock FM, Lu G, Shah N, Bashir Q, Patel KK, Popat UR, Hosing CM, Gaballa S, Korbling M, Delgado R, Rondon G, Orlowski RZ, Shah JJ, Manasanch EE, Champlin RE and Qazilbash MH. Clinical characteristics and outcomes in patients with multiple myeloma and CKS1B gene gain/amplification after autologous stem cell transplantation. Blood 2014; 124: 2521-2521.

[19] Grzasko N, Hus M, Pluta A, Jurczyszyn A, Walter-Croneck A, Morawska M, Chocholska S, Hajek R and Dmoszynska A. Additional genetic abnormalities significantly worsen poor prognosis associated with 1q21 amplification in multiple myeloma patients. Hematol Oncol 2013; 31: 41-48.

[20] Jung HA, Jang MA, Kim K and Kim SH. Clinical utility of a diagnostic approach to detect genetic abnormalities in multiple myeloma: a single institution experience. Ann Lab Med 2018; 38: 196-203.

[21] An G, Xu Y, Shi L, Shizhen Z, Deng S, Xie Z, Sui W, Zhan F and Qiu L. Chromosome 1q21 gains confer inferior outcomes in multiple myeloma treated with bortezomib but copy number variation and percentage of plasma cells involved have no additional prognostic value. Haematologica 2014; 99: 353-359.

[22] Varma A, Sui D, Milton DR, Tang G, Saini N, Hasan O, Mukherjee A, Joseph JJ, Bashir Q, Rondon G, Srour S, Popat UR, Hosing CM, Nieto Y, Kebriaei P, Alousi AM, Ahmed S, Mehta R, Khouri IF, Ahmed H, Iyer S, Weber DM, Thomas SK, Manasanch E, Lee HC, Patel K, Ciurea SO, Shpall EJ, Orlowski RZ, Champlin RE and Qazilbash MH. Outcome of multiple myeloma with chromosome 1q gain and 1p deletion after autologous hematopoietic stem cell transplantation: propensity score matched analysis. Biol Blood Marrow Transplant 2020; 26: 665-671.

[23] Hanamura I, Stewart JP, Huang Y, Zhan F, Santra M, Sawyer JR, Hollmig K, Zangarri M, Pineda-Roman M, van Rhee F, Cavallo F, Burington B, Crowley J, Tricot G, Barlogie B and Shaughnessy JD. Frequent gain of chromosome band 1q21 in plasma-cell dyscrasias detected by fluorescence in situ hybridization: incidence increases from MGUS to relapsed myeloma and is related to prognosis and disease progression following tandem stem-cell transplantatio. Blood 2006; 108: 1724-1732.

[24] Neben K, Lokhorst HM, Jauch A, Bertsch U, Hielscher T, van der Holt B, Salwender H, Blau IW, Weisel K, Pfreundschuh M, Scheid C, Dührsen U, Lindemann W, Schmidt-Wolf IG, Peter N, Teschendorf C, Martin H, Haenel M, Derigs HG, Raab MS, Ho AD, van de Velde H, Hose D, Sonneveld P and Goldschmidt H. Administration of bortezomib before and after autologous stem cell transplantation improves outcome in multiple myeloma patients with deletion 17p. Blood 2012; 119: 940-948.

[25] Schmidt TM, Barwick BG, Joseph N, Heffner LT, Hofmeister CC, Bernal L, Dhodapkar MV, Gupta VA, Jaye DL, Wu J, Goyal S, Chen Z, Boise LH, Lonial S, Nooka AK and Kaufman JL. Gain of Chromosome 1q is associated with early progression in multiple myeloma patients treated with lenalidomide, bortezomib, and dexamethasone. Blood Cancer J 2019; 9: 94.

[26] Smetana J, Berankova K, Zaoralova R, Nemec P, Greslikova H, Kupska R, Mikulasova A, Frohlich J, Sevcikova S, Zahradova L, Krejci M, Sandecka V, Almasi M, Kaisarova P, Melicharova H, Adam Z, Penka M, Jarkovsky J, Jurczyszyn A, Hajek R and Kuglik P. Gain(1)(q21) is an unfavorable genetic prognostic factor for patients with relapsed multiple myeloma treated with thalidomide but not for those treated with bortezomib. Clin Lymphoma Myeloma Leuk 2013; 13: 123-130.

[27] Shi L, Wang S, Zangari M, Xu H, Cao TM, Xu C, Wu Y, Xiao F, Liu Y, Yang Y, Salama M, Li G, Tricot G and Zhan F. Over-expression of CKS1B activates both MEK/ERK and JAK/STAT3 signaling pathways and promotes myeloma cell drug-resistance. Oncotarget 2010; 1: 22-33.

[28] Huang J, Zhou Y, Thomas GS, Gu Z, Yang Y, Xu H, Tricot G and Zhan F. NEDD8 inhibition overcomes CKS1B-induced drug resistance by upregulation of p21 in multiple myeloma. Clin Cancer Res 2015; 21: 5532-5542.

[29] Marchesini M, Ogoti Y, Fiorini E, Aktas Samur A, Nezi L, D'Anca M, Storti P, Samur MK, Ganan-Gomez I, Fulciniti MT, Mistry N, Jiang S, Bao N, Marchica V, Neri A, Bueso-Ramos C, Wu CJ, Zhang L, Liang H, Peng X, Giuliani N, Draetta G, Clise-Dwyer K, Kantarjian H, Munshi N, Orlowski R, Garcia-Manero G, DePinho RA and Colla S. ILF2 is a regulator of RNA splicing and dna damage response in 1q21-amplified multiple myeloma. Cancer Cell 2017; 32: 88-100.

[30] Boyd KD, Ross FM, Chiecchio L, Dagrada GP, Konn ZJ, Tapper WJ, Walker BA, Wardell CP, Gregory WM, Szubert AJ, Bell SE, Child JA, Jackson GH, Davies FE and Morgan GJ. A novel prognostic model in myeloma based on cosegregating adverse FISH lesions and the ISS: analysis of patients treated in the MRC Myeloma IX trial. Leukemia 2012; 26: 349-355.

[31] Rajkumar SV. Multiple myeloma: 2018 update on diagnosis, risk-stratification, and management. Am J Hematol 2018; 93: 981-1114.

[32] Rajkumar SV, Kyle RA and Buadi FK. Advances in the diagnosis, classification, risk stratification, and management of monoclonal gammopathy of undetermined significance: implications for recategorizing disease entities in the presence of evolving scientific evidence. Mayo Clin Proc 2010; 85: 945-948.

[33] Lakshman A, Rajkumar SV, Buadi FK, Binder M, Gertz MA, Lacy MQ, Dispenzieri A, Dingli D, Fonder AL, Hayman SR, Hobbs MA, Gonsalves WI, Hwa YL, Kapoor P, Leung N, Go RS, Lin Y, Kourelis TV, Warsame R, Lust JA, Russell SJ, Zeldenrust SR, Kyle RA and Kumar SK. Risk stratification of smoldering multiple myeloma incorporating revised IMWG diagnostic criteria. Blood Cancer J 2018; 8: 59-69.

[34] Durie BG, Harousseau JL, Miguel JS, Bladé J, Barlogie B, Anderson K, Gertz M, Dimopoulos M, Westin J, Sonneveld P, Ludwig H, Gahrton G, Beksac M, Crowley J, Belch A, Boccadaro M, Cavo M, Turesson I, Joshua D, Vesole D, Kyle R, Alexanian R, Tricot G, Attal M, Merlini G, Powles R, Richardson P, Shimizu K, Tosi P, Morgan G and Rajkumar SV; International Myeloma Working Group. International uniform response criteria for multiple myeloma. Leukemia 2006; 20: 1467-1473.

[35] Rajkumar SV, Dimopoulos MA, Palumbo A, Blade J, Merlini G, Mateos MV, Kumar S, Hillengass J, Kastritis E, Richardson P, Landgren O, Paiva B, Dispenzieri A, Weiss B, LeLeu X, Zweegman S, Lonial S, Rosinol L, Zamagni E, Jagannath S, Sezer O, Kristinsson SY, Caers J, Usmani SZ, Lahuerta JJ, Johnsen HE, Beksac M, Cavo M, Goldschmidt H, Terpos E, Kyle RA, Anderson KC, Durie BG and Miguel JF. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. Lancet Oncol 2014; 15: e538-548.

[36] Flores-Montero J, Sanoja-Flores L, Paiva B, Puig N, García-Sánchez O, Böttcher S, Van Der Velden VHJ, Pérez-Morán JJ, Vidriales MB, García-Sanz R, Jimenez C, González M, Martínez-López J, Corral-Mateos A, Grigore GE, Fluxá R, Pontes R, Caetano J, Sedek L, Del Cañizo MC, Bladé J, Lahuerta JJ, Aguilar C, Bárez A, García-Mateo A, Labrador J, Leoz P, Aguilera-Sanz C, San-Miguel J, Mateos MV, Durie B, Van Dongen JJM and Orfao A. Next generation flow for highly sensitive and standardized detection of minimal residual disease in multiple myeloma. Leukemia 2017; 31: 2094-2103.

[37] Kumar S, Paiva B, Anderson KC, Durie B, Landgren O, Moreau P, Munshi N, Lonial S, Bladé J, Mateos MV, Dimopoulos M, Kastritis E, Boccadoro M, Orlowski R, Goldschmidt H, Spencer A,

Hou J, Chng WJ, Usmani SZ, Zamagni E, Shimizu K, Jagannath S, Johnsen HE, Terpos E, Reiman A, Kyle RA, Sonneveld P, Richardson PG, McCarthy P, Ludwig H, Chen W, Cavo M, Harousseau JL, Lentzsch S, Hillengass J, Palumbo A, Orfao A, Rajkumar SV, Miguel JS and Avet-Loiseau H. International Myeloma Working Group consensus criteria for response and minimal residual disease assessment in multiple myeloma. Lancet Oncol 2016; 17: e328-346.

[38] Pérez-Persona E, Vidriales M-B, Mateo G, García-Sanz R, Mateos MV, de Coca AG, Galende J, Martín-Nuñez G, Alonso JM, de Las Heras N, Hernández JM, Martín A, López-Berges C, Orfao A and San Miguel JF. New criteria to identify risk of progression in monoclonal gammopathy of uncertain significance and smoldering multiple myeloma based on multiparameter flow cytometry analysis of bone marrow plasma cells. Blood 2007; 110: 2586-2592.

[39] Periago A, Campillo JA, Mrowiec A, Gimeno L, Montes NR, Martínez-Sánchez MV, Cabañas-Perianes V, García-Garay C, Bolarin JM, Blasco-Mogorrón A, Muro M, Berenguer M, Moraleda JM, García-Alonso AM and Minguela A. Circulating aberrant plasma cells allow risk stratification of patients with myeloma. Am J Hematol 2016; 91: E353-355.

[40] Chng WJ, Goldschmidt H, Dimopoulos MA, Moreau P, Joshua D, Palumbo A, Facon T, Ludwig H, Pour L, Niesvizky R, Oriol A, Rosiñol L, Suvorov A, Gaidano G, Pika T, Weisel K, Goranova-Marinova V, Gillenwater HH, Mohamed N, Feng S, Aggarwal S and Hájek R. Carfilzomib-dexamethasone vs. bortezomib-dexamethasone in relapsed or refractory multiple myeloma by cytogenetic risk in the phase 3 study ENDEAVOR. Leukemia 2017; 31: 1368-1374.

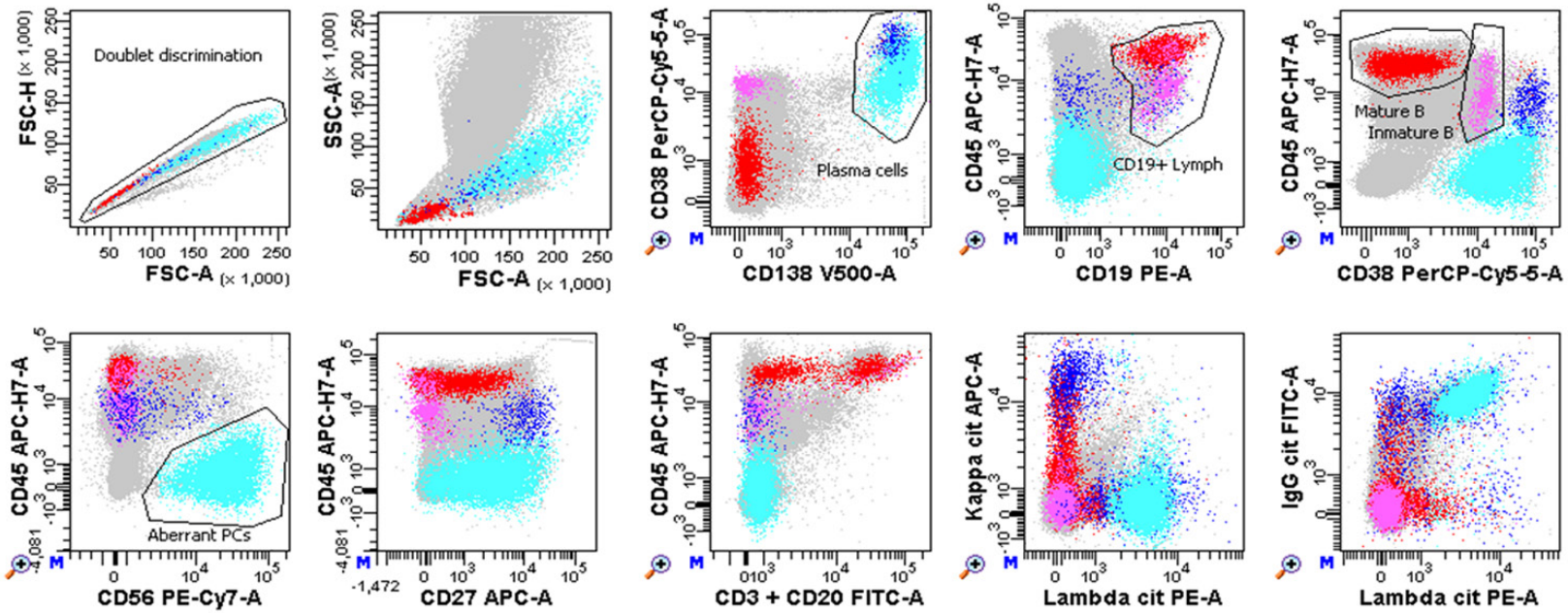
[41] Bergsagel PL, Mateos MV, Gutierrez NC, Rajkumar SV and San Miguel JF. Improving overall survival and overcoming adverse prognosis in the treatment of cytogenetically high-risk multiple myeloma. Blood 2013; 121: 884-892.

[42] Dimopoulos MA, Weisel KC, Song KW, Delforge M, Karlin L, Goldschmidt H, Moreau P, Banos A, Oriol A, Garderet L, Cavo M, Ivanova V, Alegre A, Martinez-Lopez J, Chen C, Spencer A, Knop S, Bahlis NJ, Renner C, Yu X, Hong K, Sternas L, Jacques C, Zaki MH and San Miguel JF. Cytogenetics and long-term survival of patients with refractory or relapsed and refractory multiple myeloma treated with pomalidomide and low-dose dexamethasone. Haematologica 2015; 100: 1327-1333.

[43] Leleu X, Karlin L, Macro M, Hulin C, Garderet L, Roussel M, Arnulf B, Pegourie B, Kolb B, Stoppa AM, Brechiniac S, Marit G, Thielemans B, Onraed B, Mathiot C, Banos A, Lacotte L, Tiab M, Dib M, Fuzibet JG, Petillon MO, Rodon P, Wetterwald M, Royer B, Legros L, Benboubker L, Decaux O, Escoffre-Barbe M, Caillot D, Fermand JP, Moreau P, Attal M, Avet-Loiseau H and Facon T; Intergroupe Francophone du Myélome (IFM). Pomalidomide plus low-dose dexamethasone in multiple myeloma with deletion 17p and/or translocation (4;14): IFM 2010-02 trial results. Blood 2015; 125: 1411-1417.

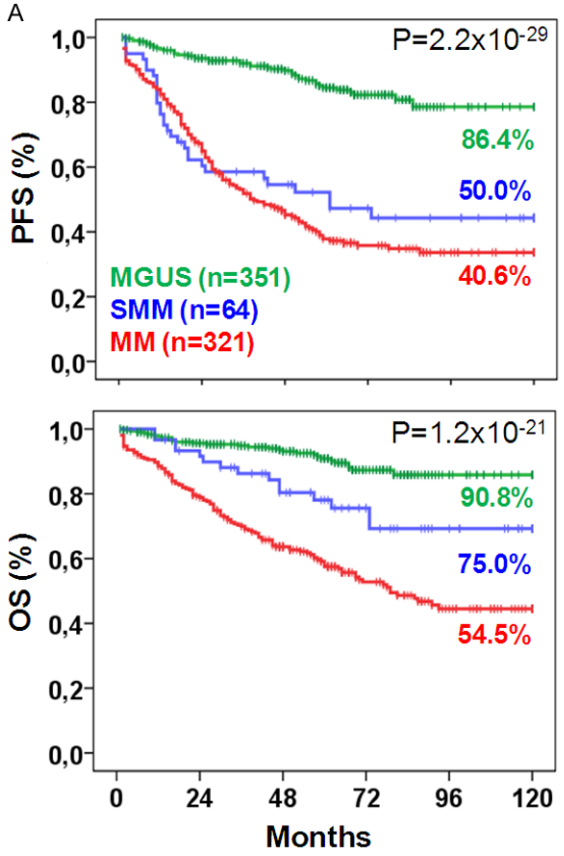
[44] Jasiński M, Basak GW and Jedrzejczak WW. Perspectives for the use of CAR-T cells for the treatment of multiple myeloma. Front Immunol 2021; 12: 632937.

[45] Pawlyn C, Melchor L, Murison A, Wardell CP, Brioli A, Boyle EM, Kaiser MF, Walker BA, Begum DB, Dahir NB, Proszek P, Gregory WM, Drayson MT, Jackson GH, Ross FM, Davies FE and Morgan GJ. Coexistent hyperdiploidy does not abrogate poor prognosis in myeloma with adverse cytogenetics and may precede IGH translocations. Blood 2015; 125: 831-840.



Supplementary Figure 1. Plasma cell (PC) immunophenotyping. Flow cytometry analysis of bone marrow samples performed with FACSCanto-II and DIVA Software (Becton Dickinson, San Jose, CA, BD). Photomultiplier (PMT) voltages were adjusted daily using CS&T beads (BD). Fluorescence compensations were finely adjusted using negative events as reference for each fluorochrome. A total of three million white cells were stained for each tube, tube-1: CD3+CD20 FITC, CD19 PE, CD38 PerCP-Cy5.5, CD56 PE-Cy7, CD27 APC, CD45 APC-Cy7, Annexin-V V450, and CD138 BV510 (BD); and tube-2: cylgG, cylgA, cylgD, or cylgM FITC, cyLambda PE, CD38 PerCP-Cy5.5, CD56 PE-Cy7, cyKappa APC, CD45 APC-Cy7, CD19 BV421, and CD138 BV510 (BD). One million cells were recorded for each tube. After doublet discrimination (in a FSC-H/SSC-A dotplot), total PC (Blue) were identified as $CD38^{+++}CD138^{+/++}$ events. Aberrant PC (cyan) were identified as $CD45^{low/negative}$, $CD19^{low/negative}$, $CD20^{+}$, $CD27^{low/negative}$, $CD56^{+}$ and/or monoclonal restriction for the heavy and/or light immunoglobulin chains (specific gating strategy was followed for each patient based on their phenotype). Mature B cells (red) were identified as lymphocytes (FSC/SSC^{low}) $CD19^{+}CD45^{++}CD38^{-/+low}$. Immature B lymphocytes (pink) were defined as $CD19^{+}CD45^{low}CD38^{++}$. Grey cells are non-B non-PC cells.

A



B

Standard risk stratification (sRisk)

10-year PFS (%)

	MGUS	SMM	MM
sRisk-I	94.4	58.3	55.9
sRisk-II	77.9	50.0	39.0
sRisk-III	63.6	20.0	15.6
P <	0.0001	0.05	0.0001

10-year OS (%)

	MGUS	SMM	MM
sRisk-I	96.3	87.5	83.8
sRisk-II	85.5	75.0	50.0
sRisk-III	72.7	40.0	18.7
P <	0.0001	0.01	0.0001

Supplementary Figure 2. Survival of patients according to the type of plasma cell neoplasm (PCN) and their standard risk. A. Kaplan-Meier and Log-rank tests for Progression-Free (PFS) and Overall Survival (OS) according to PCN stage: monoclonal gammopathy of undetermined significance (MGUS), smoldering multiple myeloma (SMM), or multiple myeloma (MM). Ten-year survivals are shown for each PCN type. B. Ten-year PFS and OS rates (estimated with Kaplan-Meier and Log-rank tests) according to the low, intermediate (mid) and high standard risk for MGUS [30], SMM [31] and MM [29] patients.