An acquired high-risk chromosome instability phenotype in multiple myeloma: Jumping 1q Syndrome

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Abstract
Patients with multiple myeloma (MM) accumulate adverse copy number aberrations (CNAs), gains of 1q21, and 17p deletions during disease progression. A subset of these patients develops heightened 1q12 pericentromeric instability and jumping translocations of 1q12 (JT1q12), evidenced by increased copy CNAs of 1q21 and losses in receptor chromosomes (RC). To understand the progression of these aberrations we analyzed metaphase cells of 50 patients with ≥4 CNAs of 1q21 by G-banding, locus specific FISH, and spectral karyotyping. In eight patients with ≥5 CNAs of 1q21 we identified a chromosome instability phenotype similar to that found in ICF syndrome (immunodeficiency, centromeric instability, and facial anomalies). Strikingly, the acquired instability phenotype identified in these patients demonstrates the same transient structural aberrations of 1q12 as those found in ICF syndrome, suggesting similar underlying pathological mechanisms. Four types of clonal aberrations characterize this phenotype including JT1q12s, RC deletions, 1q12-21 breakage-fusion-bridge cycle amplifications, and RC insertions. In addition, recurring transient aberrations include 1q12 decondensation and breakage, triradials, and 1q micronuclei. The acquired self-propagating mobile property of 1q12 satellite DNA drives the continuous regeneration of 1q12 duplication/deletion events. For patients demonstrating this instability phenotype, we propose the term “Jumping 1q Syndrome.”

Introduction
Chromosome instability (CIN) is defined as an increased rate and ongoing acquisition and accumulation of copy number aberrations (CNAs), and not simply the existence (state) of structurally and numerically abnormal aneuploid clones1,2. The most common CIN phenotypes are either whole-chromosome gains or losses or unbalanced structural aberrations leading to segmental focal amplifications and deletions. CIN phenotypes are thought to contribute to tumor progression and drug resistance through the amplification of oncogenes and/or loss of tumor suppressor genes and will require novel therapeutic interventions3,4. In multiple myeloma (MM), numerical and structural chromosome aberrations are most commonly identified by interphase fluorescence in situ hybridization (iFISH)5, which provides guidance for patient stratification and therapeutic intervention6. The International Myeloma Working Group (IMWG) has designated a group of adverse IgH translocations including t(4;14), t(14;16), and t(14;20), and adverse CNAs including deletions of 17p and gains of 1q217,8. The accumulation of different combinations of these adverse iFISH lesions during tumor progression has been reported to be a factor contributing to high-risk disease and eventual relapse9.
In MM, whole-chromosome CNAs are reflected in either hyperdiploid or nonhyperdiploid clones, which are not indicative of CIN, since both of these aneuploidy clones generally remain stable during the course of the disease. CIN phenotypes also include arm length and smaller segmental CNAs involving focal amplifications and deletions that are best identified by single-nucleotide polymorphism (SNP) microarrays. SNPs show that segmental CNAs are frequent in MM, and that 1q21 is not only one of the most frequent CNAs in this disease but in all cancers. Microarray studies have shown that in a typical cancer 25% of the genome is affected by arm-level CNAs, while only 10% by focal CNAs. Importantly, in MM the gains of the 1q21 region occur most commonly as arm-level aberrations, but also occur as focal amplifications, both of which have been shown to result from 1q12 instability. The 1q21 region is known to contain a number of putative oncogenes and genes that may show the simultaneous amplification and/or deregulated expression, including MCL1, IL6R, BCL9, KCS1B, ANP32E, ILF2, and ADAR1. Gain of a single copy of 1q (CN 1q21 of 3) are found in about 30% of newly diagnosed patients, while in highly proliferative disease or relapsed patients the accumulation of additional 1q21 CNAs result in as many as 70% of patients having four or more 1q21 aberrations. Increasing copy numbers of 1q21 are associated with a worse prognosis. The accumulation of CNAs of 1q21 evidenced by the progressive gain of 1q21 from 3 to 4 and 5 copies is significant because it serves as a marker for chromosomal instability, including whole-arm gains and losses, breakage-fusion-bridge (BFB) cycle gene amplification, and dispersed insertions. Recent comprehensive genomic analysis indicates that the nonrandom accumulation of genetic hits occurs on top of the primary events in myeloma, and that high-risk subgroups can be defined by either biallelic deletion of TP53 or amp 1q21 (≥4 copies) on the background of ISS III. Unfortunately, the underlying mechanisms for the genomic CNAs, such as del(17p) and gain of 1q21, are still poorly understood.

We have previously reported increasing CNAs of 1q21 occurring as jumping translocations of 1q12 (JT1q12) and that these translocations are the cause of unbalanced aberrations in the receptor chromosomes (RCs). A subset of these patients with CNAs ≥ 5 1q21 has shown an increased level of site-specific instability in 1q12 satellite DNA. Importantly, the 1q12 satellite DNA region in these patients acquires a self-replicating mobile property that is demonstrated by the ability to duplicate the 1q12 region and to “jump” to different nonhomologous chromosomes. This 1q12 CIN is continually regenerated in subsequent cell cycles, resulting in both novel transient and clonal 1q12 aberrations. This intrachromosomal CIN strongly suggests a mechanistic link to the CIN phenotypes found in the autosomal recessive (AR) CIN syndromes, most notably ICF syndrome (immunodeficiency, centromeric instability, and facial anomalies) and AR CIN syndromes showing unbalanced gains of 1q as part of their cytogenetic phenotypes. In particular, ICF patients show hallmark aberrations including site-specific 1q12 pericentromeric instability involving 1q12 decondensation, 1q12 triradials, multibranch chromosomes 1q, isochromosomes 1q (iso 1q), unbalanced translocations with 1q6, and 1q micronuclei in blood cultures. Strikingly, myeloma patients with highly proliferative disease and high CNAs of 1q21 share these same transient 1q12 chromosome aberrations, but also display a spectrum of clonal 1q21 CNAs. A defining feature of this CIN phenotype in MM is the continuous regeneration of unstable aberrations of 1q12 that results in an ongoing accumulation of both arm-level and focal CNAs. The distinction between the 1q12 instability in ICF syndrome and that seen in MM is that in ICF the instability is transient, found only in blood, while in MM the 1q12 aberrations become clonal and continue to drive subclonal copy number heterogeneity.

To investigate the structural relationships of 1q12 aberrations to the accumulation of 1q21 CNAs during disease progression, we undertook a metaphase FISH study of 50 patients with ≥ 4 copies of 1q by G-banding. This analysis revealed a subset of patients with high-risk IgH aberrations and/or 17p deletions and ≥ 5 copies of 1q21. We now recognize and report the cytogenetic features of a distinct acquired 1q12 CIN phenotype. These patients show profound chromosome 1q12 instability including the major clonal features of JT1q12 gains, losses of RC arms including 17p, amplification of the 1q12-23 region by BFB cycles, and dispersed insertions of non-homologous chromosome segments. Transient features of this phenotype include the decondensation and breakage of 1q12, acentric lagging chromosomes 1q, 1q21 micronuclei, and localized shattering of 1q distal to 1q12. In addition, the identification of different copy numbers or the switching of copy numbers can occur in different subclones. For this constellation of 1q12 cytogenetic findings, we propose the term “Jumping 1q Syndrome.” To provide guidance for patient stratification and therapeutic intervention this phenotype can be identified by IMWG iFISH panels by the presence of ≥ 5 CNAs of 1q21 concurrent with the detection of at least one other adverse iFISH lesion.
Patients and methods
The Institutional Review Board of the University of Arkansas for Medical Sciences approved the research studies, and all subjects provided written informed consent approving the use of their samples for research purposes. Metaphase chromosomes were prepared from bone marrow, fine needle aspirate, and pleural effusion specimens, and processed for G-band analysis as previously described. Patient sample selection was based on the suspected presence of at least two additional copies of 1q (≥4); only patients with ≥4 copies of 1q21 confirmed by metaphase FISH were included in this study. Specimens with unresolved aberrations by FISH were further scrutinized by spectral karyotyping (SKY).

FISH and SKY methods
Probes used to demark the pericentromeric regions of chromosomes including sat III (1q12), sat II (D16Z3) (16q11), and alpha sat (9q12) were used according to the manufacturer’s protocol (Vysis, Downers Grove, IL). Two probe sets for the detection of IgH translocations included IGH/FGFR3 dual color, dual fusion probe set and the IgH dual color, break-apart rearrangement probe (Vysis). FISH probes for MCL1 (1q21), CKS1B (1q21), TP53 (17p13), and ERBB2 (17q12) were prepared as previously described. The probe mixture and hybridization reagents were prepared by Applied Spectral Imaging (Carlsbad, CA) and procedure performed as previously described. Image acquisition for FISH and SKY was performed using an SD200 Spectracube (Applied Spectral Imaging, Inc.) mounted on a Zeiss Axioplan II microscope (Gottingen, Germany). DAPI images were captured, and then inverted and enhanced by SKY View software to produce G-band-like patterns on the chromosomes, which propagates the extra copy to 1q21 (Fig. 1a).

Results
Metaphase FISH results and clinical characteristics for all 50 patients are provided in Supplemental Table 1, and complete G-band karyotype designations are provided in Supplemental Table 2. FISH analysis showed 27 patients with four copies of 1q21 (nos 3, 9, 10, 12, 13, 15, 16, 17, 19, 24, 25, 26, 27, 28, 29, 33, 34, 35, 36, 39, 40, 41, 43, 44, 45, 48, and 50), while 23 patients had ≥5 copies (nos 1, 2, 4, 5, 6, 7, 8, 11, 14, 18, 20, 21, 22, 23, 30, 31, 32, 37, 38, 42, 46, 47, and 49). Patients with the IgH translocations t(4;14), t(14;16), and t(14;20) were grouped together and designated as adverse IgH lesions. Seventeen patients showed adverse IgH lesions (nos 2, 4, 7, 16, 17, 18, 19, 20, 21, 25, 27, 39, 43, 45, 46, 47, and 49). Six patients (nos 6, 8, 9, 24, 41, and 50) showed t(11;14), eight (nos 1, 11, 13, 32, 33, 37, 38, and 44) showed del 14q32 or -14, and one (14) with t(8;14). Eighteen patients (nos 3, 5, 10, 12, 15, 22, 23, 26, 28, 29, 30, 31, 34, 35, 36, 40, 42, and 48) were normal for IgH FISH. FISH identified 22 patients with deletion 17p (nos 3, 4, 5, 8, 9, 14, 16, 19, 22, 24, 29, 30, 34, 36, 37, 39, 40, 42, 43, 45, 49, and 50) and 28 normal. Five showed RC deletions of 17p by JT1q12 (nos 3, 30, 42, 43, and 49) (Supplemental Table 1).

Jumping translocations of 1q12 and concomitant deletions in RCs
Among the total group of 50 patients, 36 with JT1q12 showed a concomitant deletion in an RC, while 14 showed no deletion in an RC (Supplemental Table 1). Recurring arm-length deletions in five or more patients were identified in chromosome arms 1p, 19q, 6q, 16q, and 17p (Supplemental Table 1). Among the 23 patients with ≥5 CNAs of 1q21 recurring arm-length losses were found in 1p (nos 2, 4, 6, and 7), 6q (nos 1, 14, and 46), 17p (nos 30, 42, and 49), 16q (nos 20, 32, 37, and 38), 19q (nos 22 and 31), and six with no deletion (Table 1).

We identified a subset of eight patients with ≥5 of 1q21, arm-length deletions (nos 2, 4, 7, 14, 22, 30, 42, and 49), and at least one other high-risk iFISH lesion (Table 1) that showed ongoing instability of 1q12 in their metaphase cells. These patients showed continuous regeneration of novel 1q12 and 1q21 CNAs, with concomitant losses in RCs. For example, patient no 22 showed regenerating JT1q12 instability with a range of CNAs for 1q21 from 5 to 9 and multiple sequential aberrations to chromosomes 19 (Fig. 1a–h). The driver of 1q21 CNAs in this patient is evidenced by the presence of the hallmark triradiial of 1q12, which propagates the extra copy to 1q21 (Fig. 1a). In this patient, the JT1q12 jumped to RC19 deleting the 19q (Fig. 1b), which subsequently reduplicates and shows two copies of 1q21 (Fig. 1c). In a second more complex subclone a multichromosome rearrangement distal to 1q21 is identified by SKY (Fig. 1d), and continued decondensation and breakage of 1q12 is seen (Fig. 1e). In a third subclone (Fig. 1f–h), an isochromosome 1q is identified containing four copies of 1q21 (Fig. 1h). The continuous 1q12 instability on the RCs 19q resulted in subclones containing different copy numbers of 1q21, in different configurations (Fig. 1a–h).

The loss of chromosome 1p by the formation of isochromosome 1q was identified in four patients (nos 4, 6, 7, and 8) with ≥5 of 1q21, with one of these showing complex intra-arm amplification of the 1q12–23 region generated by BFB cycle amplification. Patient no 7 showed the specific progressive expansion of the 1q12–23 region by BFB amplification with 1q12 as the recurring breakpoint in the amplification cycles. In this patient the 1q12–23 amplicon expanded, demonstrating increasing CNAs with four, six, eight, and twelve copies of 1q21
In addition to the ladder-like amplification pattern resulting in higher CNAs of 1q12-23, some cells also displayed small extra-chromosomal rings (Fig. 2d [top]). In a second patient (no 4) with iso 1q, extensive cell to cell heterogeneity was found for both copy number and the positioning of 1q12 and 1q21 within the long arm of 1q (Fig. 2a–e [bottom]). These cells showed CNAs for 1q21 which ranged from 4 to 7 and also showed transient features of instability associated with catastrophic chromosome aberrations including localized 1q pulverization and 1q21 micronuclei (Supplemental Fig. 1a–c).

Deletion of 17p and concomitant gain of 1q21, der(1;17)q12q10

Deletion of 17p is the single most important iFISH indicator of poor prognosis5,32,33 and has previously been found to occur by the JT1q1220. In this study five patients showed del(17p) resulting from JT1q12s (nos 3, 30, 42, 43, and 49). Two of these patients had four copies of 1q21, and three showed five copies (nos 30, 42, and 49), all of which showed additional CNAs of 1q21 involving different RCs (Fig. 3a–c). These cases demonstrate that the der(1;17) is a recurring secondary aberration, which involves both an amplification of 1q21 and concomitant deletion of TP53. The additional copies of 1q21 involving different RCs demonstrate the ongoing subclonal heterogeneity introduced by the 1q12 instability.

Amplification and insertions of MYC and MET

The amplification and distribution of insertions in cancer cells is poorly understood34. In this study we found two patients with cryptic unbalanced insertions resulting from 1q12 instability. Patient no 2 showed multiple subclones demonstrating both transient and clonal 1q12 aberrations (Fig. 4). The initial 1q12 aberrations involved a JT1q12 to distal 8q (Fig. 4a). SKY detected a cryptic t(8;16)(q24;p13) had taken place prior to the JT1q12, resulting in the presence of 16p13 inserted between MYC and MET.

### Table 1 Ploidy levels and metaphase FISH results for 23 patients with ≥5 copies for 1q21

| Ploidy  | IgH trans | 1q12 CN | 1q21 CN | 17p CN | 1q12 trans to RC | 1q12 deletion in RC |
|---------|-----------|---------|---------|--------|-----------------|-------------------|
| 1       | NHRD      | del(14q32) | 6       | 6      | 2    | der(1;6) | del 6q |
| 2       | NHRD      | t(4;14)   | 5–10    | 5–6    | 2    | der(1;8) | del 1p |
| 4       | NHRD      | t(4;14)   | 4–6     | 5–7    | 1    | iso 1q   | del 1p |
| 5       | HRD       | Normal    | 5       | 5      | 1    | der(1;12) | None  |
| 6       | NHRD      | t(11;14)  | 5–6     | 5–6    | 2    | iso 1q   | del 1p |
| 7       | NHRD      | t(4;14)   | 5–9     | 5–9    | 2    | iso 1q   | del 1p |
| 8       | NHRD      | t(11;14)  | 6–8     | 6–8    | 1    | iso 1q   | del 1p |
| 11      | NHRD      | del(14)   | 5–7     | 5–7    | 2    | quad 1q, trip 1q21 | None  |
| 14      | HRD       | t(8;14)   | 5–6     | 5–6    | 1    | der(1;19) | del 6q |
| 18      | NHRD      | t(4;14)   | 5–7     | 5–7    | 2    | dup 1q, der(1;7)dup 2 | None  |
| 20      | NHRD      | t(4;14)   | 6       | 6      | 2    | der(1;16) | del 16q |
| 21      | NHRD      | t(4;14)   | 5       | 5      | 2    | der(1;7) | None  |
| 22      | HRD       | Normal    | 5–9     | 5–9    | 1    | der(1;19) | del 19q |
| 23      | HRD       | Normal    | 5       | 5      | 2    | dup 1q, der(1;13) | None  |
| 30      | NHRD      | Normal    | 5–6     | 5–6    | 1    | der(1;9p)der(1;17) | del 17p |
| 31      | NHRD      | Normal    | 7       | 7      | 2    | der(1;19) | del 19q |
| 32      | NHRD      | del(14q32) | 6       | 6      | 2    | der(16), der(1;22) | del 16q |
| 37      | NHRD      | del(14q32) | 5       | 5      | 1    | der(1;16),der(1;20) | del 16q |
| 38      | NHRD      | del(14q32) | 5       | 5      | 2    | der(1;16) | del 16q |
| 42      | HRD       | Normal    | 5       | 5      | 1    | der(1;17) | del 17p |
| 46      | NHRD      | t(4;14)   | 5       | 5      | 2    | der(1;6), dup 1q | del 6q |
| 47      | HRD       | t(4;14)   | 5       | 5      | 3    | der(1;2), der(19), der(22) | None  |
| 49      | NHRD      | t(4;14)   | 5       | 5      | 1    | der(1;17) | del 17p |

IgH translocations, CNAs for 1q12, 1q21, 17p, and receptor chromosome gains and losses related to 1q12. Chromosome ploidy levels defined by hyperdiploid (HRD) equaling 47–75 chromosomes and nonhyperdiploid (NHRD) equaling 46 and/or >75 chromosomes.
and 1q12 on the der(8) (Fig. 4a). Additional progression events included a direct duplication of 1q12-21 on the 8q resulting in extra copies of 1q12, 1q21, MYC, and 16p13 (Fig. 4b). BFB cycle amplification (Fig. 4c–e) of both MYC and 16p was found, with the initial step being the loss of 1q, followed by a sister chromatid fusion (SCF) at 1q12 (Fig. 4c). The SCF resulted in the formation of an unstable 8q dicentric with 1q12 as the bridging point (Fig. 4d) and subsequent BFB cycle amplification of MYC and 16p (Fig. 4e). In a different subclone, a translocation/insertion of both MYC and 16p into the 1q12 region of one of the previously normal chromosome 1 homologs was found (Fig. 4f)16,20. This insertion could arise following the ongoing instability of 1q12 demonstrated by the presence of the hallmark 1q12 triradial on the distal 8q in the same cell as an extra acentric lagging copy of 1q (Fig. 4g). These transient 1q12 aberrations demonstrate first, that the continuous regeneration of 1q12 triradials occurs on nonhomologous RCs, and secondly this mechanism can cause dispersed insertions of nonhomologous chromosome segments, such as MYC translocations. Additional transient aberrations in this patient included 1q12 micronuclei, interphase bridging of 1q12, and acentric iso(1q)s (Supplemental Fig. 2).

The amplification and dispersed insertion of the 7q31 region containing the MET loci was identified in patient no 14. This patient demonstrated the amplification of both the MET region and 1q21 to 6q and 1q (Fig. 5). In this patient a JT1q12 to 7q occurred where a direct duplication of 1q12-23 occurred (Fig. 5a). Subsequently, a JT1q12 to 6q carrying the MET region caused a deletion in the 6q (Fig. 5b). A second JT1q12 resulted in an insertion of MET into the 1q12 region of one of the previously normal chromosomes 1 (Fig. 5c). Of note,
patient no 14 also showed nuclear budding, interphase bridging of 1q12, and multiple variations of acentric lagging 1qs leading to cells with 1q21 counts of 7–8 (Supplementary Fig. 3). Both patients, nos 2 and 14, add to the previous reports demonstrating insertions of MYC, BCL2, 16q11, and 20q11 into the 1q12 region, are recurring aberrations of this phenotype18,20.

Major and minor metaphase aberrations identifying the 1q12 instability phenotype

From this group of eight patients with ≥5 1q21s (nos 2, 4, 7, 14, 22, 30, 42, 49), and 21 patients from previous reports18–21, we identify four major recurring types of clonal cytogenetic aberrations that can be used to define this 1q12 site-specific instability phenotype. These include: (1) JT1q12s to an RC, (2) arm-length deletions in RC, (3) BFB cycle amplifications, and (4) 1q12 insertions. In addition, five types of recurring transient aberrations that define this phenotype include: (1) 1q12 decondensation and breakage, (2) 1q12 triradials, (3) acentric lagging 1q12, (4) 1q12 micronuclei, and (5) localized pulverization of 1q. The key structural chromosome rearrangements defining this CIN phenotype are depicted in Fig. 6. In normal cells, the 1q12 pericentromeric region remains condensed in metaphase (Fig. 6a). Cells showing the transient decondensation and breakage of 1q12 (Fig. 6b) are precursors to triradial formation at 1q12 as seen in patient nos 2 and 22 (Fig. 6c). The extra copy of 1q generated by the triradial results in a JT1q12 and can become a clonal arm-length duplication/deletion as depicted for loss of 17p (Fig. 6d). Alternatively, breakage at 1q12 can result in an acentric copy of 1q12 (Fig. 6e) which subsequently is lost as a micronucleus. JT1q12s to telomeres (Fig. 6f) can remain stable or can further evolve to different subclones. For example, in patient no 2 the der(8) in subclone 1 (Fig. 6g) evolved by breakage at 1q12 and the loss of 1q, which initiated the formation of a SCF at 1q12. This SCF resulted in the formation of a dicentric der(8) (Fig. 6h), which in turn resulted in BFB cycles amplifying MYC and 16p (Fig. 6i). In subclone no 2, a triradial on the der(8) (Fig. 6j), is depicted with the subsequent 1q12 insertion of MYC and 16p into the 1q
In patient no 14 unbalanced dispersed insertions of the MET region of chromosome 7q31 (Fig. 6l) into different RCs result in a duplication of MET and deletion involving 6q (Fig. 6m), and an insertion of MET into 1q12 (Fig. 6n).

**iFISH Identification of 1q12 Instability Phenotype**

The jumping 1q12 instability phenotype is identified by iFISH by the presence of ≥5 CNAs of 1q21 and at least one other high-risk iFISH lesion, including high-risk IGH translocations and del(17p). In addition, deletions of 1p detected by iFISH can indicate either an interstitial deletion of 1p or iso(1q), both of which are associated with 1q12 instability.

**Discussion**

Chromosomal instability contributes to malignant transformation by altering gene dosage and is known to play a role in tumor progression, relapse, and therapeutic failures. The hallmark feature of CIN is the continuous regeneration of either whole-chromosome or segmental CNAs, the causes of which include mitotic segregation errors, aberrant DNA repair, DNA replication stress, and fragile sites, among others. Although these mechanisms can account for many of the numerical and structural aberrations found in MM, accumulating evidence indicates that additional mechanisms, such as epigenetic modifications and transcriptome remodeling, likely play a role in the spectrum of segmental CNAs found in relapsed...
and refractory patients. The presence of 1q12 DNA breaks, localized chromosome pulverization distal to 1q12, acentric lagging chromosomes 1q, and 1q12 micronuclei as described here are part of a spectrum of catastrophic chromosome aberrations associated with chromothripsis. In fact, localized pulverization of chromosome arms in micronuclei has been suggested as one mechanism for chromothripsis. Interestingly, chromothripsis has been reported in MM involving a rare, aggressive entity in some patients with 1q aberrations.

The CIN phenotype described here is acquired during disease progression or at relapse with the accumulation of a combination of adverse CNAs of 1q21, 17p, and 1q12 breakpoints. This cytogenetic phenotype is characterized by the continuous regeneration of site-specific 1q12 aberrations that propagate and induce an apparently unlimited number of both transient and clonal 1q12 aberrations (Figs. 1 and 3). Four recurring 1q12 cytogenetic features of this phenotype include: (1) JT1q12s to the RCs, (2) 1q12 induced deletions in RCs, (3) 1q12 induced BFB cycle amplifications, and (4) 1q12 insertions (Fig. 6). In addition, five transient features include: (1) 1q12 decondensation and breakage, (2) 1q12 triradials, (3) acentric lagging 1q12, (4) 1q12 micronuclei, and (5) localized pulverization of 1q distal to 1q12.

The cause of this cytogenetic phenotype is currently unknown; however, it has been speculated that hypomethylation or other modifications to the 1q12 region are responsible. The 1q12 pericentromeric heterochromatin is made up of the single largest block of late-replicating satellite DNA in the human genome, contains a 1q12 fragile site, and undergoes satellite repeat expansions in cancer. In normal cells this region remains highly condensed; however, in tumor cells this

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**Fig. 4 Partial karyotypes of patient no 2 demonstrating the different types of transient 1q12 aberrations that continually regenerate gains of 1q21 and MYC.**

A. Chromosome 8 showing metaphase FISH of JT1q12 to the der(8) hybridized on left with 1q12 (red) and 1q21 (green) and on right the same chromosome hybridized with MYC (red) and 16p (green). The der(8) (left) hybridized first with chromosome 1 probes demonstrates a direct duplication of 1q12 (red) and 1q21 (green). Importantly, the hybridization with FISH probes for MYC and 16p (on right) shows a cryptic duplication and concomitant amp of the region spanning 1q12-1q21, MYC, and 16p (arrows). C. Breakage at 1q12 and the loss of 1q distal to 1q12 resulted in a sister chromatid fusion (SCF) at 1q12 on the distal 8q. D. The SCF aberration resulted in the subsequent formation of a dicentric 8 showing 1q12 (arrow) as the bridging point connecting the dicentric 8. E. The outcome of BFB cycles of the dicentric (8) is the amplification of MYC and 16p in an inverted duplication pattern typical of BFB amplification. This chromosome has three copies of MYC and two of 16p. F. A dispersed insertion of MYC and 16p into one of the previously normal chromosomes 1. G. The regeneration of additional copies of 1q21 is demonstrated by a triradial of 1q12 (red) on distal der(8q) showing an extra copy of 1q21 (green) transiently attached to each chromatid of the der(8q). Note also in this cell the presence of an extra acentric lagging copy of 1q with signals for both 1q12 (red) and 1q21 (green).
region can become perturbed, decondensed and prone to breakage and jumping as occurs in ICF syndrome. ICF patients have a known mutation in DNA methyltransferase (DNMT) 3B, which is thought to be responsible for the site-specific hypomethylation of the 1q12 pericentromeric heterochromatin and 1q12 instability. In MM and other B-cell disorders no DNMT mutations known to affect 1q12 have been reported, although global DNA hypomethylation of repetitive elements and aberrant methylation patterns have been reported as associated with disease progression.

Accumulating evidence also now suggests modifications of the 1q12 region by other genetic and epigenetic factors. For example, reprogramming of 1q12 pericentromeric satellite DNA domain has been reported to be associated with polycomb body formation on the 1q12 satellite DNA. This epigenetic conversion coincides with both global and satellite DNA demethylation, and has been shown to be induced by inhibition of DNMTs. Another possible cause of instability is the overexpression of the chromatin modifying enzymes, such as KDM4A. KDM4A is a histone demethylase, which binds the BCL9 locus and causes the rereplication and site-specific copy number gains of 1q12 and 1q21, altered expression of microRNAs, and selective pressures, such as hypoxic stress.

The induced gains of 1q12 and 1q21 reported in these studies are not stably inherited but transiently regenerates in subsequent S phases, just as in ICF syndrome and MM. The in vitro modification of the 1q12 region by 5-azacytidine also provides evidence that the site-specific hypomethylation of the 1q12 region can induce copy number gains of 1q21 and regions translocated next to it. This finding demonstrates that 1q12 pericentromeric heterochromatin can potentially drive CNAs of any genomic region juxtaposed to it.

Alterations in RNA processing have also been linked to 1q21 CNAs through amplification and overexpression of ILF2. ILF2 is a gene, which promotes tolerance of genomic instability and the stabilization of transcripts involved in homologous recombination (HR). The upregulation of 1q21 by ILF2 deregulates HR resulting in increased abnormal nuclear morphologies including nucleoplasmic bridges, nuclear buds, and micronuclei, demonstrating the same types of chromosomal instability characterized here (Supplemental Figs. 1–3). Elevated levels of HR are thought to mediate genomic instability, an increase in mutations, and the accumulation of genetic alterations in MM. Finally, RNA editing in MM of ADAR1, an
adenosine deaminase acting on RNA-1 in the 1q21 region, has been reported to promote malignant regeneration resulting in reduced survival in MM49. Recent genomic studies have shown that most patients already have complex subclonal structure at diagnosis that undergoes further evolution over time26,27. The chronology of CNAs in MM suggests a greater degree of instability is associated with complex deletions and a punctuated evolutionary process50. This is in agreement with our findings that patients with more advanced disease show the onset of chromosome 1q12 instability and the accumulation of ≥5 copies of 1q21 in association with the deletions of 17p and other regions17–21. Here we define a high-risk cytogenetic phenotype as a constellation of cytogenetic findings identifiable by current iFISH panels. In this subset of patients, the 1q12 satellite DNA acquires a self-propagating mobile property that adversely impacts the genome by initiating whole-arm JT1q12 and concomitant deletions in RCs. As part of this phenotype, additional smaller focal duplications/triplications of 1q21 and other regions occur, evidenced by BFB cycles and insertions. We propose the term “Jumping 1q Syndrome” for this cytogenetic phenotype type based on the two factors. First, the striking mechanistic relationships of the 1q12 aberrations shared with ICF syndrome and second on the four characteristic patterns of aberrations associated with induced by 1q12 instability. CIN phenotypes enable cells to enter different evolutionary trajectories and adapt to selective pressures of therapies, which underlies treatment failures4. Given the low prevalence of mutated driver genes in MM, and the stronger impact of cytogenetic aberrations on prognosis over gene mutations51, it may be that the apparently unlimited combination of unbalanced 1q12 amplifications/deletions is in part responsible. This study suggests that these patients may exhibit a previously unrecognized form of ultra-high-risk disease52,53, and provides evidence that patients with ≥5 CNAs of 1q21 have a type of emerging epigenetic
instability in need of novel types of therapeutic intervention\textsuperscript{54–56}.

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Author contribution
J.R.S. conceived hypothesis, analyzed and interpreted data, and wrote the manuscript. E.T. and J.L.L. performed FISH and SKY studies. C.D.S., S.T., F.E.D., G.M., B.B., M.Z. and F.V.R. provided patient samples and reviewed manuscript. All authors read and approved the final submission of the manuscript.

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