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Amp(1q) and tetraploidy are commonly acquired chromosomal abnormalities in relapsed multiple myeloma

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Abstract

Long-term disease control in multiple myeloma (MM) is typically an unmet medical need, and most patients experience multiple relapses. Fluorescence in situ hybridization (FISH) is the standard technique to detect chromosomal abnormalities (CAs), which are important to estimate the prognosis of MM and the allocation of risk adapted therapies. In advanced stages, the importance of CAs needs further investigation. From 148 MM patients, two or more paired samples, at least one of which was collected at relapse, were analyzed by FISH. Using targeted next-generation sequencing, we molecularly investigated samples harboring relapse-associated CAs. Sixty-one percent of the patients showed a change in the cytogenetic profile during the disease course, including 10% who acquired high-risk cytogenetics. Amp(1q) (≥4 copies of 1q21), driven by an additional increase in copy number in patients who already had 3 copies of 1q21, was the most common acquired CA with 16% affected patients. Tetraploidy, found in 10% of the samples collected at the last time-point, was unstable over the course of the disease and was associated with *TP53* lesions. Our results indicate that cytogenetic progression is common in relapsed patients. The relatively high frequency of amp(1q) suggests an active role for this CA in disease progression.

KEYWORDS
chromosome aberrations, multiple myeloma, recurrence, tetraploidy

Novelty statements

What is the new aspect of your work?

In a longitudinal multiple myeloma study, we examined the occurrence of chromosomal amplifications such as tetraploidy.

What is the central finding of your work?

Amp(1q) and tetraploidy are acquired relatively frequently in relapsed multiple myeloma.

What is (or could be) the specific clinical relevance of your work?

Therapeutically targeting chromosomal features such as amp(1q) and tetraploidy could potentially help overcome resistance mechanisms in the relapse setting.

1 | INTRODUCTION

Multiple myeloma (MM) is caused by monoclonal proliferation of malignant plasma cells.[1] The disease shows marked heterogeneity, which is reflected in the survival range from few months to over 10 years.[2] It was shown that disease progression is linked to genetic events.[3] Due to primary oncogenic events of either an immunoglobulin heavy chain gene (*IGH*) translocation or gains of odd-numbered chromosomes (hyperdiploidy), MM can be divided into two nearly equal sized groups with little overlap.[4–6] Secondary events, including gain of 1q, del(17p), del(1p) and *MYC* translocations, accumulate along the disease course. Recently, large datasets and computational approaches have been used to refine the genomic classification of MM by accounting for numerous features, resulting in a granular view of the disease.[7–9] In the context of genetic risk stratification of newly diagnosed MM (NDMM), the *IGH* translocations t(4;14), t(14;16) and t(14;20) as well as del(17p), all established unfavorable markers, are usually analyzed using fluorescence in situ hybridization (FISH).[2,10,11] Additional copies of 1q, detected in approximately 40% of NDMM, are also considered as prognostically unfavorable.[12] Amp (1q) (≥4 copies of 1q) appear to be correlated with shorter survival than gain(1q) (3 copies of 1q).[13,14] In longitudinal analyses, samples from the same patient obtained at different stages of disease (e.g., paired samples from diagnosis and relapse) are analyzed to study clonal evolution and disease progression.[3,15,16] This approach identified different evolution patterns after therapy: linear increase of abnormalities, losses and gains of abnormalities indicative of branching evolution, and clonal stability.[15,17] Longitudinal analyses by FISH provided evidence that clonal instability may be associated with an adverse outcome.[18,19] In particular, gain/amp(1q) and del(17p), which are among the most commonly acquired chromosomal abnormalities (CAs) at relapse, have been associated with an adverse prognosis,[19–22] underscoring the utility of repeated FISH testing during the disease course.[23] Limited data are available on near-tetraploidy/tetraploidy, which refers to 4 copies of (almost) every genomic region and results from genome doubling.[24]

In our cohort of 148 patients with at least two longitudinal samples we aimed to investigate the evolution of CAs such as amp (1q) and near-tetraploidy/tetraploidy. In addition, we investigated in which cytogenetic subgroup and therapy context, cytogenetic evolution or stable progression took place.

2 | METHODS

2.1 | Patients

Our database was reviewed to identify 148 MM patients for whom two or more samples were analyzed by Interphase FISH. The FISH analyses were performed between January 2010 and June 2021. In 120 patients, the first sample was analyzed at diagnosis and in 28 patients at relapse. The following samples were obtained at subsequent relapses or refractory stages. The number of therapy lines was determined according to standard guidelines.[25] The study was approved by the local ethics committee of the Medical University of Innsbruck (protocol #1348/2020).

2.2 | Interphase FISH

Interphase FISH analysis was carried out either on unsorted samples or on CD138+ enriched plasma cells after purification by magnetic-activated cell sorting (Miltenyi Biotec, Bergisch Gladbach, Germany) or RoboSep (STEMCELL Technologies, Vancouver, Canada). Probes for the chromosomal regions 1q21 (*CKS1B*), 11q22 (*ATM*), 13q14 (*DLEU1*) and 17p13 (*TP53*) as well as a break-apart probe for the region 14q32 (*IGH*) were used. If the results showed an *IGH* split, reflex testing was performed with the translocation probes t(4;14) (*FGFR3/IGH*), t(11;14) (*CCND1/IGH*), and t(14;16) (*IGH/MAF*). The 1p probe was changed in August 2016 from 1p36 (D1S2795, D1S253) to 1p32 (*CDKN2C*). Hybridization was carried out according to the manufacturer's instructions (Kreatech, Amsterdam, Netherlands; MetaSystems, Altlussheim, Germany; Vysis/Abbott, Downers Grove, IL). The thresholds for anomaly detection were set at 5% for gains and translocations and 10% for deletions. ≥3 and ≥4 copies of a particular region were defined as gain and amplification (amp), respectively. Near-tetraploidy/tetraploidy (denoted here as tetraploidy or 4N) was predicted when 3 or more chromosomal regions indicated a doubled genome. A region was counted as indicative if either 4 copies were present or a previous deletion was lost. Cytogenetic subgroup designation was performed as previously published.[14]

2.3 | Next-generation sequencing

Samples from 53 patients (10 with tetraploidy, 25 with amp(1q) and 18 control MM as determined by FISH) were analyzed by custom

hybridization-based sequencing panel of 28 genes known to be mutated in MM (Table S1). Of these patients, 39 were treatment-naive and 14 were relapsed/refractory. To be considered, the unsorted samples or the isolated CD138+ plasma cells had to show CAs in at least 40% of the cells as determined by prior FISH analysis. Unsorted samples were prepared from methanol:acetic acid-fixed cells as previously described.[26] Libraries were sequenced on a NextSeq 500 using 150-bp paired-end reads (Illumina, San Diego, CA). Using DRAGEN Somatic app (v3.8.4) with default parameters (tumor-only mode) on BaseSpace (Illumina), reads were mapped to the human reference genome (hg38) and single-nucleotide variants (SNVs) and indels were called. Sequencing artifacts were flagged using a panel of normals and filtered out. Variant Interpreter (Illumina) was used to annotate passed variants with coding consequences, a variant allele frequency of ≥5% and a frequency of less than 0.05% in the Genome Aggregation Database. The passed variants were visually inspected in the Integrative Genomics Viewer.

2.4 | Data analysis

Data analysis was performed in R version 4.1.1 (www.r-project.org/). The McNemar test was used to examine the changes in CAs from the first to the last cytogenetic evaluation. Fisher's exact test was used to test association between categorical parameters. *P* values of ≤.05 were considered statistically significant.

3 | RESULTS

3.1 | Patient characteristics

Our cohort included 148 patients of whom two to five samples were analyzed. Median time from first cytogenetic evaluation to last sampling was 729.5 days. At baseline, 35 patients (24%) had high risk cytogenetics.[2] During follow-up the number of patients with high risk abnormalities increased to 50 (34%). Similarly, the number of patients with co-occurrence of two and three adverse abnormalities (double hit and triple hit)[27] increased from 21 (14%) to 34 (23%) and 0 to 4 (3%), respectively. Patient characteristics are shown in Table S2.

3.2 | Stability of CAs during the disease course

Seventy-eight of 148 cases (53%) had acquired and/or lost CAs and were considered as cytogenetically unstable. The most frequently gained CA was del(17p) in 13% (19/148), gain/amp(1q) (≥3 copies of 1q21) in 12% (18/148), gain/amp(11q) in 9% (14/148), and del(13q) in 9% (13/148) of cases (Table S3). We found an increased risk of gain/amp(1q) ($p = .02$, McNemar's test) and del(17p) ($p < .001$, McNemar's test) occurring in the samples collected at the last time-point compared to the baseline samples. In six patients, del(13q) was acquired together with del(17p) in the same sample (Table S4), which suggests that these two abnormalities may function cooperatively in tumor progression. By considering amplifications such as amp(1q) as distinct from gains, the percentage of cytogenetically unstable cases increased to 61% (90 of 148 cases). Moreover, with this counting approach, amp(1q) (≥4 copies of 1q21) was significantly enriched in the group of samples obtained last ($p = .01$, McNemar's test) and, with 16% (23/148) affected cases, the most frequently gained CA in our cohort (Tables S3 and S4). In 26% of these cases (6/23), amp(1q) was gained in the context of an acquired tetraploidy. In 7% of cases (10/148) ≥5 copies of 1q21 were acquired during the disease course. Nine percent of patients (13/148) acquired a tetraploidy, a condition, which happened to be particularly unstable during the disease course (Figure 1A,B). In the majority of cases with tetraploidy (13/21), tetraploidy was acquired and this mostly occurred in the last samples analyzed. Patients who showed tetraploidy in their first sample almost always lost it, usually between the first and second sampling. Only patient MMP-46581 showed tetraploidy over the whole analyzed disease course (137 days) (Table S4). Patient MMP-69541 had a temporary loss of tetraploidy before it was detected again in the last sample collected. Interestingly, 7 of 13 patients with acquired tetraploidy had an amp(1q) in a previous sample. In two other cases with lost tetraploidy, amp(1q) persisted, indicating that amp(1q) preceded the tetraploid clone also in these patients.

As previously reported[21,28,29] and in contrast to the unstable tetraploidy, the status of the primary *IGH* translocations t(4;14), t(11;14), and t(14;16) did not change over the disease course. The most frequent losses of CAs were gain/amp(17p), del(13q) and gain/amp(1q) in 7%, 5%, and 4% of cases, respectively (Table S3). Four of the seven supposed del(13q) losses and the only del(17p) loss occurred in connection with an acquired tetraploid karyotype and therefore presumably do not represent true losses, but rather masked deletions in a doubled genome (Table S4).

3.3 | Cytogenetic subgroups

We and others have previously reported on the role of subgroups in MM in acquiring CAs.[7,14,21,30] Tumors with t(4;14) were characterized by stable del(13q) (74% [14/19] of cases) and gain(1q) (68% [13/19]) (Table S5). One t(4;14) case (MMP-61227), initially tetraploid with 3 copies of 13q, showed the del(13q) only after loss of tetraploidy. A second t(4;14) case (MMP-80274), lacking del(13q) in the first sample, was also accompanied by tetraploidy; the 2 copies of 13q in this patient, indicated a relative loss of 13q. In another t(4;14) tumor (MMP-53996), gain(1q) was acquired later. Furthermore, 40% (4/10) of the cases in which ≥5 copies of 1q21 were acquired belonged to the t(4;14) subgroup. Tumors with t(11;14) acquired relatively often a gain/amp(11q) (50% of cases [7/14] with acquired gain/amp(11q)) and a tetraploidy (46% of cases [6/13] with acquired tetraploidy). Seven of 11 (64%) stable del(17p) were detected in the clonal gain(11q) (CG11q) subgroup.

3.4 | Patients with detailed clinical information

We had knowledge of treatment regimens and clinical outcomes from 41 of the 148 patients (Table S6). Fish plots of eight patients who

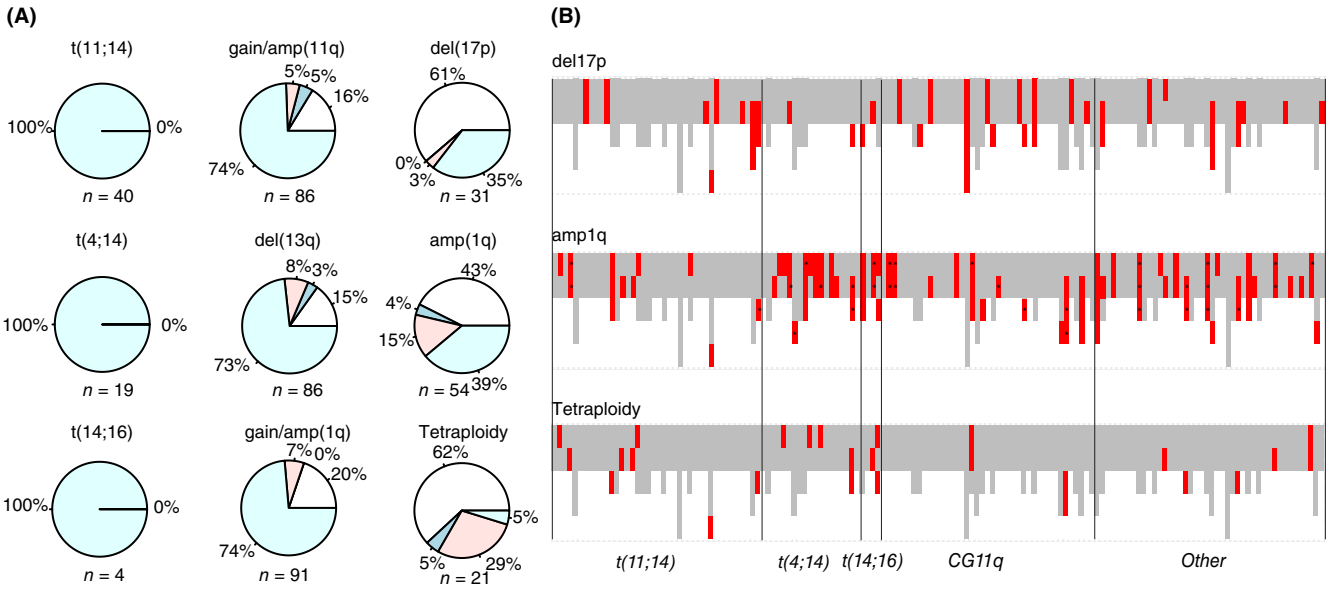

FIGURE 1 (A) Summary of the cases with a stable (represented by light blue), a lost (red), a gained and lost (dark blue), and a gained (white color) CA during the follow-up. Gain/amp(1q) and gain/amp(11q) indicate ≥3 copies of 1q21 and 11q22, respectively, and amp(1q) indicates ≥4 copies of 1q21. The percentages relate to the total number of the respective chromosome abnormalities detected in the cohort. (B) Profile of each patient with regard to frequently gained CAs (i.e., del(17p), amp(1q) and tetraploidy). Red color indicates the presence and gray color the absence of a CA. The patients are ordered from left to right according to the subgroups t(11;14), t(4;14), t(14;16), and CG11q and other. Asterisk indicates presence of ≥5 copies of 1q21.

showed tetraploidy at some point during their disease are depicted in Figure 2 and the plots of the other patients with detailed clinical data are shown in Supplemental Figure 1. Three patients (MMP-47355, MMP-73677, and MMP-98449) with acquired tetraploidy had a preceding amp(1q). Tetraploidy was accompanied by fatal outcome: patients MMP-10707, MMP-35601, MMP-73677, MMP-94267, and MMP-98449 died 49, 174, 28, 188, and 5 days after the detection of tetraploidy. An exception was patient MMP-47355 who was alive 643 days after detection of acquired tetraploidy. In three of the patients (MMP-10707, MMP-35601, MMP-73677), del(17p) and extramedullary disease were co-acquired with tetraploidy. Patient MMP-35601 showed a relative loss of 17p in the tetraploid clone before a second clone with an ordinary del(17p) appeared. Two patients (MMP-52190 and MMP-61227) showed tetraploidy already at diagnosis and lost it during follow-up. Patients with clonal gain of 11q without presence or acquisition of high risk features such as gain/amp(1q) or del(17p) showed a long survival (MMP-02871, MMP-10766, and MMP-52638; Supplemental Figure 1).

3.5 | Mutational profile of samples with amp (1q) and tetraploidy

We analyzed 53 MM cases by targeted next-generation sequencing (NGS) and found (putative) driver mutations in 44 of the cases (Table S7). Based on cytogenetic characterization, we divided the cases into three groups: tetraploid cases ($n = 10$), cases with amp (1q) ($n = 25$), and a control group of patients without tetraploidy or amp(1q) ($n = 18$). The mutational profile of these patients is shown in Figure 3. In the patients with amp(1q) and tetraploidy, the most frequently mutated gene was *NRAS* (29%), followed by *DIS3* (20%), and *KRAS*, *KMT2C*, *CREBBP*, and *ATM* (all 15%). *TP53* mutations were enriched in tetraploid cases compared to amp(1q) cases (30% [3/10] versus 0% [0/25]; $p = .02$, Fisher's exact test) and control cases (30% [3/10] versus 0% [0/18]; $p = .04$, Fisher's exact test) (Table S8). In two of the three tetraploid cases with *TP53* mutation also a del(17p) was detected by FISH, indicating a bi-allelic inactivation of *TP53*. An additional tetraploid case showed only a del(17p); thus, in 40% of tetraploid cases *TP53* was disrupted. We also found significantly more mutations in *CREBBP* in tetraploid cases compared to control cases (30% [3/10] vs. 0% [0/18]; $p = .04$, Fisher's exact test). Mutational profiles of treated ($n = 14$) and untreated patients ($n = 39$) were not significantly different (Table S9). Due to the small study population, the results should be interpreted with caution.

4 | DISCUSSION

Although the drugs used to treat MM have advanced over the past 10 years, long-term control of the disease or cure is only achieved in very few patients.[33] Therapy resistance and disease progression driven by genetic abnormalities are common features of the disease.[3,34] Understanding resistance mechanisms, including the emergence of fitter cancer clones, may ultimately help to design treatments that are more appropriate to the patient's need. While cytogenetic assessment at diagnosis is obligatory according to

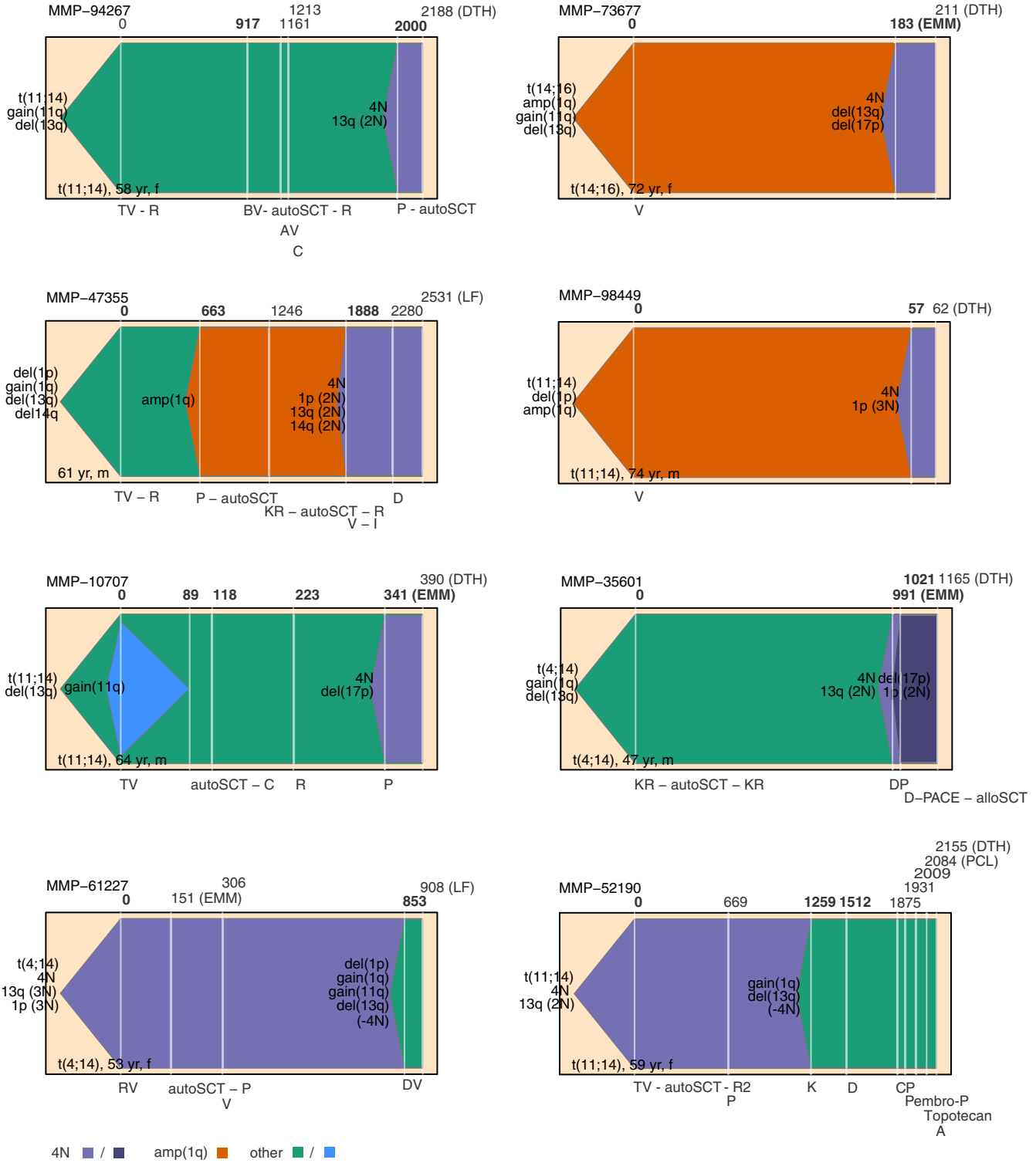

FIGURE 2 Cytogenetic evolution of patients with tetraploidy. Fish plots are drawn by the fishplot R package.[31] Days from treatment start are shown above and applied treatment schemes below. Last follow-up (LF), death (DTH), extramedullary multiple myeloma (EMM), and plasma cell leukemia (PCL) are indicated in brackets above. Vertical lines indicate performed cytogenetic analyses and/or treatment changes. Time-points with cytogenetic analyses are indicated in bold. Tetraploidy (4N) and amp(1q) are indicated by specific colors. A, anthracycline; alloSCT, allogeneic stem cell transplantation; autoSCT, autologous SCT; B, bendamustine; C, cyclophosphamide; D, daratumumab; D-PACE, dexamethasone, continuous-infusion cisplatin, doxorubicin, cyclophosphamide, and etoposide; I, ixazomib; K, carfilzomib (Kyprolis); P, pomalidomide; Pembro, pembrolizumab; R, lenalidomide (Revlimid); R2, Revlimid and rituximab; T, thalidomide; V, bortezomib (Velcade).

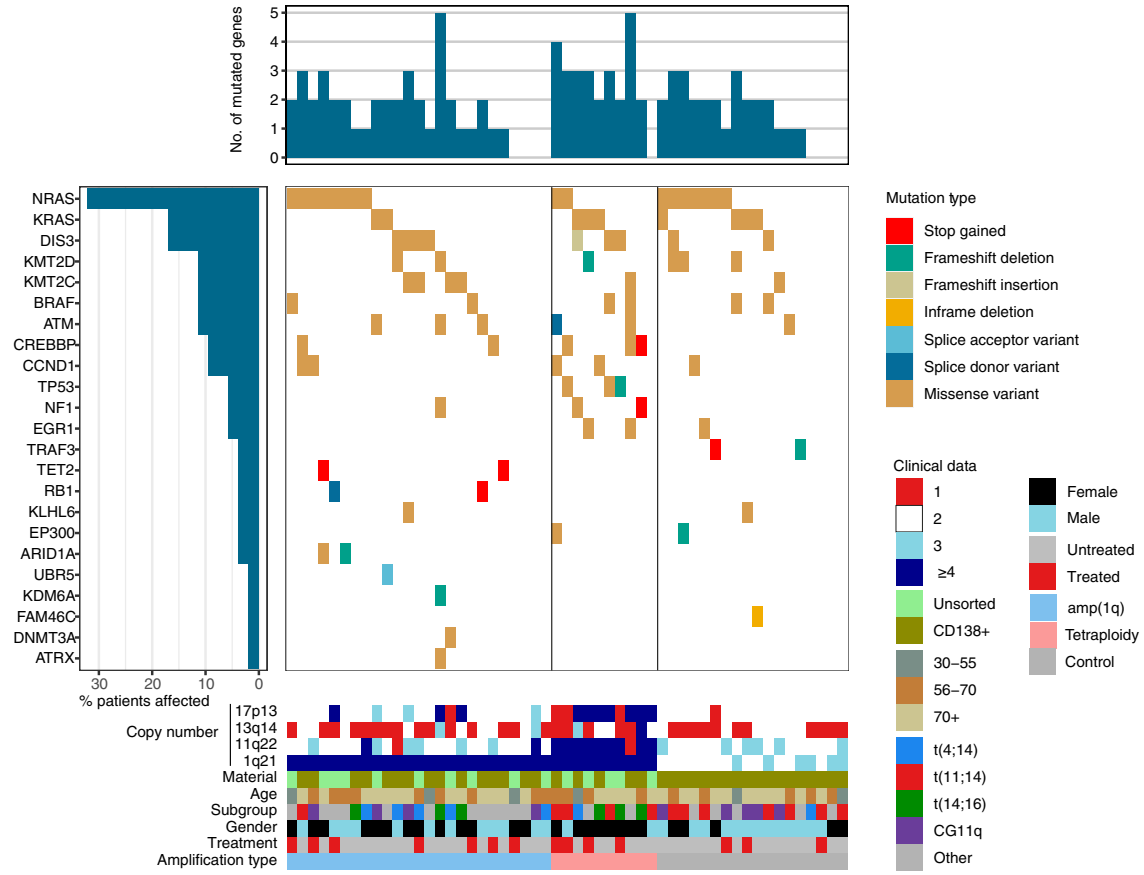

FIGURE 3 Mutational profiles. The oncoplot was created based on the GenVisR package.[32] Copy number, subgroup and amplification type were determined by FISH.

recommendations,[10] only limited cytogenetic data are available at relapse. Nonetheless, recent studies showed evidence that high-risk abnormalities acquired during the course of the disease have a significant negative impact on patient outcomes.[20,22] By comparing with a control group, Lakshman et al. found reduced progression-free survival and overall survival (OS) for MM patients who acquired del(17p) during follow-up,[22] and Audil and colleagues showed poorer OS for patients who acquired a 1q22 gain.[20]

In our cohort, similar to other reports,[35,36] we observed an increase in high-risk CAs from presentation to relapse. The most commonly gained CAs at relapse were del(17p) and gain/amp(1q) in 13% and 12% of cases, respectively, in agreement with the literature (8–23% and 13–19% of cases, respectively).[19,21,28,37] However, if only the first relapse is considered, the percentages might be lower. For example, del(17p) was acquired in 7% of cases in a homogeneously treated patient cohort (bortezomib, thalidomide, and dexamethasone induction, ASCT and consolidation therapy) of the Intergroupe Francophone du Myélome.[29] If we consider amp(1q) to be different from gain(1q), amp(1q) was the most common acquired CA (16% of cases). Consistent with previous findings,[19,21] we found an increase in 1q copy number from diagnosis to relapse in a significant proportion of gain(1q)-positive tumors. A progression from 3 copies to ≥4 copies of 1q21, which has been recently associated with poor outcome,[19] was observed in 16/148 (11%) cases (Table S4). Additionally, an amp (1q) clone was acquired in seven patients who had 2 copies of 1q at first presentation. The relatively high frequency of amp(1q) at relapses of up to ~45%[38] indicates a specific role for amp(1q) in disease progression. This is supported by reports showing that amp(1q) has an even more pronounced adverse impact than gain(1q) at diagnosis,[7,39] and relapse,[40] although an negative impact of amp(1q) at diagnosis was not consistently found across all cohorts.[41]

Several candidate genes in the commonly gained/amplified region of chromosome 1 have been implicated in drug resistance, MM cell survival, and also genomic instability.[42] In line with that, we found evidence of an antecedent amp(1q) clone in 54% of patients who developed a tetraploidy, a feature associated with genomic instability.[43] To identify potential predisposing molecular factors for tetraploidy, we sequenced tetraploid MM cases with a custom panel. In 4 out of 10 patients with tetraploidy, we detected *TP53* lesions previously described as being significantly mutated in genome doubling of

various cancers.[43,44] In addition, mutations in the histone acetyltransferase gene *CREBBP* were overrepresented in tetraploid cases, a gene found to be frequently mutated in relapsed MM.[45] As seen before,[8] tetraploidy was observed more frequently in the late stage of the disease. It was present in 10% of the samples from the last time-point and was, particularly when acquired, often followed by a rapid fatal outcome; five out of six patients died within seven months after its discovery. Treatments were similar in the patients who acquired del (17p), amp(1q), and tetraploidy as well as in patients who do not acquire any high risk cytogenetics. With the FISH panel used, ~40% of patients showed a cytogenetically stable disease course. Binder et al. found that developing new CAs over a 3-year period after diagnosis was associated with increased mortality in MM.[18] The adverse effect of new abnormalities was independent of the presence of high-risk abnormalities at the time of diagnosis. In a more recent study,[19] cytogenetic stability was associated with improved overall survival and detected in one-third of the cases. The lower proportion of cytogenetic stable cases compared to our study despite similar tested chromosomal regions could be, besides of technical differences, due to the fact that in this study shifts in subclones in follow-up samples were considered (differential evolution in ~20% of the cases). Taking into account a large number of copy number alterations and/or gene mutations may further decrease the percentage of cases with stable progression.[21,37] In the past decade, bulk tumor NGS and single-cell sequencing have revealed extensive inter- and intrapatient genetic heterogeneity and enabled characterization of patterns of clonal evolution and identification of ~80 driver mutations.[46] Connecting genomics to clinical data, as pursued in the large, observational CoMMpass study of the Multiple Myeloma Research Foundation,[47] contributes to the understanding of outcome heterogeneity and ultimately holds promise for exploring precision medicine approaches. Due to the longitudinal nature of CoMMpass, this study provides insights into relapses. At progression events, samples will be analyzed comprehensively using whole genome sequencing (WGS), whole exome sequencing and RNA sequencing (RNA-seq). Recently, a novel resistance signature present in a large subset of highly therapy-resistant MM patients was identified in a single-cell RNA-seq study and validated in the CoMMpass dataset.[48] Since this signature was highly correlated with poor outcome, it outperformed a current risk stratification strategy by FISH. In the context of new methods such as WGS and RNA-seq, which allow detection of gene mutations, CAs and transcriptional changes, the role of (serial) FISH is questioned.[49,50] In addition, patient material in relapsed MM may be scarce and occasionally insufficient for all examinations. However, apart from being a valid routine method for minimal residual disease detection, NGS has mainly been used in research.[46] After overcoming challenges such as cost, lack of standardization, emergence of new predictive models, it is expected that the importance of the new sequencing methods in the clinical routine of MM will increase. To date, FISH is still a widely used and accepted tool to stratify MM patients.[2] Although only a small proportion of drivers are usually tested with FISH, some of the tested abnormalities are clinically highly significant, as also shown by the sequencing of a large data set from the Myeloma Genome Project.[13] Specifically, amp(1q) and del(17p) along with *TP53* mutations are associated with an adverse outcome in this study. Compared to bulk analysis, FISH has the advantage to detect CAs at the single-cell level.[19] Moreover, depending on the method and bioinformatic tools used, tetraploidy might be missed with NGS.[50] As we show in this work, a common and potentially clinically important feature of relapse can be thereby overlooked.

This retrospective study has several limitations. Clinical information was missing for the majority of patients who were treated at other centers and referred only for cytogenetic analysis to our center. The treatment schemes were heterogeneous, which makes it difficult to draw conclusions about possible drug-related abnormalities. The samples were partially unsorted, which reduced the sensitivity for detecting subclonal abnormalities. This may have led to underreporting of changes in patients with unsorted samples. In patients with an unsorted sample at baseline and a subsequent sample with CD138+-enriched cells, changes may have been overestimated. No distinction was made between clonal and subclonal CAs in our study. Molecular lesions and some recurrent CAs (such as relapse-associated *MYC* rearrangements) were not systematically analyzed and therefore not included in the study. Our cohort may exhibit sampling biases. Standard-risk patients with a favorable course of disease who have not suffered a relapse in follow-up and high-risk patients with early death are likely to be underrepresented in our cohort. Moreover, frail patients are also likely to have fewer bone marrow biopsies than younger, fitter patients. We did not correct for multiple comparisons, because of the retrospective nature of the study. Any results need confirmation in larger data sets.

In summary, our study confirmed that cytogenetic evolution is common in relapses. Since amp(1q) is frequently acquired and associated with an adverse prognosis, it may be important to evaluate this marker in follow-up. As shown in this study, a common feature of relapse is the gain of tetraploidy. In the future, deregulated genes of the 1q21 amplicon[42] and tetraploidy-associated vulnerabilities[44] may be therapeutically addressed after relapse.

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CONFLICT OF INTEREST

None.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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