Multiple myeloma (MM) is a malignant disorder of plasma cells representing the second most common hematological malignancy. The currently accepted MM pathogenetic model includes two different types of primary events, namely chromosome translocations or chromosome number alterations resulting in hyperdiploidy. Primary translocations, often associated with hypodiploid karyotype, can be found in at least 40% of patients. Among these translocations, t(4;14) and t(14;16), found in 10–15% and 2–3% of patients, respectively, are associated with a worse outcome, and included in the revised ISS. More than half of MM cases, instead, have a hyperdiploid karyotype, characterized by trisomies involving odd chromosomes. In several studies, trisomic MM is associated with a favorable outcome.

In addition to these primary events, secondary aberrations can be detected at diagnosis, or acquired following treatments, including gain of 1q (+1q) present in about 35–40% and associated with worse prognosis, 13q deletion or monosomy 13 (del13q/−13), which were initially considered a poor prognostic marker only if present at the karyotype level, and 17p deletion (17p−) found in 15–20% of MM and considered a high-risk disease marker, predictive of reduced survival.

Cytogenetic abnormalities in MM can be studied by conventional karyotyping (CK) or fluorescent in situ hybridization (FISH) analysis, and more recently by array-comparative genomic hybridization (Array-CGH) and single-nucleotide polymorphism array (SNP-array). Although karyotyping may ideally describe all chromosome aberrations of the neoplastic clone, with the exception of some cryptic translocations, the amount of proliferating cells that are required hampered the routine use of CK. For this reason, in the latest European Myeloma Network (EMN) guidelines, FISH analysis on plasma cells is recommended, and should include at least t(4;14) and 17p− abnormalities, with t(14;16) and +1q suggested as well.

Hyperdiploid MM (HRD MM), instead, is not routinely investigated because multiple probes are required over the conventional markers. However, in the era of novel agents, the identification of hyperdiploidy was proved to be helpful in MM prognostication since trisomic MM can benefit the most from lenalidomide treatment, and retains a favorable outcome.

Starting from CK, our study aims at identifying distinctive genetic features of hyperdiploid MM that are associated with a favorable outcome, pursuing the goal of their inclusion in the routine FISH assessment with a step-by-step approach.

Bone marrow of 292 newly diagnosed MM patients was studied by both CK and FISH on separated plasma cells for detecting high-risk (HR) cytogenetic aberrations, including t(4;14), t(14;16), 17p−, and +1q. Patient’s characteristics, including ISS stage, symptoms at diagnosis, type of treatment, and OS, were collected (Supplementary). Median follow-up of the study population was 42 months.

Among the entire cohort, 76 (26%) patients showed an abnormal karyotype, and were selected for further analysis, while the remnant 216 cases were not evaluable or not informative. Based on CK nomenclature, karyotypes were classified into hyperdiploid (HRD, 47–57 chromosomes)
and hypodiploid (HD, 35–45 chromosomes), leading to two groups of patients: 50 HRD MM (66%) and 26 HD MM patients (34%). The clinical and biological features of the two groups are reported in Supplementary Table 1. All patients received treatment with novel agents, including bortezomib (74/76, 97%), lenalidomide (52/76, 68%), or both (51/76, 67%), and 14 patients (14/76, 18%) were treated with pomalidomide. Moreover, 29 (38%) patients received autologous stem cell transplantation (ASCT). No significant differences were found in age, sex, stage, and clinical presentation between the two subsets of patients, although a trend to a more aggressive disease characterized HD MM, with a higher frequency of ISS III (57.7% vs. 38.8%), higher LDH levels (32% vs. 12.8%), and higher frequency of renal injury (30.8% vs. 14.0%) and hypercalcemia at diagnosis (23.1% vs. 6%). Concerning cytogenetics, HD MM patients were characterized by a significantly higher frequency of HR FISH alterations \([t(4;14), t(14;16), p - 1q - (63.2\% vs. 20.5\%, p = 0.0028)],\) of del13q/−13 by CK (64.0% vs. 22.4%, \(p = 0.0008\)), and immunoglobulin heavy locus (IGH) translocation (66.7% vs. 29.2%, \(p = 0.0053\)), but no difference in +1q frequency was found (36.0% vs. 42.9%, \(p = 0.624\)). Despite the fact that hypodiploidy is a recognized adverse prognostic factor\(^{11}\), in our cohort OS was not significantly different between HD MM and HRD MM patients (42 vs. 53 months, \(p = 0.5\), Supplementary Fig. 1). This unexpected result led us to focus on the cytogenetic features of HRD MM patients.

Overall, within HRD MM cases, trisomies of chromosomes 3 (31/49, 63%), 5 (29/49, 59%), 9 (37/48, 77%), 11 (34/49, 69%), 15 (27/49, 55%), and 19 (31/49, 63%) were the most represented. HR chromosomal changes were also detected in 30/50 (57.7%) cases, including 21/49 (42.9%) with +1q, 1/44 (2%) with t(4;14), and 8/45 (18%) with 17p−. In addition, in 14/48 patients (29.2%), a IGH rearrangement was detected by both karyotype and FISH analysis (Supplementary Table 1).

In this subset, the major features associated with decreased OS were ≥2 alterations by FISH (32 vs. 57 months, \(p = 0.0123\)), IGH rearrangement (32 vs. 57 months, \(p = 0.0319\)), and +1q (39 vs. 56 months, weakly significant with \(p = 0.0929\)). On the contrary, features associated with a better outcome were co-occurrence of trisomy of 9/11/15 chromosomes (62 vs. 39 months, \(p = 0.0218\)) and ASCT (80 vs. 43 months, \(p = 0.0465\)) (Fig. 1a–e). According to these results, we classified HRD MM based on the number of trisomies into trisomic HRD MM (T-HRD MM, \(n = 26\)) with ≥5 trisomies and a non-trisomic group (N-HRD MM, \(n = 16\)), with <5 trisomies.

Interestingly, T-HRD MM patients were characterized by a better outcome than N-HRD MM (57 vs. 32 months, \(p = 0.0105\), Fig. 1f). Moreover, T-HRD MM was associated with lower rates of FISH alterations (≥2 alterations, 7.7% vs. 68.7%, \(p = 0.0001\)) and HR FISH aberrations (4.8% vs. 50%, \(p = 0.0023\)). Although not supported by a statistical significance, both del13q/−13 (15.4% vs. 37.5%, \(p = 0.1422\)) and IGH translocations (20.0% vs. 43.8%, \(p = 0.1606\)) were less represented in T-HRD MM. Overall, these observations support the hypothesis that T-HRD MM accounts for the classical definition of HRD MM, and more specifically that concurrent trisomy of 9/11/15 represents a surrogate marker of true HRD MM, being present in 53.8% of T-HRD MM patients and completely absent in N-HRD MM (\(p = 0.0002\), Table 1).

By univariate analysis, IGH translocations, concomitant trisomies of 9/11/15, ≥2 FISH alterations, and stratification in T-HRD and N-HRD were the most important prognostic factors, while ASCT and +1q were only weakly significant (Supplementary Table 2). In multivariate analysis, concomitant trisomies 9/11/15 was the most powerful prognostic factor (\(p < 0.01\), HR = 0.28; 95% CI: 0.10–0.74), followed by IGH rearrangement (\(p < 0.04\), HR = 2.42; 95% CI: 1.04–5.64), although in the opposite direction (Supplementary Table 3).

In our cohort, HRD MM OS was not significantly different with respect to HD MM, despite an increased presence of HR genetic and clinical features in HD MM patients. This can be only partially related to the not negligible presence in HRD MM patients of HR alterations, like those detected by FISH. It is also possible that a cytogenetic clone unintentionally selects a more aggressive form of MM, therefore making the difference elusive between the two groups\(^{11,12}\). In fact, the possibility of identifying an abnormal clone by CK has been related to a high mitotic rate and high percentage of bone marrow plasma cells, these variables also being correlated with the percentage of abnormal metaphases. Alternatively, it is also possible that the development of chromosome abnormalities in the malignant plasma cell may lead to a more aggressive tumor cell growth. Despite the limitations of the study (retrospective nature, small sample size, and heterogeneous treatments received), in our cohort, it clearly appears that cytogenetically defined HRD MM represents a heterogeneous group of MM where numerical changes are coupled with structural aberrations. The same evidence was reported by other groups that demonstrated that trisomies and high-risk cytogenetic alterations could coexist\(^{11,13,14}\), although with conflicting results on the outcome. Consequently, it is evident that the number of chromosomes or the ploidy level could not be enough to define the HRD MM, but rather the whole pattern of chromosome aberrations is needed to identify myeloma with hyperdiploidy. Indeed, in our cohort, we confirmed that the type and number of trisomies detected by CK have a relevance since concomitant 9/11/15...
Fig. 1 (See legend on next page.)
patients had an improved OS as compared with those
DNA index. With this method, high-hyperdiploidy
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situ hybridization.

Table 1 Biological features of T-HRD MM and N-HRD MM.

|                     | T-HRD (n = 26) | N-HRD (n = 16) | p Value* |
|---------------------|----------------|----------------|----------|
| iGH translocation   | 5/25 (20%)     | 7/16 (43.8%)   | 0.1606   |
| High-risk FISH**    | 1/21 (4.8%)    | 8/16 (50%)     | 0.0023   |
| Gain 1q             | 11/26 (42.3%)  | 9/16 (56.2%)   | 0.5265   |
| Del13q/−13          | 4/26 (15.4%)   | 6/16 (37.5%)   | 0.1422   |
| ≥2 FISH alterations | 2/26 (7.7%)    | 11/16 (68.7%)  | 0.0001   |
| 9–11–15 trisomy     | 14/26 (53.8%)  | 0/16 (0%)      | 0.0002   |

*p Values are calculated using Fisher’s exact test.
**Includes t(4;14), t(14;16), and 17p−.

trisomies correlate to a better outcome, and represent an
independent prognostic factor together with the absence of iGH rearrangement. Moreover, this association was
particularly evident in T-HRD patients, where trisomies correlate with low frequencies (≤ 2) of FISH alterations
and HR FISH.

Although at least half of MM patients belong to the
HRD subset, the latest EMN guidelines do not recom-
end the assessment of hyperdiploid status at diagnosis7. The major issue of evaluating hyperdiploidy by FISH is
the need of multiple probes for odd chromosomes that
increases the costs, the effort of testing, and the amount of
plasma cells required. Indeed, our results suggest to
restrict the investigation of hyperdiploidy to those cases
that in the beginning are negative for HR FISH, and
simultaneously have ≤ 2 FISH abnormalities and no iGH rearrangement. This approach would save resources, and
at the same time, would make the characterization of
most of not high-risk samples possible.

The detection of ploidy status is an emerging issue,
especially for HRD MM, and in a recent paper, Sidana
et al. developed a flow cytometry approach based on the
DNA index. With this method, high-hyperdiploidy
patients had an improved OS as compared with those
with low-hyperdiploidy15, consistent with our CK-based
results.

In conclusion, the identification of T-HRD MM rep-
resents a new challenge within the heterogeneous group of
HRD patients, with relevant prognostic implications. The
combination of banding analysis and FISH for HR aber-
rations contributes to better define the complexity of
HRD MM. Finally, the concomitant presence of trisomies
of chromosomes 9/11/15, after exclusion of HR features,
is a surrogate marker of true hyperdiploid myeloma.

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Author contributions
G.B. and L.B. designed the research, analyzed the data, and wrote the paper,
A.G. performed statistical analysis and wrote the paper. A.M., S.N., and N.M.
performed conventional karyotyping and FISH analysis. A.L., T.B., A.B., and L.P.
provided patient’s samples and patient’s data. S.M., F.P., A.T., M.C., and G.C.
contributed to analyze the data. G.S. participated in the analysis of data and
critically reviewed and edited the paper. R.Z. designed the study, analyzed the
data, wrote the paper, and supervised the study.

Conflict of interest
The authors declare that they have no conflict of interest.

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