A longitudinal analysis of chromosomal abnormalities in disease progression from MGUS/SMM to newly diagnosed and relapsed multiple myeloma

Original article

A Longitudinal Analysis of Chromosomal Abnormalities in Multiple Myeloma Disease Progression

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SO has received honoraria from Amgen, Celgene, and Janssen; has served on the advisory boards for Adaptive Biotechnologies, Janssen, Amgen, and Takeda.
MD has served on the advisory board for GSK.
RM has received honoraria from Amgen, Celgene, Takeda and Janssen; has served on the advisory boards for Janssen.
SB has received honoraria from Celgene, Amgen, Janssen, and Bristol-Myers Squibb; has served on the advisory boards for Celgene, Amgen, Janssen, and Karyopharm; has received consultancy fees from Janssen and Takeda.
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PO has served on the advisory boards for Janssen.
The other authors declare no competing financial interests.

Ethics approval
All procedures followed were in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Patients gave written informed consent before bone marrow aspiration and before starting therapy.

Availability of data and material
After the publication of this article, data collected for this retrospective analysis and related documents will be made available to others upon reasonably justified request, which has to be written and addressed to the attention of the corresponding author Dr. Stefania Oliva at the following e-mail address: stefania.olivamolinet@gmail.com. The corresponding author Dr. Stefania Oliva is responsible to evaluate and eventually accept or refuse every request to disclose data and their related documents, in compliance with the ethical approval conditions, in compliance with applicable laws and regulations, and in conformance with the agreements in place with the

involved subjects, the participating institutions, and all the other parties directly or indirectly involved in the participation, conduct, development, management and evaluation of this analysis.

Authors' contributions

SO and PO conceived and designed the work that led to the submission.
All the authors acquired the data and interpreted the results.
SO drafted the first version of the manuscript.
All the authors revised the manuscript and approved the final version.
All the authors agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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Abstract

We analyzed variations in terms of chromosomal abnormalities (CA) by fluorescence in situ hybridization (FISH) analysis on purified bone marrow plasma cells throughout the progression from monoclonal gammopathy of undetermined significance/smoldering multiple myeloma (MGUS/SMM) to newly diagnosed MM/plasma cell leukemia (NDMM/PCL) at diagnosis and from diagnostic samples to progressive disease. High risk was defined by the presence of at least del(17p), t(4;14) and/or t(14;16). 1p/1q detection (in the standard FISH panel from 2012 onward) was not available for all patients. We analyzed 139 MM/PCL diagnostic samples from 144 patients, with a median follow-up of 71 months: high-risk CA at diagnosis (MGUS/SMM or NDMM) was present in 28% of samples, whereas 37-39% showed high-risk CA at relapse. In 115 patients with NDMM who evolved to relapsed/refractory MM, we identified 3 different populations: (1) 31/115 patients (27%) with gain of new CA (del13, del17p, t(4;14), t(14;16) or 1q CA when available); (2) 10/115 (9%) patients with loss of a previously identified CA; and (3) 74 patients with no changes. The CA-gain group showed a median overall survival of 66 months vs. 84 months in the third group (HR 0.56; 95% CI 0.34-0.92, p=0.023). Clonal evolution occurs as disease progresses after different chemotherapy lines. Patients who acquired high-risk CA had the poorest prognosis. Our findings highlight the importance of performing FISH analysis both at diagnosis and at relapse.

Keywords: multiple myeloma; cytogenetic abnormalities; fluorescence in situ hybridization; FISH; clonal evolution; disease progression;

Introduction

In multiple myeloma (MM), the heterogenous biology of plasma cells (PCs) confers an inter- and intra-patient complexity both at clonal and sub-clonal level [1–3]. Cytogenetic abnormalities (CA) may change throughout the progression from the premalignant stage of monoclonal gammopathy of undetermined significance (MGUS) to smoldering MM (SMM) to symptomatic MM, and the major subclone at the time of diagnosis can be different from the one at first or subsequent relapses [4, 5]. A Darwinian model has been proposed to explain a possible competition among the survival clones in the bone marrow (BM) niche [6–8]. In this view, clonal evolution may occur in different ways: stable evolution (same genomic profile at diagnosis and relapse), linear evolution (novel mutations at relapse, with the same mutational architecture), and branching evolution ('disappearance' of some mutations revealing evolution from a minor undetected subclone, or appearance of novel different subclones) [9, 10]. Different characteristics could explain why clone selection and chemotherapy may play a major role, for instance in killing the most sensitive cells and selecting the more resistant ones.

Prognosis is influenced by CA such as del(17p), t(4;14) and/or t(14;16) and amp1q [11–13]; however, it remains unclear whether the CA changes during disease progression preserve the same impact showed at diagnosis. In this study, we investigated the CA changes in BM PCs from MGUS/SMM to MM/plasma cell leukemia (PCL) and from diagnostic samples to progressive disease.

Patients and methods

Patients

From 2002 to 2018, 144 patients were retrospectively analyzed by fluorescence in situ hybridization (FISH) during the MGUS/SMM phase, at MM/PCL diagnosis, and at MM relapse (RRMM). Patients were diagnosed in Turin and Novara (Italy); samples and data were stored

and analyzed in Turin. All procedures followed were in accordance with the ethical standards of the responsible committees on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2008. Patients gave written informed consent before BM aspiration and before starting therapy. In conformance with the International Myeloma Working Group guidelines, MGUS/SMM patients were not considered for any therapy. Almost all patients with newly diagnosed MM (NDMM) or PCL received new drugs (immunomodulatory agents [IMiDs] or proteasome inhibitors [PIs]) at diagnosis in combination with conventional chemotherapy as upfront treatment or incorporated into pre-transplant induction or post-transplant maintenance strategies inside or outside clinical trials. Almost all patients evaluable at relapse received new drugs with or without transplantation both inside or outside clinical trials (**Table 1**). Baseline data included age, sex, isotype, laboratory values, International Staging System (ISS) stage, CA detected by iFISH, serum level of lactate dehydrogenase (LDH), and bone lytic lesions.

FISH analysis

FISH was performed on BM aspirates after red blood cell lysis. Bone marrow plasma cells (BMPCs) were enriched using anti-CD138-coated magnetic microbeads and AutoMACS Pro Separator (Miltenyi Biotech GmbH, Bergisch Gladbach, Germany) following manufacturer's instructions. Samples were then centrifuged at 1600 rpm for 5'; afterwards, the obtained dry pellets were fixed in Carnoy's solution. Purity was assessed by multiparameter flow cytometry (plasma-cell purity always exceeded 90%). Finally, the samples were stored at -80 °C without any technical difference in storage process over time.

DNA probes (Cytocell Lt, Cambridge, UK) were used to detect del13, del(17p), del1p32.3, t(11;14)(q13;q32), t(4;14)(p16;q32), t(14;16)(q32;q23) and gain1q21. The same panels for

DNA probes were used for the same patient at different stages of testing, from MGUS/SMM to NDMM/PCL to RRMM,

Three hundred BMPC nuclei from each sample were scored. Cut-off levels for positive values represented the mean plus three standard deviations (SDs) of abnormal cells from 15 healthy donors' BMPC samples, and were adjusted at 15% for single fusion, 3% for 2 or more fusion signals in IgH translocations and 10% for deletions/gains. "Abnormal Chr1" category represented patients carrying at least del(1p) and/or gain(1q) and was implemented in the standard FISH panel from 2012 onward. As compared to standard risk, high risk was defined by the presence of at least del(17p), t(4;14) and/or t(14;16).

Statistical methods

Differences in patient and disease characteristics for comparison between groups were investigated using Kruskal-Wallis and Fisher's exact tests, as appropriate, for continuous and categorical variables, respectively. Overall survival (OS) was calculated from the date of diagnosis to the date of death or the date the patient was last known to be alive. Time-to-event data were analyzed using the Kaplan–Meier method; groups were compared with the log-rank test. Adjusted hazard ratios (HRs), 95% confidence intervals (CIs) and two-sided p-values were estimated using Cox proportional hazards models. Data were analyzed as of January 2020 using R (v3.6.1).

Results

Patients

The median follow-up was 71 months (IQR 38-96); 139/144 patients had MM/PCL diagnostic sample, of which 29 had also MGUS/SMM evaluable FISH; 115/139 had relapse samples: 84

had the 1st relapse sample, whereas the other patients had ≥2nd relapse sample. Five out of 144 total patients were evaluable at 2 subsequent relapses.

Patient characteristics of the 139 NDMM/PCL patients are illustrated in **Table 1**: median age was 64 years (IQR 57-72) and males were 70 (50%). The ISS scores were respectively 41%, 39% and 17% for stages I, II and III.

Sixty-seven (48%) patients received front-line PI-based therapy; 66 (47%) lenalidomide-based therapy, 5 (4%) conventional chemotherapy/other; 1 patient did not start any therapy. Fifty patients (36%) received autologous stem-cell transplantation (ASCT).

A total of 293 samples were analyzed by FISH at different disease evolution stages (**Table 2**). Median time to progression (TTP) from MGUS/SMM to NDMM/PCL was 24 vs. 40 months for high-risk vs. standard-risk CA (p=0.04). Median TTP from NDMM/PCL to 1st relapse was 25 vs. 30 months for high-risk vs standard-risk CA (p=0.14).

Clonal evolution

Clonal evolution in terms of CA changes was evaluated in 29 patients evolving from MGUS/SMM to NDMM/PCL, 115 from NDMM/PCL to relapse or from 1st to ≥2nd relapse. One-third of patients showed CA changes during disease progression. Three patient populations were identified:

A. 29 MGUS/SMM → NDMM/PCL:
   - Group 1: 5/29 patients (17%) gained new CA [2 gained del13, 2 del(17p) and 1 t(14; 16)].
   - Group 2: 3/29 patients (10%) lost previously detected CA.
   - Group 3: the other 21/29 (72%) patients showed no CA changes.
B. 115 NDMM/PCL→ RRMM

- Group 1: 31/115 patients (27%) gained new CA among del13, del(17p), t(4;14), t(14;16) and abnormal Chr1 [only 1 patient gained t(11;14)].
- Group 2: 10/115 patients (9%) lost previously detected CA.
- Group 3: the other 74/115 (64%) patients showed no CA changes.

The most frequent gains or losses were associated with del13 or del(17p) (**Figure 1**). A proportion of subclones emerged during disease progression: 40% of deletions/gains were detected in <10% of BMPC nuclei; 65% of single fusion abnormalities were detected in <15% of BMPC nuclei at the MGUS/SMM or NDMM/PCL phases.

Throughout the progression from NDMM to relapse, no baseline characteristics were significantly associated with CA gains vs. losses vs. no changes after progression (e.g. plasmacytoma 10% vs. 0% vs. 4% [p=0.73], ISS III 26% vs. 10% vs. 16% [p=0.57], LDH>ULN 10% vs. 20% vs. 12% [p=0.36], respectively; **Table 3**).

No statistically significant differences among therapies in terms of CA gains vs. losses vs. no changes after progression were observed (IMiDs 61% vs. 60% vs. 49%; PIs 35% vs. 30% vs. 49% [p=0.36]; ASCT 32% vs. 50% vs. 43% [p=0.50], respectively; **Table 3**).

Patients gaining new CA during disease evolution (group 1) showed the worst median OS with 66 months (95% CI 58-82) vs. 84 months (95% CI 73-NR) in group 3 (group 1 vs. 3, HR 0.56; 95% CI 0.34-0.92, p=0.023; **Figure 2**).

Discussion

Our results confirmed that genetic instability increases as disease progresses after different chemotherapies, with a group with acquired high-risk CA having the poorest prognosis. This suggests that some clones that might have existed at the diagnosis as a minor population could expand during treatment and drive relapse.

Although MGUS and SMM are genetically less complex than MM, clonal heterogeneity is already present at these early pre-malignant stages. Indeed, early reports detected fewer aneuploidies in MGUS than in MM [14–16], whereas next-generation sequencing (NGS) analysis of sample series showed variable degrees of spontaneous evolution of genes mutations, cytogenetic lesions and mutational signatures [17–24]. This suggests that MM evolves in discrete steps not just clinically but also from a molecular point of view, with the acquisition of subsequent genomic lesions underlying an increasingly aggressive clinical behavior. In this study, we observed a clonal evolution by FISH in a subgroup of patients progressing from MGUS/SMM to NDMM, although a larger series of patients and the analysis of mutational profiles could add biological significance to these findings.

Our study confirmed the importance of performing FISH analysis both at diagnosis and relapse, since the acquisition of structural genetic lesions has been associated with a dismal outcome, suggesting that clonal selection during the MM course is fundamental in the evolution of subsequent refractoriness and inferior survival.

This retrospective analysis has some limitations. The patient inclusion period was very long, from 2002 to 2018, with clearly different treatment approaches available over time. The heterogeneity of therapies could suggest that therapy is apparently not a significant factor influencing clonal evolution (e.g. ASCT vs. no ASCT, IMiDs, PIs) nor affecting the clone selection responsible for relapse. Indeed, this selection could be patient-dependent or related to internal factors, such as local BM and/or immune conditions. This was demonstrated by Corre et al., who observed no significant changes in the mutational burden of MM patients undergoing the same treatment [25]. Future prospective clinical trials with homogeneously treated MM patients (e.g. young, transplant-eligible NDMM patients, or elderly NDMM patients treated with PIs or IMiDs) could include pre-planned BM aspirates with FISH at different time points in order

to perform a longitudinal comparison of changes in the genetic profiles of patients in remission (as control arm) and at relapse.

Corre et al. did not observe any differences between primary diagnosis and relapse in terms of defined IGH translocations, including t(4;14). These findings are in line with a model suggesting that trisomies of odd chromosomes and rearrangements involving the IGH gene are primary events occurring in the early disease phase, whereas other abnormalities (e.g. loss of chromosome 13, 17p deletions or MYC rearrangements) are considered secondary events. Accordingly, IGH translocations were observed in the large majority of PCs at diagnosis, whereas monosomy 13 or del(17p) were frequently detected only in subclones [26]. On the contrary, as also shown in 2013 by Hébraud et al. for t(4:14) [27], we observed a small fraction of patients gaining or losing translocations after progression, even if the gain or loss of chromosome 13 and the 17p deletions were the most frequent. One hypothesis is that small positive subclones involving IgH translocations at the time of diagnosis could exist, since most of our patients were considered negative at diagnosis with a translocation in a subclonal fraction (<15% of PCs) and were subsequently considered positive because these subclones appeared with higher frequency at relapse (≥15% of PCs).

In conclusion, we retrospectively confirmed the occurrence of CA changes, which may reflect a clonal evolution during the disease course, and the importance of implementing FISH analysis at different phases of the disease. We need to understand whether different therapies may select resistant clones and favor the aggressiveness of MM at relapse and whether the evolution of FISH techniques will help tailoring clinical decision making.

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subclones in multiple myeloma. Clin Cancer Res 19:4634–7. https://doi.org/10.1158/1078-0432.CCR-12-3866

Tables

Table 1. Patient characteristics of NDMM patients

	N=139
Age, years	
Median (IQR)	64 (57-72)
Gender, N (%)	
M	70 (50)
ISS, N (%)	
I	57 (41)
II	54 (39)
III	24 (17)
Missing	4 (3)
Creatinine	
Median, mg/dL (IQR)	0.96 (0.8-1.22)
Missing, N (%)	3 (2)
LDH	
Median, U/L (IQR)	330 (279-84)
>ULN, N (%)	14 (10)
Missing, N (%)	34 (24)
Front-line therapies, N (%)*	
PIs	67 (48)
IMiDs	66 (47)
Conventional chemotherapy/other	5 (4)
ASCT	50 (36)
None	1 (1)
Therapy at 1st relapse, N (%)*	
PIs	68 (49)
IMiDs	32 (23)
Conventional chemotherapy/other	5 (4)
ASCT	12 (9)
Therapy at ≥2nd relapse, N (%)*	
PIs	24 (17)
IMiDs	23 (17)
Conventional chemotherapy/other	5 (4)
ASCT	8 (6)

Percentages may not total 100 because of rounding.

**Percentages may not total 100 because patients may have received more than one type of treatment.*

Abbreviations. ASCT, autologous stem-cell transplantation; IMiDs, immunomodulatory drugs; IQR, interquartile range; ISS, International Staging System; LDH, lactate dehydrogenase; M, male; N, number; NDMM, newly diagnosed multiple myeloma; U/L, units per liter; ULN, upper limit of normal; PIs, proteasome inhibitors.

Table 2. FISH results at baseline (first sample) and at relapse

FISH risk* N (%)	MGUS/SMM N=29	NDMM N=139	1st relapse N=84	≥2nd relapse N=70
Standard	19 (66)	100 (72)	53 (63)	43 (61)
High	10 (34)	39 (28)	31 (37)	27 (39)
del(17p)	4 (14)	23 (17)	23 (27)	21 (30)
t(4;14)	7 (24)	20 (14)	10 (12)	11 (16)
t(14;16)	2 (7)	1 (1)	1 (1)	1 (1)
del(13p)	9 (31)	66 (47)	47 (56)	42 (60)
t(11;14)	6 (21)	32 (23)	19 (23)	20 (29)
1q/p CA**	6/8 (75)	20/27 (74)	7/16 (44)	24/38 (63)

**FISH risk defined by the presence of at least one among del(17p), t(4;14)] and/or t(14;16).*
***Only in evaluable patients.*
Percentages may not total 100 because of rounding.
Abbreviations. FISH, fluorescence in situ hybridization; CA, chromosomal abnormalities; MGUS, monoclonal gammopathy of undetermined significance; MM, multiple myeloma; N, number; NDMM, newly diagnosed MM; SMM, smoldering MM.

Table 3. Baseline characteristics and therapies in the three groups in 115 NDMM/RRMM patients

	Group 1 [CA gain] N=31	Group 2 [CA loss] N=10	Group 3 [Unchanged] N=74	P-value
Median age, years (IQR)	67 (58-71)	63 (60-64)	62 (57-70)	0.32
Sex				0.28
Male	18 (58%)	3 (30%)	35 (47%)	
Female	13 (42%)	7 (70%)	39 (53%)	
Plasmacytoma	3 (10%)	0	3 (4%)	0.73
LDH>ULN	3 (10%)	2 (20%)	9 (12%)	0.36
ISS				0.57
I	13 (42%)	3 (30%)	32 (43%)	
II	10 (32%)	6 (60%)	27 (36%)	
III	8 (26%)	1 (10%)	12 (16%)	
Missing	-	-	3 (4%)	
Median Hb value	10.6	10.6	10.6	0.82
Median creatinine value	1.04	0.86	0.95	0.67
Bone lytic lesions ≥2	12 (39%)	4 (40%)	22 (30%)	0.85
IMiDs	19 (61%)	6 (60%)	36 (49%)	0.36
PIs	11 (35%)	3 (30%)	36 (49%)	
Conventional chemotherapy/other	1 (3%)	1 (10%)	2 (3%)	
ASCT	10 (32%)	5 (50%)	32 (43%)	0.50

Group 1: patients who gained new CA among del13, del(17p), t(4;14), t(14;16) and abnormal Chr1 [only 1 patient gained t(11;14)]; Group 2: patients who lost previously detected CA; Group 3: patients who showed no CA changes. Percentages may not total 100 because of rounding.
Abbreviations. ASCT, autologous stem-cell transplantation; CA, chromosomal abnormalities; Chr1, chromosome 1; Hb, hemoglobin; IQR, interquartile range; IMiDs, immunomodulatory drugs; ISS, International Staging System

stage; LDH, lactate dehydrogenase; NDMM, newly diagnosed multiple myeloma; PIs, proteasome inhibitors; RRMM, relapsed/refractory multiple myeloma; ULN, upper limit of normal.

Figure titles and legends

Figure 1. Frequency of FISH changes
Panel A: Gain frequency (N=31)*
Panel B. Loss frequency (N=10)**

**Chr1 abnormalities only in evaluable patients [N=3/10].*
***Chr1 abnormalities only in evaluable patients [N=0/4].*
N.b. A single patient may present with more than 1 CA change.
Abbreviations. FISH, fluorescence in situ hybridization; CA, chromosomal abnormalities; Chr1, chromosome 1.

Figure 2. Overall survival of 3 longitudinal FISH groups

Abbreviations. FISH, fluorescence in situ hybridization; CI, confidence interval; HR, hazard ratio; p, p-value; unch. unchanged.

408 Figure 1
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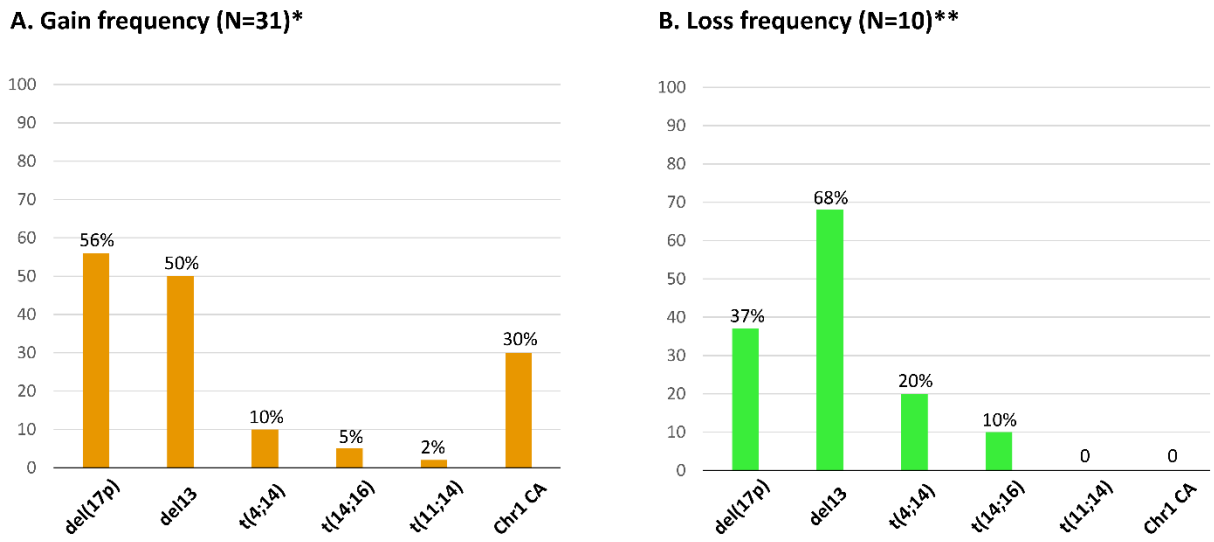

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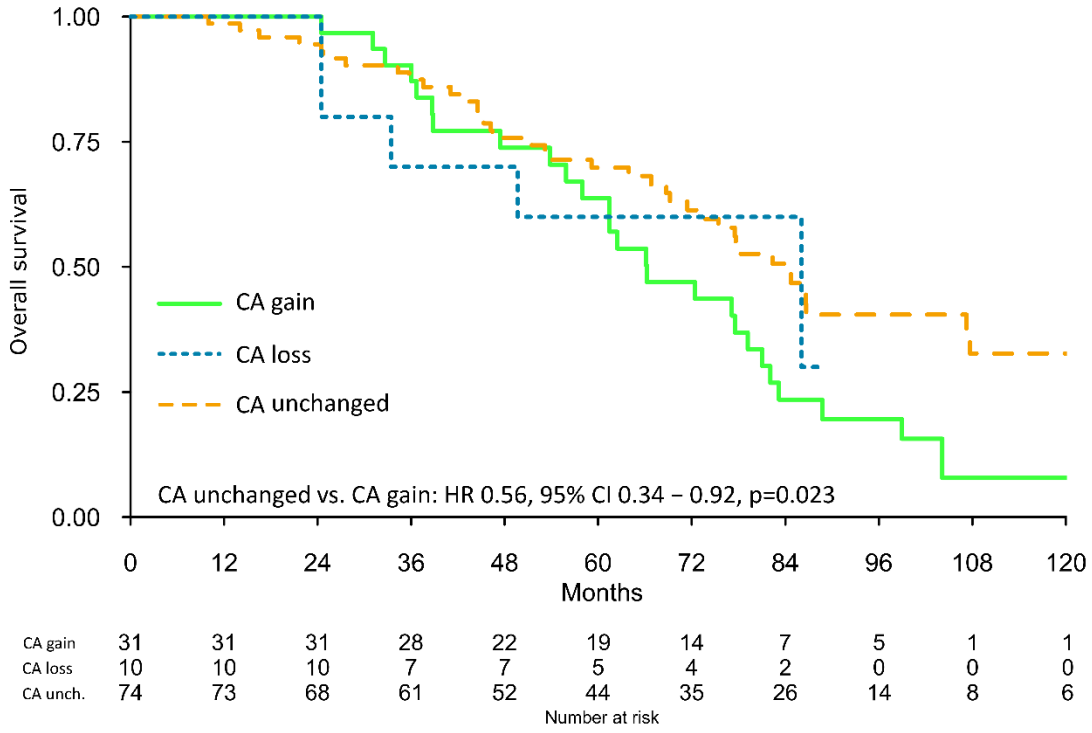

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