Favorable outcome of patients who have 13q deletion: a suggestion for revision of the WHO 'MDS-U' designation

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ABSTRACT

To characterize bone marrow failure with del(13q), we reviewed clinical records of 22 bone marrow failure patients possessing del(13q) alone or del(13q) plus other abnormalities. All del(13q) patients were diagnosed with myelodysplastic syndrome-unclassified due to the absence of apparent dysplasia. Elevated glycosylphosphatidylinositol-anchored protein-deficient blood cell percentages were detected in all 16 with del(13q) alone and 3 of 6 (50%) patients with del(13q) plus other abnormalities. All 14 patients with del(13q) alone and 2 of 5 (40%) patients with del(13q) plus other abnormalities responded to immunosuppressive therapy with 10-year overall survival rates of 83% and 67%, respectively. Only 2 patients who had abnormalities in addition to the del(13q) abnormality developed acute myeloid leukemia. Given that myelodysplastic syndrome-unclassified with del(13q) is a benign bone marrow failure subset characterized by good response to immunosuppressive therapy and a high prevalence of increased glycosylphosphatidylinositol-anchored protein-deficient cells, del(13q) should not be considered an intermediate-risk chromosomal abnormality.

Key words: glycosylphosphatidylinositol-anchored protein-deficient, cells, bone marrow failure, 13q deletion, immunosuppressive therapy.

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Introduction

Numerical karyotypic abnormalities such as -7/del(7q) and del(13q) are occasionally seen in patients with bone marrow (BM) failure who do not exhibit typical signs of myelodysplasia. The 2008 World Health Organization (WHO) criteria defined this subset of BM failure as myelodysplastic syndrome-unclassified (MDS-U) because patient progression to leukemia was still possible. However, no large patient study has been conducted to explore an association between del(13q) and pre-leukemia.[1] Several anecdotal reports have shown that BM failure patients with del(13q) responded to immunosuppressive therapy (IST) and had a favorable prognosis.[2,3] However, the incidence of BM failure with del(13q) and its relationship with immune pathophysiology of BM failure remain unclear.

Several studies have identified the presence of small populations of glycosylphosphatidylinositol-anchored protein-deficient (GPI-AP$^-$) blood cells as a significant factor predicting a good response to IST in patients with aplastic anemia (AA) and low-risk myelodysplastic syndromes (MDS).[4,5] Immune mechanisms are, therefore, thought to be involved in the increase in the GPI-AP$^-$ cells in this type of BM failure, though the exact mechanisms responsible for the increase in the GPI-AP$^-$cells remain unknown. Given that BM failure with del(13q) is likely to respond to IST, this type of BM failure may be associated with the presence of small populations of GPI-AP$^-$ cells. It is essential to precisely characterize BM failure with del(13q) because the present WHO definition of an intermediate-risk abnormality may lead to inappropriate treatment of potentially benign BM failure with hypomethylating agents or allogeneic stem cell transplants from unrelated donors. To address this issue, the present study analyzed clinical and genetic features of 22 BM failure patients possessing del(13q) by comparing them to BM failure patients with a normal karyotype.

Design and Methods

Study subjects

Clinical records were analyzed for 1,228 BM failure patients: 733 with aplastic anemia (AA), 495 with low-risk MDS, including 286 with refractory cytopenia with unilineage dysplasia (RCUD), 149 with

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refractory cytopenia with multilineage dysplasia (RCMD) and 60 with MDS-U, whose blood samples were sent to our laboratory between May 1999 and July 2010 for screening of GPI-AP$^-$ granulocytes and erythrocytes. BM smear slides were reviewed by 2 independent hematologists. BM cellularity was defined as the percentage of BM volume occupied by hematopoietic cells in the trephine biopsy specimens. Hypocellular marrow was defined as less than 30% cellularity in patients under the age of 70 years, or less than 20% cellularity in patients 70 years and over.[6] Chromosomal analysis was performed and described according to the International System for Human Cytogenetic Nomenclature (ISCN).[7] Responses to IST were defined according to the established criteria.[8] The ethics committee of Kanazawa University Graduate School of Medical Science approved the study protocol, and all patients provided their informed consent prior to sampling.

Monoclonal antibodies

Monoclonal antibodies (mAbs) used for flow cytometry are shown on the *Online Supplementary Table S1*.

Flow cytometry for detecting GPI-AP$^-$ cells

All blood samples were analyzed within 24 h of collection to avoid false positive results due to cell damage. Staining with each mAb was performed according to the lyse-stain protocol as previously described.[5,9] The presence of CD55$^-$CD59$^-$glycophorin A$^+$ erythrocytes at the level of 0.005% and over and/or CD55$^-$CD59$^-$CD11b$^+$ granulocytes at the level of 0.003% or over was defined as an abnormal increase ('positive') based on the results obtained from 183 healthy individuals.[10] With careful handling of samples and elaborate gating strategies, cut-off values can be lowered to these levels without producing false positive results.[10-12]

Cell sorting and FISH analysis

GPI-AP$^+$ and GPI-AP$^-$ granulocytes from 2 patients with del(13q) (unique patient numbers (UPNs) 3 and 7) were sorted using a FACSAria III cell sorter (BD Bioscience, Franklin Lakes, NJ, USA) and subjected to fluorescence *in situ* hybridization (FISH) analysis using a D13S319-specific probe (Vysis, Voisins-le-Bretonneux, France) as previously described.[13]

Genome analysis of deleted region in patients with del(13q)

Genomic DNA was isolated from peripheral blood cells of 7 patients with del(13q) (UPNs 1, 3, 4, 5, 7, 8 and 22) and subjected to SNP array-based genome-wide analysis of genetic alterations using GeneChip® 250K arrays (Affymetrix, Santa Clara, California, USA) according to the manufacturer's protocol. Genomic and allele-specific copy numbers were calculated using Copy Analyser for GeneChip® (CNAG) software as previously described.[14,15]

Statistical analysis

Prevalence of increased GPI-AP$^-$ cells among different patient populations was compared using the χ^2 test. Time-to-event variables were analyzed using the Kaplan-Meier method, and groups were compared with the log rank test. Two-sided *P* values were calculated and $P<0.05$ was considered statistically significant. All statistical analyses were performed using the JMP software program version 8.0 (SAS Institute, Cary, NC, USA).

Results and Discussion

Of the 1,228 patients with BM failure, 22 possessed del(13q) (1.8%) that were demonstrated by G-banding; their clinical features are summarized in Table 1. Sixteen patients had only the del(13q) abnormality (which we define as 13q-^{alone}) while the remaining 6 patients had other abnormalities, which we define as 13q$^{-+other}$. Of these 6, 2 had -Y, one had -20, one had del(7q), one had +8, and one had +mar in addition to the del(13q) abnormality. The presence of the del(13q) clone was confirmed by FISH when the number of del(13q) revealed by the G-banding method was less than or equal to two. Median age was 64.5 years old, and BM was hypocellular in 16 patients (12 with 13q^{-alone} and 4 with 13q$^{-+other}$), normocellular in 4 (2 with 13q^{-alone} and 2 with 13q$^{-+other}$), not evaluable in 2 with 13q^{-alone}. All patients with del(13q) were diagnosed with MDS-U due to the absence of significant dysplasia that would fullfill the criteria for MDS as defined by the 2008 WHO classification. All patients were classified as Int-1 according to the International Prognostic Scoring System (IPSS), except for UPN17 who had an IPSS score of 1.5 (Int-2).

As shown in Table 1, GPI-AP$^-$ cells that accounted for from 0.006% to 12.342% (median 0.137%) of granulocytes were detected in all 16 13q^{-alone} patients. FISH analysis of sorted GPI-AP$^-$ and GPI-AP$^+$ granulocytes revealed that del(13q) cells were derived from non-*PIGA* mutant hematopoietic stem cells (HSCs) (Figure 1A). On the other hand, the prevalence of elevated GPI-AP$^-$ cell percentages in 13q$^{-+other}$ patients and those with a normal karyotype (637 patients with AA and 300 with MDS) was 50% (3 of 6) and 43% (405 of 937), respectively ($P<0.001$).

Fourteen 13q^{-alone} patients were treated with cyclosporine (CsA) alone,[6] CsA and antithymocyte globulin (ATG)[6] or CsA and anabolic steroids;[2] all achieved either a hematologic improvement in two or three lineages or complete remission (CR), while the response rate to IST in 13q$^{-+other}$ patients was 40%. No case was IST-dependent, and response was durable after the cessation of the treatment after patients achieved CR. The clinical course of one patient (UPN 4) who responded to CsA alone and entered CR, despite the fact that G-banding of BM cells showed all 20 dividing cells to be del(13q), has been previously reported.[16] Ninety-six AA patients with the normal karyotype were treated with CsA and ATG (n=47) or CsA±anabolic steroids (n=49). Seventy-eight percent of AA patients responded to IST. Among 19 MDS patients (RCUD, n=14; RCMD, n=5) with a normal karyotype who have been treated with ATG plus CsA (n=3) or CsA with or without anabolic steroids (n=16), 63% responded to IST.

None of the 17 13q^{-alone} patients progressed to advanced MDS or acute myeloid leukemia (AML) during the follow-up period of 3-108 months (median 52 months), while 2 of 6 13q$^{-+other}$ patients (one with -20, one with del(7q)) developed AML. The 10-year overall survival rates of patients with 13q^{-alone}, patients with 13q$^{-+other}$, AA patients with a normal karyotype and MDS (RCUD, n=38; RCMD, n=20; MDS-U, n=8) patients with a normal karyotype were 83%, 67%, 85% and 57%, respectively ($P=0.0003$, log rank test on 3 degrees of freedom) (Figure 1B). The 10-year overall survival rates of AA patients with a normal karyotype with and without increased GPI-AP$^-$ cells and MDS (38 with RCUD, 20 with RCMD and 8 with MDS-U) patients with a normal karyotype with and without increased GPI-AP$^-$ cells were 85%, 84%, 66% and 55%, respectively ($P=0.0011$, log rank test on 4 degrees of freedom) (Figure 1C). The percentage of del(13q) clones revealed by G-banding increased in 5 patients and decreased in 3 after successful IST. (*Online Supplementary*

Figure S1) No patient developed clinical features of paroxysmal nocturnal hemoglobinuria (PNH).

SNP array analysis of peripheral blood cells from 7 $13q^{-alone}$ and $13q^{+other}$ patients indicated the region from 13q13.3 to 13q14.3 to be commonly deleted (Figure 1D).

The current retrospective study with a large number of BM failure patients revealed distinctive clinical features of BM failure with del(13q) abnormalities. The 1.8% incidence of del(13q) patients was comparable to that of a recent study (1.9%) based on 2,072 patients with MDS,[17] for which detailed diagnoses of patients with del(13q) were not provided. All del(13q) patients in our study were classified as MDS-U due to the absence of significant dysplasia. We have previously reported that response to IST was remarkably high in 9 patients with del(13q). The present study, which used a different patient cohort, confirmed our previous finding.[2] Between these 22 patients and the 9 patients that we reported in 2002, only 2 developed AML and 22 responded to IST. The overall and leukemia-free survival spans of del(13q) patients treated with IST were as long as AA patients with normal karyotypes treated with IST. These findings suggest that the del(13q) clone in BM failure patients represents the presence of immune pathophysiology rather than clonal disorder associated with AML risk.

Table 1. Clinical features of bone marrow failure patients with del(13q) alone (patients 1-16) or del(13q) plus other abnormalities (patients 17-22).

UPN	Age (years)	Sex	Months from diagnosis to sampling	Dysplasia	Cellularity	Cytogenetics	% of del(13q) cells	Break point	% GPI(-) granulocytes	% GPI(-) RBCs	Previous therapy	Treatment	Response	Outcome	AML transformation	LFS (months)
1	64	F	54	None	hypo	46,XX,del 4/20 (13)(q?)	20	13q(?)	0.042	0.015	No	CsA+AS	HI-2	alive	No	67+
2	42	M	0	None	hypo	46,XY,del(13)(q12q14) 1/20	5	13(q12q14)	3.511	0.562	No	CsA	CR	alive	No	79+
3	47	F	0	None	hypo	46,XX, del(13)(q?) 2/20	10	13q(?)	2.101	0.601	No	ATG+CsA	HI-3	alive	No	24+
4	50	F	4	Erythroid	hypo	46,XX,del(13)(q12q22) 20/20	100	13(q12q22)	0.111	0.013	No	CsA	CR	alive	No	44+
5	65	F	5	None	hypo	46,XX,del(13)(q12q14) 3/20	15	13(q12q14)	0.009	0.008	No	ATG+CsA	CR	alive	No	43+
6	21	M	1	None	hypo	46,XY, del(13)(q?) 6/20	30	13q(?)	0.038	0.003	No	ATG+CsA	HI-3	alive	No	15+
7	52	M	1	Erythroid	NE	46,XY, del(13)(q?) 19/20	95	13q(?)	12.342	0.524	PSL	CsA	HI-3	alive	No	3+
8	87	F	1	None	normo	46,XX,del(13)(q12q22) 9/20	45	13(q12q22)	0.37	0.095	No	CsA	HI-3	alive	No	15+
9	63	F	16	None	hypo	46,XX,del(13)(q12q14) del(13)(q21q31) 5/20	25	13(q12q14), 13(q21q31)	0.006	0.665	PSL	ATG+CsA	HI-3	alive	No	29+
10	74	F	3	None	hypo	46,XX, del(13)(q12q14) 7/13	54	13(q12q14)	0.504	N/A	No	ATG+CsA	HI-3	death (cancer)	No	38
11	54	F	0	None	hypo	46,XX,del(13)(q14q22) 40/40	100	13(q14q22)	0.125	0.008	No	Allo-BMT	NE	alive	No	74+
12	53	M	43	None	hypo	46,XY,del(13)(q14.3)	14	13q14.3	0.281	0.539	No	ATG+CsA	HI-3	alive	No	108+
13	85	M	1	None	hypo	46,XY,del(13)(q?) 2/20	10	13q(?)	0.031	0.01	No	No treatment	NE	death	No	10
14	77	F	3	Erythroid	NE	46,XX,del(13)(q?) 8/20	40	13q(?)	3.125	1.65	No	CsA	CR	alive	No	45+
15	56	M	1	Erythroid	normo	46,XX,del(13)(q12q14) 6/20	30	13(q12q14)	0.069	0.036	No	CsA	HI-2	alive	No	24+
16	74	M	37	None	hypo	46,XY,del(13)(q?) 7/20, 47,X,+Y 7/20	35	13q(?)	0.171	0.441	No	CsA+AS	HI-2	alive	No	52+
17	69	M	1	None	hypo	46,XY,del(7)(q22), del(13)(q12q14) 3/20	15	13(q12q14)	0	0	No	CsA+AS	NR	death	Yes	8
18	68	F	1	None	normo	45,XX,del(13)(q12q22),-20 2/20	10	13(q12q22)	0	0	No	VitK	NE	death	Yes	7
19	75	M	2	None	hypo	45,X,-Y.del(13)(q?) 2/20	10	13q(?)	0	0.003	PSL	CsA	NR	alive	No	71+
20	81	M	17	None	hypo	47,XY,+8,del(13)(q?) 19/20	95	13q(?)	6.851	0.272	No	CsA	NE	alive	No	67+
21	57	F	122	Erythroid	normo	46,XX,del(13),+mar 10/20	50	del(13)	0.522	1.075	AS	CsA+AS	HI-3	alive	No	146+
22	66	M	1	Erythroid	hypo	45,X.-Y,del(13)(q12q14) 15/20	75	13(q12q14)	0.149	0.209	No	CsA	HI-2	alive	No	11+
Median	**65**						**30**		**0.137**	**0.095**						

UPN: unique patient number; M: male; F: female; normo: normocellular marrow; hypo: hypocellular marrow; GPI-AP-granulocytes, glycosylphosphatidyl-inositol anchored protein-deficient granulocytes; GPI-AP-erythrocytes, glycosylphosphatidyl-inositol anchored protein-deficient erythrocytes; CsA: cyclosporine; ATG: antithymocyte globulin; AS: anabolicsteroid; Allo-BMT: allogeneic bone marrow transplant; VitK: vitamin K; CR: complete remission; HI-2: hematologic improvement in two lineages; HI-3: hematologic improvement in three lineages; NR: no response; NE: not evaluable; AML: acute myeloid leukemia; LFS: leukemia-free survival.

Transformation of patient 17 (UPN17) to AML could be attributed to the coexistence of del(7q), which is associated with high risk of AML evolution.[18]

The percentage change of del(13q) clone following IST varied from one patient to another (*Online Supplementary Figure S1*) in a similar way in which the percentage of GPI-AP$^-$ cells changed in the present study (*data not shown*), which is consistent with our previous findings regarding *PIGA* mutant HSCs.[10] Given that effective removal of immune mechanisms by IST does not consistently lower the percentage of del(13q) clone, it is speculated that preferential expansion of del(13q) clones by the immune mechanisms at the onset of BM failure[10] may lead to the escape from immunological pressure, as in the case of *PIGA*

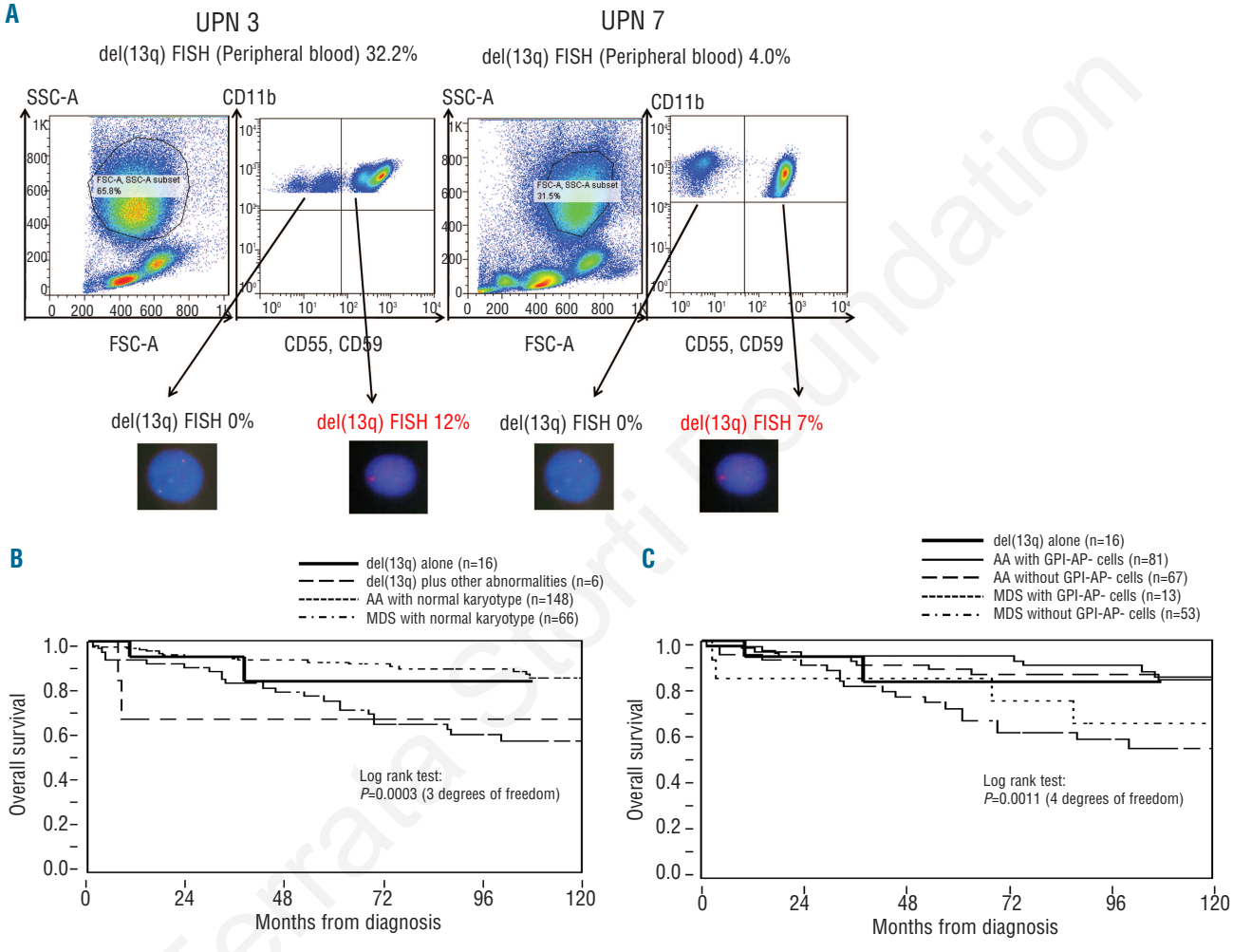

Figure 1. (A) Glycosylphosphatidylinositol-anchored protein (GPI-AP) expression in granulocytes with del(13q). GPI-AP$^-$ and GPI-AP$^+$ granulocytes were separated from peripheral blood cells of 2 patients with del(13q), one (Unique Patient Number (UPN) 3) comprised of 2.1% GPI-AP$^-$/ 32.2% del(13q) granulocytes and one (UPN 7) comprised of 12.3% GPI-AP$^-$/ 4.0% del(13q) granulocytes. Both GPI-AP$^-$ and GPI-AP$^+$ fractions were subjected to FISH analysis using a 13q probe (13q14.3); del(13q) clones were detected only in the GPI-AP$^+$ fraction of each patient (red), suggesting that del(13q) cells are derived from non-PIGA mutant hematopoietic stem cells. (B) Ten-year overall survival rates of patients with del(13q), patients with del(13q) plus other abnormalities, aplastic anemia (AA) patients with a normal karyotype and myelodysplastic syndrome (MDS) patients with a normal karyotype. All 66 MDS patients with a normal karyotype had Int-1-risk IPSS scores. (C) Ten-year overall survival rates of patients with del(13q), aplastic anemia (AA) patients with a normal karyotype with and without increased GPI-AP$^-$ cells and myelodysplastic syndrome (MDS) patients with a normal karyotype with and without increased GPI-AP$^-$ cells. (D) Deleted gene loci regions of 7 patients with del(13q), shown as bold horizontal lines for each UPN under the gene. The shaded box represents the deleted region common to patients, 13q13.3 to 13q14.3, which encodes proteins involved in cytokine signal transduction.

mutant HSCs. It is necessary to identify common mechanisms leading to preferential activation of both PIGA mutant HSCs and HSCs with del(13q) in immune-mediated BM failure to verify these hypotheses.

A possible immune pathophysiology in $13q^{-alone}$ patients is supported by the markedly high prevalence (100%) of elevated GPI-AP$^-$ cell levels which is linked to the escape of *PIGA* mutant HSCs from an immune system attack.[19] Because the del(13q) abnormality occurs in the GPI-AP$^+$ population, it may play a similar role to the GPI-AP$^-$ cells. SNP array analysis revealed the common deletion of a 15 Mb (13.3 to 14.3) region of 13q in 7 $13q^{-alone}$ and $13q^{-+other}$ patients. This segment encodes several proteins that regulate cell proliferation and the cell cycle, such as SMAD9 and RB1; both are involved in the signal transduction pathway of transforming growth factor-beta (TGF-β, an important cytokine in regulating HSC dormancy. Cytokine-mediated selection of *PIGA* mutant HSCs has been proposed as a mechanism for preferential proliferation of GPI-AP$^-$ cells,[20] but no supporting evidence has been presented. A previous study demonstrated that GPI-AP$^-$ T cells show decreased sensitivity to herpes virus entry mediator (HVEM) ligands that transmit inhibitory signals through receptors for CD160(21) and TGF-β.[22,23] The presence of del(13q) represents a unique subgroup of immune-mediated BM failure associated with an increase in the percentage of GPI-AP$^-$ cells, where del(13q) and *PIGA* mutant HSCs undergo preferential expansion, possibly due to their decreased sensitivity to cell-cycle inhibitory molecules, such as TGF-β compared to normal HSCs.

In conclusion, MDS-U with del(13q) alone is a benign BM failure syndrome characterized by a good response to IST and a markedly high prevalence of elevated GPI-AP$^-$ cell percentages. Therefore, del(13q) should be eliminated from the list of karyotypic abnormalities representing the intermediate group defined by IPSS,[24] and BM failure with del(13q) should be managed as AA.

Authorship and Disclosures

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