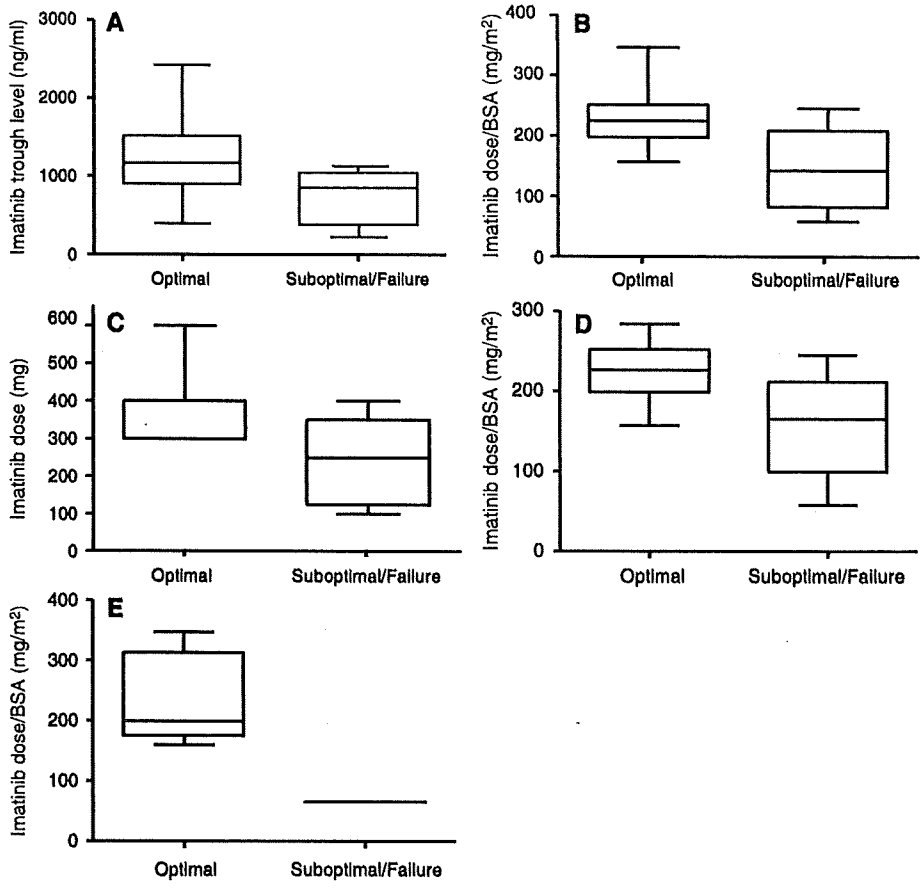

Fig. 4 Imatinib trough levels (**a**), imatinib dose/BSA (**b**), and imatinib dose (**c**) categorized as optimal response, and suboptimal response or failure based on European LeukemiaNet criteria. The trough level of cases with optimal response, mean = 1242 ng/ml, $n = 25$; suboptimal response/failure, mean = 736 ng/ml, $n = 8$; $P = 0.0087$. There were also significant differences in imatinib dose/BSA (**b**, $P = 0.01$) and in imatinib dose (**c**, $P = 0.01$) between the two response categories. This tendency remained even after cases were divided into chronic (**d**, Optimal, 18 cases; suboptimal/failure, 7 cases, $P = 0.0272$) and accelerated phase (**e**, Optimal, 5 cases; suboptimal/failure, 1 case). BSA was not available in 2 cases

Table 3 Number of patients in each quartile group

Quartile group	Total	Average dose (mg/day) of imatinib (range)	Response category[a]	
			Optimal	Suboptimal/ failure
Q1	8	275 (100–400)	4	4
Q2	9	361 (150–400)	6	3
Q3	8	325 (300–400)	7	1
Q4	8	425 (400–600)	8	0

[a] Distribution of patients in Q1/Q2 and Q3/Q4 is significantly different ($P = 0.04$)

imatinib as judged by the ELN criteria, was related to its trough concentration in the current analysis. These results strongly suggest that the high trough level in our patients resulted in an excellent imatinib response. Although body weight and BSA per se were not clinically significant determinants of the trough concentration [12, 13], the smaller body size of the Japanese population as compared to foreign populations might have influenced the results. The similar trough concentration despite smaller dose could be explained, at least in part, by the difference in the BSA between the IRIS (male 2.0 m^2, female 1.8 m^2) and the Nagasaki (male 1.77 m^2, female 1.45 m^2) studies [9].

The imatinib trough concentration is dependent on a variety of factors including prescribed dose, compliance, drug–drug interaction, serum-binding proteins, genetic differences in enzymatic pathways, and concomitant diseases [11–13]. Although not clearly mentioned previously, BSA might also affect trough imatinib concentration, in particular when BSA is small. It is well known that molecular monitoring of the *bcr-abl* fusion transcripts is necessary to manage CML patients for the appropriate choice of treatment: the conversion of tyrosine kinase inhibitors, or the indication of other treatment including IFN or stem cell transplantation [11, 14–17]. Because a plasma level above 1040 ng/ml (or 1000 ng/ml suggested by the IRIS study, or 1002 ng/ml reported from French study) seemed necessary to obtain a significant effect from imatinib, our results suggest that monitoring trough imatinib concentration in addition to molecular monitoring would be useful for the management of CML patients. For example, patients with an imatinib blood concentration lower than the optimal level could be candidates for an increased imatinib dose or for other treatment than imatinib

such as a second generation of BCR-ABL inhibitor. Given the fact that more than 30% of patients are treated with less than 400 mg/day in a practical setting, it would be useful to measure trough concentration of imatinib when it might be necessary to make a dosage change, such as to consider increase of imatinib with an unsatisfactory response, or to consider decrease of imatinib with a fair response but intolerable side effects. Maximizing the efficacy and minimizing the side effects of imatinib could be achieved by the dose adjustment based on its trough data, reducing the cost of treatment at the same time. Further research should include an evaluation of imatinib-binding proteins and genetic differences in metabolic enzymes, such as CYA3A5 [12, 13]. These types of studies would provide clinically important information for the prediction of imatinib efficacy in CML patients.

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Appendix

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Relationship between monoclonal gammopathy of undetermined significance and radiation exposure in Nagasaki atomic bomb survivors

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Radiation exposure is a possible predisposing factor for monoclonal gammopathy of undetermined significance (MGUS), but the association has been uncertain. We investigated the relationship between radiation exposure and MGUS prevalence by using data from the M-protein screening for Nagasaki atomic bomb survivors between 1988 and 2004. Radiation exposure was assessed by exposure distance from the hypocenter and exposure radiation dose. We computed prevalence ratios (PRs) and the 95% confidence intervals (CIs) adjusting for exposure age and sex. A total of 1082 cases of MGUS were identified from 52 525 participants. MGUS prevalence was significantly higher in people exposed at distance within 1.5 km than beyond 3.0 km (PR, 1.4; 95% CI, 1.1-1.9) among those exposed at age 20 years or younger, but it was not found among those exposed at age 20 years or older. MGUS prevalence was also significantly higher in people exposed to more than 0.1 Gy than those exposed to less than 0.01 Gy (PR, 1.7; 95% CI, 1.0-2.8) among those exposed at age 20 years or younger. Thus, people exposed at younger age exhibited a significantly high risk of MGUS when exposed to a high radiation dose. There was no clear association between radiation exposure and the malignant progression of MGUS. Further detailed analysis is needed. (Blood. 2009;113:1639-1650)

Introduction

Monoclonal gammopathy of undetermined significance (MGUS) is a premalignant plasma cell disorder, which is defined by a serum monoclonal protein (M-protein) concentration of 3 g/dL or less, 10% or fewer plasma cells in the bone marrow, and the absence of anemia, osteolytic lesions, hypercalcemia, and renal dysfunction.[1] Although the majority of patients with MGUS remain stable for prolonged periods, malignant transformation to multiple myeloma occurs at a constant rate of 1% per year.[2] Given that myeloma is an incurable hematologic malignancy, it is important to elucidate etiology and predisposing factors of MGUS.

Etiologic factors for MGUS have not been investigated fully.[3-5] There are currently no consistent risk predictors beyond age, sex, and race for developing MGUS. Although radiation exposure is well known to initiate leukemogenesis, there have been conflicting reports about the association between radiation exposure and plasma cell disorders.[6-11] An Italian case reference study reported an increased risk of MGUS among people exposed to occupational radiation exposure.[12] However, a small survey for atomic bomb survivors showed no association between radiation dose and the relative risk of MGUS.[13] Sample sizes of these previous studies were too small to obtain reliable results for association between radiation exposure and incidence of the disease.

We have recently reported the age-specific MGUS prevalence in the Japanese population, indicating 2.4% in those older than 50 years.[14] The report used an M-protein screening data from approximately 52 000 atomic bomb survivors but did not report the relationship between radiation exposure and MGUS risk. The large number of study participants from the radiation-exposed population could provide a great opportunity to investigate the relationship between radiation exposure and the risk of MGUS. Our preliminary analysis observed that MGUS risk was higher in those exposed to higher radiation at a young age.[15] However, the preliminary observation lacked detailed analyses for the relationship and did not include clinical characteristics. In the present study, we performed comprehensive analyses for the screening data by considering distance from the hypocenter of the nuclear explosion, radiation dose, age at exposure, age at diagnosis, and M-protein level to elucidate whether radiation exposure is related with the development of MGUS and the progression.

Methods

Data source

Screening for M-protein was initiated in October 1988 for atomic bomb survivors at the Health Management Center of Nagasaki Atomic Bomb Casualty Council, where a comprehensive medical check-up has been offered twice a year since 1968; several cancer screenings have been

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offered once a year since 1988. All examinations are free of charge and supported by the Nagasaki City government based on the Law Concerning the Relief for Atomic Bomb Survivors. Data of all medical check-ups and cancer screenings have been stored online in the computer database at the Data Center in the Atomic Bomb Disease Institute at Nagasaki University Graduate School of Biomedical Sciences since 1977. The ongoing database keeps data from approximately 120 000 atomic bomb survivors who have the Atomic Bomb Victim's Handbook, including fundamental information, age at exposure, city at the time of the bombings, exposure categories, exposure distance from the hypocenter in kilometers, date of the certificate handbook acquisition, date of examination, date of death, and date of moving to or away from Nagasaki City, and all laboratory results.[16] Data of participants who underwent the M-protein screening were extracted as anonymous data from the computer database in the Data Center of Atomic Bomb Disease Institute. Use of the database for this study was approved by the Atomic Bomb Disease Institute in June 2004 (No. 224).

Screening procedure

Screening procedures were described in detail previously.[14] Briefly, routine laboratory tests including the first-step M-protein screening were offered every year for atomic bomb survivors who visited the Health Management Center. Results of the first-step M-protein screening were evaluated on the sheet in the double-checking system by hematologists of Nagasaki University Medical Hospital regardless of exposure condition. Survivors with the presence of possible M-protein or low gammaglobulinemia were informed by mail or telephone to take the second-step screening. The second-step screening procedure consisted of physical examination by hematologists, immunoelectrophoresis of serum and urine, a qualitative test for Bence-Jones (BJ) protein, and a quantitative determination of serum concentration of immunoglobulins (Igs) using nephelometry. Subjects with a high level of M-protein or with other abnormal laboratory data were referred to the tertiary hospitals to undergo further examination with bone marrow aspiration, bone surveys, and other investigations. Skilled hematologists made a final diagnosis comprehensively based on screening data, routine laboratory data, physical examinations, and feedback letters from the reference hospitals. The diagnostic criteria used for MGUS were based on an M-protein level lower than 3.0 g/dL in serum Igs, no symptom of multiple myeloma or Waldenström macroglobulinemia (WM), no anemia, no hypercalcemia, no osteolytic lesion, and less than 10% marrow plasma cells if done.[1] Patients with high M-protein levels of more than 3.0 g/dL on the first-time detection day but showing the "reconfirmed" M-protein levels of less than 3.0 g/dL were also treated as MGUS.

Radiation exposure

Radiation exposure was assessed by exposure distance and exposure dose. In the database, exposure categories were divided into 4 categories: "directly exposed" indicates those who were exposed to atomic bomb radiation within 10 km from the hypocenter at the time of the bombing; "early entrants" indicates those who entered the city within approximately 2 km from the hypocenter within 2 weeks of the explosion; "relief" indicates those who were engaged in disposal of the dead or relief works for atomic bomb victims; and "exposed in utero" indicates children who were exposed prenatally at the time of the bombing. Although information of exposure distance was available for "directly exposed" and "exposed in utero," we used only "directly exposed" people for the analysis to investigate the relationship between radiation exposure and MGUS risk. Information on whole-body radiation dose estimates by the Atomic Bomb Survivors 1993 Dose (ABS93D) was available for a limited number of Nagasaki atomic bomb survivors.[17] The ABS93D was calculated according to 3 parameters: free-in-air kerma, shielded kerma, and organ kerma, which is the same as Dosimetry System (DS) 86,[18] which was used for the Life Span Study (LSS) cohort of the Radiation Effects Research Foundation (RERF).[11] Because a strong correlation between DS86 and ABS93D was fully documented,[19] we used ABS93D as a substitute for the DS series to estimate radiation-dose response.

Participants

The target population for the M-protein screening was 74 411 atomic bomb survivors exposed in Nagasaki City, consisting of 71 675 people who were alive at the time of the start of the M-protein screening in October 1988 and 2736 people who were included in the database after 1988 to 2004 because some newly obtained the Atomic Bomb Victim's Handbook and others moved to Nagasaki City from elsewhere. Those exposed in Hiroshima City who moved to Nagasaki City were excluded. Among 74 411 people, ABS93D dose information was available for 6837 (9.2%). Table 1 presents the breakdown of participants and nonparticipants by demographic characteristics. The participant rates were around 70% in all categorized groups except in those aged 30 years or older at exposure (36%). Finally, a total of 52 525 Nagasaki atomic bomb survivors underwent the M-protein screening between 1988 and 2004 (overall participation rate, 70.6%) and were used for analyses to examine the relationship between MGUS risk and exposure distance from the hypocenter. Among those with information on ABS93D dose, 4758 (participation rate, 69.6%) underwent the screening and were used for the dose-response analyses.

Follow-up procedure

Participants who were once diagnosed as having MGUS also annually underwent the M-protein screening in the same way as described in the screening procedure. They were followed to check the change in M-protein levels on an individual M-protein chart, which was reviewed by skilled hematologists in the Health Management Center. Participants with a high level of M-protein or with other abnormal laboratory data were referred to the tertiary hospitals to undergo further examination. Diagnoses of multiple myeloma or other related diseases were obtained from the tertiary hospitals.

Statistical analysis

Statistical analyses for prevalence were performed using all the screening data accumulated during the period from October 1, 1988, to March 31, 2004. Patients who were diagnosed with multiple myeloma or WM at the first-time screening were excluded from the analyses. Patients with MGUS were also analyzed for the risk of malignant progression during the period from the date of diagnosis to July 31, 2008. All statistical analyses were performed using SAS 8.2 software (SAS Japan Institute, Tokyo, Japan). All tests were 2-tailed, and the level of statistical significance (P) was .05.

Age at exposure was treated as a continuous data or stratified into 4 categories (0-9 years, 10-19 years, 20-29 years, and 30 years or older). Exposure distance from the hypocenter in kilometers was treated as a continuous data or stratified into 3 categories (within 1.5 km, 1.5-3.0 km, and 3.0 up to 10.0 km). The cutoff values for exposure distance were chosen based on previous reports.[20,21] Among those in the exposure category of "directly exposed," participants with no information on distance position were treated as those exposed at an unknown distance. The ABS93D dose estimate in grays was treated as continuous data or stratified into 3 categories (lower than 0.01 Gy, 0.01-0.1 Gy, and 0.1 Gy or higher). Age at diagnosis of MGUS was stratified into 5 categories ($<$ 50 years, 50-60 years, 60-70 years, 70-80 years, and $\geq$ 80 years). Basic demographic analyses were assessed using the χ^2 test or trend test for categoric variables and the nonparametric test for continuous variables, if necessary. Simple prevalence (percentage) of MGUS and the 95% confidence intervals (CIs) were calculated using the exact binomial method in each category. Exposure-response analyses were performed for 2 datasets, one for people with assured exposure distance from the hypocenter to examine the relationship between MGUS risk and the exposure distance, and another for people with assured ABS93D dose to examine the relationship between MGUS risk and exposure dose. To evaluate the relationship between MGUS risk and exposure distance or exposure dose, we calculated prevalence ratios (PRs) and the 95% CI with the log-binomial regression model using PROC GENMOD in SAS.[22,23] Univariate and multivariate analyses were performed, including relevant factors and/or interaction terms to test effect modification. To obtain the best-fit model for dose-response effect, we ran additional analyses, including sex, continuous age at exposure per year, continuous radiation dose (linear or quadratic term), and

Table 1. Demographic characteristics of participants and nonparticipants among Nagasaki atomic bomb survivors between 1988 and 2004

	Whole population (N=74411)			Population with ABS93D (n=6837)		
	No. of participants	No. of nonparticipants	Rates of participation, %*	No. of participants	No. of nonparticipants	Rates of participation, %*
Total	52 525	21 886	70.6	4 758	2 079	69.6
Sex						
Male	20 450	9 021	69.4	1 652	794	67.5
Female	32 075	12 865	71.4	3 106	1 285	70.7
Age at exposure						
< 10 y	16 993	5 522	75.5	1 636	515	76.1
10 to < 20 y	20 569	4 967	80.5	1 735	473	78.6
20 to < 30 y	10 554	3 768	73.7	961	348	73.4
30 y or older	4 409	7 629	36.6	426	743	36.4
Exposure status†						
Directly exposed, all	40 814	16 808	70.8	4 674	2 079	69.2
< 1.5 km	2 496	1 035	70.7	614	277	68.9
1.5 to < 3.0 km	10 457	4 771	68.7	4 055	1 797	69.3
3.0 to 10 km	27 857	11 000	71.7	5	5	50.0
Unknown distance	4	2	66.7	0	0	
Early entrants	9 399	3 713	71.7	5	0	100.0
Relief	714	940	43.2	0	0	
Exposed in utero	885	392	69.3	79	0	100.0
Unknown	713	33	95.6	0	0	
Exposed dose of ABS93D‡						
Available for directly exposed, all	4 674	2 079	69.2	4 674	2 079	69.2
0 to < 0.01 Gy	1 673	767	68.6	1 673	767	68.6
0.01 to < 0.1 Gy	1 720	734	70.1	1 720	734	70.1
≥ 0.1 Gy	1 281	578	68.9	1 281	578	68.9
Available for early entrants§	5	0	100.0	5	0	100.0
Available for exposed in utero§	79	0	100.0	79	0	100.0
Not available	47 767	19 807	70.7			

*Rates were calculated as the number of participants divided by the number of target population in each stratum.

†"Directly exposed" indicates those who were directly exposed to atomic radiation within 10 km from the hypocenter; "Early entrants," those who entered the city within approximately 2 km from the hypocenter within 2 weeks of the explosion; "Relief," those who were engaged in disposal of the dead or relief works for atomic bomb victims; and "Exposed in utero," children who were exposed prenatally at the time of the bombing.

‡ABS93D indicates the Atomic Bomb Survivors 1993 Dose, which is calculated for a limited number of Nagasaki atomic bomb survivors.

§Some people exposed in utero and early entrants also have ABS93D dose information, but the information was not presented in this study.

interaction terms between covariates. The most appropriate model was selected on the basis of the Akaike Information Criterion (AIC).[24] The cumulative probability of developing multiple myeloma or other lymphoid malignancies among patients with MGUS was calculated using the Kaplan-Meier method and compared using the log-rank test. Patients who died or were lost to follow-up were censored in the analysis.

Results

Of 52 525 participants, 1103 were confirmed as having monoclonal immunoglobulin, of which 1082 were diagnosed with MGUS, 19 with multiple myeloma, and 2 with WM. The 21 patients with multiple myeloma or WM were excluded from analyses. Therefore, a total of 52 504 participants were used for analyses by exposure distance. Of the 21 patients excluded, 3 had ABS93D dose information. Therefore, a total of 4755 participants with ABS93D dose were used for dose-response analyses.

Clinical characteristics of MGUS at diagnosis

Table 2 shows the clinical characteristics of 1082 patients with MGUS. The median age at diagnosis was 68.5 years (range, 45.0-100.9 years). Age at diagnosis was significantly older in women (median, 68.3 years) than in men (median, 66.3 years; $P = .003$). The distribution of age at diagnosis by exposure categories is presented in Table 3. Although patients exposed at a younger age tended to be younger at diagnosis (Figure 1C), there was no difference in age at diagnosis across exposure distance groups ($P = .65$); however, there was some tendency for age at diagnosis to be younger in those exposed to the higher dose (> 0.1 Gy) than those exposed to the lower dose in each exposure age group, though the differences were not statistically significant ($P = .46$ among 3 dose categories and $P = .23$ between the dose group of 0-0.01 and > 0.1 Gy; Figure 1A,B). Median serum M-protein level at diagnosis was 1.5 g/dL (range, 0.1-3.4 g/dL). The distribution of serum M-protein level by demographic characteristics is summarized in Table 4 and Table S1 (available on the *Blood* website; see the Supplemental Materials link at the top of the online article). MGUS with M-protein levels greater than 1.5 g/dL were highly frequent in those exposed at 20 years or older. However, the level was not different among age at diagnosis, exposure distance, or exposure dose.

Prevalence of MGUS by exposure distance from the hypocenter

MGUS prevalence in 52 504 participants by sex and exposure status is shown in Table 5. The overall prevalence of MGUS in participants was 2.1% (95% CI, 1.9-2.2), 2.8% (95% CI, 2.6-3.0) in men, and 1.6% (95% CI, 1.5-1.7) in women. MGUS prevalence was 2.7% (95% CI, 2.1-3.4) in those directly exposed within 1.5 km from the hypocenter, 1.9% (95% CI, 1.7-2.2) at 1.5 to 3.0 km, 2.0% (95% CI, 1.8-2.1) at more than 3.0 km, and 2.3% (95% CI, 2.0-2.6) in other exposure categories. Table 6 summarizes the results of univariate and multivariate regression analyses. The

Table 2. Clinical characteristics of patients with MGUS

	MGUS among all participants (n = 1082)	MGUS among participants with dose (n = 93)
Sex, no. (%)		
Male	569 (53)	48 (52)
Female	513 (47)	45 (48)
Age at diagnosis, no. (%)		
< 50 y	25 (2)	3 (3)
50-59 y	166 (15)	16 (17)
60-69 y	407 (38)	38 (41)
70-79 y	349 (32)	26 (28)
≥ 80 y	135 (13)	10 (11)
Median y, (range)	68.5 (45.0-100.9)	67.5 (48.2-100.9)
M-component heavy chain, no. (%)		
IgG	796 (74)	75 (81)
IgA	191 (18)	16 (17)
IgM	82 (7)	1 (1)
IgD	1 (0.1)	0
Biclonal	12 (1)	1 (1)
M-component light chain, no. (%)		
κ	609 (56)	52 (56)
λ	440 (41)	40 (43)
Biclonal	12 (1)	0
Not determined	21 (2)	1 (1)
Serum M-protein level, no. (%)*		
< 1.5 g/dL	496 (48)	31 (34)
1.5 to < 3.0 g/dL	525 (50)	60 (65)
3.0 to < 3.5 g/dL†	22 (2)	1 (1)
Median (range), g/dL	1.5 (0.1-3.4)	1.6 (0.4-3.1)
Other laboratory values		
Median serum albumin level, g/dL (range)	4.5 (3.0-5.8)	4.5 (38-58)
Median serum calcium level, mM (range)	2.4 (2.0-3.0)	2.3 (2.1-2.7)
Median serum creatinine level, μM (range)	88.4 (44.2-663.0)	88.4 (44.2-176.8)
Median hemoglobin level, g/L (range)	135 (67-182)	135 (83-178)

*Data from 12 patients with biclonal gammopathy were not included and 27 patients were not available for M-protein level at the first-time diagnosis, but were available for data at the next follow-up year.

†These patients were diagnosed with MGUS in the referral hospitals based on the "recurrent" examination of immunoglobulin and the plasma cell percentage in the bone marrow.

unadjusted PR was significantly higher in men, in those at an older age at exposure, and in those exposed at within 1.5 km compared with those exposed at more than 3.0 km. A multivariate analysis including interaction terms among all variables showed a significant interaction ($P < .03$) between age at exposure and the exposure distance, but no significant interaction between sex and age at exposure ($P < .7$) or exposure distance ($P < .9$), suggesting that the effect of exposure distance on MGUS prevalence might be different by age at exposure. Therefore, we analyzed data by dividing into 2 age categories: those exposed at younger than 20 years and those exposed at age 20 years or older. Because age function is a strong risk factor for MGUS, we included age at exposure as a continuous variable in both stratified multivariate analyses. In the multivariate analysis for those exposed when

Table 3. Age at diagnosis of MGUS by exposure distance, exposure dose, and age at exposure

	Distance from the hypocenter, median (range)			Total,
Overall population	**< 1.5 km**	**1.5-3.0 km**	**> 3.0 km**	**median (range)**
Age at exposure				
0-9 y	57.0 (50.3-67.1)	56.4 (47.4-67.5)	57.5 (45.0-68.0)	57.2 (45.0-68.0)
10-19 y	68.5 (56.0-77.5)	65.8 (56.4-75.7)	65.1 (54.8-77.8)	65.9 (54.8-77.9)
20-29 y	74.6 (68.3-78.5)	74.2 (64.5-86.4)	74.2 (64.5-87.4)	73.6 (63.7-87.4)
≥ 30 y	86.4 (79.5-88.4)	81.3 (74.9-100.9)	81.4 (73.3-93.7)	82.1 (73.3-100.9)
All ages	69.7 (50.3-88.4)	67.5 (47.4-100.9)	67.5 (45.0-93.7)	68.5 (45.0-100.9)
	Radiation dose of ABS93D			**Total,**
Subpopulation with radiation dose	**> 0.1 Gy**	**0.01-0.1 Gy**	**0-0.01 Gy**	**median (range)**
Age at exposure				
0-9 y	55.2 (49.7-67.1)	58.1 (48.2-64.0)	59.5 (49.3-67.5)	58.1 (48.2-67.5)
10-19 y	64.5 (56.0-70.7)	65.2 (58.2-73.7)	67.7 (57.4-70.5)	65.5 (56.0-73.7)
20-29 y	72.6 (71.1-77.1)	72.9 (67.7-79.5)	74.8 (67.4-86.4)	73.0 (67.4-86.4)
≥ 30 y	83.0 (77.3-87.5)	86.1 (74.9-91.9)	87.2 (79.9-100.9)	86.4 (74.9-100.9)
All ages	65.5 (49.7-87.5)	68.0 (48.2-91.9)	68.1 (49.3-100.9)	67.5 (48.2-100.9)

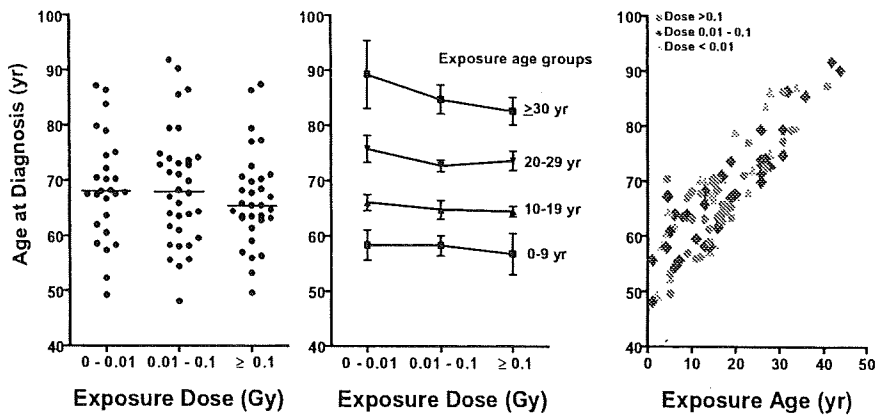

Figure 1. Distribution of age at diagnosis. (A) By exposure dose categories. The horizontal bar indicates the median age at diagnosis: 68.1 years in those exposed to 0 to 0.01 Gy; 68.0 years in those exposed to 0.01 to 0.1 Gy; and 65.5 years in those exposed to more than 0.1 Gy. (B) By exposure dose categories and exposure age categories. The points indicate the mean values and the whiskers indicate the standard errors. (C) By age at exposure. Red circles indicate each patient exposed to doses of more than 0.1 Gy; diamonds, each patient exposed to doses between 0.01 and 0.1 Gy; and triangles, each patient exposed to doses less than 0.01 Gy.

younger than 20 years, the adjusted PR of MGUS showed a 40% increase per every 5-year increase of age at exposure (adjusted PR, 1.4; 95% CI, 1.3-1.5), and the probability of MGUS among participants who were exposed within 1.5 km was overall 40% higher than among those exposed further than 1.5 km (adjusted PR, 1.4; 95% CI, 1.1-1.9). The adjusted PR of MGUS showed no difference among exposure distance categories in those of age at exposure older than 20 years.

Prevalence of MGUS by radiation dose

Table 7 presents the breakdown of MGUS prevalence in people with information on ABS93D dose. Among dose categories, the prevalence was 2.5% (95% CI, 1.7-3.5) in those exposed to 0.1 Gy or more, 2.0% (95% CI, 1.4-2.8) in those exposed to 0.01 to 0.1 Gy, and 1.6% (95% CI, 1.1-2.3) in those exposed to 0.01 Gy or less. Before applying dose as continuous data, doses are truncated to correspond to the 4 Gy level according to previous RERF studies.[11,25] Table 8 summarizes results of univariate and multivariate regression analyses for PRs. For those exposed when younger than 20 years, univariate analyses showed significantly higher PR in those exposed to 0.1 Gy or more compared with those exposed to a lower-dose category. However, no significant dose effect was observed when dose was treated as a continuous variable. After adjusting sex and age at exposure, the PR of MGUS in those exposed to 0.1 Gy or more was estimated at 1.66, suggesting that radiation exposure over 0.1 Gy had a 1.66 times higher risk of MGUS compared with the dose of less than 0.1 Gy. However, the linear-dose model failed to find a clear dose-response effect even after controlling sex and age at exposure (multivariate analysis 1 in Table 8). We performed additional models, including dose as a treated quadratic transformation. The AIC value in each multivariate analysis was 587.7646 for a model using a linear term (the parameter estimate [beta] for dose, 0.2179; standard error [SE], 0.1651; $P = .2$), 588.0652 for a simple quadratic term (dose squared; beta, 0.0569; SE, 0.0469; $P = .2$), and 589.7468 for a quadratic term (beta, 0.2794; SE, 0.4867; $P = .5$). For those exposed when older than 20 years, both univariate and multivariate analyses showed no effect of radiation dose on MGUS prevalence even after controlling other covariates. Figure 2A shows the PR of MGUS by exposure dose squared, adjusting for sex and age at exposure. PR at 1 Gy was 1.06 (95% CI, 0.97-1.16; $P = .2$) among those aged younger than 20 years at exposure. Figure 2B shows the PR of MGUS by age at exposure adjusting for sex and exposure dose squared. Advanced age was significantly associated with an increased prevalence of MGUS among those aged younger than 20 years at exposure (PR, 2.24 for 10-year increase; 95% CI, 1.39-3.62; $P = .001$) and those older than 20 years (PR, 1.77 for 10-year increase; 95% CI, 1.03-3.03; $P = .04$).

Table 4. Comparison of M-protein level by sex, age at diagnosis, and exposure status

	MP < 1.5 g/dL	MP ≥ 1.5 g/dL	P*
Total, no. (%)	378 (48.4)	403 (51.6)	
Sex, no. (%)			
Male	195 (50.7)	190 (49.4)	.21
Female	183 (46.2)	110 (27.8)	
Age at exposure, no. (%)			
0-9 y	83 (51.2)	79 (48.8)	.004
10-19 y	183 (53.2)	161 (46.8)	
20-29 y	78 (42.9)	104 (57.1)	
≥ 30 y	34 (36.6)	59 (63.4)	
Age at diagnosis, no. (%)			
< 50 y	7 (35.0)	13 (65.0)	.93
50-59 y	68 (50.8)	66 (49.2)	
60-69 y	141 (47.8)	154 (52.2)	
70-79 y	125 (50.8)	121 (49.2)	
≥ 80 y	37 (43.0)	49 (57.0)	
Exposure distance, no. (%)			
< 1.5 km	34 (52.3)	31 (47.7)	.25
1.5-3.0 km	79 (40.5)	116 (59.5)	
≥ 3.0 km	265 (50.9)	256 (49.1)	
Exposure dose, no. (%)			
> 0.1 Gy	9 (34.6)	17 (65.4)	.92
0.01-0.1 Gy	10 (29.4)	24 (70.6)	
0-0.01 Gy	11 (35.5)	20 (64.5)	

Data were used for only patients with MGUS with a heavy-chain class of A, G, and M and with available information on exposure distance and exposure dose. MP indicates M-protein concentration.

*P values were calculated using the χ^2 test or the Fisher exact test.

Risk of progression

Patients with MGUS were followed for a total of 8822.5 person-years (median, 7.4 years; range, 0-19.6 years). There were 365 (33.7%) patients who were followed until death. During this period of observation, 44 (4.1%) patients experienced the progression to multiple myeloma (41 patients) and WM (3 patients). All cases of myeloma were developed from IgG or IgA MGUS. Among 3 cases of WM, 2 were developed from IgM MGUS, and one was developed from IgG MGUS.[26] The median latency period between the diagnosis of MGUS and the development of multiple myeloma or WM was 5.3 years (range, 0.1-15.9 years). The overall cumulative probability of the progression was 6.9% (95% CI, 4.9-9.6) at

Table 5. Prevalence of MGUS by sex, age at exposure, and distance from the hypocenter

	Age at exposure													
	Male					Female					Total			
	0-9 y	10-19 y	20-29 y	≥ 30 y	All ages	0-9 y	10-19 y	20-29 y	≥ 30 y	All ages	0-9 y	10-19 y	20-29 y	≥ 30 y	All ages
< 1.5 km from hypocenter															
No. participants	195	522	164	97	978	225	861	363	68	1 517	420	1 383	527	165	2 495
No. cases	7	22	4	3	36	2	21	5	2	30	9	43	9	5	66
Prevalence, %	3.6	4.2	2.4	3.1	3.7	0.9	2.4	1.4	2.9	2.0	2.1	3.1	1.7	3.0	2.7
1.5 to < 3.0 km from hypocenter															
No. participants	1 452	1 726	367	272	3 817	1 825	2 411	1 753	650	6 639	3 277	4 137	2 120	922	10 456
No. cases	25	46	14	12	97	21	40	33	10	104	46	86	47	22	201
Prevalence, %	1.7	2.7	3.8	4.4	2.5	1.2	1.7	1.9	1.5	1.6	1.4	2.1	2.2	2.4	1.9
≥ 3.0 km from hypocenter															
No. participants	4 639	4 207	782	575	10 203	5 603	6 231	4 240	1 569	17 643	10 242	10 436	5 022	2 144	27 846
No. cases	70	133	30	34	267	46	95	98	42	281	116	228	128	76	548
Prevalence, %	1.5	3.2	3.8	5.9	2.6	0.8	1.5	2.3	2.7	1.6	1.1	2.2	2.6	3.5	2.0
Others*															
No. participants	1 419	2 483	1 043	497	5 442	1 633	2 121	1 835	676	6 265	3 052	4 604	2 878	1 173	11 707
No. cases	16	81	38	34	169	10	33	37	18	98	26	114	75	52	267
Prevalence, %	1.1	3.3	3.6	6.8	3.1	0.6	1.6	2.0	2.7	1.6	0.9	2.5	2.6	4.4	2.3
Total															
No. participants	7 705	8 938	2 356	1 441	20 440	9 286	11 624	8 191	2 983	32 064	16 991	20 562	10 547	4 404	52 504
No. cases	118	282	86	83	569	79	189	173	72	512	197	471	259	155	1 082
Prevalence, %	1.5	3.2	3.7	5.8	2.8	0.9	1.6	2.1	2.4	1.6	1.2	2.3	2.5	3.5	2.1

*Others included survivors with unknown exposure distance, those who entered the city early, those who were engaged in disposal of the dead or in relief works for atomic bomb victims, those exposed in utero, and those with unknown exposure status.

10 years and 8.0% (95% CI, 5.4-11.9) at the latest follow-up (Figure 3A). Among the 44 patients, 36 had information on exposure distance, and only 2 had information on exposure dose. Therefore, risk analyses were performed only by exposure distance. The frequency of malignant progression by factors is summarized in Table 9. The cumulative probability of the progression was greater in those exposed within a 1.5-km distance than those exposed at 1.5 to 3.0 km and at 3.0 km or more, but the difference was not statistically significant (13.9% vs 6.7% vs 7.7%; log-rank test $P = .34$; Figure 3B). The probability was significantly higher for those exposed at 20 years of age and older than those exposed at younger than 20 years (18.1% vs 5.4%; $P = .04$; Figure 3C). Among those exposed at 20 years old or older, there was no difference in the progression between those exposed within 3 km and greater than 3 km from the hypocenter ($P = .90$), but among those aged younger than 20 years at exposure, the probability had a

Table 6. PRs for MGUS in relation to sex, age at exposure, and distance from the hypocenter in participants with information of exposure distance

	All		Age at exposure < 20 y		Age at exposure ≥ 20 y	
	PR (95% CI)	P	PR (95% CI)	P	PR (95% CI)	P
Univariate analysis						
Sex						
Male	1.7 (1.5-1.9)	< .001	1.8 (1.5-2.1)	< .001	2.0 (1.5-2.5)	< .001
Female	Referent		Referent		Referent	
Age at exposure						
Per y	1.4 (1.3-1.5)	< .001	1.1 (1.1-1.1)	< .001	1.0 (1.0-1.1)	.001
Per 5 y	1.2 (1.1-1.2)	< .001	1.4 (1.3-1.5)	< .001	1.2 (1.1-1.3)	.001
Age at exposure group						
30 y or older	2.6 (2.0-3.3)	< .001			1.3 (1.0-1.7)	.02
20 to < 30 y	2.0 (1.6-2.4)	< .001			Referent	
10 to < 20 y	1.8 (1.5-2.2)	< .001	1.8 (1.5-2.2)	< .001		
< 10 y	Referent		Referent			
Exposure distance group						
< 1.5 km	1.3 (1.0-1.7)	.02	1.7 (1.3-2.3)	< .001	0.7 (0.4-1.2)	.2
1.5 to < 3.0 km	1.0 (0.8-1.1)	.7	1.1 (0.9-1.3)	.5	0.8 (0.6-1.0)	.1
3.0 to 10.0 km	Referent		Referent			
Multivariate analysis						
Male sex			1.9 (1.6-2.3)	< .001	1.9 (1.5-2.4)	< .001
Age at exposure per 5 y			1.4 (1.3-1.5)	< .001	1.1 (1.0-1.2)	.03
Exposure distance group						
< 1.5 km			1.4 (1.1-1.9)	.02	0.6 (0.4-1.1)	.1
1.5 to < 3.0 km			1.0 (0.8-1.2)	.9	0.8 (0.6-1.0)	.1
3.0 to 10.0 km			Referent		Referent	

Table 7. Prevalence of MGUS by sex, age at exposure, and exposure dose by ABS93D

Sex	Male					Female					Total				
Age at exposure	0-9 y	10-19 y	20-29 y	≥ 30 y	All ages	0-9 y	10-19 y	20-29 y	≥ 30 y	All ages	0-9 y	10-19 y	20-29 y	≥ 30 y	All ages
ABS93D dose															
0 to < 0.01 Gy															
No. participants	238	207	57	35	537	336	382	306	111	1135	574	589	363	146	1672
No. cases	3	7	3	0	13	3	3	5	3	14	6	10	8	3	27
Prevalence, %	1.3	3.4	5.3	0	3.7	0.9	0.8	1.6	2.7	1.2	1.1	1.7	2.2	2.1	1.6
0.01 to < 0.1 Gy															
No. participants	288	245	34	40	607	336	357	292	127	1094	624	602	326	167	1719
No. cases	5	4	3	4	16	4	6	6	2	18	9	10	9	6	34
Prevalence, %	1.7	1.6	8.8	10	2.7	1.2	1.7	2.1	1.6	1.6	1.4	1.7	2.8	3.6	2.0
≥ 0.1 Gy															
No. participants	164	225	51	29	469	195	315	219	82	811	359	540	270	111	1280
No. cases	3	14	0	2	19	1	7	3	2	13	4	21	3	4	32
Prevalence, %	1.8	6.2	0	6.9	4.1	0.5	2.2	1.4	2.4	1.6	1.1	3.9	1.1	3.6	2.5
Total															
No. participants	690	677	142	104	1613	867	1054	817	320	3058	1557	1731	959	424	4671
No. cases	11	25	6	6	48	8	16	14	7	45	19	41	20	13	93
Prevalence, %	1.6	3.7	4.2	5.8	3.0	0.9	1.5	1.7	2.2	1.6	1.2	2.4	2.1	3.1	2.0

tendency to be high in those exposed at within 3 km than those exposed more distantly (7.4% vs 4.2%; $P = .17$; Figure 3D). Among those aged 20 years or older at exposure, those diagnosed younger than the median age of 68.5 years significantly progressed to myeloma more than those older than 68.5 years (35.4% vs 7.6%; $P = .02$; Figure 3E). The cumulative probability was significantly higher in those with higher M-protein levels at diagnosis (≥ 1.5 g/dL) than those with lower levels (< 1.5 g/dL; 12.5% vs 2.0%; $P < .001$; Figure 3F). The older age at exposure showed the greater risk of progression among those with higher M-protein levels at diagnosis ($P = .06$), but there was not a different risk in age categories among those with the lower M-protein levels ($P = .80$; Figure 3G). There was no risk difference between the exposure distance categories among those with higher M-protein levels at diagnosis ($P = .60$), but there was a tendency for greater risk in those exposed within 3 km of the hypocenter among those with lower M-protein levels ($P < .001$; Figure 3H).

Discussion

The present study is the first comprehensive evaluation of the effects of radiation exposure on MGUS prevalence using a large number of atomic bomb survivors. We observed that, among those exposed when younger than 20 years, the probability of MGUS was 1.4 times greater in those exposed near the hypocenter than those exposed far from the hypocenter, and 1.7 times greater in those exposed to radiation doses of 0.1 Gy or more than those

Table 8. PRs for MGUS in relation to sex, age at exposure, and radiation dose in participants with ABS93D dose

	Age at exposure < 20 y		Age at exposure ≥ 20 y	
	PR (95% CI)	P	PR (95% CI)	P
Univariate analysis				
Sex				
Male	2.11 (1.26-3.52)	.004	2.64 (1.31-5.30)	.006
Female	Referent		Referent	
Age at exposure				
Per 1 y	1.08 (1.03-1.13)	.002	1.07 (1.02-1.13)	.01
Per 5 y	1.44 (1.14-1.82)	.002	1.40 (1.08-1.83)	.01
ABS93D dose				
Per 0.1 Gy	1.02 (0.99-1.06)	.1	0.99 (0.63-1.57)	.9
Per 1 Gy	1.25 (0.93-1.71)	.1	0.99 (0.63-1.57)	.9
ABS93D dose group				
≥ 0.1 Gy	2.02 (1.09-3.76)	.03	0.85 (0.33-2.17)	.7
0.01 Gy to < 0.1 Gy	1.13 (0.58-2.18)	.7	1.41 (0.65-3.04)	.4
0 to < 0.01 Gy	Referent		Referent	
Multivariate analysis-1				
Male sex	2.30 (1.38-3.84)	.002	2.30 (1.13-4.68)	.02
Age at exposure per 1 y	1.49 (1.17-1.89)	.001	1.06 (1.00-1.12)	.04
ABS93D dose per 1 Gy	1.24 (0.90-1.71)	.2	0.96 (0.59-1.62)	.9
Multivariate analysis-2				
Male sex	2.24 (1.34-3.74)	.002	2.34 (1.15-4.77)	.02
Age at exposure per 1 y	1.08 (1.03-1.13)	.003	1.06 (1.00-1.12)	.04
ABS93D dose ≥ 0.1 Gy (vs < 0.1 Gy)	1.66 (0.99-2.77)	.05	0.69 (0.30-1.58)	.4

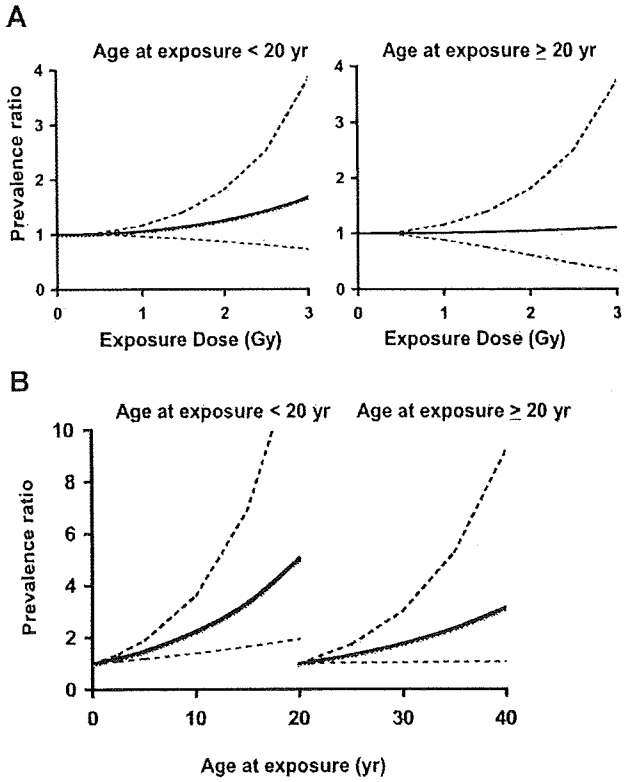

Figure 2. PR of MGUS. (A) By exposure dose in grays adjusting for sex and age at exposure among each exposure category. PR at 1 Gy was 1.06 (95% CI, 0.97-1.16; $P = .2$) among those aged younger than 20 years at exposure, and 1.01 (95% CI, 0.88-1.16; $P = .9$) among those aged 20 years and older at exposure. (B) By age at exposure, adjusting for sex and exposure dose among each exposure category. PR for 10-year increase of age was 2.24 (95% CI, 1.39-3.62; $P = .001$) among those aged younger than 20 years at exposure, and 1.77 (95% CI, 1.03-3.03; $P = .04$) among those 20 years and older. The dashed line shows 95% CI in each dose.

exposed to smaller radiation doses. We also observed that the strongest factor on the progression of MGUS was the high level of M-protein at diagnosis rather than the effect of the higher radiation exposure.

Only a few epidemiologic studies reported an effect of radiation exposure on MGUS. Pasqualetti et al observed that occupational exposure to radiation was significantly associated with an increasing risk of MGUS.[12] However, the result was based on only 13 patients, and no dose-response analysis was performed. Neriishi et al reported no association between radiation dose (DS86) and the incidence of 112 (1.7%) cases of MGUS among 6737 atomic bomb survivors who were members of the Adult Health Study (AHS) of RERF.[13] The study found that the MGUS risk was not different between those exposed to more than 0.01 Gy and those exposed to less than 0.01 Gy (relative risk [RR] = 1.35; 95% CI, 0.9-2.0). There were several differences between the AHS study and the present study in terms of analytic method and observed results. The overall prevalence was lower in the AHS study than our result (1.7% vs 2.1%) despite the same study periods. The cut-off value to compare MGUS risk by dichotomized dose category was also different, as the present study uses 0.1 Gy, but the AHS study used 0.01 Gy. This difference might affect the different interpretation of the results. In addition, the AHS study did not observe the significant interaction between age at exposure and dose, nor demonstrated dose-response analysis by age at exposure. Nevertheless, they realized marginally significant increases in MGUS risk in those younger than 80 years at onset, which might support our result that a significantly higher prevalence risk of MGUS was observed only in participants who were exposed at a younger age. Even though there were some differences between the AHS study and our present study, the estimated MGUS risk was similar; the RR was 1.603 ($P = .05$) in those younger than 80 years at diagnosis in the AHS study, and the PR was 1.66 ($P = .05$) in those exposed when younger than 20 years in our study. This suggests that it is consistent that there exists a significant weak association between radiation exposure and MGUS risk among those exposed when young.

Although we found that only younger age at exposure had a significant association between the higher-dose radiation exposure and the higher MGUS risk, the result does not necessarily deny the association in those of older exposure age. As shown in Table 1, the participation rate was lower in older ages, which suggests the data are less representative of the actual MGUS prevalence among the older target population. As is well known, older atomic bomb survivors, especially those exposed at the higher radiation dose, had higher mortality due to both cancers and noncancer diseases.[21,27] Therefore, results among those exposed at 20 years of age or older in our study might be strongly affected by detection loss.

For the association between radiation and myeloma, a number of epidemiologic studies analyzed people exposed to environmental, occupational, and medical radiation.[5,28,29] A series of reports from Hanford nuclear workers in the US and Sellafield workers of British Nuclear Fuels indicated a significant dose-response trend between death from myeloma and cumulative external radiation dose.[30-35] A recent international report of the 15-country collaborative study of nuclear workers also found a borderline significant association with radiation dose and 87 deaths from myeloma (RR = 1.61 at 100 mSv).[36] The age effect in most nuclear worker studies reported that a significant dose response was observed in those of older ages at exposure, which differs from findings in the experiences of atomic bomb survivors, including our study, that significant dose responses were observed to be more likely in those who were younger at exposure. For this discrepancy, Wing et al discussed that selection bias and basic differences in the characteristics of the study populations may be considered.[7] Another difference might be due to the differences in the type of exposure to radiation; nuclear workers received chronic exposures to cumulative lower doses over their lifetimes, in contrast with atomic bomb survivors, who received acute exposure to high doses of radiation.

Unlike nuclear workers, there is no epidemiologic evidence supporting an increased risk of myeloma among atmospheric nuclear test participants.[8,9,37-40] All of these studies had less power to evaluate dose-response association because the observed number of myeloma cases was too small (less than 8). A mixed association has been observed between risk of myeloma and diagnostic or therapeutic radiation.[41-43] In a large international study of radiation treatment for cervical cancer, there was no difference in risk of myeloma between those who were received less than 2 Gy and 2 Gy or greater; however, increased risks were observed among patients followed long term and those irradiated at relatively younger ages.[44] The observation supports our result that the higher MGUS risk was observed in those exposed to the higher radiation at a younger age.

Among atomic bomb survivors, the relationship between exposed radiation dose and myeloma has been also inconsistent.

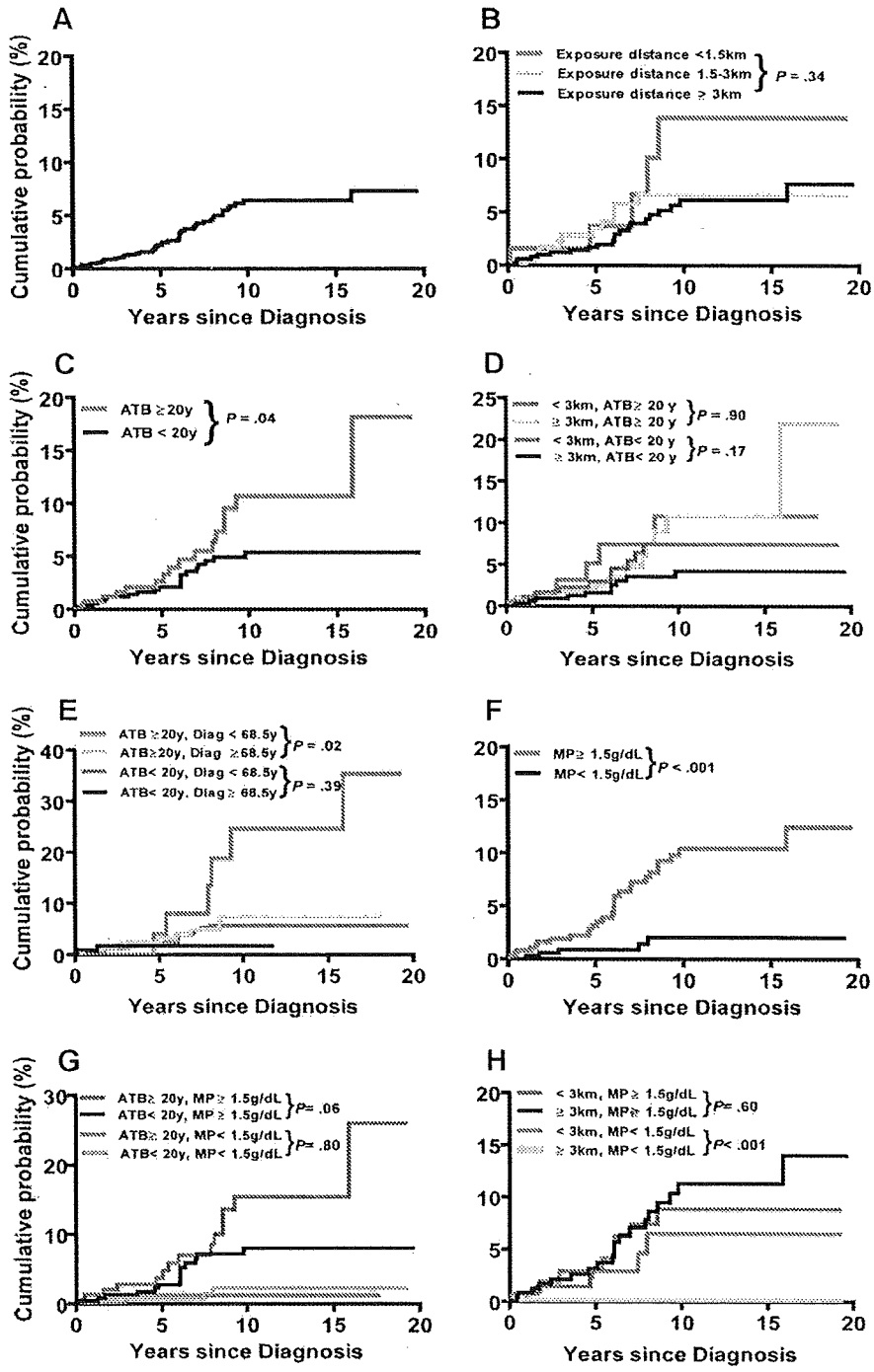

Figure 3. Risk of progression of MGUS to myeloma or related disorders. (A) The overall cumulative probability of the progression was 6.9% (95% CI, 4.9-9.6) at 10 years and 8.0% (95% CI, 5.4-11.9) at the latest follow-up. (B) By exposure distance. (C) By exposure age. (D) By exposure distance and exposure age. (E) By exposure age and age at diagnosis. (F) By the dichotomized serum M-protein level. (G) By exposure age and the serum M-protein level. (H) By exposure distance and serum M-protein level. The *P* values were calculated using the log-rank test. MP indicates M-protein. ATB indicates age at the time of bombing.

Ichimaru et al analyzed 29 patients with myeloma accumulated between 1950 and 1976, and found a statistically significant increase in the incidence among the higher-dose group (more than 0.5 Gy) since 1965, suggesting a prolonged latency period for radiation-induced myeloma.[10] The study also indicated a different dose effect by age of exposure; the positive effect was seen only in those aged 20 to 59 years at exposure, which was very similar to our present study. Shimizu et al also reported a statistically significant excess risk for myeloma from 1950 to 1985.[45] However, the latest report did not observe a significant dose response ($P = .12$) when analyses were limited to first-primary myeloma cases, though a statistically significant increase was observed when excluded cases were included in the analysis ($P = .02$).[11] In the latest report, only 59 of 94 patients were used for the analysis because many patients were excluded for a variety of reasons. The report explained the discrepancy within the same cohort might be affected by differences in the inclusion criteria of case and dosimetry system.

The majority of patients with MGUS will never develop MM. So far, the size of serum M-protein, the IgA isotype, an abnormal serum free light-chain ratio, detectable BJ protein excretion, and more than 5% of plasma cells in bone marrow have been identified as predictors of MM progression.[46-48] Nevertheless, precise predictors to define patients with high-risk MGUS should be identified. In the present study, we confirm that the strongest factor on the progression of MGUS is the high level of M-protein at diagnosis rather than the effect of radiation exposure. Exposure age and age at diagnosis showed complicated effects on the prognosis. Those exposed at 20 years or

Table 9. Frequency of malignant progression among MGUS with information of exposure distance

	No. MGUS with distance information	No. of progressions, %	P*
Total	815	36 (4.4)	
Sex			
Male	400	14 (3.5)	.21
Female	415	22 (5.3)	
Age at exposure			
< 10 y	171	6 (3.5)	.43
10 to < 20 y	357	14 (3.9)	
20 to < 30 y	184	12 (6.5)	
30 y or older	103	4 (3.9)	
Exposure distance			
< 1.5 km	66	5 (7.6)	.16
1.5-3.0 km	201	10 (5.0)	
3.0-10.0 km	548	21 (3.8)	
Age at diagnosis			
< 59 y	165	7 (4.2)	.11
60-69 y	304	21 (6.9)	
≥ 70 y	346	8 (2.3)	
M-component heavy chain			
IgG	148	29 (4.8)	.77
IgA	599	5 (3.4)	
IgM	61	2 (3.3)	
Biclonal	7	0	
Serum M-protein level			
< 1.5 g/dL	385	5 (1.3)	< .001
≥ 1.5 to < 3.0 g/dL	387	24 (6.2)	
3.0 to < 3.5 g/dL*	16	4 (25.0)	

*P values were calculated using the χ^2 test or the Fisher exact test for sex and M-compornent and using the Mantel-Haenszel trend test for age at exposure, exposure distance, age at diagnosis, and serum M-protein level.

older progressed more than those exposed at younger ages (Figure 3C), but those diagnosed younger than 68.5 years were more likely to progress to myeloma in both exposure age categories (Figure 3E). These results might be affected by the competing cause of death; the older patients would die before the progression of MGUS, which could introduce the underestimate of the progression risk among older patients. Although the present study did not find confident evidence that radiation exposure was related to the malignant progression of MGUS, there was a tendency for a greater risk of progression among patients exposed proximally. Neriishi et al also reported that the multiple myeloma mortality rate was higher among the exposed group (> 0.01 Gy, 10 patients) than the nonexposed group (0-0.01 Gy, 4 patients), though the difference was not significant.[13] Both studies suggested a potential adverse effect of radiation exposure on the progression from MGUS to multiple myeloma.

The present study has several limitations. Dose analyses were performed for a limited number of subjects. A healthy screenee bias[49] might affect the results, especially in older age group. Indeed, the participation rate decreased by age (Table 1). Overdiagnosis bias surely exists because of the long-term prognosis of MGUS in nature. Potential factors included in analyses were also insufficient. These limitations would have introduced over- or underestimates of the association. Further researches including other potential factors as covariate together are needed to confirm the effect of radiation on MGUS.

The mechanism of how radiation exposure affects the increasing risk of MGUS is still unknown. As is well known, radiation exposure induces chromosomal and genomic instabilities by direct and indirect ways.[50] Meanwhile, a variety of chromosome abnormalities have been reported even though MGUS is a benign hematologic disorder.[51,52] These facts might explain that MGUS risk increases when exposed to the higher level of radiation dose through radiation-induced chromosomal and genomic instabilities. Beyond the effect of radiation on MGUS risk, recent epidemiologic studies provided clear evidence of a significant racial disparity in MGUS prevalence[3,4,14] and familial aggregation for multiple myeloma/MGUS,[53] both of which suggest a role for genetic susceptibility as MGUS etiology. More recently, Brown et al reported a possible role for immune-related and inflammatory conditions in the causation of MGUS.[54] This report may also suggest another perspective on radiation-induced MGUS because recent molecular studies have revealed that radiation-induced inflammatory reaction and radiation-induced genomic instability may be interrelated with a predisposition to radiation carcinogenesis.[50,55]

We previously reported that, even allowing for atomic bomb survivors, our Japanese population had a lower prevalence of MGUS compared with the white population.[14] Although the conclusion is solid evidence, the present findings suggest that the prevalence data of atomic bomb survivors may not be generalizable to other Japanese populations, but rather suggest that MGUS prevalence in a general Japanese population might be lower than our population because the present study showed that those who were exposed to lower radiation had a significantly lower prevalence. Further population-based epidemiologic studies using the general population are needed to estimate a more reliable MGUS prevalence in Japanese and other Asians.

In conclusion, the present study suggests that atomic bomb survivors exposed to high levels of radiation at young ages are at high risk of the evolution of MGUS, even many years after radiation exposure. During the screening period from 1988 to 2004, the population of atomic bomb survivors became older, with the youngest atomic bomb survivors around 60 years of age. Unlike leukemia, the risk of solid cancers following exposure to ionizing radiation becomes manifest after a relatively long latency period,[27] after which the excess risk persists for decades. MGUS and myeloma are also diseases with long latencies. Further investigations of MGUS and myeloma are needed for this large and long-followed population, especially for people exposed at younger ages.

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Authorship

Contribution: M.I. was involved in the screening procedure, analyzed data, and wrote the manuscript; M. Tagawa established and managed the screening procedure; T.M. managed the screening procedure; K.Y. administrated and extracted data from the Data Center in the Atomic Bomb Disease Institute; Y.M., T.F., T.H., Y.I., D.I., and J.T. were responsible for the first screening procedure; S.K., S.M., and K.T. were responsible for the final diagnosis of the screening procedures; M. Tomonaga established the screening procedure and managed the database of the Atomic bomb survivors; and all authors revised the article critically and approved the final version.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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The Cytotoxic Effects of Gemtuzumab Ozogamicin (Mylotarg) in Combination with Conventional Antileukemic Agents by Isobologram Analysis *In Vitro*

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Abstract. *Background: The CD33 antigen is expressed on leukemia cells in most patients with acute myeloid leukemia (AML) and acute promyelocytic leukemia (APL), and in 20% of patients with acute lymphoblastic leukemia (ALL), while it is absent from pluripotent hematopoietic stem cells and nonhematopoietic cells. Gemtuzumab ozogamicin (GO) is an immunoconjugate of an anti-CD33 antibody linked to calicheamicin, which is a potent cytotoxic agent that causes double-strand DNA breaks, resulting in cell death. GO was developed against CD33 antigen-positive leukemias. The aim of this study was to investigate the cytotoxic effects of this agent in combination with conventional antileukemic agents. Materials and Methods: The cytotoxic effects of GO in combination with antileukemic agents were studied against human CD33 antigen-positive leukemia HL-60, U937, TCC-S and NALM20 cells. The leukemia cells were exposed simultaneously to GO and to the other agents for 4 days. Cell growth inhibition was determined using a MTT reduction assay. The isobologram method was used to evaluate the cytotoxic interaction. Results: GO produced synergistic effects with mitoxantrone, additive effects with cytarabine, daunorubicin, idarubicin, doxorubicin, etoposide and 6-mercaptopurine, and antagonistic effects with methotrexate and vincristine. Conclusion: Our findings suggest that the simultaneous administration of GO with most agents studied would be advantageous for antileukemic activity. The simultaneous administration of GO with methotrexate or vincristine would have little cytotoxic effect, and this combination may be inappropriate. These findings may be useful in clinical trials of combination chemotherapy including GO or other monoclonal antibodies linked to calicheamicin.*

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Key Words: Gemtuzumab, calicheamicin, isobologram, CD33, leukemia.

Gemtuzumab ozogamicin (GO) is a humanized anti-CD33 antibody conjugated with the cytotoxic antibiotic calicheamicin (1, 2), which is a potent chemotherapeutic agent with a low therapeutic index that requires targeting to tumor cells for clinical use. On binding to target cells, the antibody-antigen complex is internalized into the cells, and hydrolytic release of the toxic calicheamicin moiety occurs, which subsequently causes DNA double-strand breaks that lead to apoptosis (1, 3, 4).

Acute myeloid leukemia (AML) is a major target of GO, since the CD33 antigen is expressed on blast cells in most patients with AML, while it is absent from pluripotent hematopoietic stem cells and nonhematopoietic cells (5-8). In spite of positive expectations, GO only has a moderate antileukemic activity. It produces a complete response (CR) rate of 10-16% of cases, with another 7-15% achieving CR with inadequate platelet recovery in relapsed CD33-positive AML (9-16). The median survival of patients treated with GO alone is less than 6 months. GO in monotherapy at 9 mg/m^2 is complicated by hepatic veno-occlusive disease in 5-10% of patients. Acute promyelocytic leukemia (APL) cells express large amounts of CD33 and GO is also effective as a single agent with relapsed APL, including those cases with very advanced disease (17).

Around 20% of acute lymphoblastic leukemia (ALL) is also observed to express CD33 and is considered as a target of GO (5-8). Preclinical studies have shown that CD33-positive ALL cells are much more sensitive to GO than are AML cells (18). In clinical studies, several cases of relapsed CD33-positive ALL were reported to achieve complete remission following GO administration (19-20).

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Combination of lower doses of GO with other agents is the next strategy for improving the response and avoiding toxicity, and clinical studies are in progress for fresh and relapsed AML, APL and CD33-positive ALL cases as remission induction and consolidation therapies with other agents with a variety of schedules (15, 16, 21-26). However, to our knowledge, there are no experimental data available about the cytotoxic effects of GO in combination with conventional antileukemic agents. In the present study, we investigated the *in vitro* effects of GO in combination with antileukemia agents against CD33-positive human leukemia cell lines.

Materials and Methods

Cell lines. Experiments were conducted with CD33-positive human acute myeloid leukemias, U937 and HL-60, and Philadelphia chromosome-positive myeloid leukemia TCC-S, and acute lymphoblastic leukemia NALM20 cells. HL-60 and U937 were obtained from Health Science Research Resources Bank (Osaka, Japan). TCC-S was established in our laboratory (27). NALM20 was kindly donated by Yoshinobu, Matsuo, Hayashibara Biochemical Laboratories Inc., Fujisaki Cell Centre (Okayama, Japan). Cells were maintained in 75-cm^3 plastic tissue culture flasks containing RPMI-1640 medium (Sigma, St. Louis, MO, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Grand Island Biological Co. Grand Island, NY, USA) and antibiotics. The cell cycle times of rapidly growing U937, HL60 and TCC-S cells were around 24 h, while that of slowly growing NALM20 cells was 70-80 h.

Drugs. Anticancer agents used and their sources were: GO (Wyeth Laboratories, Philadelphia, PA, USA), cytarabine (Nihon Shinyaku Co. Ltd., Tokyo, Japan), daunorubicin (Meiji Co. Ltd., Tokyo, Japan), doxorubicin (Meiji Co. Ltd., Tokyo, Japan), idarubicin (Pfizer Japan Inc. Tokyo, Japan), etoposide (Nihon Kayaku Co. Ltd., Tokyo, Japan), 6-mercaptopurine (Takeda Co. Ltd., Tokyo, Japan), vincristine (Shionogi Co. Ltd., Tokyo, Japan), and methotrexate (Wyeth Lederle Japan Ltd., Tokyo, Japan). All drugs were dissolved in RPMI-1640. Appropriate drug concentrations were made by dilution with fresh medium immediately before each experiment.

Inhibition of cell growth by combination of GO and other agents. Two to four leukemia cell lines were used for the each study of GO in combination with other agents. Leukemia cells lines were harvested from the media and resuspended to a final density of 1×10^5 cells/ml for U937, HL-60, and TCC-S cells, and of 5×10^5 cells/ml for NALM20. Cell suspensions (100 μl) were dispensed into individual wells of 96-well tissue culture plates with lids (Falcon, Oxnard, CA, USA). Eight plates were prepared for the testing of each drug combination. Each plate had one 8-well control column containing medium alone and one 8-well control column containing cells but no drugs. Cells were incubated in a humidified atmosphere of 95% air/5% CO_2 at 37°C overnight. Drug solutions of GO and other drugs at different concentrations were then added (50 μl) to 8 wells containing cell suspensions and the plates were then incubated under the same conditions for 4 days for U937, HL-60 and TCC-S cells, and for 8 days for NALM20 cells.

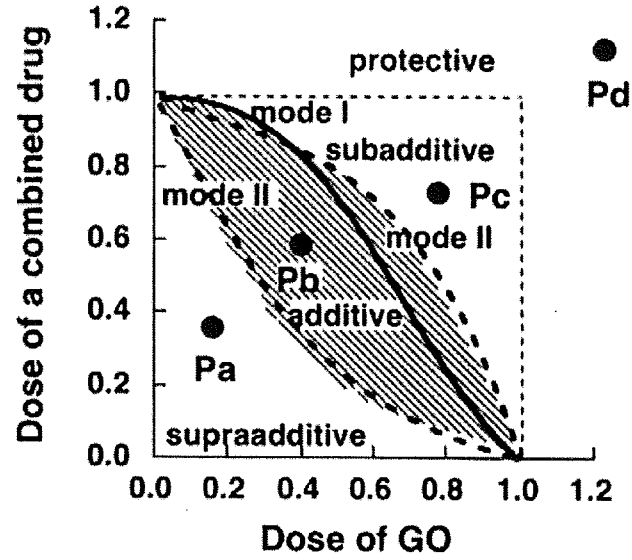

Figure 1. *Schematic representation of isobologram. Envelope of additivity (shaded area), surrounded by mode I (solid line) and mode II (dotted lines) isobologram lines, was constructed from the dose-response curves (shaded area) of GO and a combined drug. The concentrations that produced 80% cell growth inhibition were expressed as 1.0 on the ordinate and the abscissa of the isobolograms. Combined data points Pa, Pb, Pc, and Pd show supraadditive, additive, subadditive, and protective effects, respectively.*

3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay (MTT assay). Viable cell growth was determined using a modified MTT assay as described previously (28).

Isobologram method of Steel and Peckham. Cytotoxic interactions of GO with other agents at the 80% inhibitory concentration (IC_{80}) level were evaluated by the isobologram method of Steel and Peckham (Figure 1) (29). The theoretical basis of the isobologram method and the procedure for making isobolograms have been described in detail previously (30, 31).

Based upon the dose-response curves of GO and the other agents, three isoeffect curves were constructed (Figure 1). If the agents were acting additively by independent mechanisms, the combined data points would lie near the mode I line (hetero-addition). If the agents were acting additively by similar mechanisms, the combined data points would lie near the mode II lines (iso-addition).

Since it is unknown in advance whether the combined effects of two agents will be hetero-additive, iso-additive or an effect intermediate between these extremes, all possibilities should be considered. Thus, when the data points of the drug combination fell within the area surrounded by three lines (envelope of additivity), the combination was regarded as additive. When the data points fell to the left of the envelope, *i.e.* the combined effect was caused by lower doses of the two agents than was predicted, we regarded the drug combination as having a supraadditive effect (synergism). When the points fell to the right of the envelope, *i.e.* the combined effect was caused by higher doses of the two agents than was predicted, but within the square or on the line of the square, we regarded the combination as having a subadditive effect, *i.e.* the combination was

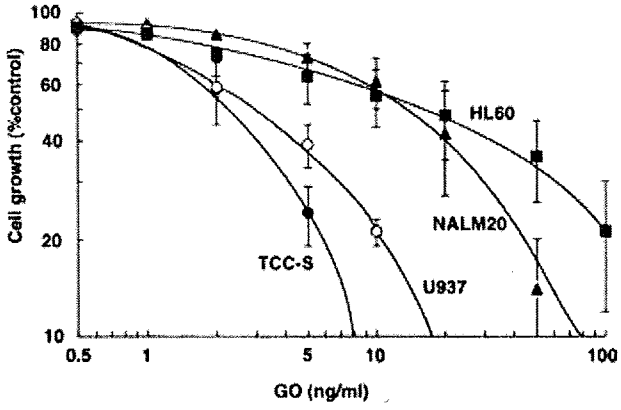

Figure 2. *The dose-response curves of GO in U937, HL-60, TCC-S and NALM20 cells. Cell growth inhibition was measured using the MTT assay and was plotted as a percentage of the control (cells not exposed to drugs). Each point represents the mean±SEM (n>10).*

superior or equal to the single agents but was less than additive. When the data points were outside the square, the combination was regarded as having a protective effect, *i.e.* the combination was inferior in cytotoxic action to the single agents. Bothsubadditive and protective interactions were regarded as antagonism.

Data analysis. To determine whether the condition of synergism (or antagonism) truly existed, statistical analysis was performed. The Wilcoxon signed-ranks test was used for comparing the observed data with the predicted minimum (or maximum) values for additive effects, which were closest to the observed data (*i.e.* the data on the boundary (mode I or mode II lines) between the additive area and supraadditive area (or subadditive and protective areas) (32). Probability (P) values ≤0.05 were considered to be significant. Combinations with $p>0.05$ were regarded as indicating additive/synergistic (or additive/antagonistic) effects. All statistical analyses were performed using the Stat View 4.01 software program (Abacus Concepts, Berkeley, CA, USA).

Results

Figure 2 shows the dose-response curves of GO in U937, HL-60, TCC-S and NALM20 cells. The IC_{80} values of GO alone against U937, HL-60, TCC-S, and NALM20 cells were 10.9±1.1 ng/ml, 100±36 ng/ml, 5.6±1.1 ng/ml, and 41±9 ng/ml, respectively (n>10). Figure 3 shows the dose-response curves for GO in combination with cytarabine, doxorubicin, and vincristine in U937 cells. Each isobologram was generated based on such dose-response curves.

Cytotoxic effects of GO in combination with cytarabine. U937, HL-60 and TCC-S cells were used for this combination study. Figure 4A-C shows the isobolograms of the combination of GO and cytarabine in these cells. In the U937 cells, the combined data points fell within the envelope of additivity (Figure 4A). The mean value of the data (0.55) was larger than that of the predicted minimum value (0.39) and smaller than that of the predicted maximum value for an additive effect (0.74) (Table I), indicating that the simultaneous exposure to GO and cytarabine produced an additive effect. In HL-60 and TCC-S cells, most data points for the combination also fell within the envelope of additivity (Figure 4B, and C). These findings suggest that the simultaneous administration of GO and cytarabine produced additive effects.

Cytotoxic effects of GO in combination with doxorubicin, daunorubicin, idarubicin, or etoposide. Figure 5A-C shows the isobolograms of the combination of GO with doxorubicin in U937, HL-60 and TCC-S cells, respectively. In all cell lines, all combined data points fell within the envelope of additivity, indicating that the simultaneous exposure to GO and doxorubicin produced additive effects (Table I). The simultaneous exposure to GO and daunorubicin, idarubicin, and etoposide showed quite similar effects (isobolograms not shown) in the cell lines studied (Table I)

Cytotoxic interaction between GO and mitoxantrone. U937 and HL60 cells were used for this study and showed similar effects. Most data points for the combination fell in the area of supraadditivity (isobolograms not shown). The mean values of the data were slightly smaller than those of the predicted minimum values for an additive effect (Table I). Statistical analysis showed that the difference was significant, indicating that the simultaneous exposure to GO and mitoxantrone produced marginally synergistic effects.

Cytotoxic effects of GO in combination with 6-mercaptopurine. U937, HL60 and TCC-S cells were used for this study. U937 and TCC-S cells were resistant to 6-mercaptopurine and the cytotoxic effects of this combination were evaluated at the IC_{50} level. In all three cell lines studied, most combined data points fell within the envelope of additivity, indicating that the simultaneous exposure to GO and 6-mercaptopurine produced additive effects (Table I).

Cytotoxic interaction between GO and methotrexate. In all four cell lines studied, most data points for the combination fell in the areas of sub-additivity and protection (isobolograms not shown). The mean values of the observed data were larger than those of the predicted maximum additive values (Table I). The difference was statistically significant, indicating antagonistic effects of the simultaneous exposure to these two agents.

Cytotoxic interaction between GO and vincristine. All four cell lines were used for this study. Figure 6A-C shows the isobolograms of this combination of this combination in U937, HL-60, and TCC-S cells, respectively. In U937, TCC-

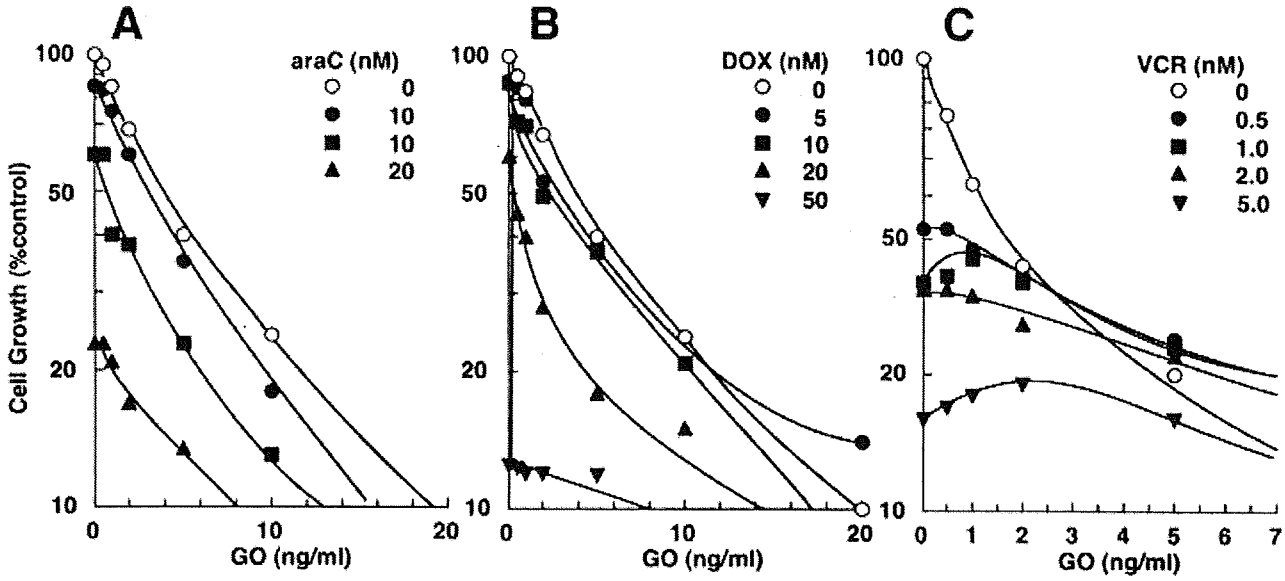

Figure 3. *Dose-response curves for GO in combination with cytarabine (ara-C) (A), doxorubicin (DOX) (B) and vincristine (VCR) (C) in U937 cells. Cell growth was measured using the MTT assay after 4 days and was plotted as a percentage of the control (cells not exposed to drugs). Each point represents the mean value for at least three independent experiments; the SEs of the means were less than 25% and are thus omitted.*

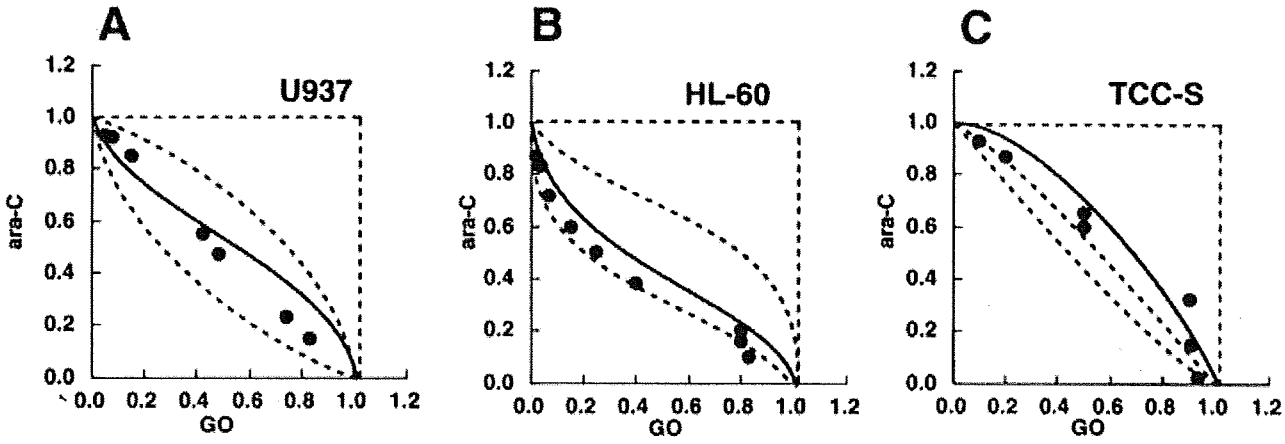

Figure 4. *Isobolograms of simultaneous exposure to GO and cytarabine (ara-C) in U937 (A), HL-60 (B) and TCC-S (C) cells. Data are presented as mean values of at least three independent experiments. In all three cell lines, all or most data points of the combinations fell within the envelope of additivity, suggesting additive interactions.*

S and NALM20 cells, the data points fell in the areas of subadditivity and protection. The mean values of the observed data were larger than those of the predicted maximum additive values. Statistical analysis showed that the difference was significant, indicating antagonistic effects (Table I). For HL60 cells, the data points fell within the envelope of additivity and in the area of subadditivity. The mean value of the observed data was slightly smaller than that of the predicted maximum additive value, indicating additive effects.

Discussion

Linking anticancer agents to an antibody that recognizes a tumor-associated antigen can improve the therapeutic index of the drug. The most promising results have been obtained with GO ozogamicin, a CD33 monoclonal antibody joined to the potent cytotoxin calicheamicin. The purpose of this study was to assess the cytotoxic effects of GO alone or in combination with commonly used antileukemic agents against CD33-positive leukemia cell lines.

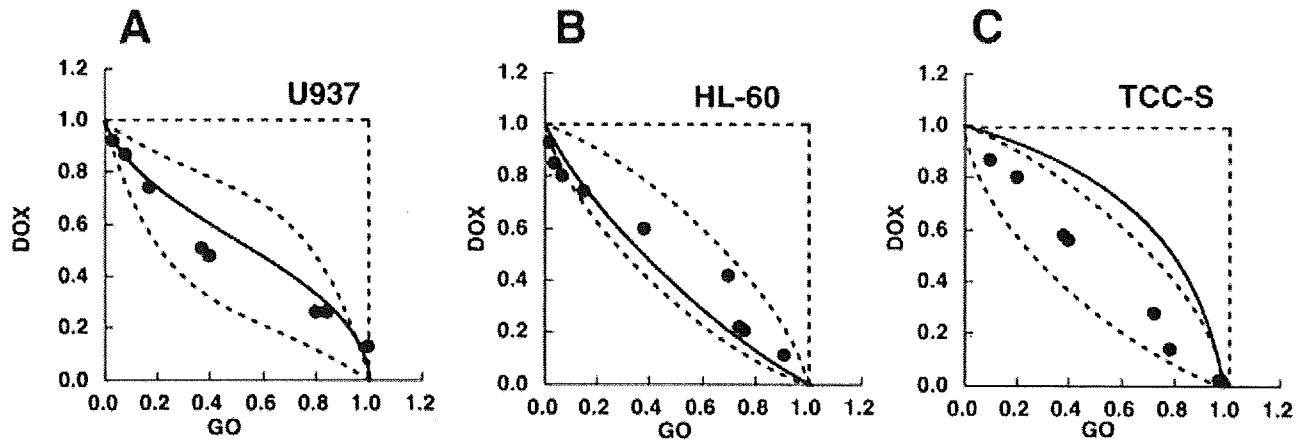

Figure 5. *Isobolograms of simultaneous exposure to GO and doxorubicin (DOX) in U937 (A), HL-60 (B) and TCC-S (C) cells. Data are presented as mean values of at least three independent experiments. In all three cell lines, all or most data points of the combinations fell within the envelope of additivity, suggesting additive interactions.*

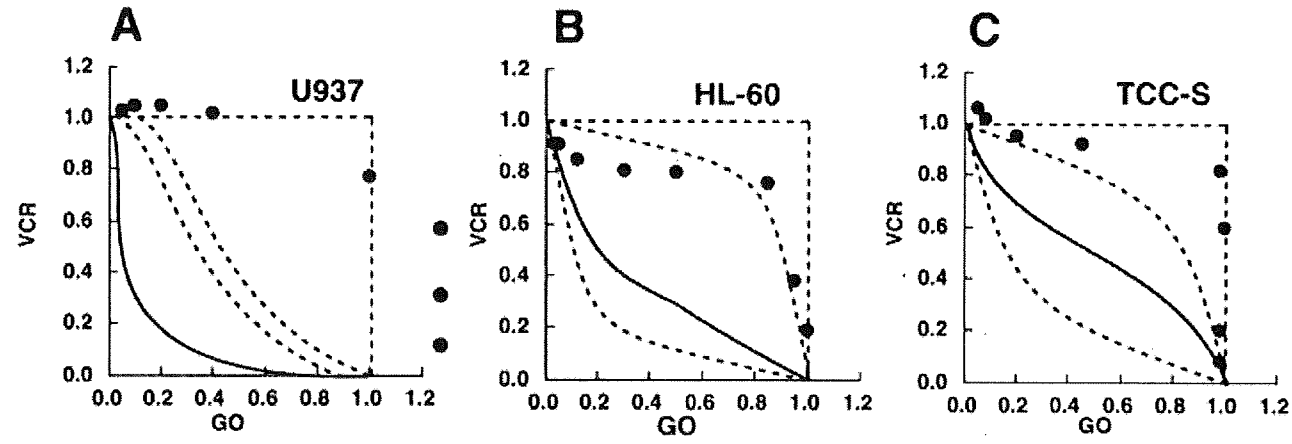

Figure 6. *Isobolograms of simultaneous exposure to GO and vincristine (VCR) in U937 (A), HL-60 (B) and TCC-S (C) cells. Data are presented as mean values of at least three independent experiments. In U937 and TCC-S lines, all or most data points fell in the areas of sub-additivity and protection, suggesting antagonistic interactions, while, in HL-60 cells, data points of the combinations fell within the envelope of additivity and in the area of subadditivity, suggesting additive interactions.*

The IC_{80} values of GO alone against U937, HL-60, TCC-S and NALM20 cells were approximately 10 ng/ml, 100 ng/ml, 5 ng/ml and 10 ng/ml, respectively. From the pharmacokinetic study, these concentrations are clinically achievable as the peak plasma concentration of GO was 2.86±1.35 mg/l and the half life of GO was 72.4±42.0 h after administration of the first 9 mg/m^2 dose of GO (33).

We studied the cytotoxic effects of GO in combination with conventionally used antileukemic agents. Cytarabine and anthracyclines such as daunorubicin and idarubicin are most widely used for remission induction or consolidation therapy of AML. At present, clinical trials of remission induction or consolidation therapy, with or without GO, are in progress. In our study, GO in combination with cytarabine and anthracyclines showed additive effects for all three cell lines studied.

The combination of cytarabine and an anthracenedione anticancer agent, mitoxantrone, is also used for the treatment of AML. Both anthracyclines and mitoxantrone inhibit topoisomerase-II and disrupt DNA synthesis and DNA repair in cancer cells. Mitoxantrone produced marginally synergistic effects with GO. These findings suggest that the simultaneous administration of GO with cytarabine or topoisomerase-II inhibitors could produce the expected (or more than expected) clinical activity. However, since the dose-limiting toxicity of GO, cytarabine, and topoisomerase-

Table I. *Mean values of observed data, predicted minimum, and predicted maximum of gemtuzumab ozogamicin in combination with other anticancer agents.*

Combined drug	Cell line	No. of data points	Observed data*	Predicted min.**	Predicted max.***	Effect
Cytarabine	U937	6	0.55	0.39	0.74	Additive
	HL60	9	0.67	0.49	0.85	Additive
	TCC-S	7	0.81	0.67	0.83	Additive
Doxorubicin	U937	8	0.68	0.50	0.84	Additive
	HL60	9	0.67	0.49	0.85	Additive
	TCC-S	8	0.74	0.58	0.91	Additive
	NALM-20	9	0.67	0.64	0.81	Additive
Daunorubicin	U937	6	0.66	0.49	0.86	Additive
	HL60	5	0.47	0.32	0.70	Additive
Idarubicin	U937	7	0.63	0.56	0.84	Additive
	HL60	6	0.51	0.37	0.81	Additive
Mitoxantrone	U937	6	0.54	0.60	0.82	Synergism ($p<0.05$)
	HL60	7	0.51	0.57	0.68	Synergism ($p<0.05$)
Etoposide	U937	7	0.53	0.51	0.63	Additive
	HL60	9	0.60	0.56	0.79	Additive
	TCC-S	7	0.53	0.49	0.75	Additive
6-Mercaptopurine	U937	7	0.66	0.60	0.69	Additive (IC_{50})
	HL60	7	0.54	0.46	0.66	Additive
	TCC-S	5	0.52	0.53	0.58	Additive (IC_{50})
Methotrexate	U937	6	>1.19	0.23	0.84	Antagonism ($p<0.01$)
	HL60	7	0.92	0.23	0.81	Antagonism ($p<0.05$)
	TCC-S	7	0.81	0.05	0.40	Antagonism ($p<0.02$)
	NALM-20	9	0.86	0.32	0.75	Antagonism ($p<0.01$)
Vincristine	U937	8	>1.09	0.26	0.66	Antagonism ($p<0.01$)
	HL60	8	0.90	0.36	0.93	Additive
	TCC-S	10	0.97	0.36	0.87	Antagonism ($p<0.01$)
	NALM-20	9	0.91	0.40	0.85	Antagonism ($p<0.05$)

*Mean value of observed data; **mean value of the predicted minimum values for an additive effect; ***mean value of predicted maximum values for an additive effect.

II inhibitors involves myelosuppression, there must be careful monitoring for myelosuppression during the combination treatment.

About 20% of ALL is observed to express CD33 and is considered as a target of GO (5-8) and encouraging data have been obtained from preclinical and clinical stuies (18-20). Recently, a CD22-targeted immunoconjugate of calicheamicin (CMC-544) has been developed for B-cell non-Hodgkin's lymphoma and ALL. CMC-544 has shown significant preclinical potential in studies in a mouse model (34-36).

We also studied the cytotoxic effects of GO in combination with methotrexate and vincristine, which are mainly used for lymphoid malignancies. GO showed definite antagonistic effects with methotrexate and vincristine in four out of four, and three out of four cell lines, respectively (Table I). The observed data values of GO in combination with methotrexate and vincristine were greater than 0.80 in all cell lines. These combinations also produced protective effects in the Philadelphia chromosome-positive leukemia cell line KU812 (data, not shown). Our findings suggest that the simultaneous administration of GO with methotrexate or vincristine may have almost no cytotoxic advantage over the administration of either agent alone, and thus may be inappropriate for the treatment of CD33-positive ALL. When CMC-544 is clinically available, the simultaneous administration of CMC-544 with methotrexate or vincristine would be also inappropriate.

There are a number of difficulties in the translation of results from *in vitro* to clinical therapy, and the pharmacokinetic profiles are significantly different between them. The toxic effects of the combination cannot be measured by *in vitro* systems, and the cell kinetics and cell biochemistry may be quite different. These differences between *in vitro* and clinical systems may influence the cytotoxic interaction of GO and other agents. In addition, we tested only simultaneous exposure to GO and other agents. Since cytotoxic effects are often schedule dependent, sequential exposure to GO followed by other agents or the reverse sequence may not show the same effects as simultaneous exposure to these agents. Continued preclinical and clinical studies would be necessary to assist in determining the optimal combination and schedule of GO in clinical use.