myelogenous leukemia (AML) has been observed in clinical trials of romiplostim (6). The prescribing information for romiplostim states that a randomized, double-blind, placebo-controlled trial enrolling patients with severe thrombocytopenia and International Prognostic Scoring System (IPSS) low- or intermediate-1-risk MDS was terminated due to the increased number of cases of AML observed in the romiplostim treatment arm. At the time of an interim analysis, among 219 MDS patients randomized 2:1 to receive treatment with romiplostim or a placebo (147 romiplostim: 72 placebo), 11 patients exhibited progression to AML, including nine patients in the romiplostim arm (6.21% [95%CI; 2.83-11.3]) versus two patients in the placebo arm (2.78% [95%CI; 0.34-9.68]). Moreover, Olnes et al. recently reported that as many as three of 25 aplastic anemia patients (12.0%[95%CI; 2.5-31.2%]) treated with eltrombopag developed clonal evolution, including two cases of monosomy 7 and one case of myeloid leukemia (7, 8). Meanwhile, only one case of AML among 653 (0.15% [95%CI; 0.00-0.85]) immune thrombocytopenic purpura (ITP) subjects was reported in a cumulative analysis of clinical trials (http:www.info.pmda.go.jp/shinyaku/P201100020/index.html). Since the risk appeared to be relatively smaller in the ITP patients than in the MDS patients, there were no statistically significant differences between the study drug and placebo groups. Nevertheless, it is noteworthy that the patient who developed AML had been assigned to receive the study drug. Therefore, no statistically significant increases in the incidence of myeloid malignancies have been reported thus far in ITP patients treated with MPL agonists (5).

We routinely run queries to detect signals of adverse reaction risks for whole drugs registered in the Food and Drug Administration (FDA) Adverse Event Reporting System (FAERS) and happened to find various potential risks. Some of the detected signals are well known, such as myelosuppression associated with cytotoxic antineoplastic agents or hypoglycemia associated with human insulin or its derivatives. With respect to MPL agonists, we found AML, as well as myelofibrosis, including bone marrow reticulin fibrosis. During the routine queries, not insignificant proportions of patients with myelofibrosis were detected among those receiving romiplostim (2.54%[95%CI; 2.01-3.16]) and eltrombopag (3.81%[95%CI; 2.48-5.57]). An activating mutation of the MPL gene is associated with myelofibrosis rather than AML. In addition, prescriber information for MPL agonists recommends caution regarding the possibility for bone marrow reticulin fibrosis. Therefore, the signals detected for myelofibrosis appear to be relevant. As for AML, it is worth further analyzing the database because MPL agonists are approved for the treatment of ITP, not MDS. We herein further assess the signals of a risk of AML in ITP patients treated with MPL agonists.

Analyzing existing databases, such as the FAERS, can be quickly performed at an affordable cost. Spontaneous reporting databases can be viewed as sources of case and control data for case-controlled studies. However, due to the incompleteness and inconsistencies in spontaneous adverse event reporting, analyses of spontaneous reporting databases are usually regarded as being surrogates for controlled epidemiologic studies. This approach will provide hypotheses to interested scientists. Before conducting conventional controlled epidemiologic research, the use of preliminary and exploratory screening may be helpful, although screening may not yield conclusive results.

Our primary objective was to clarify whether the possibility of the development of AML in patients with ITP is worth further investigation. As a secondary objective, we herein describe the demographic characteristics of the AML patients registered in the database.

Materials and Methods

Study design

A case-controlled study comparing exposure to MPL agonists between patients with myeloid leukemia and a comparison group.

Setting

We used the Japan Pharmaceutical Information Center (JAPIC) AERS database, a version of the FAERS database whose data format was arranged by the JAPIC (Tokyo, Japan). Duplicates and multiple records, a well-known drawback of the FAERS (9), were excluded using a semiautomatic multistep process (10). In order to detect and exclude as many as duplicates as possible in the database, an automated multistep process was applied. The process was carried out using a record-linkage strategy that groups records overlapping in four key fields: event date, patient age and sex and the reporting country, as previously reported (9, 10). Records with three overlaps and just one missing datum were considered to be duplicates.

Subjects

We extracted ITP subjects by searching for their indication for therapeutic drug treatment in the FAERS between 2002 and 2011. The row data handling and basic tabulations were performed as previously reported (11-15). In brief, the codes, "AUTOIMMUNE THROMBOCYTOPENIA," "IDIOPATHIC THROMBOCYTOPENIC PURPURA" or "THROMBOCYTOPENIC PURPURA," coded according to the medical terminology for the safety database, called MedDRA, were assumed to be used to register the ITP subjects. This extraction resulted in the identification of 4,821 ITP subjects after removing duplicate case IDs. Among the ITP subjects, we identified AML patients by searching for the terms, "ACUTE MYELOID LEUKAEMIA," "ACUTE MYELOID LEUKAEMIA (IN REMISSION)," "ACUTE MYELOID LEUKAEMIA AGGRAVATED," "ACUTE MYELOID LEUKAEMIA NOS" and "ACUTE MYELOID LEUKAEMIA RECURRENT," in the adverse reaction fields of the FAERS. Among the 4,281 ITP subjects, there were

Table 1. Characteristics of ImmuneThrombocytopenia Patient (n=4,821)

	AML† (n=62)		Other AE (n=4,759)		Unadjusted Odds Ratio		
variables	number	(%)	number	(%)	[95% confidence interval]		p value¶
Age							
Mean (SD), years	66.3	(13.2)	58.9	(21.5)			<0.001
0-9	0	0.0	93	2.0	0.00	[0.00 - 3.14]	0.634
10-19	0	0.0	129	2.7	0.00	[0.00 - 2.23]	0.414
20-29	0	0.0	146	3.1	0.00	[0.00 - 1.96]	0.264
30-39	3	4.8	153	3.2	1.53	[0.30 - 4.78]	0.453
40-49	1	1.6	312	6.6	0.23	[0.01 - 1.36]	0.186
50-59	5	8.1	455	9.6	0.83	[0.26 - 2.07]	0.830
60-69	16	25.8	593	12.5	2.44	[1.28 - 4.43]	0.006
70-79	10	16.1	622	13.1	1.28	[0.58 - 2.56]	0.450
80-89	7	11.3	404	8.5	1.37	[0.52 - 3.05]	0.366
90-99	0	0.0	71	1.5	0.00	[0.00 - 4.15]	>0.999
100-109	0	0.0	1	0.0	0.00	[0.00 - 2,860]	>0.999
Age Missing	20	32.3	1,780	37.4	0.80	[0.44 - 1.39]	0.431
Gender							
Male	36	58.1	1,890	39.7	2.10	[1.23 - 3.64]	0.004
Female	18	29.0	2,068	43.5	0.53	[0.29 - 0.94]	0.028
Gender Missing	8	12.9	801	16.8	0.73	[0.30 - 1.56]	0.496
Reporting Year							
2002	0	0.0	53	1.1	0.00	[0.00 - 5.63]	>0.999
2003	0	0.0	78	1.6	0.00	[0.00 - 3.77]	0.627
2004	0	0.0	86	1.8	0.00	[0.00 - 3.40]	0.629
2005	0	0.0	71	1.5	0.00	[0.00 - 4.15]	>0.999
2006	0	0.0	78	1.6	0.00	[0.00 - 3.77]	0.627
2007	0	0.0	123	2.6	0.00	[0.00 - 2.35]	0.409
2008	0	0.0	152	3.2	0.00	[0.00 - 1.88]	0.266
2009	3	4.8	571	12.0	0.37	[0.07 - 1.15]	0.111
2010	34	54.8	2,205	46.3	1.41	[0.82 - 2.42]	0.201
2011	25	40.3	1,342	28.2	1.72	[0.99 - 2.95]	0.046
Reportin Country							
USA	57	91.9	3,595	75.5	3.69	[1.49 - 11.82]	0.002
DEU	4	6.5	97	2.0	3.31	[0.86 - 9.22]	0.040
Other countries	1	1.6	810	17.0	0.08	[0.00 - 0.46]	<0.001
Country Missing	0	0.0	257	5.4	0.00	[0.00 - 1.08]	0.078

¶ p values are calculated using Fisher's Exact Test except one for Age that is calculated using two-tailed t-test. Due to various limitations of spontaneously-reported adverse event database, p value is not intended to test but to hilight dfferences in characteristics between AML and comparison groups. † Abbreviations, AML: acute myeloid leukemia, AE: adverse event, USA: United States of America, DEU: Germany, MPL, Myeloproliferative Leukemia virus proto-oncogene product

62 patients with AML. The control/comparison group consisted of the remaining ITP subjects. We compared the identified AML patients (cases) versus the subjects (non-cases) in the comparison group.

Analysis

The indicated numbers are the number of cases or non-cases of the indexed conditions. The number of identified patients and comparison subjects was tabulated according to the reporting year, gender, age and drug. The reporting year was extracted from the reporting date, that is, the date received by the FDA. The demographic data are presented with the number of cases, unadjusted odds ratios (uaOR) with 95% confidence intervals (CIs) and p values calculated with two-sided Fisher's exact test in Table 1. Due to the nature of spontaneous reports, the calculated p values are shown to highlight the differences in characteristics between the AML group and the comparison group; however, they are not intended to test statistical differences. The proportion (%) and 95% CI of cases of AML among the individual drugs used for ITP are tabulated in Table 2. Table 2A shows the number of cases associated with each drug regardless of the concomitant use of other drugs. Table 2B shows the number of cases associated with a single agent or a combination of drugs. Variables identified to be associated with AML in the univariate analyses were further analyzed with an unconditional logistic regression model using the R-statistical software package. There was an increase in the number of AML cases in the years 2010-2011 following the approval of MPL agonists as ethical pharmaceuticals in the USA. We did not place the reporting year in the proposed model to prevent multiple colinearity with the MPL agonists. In order to calculate the adjusted odds ratio, there must be a certain number of cases and controls. Since the number of cases was relatively small, we selected three drugs for the multiple logistic regression analysis based on the number of cases and individuals exposed to romiplostim, eltrombopag and prednisolone. Age was entered into the logistic regression model as a continuous variable with one increment of 10 years. Other variables were treated as logical variables, including gender (male=True, female=False) and drugs (exposure=True, no exposure=False). All combinations of variables were calculated for the Akaike's Information Criterion (AIC) value of the logistic regression models and then sorted according to the AIC value. We selected the model with the lowest AIC value among the models harbor-

Table 2A. Reporting of AML in Each Drug for ITP (n=4,821)

Drug ‡	Number of Patient		Proportion of AML (%)	
	AML	total	[95% confidence interval] ¶	
romiplostim	54	3,102	1.74	[1.31 - 2.27]
immunoglobulin	3	861	0.35	[0.07 - 1.01]
eltrombopag	9	594	1.52	[0.70 - 2.86]
prednisolone	5	343	1.46	[0.47 - 3.37]
rituximab	3	318	0.94	[0.19 - 2.73]
prednisone	0	74	0.00	[0.00 - 4.86]
danazol	2	54	3.70	[0.45 - 12.8]
unknown†	0	30	0.00	[0.00 - 11.6]
azathioprine	2	28	7.14	[0.88 - 23.5]
cyclophosphamide	0	20	0.00	[0.00 - 16.8]
methylprednisolone	0	19	0.00	[0.00 - 17.7]
ciclosporin	0	18	0.00	[0.00 - 18.5]
vincristine	0	15	0.00	[0.00 - 21.8]
platelets	0	10	0.00	[0.00 - 30.9]

Table 2B. Reporting of AML in Monotherapy or in Combination Therapy for ITP (n=4,821)

Drug ‡	Number of Patient		Proportion of AML (%)	
	AML	total	[95% confidence interval]¶	
Monotherapy (n=4,337)	55	4,337	1.27	[0.96 - 1.65]
romiplostim	52	2,893	1.80	[1.35 - 2.35]
immunoglobulin	0	699	0.00	[0.00 - 0.53]
eltrombopag	3	362	0.83	[0.17 - 2.40]
rituximab	0	189	0.00	[0.00 - 1.93]
prednisolone	0	52	0.00	[0.00 - 6.85]
prednisone	0	37	0.00	[0.00 - 9.49]
danazol	0	15	0.00	[0.00 - 21.8]
More than one agent (n=484)	7	484	1.45	[0.58 - 2.96]
Romiplostim and (n=209)	2	209	0.96	[0.12 - 3.41]
immunoglobulin	1	62	1.61	[0.04 - 8.66]
eltrombopag	1	27	3.70	[0.09 - 19.0]
prednisolone	1	116	0.86	[0.02 - 4.71]
rituximab	1	56	1.79	[0.05 - 9.55]
prednisone	0	11	0.00	[0.00 - 28.5]
danazol	0	13	0.00	[0.00 - 24.7]
Immunoglobuline and (n=162)	3	162	1.85	[0.38 - 5.32]
romiplostim	1	62	1.61	[0.04 - 8.66]
eltrombopag	3	80	3.75	[0.78 - 10.6]
prednisolone	2	86	2.33	[0.28 - 8.15]
rituximab	2	50	4.00	[0.49 - 13.7]
Eltrombopag and (n=232)	6	232	2.59	[0.95 - 5.54]
romiplostim	1	27	3.70	[0.09 - 19.0]
immunoglobulin	3	80	3.75	[0.78 - 10.6]
prednisolone	4	142	2.82	[0.77 - 7.06]
rituximab	3	45	6.67	[1.40 - 18.3]
prednisone	0	12	0.00	[0.00 - 26.5]
danazol	2	17	11.76	[1.46 - 36.4]
Rituximab and (n=129)	3	129	2.33	[0.48 - 6.65]
romiplostim	1	56	1.79	[0.05 - 9.55]
immunoglobulin	2	50	4.00	[0.49 - 13.7]
eltrombopag	3	45	6.67	[1.40 - 18.3]
prednisolone	2	61	3.28	[0.40 - 11.4]
danazol	1	11	9.09	[0.23 - 41.3]
azathioprine	0	11	0.00	[0.00 - 28.5]

‡ Therapy or Drugs with at least 10 reports are shown ¶ Confidence intervals are obtained by a procedure first given in Clopper and Pearson (24). † unknown may possibly contain more than one agent

ing both MPL agonists as variables (Table 3), because the objective of this analysis was to study the role of MPL agonists. We tested for confounding among factors, including age, gender and drugs. No confounding was detected, except between romiplostim and eltrombopag. We compared the residual error between the models in order to test for interactions one by one, and no interactions were detected. In one combination, we were unable to estimate the interaction because there were zero cells between romiplostim and eltrombopag. As a reference, we created one dummy case with AML without treatment with any of the MPL agonists in addition to the original dataset. The dataset with the dummy case did not indicate any interactions between romiplostim and eltrombopag. We believe that five variables compared to 62 events is a small enough number to avoid overfitting.

Missing data

The number of missing data is shown in Table 1. Records

Table 3. Logistic Regression Model for AML†

Variable	Coefficient beta	Standard Error	Wald kai square	Adjusted Odds Ratio	[95% Confidence Interval]	p value
(Intercept)	-7.44	0.84	-8.88	0.00	[0.00 - 0.00]	<0.001
Age --10 year increment--	0.12	0.09	1.26	1.12	[0.94 - 1.34]	0.209
Gender						
Female (reference)				1.00		
Male	0.74	0.33	2.21	2.09	[1.09 - 4.01]	0.026
Drugs						
romiplostim	2.36	0.60	3.93	10.5	[3.25 - 34.2]	<0.001
eltrombopag	1.77	0.58	3.07	5.90	[1.90 - 18.3]	0.002
prednisolone	0.17	0.66	0.26	1.19	[0.33 - 4.29]	0.793

† Record with missig data in gender or age are removed from the modeling resulting in 2,994 degree of freedom.

Table 4. Reporting of AML in Various Drugs in FAERS (2002-2011)

Drug	Number of Reports		Proportion of AML	
	AML	total	[95% confidence interval]¶	
Ranimustine	20	122	16.4%	[10.3 - 24.2]
Aclarubicin	7	50	14.0 %	[5.82 - 26.7]
Mitoxantrone	183	2,761	6.63 %	[5.73 - 7.62]
Idarubicin	15	341	4.40 %	[2.48 - 7.15]
Busulfan	96	2,514	3.82 %	[3.10 - 4.64]
Cytarabine	314	8,718	3.60 %	[3.22 - 4.01]
Fludarabine	238	6,803	3.50 %	[3.07 - 3.96]
Etoposide	297	10,203	2.91 %	[2.59 - 3.26]
Lenograstim	18	880	2.05 %	[1.22 - 3.21]
Cyclophosphamide	524	26,582	1.97 %	[1.81 - 2.15]
Melphalan	93	4,952	1.88 %	[1.52 - 2.30]
Actinomycin D	13	698	1.86 %	[1.00 - 3.16]
Epirubicin	68	3,806	1.79 %	[1.39 - 2.26]
Romiplostim	64	3,755	1.70 %	[1.32 - 2.17]
Eltrombopag	18	1,061	1.70 %	[1.01 - 2.67]
Doxorubicin	288	17,321	1.66 %	[1.48 - 1.86]
Nimustine	1	66	1.52 %	[0.04 - 8.16]
Cladribine	7	479	1.46 %	[0.59 - 2.99]
Filgrastim	167	12,361	1.35 %	[1.15 - 1.57]
Mitomycin C	8	980	0.82 %	[0.35 - 1.60]
Nelarabine	1	125	0.80 %	[0.02 - 4.38]
Methotrexate	306	65,441	0.47 %	[0.42 - 0.52]
Imatinib	44	10,044	0.44 %	[0.32 - 0.59]
Azathioprin	51	12,623	0.40 %	[0.30 - 0.53]
Temozolomide	17	4,602	0.37 %	[0.22 - 0.59]
Nilotinib	5	1,523	0.33 %	[0.11 - 0.76]
Tretinoin	46	16,568	0.28 %	[0.20 - 0.37]
Dasatinib	5	1,818	0.28 %	[0.09 - 0.64]
Interferon alpha	26	19,610	0.13 %	[0.00 - 0.09]
Phenytoin	19	15,518	0.12 %	[0.07 - 0.19]
Lapatinib	1	5,158	0.02 %	[0.00 - 0.11]
Ethotoin	0	4	0.00 %	[0.00 - 60.2]
Fosphenytoin	0	403	0.00 %	[0.00 - 0.91]
Golimumab	0	1,110	0.00 %	[0.00 - 0.33]

¶ Confidence intervals are obtained by a procedure first given in Clopper and Pearson (24).

with missing data were removed from the multiple logistic regression model. We constructed the model using the resulting 3,000 records.

Next, we tabulated the proportion of cases of AML among the patients treated with other drugs that are known to cause secondary AML. For this purpose, we created another dataset in which there were no restrictions regarding the indications for the use of the drugs. Drugs "known to cause secondary AML" were selected if their labels contained a warning of AML or its adverse reactions by searching the label database maintained by the Japanese health authority, the Pharmaceuticals and Medical Devices Agency (PMDA) (http://www.pmda.go.jp/). We tabulated the data to demonstrate the number and proportion of AML cases for each drug, as shown in Table 4.

Results

A total of 4,821 subjects were identified as having ITP in the FAERS. We found 62 AML patients (1.29% [95%CI; 0.99-1.65]) among the ITP subjects. The remaining 4,759 subjects served as the comparison group. The ages (mean

(SD)) of the AML and comparison groups were 66.4 (13.2) and 58.9 (21.5) years, respectively. All AML patients were treated with at least one MPL agonist. Among the comparison group subjects, the ages of the patients treated with and without MPL agonists were 62.3 (18.8) and 50.0 (25.1) years, respectively. There was a single peak in the sixties to seventies among the MPL agonist users, whereas there were two peaks around the teens and sixties to seventies in the comparison group. The younger peak in the comparison group most likely corresponds to patients with acute ITP. As shown in Table 1, factors found to be associated with AML in the univariate analysis were an age between 60 and 69 (16 AML patients among 609 ITP subjects, 2.63% [95%CI; 1.51-4.23], uaOR 2.44 [95%CI; 1.28-4.43]), male gender (36 AML patients among 1,926 ITP subjects, 1.87% [95% CI; 1.31-2.58], uaOR 2.10 [95%CI; 1.23-3.64]), a reporting year of 2010 (34 AML patients among 2,239 ITP subjects, 1.52% [95%CI; 1.05-2.12], uaOR 1.41 [95%CI; 0.82-2.42]) or 2011 (25 AML patients among 1,367 ITP subjects, 1.83% [95%CI; 1.19-2.79], uaOR 1.72 [95%CI; 0.99-2.95]) and reporting from the USA (57 AML patients among 3,652 ITP subjects, 1.56% [95%CI; 1.18-2.02], uaOR 3.69 [95%CI; 1.49-11.82]). As shown in Tables 2A, 3, the use of romiplostim (54 AML patients among 3,102 ITP subjects, 1.74% [95%CI; 1.31-2.27], aOR 3.70 [95%CI; 1.18-11.6]), male gender (36 AML patients among 1,926 ITP subjects, 1.87% [95%CI; 1.31-2.58], aOR 2.01 [95%CI; 1.03-3.91]) and an age between 60 and 69 (16 AML patients among 609 ITP subjects, 2.63% [95%CI; 1.51-4.23], aOR 2.22 [95%CI; 1.16-4.24]) were associated with AML. The adjusted odds ratio (aOR) for the use of eltrombopag in the logistic regression analysis was 1.92 [95%CI; 0.65-5.70] (9 AML patients among 603 ITP subjects, 1.52% [95%CI; 0.70-2.86]). Since the number of individuals treated with eltrombopag was relatively small (n=594) compared to that treated with romiplostim (n=3,102), the calculated proportion was associated with a wider confidence interval. We also attempted to further investigate the effects of multiple medications. However, 4,337 (90.0%) of the 4,821 ITP subjects were treated with a single agent. Therefore, the combination use of drugs for ITP was limited, as shown in Table 2B.

We compared the proportion of cases of AML among the patients taking drugs that are "known to cause secondary AML" and MPL agonists. After searching the label database maintained by the PMDA, we found the following drugs: ranimustine, aclarubicin, mitoxantrone, idarubicin, busulfan, cytarabine, fludarabine, etoposide, lenograstim, cyclophosphamide, melphalan, actinomycin D, epirubicin, doxorubicin, nimustine, cladribine, filgrastim, mitomycin C, nelarabine, methotrexate, imatinib, azathioprine, temozolomide, nilotinib, tretinoin, dasatinib, interferon alpha, phenytoin, lapatinib, ethotoin, fosphenytoin, golimumab, live varicella-zoster vaccine and mesna. We excluded the live varicella-zoster vaccine from the tabulation because adverse reactions to vaccines are registered in another database called "VAERS," which is maintained by the US FDA and Centers for Disease Control and Prevention (CDC). In addition, we excluded mesna because this drug is used in combination with high-dose cyclophosphamide, which is known to cause secondary AML. As shown in Table 4, among the 32 drugs that are known to cause secondary AML and the two MPL agonists, 25 (73.5%) drugs were antineoplastic drugs. Four drugs (12.5%) were cytokines, including two granulocyte colony-stimulating factors and the two MPL agonists. Three drugs (8.8%) were antiepileptic agents. The proportions of AML patients taking antiepileptic drugs and interferon were relatively small, although these drugs carry warnings in their prescribing information. On the other hand, the proportion of AML patients taking cytokines, including MPL agonists, was comparable to that of antineoplastic drugs.

Discussion

As shown in the results, some factors, including the use of romiplostim, were found to be associated with the development of AML in the ITP subjects. The number of adverse events, including AML and other adverse events (AEs), increased in 2010 and 2011 (Table 1), a few years after the approval of MPL agonists in 2008, which may be explained by the market penetration of MPL agonists after that event. This increase possibly suggests the duration of time to onset, although the dose in each subject and the duration between the use of the suspected drug and the onset of AML were not examined in detail in our study. Similarly, reporting from the USA was suggested to be a possible risk factor in a univariate analysis but not in a multivariate logistic regression analysis. Therefore, the high proportion of cases in the USA may reflect market penetration as well. In this analysis, an age between 60 and 69 years was identified as a possible risk factor in both a univariate and multivariate analysis. This finding may be affected by the peak ages of adult ITP (40-45 years) (16) and AML (70 years and older) (17). Since the total number of AML cases was small, the number of cases in each group when analyzed according to age was very small. Therefore, the data of the high-risk age group should be interpreted with caution. Additionally, the higher incidence of reporting of AML in men is compatible with the gender distribution of the disease. The high incidence of AML in the elderly as well as men is also compatible with the distribution of de novo AML. These findings can be explained from the viewpoint of multistep pathogenesis based on the accumulation of various genetic alterations. Preleukemic individuals and those who have not yet developed AML but have accumulated genetic alterations exhibit a similar demographic distribution. The use of MPL agonists may possibly open the final gate, stimulating the development of AML in preleukemic individuals. Therefore, it is likely that MPL agonists play a role in these events. Since most cases of AML involve adults, the underlying ITP observed in this analysis was most likely chronic. This finding is compatible with the approved indications for the use of these drugs.

Both MPL agonists were associated with a similarly high proportion of cases of AML (1.74% [95%CI; 1.31-2.27%] and 1.52% [95%CI; 0.70-2.86%] for romiplostim and eltrombopag, respectively) (Table 2A). On the other hand, the logistic regression analysis demonstrated a relatively higher adjusted odds ratio for romiplostim (10.5 [95%CI; 3.25-34.2]) than for eltrombopag (2.09 [95%CI; 1.90-18.3]) (Table 3). This difference between the two MPL agonists must have been due to a kind of artifact. The odds ratio reflects the relative number of each type of AE associated with a drug of interest versus the other drugs. Since the number of cases of AML and other types of AEs associated with romiplostim accounted for more than half of the database, the large number of AML cases associated with romiplostim decreased the odds ratio for eltrombopag. In other words, most cases of AML associated with romiplostim served as "AML cases associated with other drugs" in the calculation of the odds ratio for eltrombopag. Therefore, we think that both MPL agonists are similarly associated with the development of AML. Indeed, all AML patients were exposed to one or more MPL agonists.

From the time human MPL, which is known to be a proto-oncogene of v-mpl, a truncated form of a cytokine receptor (1), was first cloned, its potential oncogenicity has been a concern with respect to its therapeutic use. Initially, v-mpl was originally recognized to be a viral oncogene that transforms myeloproliferative leukemia virus-infected hematopoietic progenitors (2). Since v-mpl alone is sufficient to promote leukemic transformation, activation of the MPL signal pathway is thought to play a role in leukemogenesis. Indeed, among 538 healthy volunteers evaluated in an early clinical study who received pegylated recombinant human thrombopoietin, an MPL agonist, three subjects developed B-cell malignancies. Although the incidence of malignancy was high, a causal relationship between the development of B-cell malignancy and the use of the study drug could not be established (18). In addition to the clinical experience of myelodysplastic syndrome (6) and aplastic anemia (5, 8) described in the background of this paper, data supporting concerns regarding potential myeloid malignancy associated with MPL agonists have been accumulated with respect to MPL agonists and a natural ligand, thrombopoietin. In a report on mice induced to express murine thrombopoietin using a retrovirus vector, leukemic transformation of bone marrow cells was noted in two individual mice. It is not clear whether this transformation was caused by thrombopoietin exposure at a high concentration or mutagenesis induced by the retrovirus vector.

As shown in Table 4, the proportion of cases of AML associated with MPL agonists was comparable to that of antineoplastic drugs. Since no drugs other than MPL agonists are usually used in the treatment of ITP, the underlying disease is quite different from that of patients treated with MPL agonists. Therefore, further interpretation of the data is difficult. However, it is noteworthy that cases of MPL agonists are required to be registered until December 2011. This processes is assumed to enhance reporting and increase the total number of reported adverse events. In other words, this condition may increase the denominators for MPL agonists and decrease the proportion of adverse events. Therefore, the calculated proportion of cases of AML associated with MPL agonists may be underestimated compared to that of other drugs. It is also noteworthy that the proportion of AML cases associated with drugs "known to cause AML". This means that a search of the FAERS would provide relevant information to some extent.

Additional findings were obtained from an analysis of hereditary thrombocytosis. As shown in Table 5, two cases of myeloid leukemia (3.2%, [95%CI; 0.4-11.2]) including one case of AML (1.6%, [95%CI; 0.04-8.66]) were noted among 62 individuals with hereditary thrombocytosis whose pathogenesis was determined to involve the overexpression of thrombopoietin due to a gene mutation. Therefore, it is reasonable to be concerned that continuous exposure to thrombopoietin at a high concentration will promote leukemic transformation. Other studies have accumulated pedigrees of essential thrombocythemia patients with mutant c-MPL genes (Table 6). While the mechanisms by which the c-MPL mutations caused thrombocytosis in these cases are not entirely clear, the mutations were reported to be activated without the ligand. No cases of myeloid leukemia or AML (0.0%, [95%CI: 0.0-3.7]) were reported among 97 affected individuals listed in these pedigrees. Since there is a wide interval estimate due to the limited number of patients, this tabulation does not negatively influence the postulated concern.

By searching the FAERS database, we found a signal of the development of AML in ITP subjects treated with MPL agonists. One of the essential limitations of spontaneous reports is that the diagnosis of reported adverse events is not based on a predefined diagnostic criterion. However, the diagnosis of AML may be assumed to be generally reliable because ITP patients are diagnosed and treated by specialists in hematology who are assumed to be specialists in AML compared to general practitioners. Finally, our analysis is affected by various limitations of spontaneous reports conducted in postmarketing settings, such as underreporting, the effects of publicity and media attention and the temporal relationship between drug launch and the rate of reporting (19-22). Moreover, detailed information, such as the type of AML based on the FAB classification, is not included in the FAERS. Information such as the dose used in each subject or the duration between the use of the suspected drug and the onset of AML was also not examined in detail. Case-controlled studies conducted using spontaneous reporting databases are sometimes called 'disproportionality analyses.' When a spontaneous reporting database is viewed as the source of case and control data for a case-controlled study, then the reporting odds ratio (ROR) is an estimate of the relative risk (23). This approach provides hypotheses or signals of risks for drugs. Due to the above mentioned limitations, the results may not be interpreted as results obtained

Table 5. Summary of THPO Gene Mutation Associated with THPO Over Experssion and Thrombocytosis

Reference	Year	Mutation	Number of Family	Number of Affected Individual	Malignancy in Affected Individual
25	1998	deletion of G in 5'-UTR	1	5	
26	1998	G>C in intron3 position +1	1	11	
27	1999	deletion of G in 5'-UTR	1	1	
28	1999	G>T in 5'-UTR	1	4	
29	2004	G>A in intron3 position +1	1	4	1 Ph1(+) chronic myeloid leukemia
30	2008	G>C in intron3 position +1	1	11	1 melanoma
31	2009	G>T in 5'-UTR	1	4	
32	2010	G>C in intron3 position +1	1	12	1 acute myeloid leukemia
33	2011	T>C in intron2 position +2	1	3	
34	2012	G>C in intron3 position +1	2	7	1 multiple myeloma

THPO: thrombopoietin, UTR: untranslated region, Ph1: Philadelphia chromosome

Table 6. Summary of Reported MPL Gene Mutation Associated with Hereditary Thrombocythemia

Reference	Year	Aminoacid substitution	Number of Family	Number of Affected Individulal	Malignancy in Affected Individual ¶
35	2004	S505N	1	8	
36	2006	W515L	§	4	
37	2007	S505N	4	11	
38	2007	S505N	§	10	
39	2008	P106L	4	4	
40	2009	S505N	9	39	
41	2010	S505N	7	21	

¶ No malignancy has been reported in affected individuals, § Not clearly described in the literature

from controlled epidemiologic studies.

Conclusion

We found an association between the development of AML and the use of MPL agonists in ITP patients. As mentioned above, due to the limitations of spontaneous reporting, the data presented in this paper are not conclusive, but rather simply suggest a signal of a risk of AML. Further studies are warranted to determine whether the detected signal is a real risk. As a result, prescribing physicians should not alter their prescribing behavior based only on this preliminary analysis alone.

Author's disclosure of potential Conflicts of Interest (COI).
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References

1. Vigon I, Mornon JP, Cocault L, et al. Molecular cloning and characterization of MPL, the human homolog of the v-mpl oncogene: identification of a member of the hematopoietic growth factor receptor superfamily. Proc Natl Acad Sci USA **89**: 5640-5644, 1992.
2. Souyri M, Vigon I, Penciolelli JF, Heard JM, Tambourin P, Wendling F. A putative truncated cytokine receptor gene transduced by the myeloproliferative leukemia virus immortalizes hematopoietic progenitors. Cell **63**: 1137-1147, 1990.
3. Wendling F, Vigon I, Souyri M, Tambourin P. Myeloid progenitor cells transformed by the myeloproliferative leukemia virus proliferate and differentiate in vitro without the addition of growth factors. Leukemia **3**: 475-480, 1989.
4. Wendling F, Penciolelli JF, Charon M, Tambourin P. Factor-independent erythropoietic progenitor cells in leukemia induced by the myeloproliferative leukemia virus. Blood **73**: 1161-1167, 1989.
5. Liebman HA, Pullarkat V. Diagnosis and management of immune thrombocytopenia in the era of thrombopoietin mimetics. Hematology Am Soc Hematol Educ Program **2011**: 384-390, 2011.
6. Kantarjian H, Fenaux P, Sekeres MA, et al. Safety and efficacy of romiplostim in patients with lower-risk myelodysplastic syndrome and thrombocytopenia. J Clin Oncol **28**: 437-444, 2010.
7. Olnes MJ, Scheinberg P, Calvo KR, et al. Eltrombopag and improved hematopoiesis in refractory aplastic anemia. N Engl J Med **367**: 11-19, 2012.
8. Oshima Y, Yuji K, Tojo A. Eltrombopag in refractory aplastic anemia. N Engl J Med **367**: 1162-1163, 2012.
9. Hauben M, Reich L, DeMicco J, Kim K. 'Extreme duplication' in the US FDA Adverse Events Reporting System database. Drug Saf **30**: 551-554, 2007.
10. Poluzzi E, Raschi E, Motola D, Moretti U, De Ponti F. Antimicrobials and the risk of torsades de pointes: the contribution from data mining of the US FDA Adverse Event Reporting System. Drug Saf **33**: 303-314, 2010.
11. Oshima Y, Tsukamoto H, Tojo A. Association of hepatitis B with antirheumatic drugs: a case-control study. Mod Rheumatol **23**: 694-704, 2013.
12. Oshima Y. Characteristics of drug-associated rhabdomyolysis: analysis of 8,610 cases reported to the U.S. Food and Drug Administration. Intern Med **50**: 845-853, 2011.

13. Oshima Y, Tojo A. Attractive tools for systematic case accumulation. Int J Hematol **94**: 413-414, 2011.
14. Oshima Y, Tojo A. Pulmonary alveolar hemorrhage possibly associated with lenalidomide use. Int J Hematol **94**: 296-297, 2011.
15. Oshima Y. Characteristics of 1,544 Drug-induced Hypersensitivity Syndrome Cases Reported to Food and Drug Administration. DMC DERMATOLOGY 2: WMC001980, 2011.
16. Levine SP. IDIOPATHIC AUTOIMMUNE THROMBOCYTOPENIC PURPURA. In: Wintrobe's Clinical Hematology. 53rd ed. Greer JP, Foerster J, Lukens JN, Rodgers GM, Paraskevas F, Glader B, Eds. Lippincott Williams & Wilkins, Philadelphia, 2004: 1534-1544.
17. American Cancer Society. Cancer facts & figures 2008, 2009.
18. Bennett CL, Evens AM, Andritsos LA, et al. Haematological malignancies developing in previously healthy individuals who received haematopoietic growth factors: report from the Research on Adverse Drug Events and Reports (RADAR) project. Br J Haematol **135**: 642-650, 2006.
19. Brewer T, Colditz GA. Postmarketing surveillance and adverse drug reactions: current perspectives and future needs. JAMA **281**: 824-829, 1999.
20. Miremont G, Haramburu F, Begaud B, Pere JC, Dangoumau J. Adverse drug reactions: physicians' opinions versus a causality assessment method. Eur J Clin Pharmacol **46**: 285-289, 1994.
21. Wallenstein EJ, Fife D. Temporal patterns of NSAID spontaneous adverse event reports: the Weber effect revisited. Drug Saf **24**: 233-237, 2001.
22. Stang PE, Fox JL. Adverse drug events and the Freedom of Information Act: an apple in Eden. Ann Pharmacother **26**: 238-243, 1992.
23. Rothman KJ, Lanes S, Sacks ST. The reporting odds ratio and its advantages over the proportional reporting ratio. Pharmacoepidemiol Drug Saf **13**: 519-523, 2004.
24. Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of the binomial. Biometrika **26**: 404-413, 1934.
25. Kondo T, Okabe M, Sanada M, et al. Familial essential thrombocythemia associated with one-base deletion in the 5'-untranslated region of the thrombopoietin gene. Blood **92**: 1091-1096, 1998.
26. Wiestner A, Schlemper RJ, van der, Maas AP, Skoda RC. An activating splice donor mutation in the thrombopoietin gene causes hereditary thrombocythaemia. Nat Genet **18**: 49-52, 1998.
27. Ghilardi N, Skoda RC. A single-base deletion in the thrombopoietin (TPO) gene causes familial essential thrombocythemia through a mechanism of more efficient translation of TPO mRNA. Blood **94**: 1480-1482, 1999.
28. Ghilardi N, Wiestner A, Kikuchi M, Ohsaka A, Skoda RC. Hereditary thrombocythaemia in a Japanese family is caused by a novel point mutation in the thrombopoietin gene. Br J Haematol **107**: 310-316, 1999.
29. Oshima Y, Komatsu N, Ozawa K, Fujimura A. CML Developed in a Japanese Family Transmitting a Novel Point Mutation in the Thrombopoietin Gene (TPO). ASH Annual Meeting Abstracts **104**: 4220, 2004.
30. Liu K, Kralovics R, Rudzki Z, et al. A de novo splice donor mutation in the thrombopoietin gene causes hereditary thrombocythemia in a Polish family. Haematologica **93**: 706-714, 2008.
31. Graziano C, Carone S, Panza E, et al. Association of hereditary thrombocythemia and distal limb defects with a thrombopoietin gene mutation. Blood **114**: 1655-1657, 2009.
32. Posthuma HL, Skoda RC, Jacob FA, van der Maas AP, Valk PJ, Posthuma EF. Hereditary thrombocytosis not as innocent as thought? Development into acute leukemia and myelofibrosis. Blood **116**: 3375-3376, 2010.
33. Zhang B, Ng D, Jones C, et al. A novel splice donor mutation in the thrombopoietin gene leads to exon 2 skipping in a Filipino family with hereditary thrombocythemia. Blood **118**: 6988-6990, 2011.
34. Stockklausner C, Echner N, Klotter AC, Hegenbart U, Dreger P, Kulozik AE. Hereditary thrombocythemia caused by a thrombopoietin (THPO) gain-of-function mutation associated with multiple myeloma and congenital limb defects. Ann Hematol **91**: 1129-1133, 2012.
35. Ding J, Komatsu H, Wakita A, et al. Familial essential thrombocythemia associated with a dominant-positive activating mutation of the c-MPL gene, which encodes for the receptor for thrombopoietin. Blood **103**: 4198-4200, 2004.
36. Pardanani AD, Levine RL, Lasho T, et al. MPL515 mutations in myeloproliferative and other myeloid disorders: a study of 1182 patients. Blood **108**: 3472-3476, 2006.
37. Teofili L, Giona F, Martini M, et al. Markers of myeloproliferative diseases in childhood polycythemia vera and essential thrombocythemia. J Clin Oncol **25**: 1048-1053, 2007.
38. Teofili L, Giona F, Martini M, et al. The revised WHO diagnostic criteria for Ph-negative myeloproliferative diseases are not appropriate for the diagnostic screening of childhood polycythemia vera and essential thrombocythemia. Blood **110**: 3384-3386, 2007.
39. El-Harith el HA, Roesl C, Ballmaier·M, et al. Familial thrombocytosis caused by the novel germ-line mutation p.Pro106Leu in the MPL gene. Br J Haematol **144**: 185-194, 2009.
40. Liu K, Martini M, Rocca B, et al. Evidence for a founder effect of the MPL-S505N mutation in eight Italian pedigrees with hereditary thrombocythemia. Haematologica **94**: 1368-1374, 2009.
41. Teofili L, Giona F, Torti L, et al. Hereditary thrombocytosis caused by MPLSer505Asn is associated with a high thrombotic risk, splenomegaly and progression to bone marrow fibrosis. Haematologica **95**: 65-70, 2010.

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ORIGINAL ARTICLE

Association of hepatitis B with antirheumatic drugs: a case–control study

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Abstract
Background Though concern of hepatitis B virus (HBV) reactivation by antirheumatic agents has limited therapeutic opportunities in HBV-infected rheumatoid arthritis (RA) patients, the relative risks (RR) among such agents have not been clarified.
Objective We compared the reporting of antirheumatic-agent-associated hepatitis B.
Patients We assessed 92 hepatitis B cases and 98,069 controls from a population of 98,161 RA patients registered into the US Food and Drug Administration's (FDA's) adverse event database between 2004 and 2010.
Measurements A reporting odds ratio (ROR), a signal suggesting a risk for hepatitis B among antirheumatic agents, was measured.
Results Treatment with corticosteroids [ROR 2.3 (95 % confidence interval 1.3–4.0)], methotrexate [4.9 (3.9–6.0)], rituximab [7.2 (5.3–9.9)], tacrolimus [4.2 (1.5–11.9)], or reporting from Japan [2.2 (1.1–4.2)] were associated with higher signal, whereas adalimumab had a lower ROR [0.2 (0.1–0.4)].
Limitations There are known limitations of spontaneous reporting, such as underreporting, the Weber effect, reporting bias, indication bias, and limited clinical information such as HBV status.
Conclusions Adalimumab's low reporting rate is most likely be due to notoriety. However, the possibility that adalimumab might suppress reactivation of HBV cannot be denied. Until the possibility is clarified in well-designed clinical studies, physicians should use adalimumab cautiously in patients with HBV.

Keywords Hepatitis B · Rheumatoid arthritis · Antirheumatic drug · Adverse event reporting system (AERS) · Spontaneous report

Introduction

Progresses in pathophysiological knowledge, especially in cytokine cascades and their effector cells in rheumatoid arthritis (RA), have brought various developments of new antirheumatic agents. The classes of therapeutic agents directed against specific cytokines or effector cells in the disease process of RA, are: (1) disease-modifying antirheumatic drugs (DMARD), such as methotrexate (MTX), hydroxychloroquine (HCQ), leflunomide (LEF), and sulfasalazine (SSZ); (2) biological DMARDs, such as adalimumab (ADA), etanercept (ETA), infliximab (IFX), and rituximab (RTX); and (3) immunosuppressants, such as tacrolimus (TAC), azathioprine (AZT), cyclosporine (CSA), and mizoribine (MZB). These antirheumatic agents have greatly improved and expanded therapeutic options for RA. However, RA patients infected with hepatitis B virus (HBV) have been excluded from the benefit of therapeutic opportunities with these new agents. Reports about severe hepatitis case with increasing HBV-DNA after methotrexate (MTX) and corticosteroid therapy [1, 2]

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alerted the medical community about the use of antirheumatic agents in patients with HBV carrier status and proposed an algorithm for assessment and prevention of HBV reactivation in RA patients. The American College of Rheumatology (ACR) made recommendations on the use of DMARDs and biologics based on hepatitis type, Child–Pugh grade, and whether or not antiviral agents to treat hepatitis had been initiated. The college also asked physicians to consider the risks and benefits of all DMARDs [3]. The Japan College of Rheumatology (JCR) more strictly limited the use of tumor necrosis factor-alpha (TNF-α)-blocking biologics and MTX in HBV-carrying RA patients in 2008 (http://www.ryumachi-jp.com/english/index.html).

Serum hepatitis B surface antigen (HBsAg) is infrequent (0.1–0.5 %) in the normal population in the United States and western Europe. However, a prevalence of up to 5–20 % has been found in the Far East and in some tropical countries in patients with Hodgkin's disease, polyarteritis nodosa, and chronic renal disease [4]. Since HBV reactivation was reported not only in HBV carriers but also in RA patients with resolved or past HBV infection [5, 6], and the prevalence of concurrent and resolved HBV infection among RA patients in Japan was reported to be 0.8 % and 25.1 %, respectively [6], approximately one fourth of RA patients appear to be at risk for reactivation of HBV in Japan. As the reasons for restricting the use of certain DMARDs in HBV carriers by the colleges are mainly based on case series, case reports, and reviews of them, and the relative strength of risks still remain to be clarified. Recent reports suggest that screening for HBV infection and careful monitoring during the use of nonbiologic and biologic DMARDs may ameliorate the risk of severe hepatitis [5, 7, 8].

Guideline for the use of immunosuppressants and chemotherapy for malignant neoplasm in patients with HBV carriers is available in Japan [9]. The guidelines are not restricted to antirheumatic agents, but those who want to treat HBV-carrier RA patients may refer to the recommendations mentioned in the guidelines. The JCR also released recommendations regarding immunosuppressant use for RA patients with HBV infection in 2010 (http://www.ryumachi-jp.com/english/index.html). They describe HBV screening and the use of nucleoside analogs prior to immunosuppressive therapies. However, their recommendations lack a description regarding selection of antirheumatic agents. In Japan, one of the HBV-epidemic areas, no useful information appeared to be available for selecting antirheumatic agents for treating HBV-infected patients from the standpoint of relative risk (RR) for HBV reactivation.

There is no doubt that results from prospectively randomized clinical trial yield high-level evidence comparing risk of drugs. In order to assess risk level, prospective intervention studies using randomly assigned nonbiologic DMARDs or biologics to patients selected based on eligibility criteria and standardized assessment of occurrence of hepatitis are, of course, useful. However, ethical limitations and time/cost may make such a study unfeasible. Testing the risk of drugs with concern for severe adverse reactions may not be ethically acceptable. As clinical trials require exhaustive efforts and extensive costs and time, they may not provide timely information with a reasonable cost. Here, we propose the use of an adverse event reporting system (AERS) to rapidly estimate possible risks in these patients, as mentioned elsewhere [10, 11]. Despite limitations of the AERS, it may provide timely information with fewer costs [12, 13]. In this study, we compared reporting odds ratio (ROR) as a signal of risk for HBV reactivation associated with antirheumatic agents use. One can more effectively design clinical trials with less ethical concern to clarify crucial points based on the estimated results from AERS research.

Methods

Study design

A nested case–control analysis of antirheumatic-agent-associated HBV reported to the FDA between January 2004 and December 2010 was conducted. The subcohort study participants were individuals registered in the FDA AERS, with RA as an indication for drug use. Cases and controls were respectively defined as individuals with and without drug-associated HBV among the subcohort. The analyses included the number of unique cases and ROR among antirheumatic agents. In addition to monovariate analyses, a multivariate assessment by unconditional logistic regression was performed.

Datasource

The AERS database was downloaded from the FDA AERS Web page (http://www.fda.gov/), between first quarter 2004 and fourth quarter 2010.

Case identification

Drugs used to treat RA were identified as follows: First, the table for therapeutic indications was searched, which recorded an individual drug identifier (drug code) and corresponding indication for its use. As reported indications in the AERS database are coded according to the Medical Dictionary for Regulatory Activities (MedDRA) (Maintenance and Support Services Organization, Chantilly, VA, USA), we identified drugs with RA as their