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ORIGINAL REPORT

Pathologic and Molecular Features Correlate With Long-Term Outcome After Adjuvant Therapy of Resected Primary GI Stromal Tumor: The ACOSOG Z9001 Trial

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ABSTRACT

Purpose
The ACOSOG (American College of Surgeons Oncology Group) Z9001 (Alliance) study, a randomized, placebo-controlled trial, demonstrated that 1 year of adjuvant imatinib prolonged recurrence-free survival (RFS) after resection of primary GI stromal tumor (GIST). We sought to determine the pathologic and molecular factors associated with patient outcome.

Patients and Methods
There were 328 patients assigned to the placebo arm and 317 to the imatinib arm. Median patient follow-up was 74 months. There were 645 tumor specimens available for mitotic rate or mutation analysis.

Results
RFS remained superior in the imatinib arm (hazard ratio, 0.6; 95% CI, 0.43 to 0.75; Cox model–adjusted $P < .001$). On multivariable analysis of patients in the placebo arm, large tumor size, small bowel location, and high mitotic rate were associated with lower RFS, whereas tumor genotype was not significantly associated with RFS. Multivariable analysis of patients in the imatinib arm yielded similar findings. When comparing the two arms, imatinib therapy was associated with higher RFS in patients with a *KIT* exon 11 deletion of any type, but not a *KIT* exon 11 insertion or point mutation, *KIT* exon 9 mutation, *PDGFRA* mutation, or wild-type tumor, although some of these patient groups were small. Adjuvant imatinib did not seem to alter overall survival.

Conclusion
Our findings show that tumor size, location, and mitotic rate, but not tumor genotype, are associated with the natural history of GIST. Patients with *KIT* exon 11 deletions assigned to 1 year of adjuvant imatinib had a longer RFS.

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INTRODUCTION

GI stromal tumors (GISTs) are the most common mesenchymal tumors in the GI tract. Approximately 85% of these tumors are driven by an oncogenic mutation in either of two homologous kinase genes, *KIT* or *PDGFRA*. On the basis of clinical trials conducted during the past decade, the use of inhibitors targeted to these two receptor tyrosine kinases is now the established therapy for patients with unresectable or advanced disease.[1-5] Specifically, imatinib is used as the initial therapy. Sunitinib is indicated for patients who experience disease progression during imatinib therapy or cannot tolerate the drug. The use of these inhibitors has extended the overall survival (OS) of patients with advanced disease from an average of 18 months to almost 5 years,[6] and there are long-term survivors (more than 12 years of active treatment) with measurable residual disease.

The results of the Z9001 trial led to approval of the use of imatinib in the adjuvant setting by the US Food and Drug Administration and the European Medicines Agency. However, questions remained as to which patients with GISTs were most likely to benefit from adjuvant treatment, because surgery alone cures 45% to 60% of patients with primary GISTs.[7-9] On the basis of many retrospective series, the three pathologic parameters that best define the risk of disease recurrence are mitotic rate, tumor size, and tumor location.[7,10,11] Several risk assessment strategies based on these parameters have been

proposed,[12,13] including the scheme developed at the Armed Forces Institute of Pathology (AFIP), which has been endorsed by the American Joint Commission on Cancer and is widely used.[14,15] In addition to these three parameters, several studies have shown that recurrence is more likely when tumors harbor a *KIT* exon 11 deletion as compared with another type of exon 11 mutation (point mutation or insertion), harbor a mutation in *KIT* exon 9, or lack a *KIT* or *PDGFRA* mutation.[16,17] In particular, *KIT* exon 11 deletions involving codons 557 and/or 558 were associated with aggressive disease.[17-20]

In our initial report on the Z9001 trial, median follow-up was 19.7 months.[21] Here we present long-term (median, 74 months) data and include an analysis of mitotic rate and tumor mutation status. Central pathology review and screening for *KIT* and *PDGFRA* gene mutations were part of the study protocol. Of 713 enrolled patients, complete pathologic data were obtained for 90% of the tumors, and genotypes were determined for 71%. The placebo arm of the trial included more than 300 patients, providing a unique opportunity to examine the natural history of GIST in a prospective manner and to determine if pathologic and genotypic characteristics were associated with disease recurrence. The relationship between tumor genotype and benefit of adjuvant imatinib was also examined.

PATIENTS AND METHODS

Patients

Enrollment criteria and details on the study population for the Z9001 trial were previously reported.[20] Briefly, patients who underwent complete gross resection (R0 or R1) of a ≥ 3-cm primary GIST that was KIT positive by immunohistochemistry were eligible for the trial if they were registered within 70 days after surgery. Each participant signed an institutional review board–approved, protocol-specific informed consent for specimen collection in accordance with federal and institutional guidelines. Patients were assigned, in a double-blind manner, to receive either imatinib at 400 mg daily or placebo for a period of 1 year. Patients underwent follow-up with computed tomography or magnetic resonance imaging scans every 3 months for the first 2 years, every 6 months for the next 3 years, and then yearly until year 10. Patients in the placebo arm were able to receive imatinib on tumor recurrence, and patients in the imatinib arm were able to resume imatinib if they developed recurrence after completing the study drug.

Pathology

A retrospective central review of all primary resection specimens was included in the initial part of the trial.[21] Recorded pathologic parameters included tumor size (assessed at the originating institution either before or after fixation) and anatomic location. Subsequently, one hematoxylin and eosin–stained slide from each specimen was used to determine the mitotic rate. Mitotic counts were performed by one of two pathologists (C.L.C., V.K.) on a Leica DMLB microscope (Leica Microsystems, Wetzlar, Germany); 65% of the specimens were counted by C.L.C. Tumor sections were first surveyed to identify areas with the greatest mitotic activity, and mitoses were then counted using a 40× objective lens across 50 high-power fields (hpfs), which totaled 11.87 mm^2. Only unequivocal mitotic figures in areas of well-fixed tumor were counted; care was taken to avoid counting artifacts resembling mitotic figures, such as apoptotic nuclei or infiltrating lymphocytes. Forty-five randomly selected slides were separately counted by both pathologists, and the interobserver correlation was excellent (Pearson $r = 0.94$; no statistical difference by Wilcoxon signed rank test [$P = .49$]).

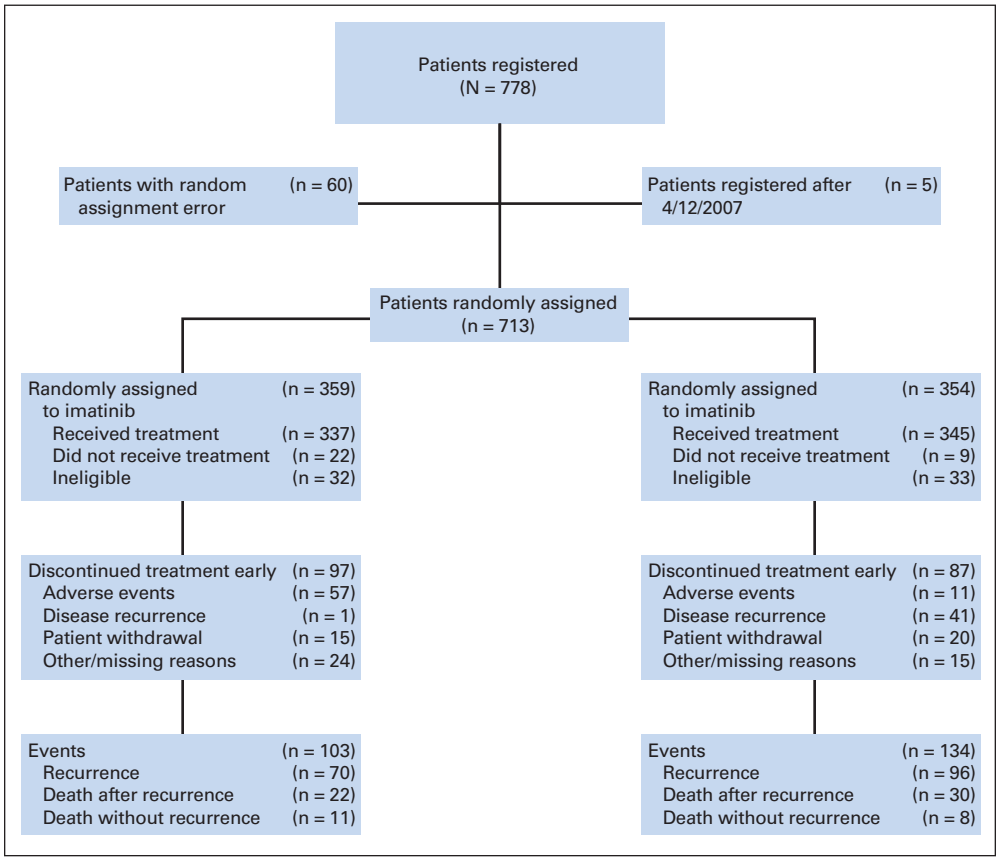

Fig 1. CONSORT diagram.

Genotyping

Genotyping was carried out in a Clinical Laboratory Improvement Amendments–licensed, College of American Pathologists–accredited laboratory. DNA was extracted from tumor-rich areas macrodissected from unstained sections of archival formalin-fixed, paraffin-embedded tissue. Selected exons of *KIT* (9,11,13,17) and *PDGFRA* (12,14,18) were amplified by polymerase chain reaction and screened for sequence alterations by high-resolution melting curve analysis on an LC480 Lightcycler (Roche Applied Science, Penzberg, Germany). Primer sequences and polymerase chain reaction conditions are detailed in the Data Supplement. All mutations were confirmed by bidirectional Sanger sequencing as previously described.[22]

Statistics

Statistical analyses were performed by the Alliance Statistics and Data Center. The database was locked on December 5, 2012. The primary end point for this analysis was recurrence-free survival (RFS), which was defined as the time from patient registration to the development of tumor recurrence or death resulting from any cause. If patients were recurrence free, they were censored at the time of last follow-up for disease recurrence. OS was defined as the time from study registration to death resulting from any cause. Patients who were alive at the time of last follow-up were censored. Patients who crossed over to the imatinib arm were not censored at the time of crossover for either RFS or OS.

Categorical variables between groups were compared with a χ^2 test, and continuous variables were compared with a two-sample t test. RFS and OS experiences were summarized with Kaplan-Meier curves and compared with log-rank tests. Univariable and multivariable Cox regression models were used to determine associations between variables of interest (tumor size, tumor location, mitotic rate, and tumor genotype) and outcome variables (RFS and OS). All of the Cox regression models included treatment as a time-dependent variable for patients who crossed over from placebo to imatinib. The strengths of the associations were summarized with a hazard ratio (HR) and corresponding 95% CI. The analyses were performed with SAS software (version 9.3; SAS Institute, Cary, NC), and all tests were two sided. P values less than .05 were considered statistically significant.

RESULTS

Adjuvant Imatinib Improves RFS But Not OS

Of the 713 patients in the intent-to-treat population (Fig 1), 68 did not have either mitotic rate or genotype available and were removed, leaving 645 patients (328 in the placebo arm, 317 in the imatinib arm) for additional analyses. Patient characteristics and pathologic features of the tumors were comparable between the two arms, including tumor size, location, and mitotic rate (Table 1). As illustrated in Figure 2A, RFS remained superior in the imatinib arm at longer follow-up (HR, 0.6; 95% CI, 0.43 to 0.75; Cox model adjusted $P < .001$). These data were not censored for crossover after the trial was unblinded. However, the findings were similar if censoring was included (data not shown). There was no significant difference in OS between the two arms (Fig 2B).

Pathologic Parameters Correlating With RFS in the Placebo Group

In our initial report, RFS was related to tumor size in both study arms.[7] With longer follow-up in the placebo group, large tumor size continued to be associated with shorter RFS on multivariable analysis ($P < .001$; Table 2; Data Supplement). In the multivariable model, patients who had tumors in the small intestine had shorter RFS than patients with gastric tumors ($P = .023$). Meanwhile, mitotic rate was

Table 1. Clinicopathologic Characteristics

Characteristic	All Patients (N = 645) No.	%	Placebo Arm (n = 328) No.	%	Imatinib Arm (n = 317) No.	%	P
Age, years							
Median	58		58		59		
Range	18-91		18-91		18-88		
Male sex	334	51.8	180	54.9	154	48.6	.11
Tumor size, cm							.94
Median	6.5		6.5		6.5		
Range	3-43		3-43		3-37		
< 5	174	27.0	89	27.1	85	26.8	
5-10	307	47.6	154	47.0	153	48.3	
> 10	164	25.4	85	25.9	79	24.9	
Mitotic rate, No. per mm^2	(n = 620)		(n = 317)		(n = 303)		.95
Median	3		3		3		
Range	0-351		0-351		0-289		
< 5 per 11.87	390	62.9	199	62.8	191	63.0	
≥ 5 per 11.87	230	37.1	118	37.2	112	37.0	
Tumor location	(n = 644)		(n = 328)		(n = 316)		.16
Stomach	402	62.4	218	66.5	184	58.2	
Small intestine	204	31.7	93	28.4	111	35.1	
Rectum	9	1.4	5	1.5	4	1.3	
Other	29	4.5	12	3.7	17	5.4	
Margins	(n = 644)		(n = 327)		(n = 317)		.16
R0 (microscopic margin negative)	589	91.5	304	93.0	285	89.9	
R1 (microscopic margin positive)	55	8.5	23	7.0	32	10.1	

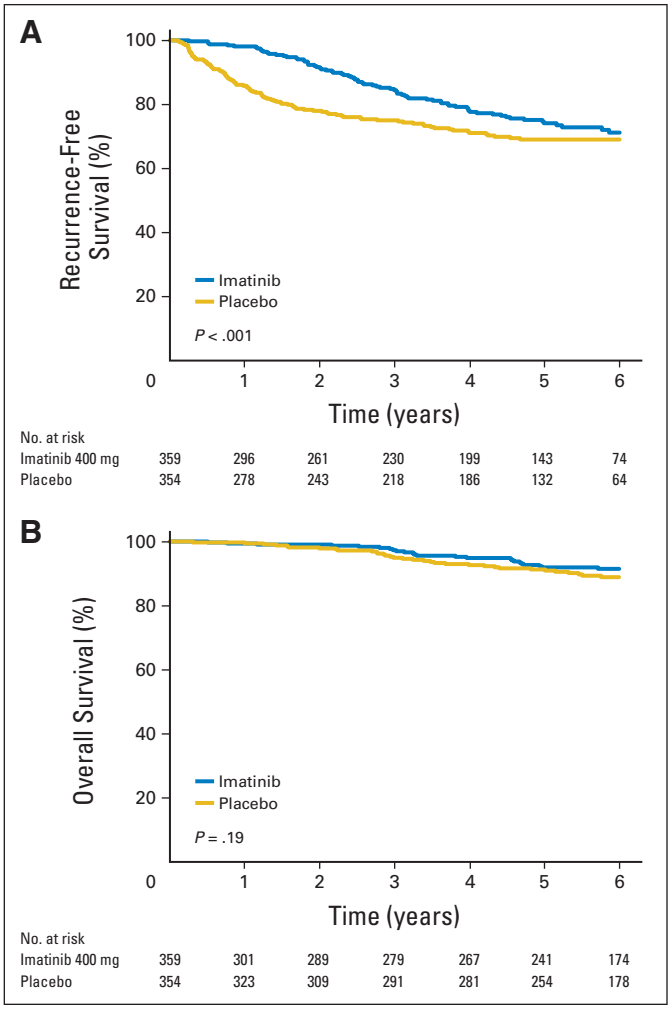

Fig 2. (A) Recurrence-free and (B) overall survival in entire population.

strongly associated with RFS ($P < .001$; Table 2; Fig 3A). On multivariable analysis, patients with tumors with more than 10 mitoses had an HR of 7.81 (95% CI, 4.42 to 13.83).

A protocol for assessing the risk of GIST recurrence published by Miettinen et al[15] at the AFIP has been endorsed by the American Joint Committee on Cancer for use in routine pathology reports on primary GISTs. A critical element of the AFIP protocol, which was developed from large retrospective studies, is a mitotic-rate cutoff of $\leq$ five mitoses per 50 hpfs, where 50 hpfs is defined as an area of 5 mm^2. Our analysis was performed using a more modern model of microscope with a larger field of view, such that 50 hpfs corresponded to 11.87 mm^2. By recursive partitioning, the single best mitotic-rate cutoff for predicting the risk of recurrence in the Z9001 placebo group was $\geq$ 9.5 per 11.87 mm^2. Notably, the HR at this cutoff was not that different from $\geq$ 5 per 11.87 mm^2 (9.3 *v* 8.8 on univariable analysis). Interestingly, 9.5 mitoses across 11.87 mm^2 correspond to 4.0 mitoses in 5 mm^2, which is similar to $\leq$ 5 mitoses per 50 hpfs defined in the AFIP protocol. Thus, the outcomes of the prospectively observed Z9001 placebo group seem to confirm the AFIP cutoff of $\leq$ 5 mitoses per 5 mm^2.

Analysis of Tumor Mutation Status in the Placebo Group

The frequency of *KIT* and *PDGFRA* mutations seemed to be similar between the trial arms (Table 3). As expected, *KIT* exon 11 mutations were the most common, followed by wild-type and *PDGFRA*-mutant tumors.[2,3] *KIT* exon 9 mutations (6.9% overall) were somewhat less common than reported in trials conducted among patients with advanced disease (9% to 11%), but consistent with studies of primary tumors.[2,3,23]

We performed a number of analyses of patients on the placebo arm. The differences in RFS among patients grouped as having a *KIT* exon 9–mutant, *KIT* exon 11–mutant, *PDGFRA*-mutant, or wild-type tumor (Fig 3B) were not statistically significant. However, patients with a *KIT* exon 11 deletion seemed to have worse outcome on univariable analysis (HR, 2.46; 95% CI, 1.19 to 5.10; $P = .005$), but this association was lost on multivariable analysis (HR, 1.44; 95% CI, 0.68 to 3.06; $P = .41$; Table 2; Fig 3C). There did not seem to be a difference in RFS between patients with *KIT* exon 11 insertions or *KIT* exon 11 point mutations and those with wild-type tumors (Fig 3C). There were two recurrences among 12 patients with *PDGFRA* D842V–mutant tumors, as compared with four recurrences among 15 patients with other *PDGFRA* mutations.

Pathologic Parameters Correlating With RFS in the Imatinib Group

As in the placebo group, tumor size, small bowel location, and mitotic rate were independently associated with RFS on multivariable analysis (Table 2). Mitotic rate had the largest observed effect, with those with a mitotic rate $\geq$ 10 per 11.87 mm^2 having an HR of 4.97 (95% CI, 2.77 to 8.94). However, as before, tumor genotype was not significantly associated with RFS on multivariable analysis ($P = .13$).

Effect of Adjuvant Imatinib Depends on Tumor Genotype

RFS for patients with a *KIT* exon 11 mutation was longer in the imatinib group than in the placebo group ($P < .001$; Fig 4). However, the differences in RFS between patients with a *KIT* exon 9 mutation and wild-type tumor did not seem to be significantly associated with treatment (Data Supplement), although the lack of association may have been the result of limited power. Meanwhile, there was a trend toward imatinib benefit in patients with *PDGFRA*-mutant tumors (Data Supplement). Among the subsets of *KIT* exon 11 mutations, adjuvant imatinib seemed to increase RFS in patients with deletions covering codons 557 and/or 558 ($P = .0027$; Data Supplement), as well as other types of deletions not including these codons ($P = .0036$; Data Supplement). In contrast, adjuvant imatinib did not significantly alter RFS in patients with a *KIT* exon 11 insertion or point mutation (Data Supplement).

DISCUSSION

The initial results of the Z9001 trial showed that the risk of recurrence after resection of a primary GIST was significantly reduced after assignment to 1 year of postoperative imatinib compared with placebo. These results are now confirmed with a longer median follow-up of 74 months. Although it is clear that adjuvant treatment can delay recurrence, our data do not demonstrate that recurrence can be altogether

Table 2. Impact of Tumor Size, Location, Mitotic Rate, and Mutation Status on RFS

	Placebo Arm						Imatinib Arm					
	Univariable			Multivariable			Univariable			Multivariable		
Variable	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*	HR	95% CI	*P*
Tumor size, cm			< .001			< .001			< .001			< .001
< 5	1.0 (reference)			1.0 (reference)			1.0 (reference)			1.0 (reference)		
5-10	2.11	1.11 to 4.21		1.49	0.72 to 3.06		6.06	1.87 to 19.67		2.90	0.86 to 9.74	
> 10	6.34	3.34 to 12.03		3.25	1.56 to 6.67		15.05	4.63 to 48.89		6.51	1.91 to 22.08	
Tumor location			.42			.023			.023			.045
Stomach	1.0 (reference)			1.0 (reference)			1.0 (reference)			1.0 (reference)		
Small intestine	1.18	0.76 to 1.82		1.97	1.14 to 3.41		1.98	1.23 to 3.19		2.03	1.12 to 3.68	
Rectum	3.37	0.82 to 13.88		4.88	0.59 to 40.57		2.42	0.58 to 10.11		1.04	0.24 to 4.62	
Other	0.90	0.28 to 2.86		0.29	0.04 to 2.09		2.35	0.91 to 6.04		4.01	1.09 to 14.72	
Mitotic rate*			< .001			< .001			< .001			< .001
< 5	1.0 (reference)			1.0 (reference)			1.0 (reference)			1.0 (reference)		
5-10	2.85	1.44 to 5.63		2.00	0.90 to 4.45		0.61	0.18 to 7.95		0.84	0.25 to 2.84	
≥ 10	9.27	5.83 to 14.74		7.81	4.42 to 13.83		6.16	3.85 to 9.84		4.97	2.77 to 8.94	
Mutation			.005			.41			.12			.13
WT	1.0 (reference)			1.0 (reference)			1.0 (reference)			1.0 (reference)		
Exon 9	1.30	0.48 to 3.52		0.89	0.32 to 2.53		1.37	0.39 to 4.86		1.06	0.29 to 3.84	
Exon 11 deletion	2.46	1.19 to 5.10		1.44	0.68 to 3.06		1.42	0.59 to 3.42		0.84	0.33 to 2.16	
Exon 11 no deletion	1.00	0.45 to 2.26		0.84	0.37 to 1.92		1.08	0.43 to 2.75		0.80	0.31 to 2.06	
PDGFRA	1.00	0.36 to 2.85		1.33	0.45 to 3.92		0.26	0.05 to 1.26		0.15	0.03 to 0.90	

Abbreviations: HR, hazard ratio; RFS, recurrence-free survival; WT, wild type.
*Per 11.87 mm^2.

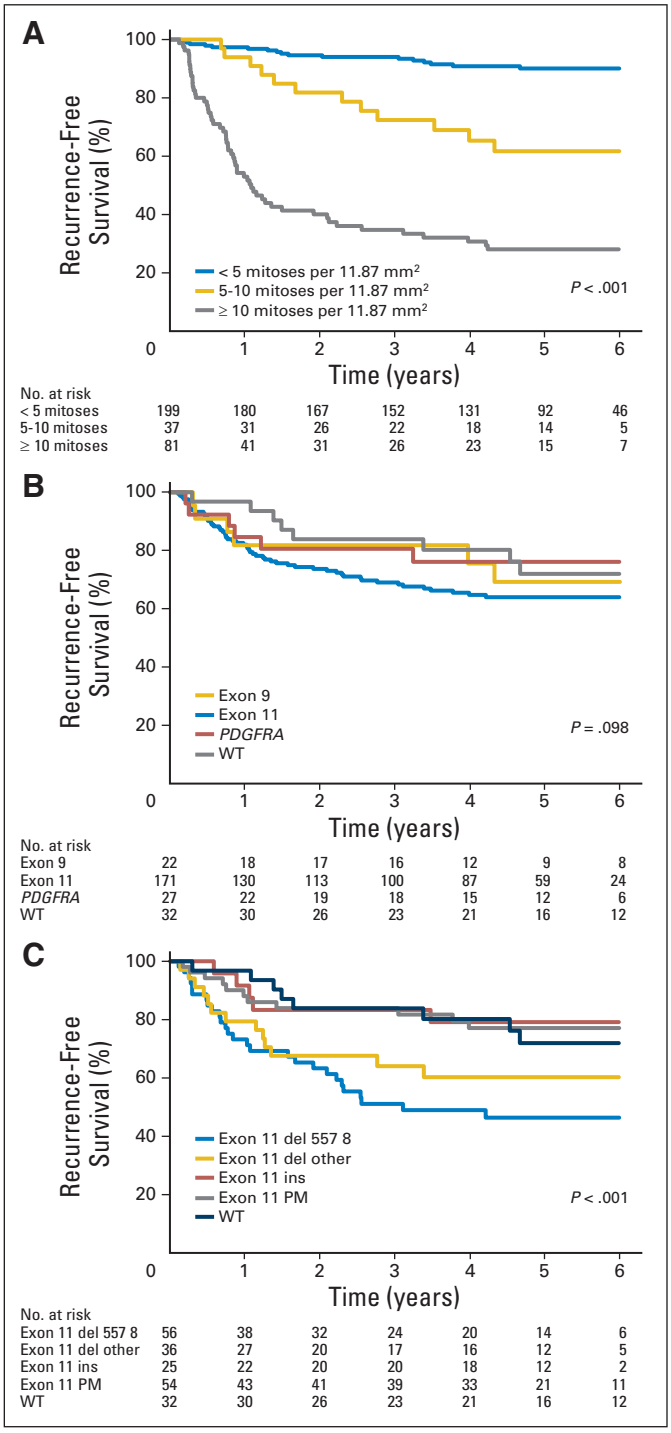

Fig 3. Effect of (A) mitotic rate and (B, C) genotype on recurrence-free survival in placebo group. del, deletion; ins, insertion; PM, point mutation; WT, wild type.

prevented with just 1 year of treatment. OS was not different between the two groups, most likely because imatinib is an effective salvage therapy on disease recurrence, and recurrence is detected early when patients undergo serial radiologic surveillance. In this regard, it is interesting to compare the results of the SSG (Scandinavian German) XVIII adjuvant trial of 1 year versus 3 years of imatinib.[9] In that trial, RFS was significantly better in the 3-year arm. OS was increased, but the event rate was low (9.3%), and there was no statistical difference in disease-specific survival. Therefore, although adjuvant imatinib can suppress residual microscopic disease for certain tumor genotypes, it may not be curative, at least in any substantial proportion of patients.

Determining which patients are mostly likely to experience disease recurrence or spread is the major challenge when considering the use of adjuvant therapy. The placebo group in the Z9001 trial provided a unique opportunity to assess the pathologic and genotypic features that best correlate with RFS, because this was the largest prospectively observed cohort reported to date, to our knowledge. As expected, we found that mitotic rate was the single best predictor of tumor behavior. Our statistically derived cutoff of 9.5 mitoses per 11.87 mm^2 is equivalent to four mitoses per 5 mm^2 tumor area, which is similar to the cutoff of $\leq$ five mitoses per 5 mm^2 used in the AFIP risk assessment scheme. In addition, we confirmed that tumor size was a significant risk factor, as was small bowel location, consistent with prior reports.[11,17]

With regard to tumor genotype and disease recurrence in the placebo group, we observed that tumors with a *KIT* exon 11 deletion of any type were significantly more likely to recur, as compared with wild-type tumors. However, this finding was not present on multivariable analysis. Among patients with a *KIT* exon 11–mutant tumor, those in the imatinib arm had significantly longer RFS compared with patients in the placebo arm. However, this effect was accounted for by those with *KIT* exon 11 deletions. In contrast, RFS for the subsets of patients with exon 11 point mutations and insertions was not statistically affected by treatment, even though these types of mutations have been shown to respond in the advanced disease setting.[2,3]

There was no statistical difference in RFS for patients with *KIT* exon 9–mutant GISTs treated with imatinib versus placebo. However, the number of patients was relatively small, and patients were not evenly distributed between the two arms (placebo, n = 22 *v* imatinib, n = 13). Interestingly, in the SSG XVIII adjuvant trial, there was no statistical difference between 1 year versus 3 years of imatinib among patients with a *KIT* exon 9 mutation, whereas the *KIT* exon 11–mutant patients clearly benefited from longer therapy.[9] It should be noted, however, that in both the SSG XVIII and Z9001 trials, patients were treated with only the standard dose of 400 mg per day. In the setting of advanced disease, RFS for patients with a *KIT* exon 9–mutant GIST is significantly longer when patients are treated with 800 mg per day of imatinib as compared with 400 mg per day.[4] The Z9001 trial was initiated before this information was available, and it remains possible that use of a higher imatinib dose would be more effective in the adjuvant setting.

Patients with wild-type GISTs did not seem to benefit from adjuvant therapy. The two arms were well balanced for these tumors, and one might therefore conclude that wild-type patients should be excluded from treatment after primary surgery. However, a number of recent publications have established that wild-type GISTs constitute a heterogeneous group. Between 7% and 15% of these tumors harbor an activating mutation in *BRAF*, and additional small percentages have an *NF1* or *RAS* gene mutation.[24-27] Approximately 40% of wild-type GISTs show loss of SDHB protein expression, and half of these tumors harbor one or more mutations in *SDHA*, *SDHB*, *SDHC*, or *SDHD*.[28-32] Sensitivities of these different molecular subtypes of wild-type GIST to imatinib treatment are not established. Additional studies are needed to better define the management of wild-type tumors in both the adjuvant and advanced disease settings.

Table 3. Tumor Genotypes

Mutation Status	Placebo Arm (n = 258)		Imatinib Arm (n = 249)		All Patients (n = 507)	
	No.	%	No.	%	No.	%
KIT exon 9	22	8.5	13	5.2	35	6.9
KIT exon 11	171	66.3	170	68.3	341	67.3
Any deletion	92	35.7	92	37.0	184	36.3
Deletion of codons 557 and/or 558	56	21.7	51	20.5	107	21.1
Deletion not including codon 557 or 558	36	14.0	41	16.5	77	15.2
Insertion	25	9.7	21	8.4	46	9.1
Point mutation	54	20.9	57	22.9	111	21.9
KIT exon 13	6	2.3	3	1.2	9	1.8
KIT exon 17	0	0.0	1	0.4	1	0.2
PDGFRA	27	10.5	30	12.0	56	11.2
D842V	12	4.6	15	6.0	27	5.3
Not D842V	15	5.8	15	6.0	30	5.9
WT	32	12.4	32	12.8	64	12.6

Abbreviation: WT, wild type.

Recent reports of treatment-naive, *KIT*-mutant GISTs have uncovered coexisting downstream mutations in some patients.[27,33] Although infrequent, mutations in *KRAS* (5%), *BRAF* (2%), and *PIK3CA* (1%) may confer primary resistance to imatinib and other inhibitors. These observations add to the complexity of the molecular genetics of GISTs and underscore the importance of routine genotyping in the management of these tumors, whether in the adjuvant or advanced disease setting. There were too few *PDGFRA*-mutant GISTs in our study to determine a benefit of adjuvant therapy in this group, but the most common *PDGFRA* mutation, D842V, is fully resistant to imatinib in vitro and correlates with lack of response to this drug in patients with advanced disease.[34,35]

In summary, the Z9001 trial has demonstrated the importance of tumor size, location, and mitotic rate in the risk of disease recurrence, both in patients in the placebo arm and the imatinib arm. Surprisingly, tumor mutation status did not independently affect RFS in either the placebo or imatinib arm. Furthermore, there was a clear benefit of adjuvant imatinib in patients with *KIT* exon 11 deletions.

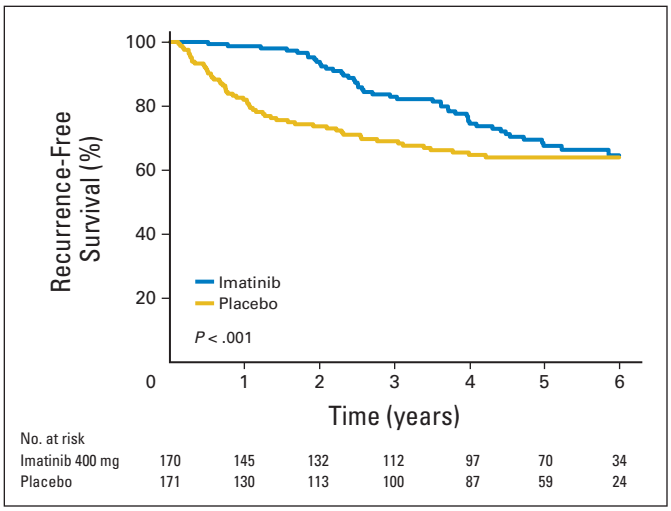

Fig 4. Relationship between *KIT* exon 11 mutation and effect of imatinib treatment.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Although all authors completed the disclosure declaration, the following author(s) and/or an author's immediate family member(s) indicated a financial or other interest that is relevant to the subject matter under consideration in this article. Certain relationships marked with a "U" are those for which no compensation was received; those relationships marked with a "C" were compensated. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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AUTHOR CONTRIBUTIONS

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