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ORIGINAL ARTICLE

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Sunitinib-resistant gastrointestinal stromal tumors harbor *cis*-mutations in the activation loop of the *KIT* gene

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Abstract

Background. Although sunitinib malate has shown significant clinical effect on imatinib-resistant gastrointestinal stromal tumors, with acceptable tolerability and improved prognosis for the patients, the mechanism of resistance to the drug is still under investigation.

Methods. We analyzed findings in 8 patients (seven men and one woman, median age, 59 years) out of 17 patients with imatinib-resistant gastrointestinal stromal tumors who had been treated with sunitinib. Sunitinib was orally administered once a day at a starting dose of 37.5 mg/day, 50 mg/day, or 75 mg/day, with 4 weeks on and 2 weeks off.

Results. All imatinib- as well as sunitinib-resistant lesions showed viable tumor cells strongly re-expressing the KIT protein. Pre-imatinib samples had heterogeneous *KIT* mutations either in exon 9 ($n = 1$) or exon 11 ($n = 7$), and seven imatinib-resistant tumors carried a secondary mutation either in the ATP-binding domain or in the activation loop in the same allele as the primary mutation. Most patients with imatinib-resistant tumors carrying secondary mutations in the ATP-binding domain obtained clinical benefits from sunitinib, whereas some tumors with mutations in the activation loop showed resistance to the drug. A tumor with mutations in exon 11 and 13 of the *KIT* gene, and showing partial response to sunitinib, harbored a third mutation in the activation loop when sunitinib resistance was shown. All additional secondary and tertiary mutations were located on the same allele as the primary mutation (*cis*-mutation).

Conclusion. These findings indicate that an additional *cis*-mutation in the activation loop of the *KIT* gene could be a potential cause of sunitinib resistance in gastrointestinal stromal tumors.

Key words Acquired resistance · KIT · Imatinib · Sunitinib · Mutation

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Introduction

Gain-of-function mutations in the *KIT* gene or the platelet-derived growth factor alpha (*PDGFRA*) gene induce proliferation of gastrointestinal stromal tumors (GISTs) and are major causes of sporadic as well as familial GIST.[1–3] These mutations are mutually exclusive and are predominantly found in the *KIT* juxtamembrane domain (exon 11), sometimes in the extracellular domain of the *KIT* gene (exon 9), and rarely in kinase domains.[4] Approximately 5% of GISTs have mutation in neither gene.

Imatinib mesylate (Glivec or Gleevec; Novartis Pharma, Basel, Switzerland), a selective tyrosine kinase inhibitor of BCR-ABL, KIT, and PDGFRA tyrosine kinases, has a strong clinical effect on advanced and/or metastatic GIST, with substantial tolerability.[5] The action of imatinib largely depends on the genotype of GIST, i.e., GISTs with mutations in *KIT* exon 11 are most sensitive to imatinib, followed by those with *KIT* exon 9 mutations, while a small but definite number of GISTs without mutation in either the *KIT* gene or the *PDGFRA* gene, or with mutations in the kinase domain (e.g., D842V mutation in the *PDGFRA* gene) show resistance to imatinib. Although imatinib improved the prognosis of patients with advanced GIST, with a median progression-free survival (PFS) of 2 years and overall survival (OS) of 5 years,[4] resistance to the drug develops with

prolonged use. A recent clinical study has shown significant clinical activity of sunitinib malate (Sutent; Pfizer, New York, NY, USA), with acceptable tolerability, in patients with imatinib-resistant GIST or imatinib-intolerant patients,[6] of whom 7% showed a partial response (PR); 58% showed stable disease (SD); and 19% showed progressive disease (PD).

Recent studies have demonstrated that imatinib resistance is associated with the re-activation of KIT tyrosine kinase, as well as being associated with acquired mutations in the *KIT* kinase domain.[7–13] However, the detailed mechanisms of acquired mutations in GIST remain unknown. Furthermore, the mechanisms of sunitinib resistance and the relationship between genotype and the effectiveness of sunitinib are still under investigation. In this study, we conducted a preliminary investigation of the clinicopathological and molecular features of sunitinib-resistant GIST.

Patients, materials, and methods

Patient demographics

The patients analyzed in this study had been diagnosed with GIST following histological examinations of either surgical or biopsy samples in which spindle and/or epithelioid tumor cells were found to be positive for KIT and/or CD34. The patients received 400 or 600 mg/day of imatinib for more than 180 days and if their GIST subsequently showed secondary imatinib resistance, they received 37.5 to 75 mg/day of sunitinib. Tumor responses were assessed with the response evaluation criteria in solid tumors (RECIST) and the use of periodic multidetector-computed tomography (MDCT) scans with contrast enhancement. When necessary, an ^{18}F-fluoro-2-deoxy-D-glucose positron emission tomography (^{18}FDG-PET) scan was performed as part of the study, although the use of ^{18}FDG-PET had not yet been approved by the Japanese social insurance system in March 2008. After progression under imatinib and sunitinib, samples obtained at surgery or by biopsy or autopsy from eight patients (seven men and one woman; median age, 59 years) at Osaka University Hospital (Table 1) were subjected to genetic analysis. Final prognostic analysis was done at the end of March 2008. The median treatment period with imatinib was 22.2 months (range, 11.5–34 months) and the median treatment with sunitinib consisted of four cycles. The median follow-up periods from the initial diagnosis of GIST and the initiation of imatinib therapy were 50.6 months (range, 26–124 months) and 39.4 months (range, 22.7–64.1 months), respectively. One patient remains alive with the disease and the other seven patients have died of the disease.

Sunitinib and its metabolites were measured by a liquid chromatographic tandem mass spectrometric method, as reported previously.[14]

Pre-imatinib, imatinib-resistant, and sunitinib-resistant samples were examined by hematoxylin and eosin (H&E) staining and KIT immunostaining, as well as by CD34 immunohistochemistry, using paraffin-embedded sections (3-μm-thick) of formalin-fixed tissues. The proliferative activity of the imatinib-resistant lesions was evaluated by Ki-67 antigen immunohistochemistry. Immunohistochemistry was performed using the ENVISION+ KIT HRP (DAB) system (Dako Cytomation, Kyoto, Japan) with rabbit polyclonal antibody against human KIT (A4502; Dako), mouse monoclonal antibody against human CD34 (QBend10; Novocastra Laboratories, Newcastle, UK), or mouse monoclonal antibody against human Ki-67 antigen (MIB-1; Dako) as the primary antibodies, as described previously.[15]

RNA extraction, reverse transcription polymerase chain reaction (RT-PCR), and sequence analysis

All the fresh samples were snap-frozen in liquid nitrogen at the time of surgical resection or biopsy and were kept at –80°C until RNA extraction. Total RNA was extracted with the RNeasy Mini Kit (QIAGEN, Valencia, CA, USA). Complementary DNA (cDNA) was synthesized by means of reverse transcriptase (Superscript II; GIBCO-BRL, Grand Island, NY, USA), and *KIT* or *PDGFRA* cDNA was amplified by PCR (RT-PCR), after which the full sequences were determined.[16] In cases where fresh samples were not available as pre-imatinib samples, genomic DNA was extracted from formalin-fixed, paraffin-embedded specimens (10-μm-thick) with the aid of DEXPAT (Takara, Kyoto, Japan) and used for direct sequencing of the known mutated sites of *KIT* (exons 9, 11, 13, 14, and 17) and *PDGFRA* (exons 12, 14, and 18) as described previously.[15] When mutations were detected at two or more sites, amplified cDNA including the mutational sites was subcloned into pT7-blue plasmid, and the sequencing of 10 to 20 independent cloned cDNAs was performed to examine allelic distribution.

The study, as well as the genetic analysis, was performed under the guidelines of the institution the authors are affiliated with after approval was given by the institutional review board and written informed consent was obtained from all patients and/or their relatives.

Results

Clinicopathological demographics

Treatment for 17 patients with imatinib-resistant GIST was started with the oral administration of sunitinib malate once a day at a starting dose of 37.5 mg/day, 50 mg/day, or 75 mg/day, with 4 weeks on and 2 weeks off depending on the protocol of the clinical study or the patient's performance status. Histologic and genetic analysis of imatinib- as well as sunitinib-resistant lesions could be performed for 8 of the 17 patients: 2 with primary gastric GISTs, 5 with small-intestinal GISTs, and 1 with colonic GIST. The initial imatinib-target lesions comprised seven peritoneal disseminations and six liver metastases (including duplication; Table 1). All patients showed a PR as their best response

Table 1. Patients' Characteristics and GIST Genotype

Case No no.	Age (years)	GenderSex	Origin	Recurrence (imatinib target)	Imatinib dose (mg/day)	Imatinib best response	Imatinib duration (Months)	Imatinib-resistant loci	Primary mutation
1	67	M	Small intestine	Liver Peritoneum	400	PR	11.5	Peritoneum	Exon 11 del 554–570
2	63	M	Small intestine	Liver Peritoneum	400	PR	26.8	Peritoneum	Exon 11 del 556–557
3	54	M	Colon	Peritoneum	400	PR	14.6	Peritoneum	Exon 11 V560D
4	72	M	Stomach	Liver	600	PR	24.9	Liver Bone	Exon 11 del 557–558
5	46	F	Small intestine	Liver Peritoneum	400	PR	12.2	Peritoneum	Exon 11 del 557–558
6	49	M	Stomach	Liver Peritoneum	600	PR	24.6	Peritoneum	Exon 11 del 557–558
7	63	M	Small intestine	Liver Peritoneum	400	PR	34	Peritoneum	Exon 11 del 560
8	55	M	Small intestine	Peritoneum	600	PR	19.8	Peritoneum	Exon 9 dup 502+503

Table 1. Patients' Characteristics and GIST Genotype (continued)

Case No no.	Secondary Mutation mutation	Sunitinib dose (mg/day)	Sunitinib best response	PFS (months)	Third mutation	Allelic distribution	Blood level of sunitinib (ng/ml)	Outcome on Day day 28
1	Exon 13 V654A	50	PR (8 cycles)	11.2	Exon 17 del 820–821	all *cis*	48.1	Dead
2	Exon 13 V654A	75	SD (4 cycles)	7	N/E	*cis*	N/E	Dead
3	No mutation	50	PD (2 cycles)	2.3	N/E	–	11.4	Dead
	Exon 13 V654A				N/E	*cis*[a]		
4	Exon 14 T670I	37.5	SD (6 cycles)	9 ongoing	N/E	*cis*	N/E	Alive
5	Exon 17 D816H	50	PD (2 cycles)	2.3	No mutation	*cis*	N/E	Dead
6	Exon 17 W823D	75	PD (2 cycles)	3.5	N/E	*cis*	N/E	Dead
7	Exon 17 D822K, N822K	50	SD (4 cycles)	6.5	No mutation	*cis*	70.1	Dead
8	No mutation	50	PD (2 cycles)	6	No mutation	–	N/E	Dead

N/E: not examined; PFS: progression-free survival
[a]: V654A mutation is *cis*-positioned to V560D

to imatinib. Secondary resistance to imatinib occurred in peritoneal lesions in seven cases, and hepatic metastasis and bone metastasis in one case each. MDCT showed secondary resistance as a nodule in a mass in five patients and as enlargement of a pre-existing mass in three patients. Treatment with sunitinib resulted in four patients showing PD at the end of the second cycle, three patients showing SD for at least four cycles, and one patient showing PR after two cycles (Table 1). The last patient (case 1 in Table 1), who showed PR to sunitinib in the second cycle of sunitinib therapy, had an enhanced lesion in the attenuated background in the seventh cycle, and later showed PD in the eighth cycle (Fig. 1).

H&E and KIT immunostaining in case 1 showed viable spindle tumor cells with strong and uniform expression of the KIT protein in imatinib-resistant as well as sunitinib-resistant lesions (Fig. 2). Viable tumor cells with strong KIT immunoreactivity were also confirmed in pre-imatinib, imatinib-resistant, and sunitinib-resistant samples obtained from other patients.

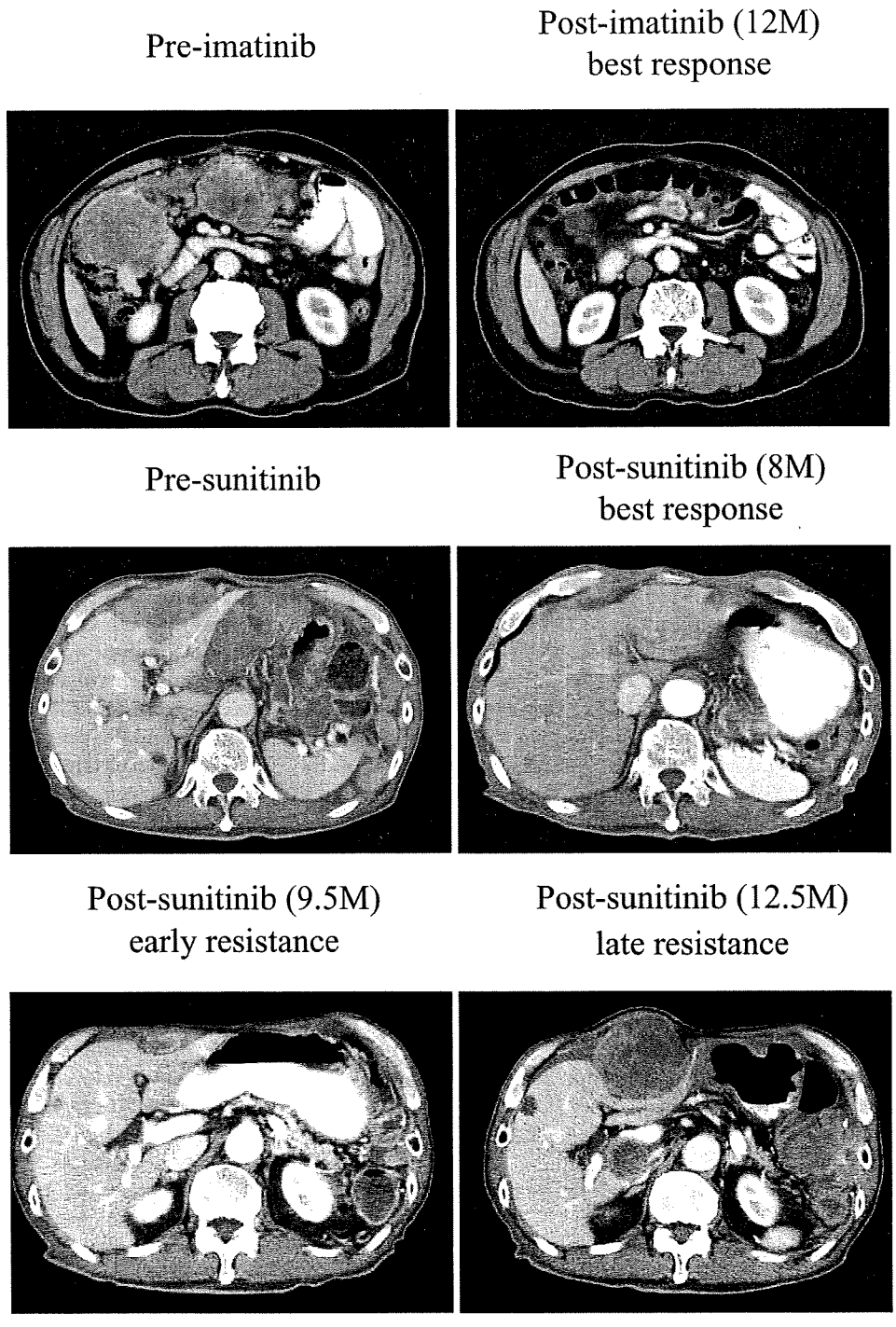

Fig. 1. Representative radiographic responses to imatinib and sunitinib treatment (case 1). *Numbers in parentheses* indicate months after the initiation of either imatinib or sunitinib therapy. The *two upper panels* show responses to imatinib (partial response; PR) when the patient's gastrointestinal stromal tumor (GIST) harbored a mutation in exon 11 of the *KIT* gene; *the middle panels* show PR to sunitinib in conjunction with mutations in exon 11 and 13; and the *lower panels* show subsequent progressive disease (PD) during sunitinib treatment, when tumors had a mutation in exon 17 in addition to those in exons 11 and 13. The initial appearance of sunitinib resistance emerged as an enhanced lesion in the attenuated background (*bottom left scan*)

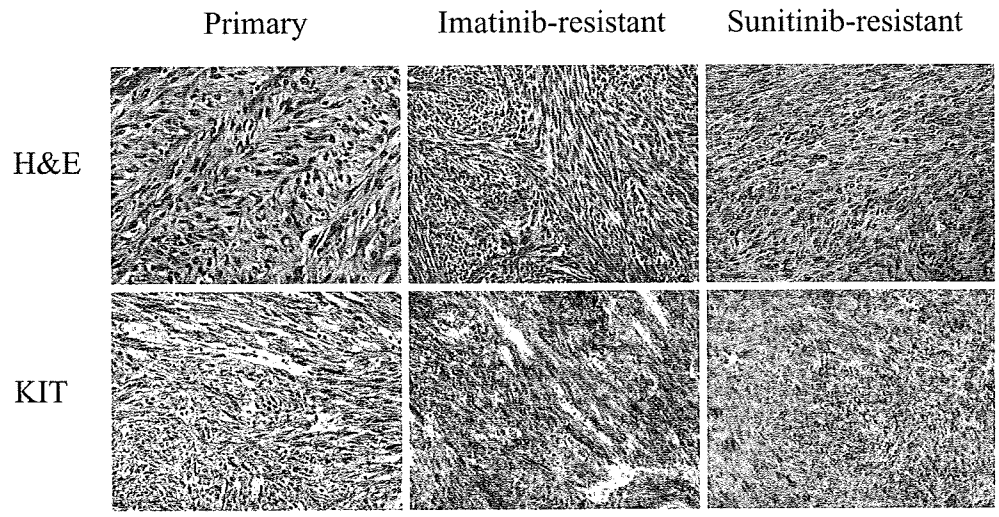

Fig. 2. Typical pathological findings (case 1). The *left panels* show the histology of the pre-imatinib (*primary*) lesion, the *middle panels*, the histology of an imatinib-resistant lesion, and the *right panels*, the histology of a sunitinib-resistant lesion. *Upper panels* H & E, original magnification ×400; *lower panels* KIT immunostaining, original magnification ×400

Additional *cis* mutation in the *KIT* gene

Next, we examined sequencing of the *KIT* and *PDGFRA* genes in pre-imatinib, imatinib-resistant, and post-sunitinib samples (Table 1). Analysis of the pre-imatinib samples revealed mutations in either exon 9 (n = 1) or exon 11 (n = 7) of the *KIT* gene, which were also detected in all resistant lesions, but no mutations were found in the *PDGFRA* gene. Imatinib-resistant tumors in seven patients showed secondary mutations in the kinase domains of the *KIT* gene, including exon 13 mutations (V654A) in three patients, exon 14 mutation (T670I) in one, and exon 17 mutations (D816H, N822K, W823D) in three. Case 3 had several resistant lesions, some of which harbored the V654A mutation and others no additional mutation. The patient with GIST carrying an exon 9 mutation (case 8) had no secondary mutation, so that two patients had imatinib-resistant lesions without secondary mutations. Case 1, who possessed mutations in both exon 11 and exon 13 before sunitinib treatment and had shown a PR to sunitinib, was found to harbor a tertiary mutation in the activation loop of the *KIT* gene when the tumor was resistant to sunitinib. The blood levels of sunitinib on day 28 of each cycle were measured in three patients. One patient (case 3) showed little increase (Table 1).

Next, we examined the allelic distribution of additional *KIT* mutations. Of interest was that all detected secondary and tertiary mutations were located on the same allele as the primary mutations (*cis*-position), while the other allele remained as wild-type after imatinib as well as sunitinib resistance was shown (Table 1). Neither *trans*-position mutation in the *KIT* gene nor mutation in the *PDGFRA* gene was found.

Discussion

Resistance to chemotherapeutic agents is critical for cancer patients and may determine treatment outcome. In molecular terms, resistance to target agents may be generated to target molecules, although several factors, including doses of drugs, tumor burden, and pharmacodynamics may be clinically involved. In fact, target mutations have been found to be associated with imatinib resistance in chronic myelogenous leukemia (CML), hypereosinophilic syndrome (HES), and GIST.[7–13,17,18] Similar mechanisms of acquired mutations in the epidermal growth factor receptor (*EGFR*) gene have been identified in secondarily gefitinib-resistant lung cancer.[19]

KIT, PDGFR, and vascular endothelial growth factor receptor (VEGFR), targets of sunitinib, are type III receptor tyrosine kinases with a split kinase domain. Sunitinib has been used for imatinib-resistant GIST because of its broad-spectrum activity, including the inhibition of VEGFR tyrosine kinases, and KIT inhibition was proven to be critical in imatinib-resistant GIST, although details of the mechanism involved are still being studied. In an in vitro study, sunitinib inhibited KIT kinase activity when the secondary mutation was in the ATP-binding domain (exon 13 or 14), whereas the drug had little effect on activation loop mutations (exon 16 or 17).[20,21] In the present study, in patients with GISTs with secondary mutations in the ATP-binding domain of the *KIT* gene, PR was shown in one patient, SD in two patients, and PD in one patient. The PD patient (case 3) had several resistant lesions with and without secondary mutations. Furthermore, this patient showed a small increase in the blood levels of sunitinib on day 28 of each cycle (Table 1), probably because of previous extensive intestinal resection, indicating that this patient was not suitable for the evaluation of sunitinib effects and genotype. Of the patients with GISTs with secondary mutations in the activation loop of the *KIT* gene, two patients (cases 5 and 6) showed PD and one showed SD (case 7). Case 7 had a secondary N822K mutation, which is relatively sensitive to imatinib.[12,22] Case 1 showed a tertiary mutation in the activation loop of the *KIT* gene after sunitinib resistance occurred. Thus, clinical benefit (CR, PR, or SD for more than 22 weeks) was obtained for all three evaluable patients with GIST possessing

secondary mutations in the ATP-binding domain of the *KIT* gene, whereas the addition of secondary or tertiary mutations in the activation loop of the gene were associated with resistance to sunitinib in three of four patients. These results indicate that mutations in the ATP-binding domain of the *KIT* gene may be sensitive to sunitinib with sufficient blood levels of sunitinib, whereas some of these mutations in the activation loop appeared to be resistant to the drug, although the number of patients analyzed was small. Recently, sorafenib tosylate (Bayer Pharmaceuticals, West Haven, CT, USA) also inhibited the activity of KIT with an ATP-binding domain mutation of T670I, a gatekeeper mutation,[23] suggesting that these two drugs may have a similar binding pattern in the KIT kinase pocket, which is postulated to be somewhat different from that of imatinib.

Although the reported frequency of secondary mutations is 43.8% to 73%, secondary mutation is considered to be a major cause of imatinib resistance.[8–10,12,13,24] Secondary mutation in the kinase domain of the *KIT* or *PDGFRA* gene is accompanied by concomitant re-activation of the corresponding tyrosine kinase even in the presence of imatinib.[9,10,12] Moreover, mutations in the kinase domain may induce conformational changes, resulting in loss of binding affinity to the drugs or in transformation from the autoinhibited to the activated form. Imatinib, and probably sunitinib and sorafenib, may bind to the autoinhibited form, and may not be stabilized in the activated form.[21,25] Another possibility is that mutations in drug-binding loci may cause loss of binding and, as a result, the inhibitory activity of the drug may be lost.[10]

One interesting finding concerns the allelic distribution of primary, secondary, and tertiary mutations. Most *KIT* mutations found in GIST are heterozygous, so that primary GIST has one wild and one mutated allele. Preliminary results of allelic analysis of imatinib-resistant GIST have shown that most secondary mutations are on the same allele as the primary mutation.[12,24] All mutations found in the present study were also grouped on one allele of the *KIT* gene, while the other allele remained wild throughout treatment with the two drugs. The findings of a previous study and our unpublished data indicate that the sensitivity of *trans*-positioned mutations to imatinib may be similar to that of corresponding *cis*-positioned mutations.[12] It is possible that some mechanism exists for the accumulation of genetic mutations on a single allele.

To summarize, we analyzed clinical cases of sunitinib-resistant GIST which had previously shown secondary resistance to imatinib. GIST with secondary mutations in the ATP-binding domain of the *KIT* gene was found to be clinically sensitive to sunitinib with sufficient blood levels of sunitinib, whereas some of the secondary mutations in the activation loop of the *KIT* gene may confer resistance to sunitinib. We also demonstrated that most of the imatinib- and sunitinib-resistant GISTs harbored secondary and tertiary mutations in the *cis* position. Because of the limited number of patients analyzed in this study, a further large-volume study may be required to clarify the detailed mechanisms of these mutations in GIST.

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References

1. Hirota S, Isozaki K, Moriyama Y, et al. (1998) Gain-of-Function mutations of *c-kit* in human gastrointestinal stromal tumors. Science 279:577–580
2. Heinrich MC, Corless CL, Duencing A, et al. (2003) PDGFRA activating mutations in gastrointestinal stromal tumors. Science 299:708–710
3. Nishida T, Hirota S, Taniguchi M, et al. (1998) Familial gastrointestinal stromal tumors with germ line mutation of the KIT gene. Nat Genet 19:323–324
4. Rubin BP, Heinrich MC, Corless CL (2007) Gastrointestinal stromal tumor. Lancet 369:1731–1741
5. Demetri GD, von Mehren M, Blanke CD, et al. (2002) Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 347:472–480
6. Demetri GD, van Oosterom AT, Garrett CR, et al. (2006) Efficacy and safety of sunitinib in patients with advanced gastrointestinal stromal tumour after failure of imatinib: a randomised controlled trial. Lancet 368:1329–1338
7. Wakai T, Kanda T, Hirota S, et al. (2004) Late resistance to imatinib therapy in a metastatic gastrointestinal stromal tumour is associated with a second KIT mutation. Br. J Cancer 90:2059–2061
8. Chen LL, Trent JC, Wu EF, et al. (2004) A missense mutation in KIT kinase domain 1 correlates with imatinib resistance in gastrointestinal stromal tumors. Cancer Res 64:5913–5919
9. Debiec-Rychter M, Cools J, Dumez H, et al. (2005) Mechanism of resistance to imatinib mesylate in gastrointestinal stromal tumors and activity of the PKC412 inhibitor against imatinib-resistant mutants. Gastroenterology 128:270–279
10. Antonescu CA, Besmar P, Tianhua G, et al. (2005) Acquired resistance to imatinib in gastrointestinal stromal tumor occurs through secondary gene mutation. Clin Cancer Res 11:4182–4190
11. Wardelmann E, Thomas N, Merkelbach-Bruse S et al. (2005) Acquired resistance to imatinib in gastrointestinal stromal tumours caused by multiple KIT mutations. Lancet Oncol 6:249–251
12. Heinrich MC, Corless CL, Blanke CD et al. (2006) Molecular correlates of imatinib resistance in gastrointestinal stromal tumors. J Clin Oncol 24:4764–4774
13. Wardelmann E, Merkelbach-Bruse S, Pauls K, et al. (2006) Polyclonal evolution of multiple secondary KIT mutations in gastrointestinal stromal tumors under treatment with imatinib mesylate. Clin Cancer Res 12:1743–1749
14. Bello CL, Sherman L, Zhou J, et al. (2006) Effect of food on the pharmacokinetics of sunitinib malate (SU11248), a multi-targeted receptor tyrosine kinase inhibitor: results from a phase I study in healthy subjects. Anticancer Drugs 17:353–358
15. Nishitani A, Hirota S, Nishida T, et al. (2005) Different expression of connexin 43 in gastrointestinal stromal tumours between gastric and small intestinal origin. J Pathol 206:377–382
16. Hirota S, Ohashi A, Nishida T, et al. (2003) Gain-of-function mutations of platelet-derived growth factor receptor alpha gene in gastrointestinal stromal tumors. Gastroenterology 125:660–667
17. Gorre ME, Mohammed M, Ellwood K, et al. (2001) Clinical resistance to STI-571 cancer therapy caused by BCR-ABL gene mutation or amplification. Science 293:876–880
18. Cools J, DeAngelo DJ, Gotlib J, et al. (2003) A tyrosine kinase created by fusion of the PDGFRA and FIP1L1 genes as a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. N Engl J Med 348:1201–1214
19. Kobayashi S, Boggon TJ, Dayaram T, et al. (2005) EGFR mutation and resistance of non-small-cell lung cancer to gefitinib. N Engl J Med 352:786–792
20. Prenen H, Cools J, Mentens N, et al. (2006) Efficacy of the kinase inhibitor SU11248 against gastrointestinal stromal tumor mutants refractory to imatinib mesylate. Clin Cancer Res 12: 2622–2627

21. Demetri GD, Gajiwala K, Christensen J, et al. (2008) Novel mechanisms of resistance to imatinib or sunitinib in KIT mutants from patients with gastrointestinal stromal tumors: structural biology and functional enzymology studies of wild-type and mutated proteins. Proceedings of the American Association for Cancer Research Annual Meeting: 12–16 April 2008; San Diego, CA. AACR 2008 Abstract No. 3184
22. Heinrich MC, Corless CL, Demetri GD, et al. (2003) Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. J Clin Oncol 21:4342–4349
23. Guo T, Agaram NP, Wong GC, et al. (2007) Sorafenib inhibits the imatinib-resistant KITT670I gatekeeper mutation in gastrointestinal stromal tumor. Clin Cancer Res 13:4874–4881
24. Nishida T, Kanda T, Nishitani A, et al. (2008) Secondary mutations in the kinase domain of the *KIT* gene are predominant in imatinib-resistant gastrointestinal stromal tumor. Int J Clin Oncol 99:799–804.
25. Nagar B, Hantschel O, Young MA, et al. (2003) Structural basis for the autoinhibition of c-Abl tyrosine kinase. Cell 112:859–871

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Vascular Endothelial Growth Factor Receptor Expression as a Prognostic Marker for Survival in Colorectal Cancer

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Objective: Vascular endothelial growth factor (VEGF) and its receptors VEGF-R1, -R2 and -R3 play important roles in tumor angiogenesis and are associated with poor prognosis in several solid tumors. However, their functional significance remains unclarified. Here, we investigated the associations between the expression of these receptors and the clinical outcomes of colorectal cancer (CRC) patients.
Methods: An immunohistochemical approach was used to detect VEGF-R1, -R2 and -R3 expression in 91 CRC patients who underwent surgery and received chemotherapy at the National Cancer Center Hospital. Statistical analysis was performed to determine the prognostic significance of these biomarkers.
Results: Immunoreactivity for VEGF-R2 and -R3 was localized in microvessels and that for VEGF-R1 in cancer cells and stromal microvessels. VEGF-R1 staining in cancer cells (>10% staining) was found in 84 patients (92%) and in stromal vessels in 75 patients (82%). VEGF-R2 staining in tumor vessels (>10% staining) was found in 84 patients (92%), whereas VEGF-R3 staining was found in 85 patients (93%). Strong positive staining (>60% staining) of VEGF-R1 in tumor cells, and VEGF-R1, -R2 and -R3 in vessels was identified in 58 (64%), 33 (36%), 52 (57%) and 60 (66%) patients, respectively. Univariate analysis revealed that VEGF-R1 strong positive staining correlated with shorter post-operative survival in patients with Stage II/III disease ($P = 0.01$), but neither VEGF-R2 nor R3 expression correlated with survival.
Conclusions: VEGF-R1, -R2 and -R3 were highly expressed in CRC cells and stromal vessels. VEGF-R1 strong positive staining correlated with shorter survival after CRC surgery.

Key words: VEGF – VEGF-R1 – VEGF-R2 – VEGF-R3 – colorectal cancer – prognostic factor

INTRODUCTION

Angiogenesis plays an important role in cancer invasion and metastasis. Vascular endothelial growth factor (VEGF) and its receptor (VEGF-R) represent important regulators of angiogenesis, and their increased expression has been documented in various cancer cell lines (1) and tissues (2,3). In the treatment of colorectal cancer (CRC) (4) and lung cancer (5), the efficacy of combining cytotoxic agents and bevacizumab, a monoclonal anti-VEGF antibody, has been reported. Some drugs that block the tyrosine kinase of VEGF-R are also being developed. However, the mechanisms controlling the expression of VEGF and VEGF-R and angiogenesis have not yet been fully elucidated. Moreover, the roles of VEGF and VEGF-R as prognostic markers and their usefulness in predicting the efficacy of anti-angiogenic agents have not been clarified to date.

In CRC, the expression of VEGF ligands and subtypes (i.e. VEGF-A, -B, -C, -D and -E) correlates with cancer stage (6,7) and prognosis (8,9), and the expression of soluble VEGF-R1 is a prognostic predictor (10). CRC cell lines have

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also been reported to express VEGF-R (11,12); however, the distribution, frequency and prognostic values of VEGF-R expression in CRC have not yet been clarified. This study investigated the relationships between VEGF-R expression and prognosis of primary CRC patients.

PATIENTS AND METHODS

Patients

Subjects were randomly selected from patients histologically diagnosed with CRC. Inclusion criteria were as follows: no prior chemotherapy or adjuvant/neoadjuvant chemotherapy; primary colorectal adenocarcinoma specimens were obtained by surgical resection before the start of chemotherapy at the National Cancer Center Hospital (NCCH); received 5-fluorouracil (5-FU)-based first-line chemotherapy for the treatment of recurrent or residual tumors at NCCH from January 1995 to December 2003; and therapeutic effects and prognoses were confirmed. Tissue samples were collected retrospectively from patients who met these criteria. Written informed consent was obtained before treatment and evaluation of tumor samples.

Immunohistochemical Staining

Serial 4 μm-thick sections were prepared from formalin-fixed paraffin-embedded tissue. One block that included the site of deepest invasion was selected from each specimen after examining the slides of the surgical specimens stained with hematoxylin and eosin. Tissue sections were dewaxed in xylene and rehydrated through graded alcohol. Antigen retrieval was performed by incubating tissue sections in target retrieval solution (Dako Japan, Tokyo, Japan) for 40 min in water bath at 95°C and cooling for at least 20 min.

After quenching endogenous peroxidase with peroxidase blocking reagent (Dako Japan) for 5 min and washing with Tris-buffered saline containing Tween 20, tissue sections were incubated with the primary antibody (Table 1).

Immunoreaction was detected using the following secondary antibody systems: CSA-II (Dako Japan) for VEGF-R1, -R2 and -R3; Envison+ kit (Dako Japan) for CD34 and D2-40 according to the instructions of the manufacturer. Sections were counterstained using Mayer's hematoxylin. As the negative controls, the primary antibody solution was substituted with a buffer containing goat IgG1 (VEGF-R1, -R2 and -R3) or mouse IgG1 (CD34 and D2-40).

Table 1. Antibodies used for immunohistochemistry

Antigen	Antibody	Dilution	Time (min)	Animal	Monoclonal (mono)/polyclonal (poly)
CD34	M 7165 (Dako)	1:100	30	Mouse	Mono
D2-40	M 3619 (Dako)	1:50	30	Mouse	Mono
VEGF-R1	AF 321 (R&D)	1:150	15	Goat	Poly
VEGF-R2	AF 357 (R&D)	1:50	15	Goat	Poly
VEGF-R3	AF 349 (R&D)	1:50	15	Goat	Poly

VEGF-R, vascular endothelial growth factor receptor.

Immunostaining Evaluation

The entire specimen was initially examined at low magnification (×40), and positive cells and vessels were counted in areas showing strong staining at higher magnification (×100). Immunostaining was assessed in three fields of view, and the average ratio was calculated. The percentage of vessels was defined as the ratio of positive vessels to the total number of CD34- and D2-40-positive vessels. Ratios >10% were considered significant (positive), and strong positive staining was defined as ≥60%. The cut-off value of strong positive staining (60%) was defined based on the median value. Microvessel densities (MVDs) of CD34- or D2-40-positive vessels were determined similarly to previous studies (13,14). However, MVD was quantified at lower magnification (×100) to compare VEGF receptors. Two investigators independently evaluated the immunostaining results without knowledge of clinical data.

Statistical Analysis

Statistical analysis was performed using SPSS version 11 software (SPSS Japan Inc., Tokyo, Japan). Spearman's rank correlation was used to assess the relationships between VEGF-R1, -R2, -R3, CD34 and D2-40. The χ^2 test was used to evaluate the relationships between expression of biomarkers and therapeutic effect. The Mann–Whitney test was used to examine the association of biomarkers with clinicopathological factors [i.e. age, sex, histological type (well-differentiated vs. others) and metastasis (lymph node metastasis and distant metastasis)]. Each factor and overall survival were determined by the Kaplan–Meier method and analyzed using the log-rank test. Multivariate analysis was performed using a Cox proportional hazard model.

RESULTS

Clinicopathological Characteristics

The clinicopathological characteristics of the patients are shown in Table 2. All patients underwent surgery to remove the primary lesion. At the time of primary resection, the tumor stage based on the TNM classification was II or III in 32 patients and distal metastasis (Stage IV) was confirmed in 59 patients. Well-differentiated carcinoma was found in 21 patients, moderately differentiated carcinoma was found in 63 patients and poorly differentiated or mucinous adenocarcinoma was identified in 7 patients histopathologically. All patients received chemotherapy, and first-line chemotherapy comprised 5-FU and leucovorin in 69 patients and other

Table 2. Patient characteristics ($n = 91$)

Factor	No. of patients	%
Age		
Median (range)	62 (27–77)	
Sex		
Man	53	58.2
Woman	38	41.8
ECOG performance status		
0/1/2	64/26/1	
Location		
Colon	60	65.9
Rectum	31	34.1
Stage		
II	3	3.3
III	29	31.9
IV	59	64.8
Differentiation		
Well	21	23.1
Moderately	63	69.2
Others	7	7.7
First-line chemotherapy		
5-FU/LV	69	75.8
5-FU c.i.	10	11.0
UFT/LV	9	9.9
S-1	2	2.2
UFT	1	1.1

5-FU, 5-fluorouracil; LV, leucovorin; UFT, uracil/tegafur; S-1, generic name.

agents in the remaining 22 patients (all 5-FU-based chemotherapy). The median follow-up time was 28.5 months (range: 5.5–88.1 months).

MVD of CD34-/D2-40-positive Vessels

The average MVD of CD34-positive vessels was 103 (44–247) and that of D2-40-positive vessels was 16 (1–43) at ×100 magnification (Fig. 1A and B). Neither CD34 MVD nor D2-40 MVD was correlated with clinicopathological factors. In addition, CD34 or D2-40 MVD showed no correlation with survival from surgery or chemotherapy.

Expression of VEGF-R1, -R2 and -R3

VEGF-R1 was stained on the tumor cell surface and stromal vessels (Fig. 1C and D). Specifically, VEGF-R1 was stained in tumor cells and vessels in 84 (92%) and 75 (82%) patients, respectively, and was strongly positively stained in tumor cells and vessels in 58 (64%) and 33 (36%) patients, respectively (Table 3).

VEGF-R2 and -R3 showed immunoreactivity mainly in tumor stromal vessels (Fig. 1F and H). VEGF-R2 was positively stained in 84 patients (92%) and strongly positively stained in 52 patients (57%). VEGF-R3 was positively stained in 85 patients (93%) and strongly positively stained in 60 patients (66%). Some CD34+ or D2-40+ vessels were immunoreactive with VEGF-R2 or -R3. VEGF-R3 was expressed not only in D2-40+ vessels, but also in CD34+ vessels. For tumor cells, VEGF-R2 and -R3 were stained in 5 (5%) and 22 (24%) patients, respectively, and were strongly stained in only 2 (2%) and 9 (10%) patients, respectively. VEGF-R1, -R2 and -R3 were not uniformly stained in some cases; however, no characteristic patterns were detected.

Correlation Between VEGF-R1, -R2 and -R3 and Clinicopathological Factors

Marked correlation was not found between VEGF-R1 staining and VEGF-R2 or -R3. A slight correlation was identified between VEGF-R2 and -R3 staining in vessels (Spearman's rank correlation coefficient: $\rho = 0.487$, $P < 0.001$). Significant correlation was not found between MVD (CD34 or D2-40) and staining of VEGF receptors. The χ^2 test showed no correlation between clinicopathological factors [i.e. age, sex, histological type (well differentiated vs. others) and metastasis (lymph node metastasis and distant metastasis)] and strong staining of VEGF receptors.

Relationship of VEGF-R Expression with Fluoropyrimidines

First-line chemotherapy based on 5-FU was administered to all patients, and the therapeutic effects were favorable (complete response or partial response) in 41 patients and unfavorable (non-responders, stable disease or progressive disease) in 50 patients. The χ^2 test showed no relationship between strong staining of VEGF receptors (R1, R2 and R3) and fluoropyrimidine efficacy (responder vs. non-responders) ($P = 0.67$, 0.67 and 0.19, respectively).

The relationship of survival with VEGF-R1, -R2 and -R3 staining was also assessed after chemotherapy. The median survival time after chemotherapy was 18.3 months for patients showing VEGF-R1strong positive staining and 22.6 months for other patients; 16.8 months for patients showing VEGF-R2 strong positive staining and 22.7 months for other patients; 18.5 months for patients showing VEGF-R3 strong positive staining and 21.1 months for other patients. Although the patients showing strong positive staining for VEGF receptors had poorer prognosis, no significant differences existed.

Relationship of VEGF-R1 Expression with Survival after Surgery in Stage II/III CRC

Among the 32 Stage II/III CRC patients, VEGF-R expression and survival after surgery were investigated. The

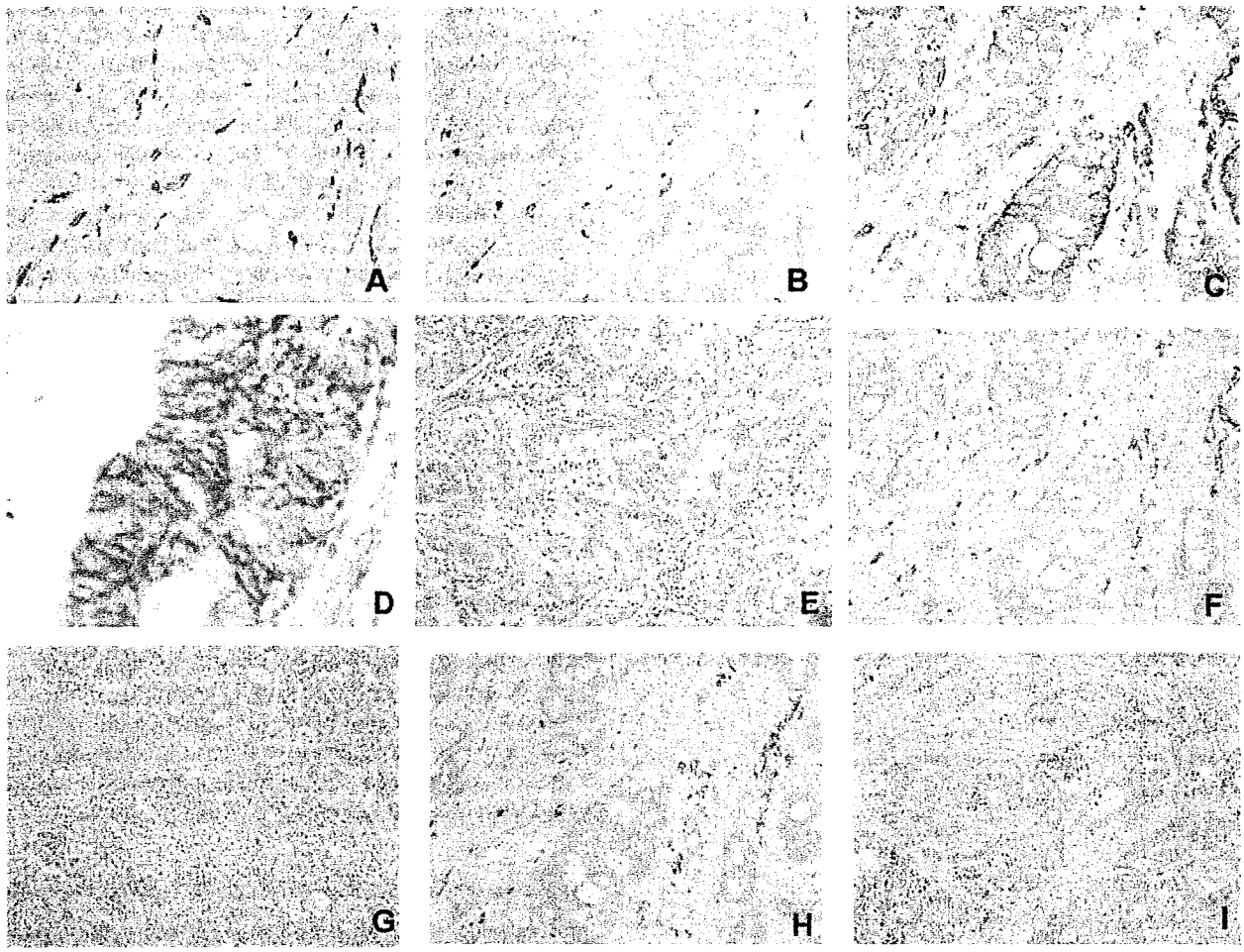

Figure 1. Typical examples of positive immunohistochemical staining for (A) CD34 (×100), (B) D2-40 (×100), (C) VEGF-R1 at low magnification (×100), (D) VEGF-R1 at high magnification (×400), (E) VEGF-R1 negative control (×100), (F) VEGF-R2 (×100), (G) negative control (×100), (H) VEGF-R3 (×100) and (I) negative control (×100). (C and D) VEGF-R1 staining is mainly observed on cancer cell surface and partially in stromal vessels. (F and H) VEGF-R2 and -R3 staining is mainly observed in stromal vessels. VEGF-R3 was expressed not only in D2-40+ vessels, but also in CD34+ vessels. VEGF-R, vascular endothelial growth factor receptor.

Table 3. Distribution of VEGF-R1, -R2 and -R3 expression

	VEGF-R1				VEGF-R2				VEGF-R3			
	Cell surface		Vessels		Cell surface		Vessels		Cell surface		Vessels	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Negative	7	8	16	18	86	95	7	8	69	76	6	7
Positive (>10%)	84	92	75	82	5	5	84	92	22	24	85	93
Strongly positive (>60%)	58	64	33	36	2	2	52	57	9	10	60	66
Weakly positive (≤60%)	26	28	42	46	3	3	32	35	13	14	25	27

median survival time of patients showing VEGF-R1 strong positive staining was 34.5 months and that of other patients was 47.4 months ($P = 0.014$, Fig. 2). Multivariate analysis

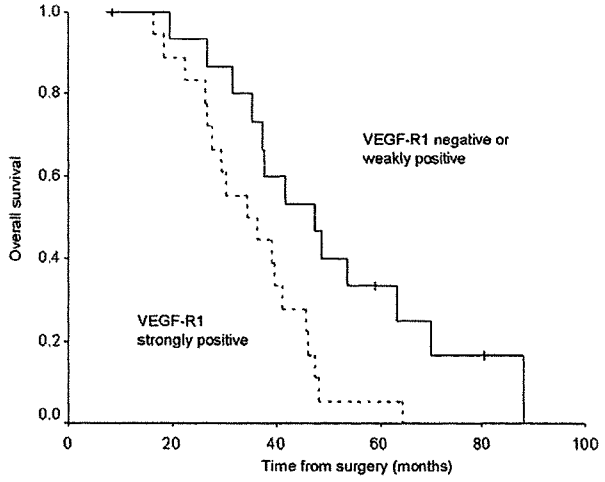

Figure 2. Impact of VEGF-R1 expression on patient survival. Kaplan–Meier estimates indicate shorter survival following surgery in patients showing VEGF-R1 strong positive staining ($P = 0.014$).

Table 4. Multivariate analyses of overall survival following surgery in 32 Stage II/III patients

Factor	Hazard ratio (95% CI)	P
VEGF-R1		
Strongly positive	1	0.02
Others	2.85 (1.16–6.99)	
Differentiation		
Well	1	0.39
Moderately and poorly	1.56 (0.57–4.26)	
Stage		
II	1	0.87
III	1.12 (0.29–4.35)	

showed VEGF-R1 strong positive staining as an independent poor prognostic factor for survival [hazard ratio, 2.85 (95% confidence interval; 1.16–6.99)] (Table 4). The median survival time of patients showing VEGF-R2 strong positive staining was 35.4 and that of other patients was 39.7 months, with no significant difference. The median survival time of patients showing VEGF-R3 strong positive staining was 35.4 and that of other patients was 39.7 months, with no significant difference.

DISCUSSION

This study showed that VEGF-R1 is mainly expressed in primary CRC cells, whereas VEGF-R2 and -R3 are mainly expressed in stromal vessels. Previous studies have shown that VEGF-R1 is expressed in CRC cell lines, such as HT-29 and KM12L4 (11,12) and an immunohistochemical study has also shown that VEGF-R1 was stained in tumor cells (11). These findings suggest that VEGF-R1 is mainly expressed in cancer cells and plays an important role in cancer proliferation. Takahashi et al. (15) reported no correlation between the expression of flt-1 (VEGF-R1) and clinicopathological factors after examining 52 patients with CRC and 10 patients with colon adenoma. Similar to the previous study, our study showed that VEGF-R1 strong staining did not correlate with clinicopathological findings. On the other hand, among Stage II/III patients, VEGF-R1 strong positive staining was an independent marker for poor prognosis. Yamaguchi et al. (10) reported that soluble VEGF-R1 expression was correlated with favorable prognosis. Some studies have reported that the expressions of VEGF, a VEGF-R1 ligand and the VEGF-A subtype correlated with advanced stage (6,7) and poor prognosis (8,9). Others have similarly shown that VEGF-R1 is important for proliferation of vascular endothelial cells (16) and migration of tumor cells (12,17). VEGF-R1 expression theoretically leads to tumor vessel proliferation and cell migration and causes cancer invasion and metastasis, thus we believe that VEGF-R1 strong positive staining is correlated with poor prognosis following surgery. The finding that VEGF-R1 was not correlated with MVD suggests that VEGF-R1 expressed on tumor cells might be more important for migration than proliferation of vascular endothelial cells. Here, the number of Stage II/III patients was small, and all patients received chemotherapy for recurrences. We therefore plan to examine a larger number of patients with Stage II/III CRC in the future.

VEGF-R2 and -R3 were mainly stained in intratumoral stromal vessels. Some CD34+ vessels were immunoreactive with VEGF-R2, suggesting that VEGF-R2 is mainly expressed in vascular endothelial cells. Meanwhile, VEGF-R3 has been thought to be expressed in lymphatic endothelia and involved in lymphangiogenesis. Here, VEGF-R3 was expressed not only in D2-40+ vessels, but also in CD34+ vessels. White et al. (18) also reported that VEGF-R3 was expressed in some CD31+ vessels, suggesting that VEGF-R3 is expressed in some lymphatic and vascular endothelial cells in the tumor stroma.

VEGF-R2 and -R3 showed no significant correlation with clinicopathological factors and prognosis. It was previously shown that VEGF-R2 expression was higher in metastatic tumors than in non-metastatic tumors in CRC, head and neck cancer (19) and breast cancer (20). Meanwhile, Yonemura et al. (21) have shown that VEGF-R3 expression demonstrated no correlation with lymph node metastasis and malignancy in gastric cancer. Importantly, a large number of patients are required to extensively clarify the interactions among VEGF-R subtypes and their clinical effects on angiogenesis and lymphangiogenesis.

Bevacizumab is a VEGF-neutralizing antibody and chemotherapy with bevacizumab and cytotoxic agents has been shown to prolong survival of CRC patients (4,22). Bevacizumab is regarded as an agent that suppresses cancer proliferation by directly blocking angiogenesis via the inhibition of VEGF, VEGF-R1 and -R2 as well as NP1/NP2 signal transduction. In breast cancer, HER-2 receptor expression was found in ~20–30% of affected patients, and studies have shown that trastuzumab, a monoclonal antibody against the HER-2 receptor, is significantly effective against HER-2-overexpressing breast cancer (23,24), with HER-2 receptor expression considered as one of the therapeutic criteria. On the other hand, fluoropyrimidine induces cell death by impairing nucleic acid synthesis and it also exerts slight effects on angiogenesis. Here, the expression of VEGF receptors showed no correlation with the therapeutic effects of fluoropyrimidine, although VEGF-R1 strong staining was correlated with shorter survival from surgery. We are going to evaluate the correlation between VEGF-R expression and the therapeutic effects of molecular-targeting agents, such as bevacizumab, containing regimens.

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Conflict of interest statement

None declared.

References

1. Senger DR, Perruzzi CA, Feder J, Dvorak HF. A highly conserved vascular permeability factor secreted by a variety of human and rodent tumor cell lines. *Cancer Res* 1986;46:5629–32.
2. Brown LF, Berse B, Jackman RW, Tognazzi K, Manseau EJ, Senger DR, et al. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in adenocarcinomas of the gastrointestinal tract. *Cancer Res* 1993;53:4727–35.
3. Brown LF, Berse B, Jackman RW, Tognazzi K, Guidi AJ, Dvorak HF, et al. Expression of vascular permeability factor (vascular endothelial growth factor) and its receptors in breast cancer. *Hum Pathol* 1995;26:86–91.
4. Hurwitz H, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, et al. Bevacizumab plus irinotecan, fluorouracil and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004;350:2335–42.
5. Sandler A, Gray R, Perry MC, Brahmer J, Schiller JH, Dowlati A, et al. Paclitaxel-carboplatin alone or with bevacizumab for non small cell lung cancer. *N Engl J Med* 2006;355:2542–50.
6. Kumar H, Heer K, Lee PWR, Duthie GS, MacDonald AW, Greenman J, et al. Preoperative serum vascular endothelial growth factor can predict stage in colorectal cancer. *Clin Cancer Res* 1998;4:1279–85.
7. Tokunaga T, Oshika Y, Abe Y, Ozeki Y, Sadahiro S, Kijima H, et al. Vascular endothelial growth factor (VEGF) mRNA isoform expression pattern is correlated with liver metastasis and poor prognosis in colon cancer. *Br J Cancer* 1998;77:998–1002.
8. Ishigami SI, Arii S, Furutani M, Niwano M, Harada T, Mizumoto M, et al. Predictive value of vascular endothelial growth factor (VEGF) in metastasis and prognosis of human colorectal cancer. *Br J Cancer* 1998;78:1379–84.
9. Ellis LM, Takahashi Y, Liu W, Shaheen RM. Vascular endothelial growth factor in human colon cancer: Biology and therapeutic implications. *Oncologist* 2000;5:11–5.
10. Yamaguchi T, Bando H, Mori T, Takahashi K, Matsumoto H, Yasutome M, et al. Overexpression of soluble vascular endothelial growth factor receptor 1 in colorectal cancer: association with progression and prognosis. *Cancer Sci* 2007;98:405–10.
11. Fan F, Wey JS, McCarty MF, Belcheva A, Liu W, Bauer TW, et al. Expression and function of vascular endothelial growth factor receptor-1 on human colorectal cancer cells. *Oncogene* 2005;24:2647–53.
12. Lesslie DP, III, Summy JM, Parikh NU, Fan F, Sawyer TK, Metcalf CA III, et al. Vascular endothelial growth factor receptor-1 mediates migration of human colorectal carcinoma cells by activation of Src family kinases. *Br J Cancer* 2006;94:1710–7.
13. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis-correlation in invasive breast carcinoma. *N Engl J Med* 1991;324:1–8.
14. Choi WW, Lewis MM, Lawson D, Yin-Goen Q, Birdsong GG, Cotsonis GA, et al. Angiogenic and lymphangiogenic microvessel density in breast carcinoma: correlation with clinicopathologic parameters and VEGF-family gene expression. *Mod Pathol* 2005;18:143–52.
15. Takahashi Y, Kitadai Y, Bucana CD, Cleary KR, Ellis LM. Expression of vascular endothelial growth factor and its receptor, KDR, correlates with vascularity, metastasis, and proliferation of human colon cancer. *Cancer Res* 1995;55:3964–8.
16. Sibuya M, Welsh LC. Signal transduction by VEGF receptors in regulation of angiogenesis and lymphangiogenesis. *Exp Cell Res* 2006;312:549–60.
17. Barleon B, Sozzani S, Zhou D, Weich HA, Mantovani A, Marme D. Migration of human monocytes in response to vascular endothelial growth factor (VEGF) is mediated via the VEGF receptor flt-1. *Blood* 1996;87:3336–43.
18. White JD, Hewett PW, Kosuge D, McCulloch T, Enholm BC, Carmichael J, et al. Vascular endothelial growth factor-D expression is an independent prognostic marker for survival in colorectal carcinoma. *Cancer Res* 2002;62:1669–75.
19. Moriyama M, Kumagai S, Kawashiri S, Kojima K, Kakihara K, Yamamoto E. Immunohistochemical study of tumour angiogenesis in oral squamous cell carcinoma. *Oral Oncol* 1997;33:369–74.
20. Valtola R, Salven P, Heikkaila P, Taipale J, Joensuu H, Rehn M, et al. VEGFR-3 and its ligand VEGF-C are associated with angiogenesis in breast cancer. *Am J Pathol* 1999;154:1381–90.
21. Yonemura Y, Endo Y, Fujita H, Fushida S, Ninomiya I, Bandou E, et al. Role of vascular endothelial growth factor C expression in the development of lymph node metastasis in gastric cancer. *Clin Cancer Res* 1999;5:1823–9.
22. Kabbinavar FF, Schulz J, McCleod M, Patel T, Hamm JT, Hecht JR, et al. Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. *J Clin Oncol* 2005;23:3697–705.
23. Vogel CL, Cobleigh MA, Tripathy D, Gutheil JC, Harris LN, Fehrenbacher L, et al. Efficacy and safety of trastuzumab as a single agent in first-line treatment of HER2-overexpressing metastatic breast cancer. *J Clin Oncol* 2002;20:719–26.
24. Seidman AD, Fornier MN, Esteva FJ, Tan L, Kaptain S, Bach A, et al. Weekly trastuzumab and paclitaxel therapy for metastatic breast cancer with analysis of efficacy by HER2 immunophenotype and gene amplification. *J Clin Oncol* 2001;19:2587–95.

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ORIGINAL ARTICLE

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Safety and pharmacokinetics of panitumumab in Japanese patients with advanced solid tumors

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Abstract
Background. Panitumumab is a fully human, monoclonal antibody against the epidermal growth factor receptor. Previous studies in non-Japanese patients with solid tumors showed that panitumumab exhibited nonlinear pharmacokinetics, was well tolerated (skin toxicities were the most common treatment-related adverse events), and had antitumor activity in some patients. This open-label, phase 1 study investigated panitumumab safety and pharmacokinetics in Japanese patients.
Methods. Japanese patients with advanced solid tumors were enrolled into one of three sequential panitumumab dose cohorts (cohort 1, 2.5 mg/kg weekly; cohort 2, 6.0 mg/kg every 2 weeks; and cohort 3, 9.0 mg/kg every 3 weeks) and received panitumumab until disease progression or drug intolerability. Safety endpoints included the incidence of adverse events, changes in laboratory values, and the appearance of anti-panitumumab antibodies. Serial pharmacokinetic samples were collected after the first and third doses of panitumumab. Tumors were assessed at week 8 and every 8 weeks thereafter.
Results. Eighteen patients (6 per cohort) were enrolled. No dose-limiting toxicities, investigator-reported infusion reactions, or deaths occurred. Seven patients had grade-3/4 adverse events; fatigue and anorexia were most common. The most common skin toxicities were rash and acneiform dermatitis. No neutralizing anti-panitumumab antibodies were detected. Panitumumab exhibited nonlinear pharmacokinetics, and antitumor activity was observed in 31% (4/13) of the patients with colorectal cancer.
Conclusion. In Japanese patients with solid tumors, panitumumab was well tolerated, demonstrated pharmacokinetic and safety profiles similar to those observed previously in non-Japanese patients, and exhibited encouraging antitumor activity in patients with colorectal cancer.

Key words Epidermal growth factor receptor · Panitumumab · Pharmacokinetics · Safety

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Introduction

Epidermal growth factor receptor (EGFR) is a transmembrane tyrosine kinase that promotes cell growth in a variety of normal and transformed tissues,[1] and EGFR overexpression has been associated with multiple types of malignancies.[2,3] Panitumumab (Vectibix; Amgen, Thousand Oaks, CA, USA) is a fully human, immunoglobulin G_2, monoclonal antibody that targets human EGFR.[4–6]

Phase 1 and phase 1/2 studies have evaluated various panitumumab doses and schedules in patients with previously treated advanced, solid tumors.[7,8] Panitumumab doses and schedules studied included 0.01 mg/kg to 5 mg/kg weekly (QW), 6 mg/kg every 2 weeks (Q2W), and 9 mg/kg every 3 weeks (Q3W). Skin-related toxicities, an expected class effect of anti-EGFR therapy,[9] were the most frequently observed adverse events. Panitumumab exhibited nonlinear pharmacokinetics characterized by decreased serum clearance with increased dose.

Phase 2 studies and a phase 3 study have examined the efficacy and safety of panitumumab monotherapy in patients with previously treated metastatic colorectal cancer.[10–14] Response rates were not generally associated with levels of EGFR (as measured by immunohistochemistry) on tumor

cell membranes.[10,13] Panitumumab is approved in the United States as a single agent for the treatment of metastatic colorectal carcinoma with disease progression on or following fluoropyrimidine, oxaliplatin, and irinotecan chemotherapy regimens.[15]

The phase 1 study presented here is the first formal evaluation of panitumumab in patients of Japanese origin living in Japan. This study examined the safety and pharmacokinetics of panitumumab in Japanese patients with advanced solid tumors.

Patients and methods

Study design and objectives

The primary objective of this open-label, phase 1 clinical study was to evaluate the safety and pharmacokinetic profiles of panitumumab in Japanese patients with solid tumors. Other objectives included the assessment of potential anti-panitumumab antibody formation and the evaluation of tumor response. The study was performed at National Cancer Center Hospital East, Kashiwa, Japan, and National Cancer Center Hospital, Tokyo, Japan. The study was approved by institutional review boards at both study sites. Written informed consent was obtained from each patient before study procedures were performed.

Patients were enrolled into one of three sequential dose cohorts. The planned sample size was 6 patients per cohort.[16] All patients enrolled in each cohort were evaluated for dose-limiting toxicities (DLTs); if none or one of the six subjects enrolled in the cohort experienced a DLT, enrollment in the next dosing cohort was initiated after a review of the safety assessments by the investigator and the sponsor. If 33% or more of the patients in the most recently initiated cohort experienced DLTs, the dose schedule was to be regarded as intolerable and initiation of the succeeding cohort was to proceed, contingent upon the outcome of consultation between the investigator and an independent safety committee. Cohort 1 received panitumumab 2.5 mg/kg QW, cohort 2 received panitumumab 6.0 mg/kg Q2W, and cohort 3 received panitumumab 9.0 mg/kg Q3W. Patients received panitumumab until disease progression, panitumumab intolerability, or other reasons for discontinuation.

Panitumumab used in this study was derived from Chinese hamster ovary cells on a commercial scale of 12 kl. Panitumumab was administered intravenously over 60 min by an infusion pump through a peripheral line or indwelling catheter, using a 0.22-micron in-line filter.

Patient eligibility

Patients of Japanese origin with documented, advanced solid tumors that were refractory to standard chemotherapy, or for which no standard therapy was available, were eligible for participation. All patients were to be between 20 and 74 years of age (inclusive) at the time of giving informed consent; have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 to 2; have adequate hematological, liver, and renal functions; and a life expectancy of 3 months or more. Patients could not have received other antibody therapy within 12 weeks prior to receiving the first dose of panitumumab. Patients who had received previous therapy with an anti-EGFR antibody were excluded; however, previous therapy with small-molecule EGFR tyrosine kinase inhibitors was permitted. Patients could not have received anticancer therapy, radiotherapy, or surgery within 4 weeks prior to the first dose of panitumumab.

Patients were not eligible to participate in this study if they had hematological malignancy, metastasis to the central nervous system, or another simultaneously active primary cancer (except for curatively treated cervical cancers, curatively resected nonmelanoma skin cancers, or other curatively treated primary solid tumors). Patients with a history of interstitial pneumonitis, pulmonary fibrosis, or evidence of such were ineligible. Patients who had concurrent diseases such as poorly controlled diabetes, infections requiring systemic administration of antibiotics, congestive heart failure, pulmonary embolism, deep vein thromboses, or a history of severe hypersensitivity to any medications were not enrolled. Patients with a myocardial infarction and/or angina within 1 year prior to registration were ineligible.

Safety assessments

Safety was monitored from the first dose of panitumumab up to 28 days after the last dose. Adverse events were coded according to a medical dictionary for regulatory activities (MedDRA, version 9.0). Toxicity was graded according to the National Cancer Institute Common Toxicity Criteria (NCI CTC, version 2.0); however, skin toxicities were graded as per modified Common Terminology Criteria for Adverse Events (CTCAE) version 3.0.

Patients were assessed for DLTs for up to 4 weeks (cohorts 1 and 2) or up to 3 weeks (cohort 3) after the first administration of panitumumab. Enrollment of cohorts 2 and 3 was initiated only after the DLT evaluation periods in prior cohorts. A DLT was defined as treatment-related NCI CTC grade 4 hematological toxicity; grade 3 or 4 diarrhea, nausea, or vomiting that developed in the presence of best supportive care; grade 3 or 4 fatigue that continued for 7 or more days; aspartate aminotransferase or alanine aminotransferase more 300 IU/l; or other grade 3 or 4 toxicities (including skin toxicities) not previously stated (with the exception of infusion reactions). Safety monitoring for all adverse events was also conducted during the DLT evaluation periods.

At the second or subsequent panitumumab infusion, panitumumab was withheld for patients in any cohort who had grade 3/4 skin toxicity or fatigue. If skin toxicity (all cohorts) and fatigue (cohort 1) had resolved to grade 2 or less by the next scheduled dose, panitumumab was restarted; otherwise the patient was withdrawn from the study. For patients in cohorts 2 and 3, if fatigue had not resolved to grade 2 or less after 7 days, the patient was withdrawn from the study.

Serum samples to be tested for anti-panitumumab antibodies were collected both before administration of panitumumab and at: weeks 1, 2, 3, 4, 7, and 23 after administration for cohort 1; weeks 1, 3, 5, 7, and 23 for cohort 2; and weeks 1, 4, 7, 10, and 25 for cohort 3. Two validated assays were used to detect the potential presence of anti-panitumumab antibodies: a screening enzyme-linked immunosorbent assay (ELISA) and a cell-based bioassay to detect neutralizing antibodies.[17,18]

Pharmacokinetics

Serial serum samples were collected after the first and third panitumumab doses for the measurement of panitumumab serum concentrations. For patients in cohort 1, samples were collected predose and at 0.5, 1, 4, 8, 24, 96, and 168 h after completion of the infusion on weeks 1 and 3. For patients in cohort 2, samples were collected predose and at 0.5, 24, 96, 168, 240, and 336 h after completion of the infusion on weeks 1 and 5. For patients in cohort 3, samples were collected predose and at 0.5, 24, 96, 168, 336, and 504 h after completion of infusion on weeks 1 and 7. A validated immunoassay with electrochemiluminescence (ECL) detection was used to measure panitumumab concentrations in the serum samples.

Noncompartmental pharmacokinetic analyses were performed using WinNonlin Professional, Version 4.1e (Pharsight, Mountain View, CA, USA). The pharmacokinetic parameters (recorded as observed) included the maximum concentration (C_{max}) after dosing and the trough concentration (predose concentration before the next panitumumab dose, C_{min}). A singular representation of half-life ($t_{1/2}$) approximated during the dosing interval was calculated as $t_{1/2} = \frac{\ln(2)}{k_{el}}$, where k_{el} was the first-order terminal rate constant estimated via linear regression of the terminal log-linear decay phase, and ln(2) is the natural log of 2. The area under the serum concentration-time curve from time 0 to the end of the dosing interval ($AUC_{0\text{-}tau}$) was estimated using the linear/log trapezoidal method.

Tumor response assessments

Imaging and clinical assessments were obtained at week 8 and once every 2 months thereafter as needed. Tumor response to treatment was assessed using the Response Evaluation Criteria in Solid Tumor (RECIST) guidelines.[19] Response was confirmed 4 weeks or more after the criteria for response were first met.

Statistical analyses

The DLT analysis set comprised patients who completed the DLT evaluation period. All patients who received one or more doses of panitumumab were included in the safety analysis set. Pharmacokinetic analyses were performed on patients who had received the correct dosage of panitumumab (within 20% of the nominal dosage) and had recorded dosing and sampling times (pharmacokinetics analysis set). The efficacy analysis set comprised patients who had measurable disease at baseline.

Descriptive statistics were provided for all endpoints by cohort. Continuous measurements were summarized with the central tendency (mean or median) and variability (standard deviation [SD] or standard error of the mean [SEM]). Categorical data were summarized using frequency counts and percentages of patients. Tumor response data were categorized using RECIST.

Results

Demographics

Eighteen patients (6 patients in each cohort) of Japanese origin were enrolled at two centers in Japan between January 2005 and March 2006. All patients received one or more doses of panitumumab. All patients completed treatment with panitumumab until disease progression. Baseline demographics and disease characteristics are shown in Table 1.

Safety

In total, 154 panitumumab infusions were administered across the three cohorts. The median number of infusions per patient was 12.5 in cohort 1, 5.5 in cohort 2, and 3.5 in cohort 3. The median average panitumumab dosage delivered (weight-adjusted cumulative dose divided by the number of infusions) was 2.55 mg/kg per infusion in cohort 1, 6.14 mg/kg per infusion in cohort 2, and 8.98 mg/kg per infusion in cohort 3. The mean (SD) weight-adjusted cumulative dose of panitumumab was 35.8 (18.0) mg/kg in cohort 1, 42.0 (29.4) mg/kg in cohort 2, and 43.5 (38.7) mg/kg in cohort 3. No adverse events led to discontinuation of panitumumab, and all patients discontinued panitumumab as a result of disease progression.

No DLTs were observed during the DLT assessment period in any dose cohort and all cohorts were fully enrolled. No maximum tolerable dose was reached. One patient in cohort 2 was excluded from the DLT analysis set (but was not replaced in the study) as a second infusion of panitumumab was not administered because of an adverse event during the DLT assessment period that was not considered to be related to panitumumab.

A summary of adverse event grades by worst grade observed is provided in Table 2. No patients died during the treatment or follow-up periods; however, one patient died of disease progression 41 days after the last dose of panitumumab. All patients had one or more treatment-related adverse events; the most common treatment-related adverse events were acneiform dermatitis and rash (type not specified). A patient in cohort 1 had a serious treatment-related adverse event of grade 3 edema, which occurred 1 day after the twenty-second (and last) panitumumab infusion.

Table 1. Baseline demographics and disease characteristics (safety analysis set)

	2.5 mg/kg QW Cohort 1 (n = 6)	6.0 mg/kg Q2W Cohort 2 (n = 6)	9.0 mg/kg Q3W Cohort 3 (n = 6)	All patients (n = 18)
Sex, n (%)				
Men	5 (83)	3 (50)	5 (83)	13 (72)
Women	1 (17)	3 (50)	1 (17)	5 (28)
Age (years)				
Median	50	58	60	55
Minimum, maximum	39, 67	25, 73	32, 70	25, 73
Weight (kg)				
Median	68	58	64	63
Minimum, maximum	48, 91	36, 81	48, 76	36, 91
ECOG status, n (%)				
0–1	5 (83)	6 (100)	6 (100)	17 (94)
2	1 (17)	0 (0)	0 (0)	1 (6)
Tumor type, n (%)				
Colon or rectum	5 (83)	5 (83)	3 (50)	13 (72)
Stomach	1 (17)	0 (0)	0 (0)	1 (6)
Ovary	0 (0)	0 (0)	1 (17)	1 (6)
Esophagus	0 (0)	0 (0)	1 (17)	1 (6)
Head and neck	0 (0)	1 (17)	1 (17)	2 (11)

QW, once weekly; Q2W, once every 2 weeks; Q3W, once every 3 weeks; ECOG, Eastern Cooperative Oncology Group

Table 2. Summary of adverse event grades[a]

	2.5 mg/kg QW Cohort 1 (n = 6)	6.0 mg/kg Q2W Cohort 2 (n = 6)	9.0 mg/kg Q3W Cohort 3 (n = 6)	All patients (n = 18)
Any adverse event – n (%)	6 (100)	6 (100)	6 (100)	18 (100)
Grade 3	2 (33)	2 (33)	1 (17)	5 (28)
Grade 4	2 (33)	0 (0)	0 (0)	2 (11)
Any serious event	5 (83)	3 (50)	1 (17)	9 (50)
Any treatment-related event – n (%)	6 (100)	6 (100)	6 (100)	18 (100)
Grade 3	1 (17)	0 (0)	1 (17)	2 (11)
Grade 4	0 (0)	0 (0)	0 (0)	0 (0)
Any serious event	1 (17)	0 (0)	0 (0)	1 (6)

QW, once weekly; Q2W, once every 2 weeks; Q3W, once every 3 weeks
[a]No grade 5 events were observed

Table 3. Adverse events,[a] excluding skin-related toxicities, occurring in 20% or more of the patients

Event – n	2.5 mg/kg QW Cohort 1 (n = 6)		6.0 mg/kg Q2W Cohort 2 (n = 6)		9.0 mg/kg/Q3W Cohort 3 (n = 6)		All patients (n = 18)	
	Any	≥Grade 3	Any	≥Grade 3	Any	≥Grade 3	Any	≥Grade 3
Fatigue	4	1	3	0	4	1	11	2
Anorexia	3	1	4	1	3	0	10	2
Constipation	5	0	1	0	2	0	8	0
Diarrhea	3	0	2	0	2	0	7	0
Nausea	2	0	3	0	1	0	6	0
Stomatitis	1	0	2	0	3	0	6	0
Weight loss	2	0	2	0	1	0	5	0
Back pain	1	0	1	0	2	0	4	0
Pyrexia	2	0	1	1	1	0	4	1
Vomiting	0	0	3	1	1	0	4	1

QW, once weekly; Q2W, once every 2 weeks; Q3W, once every 3 weeks
[a]All treatment-related and -unrelated events were summarized

The treatment-related grade 3 event in cohort 3 was fatigue, which was not deemed to be serious. All other treatment-related adverse events were reported to be less than grade 3.

Adverse events (excluding skin-related toxicities) occurring in at least 20% of patients are tabulated in Table 3. The incidence of these events was similar for each cohort; however, constipation was most frequent in cohort 1. Fatigue and anorexia were the most frequently observed nonskin-related toxicities. The incidence and frequency of skin-related toxicities occurring in at least 10% of patients was similar in all study cohorts (Table 4), and all patients

Table 4. Skin-related toxicities of any grade occurring in 10% or more of the patients

Event – *n* (%)	2.5 mg/kg QW Cohort 1 (*n* = 6)	6.0 mg/kg Q2W Cohort 2 (*n* = 6)	9.0 mg/kg Q3W Cohort 3 (*n* = 6)	All patients (*n* = 18)
Any skin toxicities[a]	6 (100)	6 (100)	6 (100)	18 (100)
Acneiform dermatitis	6 (100)	4 (67)	5 (83)	15 (83)
Worst grade of 2	2 (33)	4 (67)	4 (67)	10 (56)
Rash	4 (67)	5 (83)	6 (100)	15 (83)
Worst grade of 2	3 (50)	4 (67)	3 (50)	10 (56)
Pruritus	4 (67)	5 (83)	5 (83)	14 (78)
Worst grade of 2	1 (17)	1 (17)	1 (17)	3 (17)
Dry skin	4 (67)	4 (67)	3 (50)	11 (61)
Worst grade of 2	1 (17)	1 (17)	0 (0)	2 (11)
Paronychia	2 (33)	3 (50)	2 (33)	7 (39)
Worst grade of 2	1 (17)	3 (50)	2 (33)	6 (33)
Skin fissures	2 (33)	4 (67)	0 (0)	6 (33)
Worst grade of 2	0 (0)	2 (33)	0 (0)	2 (11)
Erythema	2 (33)	0 (0)	1 (17)	3 (17)
Nail disorder	1 (17)	0 (0)	2 (33)	3 (17)
Worst grade of 2	0 (0)	0 (0)	1 (17)	1 (6)
Conjunctivitis	1 (17)	0 (0)	1 (17)	2 (11)
Hypertrichosis	0 (0)	1 (17)	1 (17)	2 (11)
Palmar-plantar erythrodysesthesia syndrome	1 (17)	0 (0)	1 (17)	2 (11)

QW, once weekly; Q2W, once every 2 weeks; Q3W, once every 3 weeks
[a]Treatment-related toxicities were summarized. No events of grade 3 or more were observed

reported at least one event of skin-related toxicity. Acneiform dermatitis, rash, pruritus, and dry skin were the most frequently observed skin-related toxicities but there were no grade 3 or 4 skin-related toxicities reported. Categorization of skin complaints by individual investigators varied, which led to some overlap in the reported incidence of these adverse events.

Three patients (50%) in cohort 1, three patients (50%) in cohort 2, and four patients (67%) in cohort 3 had hypomagnesemia relative to their baseline serum magnesium concentration. Of these, one patient (17%) in cohort 1 had grade 3 hypomagnesemia, and one patient (17%) in cohort 2 had grade 4 hypomagnesemia.

No infusion-related reactions were reported by the investigators. A post-hoc analysis of adverse-event terms indicated that four panitumumab infusions (in 2 patients in cohort 1 and 1 patient in cohort 2) may have been associated with symptoms that might conservatively be interpreted as a "potential" infusion reaction. Symptoms of the potential infusion reactions included mild flushing and hot flush (during multiple infusions in a patient in cohort 1), mild fatigue (on the day of the twentieth infusion in another patient in cohort 1), and mild headache (with the twelfth infusion in the patient in cohort 2). All symptoms resolved without treatment except for the mild headache, which was treated with aspirin.

All patients had predose and post-dose samples available for antibody analyses. No neutralizing antibodies to panitumumab were detected.

Pharmacokinetics

After the first panitumumab dose, the mean exposure to panitumumab ($AUC_{0\text{-}tau}$) increased more than dose proportionally (from 135 to 1430 μg·day/ml as dosage increased from 2.5 mg/kg to 9.0 mg/kg), as shown in Table 5. Previous study results demonstrated that panitumumab exhibited nonlinear pharmacokinetics;[7,8] therefore, the AUC was not extrapolated to infinity, which is commonly used for clearance estimation. However, in the present study, $AUC_{0\text{-}tau}$ approximated $AUC_{0\text{-}inf}$ (Fig. 1), indicating that the time-averaged clearance decreased as the dosage increased from 2.5 to 9.0 mg/kg in this study population. Based on the mean $AUC_{0\text{-}tau}$ values, the accumulation ratios after the third dose of panitumumab were 1.92 at 2.5 mg/kg QW, 1.66 at 6.0 mg/kg Q2W, and 1.39 at 9.0 mg/kg Q3W. The mean dose-normalized $AUC_{0\text{-}tau}$ values after the third panitumumab dose were similar for 6.0 mg/kg Q2W (183 ± 16 μg·day/ml per mg per kg) and 9.0 mg/kg Q3W (221 ± 103 μg·day/ml per mg per kg).

Tumor response

All 18 patients had measurable disease at baseline and were included in the efficacy analysis set. Two patients in cohort 1 and 1 patient in each of cohorts 2 and 3 had a confirmed partial response to panitumumab. Stable disease at each assessment was observed as the best objective response for 2 patients in each cohort. Two patients in cohort 1 and 3 patients in each of cohorts 2 and 3 had progressive disease. All patients who responded to panitumumab had colorectal cancer.

Discussion

This phase 1 study evaluated the safety, efficacy, and pharmacokinetic profiles of panitumumab administered to 18 Japanese patients with advanced solid tumors at three dose

Table 5. Summary of panitumumab pharmacokinetic parameter values after the first and third doses of panitumumab

Parameter	First dose[a]			Third dose[a]		
	2.5 mg/kg QW Cohort 1 (n = 6)	6.0 mg/kg Q2W Cohort 2 (n = 6)	9.0 mg/kg Q3W Cohort 3 (n = 6)	2.5 mg/kg QW Cohort 1 (n = 6)	6.0 mg/kg Q2W Cohort 2 (n = 5)	9.0 mg/kg Q3W Cohort 3 (n = 4)
C_{max} (μg/ml)	44.1 (8.1)	118 (31)	231 (45)	68.4 (13.8)	160 (14)	291 (117)
$AUC_{0\text{-}tau}$ (μg·day/ml)	135 (35)	664 (80)	1430 (420)	259 (67)	1100 (100)	1990 (930)
C_{min} (μg/ml)	7.9 (3.1)	19.8 (3.9)	16.9 (5.9)	20.9 (7.1)	42.5 (8.5)	28.4 (24.8)
$t_{1/2}$ (day)	3.1 (0.5)	6.7 (0.7)	7.2 (1.7)	4.5 (1.0)	9.6 (2.7)	6.8 (2.6)

QW, once weekly; Q2W, once every 2 weeks; Q3W, once every 3 weeks; C_{max}, maximum concentration after dosing; $AUC_{0\text{-}tau}$, area under the serum-concentration time curve from time 0 to the end of the dosing interval; C_{min}, trough concentration; $t_{1/2}$, half-life
[a]Data are presented as means (SD)

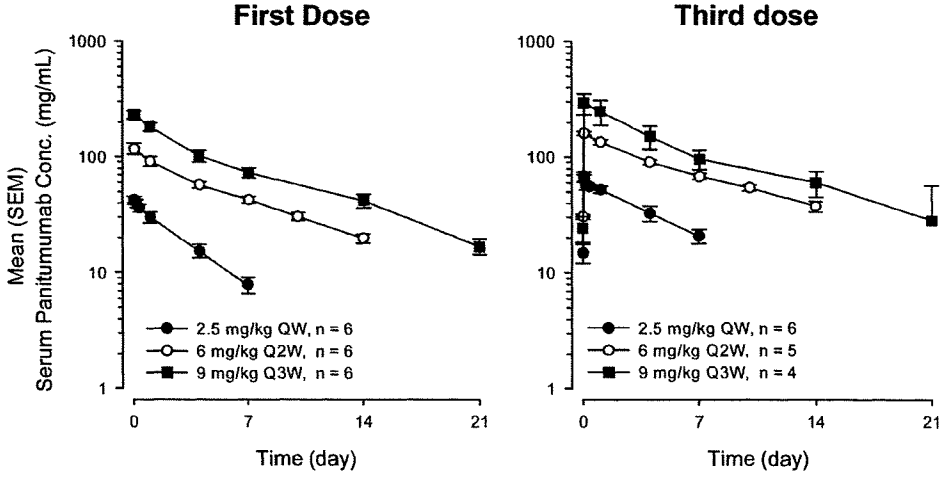

Fig. 1. Serum concentration-time profiles for panitumumab. Mean concentrations of panitumumab (mg/ml) after the first dose (*left panel*) and the third dose (*right panel*) are shown. *QW*, Every week

schedules (2.5 mg/kg QW, 6.0 mg/kg Q2W, and 9.0 mg/kg Q3W), which were generally well tolerated and exhibited nonlinear pharmacokinetic profiles.

The safety profile observed in a previous phase 1, dose-ranging study conducted in non-Japanese patients was similar to that in the present study in that no maximum tolerable dose was reached, skin toxicities were the most common adverse events, and grade 3/4 treatment-related adverse events occurred in approximately 10% of patients.[8] In the present study, the overall incidence and severity of skin toxicities and eye-related toxicities (which are characteristic of EGFR inhibition)[9] were within expectations for patients receiving anti-EGFR antibody therapy. No DLTs were observed, and no clear dose relationship was observed for adverse events, serious adverse events, or severe/life-threatening (grade 3/4) adverse events. Hypomagnesemia was observed in this study, this being a common toxicity seen in patients who receive anti-EGFR antibodies.[20–22]

No grade 3 or 4 skin toxicities were reported, in contrast with rates of up to 14% reported in prior studies of panitumumab monotherapy.[9] The lack of grade 3 or 4 skin toxicities in this study may be due to the small number of patients participating in the trial, which may not adequately represent a larger patient population. Additionally, because of the awareness of severe skin toxicities associated with anti-EGFR antibody therapies, the investigators were more likely to monitor and manage skin events. It is possible that active management of skin toxicities may therefore have prevented the development of some grade 3 or 4 events. Indeed, interim data from the Skin Toxicity Evaluation Protocol with Panitumumab (STEPP) study have shown that patients whose skin toxicities are proactively managed have significantly fewer grade 2 or higher skin-related adverse events.[23]

Similar to findings in non-Japanese patients, panitumumab exhibited nonlinear pharmacokinetics in the Japanese study population and the increase in exposure to panitumumab was more than dose-proportional. A nonlinear pharmacokinetic profile has also been observed with another anti-EGFR antibody, cetuximab; clearance of cetuximab decreases with increasing doses.[24] It has been proposed that an anti-EGFR antibody that is bound to cell-surface EGFR can be internalized and degraded; therefore, nonlinear clearance may be the result of progression saturation of the EGFR as the dosage increases.[24,25] The distribution of individual AUC values for Japanese patients was within the range observed for non-Japanese patients; therefore, these results suggested that panitumumab pharmacokinetics are comparable in Japanese and non-Japanese patients (Fig. 2).

Partial responses in 4 of 13 Japanese patients with colorectal cancer were confirmed in the present study, sug-

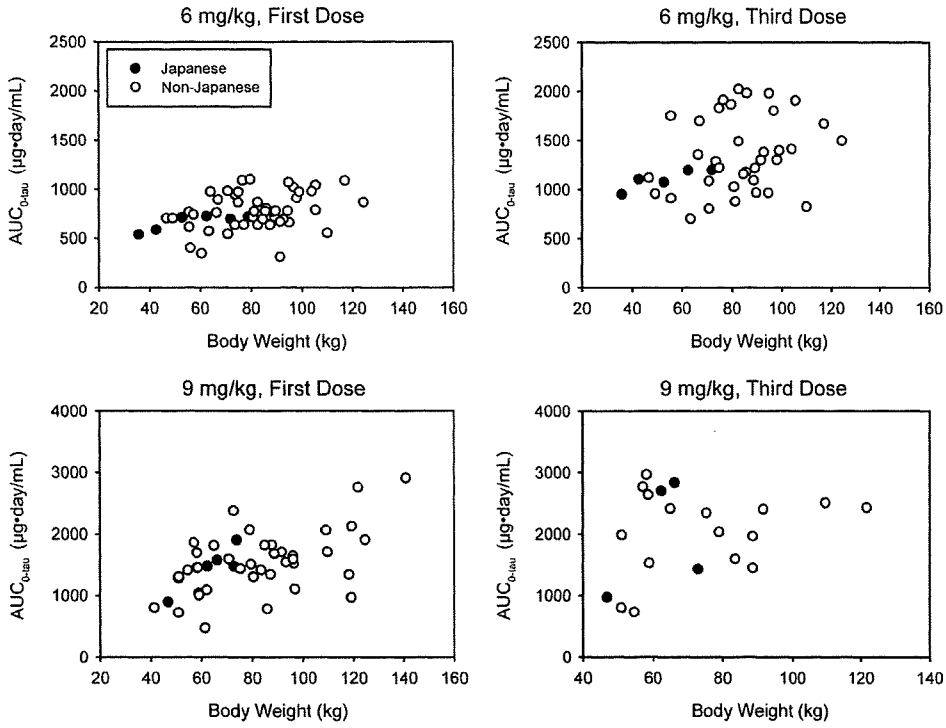

Fig. 2. Panitumumab exposure in Japanese and non-Japanese patients. *Data points* represent the area under the serum concentration-time curve from time 0 to the end of the dosing interval ($AUC_{0\text{-}tau}$) after the first (*left panels*) and third (*right panels*) doses for patients receiving panitumumab 6 mg/kg (*upper panels*) and 9 mg/kg (*lower panels*). Data are shown for both Japanese (*closed circles*) and non-Japanese (*open circles*) patients

gesting promising activity in this patient population. The patient population in this study differed from that in a study of similar design in non-Japanese patients[14] in that only 1 of the 13 patients in this study was treated with oxaliplatin. However, all 4 responders were previously treated with irinotecan and 5-fluorouracil. Therefore, panitumumab efficacy was observed in heavily pretreated Japanese patients with colorectal cancer and warrants further study.

In the present study, an additional, unconfirmed partial response was also noted in a patient with esophageal cancer who had relapsed at the perigastric lymph node after prior radiotherapy and chemotherapy. This patient achieved a partial response after receiving three cycles of panitumumab 9.0 mg/kg Q3W (after four cycles of panitumumab, the patient had disease progression). Previous studies have shown that squamous-cell carcinomas express EGFR, EGFR expression is associated with the progression of esophageal cancer,[26] and anti-EGFR agents such as gefinitib and cetuximab have efficacy for treating esophageal cancer.[27]

Recent studies have shown that the presence of a mutated *KRAS* gene in tumors from patients with metastatic colorectal cancer is a negative predictor of response to anti-EGFR antibody therapies.[28,29] The phase 1 study reported here was not designed to evaluate *KRAS* status with respect to response to panitumumab therapy in our patient population; in an ongoing analysis, *KRAS* tumor status is available for only eight patients (Amgen; data on file). Therefore, the effect of tumor *KRAS* status on the efficacy and safety of treatment with panitumumab in Japanese patients with metastatic colorectal cancer is unknown at this time.

Currently, there are no anti-EGFR antibody drugs approved in Japan for treating colorectal cancer patients. The results of the present study in Japanese patients with solid tumors suggest that panitumumab is well tolerated at doses of 2.5 mg/kg QW, 6.0 mg/kg Q2W, and 9.0 mg/kg Q3W, exhibits nonlinear pharmacokinetics similar to those seen in non-Japanese patients, and has encouraging antitumor activity in Japanese patients with colorectal cancer.

Conflict of interest statement

T. Doi, A. Ohtsu, M. Tahara, T. Tamura, K. Shirao, and Y. Yamada have no conflicts of interest to declare. S. Otani, M. Ohkura, and T. Ohtsu are compensated employees of Takeda Bio Development Center Ltd. B. Yang is a compensated employee and shareholder of Amgen Inc.

Author contributions

The authors contributed to the study as follows: T. Doi participated in data acquisition, data interpretation, and drafting and final approval of the manuscript. A. Ohtsu, M. Tahara, T. Tamura, K. Shirao, and Y. Yamada participated in data acquisition, data interpretation, and critical review and approval of the manuscript. S. Otani participated in study design, data acquisition, data interpretation, and critical review and approval of the manuscript. B. Yang provided pharmacokinetic data analysis and critical review and