instability in 95% of MSI-H CRCs whereas in 1–3% of MSI-L CRCs. Therefore, BAT25 and BAT26 were identified to be the most specific and sensitive markers to detect MSI-H CRCs. The dinucleotide marker D2S123 exhibited instability not only in 95% of the MSI-H CRCs but also in 56.7% of MSI-L. D2S123 is the most sensitive but not specific for MSI-L (Figure 1A).

*Clinicopathological features*

The association of MSI status with the clinicopathological features in the 940 CRCs is shown in Table I. Consistent with the findings of previous studies, MSI-H cancers are observed more frequently in females, in the proximal colon and in poorly differentiated or mucinous CRCs in comparison with MSS. While some differences were observed between MSI-L and MSS cancer, with regard to the female to male ratio, the site of the tumor and the stage did not reach significance.

The prognosis was assessed based on the MSI status (Figure 1B). Since no Dukes' A patients died during the follow-up period, these patients were excluded from the overall survival analysis. In total, 155 of the 731 Dukes' B–D patients (21.2%) died during a mean follow-up period of 30.3 ± 19 months after surgery. The prognosis of patients with MSI-H tumors was significantly better than that of patients with MSS tumors (log-rank test, $P = 0.0335$). The prognosis of patient with MSI-L tumors had an intermediate tendency among the three groups (Figure 1B).

In a stepwise multivariate analysis, age {hazard ratio (HR) 1.627 [confidence interval (CI) 1.216–2.301]; $P = 0.0016$}, men sex [HR 1.429 (CI 1.019–2.004); $P = 0.0388$], low-grade pathology [HR 2.029 (CI 1.231–3.343); $P = 0.0055$], *KRAS* [HR 1.69 (CI 1.215–2.351); $P = 0.0018$], *BRAF* [HR 3.593 (CI 1.933–6.678); $P < 0.0001$] and Dukes' stage [Dukes' B versus Dukes' C: HR 1.636 (CI 0.964–2.775); $P = 0.068$ and Dukes' B versus Dukes' D: HR 10.406 (CI 6.548–16.537); $P < 0.0001$] were independent variables. However, MSI was not an independent variable.

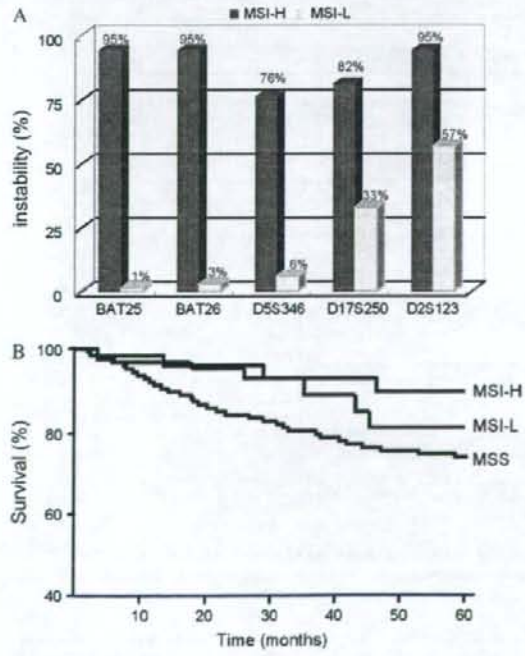


**Fig. 1.** (A) Frequency of markers demonstrated instability for MSI-H and MSI-L CRCs. (B) Overall survival of patients according to the MSI status. In total, 155 of the 731 Dukes' B–D patients (21.2%) died during a mean follow-up period of 30.3 ± 19 months after surgery.

*Mutation analysis of the KRAS and BRAF genes*

A *KRAS* mutation was detected in 39.4% and a *BRAF* V600E mutation in 4.6% of the 905 CRCs that were examined. The *BRAF* mutation was found more frequently in MSI-H cancer (32%) in comparison with MSS (3%) and MSI-L cancers (4%; $P < 0.0001$, Figure 2A). The frequency of *BRAF* mutation decreased accompanying the tumor progression in MSI-H cancer, whereas it increased in MSI-L and MSS cancers (Figure 2D–F).

The *KRAS* mutation analysis in CRCs demonstrated that MSI-L cancer showed higher frequency of the *KRAS* mutation than MSS and MSI-H cancers: MSS 39% (311/788), MSI-H 30% (16/53) and MSI-L 48% (32/67; MSI-L versus MSI-H; $P = 0.066$, MSI-L versus MSS; $P = 0.244$, MSI-H versus MSS; $P = 0.180$; Figure 2A). However, accompanying the progression from Dukes' A to Dukes' B, the frequency of the *KRAS* mutation in MSI-L cancer drastically increased from 16 to 63% (Figure 2E, MSI-L; *KRAS* mutation in Dukes A versus *KRAS* mutation in Dukes B–D, $P = 0.045$, Fisher's exact test) and was significantly higher than that in MSS or MSI-H cancers at Dukes' B–D (MSI-L versus MSS or MSI-H; $P = 0.014$, $P = 0.0394$, respectively; Figure 2C). MSI-H cancer also demonstrated an increased proportion of the *KRAS* mutation accompanying the progression from Dukes' A to Dukes' B (Figure 2F), but the number of MSI-H cases was too small to find significance (MSI-H; *KRAS* mutation in Dukes' A versus *KRAS* mutation in Dukes' B–D, $P = 0.08$, Fisher's exact test). The ratio of the *KRAS* mutation in MSI-H cancer was the same as that in MSS cancer after Dukes' B stage (Figure 2C). The ratio of tumors having either the *KRAS* or *BRAF* mutation at Dukes' B–D in MSI-H and MSI-L cancers was statistically higher than that in MSS cancer [MSS (40%) versus MSI-L (66%) or MSI-H (63%); $P = 0.0034$, $P = 0.0108$, respectively; Figure 2C].

*Type of KRAS mutation*

Of the 321 tumors with *KRAS* mutations, 196 (61%) were a G to A transition, 107 (33%) were a G to T transversion and 18 (6%) were a G to C transversion. The type of KRAS mutation was investigated in each MSI status. This revealed that 93% (14 of 15 tumors) of the *KRAS* mutations were a G to A transition in MSI-H cancer and there were significant differences between the types of *KRAS* mutations among the three MSI groups ($P = 0.0152$, chi-square test). The frequency of G to A transition mutations in MSI-L cancer was lower than in MSI-H but higher than in MSS cancer (Figure 2G). To investigate whether the high frequency of G to A transition mutation of *KRAS* gene in MSI-H and MSI-L cancer is involved in the inactivation of *hMLH1* or *MGMT*, the methylation status of the *hMLH1* and *MGMT* promoter was analyzed. Of the 30 MSI-L tumors with *KRAS* mutations, 13 (43%) had *MGMT* promoter methylation, whereas among 35 MSI-L tumors without *KRAS* mutations, 10 (29%) had it (Table II). Furthermore, 53% (10 of 19) of MSI-L tumors with G to A transition mutations in *KRAS* harbored *MGMT* promoter methylation, whereas 30% (three of 10) of MSI-L tumors with G to C or T transversion mutations in *KRAS* and 29% (10 of 35) of MSI-L tumors without a *KRAS* mutation showed *MGMT* promoter methylation (G to A versus G to C or T and wild-type, $P = 0.0705$; Table III).

These results suggest that G to A transition mutations in MSI-L tumors seem to correlate with *MGMT* promoter methylation ($P = 0.0705$). On the other hand, the frequency of *MGMT* methylation was observed in 38% of MSI-H tumors with *KRAS* mutation and 56% of MSI-H tumor without KRAS mutations. This result suggests that there is an inverse correlation between *KRAS* mutations and *MGMT* methylation in MSI-H tumors ($P = 0.2026$; Table II).

The frequency of *hMLH1* methylation was observed 25% of MSI-H tumors with *KRAS* mutations and 59% of MSI-H tumors without KRAS mutations. This result clearly shows a significant inverse correlation between *KRAS* mutations and *hMLH1* methylation in MSI-H tumors ($P = 0.0366$). The frequency of *hMLH1* methylation was

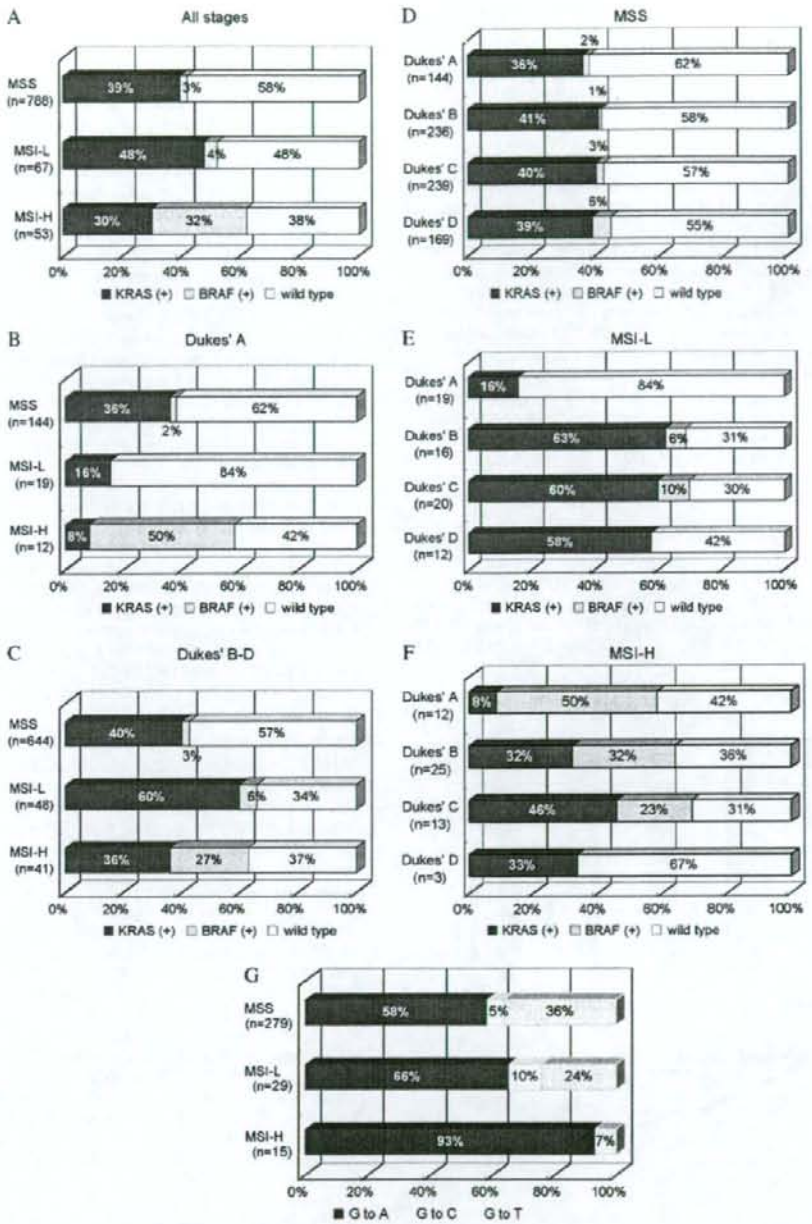


**Fig. 2.** *KRAS* and *BRAF* mutations in each MSI status. (**A**) *KRAS* and *BRAF* mutation of all CRCs. (**B**) *KRAS* and *BRAF* mutation of Dukes' A CRCs. (**C**) *KRAS* and *BRAF* mutation of Dukes' B–D CRCs. Frequency of *KRAS* and *BRAF* mutations at each stage according to the MSI status; (**D**) MSS, (**E**) MSI-L and (**F**) MSI-H. (**G**) Spectrum of KRAS mutations in each MSI status.

significantly higher in MSI-H tumors (50%) than in MSI-L tumor (6.7%; $P < 0.0001$; Table II).

*LOH of D5S346 and p53 mutation in MSI-L CRCs*

Since D5S346, one of the MSI makers, locates near the *APC* gene, MSI analysis with the Bethesda panel can also assess the LOH of *APC* gene, simultaneously. LOH of the D5S346 and *p53* mutation was detected in 75% (9/12) and 67% (12/18) of MSI-L CRC at Dukes' A, respectively (Table IV). In addition, the frequency of LOH of D5S346 and *p53* mutations in MSI-L at Dukes' B–D were 55 and 61%, respectively. These results indicate that LOH of *APC* and *p53* mutations has already occurred before Dukes' A.

**Table II.** Promoter methylation and KRAS mutation

| | | KRAS mut, *n* (%) | Wt, *n* (%) | *P* value |
|---|---|---|---|---|
| | MGMT | | | |
| MSI-L | M | 13 (43) | 10 (29) | 0.2147 |
| | U | 17 (57) | 25 (71) | |
| MSI-H | M | 6 (38) | 22 (56) | 0.2026 |
| | U | 10 (62) | 17 (44) | |
| | hMLH1 | | | |
| MSI-L | M | 2 (7) | 2 (6) | >0.9999 |
| | U | 28 (93) | 33 (94) | |
| MSI-H | M | 4 (25) | 23 (59) | 0.0366 |
| | U | 12 (75) | 16 (41) | |

M, methylated; U, unmethylated; mut, mutation; Wt, wild-type.

**Table III.** Type of KRAS mutation according to MGMT methylation

| | MGMT | KRAS mutation | | Wt, *n* (%) | *P* value |
|---|---|---|---|---|---|
| | | G to A, *n* (%) | G to C, T; *n* (%) | | |
| MSI-L | M | 10 (53) | 3 (30) | 10 (29) | 0.0705 |
| | U | 9 (47) | 7 (70) | 25 (71) | |
| MSI-H | M | 6 (43) | 0 (0) | 22 (56) | 0.4339 |
| | U | 8 (57) | 1 (100) | 17 (44) | |

M, methylated; U, unmathylated; Wt, wild type. *P* : G to A versus G to C, T + Wt.

## Discussion

### *Molecular features*

Although the *BRAF* and *KRAS* mutations are found more frequently in MSI-H and MSI-L CRC, respectively (31,34,38), the frequency of *KRAS* and *BRAF* mutation changed between each tumor stage in this study.

The development of CRC requires a multistep process characterized by the accumulation of genetic alterations. According to the well-known genetic model for colorectal tumorigenesis proposed by Fearon and Vogelstein, *KRAS* mutations occur in the early to intermediate adenomas (3). However, the frequency of *KRAS* mutations was significantly lower (16%) at Dukes' A and higher (60%) at Dukes' B–D in MSI-L CRCs. This means that most *KRAS* mutations occurred at different times in MSI-L CRC, namely, during the progression from Dukes' A to Dukes' B but not in early to intermediate adenomas. It has been reported previously that the *KRAS* mutation is found more frequently in MSI-L CRCs (31,32), but according to the current detailed study, it depends on the tumor stage. Since a large number of specimens were collected in an unbiased manner for this study, the results demonstrate representative findings of CRCs in Japan.

Meanwhile LOH of D5S346, which is located near the *APC* gene, and the *p53* mutation was observed in 75% (9/12) and 67% (12/18) of MSI-L CRC at Dukes' A, respectively. These frequencies were almost the same at Dukes' B–D in MSI-L CRC (Table IV).

Taken together, these findings indicated that LOH of *APC* and *p53* mutations have already occurred by the Dukes' A lake suppressor pathway but not the *KRAS* mutation in MSI-L CRCs.

MSI-L CRC may develop through a mild mutator pathway, which differs from the suppressor and mutator pathway and show different clinical features (31,39). However, some studies doubt the presence of the MSI-L group (27,30). In the current study, the involved genes such as LOH of *APC*, *KRAS* and *p53* mutation in MSI-L CRCs are similar to those in MSS CRCs, but at least the timing and frequency of the *KRAS* mutation is different. This may explain why the clinicopathological features of MSI-L tumors are similar to those of MSS tumors but not completely identical.

**Table IV.** LOH of D5S346 and p53 mutation in MSI-L CRCs

| Dukes' stage | D5S346 LOH | p53 mutation |
|---|---|---|
| A | 75% (9/12) | 67% (12/18) |
| B | 46% (6/13) | 57% (12/21) |
| C | 67% (8/12) | 45% (10/22) |
| D | 50% (3/6) | 92% (12/13) |

On the other hand, considering the presence of the *BRAF* mutation and methylation of the *hMLH1* promoter at the early stage in MSI-H CRC, these genetic changes should occur in the precursor of MSI-H CRC. This is not inconsistent with the concept of serrated pathways resulting from serrated polyps that were revealed in recent morphological and molecular studies (24–26,40–42).

The mechanism of the *KRAS* mutation was also analyzed and the results showed that a G to A transition mutation of *KRAS* occurs more frequently in MSI-L than MSS. Some reports demonstrated that *MGMT* inactivation by promoter methylation causes a G to A transition mutation of *KRAS* (33,43,44) and *p53* (45) and such a mutation is frequently observed in MSI-L CRCs. We attempted to determine whether or not the inactivation of *MGMT* by promoter methylation is associated with the type and frequency of *KRAS* mutation. Our results indicated that *MGMT* promoter methylation seems to affect the G to A transition and frequency of the KRAS mutation in MSI-L CRC. However, most *KRAS* mutations in MSI-H CRC show a G to A transition, *MGMT* inactivation was inversely related and the *BRAF* mutation often observed in MSI-H CRC shows a T to A transversion. Considering these results, a different mechanism might therefore be involved in mutation between MSI-L and MSI-H CRC.

### *Clinical feature*

As mention above, the genes associated with developing MSI-L CRC are similar to the suppressor pathway but the frequency and timing of KRAS mutations is different; thus, there may be different clinical and pathological features in MSI-L.

Comparing the stage distribution for each MSI status, the distribution of Dukes' B in MSI-H CRC is significantly larger than in MSS and MSI-L CRC (7). Gyef *et al.* (15) demonstrated with a logistic analysis that MSI-H CRC is less metastatic to the regional lymph nodes and distant organs than MSS CRC, even though their depth of tumor invasion is same. The same result was observed in the current study, but MSI-L CRC did not show this characteristic. This suggests that there is a mechanism restricting the progression from Dukes' B to C in MSI-H cancer. Although the precise explanation for this mechanism is still unknown, tumor-infiltrating lymphocytes, apoptosis, proliferative activity (46,47) or a mutation of *p53* (7) may lead to the 'restraining effect'.

On the other hand, the distribution of Dukes' A in MSI-L CRC is larger than MSS. Considering the *KRAS* mutation during the progression from Dukes' A to B in MSI-L CRC, tumor progression may be hindered until the occurrence of the *KRAS* mutation in MSI-L CRC.

Various studies have reported the prognosis of each MSI status. Some investigations show that the patients with MSI-H cancer demonstrate a better prognosis and the patients with MSI-L cancer have a poorer survival than patients with MSS in stage C (48,49). Considering the high frequency of the *KRAS* mutation after Dukes' B in MSI-L cancer, the worse prognosis of such patients may therefore be reasonable (50).

Although the number of cases in the current study was not sufficient to study the prognosis at each stage, among all patients with Dukes' B to D CRCs, MSI-H patients showed significantly better survival than MSS (log-rank test, $P = 0.0335$) while MSI-L patients had a slightly better prognosis than MSS. These findings may result from the fact that the proportion of Dukes' D for each MSI status is smaller, in order, MSI-H, MSI-L and MSS.

Furthermore, MSI-L tumors in the current series developed more frequently in females and in the proximal colon than MSS tumors, although no significant difference was observed.

In this study, a series of 940 patients with CRCs suggests that MSI-H, MSI-L and MSS cancer each progress through different pathways. Further study on these themes will probably attempt to clarify not only MSI cancer but also try to elucidate the true nature of CRCs itself.


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・論文・

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# 外来化学療法センター開設における取り組みと薬剤師のかかわり

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# Efforts for Establishing an Outpatient Chemotherapy Center and the Role of Pharmacists

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大阪医科大学附属病院（以下，当院）では平成18年4月に外来化学療法センターが新設され，設立にあたり標準的治療を確実にかつ安全に遂行できるよう，外来化学療法センターの下部組織としてレジメン審査委員会，クリニカルパス委員会などを発足させた。各委員会は十分に機能しており，各職種が協力しチームで治療に取り組んでいる。外来化学療法においては入院と異なり，副作用モニタリング等患者自身の自己管理によるところも多く，患者教育・指導が重要になる。当院の外来化学療法センターには専任の薬剤師が常駐し，抗がん剤の無菌調製のみならず，薬歴管理，患者の服薬指導や副作用モニタリングなど多岐にわたり活動を行っている。現在の外来化学療法における問題点を抽出したうえで，システムの構築と安全対策を模索し，患者が安心して外来で治療を継続できるような改善策を検討する必要がある。

キーワード—外来化学療法，レジメン審査委員会，服薬指導，化学療法支援システム

## 緒 言

がん化学療法は，平均在院日数の短縮並びに急性期医療診断群分類包括支払制度（DPC）導入といった医療経済的側面，患者のQOL（quality of life）向上の観点から，入院治療から外来治療へと大きくシフトし，今後ますます増加する傾向にある[1]。そこで，多くの医療機関でがん化学療法の安全性や質を維持し患者の満足度を高くするために，様々な工夫とマニュアルの整備が行われている[2-4]。大阪医科大学附属病院（以下，当院）でも外来化学療法センター設立後より，様々な問題に対応しながら，より安全に外来における治療が実施できるように検討を重ねている。当院では外来化学療法センターに専任薬剤師が終日常駐し，すべての患者を対象に服薬指導と副作用モニタリングを行っており，常に治療中の患者の傍にいる体制をとっている。そこで，当院の外来化学療法における安全への取り組みについて紹介し，薬剤師のかかわりによって安全で安心な外来化学療法が提供できたので報告する。

## 方 法

### 1．レジメン審査委員会

外来化学療法センター設立準備として，まず，EBM（evidence based medicine）に基づいた有効で安全な治療が実施できるように，臨床に精通した各診療科の医師と薬剤師を含めたレジメン審査委員会を発足させた。委員会ではエビデンスレベルの評価のみならず，各診療科間でばらつきのある制吐剤や輸液など支持療法の統一化を行い，がん種を超えて統一したレジメンの作成を行った。エビデンスレベルは，ガイドラインで推奨グレードB以上のものや，治験，臨床試験については治験審査委員会，倫理委員会で承認されたものに限り承認した。レジ

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メン審査委員会は定期的に開催され，新規レジメンの評価，現行レジメンの見直しを行っている。至急に使用が必要なレジメン等については，委員長の権限で暫定承認というかたちを採ることがあるが，次回レジメン審査委員会で必ず検討を行うことにしている。レジメン審査委員会で承認登録されたレジメンはオーダエントリシステムにセット登録され，医師の処方ミスを回避するシステムになっている。

**2．クリニカルパス**

安全性を高める目的で外来化学療法センター運営委員会の下部組織としてクリニカルパス委員会を設置し，各レジメン1クールを1シートとし，医療者用クリニカルパスを作成した。作成にあたり薬剤師は，各レジメン別にモニタリングすべき副作用をチェック項目に加えるなど積極的に作成に寄与している。作成されたクリニカルパス用紙は，主治医記入後に看護師が記録用紙として使用している。

**3．化学療法支援システム**

化学療法支援システムPicky（TOSHO製）を導入し，オーダエントリシステム（富士通㈱：HOPE/EGMAIN）と連動させた。また，当院のオーダエントリシステムにあわせ若干の改良を加えた。化学療法支援システムでは薬歴作成や体表面積から算出された投与量，配合変化など自動的に監査できるシステムになっており，このシステムを利用して薬剤師が疑義照会を行った件数の変化を比較検討した。

疑義照会率（%）＝疑義照会件数/総患者数×100

**4．患者指導**

外来化学療法センターで治療を受けているすべての患者を対象に専任薬剤師が指導を実施し，副作用のモニタリング，自宅での副作用に対する対処方法などの指導を行っている。当院では治療開始時のみならず，治療中に毎回薬剤師が訪床し，副作用モニタリング，疼痛コントロールに至るまで指導を行い，薬剤管理指導内容をカルテに記録している。外来化学療法においては在宅での患者の自己管理が重要であり，重大な副作用の初期症状などの説明を十分に行い，抗がん剤の副作用のみならず，不安なことすべてにおいて連絡するように指導を行っている。患者からの電話対応については，基本的に外来化学療法センターのスタッフが行い，状況に応じて各診療科と連携を取っている。夜間や日・祝祭日については救急外来，各診療科当直医師が対応を行うが，翌日には外来化学療法センターに報告され，患者情報の一元管理を可能にしている。外来化学療法センター利用患者100名を対象に，平成18年5月22日～6月16日までの間に満足度調査を行った。

## 結　果

**1．レジメン審査委員会**

開設前に参加診療科より提出されたレジメンは68，承認登録されたレジメンは57であった。内訳は，各科からの申請で共通化できたものが9，科より申請取り下げが3，非承認が1である。承認レジメンには，投与量，投与時間などを変更し承認になったものも含まれている。その後毎月，新規申請，暫定承認の検討など行い登録レジメンは変動しているが，そのうち稼動しているレジメンはほぼ一定数で推移しており，不要なレジメンが登録されていることが示唆される（図1）。統一したレジメンを作成することで，薬剤部での調剤ミスや混合調製ミスの防止と看護師の点滴管理での混乱を避けることが可能になると考えられた。

**2．クリニカルパス**

例としてweekly Paclitaxelのクリニカルパスを掲載する（図2）。看護記録はこのクリニカルパス用紙への記入で完結し，薬剤師は薬剤管理指導記録へ記入を行っている。医師，看護師，薬剤師各々が患者をモニタリングすることで，副作用の発現を漏れなく拾うことができている。

**3．化学療法支援システム**

導入後の疑義照会の動向を図3に示す。全処方のうち，疑義が発生する割合は17.4%まで減少しており，導入時にはオーダリングの不備が多くあったが，平成18年8月には投与スケジュールと投与量の確認がほとんどとなっている。システム導入前は調剤時に処方監査は行っていたもののスケジュール管理や休薬期間のチェックなどはできておらず，安全管理の面で不十分であった。システム導入後，患者別スケジュール管理やレジメン別の休薬期間，投与量のチェックが自動的に可能となり，誤投与や処方監査ミスは1件も発生していない。

**4．患者指導**

満足度アンケートの結果を図4に示す。「専任の薬剤師がいるので安心である」と答えた患者は85.0%（有効回答率94.0%）であり，「自宅で副作用の対処で困ったこと

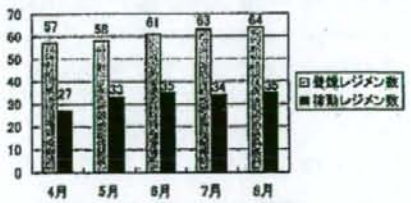


図1　稼動レジメンの変動

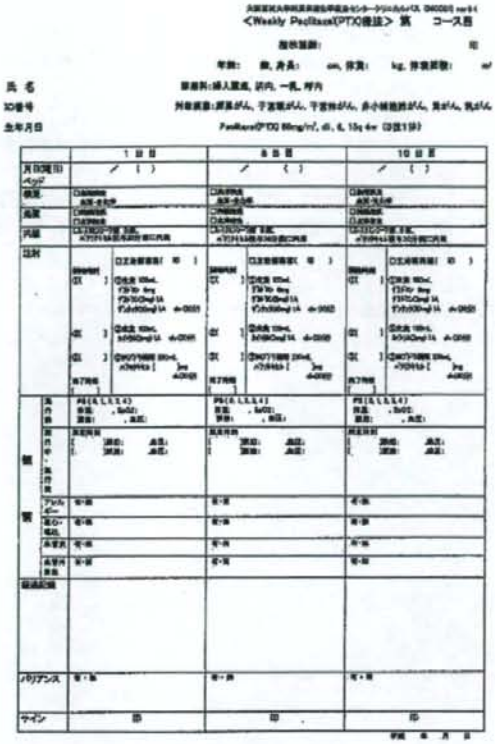


図2　医療者用クリニカルパス

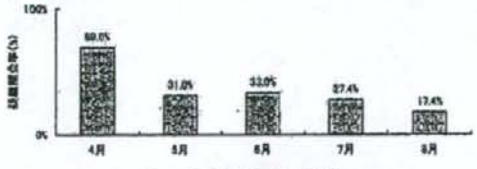


図3　疑義照会率の変動

がある」と答えた患者は23.0%（有効回答率83.0%）に留まっている。

## 考　察

レジメン審査委員会が十分に機能することでエビデンスに基づいた治療が確立した。今後は，現在稼動していないレジメンや日々変化する臨床試験の結果などの情報収集を行い，適宜再審査が必要である。

クリニカルパスを使用することで職種を超えた記録の簡略化，統一が可能になり，化学療法への知識の浅い看護師でもレジメンの特性別に副作用のモニタリングが可能になった。

化学療法支援システムの導入により薬歴管理や投与量

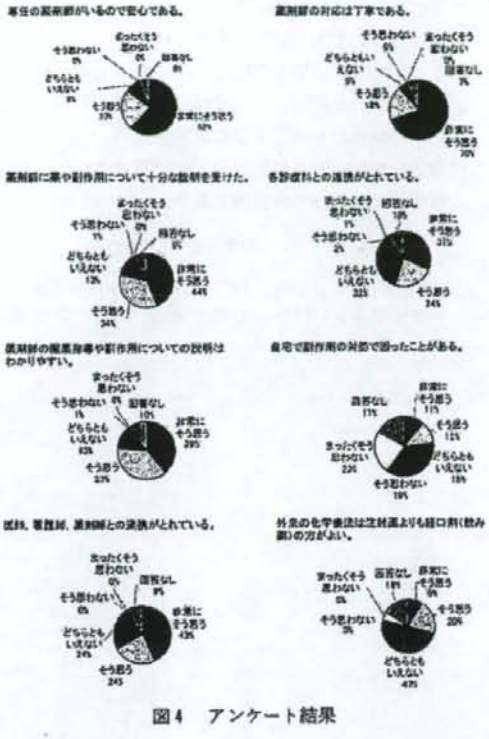


図4　アンケート結果

計算が自動的にできることで，投与予定日前の処方監査，疑義照会が薬剤師の経験年数やスキルを問わず可能となり，効果的でかつ，安全・安心な化学療法が提供できている。

外来化学療法センター開設以前は入院化学療法を施行する場合は薬剤師からの説明があるのに対し，外来で施行する場合には医師からの説明のみであった。しかし，患者アンケート結果の「専任の薬剤師がいるので安心である」と答えた85.0%と「薬剤師の服薬指導や副作用についての説明はわかりやすい」と答えた76.0%から，薬剤師に対する需要が示唆されており，外来においても化学療法施行中の患者には薬剤師による服薬指導が必要であると考えられた。また，専任薬剤師が毎回のモニタリングを通じて指導を行った結果，「自宅で副作用の対処で困ったことがある」と答えた患者は23.0%であり，前述の「薬剤師の説明はわかりやすい」と評価する結果と合わせ，薬剤師の指導により患者自身が副作用について自宅で対処不可能となる割合が軽減したと推察される。

今後，増加していく外来化学療法に対応するために，各職種間でコミュニケーションを十分にとり，各職種の専門性を活かし患者にかかわる必要がある。現在の外来

化学療法加算には治療に携わる人員数や各職種のスキルの規定はされておらず[5]，質の高い外来化学療法を遂行するうえで医療従事者の知識，技術の向上が必要である。また，外来では薬剤管理指導料の算定が不可能なために薬剤師のかかわりが不十分な施設が多い。しかし，外来化学療法において薬剤師の果たす役割は大きく，十分な人員配置とかかわりが重要である。

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# 高齢者（70歳以上）胃がん症例に対するS-1薬物体内動態

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# A Pharmacokinetic Study of an Anticancer Drug, S-1, in Elderly Patients Aged 70 Years and Over with Gastric Cancer

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ティーエスワン®カプセル（以下，S-1）の高齢者における薬物動態試験は実施されていない。そこで，高齢者胃がん症例におけるS-1投与後のテガフール，フルオロウラシル（以下，5-FU），ギメラシル（以下，CDHP）濃度を測定し，腎機能および有害事象との関連性を検討した。対象症例は5症例で，平均年齢76歳，クレアチニンクリアランス計算値は平均67.0mL/min，血中濃度-時間曲線下面積（$AUC_{0\text{-}10}$）が5-FU：755±204.5ng・h/mL，CDHP：1,029.3±389.7ng・h/mLと非高齢者の結果と大きな差はみられなかった。しかし，軽度腎機能低下症例では5-FU：958.8ng・h/mL，CDHP：1,368.6ng・h/mLと増加傾向を認め，高齢者においてもS-1は安全に投与できる薬剤であるが，腎機能低下症例は減量・慎重投与が必要であることが示唆された。

キーワード―ティーエスワン®カプセル（S-1），胃がん，高齢者，クレアチニンクリアランス（Ccr），血中濃度

## 緒　言

ティーエスワン®カプセル(以下，S-1)は，1999年1月に本邦で胃がんに対する承認を取得し，その後，頭頸部がん，結腸・直腸がん，非小細胞肺がん，手術不能または再発乳がん，膵がん，胆道がんに対する適応が承認されている。S-1単剤での胃がんに対する有効性は，2つの後期第Ⅱ相試験でそれぞれ49%（25/51例），44%（19/43例）の奏効率を示し，生存期間の中央値（MST）は250日，207日であった[1,2]。これら試験のgrade 3（NCI-CTCAE）以上の主な有害事象としては，ヘモグロビン減少，好中球減少，白血球減少や下痢などが挙げられるが，発現頻度は4%未満でいずれも高くなかった。S-1は単剤で高い奏効率が得られたことや，経口薬という利便性から広く臨床で普及しており，現在，胃がん治療の標準治療の1つと位置づけられている。

しかし従来，臨床試験においては試験の遂行性を高める目的で対象を「75歳まで」としていることが多く，高齢者の進行・再発胃がんに対する有効性および安全性は確立していない。そこで今回我々は，高齢者に対する1次治療としてのS-1の安全性を確認する目的で，高齢者胃がん症例におけるS-1投与時のテガフール（以下，FT），フルオロウラシル（以下，5-FU），ギメラシル（以下，CDHP）の血中濃度を測定し，クレアチニンクリアランス（以下，Ccr）値に準じたS-1初回投与量の妥当性の検証を行い，薬物動態結果に準じて患者個々に適したS-1投与量を設定することを目的とした。

## 方　法

下記適格条件を満たす適格例において，薬物動態学的検討に対し血液検体を提供することに関しての同意が得られた症例を対象とした。なお，本試験は大阪医科大学倫理委員会の審査において承認を得て行われた。

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### 1．適格条件

治療開始前４週間以内の検査により，(1)～(10)の基準を満たす症例

(1) 組織学的に胃がんであることが確認されている症例
(2) 測定可能病変の有無は問わない
(3) 登録時年齢が70歳以上である症例
(4) performance status（以下，PS）：０～２の症例
(5) 経口摂取が可能な症例
(6) 登録前２週間以内の臨床検査値で下記条件が確認されていること［白血球数：4,000/mm$^3$以上12,000/mm$^3$未満，血小板数：100,000/mm$^3$以上，血色素量：8.0g/dL以上，アスパラギン酸アミノトランスフェラーゼ（AST），アラニン・アミノトランスフェラーゼ（ALT）：100IU以下，総ビリルビン：施設正常値の上限値以下，血清クレアチニン（以下，Cr）：1.2mg/dL以下，Cockcroft-Gault式による推定Ccr値：30mL/min以上］
(7) 前治療（放射線治療，化学療法，ホルモン療法等）が実施されていない症例
(8) 少なくとも３ヵ月以上の生存が期待される症例
(9) 被験者本人により文書で同意を得られている症例
(10) 十分に服薬コンプライアンスを確保できると考えられる症例

### 2．投与スケジュール

身長，体重からDuBois式[3]を用いて体表面積を算出し，1.50m$^2$以上の場合120mg/body/day，1.25m$^2$以上1.5m$^2$未満の場合100mg/body/day，1.25m$^2$未満の場合80mg/body/dayを１日２回，朝夕食後に投与した。朝食の時間は午前８時，夕食の時間は午後６時半とした。また，Ccrや患者の状態により，必要に応じて減量をした。決定された投与量において４週投与し，その後２週休薬を１クールとして繰り返すこととした。もしくは，シスプラチン（以下，CDDP），塩酸イリノテカン（以下，CPT-11）併用時は３週投与２週休薬，ドセタキセル（以下，DOC）併用時は２週投与１週休薬とした。また，S-1投与は治療効果の悪化（PD）が認められない限り，少なくとも２コース以上継続することとした。

### 3．検体採取方法

採血ポイントは，S-1投与開始直前とS-1投与開始第７日目のS-1投与前，投与後２，４，６，10時間の６ポイントの採血を行った。採血方法は，ヘパリン入りシリンジで血液５mLを採取し，５℃，3,000rpmで15分間遠心分離後，血漿2.5mLを保存用チューブにて－20℃以下で保存した。

### 4．血中濃度の測定

血中濃度の測定は，Matsushimaらの方法[4]に従って契約を締結した外部委託機関ファルコバイオシステムズ㈱で，FT，5-FU，CDHPの血中濃度を測定した。

### 5．薬物学的動態において用いた指標

$T_{1/2}$：the half-life
$T_{max}$：the maximum plasma concentration time
$C_{max}$：the maximum plasma concentration
AUC：area under the curve

これらの計測についてはソフトWinNonlin Proffesional version 4.1を用い，$C_{max}$および$T_{max}$は実測値を，血中濃度-時間曲線下面積（以下，AUC）は線形台形法を用い算出した。$T_{1/2}$は血漿中濃度推移の消失相をlog変換し，直線回帰することにより算出した。

## 結　果

### 1．患者背景（表１）

平成19年10月～20年３月に登録された症例は５症例で，いずれの症例も担当医より研究の目的，方法，予測できる危険性，不測の事態，緊急時の対応法について十分な説明のうえ，血液検体を提供することに関して文書で同意を得た。

性別は男性１名，女性４名の胃がん患者で，年齢は71～81歳（平均76歳），PS０～１（平均１），治療前の血清Cr値は0.36～1.05mg/mL（平均0.60mg/mL），Ccr計算値は51.7～87.5mL/min（平均67.0mL/min），S-1の投与量は80～120mg（平均92mg，65.3mg/m$^2$）であった。効果判定はRECISTに基づき行い，総合評価はすべて不変(SD)

**表１**　患者背景

| 症例 | 性別 | 年齢 | PS (ECOG) | 血清Cr (mg/dL) | Ccr (mL/min) | 手術歴 | 化学療法歴 | 転移 |
|---|---|---|---|---|---|---|---|---|
| 1 | F | 77 | 1 | 0.51 | 68.5 | 亜全摘 | なし | 腹膜 |
| 2 | F | 77 | 0 | 0.36 | 72.3 | なし | なし | リンパ節 |
| 3 | M | 78 | 1 | 1.05 | 51.7 | 亜全摘 | なし | リンパ節 |
| 4 | F | 71 | 1 | 0.54 | 87.5 | 亜全摘 | なし | リンパ節 |
| 5 | F | 78 | 1 | 0.52 | 54.9 | なし | なし | 腹膜 |

**表２**　S-1投与量と有害事象

| 症例 | 体表面積 (m$^2$) | レジメン | S-1投与量 (mg) | コース数 | 効果 (RECIST) | 副作用 (NCI-CTC grade) |
|---|---|---|---|---|---|---|
| 1 | 1.36 | S-1/CDDP | 80 | 4 | SD | 口内炎（1） |
| 2 | 1.19 | S-1 | 80 | 3 | SD | 口内炎（1），ヘモグロビン減少（3） |
| 3 | 1.68 | S-1/DOC | 120 | 2 | SD | 口内炎（1），脱毛（1），色素沈着（1），下痢（1），手足症候群（1） |
| 4 | 1.55 | S-1/CDDP | 100 | 2 | SD | 食欲不振（1），口内炎（1） |
| 5 | 1.24 | S-1 | 80 | 2 | SD | 食欲不振（1），悪心（1），嘔吐（1），便秘（1） |

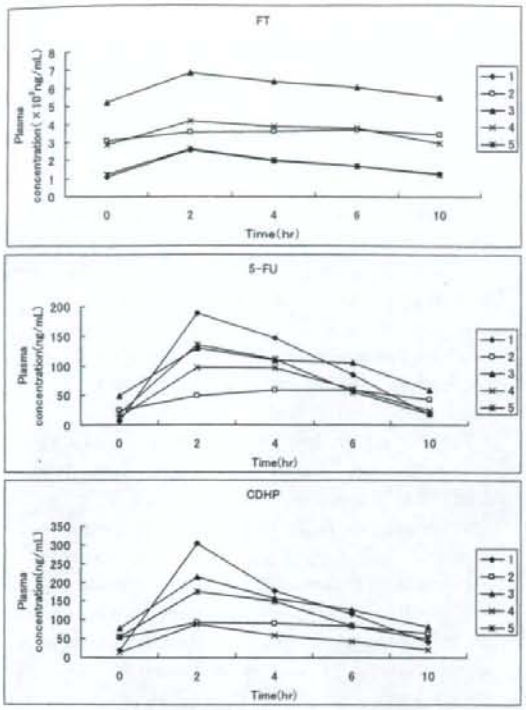


**図1**　FT，5-FU，CDHP血中濃度

**表3**　PKパラメータ

FT

| Patient No. | Ccr (mL/min) | $T_{1/2}$ (hr) | $T_{max}$ (hr) | $C_{max}$ (ng/mL) | $AUC_{0\text{-}10}$ (ng·hr/mL) |
|---|---|---|---|---|---|
| 1 | 68.5 | 9.3 | 2.0 | 2,589 | 17,844 |
| 2 | 72.3 | 36.9 | 6.0 | 3,673 | 35,187 |
| 3 | 51.7 | 28.9 | 2.0 | 6,820 | 60,566 |
| 4 | 87.5 | 14.3 | 2.0 | 4,171 | 36,154 |
| 5 | 54.9 | 8.4 | 2.0 | 2,642 | 18,020 |
| 平均 | 67.0 | 19.6±12.7 | 2.8±1.8 | 3,979±1,726 | 33,554±17,516 |

5-FU

| Patient No. | Ccr (mL/min) | $T_{1/2}$ (hr) | $T_{max}$ (hr) | $C_{max}$ (ng/mL) | $AUC_{0\text{-}10}$ (ng·hr/mL) |
|---|---|---|---|---|---|
| 1 | 68.5 | 1.9 | 2.0 | 188.3 | 967.7 |
| 2 | 72.3 | 9.1 | 6.0 | 59.2 | 503.3 |
| 3 | 51.7 | 6.2 | 2.0 | 129.1 | 958.8 |
| 4 | 87.5 | 3.1 | 2.0 | 97.0 | 630.0 |
| 5 | 54.9 | 2.4 | 2.0 | 135.4 | 715.2 |
| 平均 | 67.0 | 4.5±3.0 | 2.8±1.8 | 121.8±47.9 | 755.0±204.5 |

CDHP

| Patient No. | Ccr (mL/min) | $T_{1/2}$ (hr) | $T_{max}$ (hr) | $C_{max}$ (ng/mL) | $AUC_{0\text{-}10}$ (ng·hr/mL) |
|---|---|---|---|---|---|
| 1 | 68.5 | 2.8 | 2.0 | 303.7 | 1,414.0 |
| 2 | 72.3 | 11.5 | 2.0 | 94.8 | 810.0 |
| 3 | 51.7 | 6.3 | 2.0 | 215.2 | 1,368.6 |
| 4 | 87.5 | 3.8 | 2.0 | 89.6 | 486.4 |
| 5 | 54.9 | 3.9 | 2.0 | 177.1 | 1,067.7 |
| 平均 | 67.0 | 5.7±3.5 | 2.0±0.0 | 176.1±89.3 | 1,029.3±389.7 |

**表4**　28日間連日投与後の血漿中濃度より算出したPKパラメータ（インタビューフォームより引用）

| | $T_{1/2}$ (hr) | $T_{max}$ (hr) | $C_{max}$ (ng/mL) | $AUC_{0\text{-}48}$ (ng·hr/mL) |
|---|---|---|---|---|
| FT | 16.2±2.4 | 3.0±1.8 | 4,166.2±833.9 | 80,031.5±20,993.2 |
| 5-FU | 2.9±1.1 | 3.4±1.3 | 113.7±40.5 | 609.0±170.2 |
| CDHP | 4.2±1.4 | 2.6±2.1 | 276.0±141.8 | 1,364.0±351.6 |

Mean±S.D.，n=10
S-1投与量：FTとして体表面積1.25m²以上～1.5m²未満が100mg，1.5m²以上が120mg×28日

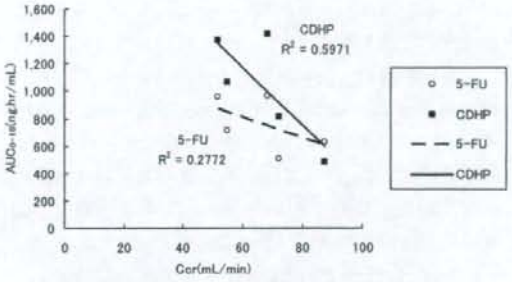


**図2**　CcrとFT，CDHPの$AUC_{0\text{-}10}$との相関

であった。副作用についてはNCI-CTCAE v3.0により評価した（**表2**）。症例2のgrade 3のヘモグロビン減少を除き，grade 1の副作用のみであった。

### 2．血中濃度

S-1投与開始第7日目における各症例のFT，5-FU，CDHPの血漿中濃度推移を**図1**に示した。5-FUのS-1投与前，投与後2，4，6，10時間値としては，それぞれ22.1±16.6，119.7±51.4，104.5±31.9，72.9±21.6，32.8±17.5ng/mLであった。S-1投与開始前の血中濃度はすべて測定限界以下であった。

### 3．薬物速度論的（pharmacokinetic）パラメータ（以下，PKパラメータ）

各症例の血漿中濃度より算出した$T_{1/2}$，$T_{max}$，$C_{max}$，AUCについて**表3**に示した。**表4**に非高齢者の薬物動態の値を今回の結果と比較するため，連日投与28日目のパラメータをティーエスワン®カプセルインタビューフォーム[5]より抜粋した。なお，各薬剤とも投与後7日目には定常状態に達しており，28日目とのパラメータ比較可能であると考えられた。

### 4．Ccrとの相関

**図2**にCcrと5-FU，CDHPの$AUC_{0\text{-}10}$との相関について示した。Ccrと腎排泄型薬剤であるCDHPの$AUC_{0\text{-}10}$は$R^2$=0.5971と相関を示しており，それに伴って，5-FUの$AUC_{0\text{-}10}$も弱いながらも相関を示していた（$R^2$=0.2772）。

## 考　察

今回の結果では，高齢者における血清Cr値から計算されるCcrは80mL/min以上が1症例，50～80mL/minが

4症例であり，加齢による軽度腎機能障害を示した。このことは，年齢とCcr値は負の相関を示すとの報告[6]と相違しなかった。

S-1の全がん種における前後期臨床第Ⅱ相試験および臨床薬理試験の結果によると，少数（14/578例）ではあるが75歳以上の症例があり，食欲不振，下痢，口内炎，白血球減少，血色素減少においてほかの年齢層よりも発現率が高い傾向がみられている。これらの結果から，75歳以上の症例を対象とする場合には，より安全性を考慮した対象の選択，投与計画の設定が求められている。今回の結果では，Ccr計算値が50～60mL/minである症例3，5において副作用の発現項目が多くみられ，5-FUの血中濃度は，$C_{max}$，$AUC_{0\text{-}10}$ともに腎機能正常者に比べ上昇していた。このことからも，5-FUの血中濃度と副作用の発現には正の相関があることが示唆される。

S-1の配合成分であるCDHPが腎排泄型薬剤であり[7]，腎機能低下が予測される高齢者においてはCDHPの血中濃度が高くなると推測されるが，今回の結果では$T_{1/2}$が4.2±1.4hrから5.7±3.5hrと延長しているものの，$C_{max}$は276.0±141.8ng/mLから176.1±89.3ng/mLと逆に低い値であり，Ccr計算値51.7～87.5mL/min（平均67.0mL/min）においては，特にCDHPの生体内蓄積は見当たらなかった。また，CcrとCDHPの$AUC_{0\text{-}10}$の相関においても，腎機能低下によるCDHPの$AUC_{0\text{-}10}$の増加が認められるものの，加齢によるCDHPの蓄積は認められなかった。

以上のことより，高齢者におけるPKパラメータは非高齢者におけるパラメータと大きな相違はなく，高齢者においても，腎機能低下に留意したうえで，通常の減量基準でS-1投与が可能であると考えられる。今回はCcr 50mL/min以下の症例がなく，高齢者の中度～高度腎機能障害における薬物動態は不明であったため，今後は高齢者かつ腎機能低下症例における薬物動態の検討が必要である。

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# Impact of vascular endothelial growth factor receptor 1, 2, and 3 expression on the outcome of patients with gastric cancer

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**Tumor angiogenesis is a multistep interactive process in which vascular endothelial growth factor (VEGF) and its receptors have a major role. However, the clinical significance of these molecules in gastric cancer (GC) remains unclear. Our study group comprised 86 patients who underwent gastrectomy and subsequently received chemotherapy for recurrent or residual tumor. Using immunohistochemical techniques, we analyzed the expression of VEGF receptors (VEGF-R) 1, 2, and 3. VEGF-R1 expression (defined as >5% staining) was found in the tumor cells of 65 tumors (76%) and in the stromal vessels of 36 tumors (42%). VEGF-R2 expression was found in tumor cells and stromal vessels of 0 and 46 tumors (0 and 53%), respectively, and VEGF-R3 expression was found in tumor cells and stromal vessels of 0 and 75 tumors (0 and 87%), respectively. Univariate analysis revealed that VEGF-R expression correlated with shorter survival (VEGF-R1 in stromal vessels, $P = 0.001$; VEGF-R2 in stromal vessels, $P = 0.009$; VEGF-R3 in stromal vessels, $P = 0.005$) and lower response to S-1 (VEGF-R1 in stromal vessels, $P = 0.039$). Multivariate analysis of potential prognostic factors showed that VEGF-R1 and VEGF-R2 in stromal vessels were independent predictors of poor outcome. Our data suggest that VEGF-R expression can be a predictor of unfavorable clinical outcome in GC. VEGF-R are promising candidates as therapeutic targets. (*Cancer Sci* 2009; 100: 310–315)**

Gastric cancer (GC) is the second leading cause of cancer-related death worldwide, accounting for over 20 deaths per 100 000 population annually in East Asia (China, Japan), Eastern Europe, and parts of Central and South America.[1] Recently, many chemotherapy regimens using new agents have been developed that show high response rates for advanced GC, and progress in basic research has revealed many factors and mechanisms implicated in sensitivity and resistance to chemotherapy.

Angiogenesis reportedly plays an important role in cancer invasion and metastasis. Vascular endothelial growth factor (VEGF) and VEGF receptor (VEGF-R) represent important regulators of angiogenesis, and increased expression of this family of molecules has been documented in various cancer cell lines[2] and tissues.[3,4] Previous clinical studies have demonstrated that increased expression of VEGF or its family is associated with the grade of angiogenesis and the prognosis for various human cancers.[5–9]

In GC, several studies have found that expression of VEGF ligands and subtypes correlates with prognosis,[10–12] and expression of soluble VEGF-R1 is also a predictor of prognosis.[13] However, the distribution, frequency, and prognostic value of VEGF-R expression in GC have not been clarified. The present study investigated relationships between VEGF-R expression and prognosis in patients with advanced GC.

## Materials and Methods

**Patients.** Subjects were 86 patients who underwent surgery for primary GC and received chemotherapy for the treatment of recurrent or residual tumors at the National Cancer Center Hospital (NCCH). Inclusion criteria were as follows: histologically proven advanced GC; unresectable, locally advanced, or metastatic disease; no prior chemotherapy and no prior adjuvant or neoadjuvant chemotherapy; specimens of primary GC were obtained before the start of chemotherapy by surgical resection or biopsy at NCCH; radiographically measurable disease; first-line chemotherapy was received from January 1995 to December 2004; tumor response and survival times were confirmed; adequate bone marrow, liver, and renal function; and written informed consent. The tissue samples were collected retrospectively from patients who met these criteria. Measurable disease was assessed by computed tomography. Response was evaluated according to the standard International Union against Cancer (UICC) guidelines as complete response (CR), partial response (PR), no change (NC), or progressive disease (PD). The response rate was calculated as the ratio of CR + PR to CR + PR + NC + PD.[14] Written informed consent was obtained before treatment and evaluation of tumor samples.

**Immunohistochemical staining.** Serial 4-μm sections were made from formalin-fixed paraffin-embedded tissue. Sections were dewaxed in xylene and rehydrated through a graded alcohol series. Antigen retrieval was carried out by incubating sections in target-retrieval solution (Dako Japan, Tokyo, Japan) for 40 min in a 95°C water bath 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, 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, VEGF-R2, and VEGF-R3; and the Envison + kit (Dako Japan) for CD34, D2-40, CD31, and factor VIII, according to the instructions of the manufacturer. Sections were counterstained using Mayer's hematoxylin.

**Evaluation of immunostaining.** The entire specimen was examined at low magnification (×40), and positive cells were counted in areas with strong immunoreactivities at high magnification (×200). The number of immunoreactive cells was counted in three fields of view that exhibited the most positive staining, and the average ratio of immunoreactive cells to the

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**Table 1. Antibodies used for immunohistochemistry**

| Antigen | Antibody | Manufacturer | Dilution | Incubation time (min) |
|---|---|---|---|---|
| CD34 | M 7165 | Dako Japan | 1:100 | 30 |
| D2-40 | M 3619 | Dako Japan | 1:50 | 30 |
| CD31 | M 0823 | Dako Japan | 1:50 | Overnight |
| Factor XIII | N 1505 | Dako Japan | 1:2 | 30 |
| VEGF-R1 | AF 321 | R&D | 1:150 | 15 |
| VEGF-R2 | AF 357 | R&D | 1:50 | 15 |
| VEGF-R3 | AF 349 | R&D | 1:50 | 15 |

total number of cancer cells per field was calculated. The number of immunoreactive vessels was counted in three fields of view that demonstrated the most positive staining, and the average ratio of immunoreactive vessels to the total number of CD34-positive and D2-40-positive vessels per field was calculated. Staining results for VEGF-R1, VEGF-R2, and VEGF-R3 were classified by estimating the percentage of epithelial cells and vessels showing specific immunoreactivity: negative (defined as <5% staining) or positive (defined as >5% staining).[7] Two researchers evaluated the immunostaining results without being informed of the clinical data.

**Statistical analysis.** We examined objective tumor response to chemotherapy overall survival. Overall survival were calculated as the period from the start of first-line chemotherapy until disease progression or death from any cause, respectively. If patients were lost to follow up, data were censored at the date of the last evaluation. Statistical analysis was carried out using Stat View version 5 software (SAS Institute, Cary, NC, USA). Pearson's correlations were used to assess VEGF and VEGF-R expression, and a $\chi^2$-test was used to assess relationships between VEGF and VEGF-R expression and therapeutic effect. Each factor and overall survival were determined by Kaplan–Meier methods and analyzed using a log-rank test. Multivariate analysis was carried out using a Cox proportional hazard model.

## Results

**Clinicopathological characteristics.** The clinicopathological characteristics of the patients are shown in Table 2. Patients comprised 69 (80%) men and 17 (20%) women, with a median age of 61 years. Tumor stage (assessed according to TNM classification at the time of surgery) was I, II, or III in 35 patients, and distant metastasis was confirmed at the time of surgery (stage IV) in 51 patients. Histopathologically, 39 patients had intestinal-type adenocarcinoma and 47 displayed diffuse-type adenocarcinoma. All patients received chemotherapy; first-line chemotherapy comprised S-1 in 29 patients, 5-fluorouracil (5-FU) in 24 patients, cisplatin (CDDP) and irinotecan (CPT-11) in 28 patients, and other agents in the remaining five patients. The median follow-up time was 13.3 months (range 1.0–71.7 months).

**Expression of VEGF-R1, VEGF-R2, and VEGF-R3.** VEGF-R1 was immunoreactive in tumor cells (not only in the membrane, but also in the cytoplasm) and tumor stromal vessels (Fig. 1a). VEGF-R1 expression was found in tumor cells of 65 tumors (76%) and in stromal vessels of 36 tumors (42%) (Table 3).

VEGF-R2 and VEGF-R3 were immunoreactive mainly in tumor stromal vessels (Fig. 1b–d). VEGF-R2 expression was found in tumor cells and stromal vessels of 0 and 46 tumors (0 and 53%), respectively, and VEGF-R3 expression was found in tumor cells and stromal vessels of 0 and 75 tumors (0 and 87%), respectively. The three types of VEGF-R were not markedly correlated with each other in terms of expression.

**Table 2. Patient characteristics ($n$ = 86)**

| Characteristic | $n$ |
|---|---|
| Sex | |
| Male | 69 |
| Female | 17 |
| Median age (years) | 61 (range 39–84) |
| Tissue type | |
| Intestinal | 39 |
| Diffuse | 47 |
| pStage† | |
| I | 2 |
| II | 11 |
| III | 22 |
| IV | 51 |
| ECOG performance status | |
| 0 | 42 |
| 1 | 41 |
| 2 | 3 |
| Metastases | |
| Liver | 25 |
| Abdominal lymph node | 43 |
| Peritoneum | 23 |
| Lung | 4 |
| Other | 4 |
| First-line chemotherapy | |
| S-1 | 29 |
| 5-Fluorouracil | 24 |
| Cisplatin + irinotecan | 28 |
| Other | 5 |

†Japanese classification. ECOG, Eastern Cooperative Oncology Group.

**Table 3. Distribution of vascular endothelial growth factor receptor (VEGF-R) 1, VEGF-R2, and VEGF-R3 expression**

| Status | VEGF-R1 | | | | VEGF-R2 | | VEGF-R3 | |
|---|---|---|---|---|---|---|---|---|
| | Cytoplasm | | Vessel | | Vessel | | Vessel | |
| | $n$ | % | $n$ | % | $n$ | % | $n$ | % |
| Negative (<5%) | 21 | 24 | 50 | 58 | 40 | 47 | 11 | 13 |
| Positive (>5%) | 65 | 76 | 36 | 42 | 46 | 53 | 75 | 87 |

**Relationship of VEGF-R expression with response to chemotherapy and survival.** The response rate was 38% (11/29) in the S-1 group, 4% (1/24) in the 5-FU group, and 43% (12/28) in the CDDP and CPT-11 group (Table 4). In the S-1 group, the response rate was lower in the 15 patients in whom stromal vessels stained positive for VEGF-R1 than in the 14 patients in whom stromal vessels did not (20 vs 57%, $\chi^2$-test $P = 0.039$). In the other groups, the response rates were not markedly affected by expression of VEGF-R.

To clarify the relevance of marker positivity in prediction of disease outcome, staining results for VEGF-R1, VEGF-R2, and VEGF-R3 were correlated with patient survival according to the log-rank test. A univariate analysis revealed that VEGF-R expression correlated with shorter survival (VEGF-R1 in stromal vessels, 11.2 vs 15.9 months, $P = 0.001$, Fig. 2a; VEGF-R2 in stromal vessels, 11.0 vs 15.6 months, $P = 0.009$, Fig. 2b; VEGF-R3 in stromal vessels, 12.8 vs 24.3 months, $P = 0.005$, Fig. 2c). Moreover, multivariate analysis of potential prognostic factors showed that VEGF-R1 and VEGF-R2 expression by stromal vessels were independent predictors of poor outcome in advanced GC (Table 5).

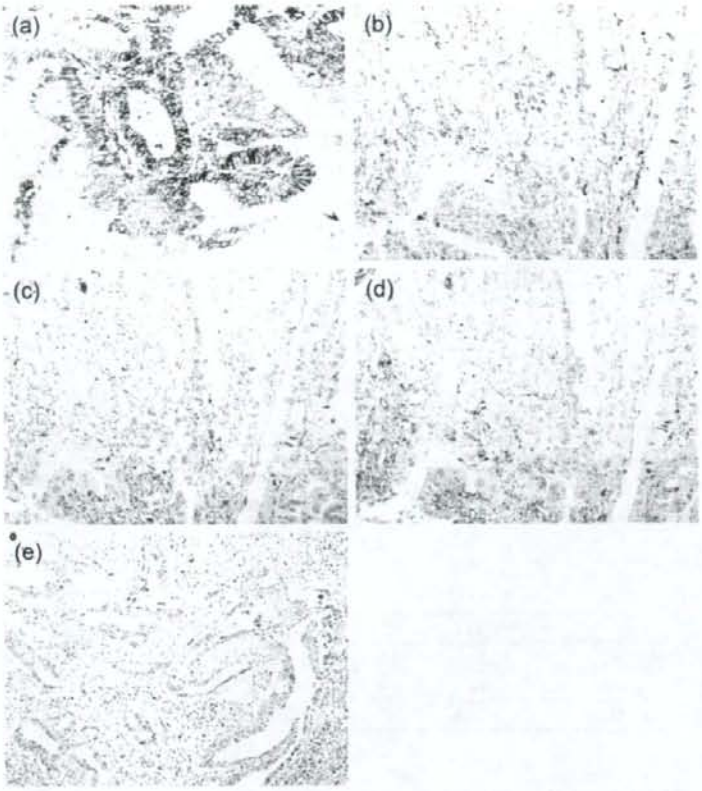


**Fig. 1.** Typical examples of (a) CD34 staining, (b) D2-40 staining, (c) CD31 staining, (d) factor VIII staining, and (e) negative controls. (a) Vascular endothelial growth factor receptor (VEGF-R) 1 is mainly expressed in tumor cells, secondarily on stromal vessels. (b–d) VEGF-R2 and VEGF-R3 are mainly expressed on stromal vessels. Original magnification, ×200.

**Table 4. Relationship between vascular endothelial growth factor receptor (VEGF-R) expression and response to chemotherapy**

| First-line regimen | *n* | Total response (%) | VEGF-R1 | | | | VEGF-R2 | | VEGF-R3 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Cytoplasm | | Stromal vessels | | Stromal vessels | | Stromal vessels | |
| | | | Positive (%) | Negative (%) | Positive (%) | Negative (%) | Positive (%) | Negative (%) | Positive (%) | Negative (%) |
| S-1 | 29 | 38 | 32 | 57 | 20 | 57 | 31 | 44 | 37 | 50 |
| | | | $P = 0.234$ | | $P = 0.039$ | | $P = 0.474$ | | $P = 0.715$ | |
| Cisplatin and irinotecan | 28 | 43 | 33 | 47 | 45 | 41 | 47 | 38 | 46 | 25 |
| | | | $P = 0.255$ | | $P = 0.570$ | | $P = 0.445$ | | $P = 0.887$ | |
| 5-Flurouracil | 24 | 4 | 0 | 4 | 0 | 4 | 4 | 0 | 4 | 0 |
| | | | – | | – | | – | | – | |

## Discussion

In the present study, we analyzed VEGF-R expression levels in primary tumors from 86 patients with advanced GC. Our goal was to determine whether such expression levels are related to treatment outcomes such as survival and response. We found that expression of VEGF-R1 and VEGF-R2 in stromal vessels in GC specimens were significant predictors of poor survival in advanced GC. Recently, several studies have reported that the genetic profile of patients is related to the outcome of cancer therapy. In colorectal cancer, VEGF-R2 expression for metastatic tumors was higher when compared to non-metastatic tumors,[5] and in head and neck cancer[15] and breast cancer,[16] some studies have documented that VEGF-R3 expression correlates with lymph node metastasis and malignancy,[7,9,14,17] whereas others have not observed this relationship.[18–20] Further investigations are needed to clarify interactions among VEGF-R subtypes and the effects of VEGF expression in stroma on angiogenesis and lymphangiogenesis. In GC, several studies have reported correlations between the expression of VEGF and poor prognosis, or lymphatic metastasis. However, most studies examined survival from the date of surgery to the time of event. In the present study, we examined the expression of VEGF-R, objective tumor response to chemotherapy, and overall survival; the latter two being calculated as the period from the start of first-line chemotherapy until disease progression or death from any cause, respectively.

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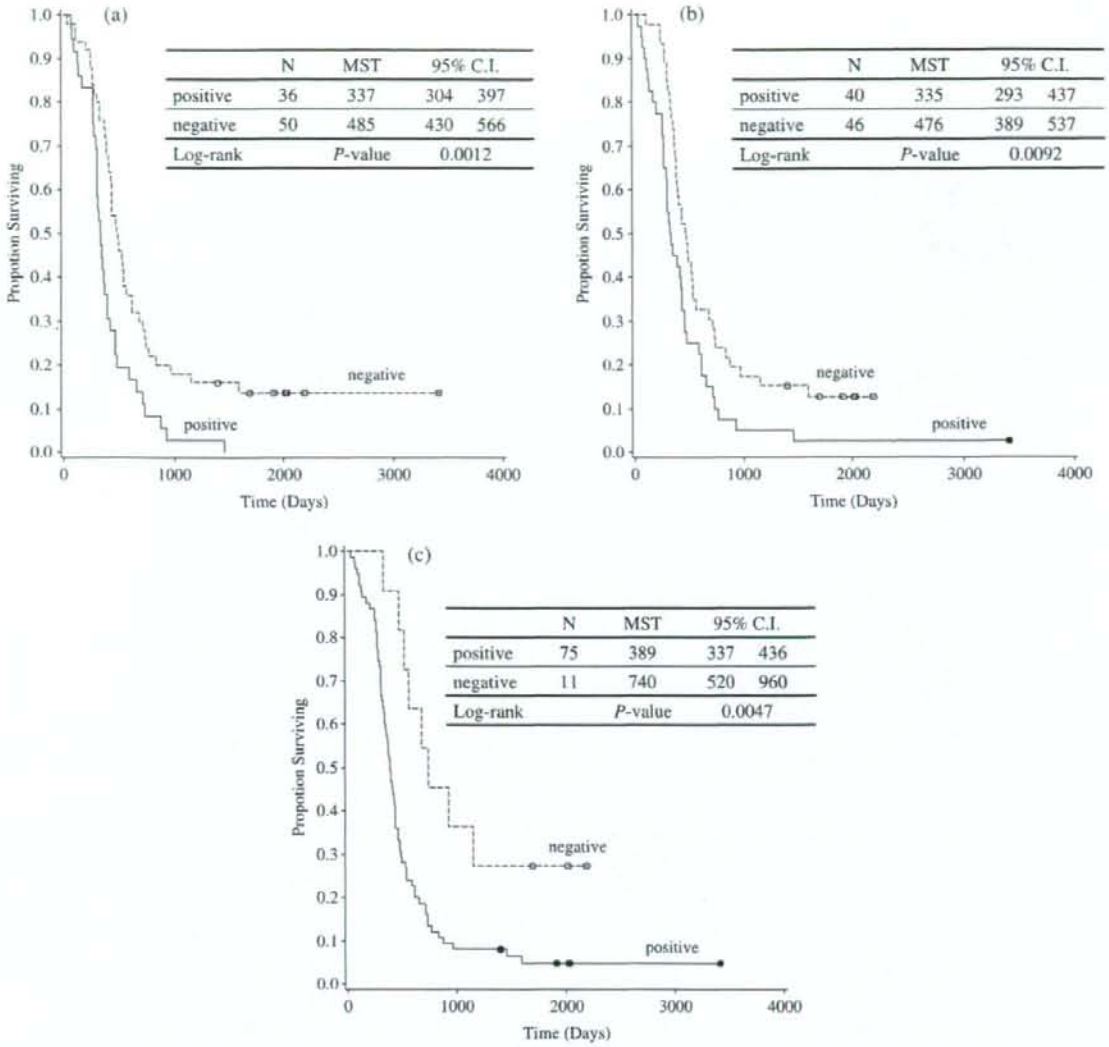


**Fig. 2.** Impact of (a) vascular endothelial growth factor receptor (VEGF-R) 1, (b) VEGF-R2, and (c) VEGF-R3 expression in stromal vessels on patient survival.

**Table 5. Impact of vascular endothelial growth factor receptor (VEGF-R) expression on patient survival from first-line chemotherapy (multivariate analysis)**

| | Parameter | Hazard ratio | 95% confidence interval. | | *P*-value |
|---|---|---|---|---|---|
| **VEGF-R1 (vessel)** | | **1.75** | **1.09** | **2.80** | **0.020** |
| PS | 1, 2 versus 0 | 1.45 | 0.62 | 2.27 | 0.109 |
| Tissue type | Diffuse vs intestinal | 0.64 | 0.64 | 1.00 | 0.052 |
| Metastasis site | 2≥ versus 1 | 1.5 | 0.89 | 2.55 | 0.132 |
| **VEGF-R2 (vessel)** | | **1.76** | **1.12** | **2.75** | **0.014** |
| PS | 1, 2 versus 0 | 1.56 | 1.00 | 2.46 | 0.052 |
| Tissue type | Diffuse versus intestinal | 0.64 | 0.41 | 1.01 | 0.055 |
| Metastasis site | 2≥ versus 1 | 1.69 | 1.01 | 2.81 | 0.045 |

PS, Performance Status.

After treatment with S-1, patients with positive staining for VEGF-R1 in stromal vessels showed a lower response rate (20 vs 57%, $P = 0.039$) and shorter survival (10.2 vs 20.2 months, hazard ratio = 3.62: data not shown) than those with negative staining, whereas there was no difference with CDDP and CPT-11. The number of patients treated with S-1 was small, but Boku *et al.* have reported the relationship between VEGF status and the effects of S-1 and 5-FU; patients expressing VEGF showed a slightly lower response rate and relatively shorter survival than those who did not.[21,22] The mechanisms behind this relationship are unclear,[23] but expression of VEGF-R may become a prognostic marker relevant in deciding on a treatment strategy of 5-FU-based drugs.

Our analysis revealed that VEGF-R expression was correlated with shorter survival (VEGF-R1 in stromal vessels, $P = 0.001$; VEGF-R2 in stromal vessels, $P = 0.009$; and VEGF-R3 in stromal vessels, $P = 0.005$), and multivariate analysis of potential prognostic factors showed that VEGF-R1 and VEGF-R2 in stromal vessels were independent predictors of poor outcome. VEGF-R2 is a potent regulator of vascular endothelial cells and has been directly linked to tumor angiogenesis and blood vessel-dependent metastasis. VEGF-R1 may contribute to pathological vascularization directly by stimulating endothelial cell function and indirectly by mediating recruitment of bone marrow progenitor cells.[24] Furthermore, Carmeliet and coworkers demonstrated synergy between the VEGF-R1- and VEGF-R2-specific ligands, indicative of cross-talk between the receptors, allowing modulation of a variety of VEGF-R-dependent signals.[25] In GC, the expression of VEGF or VEGF-C, which are intimately involved in regulation of the lymphangiogenic process, has been reported to be correlated with a poor prognosis.[10,11,26] Juttner *et al.* found that the presence of VEGF-D and its receptor VEGF-R3 was associated with lymphatic metastasis.[12] Given these results, expression of the VEGF family appears to affect the prognosis of GC.

Our immunostaining evaluation revealed that VEGF-R is expressed in tumor cells and tumor stromal vessels.VEGF-R2, which is expressed primarily in vascular endothelial cells, is believed to be the major mediator of angiogenesis in human malignancy, as it regulates activation of downstream effector molecules such as the phosphoinositide 3-kinase plus AKT and mitogen-activated protein kinase pathways. It also potentiates endothelial differentiation, DNA synthesis, and proliferation.[27,28] On the other hand, VEGF-R3 is expressed primarily in lymphatic endothelial cells and regulates lymphangiogenesis.[29] Recently, some studies have documented that the expression of VEGF-R has been observed in tumor cells in several cancers,[30–35] and in the autocrine VEGF–VEGFR loop in cancer cells. Fan *et al.* demonstrated that incubation with VEGF-A or VEGF-B significantly increased colorectal cancer cell migration; however, treatment with a VEGF-R1 antibody blocked this effect.[30] Giatromanolaki *et al.* demonstrated that phosphorylated VEGF-R2 plus KDR receptors are largely expressed in colon cancer cells and intratumoral vasculature, and their expression is associated with tumor diameter and poor histological differentiation.[31] In GC, Tian *et al.* demonstrated that VEGF-R2-positive tumor cells could be stimulated by exogenously added VEGF.[32] In our study, patients with strong positive staining (defined as >50% staining) for VEGF-R1 in the cytoplasm of tumor cells showed shorter survival (12.6 vs 14.2 m, $P = 0.044$; data not shown) than others. Thus, these results suggest that the autocrine VEGF–VEGF-R loop function may contribute to cancer cell proliferation.

In conclusion, our study provides evidence that VEGF-R expression in GC specimens is a risk factor for poor survival in patients with advanced GC. The results of our analysis can help to identify patient subgroups at higher risk for poor disease outcome in GC.

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