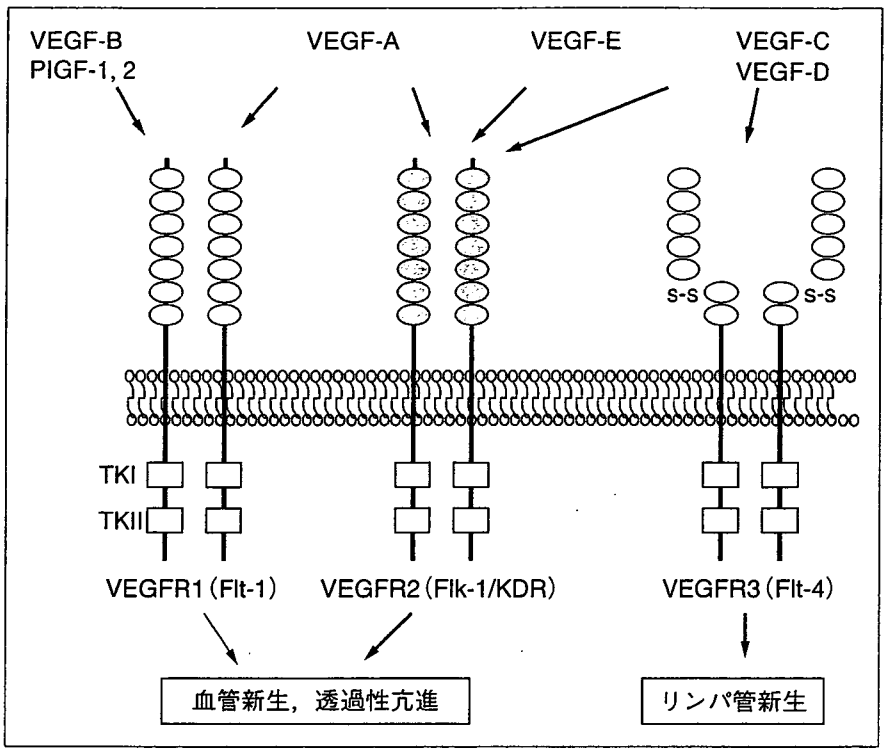

図4　VEGFとVEGFR(文献[24]より改変)

作用として，動脈，静脈そしてリンパ管の内皮細胞の増殖であるが，細胞飢餓に伴うアポトーシスから内皮細胞を守る働きや，管腔形成の促進，凝固線溶系タンパク質の産生，細胞接着因子の誘導など，さまざまな働きがあることがわかっている．VEGF関連遺伝子としては，VEGF-B,-C,-D,-Eが同定されており，同様に血管新生，リンパ管新生などに関与することがいわれている．このあたりはがんというよりも再生医学領域でも注目を集めている．

VEGFの受容体であるVEGFRには，VEGFR-1(Flt-1)とVEGFR-2(KDR/Flk-1)，VEGFR-3(Flt-4)の3種類がある(図4)．VEGFRは受容体型チロシンキナーゼで，リガンドであるVEGFの結合により細胞内にあるチロシンキナーゼ(TKI, TKII)がリン酸化され，シグナルが下流へ伝わっていく．これらの構造は，血小板由来増殖因子受容体(PDGFR)や，GISTなどで過剰発現しているc-kitチロシンキナーゼと類似しており，VEGFR2, PDGFR, c-kitを抑えるSU11248やVEGFR-1, -2, -3, PDGFR, c-kitを抑えるPTK787/ZK222584などはマルチチロシンキナーゼ阻害剤としてGISTや大腸がんをはじめとする多くのがんで臨床試験が行われている．

VEGFの過剰発現は肺がん，乳がん，消化器がん，腎細胞がん，卵巣がんと多くのがんで認められている．がん遺伝子であるsrcやras, bcr-abl, がん抑制遺伝子であるp53はVEGF発現制御機構の上流にあり，これらの遺伝子の変異により，がんにおけるVEGFの過剰発現がみられると考えられている[24]．担がんマウスの系では，抗VEGF抗体と化学療法，放射線療法との併用で相乗効果が認められており，抗VEGF抗体による血管の正常化と，それに伴う抗がん剤組織移行の上昇，酸素化などがその一因であると考えられている[25]．

2. Bevacizumab(Avastin®)

BevacizumabはVEGF-Aに対するヒト化抗体であり，血中のVEGF-Aに結合し中和することでVEGFRに結合するVEGF-Aを減少させ，結果としてVEGFR以下のシグナルを阻害する．Bevacizumabは大腸がんのみならず，肺がん[26]，乳がん[27]においても臨床開発が行われ，有効性が証明されている．大腸がんでは，IFL療法＋placebo群と，IFL療法にbevacizumab 5 mg/kgを隔週併用投与した群，そして5 FU/LVにbevacizumab 5 mg/kgを併用群の3群で比較試験(AVF2107g)を

表4 Bevacizumab関連有害事象

		Hurwitz JCO 23, 2005			Giantonio ASCO2005	
		IFL(%) (n=397)	IFL/BV(%) (n=393)	5FU/BV(%) (n=109)	FOLFOX4(%) (n=282)	FOLFOX4/BV(%) (n=286)
血栓塞栓症	Grade 1-4	16.2	19.4	13.8	2.8	4.4
出血	Grade 3,4	2.5	3.1	6.4	<1	3
高血圧	Grade 1-4	8.3	22.4	33.9	−	−
	Grade 3	2.3	11.0	18.3	2	5
	Grade 4	0	0	0	<1	1
タンパク尿	Grade 1-4	21.7	26.5	34.9	−	−
	Grade 3	0.8	0.8	1.8	1	0
消化管穿孔		0	1.5	0	1	0

(文献[28][29]より引用)

表5 CPT11不応大腸がんに対する臨床第III相試験(ECOG3200)

	ECOG3200			Rosenberg 2003
	BV (n=230)	FOLFOX4/BV (n=271)	FOLFOX4 (n=271)	FOLFOX4 (n=272)
RR(%)	3.0	21.8	9.2	9.9
		$p<0.0001$		
TTP(mon)	2.7	7.2	4.8	4.6
		$p<0.0001$		
MST(mon)	10.2	12.9	10.8	9.8
		$p=0.0018$		

(文献[29]より引用)

行い，それぞれ生存期間中央値(IFL群15.6m vs. IFL+bevacizumab群20.3m vs. 5FU/LV+bevacizumab群 18.3m)とbevacizumabの上乗せにより生存期間の延長が示された[28]．また，有害事象としては，高血圧がplacebo群で8.3%であったのに対し，bevacizumab群で22.4%と有意に高かった．これ以外にも出血やタンパク尿など，従来の抗がん剤とは毒性のプロファイルが異なるということが示唆されたが，とくに注意すべき点はbevacizumab併用群で消化管穿孔が数例に認められたことで，今後実際にbevacizumabが臨床の場で使用されるにあたり，慎重な対応が必要である(表4)．

また，CPT11不応症例に対する二次治療としてFOLFOX4単独群と，FOLFOX4+bevacizumab 10mg/kg群で行われたECOG3200試験(表5)では，生存期間中央値は(10.8m vs. 12.9m)と，bevacizumab併用群で有意な生存期間の延長が認められ，bevacizumabが大腸がんの化学療法のkey drugの一つであるという認識を確固たるものにした[29]．これを受けて，進行中であったSWOG0303などbevacizumabなしのarmをもつ臨床試験が，エントリーが少なくなり中止になった．初回治療例に対しては，mFOLFOX6療法と，bFOL療法と，XELOX(CapeOx)療法の3群にそれぞれbevacizumabを併用したランダム化第II相試験が行われた(TREE-2)[30]．プライマリーエンドポイントは毒性であり，G3/4の毒性については，各群で許容できるとの結論であった．しかし，bevacizumab群では2.8〜4.2%の消化管穿孔が認められ，創傷治癒遅延なども特徴的な毒性としてあげられた．有効性については，奏効率はそれぞれTREE1(mFOLFOX6 40.8% vs. bFOL 20.0% vs. CapeOx 27.1%)，TREE2(mFOLFOX6+bevacizumab 52.1% vs. bFOL+bevacizumab 34.3% vs. CapeOx+bevacizumab 45.8%)とbevacizumabの上乗せ効果が示唆された．

一方で，オキサリプラチン，イリノテカン不応となった大腸がんの3rd lineとして5FU/LV+bevacizumabを投与された100名では，奏効率はわずか1%[31]であり，比較的早期での使用が推奨される．

表6　現在進行中(中止含む)の抗体薬剤による大腸がん化学療法，主な臨床第III相試験

Trial	対　象	Protocol	Phase
NO16996	初回例	XELOX±BV vs. FOLFOX4±BV	PhaseⅢ
SWOG0303	初回例	XELOX±BV vs. mFOLFOX6±BV	中止
CALGB80404	初回例	FOLFOX or FOLFIRI +BV vs. +C225 vs. +BV+C225	PhaseⅢ
CALGB80203	初回例	FOLFIRI±C225 vs. FOLFOX4±C225	中止
CRYSTAL	初回例	FOLFIRI vs. FOLFIRI+C225	PhaseⅢ
EXPLORE	既治療例	FOLFOX4 vs. FOLFOX4+C225	PhaseⅢ
EPIC	FOLFOX不応	CPT11 vs. CPT11+C225	PhaseⅢ
CONCEPT	初回例	継続型mFOLFOX7+BV±Mg/Ca vs. 間欠型mFOLFOX7+BV±Mg/Ca	PhaseⅢ
BOND3	FOLFOX+BV不応	CPT11+BV+C225 vs. BV+C225	PhaseⅢ
PACCE	初回例	FOLFOX or FOLFIRI +BV vs. +BV+Panitumumab	PhaseⅢ
OPTIMOX3	初回例	(FOLFOX7+BVX6 → FOLFOX7+BVX6)+Erlotinib vs. (XELOX+BVX6 → XELOX+BVX6)+Erlotinib	PhaseⅢ

3．IMC-1C11

IMC-1C11はVEGFR-2に対するキメラ抗体である．VEGFR-2を介したシグナル伝達を抑制することで，抗腫瘍効果を発揮する．Phase Ⅰではgrade 3/4の毒性は認められず，投与した14例中4例にSDが認められている[32]．

Edrecolomab(17-1A)

Edrecolomabは，細胞表面の糖タンパク17-1Aをターゲットにした単クローンマウスIgG2a抗体である．189名のstageⅢの大腸がん術後補助療法として17-1Aを投与されたランダム化試験では，手術単独群に比べて，17-1A投与群において，有意に生存期間の延長を認めた[33]．しかしその後edrecolomab単独群，5FU/LV(Mayo regimen)単独群とそれにedrecolomabを上乗せした群とを比較したランダム化試験[34]では，毒性などには差を認めなかったものの，生存期間や無病生存期間において，edrecolomabの上乗せ効果は認められず，edrecolomab単独群は，5FU/LV群と比べて，有意に3年無病生存期間(53.0% vs. 65.5%，$p<0.0001$)で劣っていた．

現在進行中の臨床試験と今後の展望

現在進行中のcetuximab，bevacizumab，panitumumab関連の主な臨床試験を表6に示す．Bevacizumab，cetuximabともに初回治療例でのポジティブな結果が，2006年早々にも発表されると期待される．一方で，抗体療法の普及に伴う医療費の高騰はすでに欧米でも問題視されており[35]，その膨大な医療費は誰が負担するのか，はたして強力な治療を必要としているのはどのような患者群であるのか，などの議論が盛んに行われている．2006年には日本の大腸がん化学療法も分子標的治療時代へ突入すると思われる．効果のある治療法をすみやかに導入することが重要であるが，そういった社会的側面もあることを頭に入れておく必要がある．

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S-1の基礎と臨床
結腸・直腸癌

結腸・直腸癌に対するS-1単剤療法の意義

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S-1 as a Single Agent for Colorectal Cancer: Takako Eguchi and Kuniaki Shirao (*Dept. of Gastrointestinal Oncology, National Cancer Center Hospital*)

Summary

Chemotherapy for colorectal cancer is now improving rapidly due to new drugs like oxaliplatin and molecular targeting drugs. The key drug, however, is still 5-fluorouracil (5-FU). S-1 is an oral 5-FU anti-tumor drug that combines three pharmacological agents: tegafur, 5-chloro-2,4-dihydroxypyridine, which inhibits dihydropyrimidine dehydrogenase activity, and potassium oxonate, which reduces gastrointestinal toxicity.

The results of the phase II study suggested that S-1 as a single agent was active against metastatic colorectal cancer: CR rate was 36-40% and MST was about one-year. Toxicity was all tolerable. Clinical trials of S-1 with oxaliplatin or CPT-11 combination chemotherapy are ongoing in Japan. S-1 with molecular targeting drugs will also be studied. Therefore, S-1 is expected to play an important part in chemotherapy for colorectal cancer. **Key words:** S-1, Colorectal cancer, **Corresponding author:** Takako Eguchi, Department of Gastrointestinal Oncology, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

要旨 進行再発結腸・直腸癌に対する化学療法の進歩は目覚ましいものがあるが，依然としてkey drugは5-fluorouracil (5-FU) である。S-1は5-FUのprodrugであるtegafurに，DPD阻害剤である5-chloro-2, 4-dihydroxypyridineと消化管毒性軽減作用を有するpotassium oxonateとを配合した経口抗癌剤である。S-1の結腸・直腸癌に対する第II相試験の成績は良好で，奏効率36～40%，MSTは1年前後が得られ，毒性もtolerableであった。2003年12月結腸・直腸癌に対して保険適用となった後は，結腸・直腸癌に対するS-1を組み入れた新たな治療戦略が検討されるようになった。現在は，高齢者や全身状態不良例に対する選択肢として日本のガイドラインにあげられているが，今後oxaliplatin，CPT-11，分子標的薬剤などとの併用も含め，S-1が世界的な標準治療に組み込まれるか否かは今後の臨床試験の結果しだいである。

はじめに

近年，日本でも大腸癌罹患率が漸増しており死亡者数も増加している。大腸癌による年間死亡者数は2003年では年間3.9万人であったが，2015年には6万人にも上ると推定されている。大腸癌治療の中心は外科的切除であるが，Stage IVの結腸・直腸癌の5年生存率はそれぞれ12.8，13.1%と十分ではなく，外科的切除によって治癒しない患者に対する治療法を確立することが急務となっている。

irinotecan (CPT-11) やoxaliplatin (L-OHP) などの新薬，さらには分子標的薬剤まで導入され進行再発結腸・直腸癌に対する化学療法の進歩は目覚ましいものがあるが，依然としてkey drugは5-fluorouracil (5-FU) である。5-FUは生体内で特に肝臓に存在するdihydropyrimidine dehydrogenase (DPD) によりその80～90%が分解されて不活性化される。その5-FUのpro drugであるtegafurとDPD阻害剤を組み合わせることにより5-FUのF-beta-alanine (FBAL) への代謝を阻害し，効果増強を企図した薬剤をDPD inhibitory fluoropyrimidine (DIF) と総称する。DIFはDPDを阻害することにより，生物活性を高める，血中における5-

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FUの薬物動態の個人格差を少なくする，DPD発現腫瘍での抗腫瘍効果を高めかつ5-FUの代謝産物による毒性を軽減する，などの効果をもつ。DIFにはUFT，BOF-A2，S-1があるが最も強いDPD阻害活性を配合したBOF-A2は毒性のため第II相試験で開発が中止され，現在UFTとS-1が日常臨床に汎用されている。S-1はtegafurにDPD阻害剤としてuracilの約200倍の阻害活性をもつ5-chloro-2, 4-dihydroxypyridine (CDHP)と，消化管に高濃度で存在する5-FUのリン酸化酵素であるorotate phosphoribosyl tranferaseを可逆的に拮抗阻害するpotassium oxonate (Oxo)（これにより用量制限毒性である消化管毒性を軽減する）とを1：0.4：1のモル比で配合した経口抗癌剤である。S-1は進行・再発胃癌に対する第II相試験において単剤でも奏効率44.6%，MST 8か月，2年生存率17%と良好な成績を示し注目されていたため[1,2]，結腸・直腸癌でも同様の単剤での試験が行われた。その良好な結果をもって2003年12月，日本において保険適用となったが，現在では結腸・直腸癌に対するS-1を組み入れた新たな治療法が検討されるようになってきている。本稿では結腸・直腸癌に対するS-1単剤療法の意義，位置付けについて述べることとする。

I．本邦における結腸・直腸癌に対する化学療法の変遷

大腸癌領域では，世界中で多くの大規模第III相試験が行われるようになり，その結果を基に標準治療が決定されるようになった。残念ながら日本はこれら世界中で行われる開発試験に参加しておらず，海外で証明された治療が日本の標準治療として採用されずにきた。しかし最近では日本も世界の標準治療を受け入れる傾向にあり，一部未承認のために使用できない治療法があるものの基本は世界の治療体系と同様である。

数年前までの本邦における進行再発大腸癌に対する化学療法は経口フッ化ピリミジン製剤が主流であった。しかしながら経口剤の利便性のみが強調され，臨床的有用性が臨床試験で検証されずに頻用されてきた。1994年にはCPT-11が承認され，また1999年に本邦での後期第II相試験の結果を基に5-FU/LV療法がRosewell Park Memorial Institute (RPMI)のレジメンとして承認され，ごく最近まで頻用されていた。2003年には後述する第II相試験の結果を基にS-1が承認された。

このころより本邦でも世界の標準治療を受け入れる気運が高まり，本邦でもCPT-11＋5-FU/LV (IFL)の第I/II相試験で日本人における投与量の検討が行われ，標準治療として使用されるようになった。しかしその後米国で行われたIFLを含む二つの第III相試験（N 9741, C 89803)の解析で，IFL療法の60日以内の早期死亡例が高いこと，また5-FU持続静注を用いるレジメンより骨髄抑制や下痢などの副作用が強くでやすく毒性の強いレジメンであることが問題となったこともあり[3]，現在では欧米はもとより本邦でもその使用は減少しつつある。その後CPT-11＋5-FU/LV (FOLFIRI)療法の投与量に関する検討も行われ，さらにはL-OHPが承認されL-OHP/5-FU/LV (FOLFOX)療法も日本で使用できるようになった。現在bavacizmab，cetuximabなどの分子標的薬剤に関しては治験が行われている段階である。以上より現在の標準治療はFOLFOX，FOLFIRI，CPT-11単剤などであり，これが適応とならない全身状態不良例に対しては5-FU/LV，FU系単剤などを使用することと考えられる。

II．結腸・直腸癌に対するS-1単剤臨床試験

本邦で行われたS-1の結腸・直腸癌に対する第II相試験の成績を紹介する。まず各種悪性腫瘍35例を対象として行われた第I相試験の成績を最初に述べるが，75 mg/body，1日2回，28日間連日投与14日間休薬が推奨用量となり，用量制限毒性は白血球減少を主とする骨髄抑制であった[4]。その後5-FU系前治療ありの症例を含む結腸・直腸癌31例を対象として前期第II相試験が行われ，50 mg/body/day，1日2回，または75 mg/body/day，1日2回で28日間連日投与14日間休薬を1コースとし，奏効率16.7%，MST 358日であった[5]。この前期第II相試験の結果から，体表面積換算で90 mg/m²/day未満の投与が行われた症例において有害反応による中止率が低かったことから，引き続き行われた二つの後期第II相試験では体表面積換算で80 mg/m²，1日2回，28日間連日投与14日間休薬が推奨用量となった。2000年に報告されたOhtsuらの結腸・直腸癌初回治療例62例を対象として行われた後期第II相試験では，奏効率35.5%，MST 378日であった。原発巣別効果では結腸34.9%，直腸36.8%，部位別効果では肝27.5%，肺39.3%であった。毒性についてはgrade 3以上の好中球減少を13%に認めたが，その他のgrade 3以上の毒性はいずれも10%未満と軽微であった(Table 1)[6]。また，同様の対象38例に対して行われたShiraoらの後期第II相試験では奏効率39.5%，MST 358日が得られ，原発巣別効果では結腸44%，直腸33%，部位別効果では肝38%，肺27%，リンパ節30%であった。grade 3以上の毒性はいずれも10%未満であり（Table 2)，S-1の有効性と安全性が再現された[7]。以上のような後期第II相試験の成績から，S-1は胃癌に引き続き結腸・直腸癌においても承認を受けた。

Table 1 Toxicity data of S-1 phase II study

Toxicity	grade (No. of patients)				Incidence of ≥ grade 3 (%)
	1	2	3	4	
Haematological					
Leukopenia	17	10	1	2	4.8
Neutropenia	4	11	7	1	12.9
Anemia	5	11	4	0	6.5
Thrombocytopenia	5	2	0	5	8.1
Non-haematological					
Stomatitis	8	2	0	0	—
Diarrhea	2	6	1	0	1.6
Anorexia	7	11	3	0	4.8
Nausea/vomiting	7	4	1	0	1.6
Skin rash	2	4	0	0	—
Pigmentation	11	0	0	0	—
Malaise	9	2	1	0	1.6

(Ohtsu, *et al*, 2000)

Table 2 Toxicity data of S-1 phase II study

Toxicity	grade				grade ≥ 3 (%)
	1	2	3	4	
Anemia	7	7	3	0	7.9
Leukopenia	7	10	0	0	0
Neutropenia	4	10	2	0	5.3
Thrombocytopenia	4	1	0	0	0
Diarrhea	5	8	1	0	2.6
Nausea/vomiting	8	7	0	0	0
Anorexia	15	4	0	0	0
Stomatitis	11	3	0	0	0
Hand-foot syndrome	2	0	0	0	0
Pigmentation	15	0	0	0	0
Malaise	17	2	0	0	0
Bilirubinemia	—[a]	14	3	0	7.9

(Shirao, *et al*, 2004)

[a]: grade 1 bilirubinemia is not defined in the toxicity criteria of the Japan Society for Cancer Therapy. (Japan Society for Cancer Therapy: Criteria for the evaluation of the clinical effects of solid cancer chemotherapy. *J Jpn Soc Cancer Ther* 28: 101-130, 1993.)

III. 今後の展望—S-1の位置付け—

世界の標準治療という観点からみるとFOLFIRI→FOLFOX 6という群と，その逆の群の2アームの第III相試験では前者で74%，後者で62%の患者がsecond-lineを受けた上で両アームとも同等に約20か月のMSTを得ることができており[8]，またその後いくつかのRCTの結果から5-FU，CPT-11，L-OHPの3剤使用率と生存期間は正比例することがわかり，3剤をうまく組み合わせることが進行大腸癌の化学療法において重要であると考えられている[9]。開発された時期により後治療に差はあるもののS-1をはじめとする経口フッ化ピリミジン剤単剤では，いずれもMSTは1年程度である。これらは再現性のあるデータであり，結腸・直腸癌に対する化学療法においては経口フッ化ピリミジン剤単剤の限界を表すものである。そこで，最近これらの経口剤とCPT-11やL-OHPとの併用療法が注目されるようになり，有望な成績が報告されつつある[10-12]。S-1についてはいくつかのS-1+CPT-11併用第II相試験が本邦で終了しているが，その結果は他章に譲る。S-1+L-OHPについては現在第II試験が治験として進行中でありその結果が期待される。以上，大腸癌を対象にしたS-1の複数の臨床試験が日本で行われたが，いまだ第III相試験の結果は示されていない。一方，capecitabine(本邦未承認)やUFT/LVなどの経口フッ化ピリミジン薬剤はすでに第III相試験で5-FU/LVとの同等性が示されており，現在これらにCPT-11やoxaliplatinの上乗せ効果が検討されている段階である。この点からも，S-1はいまだ大腸癌領域では標準治療として認められるには不十分なデータしか得られていないといえる。しかし，単剤の試験結果で示したとおり，その成績は効果，毒性ともに大きな期待を抱かせるものであり，今後の迅速な開発が待たれるところである。現段階での本邦におけるS-1の位置付けについて明確な見解はないが，FOLFOXやFOLFIRIが適応とならない全身状態不良例における5-FU/LV，UFT/LVに次ぐ治療として考えるべきと思われる。

bavacizmab，cetuximabなどの分子標的薬剤は現在それぞれFOLFOX，CPT-11との併用第II相試験が行われている段階で，近い将来本邦でも承認される見込みである。しかし医療経済的観点から考えると，すべての患者に同じ治療を行うのではなく確実な標的，感受性をもった患者群に対し適切に治療を選択していく，すなわちテーラーメード治療が今後課題となってくるだろう。5-FUに関する感受性予測，予後予測については今までに多数の報告がされている。Metzgerらは，大腸癌の組織内のmRNAの発現と5-FU/LV療法に対する抗腫瘍効果との関係について報告しており，それによると5-FU/LV療法の奏効率は，全例の奏効率が29%であるのに対して，TS低発現例57%，TP低発現例39%，TS，TP両方の低発現例では奏効率79%であり，5-FUの効果発現には，TS，TPの発現が低いことが必要であるとしている[13]。さらに同グループのSalongaらはDPDのデータを加え，5-FU/LV療法の奏効した症例はすべてDPD，TS，TPの三つの酵素発現が低い症例であり，DPD，TSおよびTPのいずれか一つが高発現していた症例では奏効例はなかったと報告し，5-FUの効果発現にはDPD，TS，TPのすべてが低いことが必要であると

している[14]。本邦では市川らが腫瘍内の酵素発現と大腸癌に対する5-FU系薬剤の治療効果予測について，low DPDかつlow TPかつhigh OPRTの症例では，5-FU系薬剤に対する奏効率が100%であるのに対して，それ以外の症例では奏効率が23%と低く生存期間にも有意な差がみられたと報告している[15]。以上，DPDが高値の患者に対する5-FU系薬剤の投与については，S-1をはじめとするDPD阻害剤を含むDIF製剤が適切と考えられる。しかしこれらの因子はいずれも5-FUの効果予測因子としてだけでなく予後因子である可能性があり，テーラーメード治療に寄与するにはさらなる検討が必要と思われる。

おわりに

消化器癌の化学療法において，フッ化ピリミジン系薬剤はいまだkey drugであり続けている。その経口薬であるS-1は，患者のquality of lifeを低下させるような重篤な毒性の頻度が少ない，外来治療が可能である，通院回数を減らせるなどの利点をもつ有望な薬剤であり，今後第III相試験を通じて世界的標準治療のなかに組み込まれていくことが期待される。さらに今後の化学療法のめざす方向性の一つであるテーラーメード治療におけるkey drugの一つとなる可能性が高い薬剤といえる。本邦は欧米に先駆けてS-1の開発を行ってきた経緯があり，今後もその発展に寄与すべく検討を重ねていく責務があると考える。

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ORIGINAL REPORT

Pharmacodynamic Studies of Gefitinib in Tumor Biopsy Specimens From Patients With Advanced Gastric Carcinoma

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Terms in blue are defined in the glossary, found at the end of this article and online at www.jco.org.

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ABSTRACT

Purpose
Epidermal growth factor receptor (EGFR) is highly expressed in some gastric cancers and is implicated in cancer cell growth and proliferation. The objective of this study was to assess the in situ biologic activity of the EGFR tyrosine kinase inhibitor gefitinib in gastric tumor samples in a phase II study.

Methods
Patients with previously treated stage IV adenocarcinoma of the stomach or gastroesophageal junction were randomly assigned to receive gefitinib (250 or 500 mg/d). Tumor biopsies, obtained at screening and on day 28 of treatment, were assessed for biomarker expression using immunohistochemistry and analysis of apoptosis.

Results
One hundred sixteen tumor samples from 70 patients were available, 70 were baseline and 46 were on-therapy biopsies. At baseline, levels of EGFR expression significantly correlated with levels of phosphorylated EGFR (pEGFR; $P < .001$) and Ki67 expression ($P = .011$), but not with phosphorylated mitogen-activated protein kinase (pMAPK). After gefitinib treatment, levels of pEGFR in tumor cells were significantly reduced ($P = .001$); this was not the case for pMAPK and phosphorylated Akt (pAkt). However, in some cases gefitinib inhibited pAkt and these tumors had enhanced apoptosis. Likewise, there was a significant correlation between increased exposure to geftinib and enhanced apoptosis.

Conclusion
Gefitinib reached the tumors at concentrations sufficient to inhibit EGFR activation in advanced gastric carcinoma patients, although this did not translate into clinical benefit. Overall, intratumoral phosphorylation of MAPK and Akt was not significantly inhibited by gefitinib. However, the finding that decreases in pAkt correlated with enhanced apoptosis deserves further exploration.

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INTRODUCTION

Gastric cancer is one of the most common causes of cancer deaths—worldwide it is second only to lung cancer.[1] Prognosis for patients with advanced stomach cancer is poor; the 5-year survival rate for patients with localized disease is approximately 60%, whereas for those with distant disease it is only 2%.[2] Therefore, there is clearly a need for new therapeutic approaches, among them the development of agents against molecular targets.[3]

Epidermal growth factor receptor (EGFR) is a member of the ErbB family of receptors—a family of receptors that plays a major role in promoting proliferation and the malignant growth of a variety of epithelial tumors (see review in Yarden and Sliwkowski[4]). EGFR is highly expressed in approximately one third of advanced-stage gastric cancers[5] and has been shown to be a modest prognostic indicator.[6] Recently, agents targeting the EGFR have been shown to have meaningful clinical activity in a variety of tumor types (see review in Baselga and Arteaga[7]).

In order to study the effects of anti-EGFR therapy in patients with advanced gastric cancer, we conducted the current phase II study with gefitinib (Iressa; AstraZeneca, Macclesfield, Cheshire, United Kingdom), an orally active EGFR tyrosine kinase inhibitor that blocks receptor-dependent signal transduction and that shown to be active in patients with non–small-cell lung cancer.[8] Our aim was to determine the antitumor activity of gefitinib in

patients with advanced gastric cancer and to study the effects of gefitinib on EGFR phosphorylation and on the two major receptor signaling pathways, the mitogen-activated protein kinase (MAPK) pathway and the phosphatidyl-inositol-3-kinase (PI3K)/Akt pathway, as well as its effects on proliferation and apoptosis.

In the first phase II study of gefitinib in patients with advanced gastric cancer, gefitinib was well tolerated and demonstrated modest clinical activity with disease control rates of 13.9% and 22.9% at 250 mg/d and 500 mg/d, respectively.[9] The clinical results will be reported elsewhere (Van Cutsem et al, manuscript in preparation). Results from the sequential tumor biopsy study to study the effects of gefitinib on EGFR signaling are reported in this article.

METHODS

Study Design

This was a randomized, double-blind, parallel-group, multicenter, two-stage, phase II study. The exploratory objectives of this study were to investigate the pharmacodynamic parameters at steady state and to evaluate whether EGFR expression correlates with the antitumor activity of gefitinib.

Patients

Eligible patients had histologically confirmed stage IV adenocarcinoma of the stomach or gastroesophageal junction and had failed one or two prior chemotherapy regimens. In addition, patients were required to have at least one measurable lesion as defined in Response Evaluation Criteria in Solid Tumors, a WHO performance status of 0 to 2, life expectancy of 12 weeks or longer, the ability to ingest food, and to be age 18 years or older. Patients who had received more than two previous chemotherapy treatments or who had their last anticancer therapy within 21 days of the start of gefitinib treatment were excluded.

All eligible patients had to provide written informed consent and the study was conducted in accordance with good clinical practice guidelines and the Declaration of Helsinki.

Treatment

Treatment was divided into four strata: two ethnic groups of patients (Japanese and non-Japanese) randomly assigned to receive gefitinib at either 250 mg/d or 500 mg/d as an oral dose. Patients continued with their assigned dose until disease progression and any patient showing evidence of response could continue with therapy outside of the core treatment period into an extension period.

Table 1. Patient Demographics

Characteristic	No.	%
Patients	75	
Sex	83	17
Male:female	62:13	
Age, years		
Median	60	
Range	33-83	
Race	57	43
White: Japanese	43:32	
Prior therapy		
Experienced ≥ 1 failed chemotherapy regimen	39	52
Experienced 2 failed chemotherapy regimens	36	48
Dose, mg/d		
250	37	49
500	38	51

Table 2. Tumor Biopsies Available for Analysis

Type	No.
Primary tumor biopsies	70
Assessable primary tumor biopsies	67
On-therapy day 28 biopsies	35
Evaluable day 28 tumor biopsies	32
Sequential paired biopsies	32
Available paired samples with baseline biomarker expression above than 0	
EGFR	20
pEGFR	17
pMAPK	31
pAkt	26
Ki67	32
$p27^{kip1}$	32

Abbreviations: EGFR, epidermal growth factor receptor; pEGFR, phosphorylated EGFR; pMAPK, phosphorylated mitogen-activated protein kinase; pAkt, phosphorylated Akt.

Pharmacokinetic Analysis

Venous blood samples for pharmacokinetic analysis were taken at screening, pregefitinib treatment, and at 1 hour, 3 hours, 5 hours, 7 hours, 12 hours, and 24 hours postgefitinib treatment on day 28 of treatment period 1. Complete pharmacokinetic data were available from 44 treated patients. In addition, a pregefitinib treatment sample was also taken on day 28 of each subsequent treatment period. Plasma was isolated from blood samples by centrifugation (10 minutes at 1,000 g) and was analyzed using high-performance liquid chromatography with tandem mass spectrometric detection. The C_{max} and t_{max} values for each patient were derived from the plasma concentration-time profile and the area under the time-concentration curve $(AUC)_{(0-24)}$ was calculated using the linear trapezoidal rule.

Biopsy Samples

Tumor biopsies were obtained at screening (pregefitinib treatment), on day 28 of treatment, and, if possible, at disease progression. Fresh tissue samples were collected from either the primary tumor or from metastatic sites. As much tissue as possible was collected, such that the total sample volume was at least 3 × 3 × 3 mm. For endoscopic biopsies, eight to 10 samples of 1 × 1 mm were allowed. Biopsies from liver metastases were taken using a computed tomography scan-guided 18 G core needle. All samples were fixed immediately after removal in a 10% buffered formalin solution for a maximum of 48 hours at room temperature before being dehydrated and paraffin embedded under vacuum. To allow comparative biomarker studies, subsequent biopsies were taken from the same site as the screening biopsy.

Antibodies

The following antibodies were used for the detection of pharmacodynamic markers: anti-EGFR (mouse monoclonal antibody [MAb] clone

Table 3. Baseline Tumor Characteristics

Tumor Characteristic	No.	%
Location*		
Stomach	54	77.2
Gastroesophageal junction	15	21.4
Unknown	1	1.4
Type*		
Intestinal	35	50
Diffuse	27	38.6
Unknown	8	11.4

*n = 70.

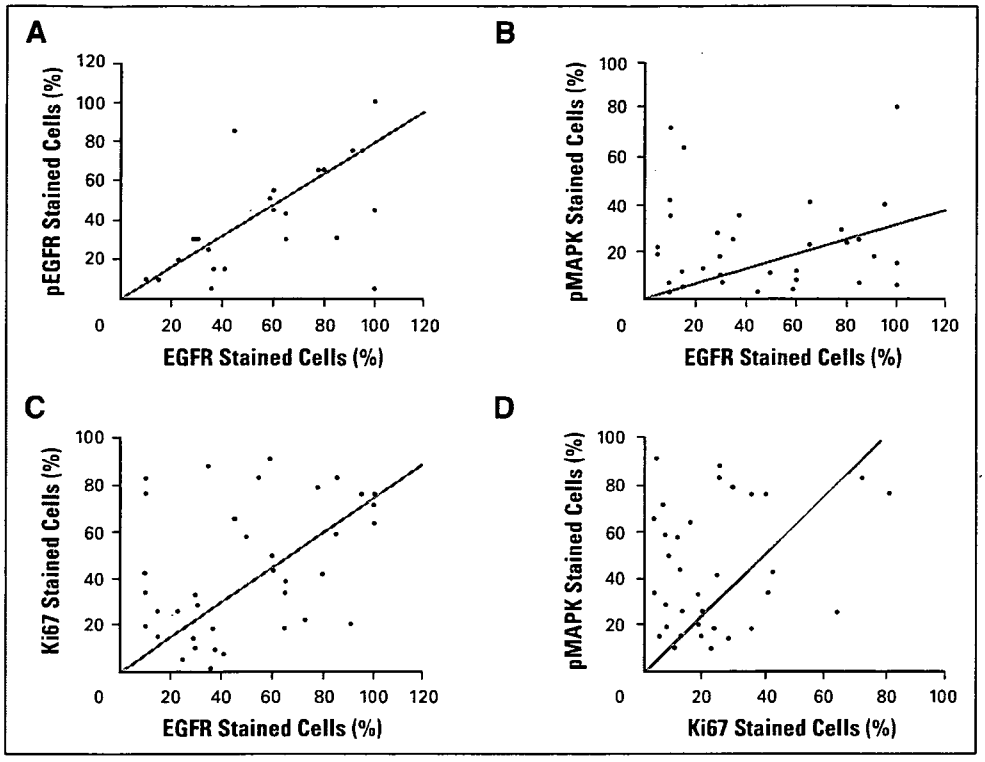

Fig 1. Baseline correlation in biomarker expression (epidermal growth factor receptor [EGFR] *v* phosphorylated EGFR [pEGFR]; EGFR *v* phosphorylated mitogen-activated protein kinase [pMAPK]; EGFR *v* Ki67; Ki67 *v* pMAPK). Significant correlations were calculated using Pearson's correlation test for EGFR versus pEGFR ($P < .001$), EGFR versus Ki67 ($P = .011$), and Ki67 versus pMAPK ($P = .01$).

2-18C9), anti-Ki67 (mouse MAb clone MIB1), and antip27^{kip1} (mouse MAb clone SX53G8) were obtained from DAKO (Carpinteria, CA), antiphosphorylated EGFR (pEGFR; mouse MAb clone 74) was obtained from Chemicon (Temecula, CA), anti-TGF-α (mouse MAb clone Ab-2) was obtained from Oncogene (San Diego, CA), antiphosphorylated p42/44 MAPK (pMAPK; rabbit polyclonal phosphorylated-p44/42 MAPK at Thr202/Tyr204) and antiphosphorylated Akt (pAkt; rabbit polyclonal phosphorylated-Akt at Ser473) were obtained from Cell Signaling Technology (Beverly, MA).

Immunohistochemistry

Immunostaining was performed on 4 μm tissue sections mounted onto positively charged glass slides. After removal of paraffin in xylene and graded alcohols, sections were hydrated. Epitope retrieval was performed when necessary in 10 mmol/L EDTA buffer, pH 8 for 10 minutes in a pressure cooker (pEGFR, pMAPK, pAkt, Ki67, and p27^{kip1}) or using proteinase K digestion for 5 minutes (EGFR) at room temperature. Tumor necrosis factor alpha (TNF-α) did not require antigen retrieval. After target retrieval, endogenous peroxidase was blocked by immersing the sections in .03% hydrogen peroxide for 5 minutes. Incubation with primary antibodies was performed at room temperature for 1 hour using the following dilutions: EGFR 1:1, pEGFR 1:500, TGF-α 1:100, pMAPK 1:80, pAkt 1:50, Ki67 1:100, and p27^{kip1} 1:100. Peroxidase-conjugated goat antirabbit (pMAPK and pAkt) or antimouse (EGFR, TGF-α, Ki67, and p27^{kip1}) were used to detect antigen-antibody reaction (En Vision+ System; DAKO) for 30 minutes at room temperature. For pEGFR, an enhanced signal amplification method (CSA; DAKO) was used as described in the manufacturer's guidelines. Sections were visualized with 3,3'-diaminobenzidine as a chromogen for 5 minutes and counterstained with Mayer's hematoxylin. Positive and negative controls were included in each experiment.

To score a tumor cell as positive, membrane staining was required for total EGFR, membrane or cytoplasmic staining for pEGFR, cytoplasmic staining for TGF-α, cytoplasmic and nuclear staining for pAkt, and nuclear staining for pMAPK, Ki67, and p27^{kip1}. Samples were assessed blind by investigators (F.R. and J.A.). For quantitative analysis, the percentage of stained tumor cells with each antibody in representative sections in 10 high power fields (×400) was used to calculate the average percentage of cells staining in every sample. A tumor was considered as positive with at least 1% of stained cells. Scoring was performed blind to clinical data (F.R.) and was used for statistical analysis.

Terminal Deoxynucleotide Transferase-Mediated dUTP Nick End Labeling Assay

Determination of apoptosis was measured by TUNEL (Terminal deoxynucleotide transferase [TdT]-mediated dUTP Nick End Labeling) assay on tissue sections using fluorescein-labeled 12-dUTP-TdT (Roche Diagnostics GmbH, Mannheim, Germany) after proteinase K digestion of the tissue. The apoptotic index was expressed as a percentage calculated from the number of green fluorescent cells in 10 high-power fields of the tumor tissue (×400 optical magnification) using a fuorescence Elipse E400 Nikon microscope (Nikon, Kanagawa, Japan). Absolute differences between apoptotic index in pre- and on-therapy samples were calculated.

Statistical Methods

Statistical analyses were carried out using the SPSS data analysis program, version 10.0 (SPSS Inc, Chicago, IL). The Spearman's correlation test was used to analyze statistical significance of continuous variables. Mann-Whitney U test was used to compare group means. Paired pre- and on-therapy samples were analyzed using the Wilcoxon signed rank test and Pearson χ^2. Significance was exclusively calculated on paired biopsies with pretherapy expression higher than 0. All statistical tests were conducted at the two sided .05 level of significance.

RESULTS

Patients and Biopsies

In total, 75 patients were randomly assigned to receive gefitinib. Of these patients, 37 received gefitinib 250 mg/d and 38 received gefitinib 500 mg/d. Patient demographics are summarized in Table 1. From this population, 105 tumor samples were available from 70

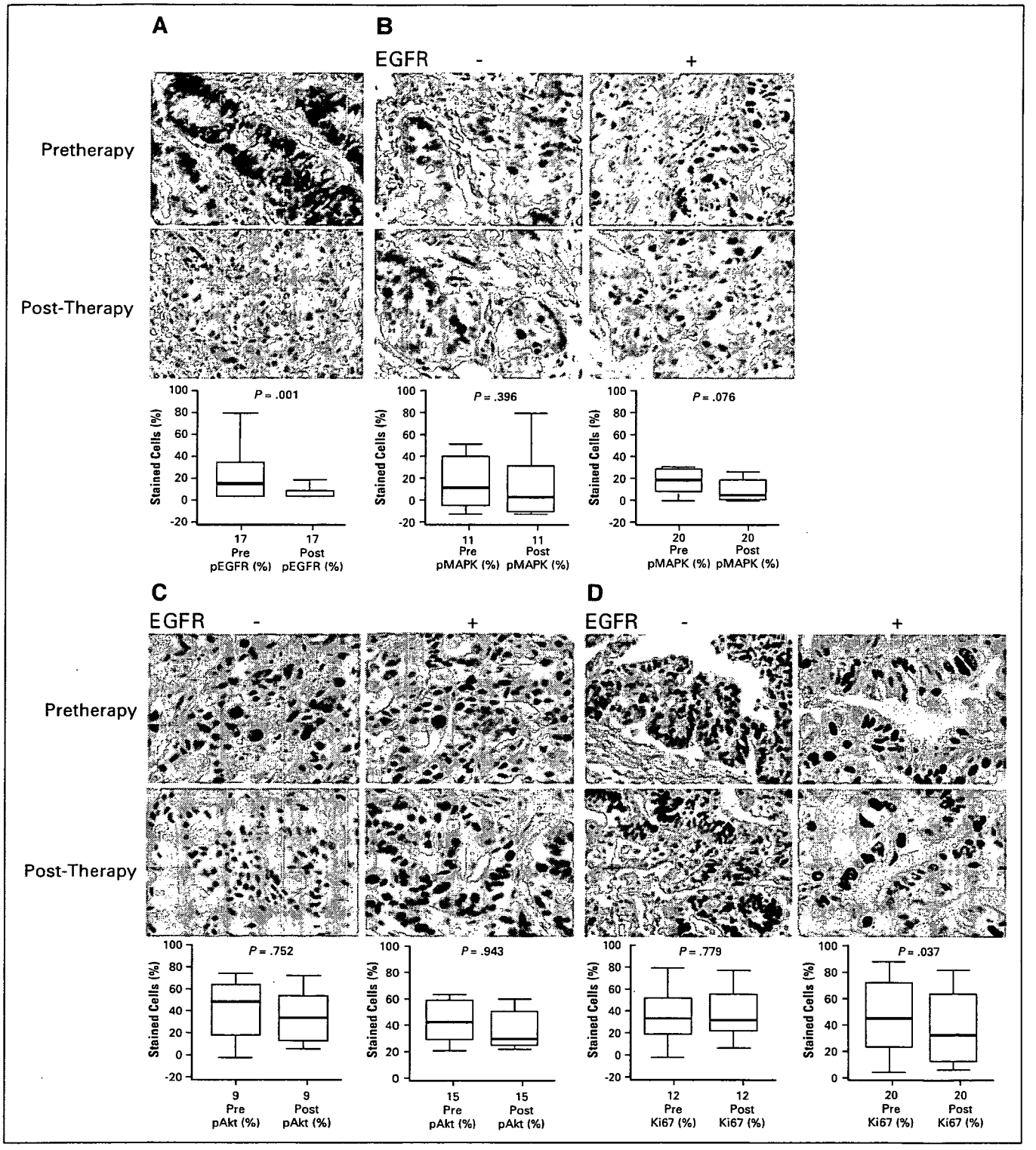

Fig 2. Basal and on-therapy levels of (A) phosphorylated epidermal growth factor receptor (pEGFR); (B) phosphorylated mitogen-activated protein kinase (pMAPK); (C) phosphorylated Akt (pAkt); and (D) Ki67. For each marker a representative case is shown in addition to the full analysis. Boxes show median and range percentage cell stained and Wilcoxon signed rank test was calculated for paired pre- and post-therapy samples.

patients (Table 2) and of these samples, 70 were baseline tumor biopsies and 35 were on-therapy biopsies. From these samples, 67 baseline tumor biopsies and 32 sequential paired biopsies (baseline and on-therapy) were of sufficient quality for immunohistochemistry assays. The baseline tumor characteristics (tumor biomarker, location, and type) are presented in Table 3. The majority of tumor samples were

obtained from the stomach (77.2%) compared with the gastroesophageal junction (21.4%). EGFR expression was detected in 41 baseline tumor biopsies (58.6%). pEGFR was observed in the same areas of tumor as EGFR expression. pMAPK expression was also mainly located in the infiltrating borders of the tumors. In samples that included gastric mucosa, EGFR, pMAPK, and pAkt were expressed in differentiated cells, as expected, while Ki67 was located in the proliferating intermediate area of gastric glands. pAkt, Ki67, $p27^{kip1}$, and TGF-α were diffusely expressed in tumors. However, the expression of pAkt appeared more intense in well differentiated areas of tumors.

Correlation Between Different Biomarkers at Baseline

Results from immunohistochemistry studies showed that, at baseline, in tumors positive for EGFR, receptor expression in tumor cells was significantly correlated with levels of pEGFR expression ($P < .001$) and Ki67 expression ($P = .011$), with expression levels of both increasing as EGFR expression levels increased. No significant correlation was identified between EGFR expression levels and pMAPK expression ($P = .720$; Figure 1). However, Ki67 expression and pMAPK expression were significantly related ($P = .01$), with expression of Ki67 increasing with increasing levels of pMAPK (Fig 1). No correlations with $p27^{kip1}$ were demonstrated in tumors.

Effects of Gefitinib Treatment on Biomarker Expression

After gefitinib treatment (day 28) at both 250 mg and 500 mg dose levels, inhibition of EGFR activation in tumor cells was observed in all 17 patients with detectable baseline EGFR activation, regardless of the dose level. The degree of EGFR inhibition was highly significant and almost complete ($P = .001$; Fig 2A). The reduction in levels of pEGFR expression occurred in tumors regardless of their level of EGFR expression, such that tumors with low, intermediate, or high expression showed a similar pattern of changes. The levels of MAPK phosphorylation at day 28 of gefitinib treatment were not significantly reduced compared with baseline phosphorylation levels in tumor samples that were considered to be EGFR negative (11 patients) nor in those considered to be EGFR positive (20 patients; $P = .396$ and $P = .076$, respectively; Fig 2B). However, in those gastric tumors with activated EGFR expression, a significant reduction of pMAPK was demonstrated ($P = .036$) after gefitinib administration at day 28. A good control in these tumors was provided by levels of pMAPK in stromal cells that remained unchanged with therapy. Interestingly, in those patients with a reduction in tumor levels of pMAPK showed a significant inhibition of tumor proliferation—Ki67—($P = .015$) in comparison to patients with increased pMAPK levels after therapy. Gefitinib treatment did not significantly reduce the levels of basal pAkt in either EGFR-negative (9 patients) or EGFR-positive tumors (15 patients; $P = .752$ and $P = .943$, respectively; Figure 2C). No significant difference was recorded between baseline and on-therapy expression of Ki67 in EGFR-negative tumors ($P = .779$); however, for tumors that expressed EGFR, Ki67 levels were significantly reduced by gefitinib ($P = .037$; Figure 2D). No significant changes in $p27^{kip1}$ expression were detected after gefitinib administration in EGFR-negative or EGFR-positive tumors ($P = .756$ and $P = .881$, respectively). Finally, analysis of tumors according to changes in tumor proliferation revealed that those patients with a reduction in tumor levels of Ki67 showed a significant inhibition of pMAPK ($P = .025$) in comparison with patients with increased Ki67 levels after therapy.

Apoptosis in EGFR-Positive Tumors: Correlation With pAkt and Pharmacokinetic Parameters

An enhanced apoptosis index was observed in some on-therapy tumor biopsies. Seeking for potential correlations between apoptosis and other pharmacodynamic markers, we observed as correlation between inhibition of Akt phosphorylation and enhanced apoptosis. As mentioned herein, there was not an overall significant decrease of pAkt with geftinib treatment; however, there were six patients whose tumors underwent a detectable decrease of pAkt with treatment. In those patients, there was an increase in apoptosis when compared to those tumors with lack of pAkt inhibition ($P = .030$; Fig 3). Among the 18 patients who did not have inhibition of Akt phosphorylation, increased apoptosis was observed in only six patients (33%). A closer look at this later group reveals that three patients had no change in pAkt and of these, two had also an increase in the apoptosis index. The remaining 15 patients had an increase in pAkt and enhanced apoptosis was only observed in three of these 15 patients (20%). It is not possible to establish a correlation between absolute values of apoptosis and inhibition of Akt inhibition given the limited size of our study. However, the tumors that displayed the highest levels of apoptosis where those with Akt inhibition. On the contrary, the majority of the tumors that had an increase in Akt phosphorylation had either no change or even a decrease in apoptosis.

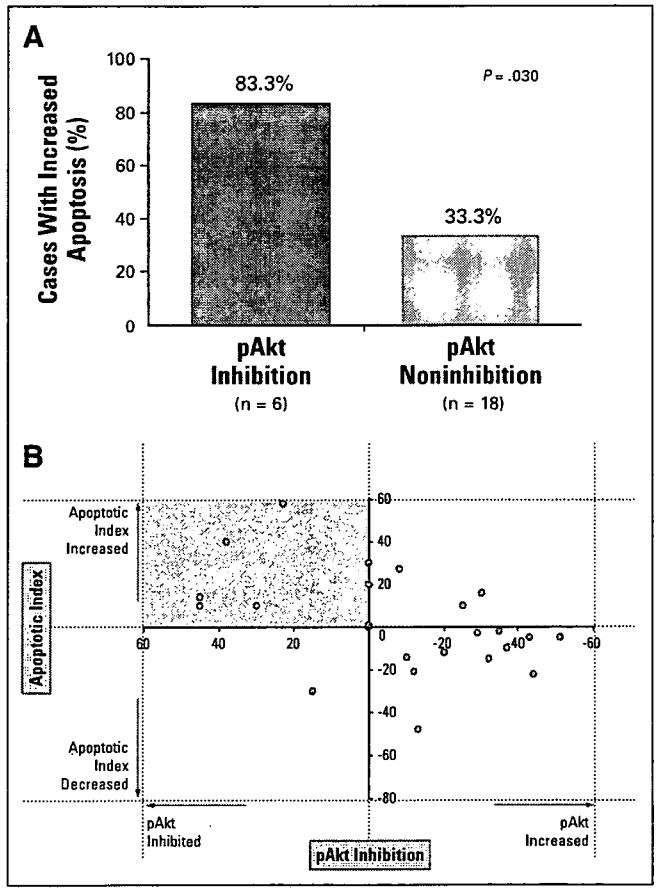

Fig 3. Positive correlation between decrease in phosphorylated Akt (pAkt) levels after gefitinib administration and tumor apoptotic index. (A) Overall correlation. (B) Individual patient display of the absolute values for pAkt inhibition and apoptosis index after gefitinib therapy. In the shadowed square the cases with increased apoptosis and pAkt reduction (five patients) are shown.

A detailed description of the clinical benefit of this study will be reported elsewhere. As a summary, and in order to be able to explore a potential association with apoptosis, disease control was observed in 13 patients (one partial response and 12 stable disease 4 weeks or longer). On-therapy biopsies were only available in six patients with clinical benefit and no on-therapy biopsy was available in the patient who achieved a partial response. Although the limited number of patients precludes any analysis, the two patients with the highest level of apoptosis as shown in Figure 3 had both a prolonged clinical benefit. The patient with an apoptotic index of 60 was on-therapy for a total of 180 days, and the patient with an apoptotic index of 40 was on-therapy for 113 days. This observation suggests a positive correlation between increased apoptosis and clinical benefit and deserves further exploration.

We also found a significant correlation between increased exposure to gefitinib and enhanced apoptosis. In those patients with assessable tumor samples for apoptotic index and pharmacokinetic assessments, there was a strong correlation between a positive apoptotic index average and enhanced gefitinib concentration, calculated as C_{max} ($P < .001$), and total exposure, calculated as $AUC_{(0\text{-}24)}$ ($P < .001$; Fig 4). No statistical differences in apoptosis and pharmacokinetic parameters were detected between Japanese and non-Japanese population, and between sex, age, and patients with disease control versus those with progression of the disease.

DISCUSSION

In this study, we evaluated evidence of biologic activity of gefitinib in gastric tumor samples. Expression of the biomarkers EGFR, pEGFR, MAPK, Akt, $p27^{kip1}$, and Ki67 was assessed before and after treatment in sequential tumor biopsy samples from 70 patients with advanced gastric cancer.

Gefitinib demonstrated activity against its target, the EGFR; in these gastric tumors, resulting in a complete and statistically significant inhibition of EGFR phosphorylation in tumors. However, inhibition of EGFR phosphorylation was not accompanied by significant inhibition of MAPK and Akt activation as measured by their phosphorylation status. However, there were hints of biologic effects of EGFR inhibition in some tumors. For example, the proliferation marker Ki67 was significantly inhibited in EGFR-positive tumors

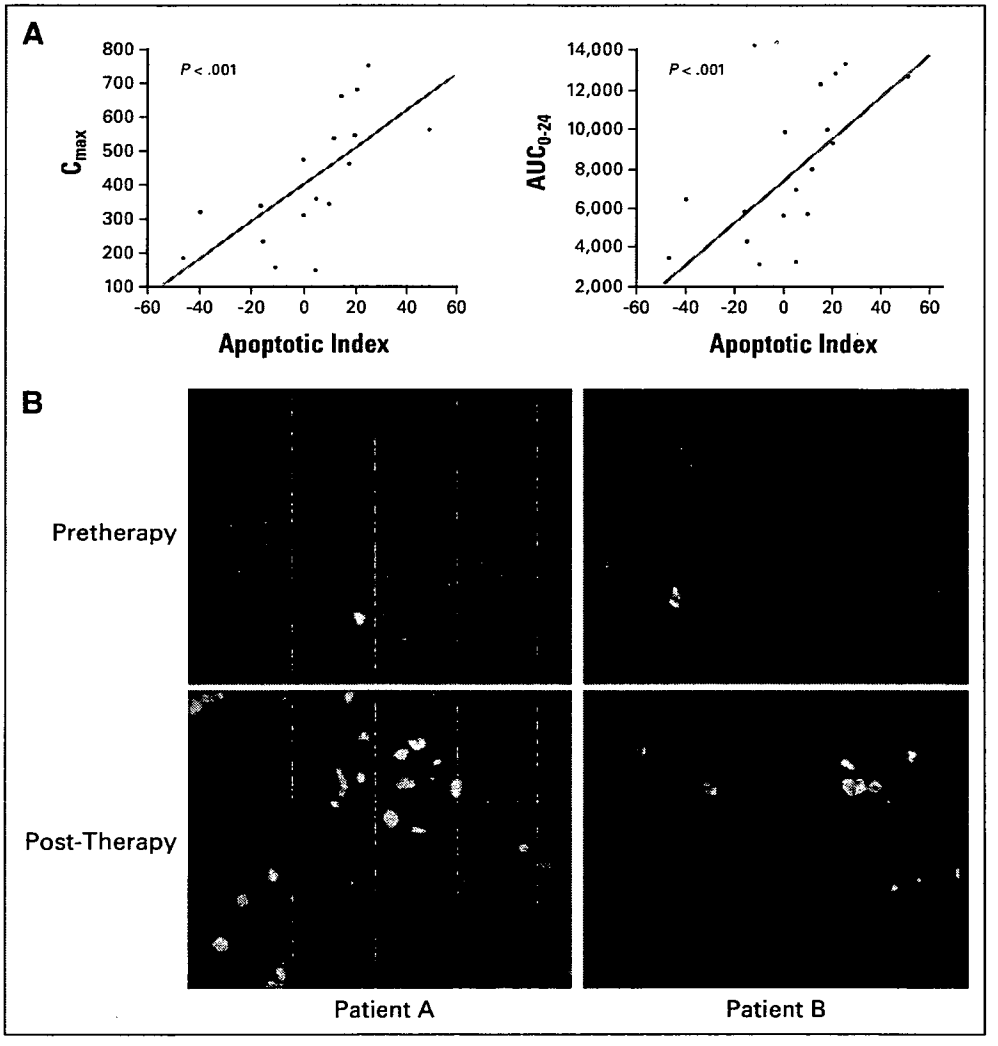

Fig 4. Correlation between pharmacokinetic parameters and apoptosis. (A) An average of apoptotic index between pre- and post-therapy samples was correlated with maximum concentration (C_{max}) and area under the time-concentration curve $(AUC)_{(0\text{-}24)}$ (B) Two representative patients are shown. Increased apoptosis is observed in patient A after gefitinib treatment in contrast with patient B. Higher gefitinib concentration and total exposure was demonstrated in patient A.

treated with gefitinib and this inhibition in proliferation correlated with a significant reduction in the activation of MAPK. Likewise, those patients who had a decrease in pAkt had enhanced apoptosis in comparison with tumors without change in pAkt. This is an indication that gefitinib may inhibit tumor proliferation and/or induce apoptosis within a subgroup of EGFR-sensitive patients; however, the insensitive patients may result in a dilution of any observable benefit.[10] One might speculate the reasons underlying the lack of Akt inhibition in the majority of tumors. Activation of PI3K/Akt is also mediated via ErbB2 through *trans*-activation and phosphorylation of ErbB3.[11] ErbB2 is highly expressed in 8% to 40% of gastric cancers, depending on disease stage, and ErbB3 is highly expressed in 72% to 86% of advanced gastric carcinomas.[5] However, some studies have shown that gefitinib does prevent EGFR and ErbB2-mediated activation of ErbB3.[12,13] Alternative mechanisms of Akt activation, independent of ErbB receptors, may be at play in gastric cancer, including loss of activity of the tumor suppressor gene *PTEN* that results in enhanced PI3K-Akt activity and resistance to EGFR inhibitors.[14] In gastric cancer, evidence suggests that a reduction in *PTEN* expression contributes to the malignant transformation of the gastric mucosa.[15,16] This ErbbB-independent Akt activation may be therapeutically exploitable.[17] Our findings would support a combined anti-EGFR and anti-PI3Kinase approach as these agents become available for clinical testing.

Other pharmacodynamic studies of gefitinib have been performed in skin biopsy samples[18] and in tumor biopsies from patients with advanced breast cancer[19] and colorectal cancer.[20] The results from skin biopsy analysis described an abolition of EGFR and most of EGFR downstream signaling pathways.[18] The pharmacodynamic study of gefitinib in breast cancer demonstrated an almost total loss of EGFR phosphorylation and a decrease in MAPK phosphorylation after gefitinib treatment. Clinical disease stabilization was observed in some patients. Furthermore, a reduction in Ki67 expression levels was observed only in tumors with low pAkt levels,[19] suggesting elements of the PI3K pathway may be involved in resistance to gefitinib therapy and this may, in part, explain the results presented herein. In colorectal carcinoma, a recent study with gefitinib has reported lack of inhibition of EGFR.[20] However, other studies with anti-EGFR agents have been reported to inhibit EGFR activation in patients with advanced colon cancer.[21,22] Therefore, there is mounting evidence that EGFR inhibition in the tumor is achieved in the different tumor types, although clinical responses and downstream signaling inhibition may depend on EGFR sensitivity of a given tumor type.

In conclusion, in this first study using sequential biopsies from patients with advanced gastric cancer, gefitinib demonstrated biologic activity to effectively inhibit EGFR activation. Gefitinib had modest clinical efficacy as well as limited effects on EGFR downstream signaling pathways. However, a subpopulation of gastric tumors have evidence of EGFR sensitivity, as demonstrated by decrease in proliferation and increased apoptosis, which warrants further study.

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Authors' Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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Dollar Amount Codes (A) < $10,000 (B) $10,000-99,999 (C) ≥ $100,000 (N/R) Not Required

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GLOSSARY

Akt: Protein kinase B belongs to a pathway that is responsible for cell survival. Following activation (ie, phosphorylation) by PI3K, activated Akt blocks the activity of molecules involved in the apoptotic pathway by in turn phosphorylating them.

EGFR (epidermal growth factor receptor): Also known as HER-1, EGFR belongs to a family of receptors (HER-2, HER-3, HER-4 are other members of the family) and binds to the EGF, TGF-α, and other related proteins, leading to the generation of proliferative and survival signals within the cell. It also belongs to the larger family of tyrosine kinase receptors and is generally overexpressed in several solid tumors of epithelial origin.

Gefitinib: Belonging to the class of tyrosine kinase inhibitors, gefitinib (also known as Iressa) binds to the cytoplasmic region of the EGFR that also binds ATP. By competing with ATP binding that is essential for tyrosine phosphorylation, gefitinib inhibits activation of EGFR and blocks the cascade of reactions leading to cellular proliferation.

Ki67: A marker of proliferation, Ki67 is a protein that is expressed in the nucleus of proliferating cells. Absent only in resting cells, cells in the G1, S, G2, and M phase of the cell cycle express this marker.

$P27^{kip1}$: Belongs to the family of cell cycle regulators, typically known as cyclin-dependent kinase inhibitors (CDKI). Kip1/p27, like other CDKIs, binds cyclin-cdk complexes, leading to cell cycle arrest in the G1 phase of growth.

PI3K: Phosphatidylinositol-3 phosphate kinase (PI3K) adds a phosphate group to PI3, which is a downstream signaling molecule involved in survival/proliferative pathways mediated by growth factors such as the EGF and the PDGFs.

pMAPK (phosphorylated mitogen-activated protein kinase): MAPKs are a family of enzymes that form an integrated network influencing cellular functions such as differentiation, proliferation, and cell death. These cytoplasmic proteins modulate the activities of other intracellular proteins by adding phosphate groups to their serine/threonine amino acids. The phosphorylated form of MAPK is being used as a surrogate to the activated form of the receptor.

Tyrosine kinase inhibitor: Molecules that inhibit the activity of tyrosine kinase receptors. They are small molecules developed to inhibit the binding of ATP to the cytoplasmic region of the receptor (eg, gefitinib), thus further blocking the cascade of reactions that is activated by the pathway.

ERRATA

The September 10, 2006, article by Rojo et al entitled, "Pharmacodynamic Studies of Gefitinib in Tumor Biopsy Specimens From Patients With Advanced Gastric Carcinoma" (J Clin Oncol 24:4309-4316, 2006) contained an error in the spelling of S. Ramon y Cajal. It was originally published as S. Ramon Cajal and should have been S. Ramon y Cajal.

DOI: 10.1200/JCO.2006.11.001

The September 10, 2006, Biology of Neoplasia article by Poulin and DeCaprio, entitled "Is There a Role for SV40 in Human Cancer?" (J Clin Oncol 24:4356-4365, 2006) contained an error in the Authors' Disclosure of Potential Conflicts of Interest section. In addition to "Novartis Pharmaceuticals (B)," "Venable LLP (A)" should have been disclosed for James A. DeCaprio in the Consultant category.

DOI: 10.1200/JCO.2006.11.002

The September 20, 2006, article by Ng et al entitled, "Prospective Study of [^{18}F]Fluorodeoxyglucose Positron Emission Tomography and Computed Tomography and Magnetic Resonance Imaging in Oral Cavity Squamous Cell Carcinoma With Palpably Negative Neck" (J Clin Oncol 24:4371-4376, 2006) contained an error. In Figure 1, the x-axis was labeled "Specificity," while it should have been "1–Specificity."

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Genetic Variations and Haplotype Structures of the *ABCB1* Gene in a Japanese Population: An Expanded Haplotype Block Covering the Distal Promoter Region, and Associated Ethnic Differences

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Summary

As functional *ABCB1* haplotypes were recently reported in the promoter region of the gene, we resequenced the *ABCB1* distal promoter region, along with other regions (the enhancer and proximal promoter regions, and all 28 exons), in a total of 533 Japanese subjects. Linkage disequilibrium (LD) analysis based on 92 genetic variations revealed 4 LD blocks with the same make up as previously described (Blocks −1, 1, 2 and 3), except that Block 1 was expanded to include the distal promoter region, and that a new linkage between polymorphisms −1789G>A in the distal promoter region and IVS5 + 123A>G in intron 5 was identified. We re-assigned Block 1 haplotypes, and added novel haplotypes to the other 3 blocks. The reported promoter haplotypes were further classified into several types according to tagging variations within Block 1 coding or intronic regions. Our current data reconfirm the haplotype profiles of the other three blocks, add more detailed information on functionally-important haplotypes in Block 1 and 2 in the Japanese population, and identified differences in haplotype profiles between ethnic groups. Our updated analysis of *ABCB1* haplotype blocks will assist pharmacogenetic and disease-association studies carried out using Asian subjects.

Keywords: *ABCB1*, P-gp, haplotype

Introduction

The *ABCB1* gene, encoding p-glycoprotein (P-gp)/multidrug resistance protein 1 (MDR1), is located on chromosome 7q21-q31 and consists of 28 exons. P-gp (1280 amino acids), a member of the

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ATP-binding cassette (ABC) transporter superfamily, is a large transmembrane glycoprotein that consists of two transmembrane domains (TMDs) and two nucleotide-binding domains (NBDs). P-gp was initially identified as a component of the multidrug resistance phenotype in cancer cells (Riordan *et al.* 1985), but was later found to be widely expressed in normal epithelial cells of tissues such as the liver, intestine, kidneys, and the blood-brain and testis barriers, as well as in lymphocytes (Fojo *et al.* 1987; Cordon-Cardo *et al.* 1989). It is thought that P-gp plays a role in the protection of these tissues against structurally-unrelated toxic xenobiotics, and can modify the oral bioavailability and renal secretion of a variety of drugs (Hoffmann & Kroemer, 2004). Multiple other physiological functions of P-gp have also been suggested in lipid transport (van Helvoort *et al.* 1996), cholesterol metabolism (Debry *et al.* 1997), inhibition of ceramide-induced apoptosis (Liu *et al.* 2001), and the initiation of immune responses by cytokine release (Drach *et al.* 1996). Moreover, reduced P-gp expression has been linked to cancer (Siegsmund *et al.* 2002) and other diseases such as Parkinson's disease (Furuno *et al.* 2002) and ulcerative colitis (Schwab *et al.* 2003).

With recent advances in genomics research there has been an increasing number of pharmacogenetic studies focused on the *ABCB1* gene. Hoffmeyer *et al.* (2000) showed that a synonymous 3435C>T mutation in exon 26 was associated with reduced P-gp expression in the duodenum, and increased plasma levels of digoxin following its oral administration in healthy volunteers. Thus, the 3435C>T single nucleotide polymorphism (SNP) has become the focus of much attention. However, reports on the role of this common SNP have been very inconsistent, which suggests that other functional polymorphisms may be linked with 3435C>T (Kim, 2002). Further studies revealed that 3435C>T was closely linked to other common polymorphisms, such as 1236C>T (silent) at exon 12 and 2677G>T (Ala893Ser) at exon 21, and that the combinations of these SNPs (i.e. haplotypes) differed greatly between ethnic groups (Kim *et al.* 2001; Kroetz *et al.* 2003; Tang *et al.* 2002, 2004). While an *in vitro* functional study on the nonsynonymous 2677G>T (Ala893Ser) SNP at exon 21 showed that 2677G>T was associated with enhanced P-gp activity (Kim *et al.* 2001), other studies found no association (Kimchi-Sarfaty *et al.* 2002; Morita *et al.* 2003; Kroetz *et al.* 2003). One of these latter studies also revealed that another nonsynonymous SNP, 2677G>A (Ala893Thr), had no impact on P-gp function (Morita *et al.* 2003). Yet several clinical studies have shown that the haplotypes 2677T-3435T and 1236T-2677T-3435T are associated with reduced P-gp activity (Johne *et al.* 2002; Kurata *et al.* 2002; Chowbay *et al.* 2003; Wong *et al.* 2005), and that 2677A-bearing subjects exhibit higher P-gp activity (Yi *et al.* 2004). Studies that found no association between these *ABCB1* SNPs and P-gp expression levels (Goto *et al.* 2002), and other conflicting results, have been summarized in recent review articles (Kim, 2002; Ieiri *et al.* 2004).

Recently, *ABCB1* gene promoter region haplotypes were reported by two Japanese research groups, and revealed the existence of functional haplotypes that resulted in altered P-gp expression (Taniguchi *et al.* 2003; Takane *et al.* 2004). In these studies, haplotypes that included −1789G>A alone or in combination with −145C>G were associated with decreased P-gp expression. However, the reported effects of haplotypes carrying −129T>C and two other linked SNPs on P-gp expression were contradictory, showing reduction and enhancement.

From these findings it is clear that the establishment of detailed *ABCB1* gene haplotype profiles specific for each ethnic group is important. We previously conducted haplotype analysis on 145 Japanese subjects by dividing the *ABCB1* gene into 4 blocks, one of which included the proximal promoter region, and revealed that the *2 haplotype in Block 2, which harbours 1236C>T, 2677G>T and 3435C>T, showed a strong association with reduced renal clearance of irinotecan and its metabolites (Sai *et al.* 2003). However, recent findings on the functional distal *ABCB1* promoter region prompted us to identify the extended haplotypes that encompassed the above promoter region in a larger Japanese population.

In this study, we sequenced the distal *ABCB1* gene promoter regions from 533 Japanese subjects. This region covered approximately 2.5 kb upstream from the translational initiation site, adjacent to the previously described Block 1 region. We found that the promoter region SNPs were closely linked with SNPs located over a relatively wide range (up to intron 5) in Block 1, such