Table 1.　入院時血液生化学検査

WBC	4300 /μl	GOT	24 IU/l	Cr	0.7 mg/dl
Seg	58.1 %	GPT	30 IU/l	BUN	16.7 mg/dl
Mono	6.5 %	LDH	263 IU/l	UA	4.0 mg/dl
Eo	7.4 %	γ-GTP	12 IU/l	Na	144 mEq/l
Baso	0.5 %	ChE	0.59 ΔpH	K	4.1 mEq/l
Lymph	27.5 %	LAP	63 IU/l	Cl	109 mEq/l
		ALP	128 IU/l	Ca	9.1 mg/dl
RBC	401×10⁴ /μl	T.Bil	0.6 mg/dl		
Hb	11.3 g/dl	D.Bil	0.1 mg/dl	CEA	0.5 ng/ml
Ht	33.8 %	T.Cho	188 mg/dl	CA19-9	< 0.6 U/ml
Plt	29.0×10⁴ /μl	T.P	7.5 g/dl		
		Alb	4.4 g/dl	HBsAg	(−)
		ZTT	4.0 Ku	HCVAb	(−)
		TTT	0.5 Ku		

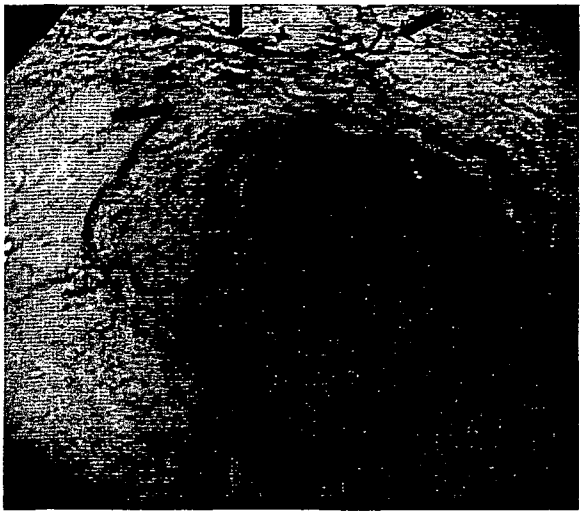

Figure 1.　上部消化管内視鏡検査所見：内視鏡検査で矢印に示す範囲の胃体下部前壁に4 cm大の浅い陥凹性病変を認めた．

N0, M0と診断した．治療は粘膜下腫瘍様病変を含めた幽門側胃切除およびD2郭清を施行した．

切除病理組織学的所見：陥凹性病変は4.0×4.5 cmの0-IIcで，signet ring cellが粘膜層に限局してみられた．脈管浸襲を認めずリンパ節転移もみられなかった．粘膜下腫瘍は胃底腺領域に存在し，長径1.7cmで，粘膜下層を中心に大小不同の嚢胞や拡張した異型のない腺管を多数認めた（Figure 5a）．拡張した腺管の間には平滑筋束の増生を認めた（Figure 5b）．粘膜下腫瘍周囲，癌および癌周囲の粘膜下層には粘膜下嚢胞はみられなかった．以上の所見よりHIPと診断した．胃癌についてはpT1, pN0, M0, H0, P0, CY0, Stage Iaであった．現在外来経過観察中であるが，2年後の現在再発を認めていない．

II　考　察

粘膜下の異所性胃腺には胃底腺と幽門腺の境界領域に好発し丈の低い比較的小さな粘膜下隆起が多発するびまん型（いわゆるびまん性胃粘膜下異所腺）と胃体上部や穹隆部など胃底腺領域に好発し比較的大きな腫瘤を形成する孤立型の2つの病態があるとされている[4]．このうち後者の粘膜下異所腺が増生，貯留，嚢胞化し孤立性腫瘤を形成するものを胃過誤腫性ポリープやHIPと呼称している[3)5)]．Aokiらは孤立型異所性胃腺でいわゆるHIPとされる病変のうち有茎性のものをポリープ型，無茎性のものを粘膜下腫瘍型と分類している[6]．本症例は粘膜下腫瘍型と考えられた．

胃HIPは極めてまれな疾患であり1990年から2004年まで医学中央雑誌にて検索した範囲では本症例を含めて13例のみ[2)3)5)~14)]報告されていた（Table 2）．平均年齢は46歳，男女比は5：8で女性に多く，主訴は貧血・消化管出血がそれぞれ2例で最も多かった．無症状のものは本症例を含めて7例であった．肉眼型はポリープ型が4例，粘膜下腫瘍型が本症例を含め10例であった．

HIPの診断についてはEUS診断の有用性が報告されている[8)~11)]．しかしながら杉山ら[8)]は第3

Figure 2. 上部消化管内視鏡検査所見：体上部小彎に2cm大の扁平な隆起性病変（矢印）を認めた．

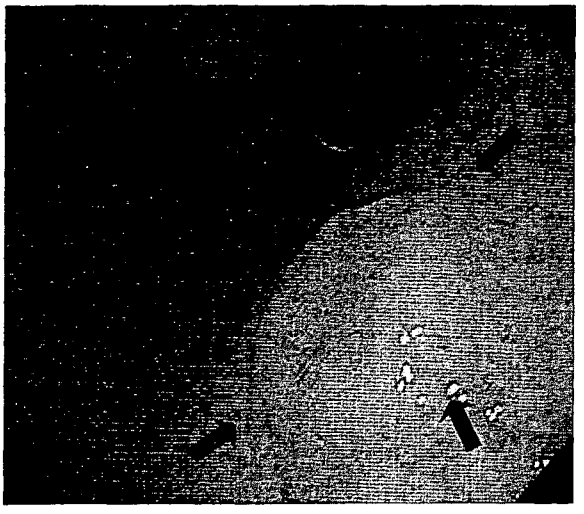

Figure 3. 上部消化管内視鏡検査所見：隆起性病変は正常粘膜に覆われており，粘膜下腫瘍（矢印）と診断した．

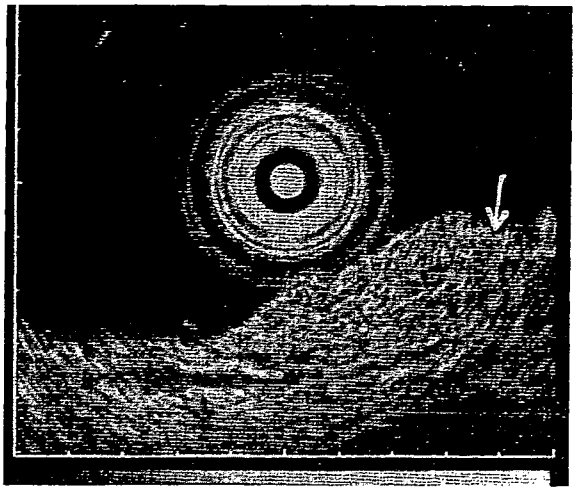

Figure 4. 超音波内視鏡検査所見：低エコーの腫瘍が第2～3層にかけて存在しており，内部に細い脈管様の無エコー域の集簇（矢印）を認めた．第4層は正常に保たれていた．

層のびまん性高エコー腫瘤，松岡ら[11]は粘膜下の大小さまざまな無エコー域の充満する海綿状腫瘤と記載しており，EUS像も症例によって異なり，EUSのみでは確定診断が困難である．Table 2のうち内視鏡でHIPと診断された例は，井上ら[10]，松岡ら[11]が報告した2例のみであった．日比野ら[3]は病巣内の数個の無エコー域を迷入膵にみられる脈管像と判断し，われわれと同様に術前診断を迷入膵としていた．胃粘膜下腫瘍のEUS像における細い脈管様の無エコーは迷入膵の導管なのか，あるいはHIPの拡張した腺管なのか，症例によって慎重に判断する必要がある．HIPはEUS下穿刺吸引生検を行えば，診断が確定する可能性があるが，腫瘤の組織が採取できていたにもかかわらず，生検組織内に腺窩上皮を多数認めたことから胃粘膜上皮のみが採取されたと誤って診断された報告[3]もあり，あらかじめ粘膜下腫瘍の鑑別診断にHIPを考慮に入れておかないと，EUS下穿刺吸引生検でも診断が困難である．

粘膜下異所性胃腺のうちびまん型では胃癌の合併（特に分化型腺癌）が多く，いわゆるparacancerous lesionとして注意を喚起されており[5)15)16)]，近年ヘリコバクターピロリ感染との関連が議論されているが[17)18)]，孤立型にも癌が存在し異所性胃腺から癌が発生したとする報告もみられる[19]．Table 2の13例のうち，加藤ら[9]の高分化型腺癌の併存をみた報告と本症例の2例にHIPと胃癌の併存がみられた．さらに本症例では合併した胃癌の組織型は印環細胞癌であり，HIPに分化型腺癌以外の癌が合併した極めてまれな症例と考えられる．癌とHIPの間に関連があるかどうか現在のところ不明であり，今後さらに症例の集積と検討が必要である．

治療に関してはポリープ型に対しては腫瘤が大きかった1例を除いて内視鏡的切除が行われてい

Table 2. 胃 hamartomatous inverted polyp の本邦報告例（1990～）

	著者	年齢	性別	報告年	初発症状	病変の位置	大きさ（cm）	形態	内視鏡診断	胃癌の併存	治療
1	瀧澤ら[7]	48	女	1990	−	体上部小彎	2.5×2.0×1.5	SMT type	粘膜下腫瘍（筋原性腫瘍）	−	手術
2	杉山ら[8]	15	女	1992	心窩部痛	体上部大彎	1.2×1.4	SMT type	記載なし	−	内視鏡的切除
3	加藤ら[9]	73	男	1993	貧血	体中部後壁	1.5×1.4×1.0	SMT type	胃のう胞	+	内視鏡的切除
						体上部大彎	1.5×1.4×1.1	SMT type		−	内視鏡的切除
4	井上ら[10]	67	男	1995	−	体上部大彎	1.0×0.9×0.5	SMT type	胃粘膜下異所腺	−	内視鏡的切除
5	松岡ら[11]	39	女	1995	−	穹隆部前壁	1.8	polyp type	過誤腫様ポリープ	−	内視鏡的切除
6	多田ら[12]	68	男	1996	食欲不振・貧血	残胃吻合部	7×6×4	polyp type	良性ポリープ	−	手術
7	Itoh et al[13]	41	女	1998	心窩部不快感	穹隆部	2.3×1.8×0.9	polyp type	記載なし	−	内視鏡的切除
8	Kubo et al[14]	21	女	2000	消化管出血	穹隆部	記載なし	polyp type	記載なし	−	内視鏡的切除
9	小沢ら[5]	40	女	2000	−	穹隆部大彎	2.5×1.7×0.9	SMT type	HIP またはリンパ管腫	−	内視鏡的切除
10	日比野ら[3]	63	男	2002	−	穹隆部大彎	2.5×2.8	SMT type	迷入膵または筋原性腫瘍	−	手術
11	梅岡ら[2]	41	男	2003	消化管出血	体上部後壁	0.5×0.5	SMT type	粘膜下腫瘍	−	手術
12	Aoki et al[6]	43	女	2004	−	体上部後壁	2.8	SMT type	診断つかず	−	手術
13	自験例	39	女	2005	−	体上部小彎	1.7×1.2	SMT type	迷入膵	+	手術

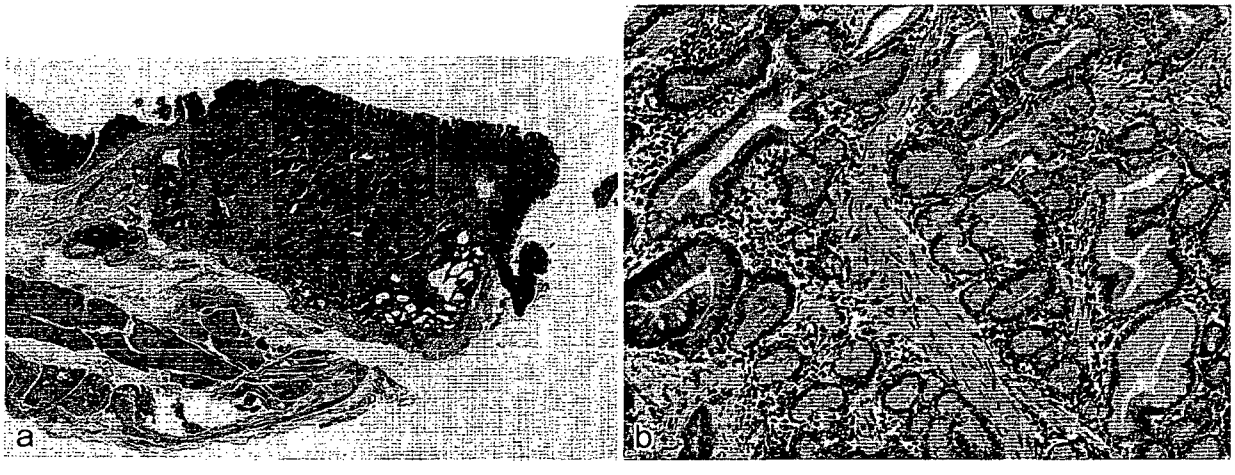

Figure 5. 手術標本組織所見　**a**：粘膜下層を中心に大小不同の嚢胞と拡張した異型のない腺管を多数認めた（HE 染色，×4）．　**b**：拡張した腺管の間には平滑筋束の増生を認めた（HE 染色，×100）．

る．粘膜下腫瘍型に対しては平滑筋肉腫を否定し得なかった例[7]，出血を繰り返した例[2]，本症例のように胃癌などの他病変に併存した例においては外科手術が行われている．粘膜下腫瘍型に対しては近年急速に普及しつつある切開剥離法（ESD）による内視鏡的切除も有効な治療手段となりうると

思われる[20]．しかしESDは出血頻度が高い治療法であり，出血の症候を呈した例[2]や内視鏡治療中に出血を生じ，外科手術を行った報告例[7]があることから，粘膜下腫瘍型胃HIPに内視鏡治療，特にESDを行う場合は特に出血に注意して慎重に行うべきであると思われた．

結　論

早期胃癌に併存し，迷入膵と鑑別困難であった粘膜下腫瘍型胃HIPの1例を報告した．胃粘膜下腫瘍の鑑別診断においてはHIPを考慮に入れておく必要があると思われた．

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●特 集●

Neoadjuvant Therapyの適応と効用・I

食道癌

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Neoadjuvant Therapy for Esophageal Cancer—Indication and Efficacy: Ken Kato, Tetsuya Hamaguchi, Yasuhide Yamada, Kuniaki Shirao and Yasuhiro Shimada (*Gastrointestinal Oncology Division, National Cancer Center Hospital*)

Summary

Some approaches such as adjuvant chemotherapy, neoadjuvant chemotherapy and neoadjuvant chemoradiotherapy have been tried to improve the efficacy of treatment for resectable esophageal cancer patients. The usefullness of neoadjuvant chemotherapy, has remained a matter of controversy. However, there is a report from JCOG9907 in Japan that two courses of neoadjuvant 5-FU/CDDP improved the survival of esophageal squamous cell cancer patients. Neoadjuvant chemoradiotherapy has not had a consistent evaluation because of the varying results of each trial. But from the results of meta-analysis and CALGB9781, the neoadjuvant chemoradiotherapy called "trimodality therapy" has been a standard treatment in the United States.

We should evaluate whether there would be similar effectiveness in Japan, where the histology and operative approach are different. Some approaches such as DNA microarray and proteomics, which can predict the treatment effect, are being tried. **Key words**: Esophageal cancer, Neoadjuvant chemotherapy, Neoadjuvant chemoradiotherapy, **Corresponding author**: Ken Kato, Gastrointestinal Oncology Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

要旨 切除可能食道癌に対して治療効果を高める方法として，術後化学療法，術前化学療法，術前化学放射線療法などの試みがされてきた。術前化学療法については定まったものではなかった。しかし，日本で行われたJCOG9907の結果，5-FU/CDDP療法2コースを術前に行うほうが，術後に行うよりもより生存に寄与するとの報告があり，食道扁平上皮癌に対するエビデンスとして注目される。術前化学放射線療法については，試験ごとに結果が異なり一定の評価がなかったが，メタアナリシスやCALGB9781の結果により，米国では術前化学放射線療法を行う，いわゆるtrimodality therapyが標準的となっている。組織型や術式の異なる日本において同様の効果が望めるのかは，今後の検討が必要であり，DNAマイクロアレイやプロテオミクスなどによる治療効果予測などが試みられている。

はじめに

1980年代後半より普及した3領域リンパ節郭清を伴う食道切除術は，腫瘍の局所制御の割合を高めることで，食道癌全体の治療成績の治療に貢献してきた。5年生存割合50%を超える成績が報告されているが，逆にいえば3〜4割再発するということであり，そのような症例に対しては手術療法単独では不十分であったといえる。再発形式としては半数以上が遠隔臓器転移であり，手術でコントロールすることができない微少な転移巣をコントロールすることが再発のリスクを下げると考えられている。手術と組み合わせて様々なmodalityを用いることで，治療成績を上げる試みは以前よりなされてきたが，2007年になって海外から，そして日本からも大きな臨床試験の結果が報告され話題を集めている。最新の知見を含め紹介する。

I．術前補助化学療法

標準治療である手術に化学療法を組み合わせて治療効果を高めることが行われてきた。本邦では，「食道癌術後化学療法（CDDP+5-FU）の無作為化比較試験（JCOG9204）」が行われ，手術単独群に対して術後にCDDP+5-FUを2コース行う群が，5年無再発生存割合において，有意に良好であった（45% vs 55%）と報告

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表 1　主な術前化学療法比較試験の結果（文献5）より改変）

報告者（年）	組織型	術前化学療法			n	MST 月	p
Maipang (1994)	SCC	CDDP 100 mg/m² d1 BLM 10 U/m² d3-8 VLB 3 mg/m² d1, 8	×2	S	22	17 m	NS
				CTx → S	24	17 m	
Law (1997)	SCC	CDDP 100 mg/m² d1 5-FU 1,000 mg/m² d1-5	×2	S	73	13 m	NS
				CTx → S	74	17 m	
Kok (1997)	SCC	CDDP 80 mg/m² d1 etoposide 200 mg/m² d1-5	×2	S	74	11 m	0.002
				CTx → S	74	19 m	
Kelsen (1998)	SCC Adeno	CDDP 100 mg/m² d1 5-FU 1,000 mg/m² d1-5	×3	S	234	16 m	NS
				CTx → S	233	15 m	
Ancona (2001)	SCC	CDDP 100 mg/m² d1 5-FU 1,000 mg/m² d1-5	×2	S	47	24 m	NS
				CTx → S	47	25 m	
MRC (2002)	SCC Adeno	CDDP 80 mg/m² d1 5-FU 1,000 mg/m² d1-4	×2	S	402	13 m	0.004
				CTx → S	400	17 m	

SCC: 扁平上皮癌，Adeno: 腺癌，S: 手術単独群，CTx → S: 術前化学療法群，MST: 生存期間中央値，NS: not significant

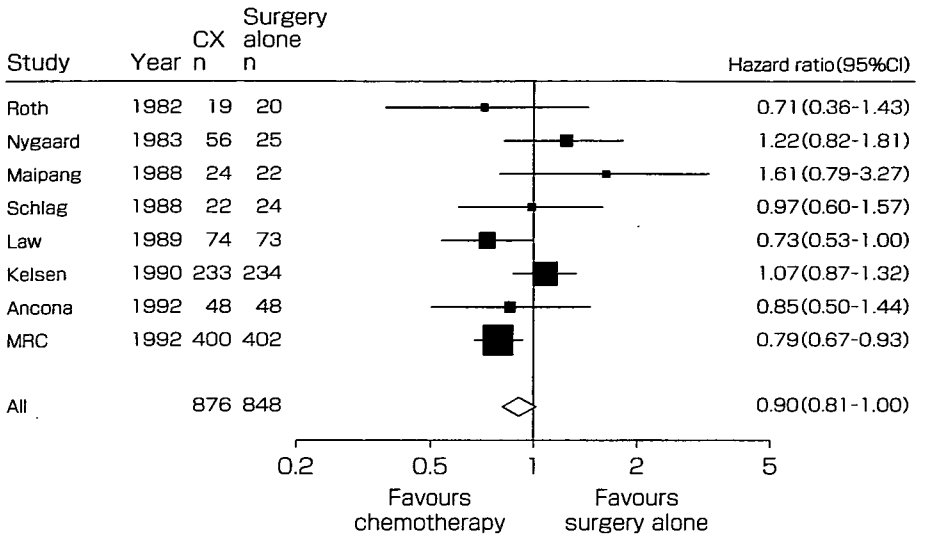
された。5年生存割合においても，有意差まで到達しないものの，単独群52%に対して，術後化学療法群で61%と術後化学療法群で良好な傾向が認められた[1]。また，サブグループ解析を行ったところ，リンパ節転移陰性症例（pN0）では両群の差を認めなかったが，リンパ節転移陽性症例（pN1）においては，より両者に差を認めている。一方，海外で行われた同様の比較試験では有意差は報告されていない[2]が，本邦での結果であることを重視し，食道癌診断治療ガイドライン2007年4月版では，治癒切除後，状態のよい患者に対しては術後補助化学療法が推奨されている。

術前補助化学療法については，海外より手術単独との比較試験がいくつか報告されている（表1）。467名を手術単独群とFP（5-FU 1,000 mg/m² day 1-5，CDDP 100 mg/m² day 1/4週ごと）3コースを術前に行う群にランダムに割り付けた試験では，primary endpointである生存期間において差を認めず（生存期間中央値16.1か月 vs 14.9か月），無再発生存期間でも同様の結果であった[3]。一方でイギリスからの報告では，802名を手術単独群とFP（5-FU 1,000 mg/m² day 1-4，CDDP 80 mg/m² day 1/3 qwk）2コースを術前に行う群に割り付け比較された。術前化学療法群において，生存期間中央値（13.3か月 vs 16.8か月），2年生存割合（34% vs 43%）ともに良好な結果を示し，術前化学療法は生存に寄与すると結論付けている[4]。それぞれの試験における腺癌の割合は52，66%とそれほど差がないにもかかわらず，異なる結果を示していることから，術前化学療法に対する明確な評価はいまだに明らかではない。

最近発表された，術前化学療法対手術単独の比較試験8報のメタアナリシスにおいては，術前化学療法が有用であると結論した報告はわずか2報であったが，サンプルサイズの大きなイギリスからの報告に引っ張られる形で，トータルでは術前化学療法は有用（p=0.05）と結論付けている（図1）[5]。組織別の解析では，興味深いことに扁平上皮癌では生存への寄与は認められず（p=0.12），腺癌では有意差あり（p=0.014）となっている。2007年ASCOにおいても下部食道～胃の腺癌を対象にした比較試験（FFCD9703）が報告され，手術群（n=111）に比して，術前術後化学療法群（n=113）が有意に生存期間を延長した（p=0.021）と報告され，腺癌においての有用性が示されようとしている[6]。

本邦においてはJCOG9204の結果を受けて，「臨床病期Ⅱ期およびⅢ期胸部食道がんに対する5FU+シスプラチン術前補助化学療法と術後補助化学療法のランダム化比較試験（JCOG9907）」が行われた。2000年5月より登録が開始され，2006年5月までに330例が登録され，166例が術前群，164例が術後群に割付けられた（図2）。第2回中間解析において，primary endpointである無増悪生存期間ではわずかに有意ではなかったものの，secondary endpointである全生存期間において，有意に術前群において有効性を認めたため，2007年5月に結果の早期公表がなされた。今後データをさらに集積し，2008年ASCOに結果が発表される予定である。海外での腺癌でのデータと比べ，本試験では全例扁平上皮癌であり，海外からどのような評価を得るのか興味が持たれる。

a: 各試験での hazard ratio



b: 組織型別 Hazard ratio

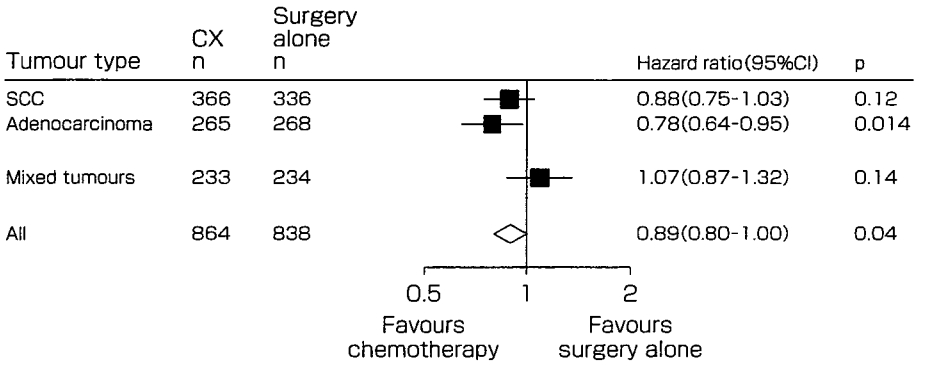


図 1　術前化学療法 vs 手術単独療法のメタアナリシス（文献5)から）

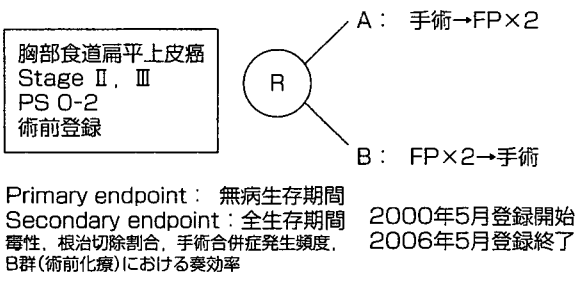


図 2　JCOG9907 シェーマ

Ⅱ．術前化学放射線療法

手術前に化学放射線療法を行う術前化学放射線療法についても，いくつかの異なるスケジュール，異なる組織型による術前化学放射線療法 vs 手術の比較試験が行われた（表2)。結果は全体的には術前化学放射線療法の有用性に傾いているが，はっきりと有意差を示した試験は二つのみで，一定の評価を得るに至ってはいない。オーストラリアより報告された，256名を手術単独群と，術前化学放射線療法1コース（5-FU 800 mg/m^2 day 2-5, CDDP 80 mg/m^2 day 1, radiation 35 Gy）を行った後に手術を行う群との比較試験では，無増悪生存期間，全生存期間ともに両群に有意な差を認めなかった。しかし，組織型別に分けたサブグループ解析では扁平上皮癌（手術群50名 vs 術前化学放射線療法群45名）において，術前化学放射線療法群が有意に増悪までの期間を延長した（p＝0.014)。全生存期間では有意差はなく，腺癌症例においても両群の差を見いだすことはできなかった[7]。2006年 ASCO にて報告された CALGB9781 試験の結果では，手術単独群に対して術前化学放射線療法群（trimodality therapy）の生存期間延長効果が示され[8]，米国においては trimodality therapy が標準的と考えられるようになった。この試験では手術群26名，術前化学放射線療法群30名のうち，75％が腺癌であった。当初400名規模のランダム化比較試験として計画されたが，先のオーストラリアでの試験結果を受けて患者のリクルートができなくなったため，このような症例数となった。

これらの試験を集めたメタアナリシスにおいても，10

表 2　主な術前化学放射線療法比較試験の結果（文献 5）より改変）

報告者（年）	組織型	術前化学放射線療法			n	MST 月	p
Apinop (1994)	SCC	CDDP 100 mg/m² d1, 5-FU 1,000 mg/m² d1-4 RT 2 Gy×20/40 Gy（concurrent）	×2	S	34	NA	NS
				CRT→S	35	NA	
Le Prise (1994)	SCC	CDDP 100 mg/m² d1, 5-FU 1,000 mg/m² d2-5 d22-25 RT 2 Gy×10/20 Gy（sequential）	×2	S	41	47%（1yrs）	NS
				CRT→S	41	47%（1yrs）	
Bosset (1997)	SCC	CDDP 80 mg/m² d0-2 RT 3.7 Gy×10/37 Gy（sequential）	×2	S	145	18.6 m	NS
				CRT→S	148	18.6 m	
Urba (2001)	SCC/ Adeno	CDDP 20 mg/m² d1-5, 5-FU 300 mg/m² d1-21 VLB 1 mg/m² d1-4 RT 1.5 Gy×30/45 Gy（concurrent）	×2	S	50	17.6 m	NS
				CRT→S	50	16.9 m	
Walsh (1995)	SCC	CDDP 75 mg/m² d7, 5-FU 15 mg/kg d1-5 RT 2.7 Gy×15/40 Gy（concurrent）	×2	S	32	NA	NS
				CRT→S	29	NA	
Burmeister (2005)	SCC/ Adeno	CDDP 80 mg/m² d1, 5-FU 800 mg/m² d2-5 RT 2.3 Gy×15/35 Gy（concurrent）	×1	S	128	19.3 m	NS
				CRT→S	128	22.2 m	
Lee (2004)	SCC	CDDP 60 mg/m² d1, 5-FU 1,000 mg/m² d3-5 RT 1.2 Gy×38/45.6 Gy（concurrent）	×2	S	50	27.3 m	NS
				CRT→S	51	28.2 m	
Walsh (1996)	Adeno	CDDP 75 mg/m² d7, 5-FU 15 mg/kg d1-5 RT 2.7 Gy×15/40 Gy（concurrent）	×2	S	55	11 m	0.01
				CRT→S	58	16 m	
Tepper (2006)	SCC/ Adeno	CDDP 60 mg/m² d1, 5-FU 1,000 mg/m² d3-5 RT 1.8 Gy×28/50.4 Gy（concurrent）	×2	S	26	21.6 m	0.02
				CRT→S	30	54 m	

S: 手術単独群，CRT→S: 術前化学療法群，MST: 生存期間中央値，1yrs: 1 年生存割合，NS: not significant

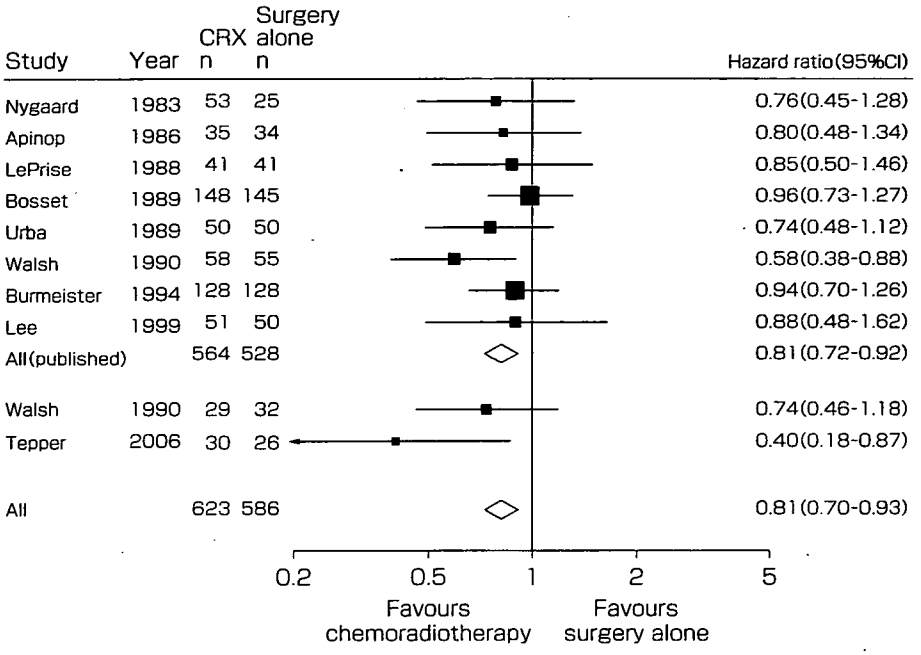
のランダム化比較試験での解析で術前化学放射線療法 vs 手術単独では，有意に術前化学放射線療法群において生存への寄与が認められた（HR＝0.81，p＝0.002）（図 3）。扁平上皮癌では HR＝0.84，腺癌では HR＝0.75 とやや腺癌において，その効果は高かった[5]。組織型により試験ごとのばらつきはあるものの，術前化学放射線療法が支持されつつあるのが現状である。また，2007 年 ASCO においてドイツより下部食道～接合部原発の腺癌に対して術前化学療法群と術前化学療法にさらに術前化学放射線療法を上乗せした群との比較試験（POET）の結果が報告された。59 名と 60 名と比較的小規模の比較試験であったため，統計学的有意差はつかなかったが，化学放射線療法を加えた群が，より生存期間が延長される傾向が認められた[9]。

これらの試験結果を本邦に外挿する上で考えなくてはいけないポイントとしては，まずは組織型のことであり，90％が扁平上皮癌である日本においては腺癌の多い海外の試験の中身を吟味し，扁平上皮癌での成績を考える必要がある。また，手術が海外と本邦では異なることも重要である。海外では食道抜去術が主流であり，リンパ節郭清を行う日本の術式とは大きく治療成績も異なる。オーストラリアの試験や，CALGB9781 においても手術単独の成績は生存期間中央値 2 年に満たないが，日本食道学会などでの報告では，国立がんセンターを含めた専門施設における Stage Ⅱ，Ⅲ食道癌症例の術後生存期間中央値は，3 年を超えている。Stage Ⅱ，Ⅲ食道癌に対する根治的化学放射線療法では，JCOG9906 の結果として 5 年生存割合 36.8％[10]と化学放射線療法のみで根治が望める場合もあり，このような対象には化学放射線療法後の手術は過剰である。また，術前化学放射線療法を行うことで 90 日以内死亡が有意に増加するというメタアナリシスの結果もある[11]。フランスからの報告（FFCD9102）では 444 名に化学放射線療法を行い，レスポンスが認められた 259 名をランダム化し，そのまま化学放射線療法を行う群と，手術を行う群に割り付け比較した。生存期間中央値は手術群で 17.7 か月，化学放射線療法群で 19.3 か月と両者に差を認めなかった[12]。ただし，化学放射線療法直後の予後予測は非常に困難であることが現状である。

まとめ

食道癌においても，もはや手術のみで治療することは

a: 各試験での Hazard ratio



b: 組織型による hazard ratio

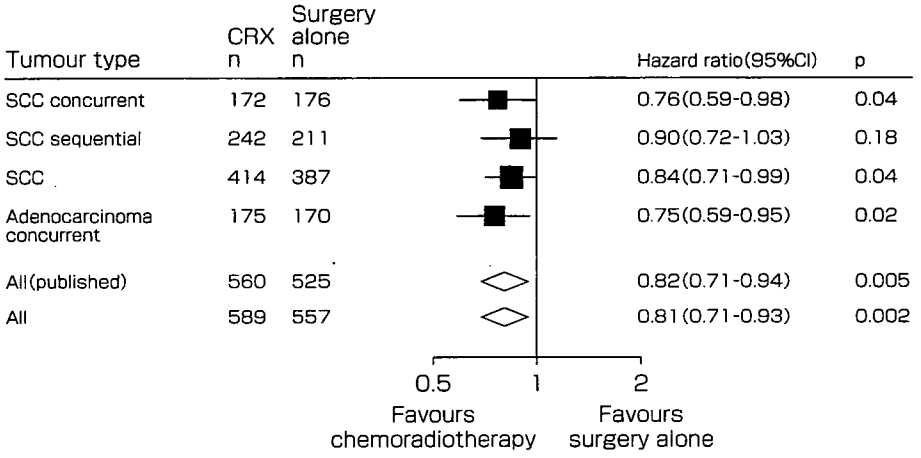


図 3　術前化学放射線療法 vs 手術単独療法のメタアナリシス（文献5)より）

少なくなってきている。JCOG9907の結果では，術前化学療法が標準的となりそうであるが，術前化学放射線療法が日本の手術に対しても上乗せ効果が認められるのか興味深いところである。いずれにしても日本での臨床試験による評価が望ましい。ただし，化学放射線療法単独でも治癒する患者がいるのも事実である。これらを予測するための一つの手段として，マイクロアレイを用いた癌の遺伝子プロファイリングや，プロテオミクスを用いた検討がなされている。また，PETなどの画像的診断方法を治療選択の手段として組み込む検討を行っているグループもある。層別化による治療戦略が功を奏するか，今後の展開が注目される。

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Significance of Biological Markers for Predicting Prognosis and Selecting Chemotherapy Regimens of Advanced Gastric Cancer Patients between Continuous Infusion of 5-FU and a Combination of 5-FU and Cisplatin

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Background: Our previous phase II study of 5-fluorouracil (5-FU) and cisplatin (FP) for treatment of advanced gastric cancer showed that strong immunoreactivity for vascular endothelial growth factor (VEGF) is associated with chemoresponse. Patients with four or five of the favorable phenotypes, p53 (–), bcl-2 (–), gluthathione *S*-transferase π (–), thymidylate synthase (–), and VEGF (+), survived longer than those with three or less of these phenotypes. The purpose of this study is to confirm our previous results and to compare the significance of those markers between continuous infusion of 5-FU (5-FUci) and FP.
Methods: Pretreatment biopsies from 131 of 210 advanced gastric cancer patients enrolled to JCOG9205 were analyzed immunohistochemically for the presence of the five markers.
Results: Median survival times of patients treated with 5-FUci ($n = 65$) or FP ($n = 66$) were 216 and 253 days, respectively ($P = 0.6953$). After FP treatment, patients with four or five favorable phenotypes ($n = 20$) survived longer than those with three or less favorable phenotypes ($n = 46$) (334 days and 243 days, respectively; $P = 0.0463$), and the survival times of 34 and 32 patients with VEGF (–) and (+) were similar (269 days and 253 days, respectively; $P = 0.6317$). After 5-FUci, 30 patients with VEGF (+) survived for a shorter time than 35 patients with VEGF (–) (142 days and 302 days, respectively; $P = 0.0043$).
Conclusion: The number of favorable phenotypes is prognostic for gastric cancer patients treated with FP, and VEGF has a different impact on survival between treatment with 5-FUci and FP.

Key words: vascular endothelial growth factor – gastric cancer – 5-fluorouracil – cisplatin

INTRODUCTION

Many combination chemotherapy regimens for treatment of advanced gastric cancer have recently been developed using new agents and shown high response rates (1–8). However, a standard chemotherapy has not been established for treatment of advanced gastric cancer because there are no reports from randomized phase III trials showing a survival benefit to the treatment with 5-fluorouracil (5-FU) alone (9,10). In the phase III study of the Gastrointestinal Oncology Study Group (GIOSG) of the Japan Clinical Oncology Group (JCOG), there was no significant difference in survival between continuous infusion of 5-FU (5-FUci) and a combination of 5-FU and cisplatin (FP) despite a higher response

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rate and longer time to progression (TTP) of FP compared with 5-FUci, associated with lower toxicity of 5-FUci than of FP (11). Therefore, 5-FUci was considered a reference regimen for the present phase III study of advanced gastric cancer patients. It has also been reported that a better response to chemotherapy contributes to longer survival and cures in some patients (12). However, severe toxicity associated with intensive chemotherapy causes deterioration of patients' quality of life, especially that of nonresponders. Thus, the ability to predict the effects of chemotherapy and to select an appropriate regimen for each patient before commencement of chemotherapy is important.

Many factors and mechanisms are involved in sensitivity and resistance to chemotherapy, of which some are clinically relevant (13–16). However, there are no reports of clinically useful biological markers for prognosis and chemotherapy regimens for advanced gastric cancer patients. In our previous phase II study of FP for advanced gastric cancer, vascular endothelial growth factor (VEGF) (+), p53 (−), bcl-2 (−), thymidylate synthase (TS) (−), and gluthathione *S*-transferase π (GST-π) (−) were shown to be favorable phenotypes for chemoresponse in 39 patients (17). Patients with VEGF (+) had significantly higher response rates than those with VEGF (−).However, there were no differences in survival times between (+) and (−) marker types. The number of favorable phenotypes was related to response rate: the 10 patients with four or five favorable phenotypes survived significantly longer than the 29 patients with three phenotypes or less.

However, because our previous study was investigational, it was considered necessary to confirm the results in a different cohort. In addition, investigation of the clinical utility of these markers is certainly warranted for selecting chemotherapy regimens in a randomized phase III trial.

In this study, we investigated relationships between expression of five biological markers and survival among patients registered for a phase III study (JCOG9205 (11)) to confirm the results of our previous study and to clarify the utility of these markers for selecting 5-FUci or FP chemotherapy regimens. The study was approved by the chair of the Japan Clinical Oncology Group.

PATIENTS AND METHODS

Patients

Two hundred and eighty patients were enrolled in the phase III study (JCOG9205 (11)); 106 patients had been treated with 5-FUci, 104 with FP and 70 with a combination of futrafur and uracil (UFT) plus mitomycin C (UFTM). Biopsy samples were obtained from 180 patients, consisting of 68 (64%) from the 5-FUci group, 67 (64%) from the FP group, and 45 (64%) from the UFTM group. Patients treated with UFTM were excluded from this study because enrollment for UFTM treatment ceased after interim analysis of the phase III study revealed that it had no survival advantage and more severe toxic effects than 5-FUci (11). Three patients in the 5-FUci group and one in the FP group were excluded because their biopsy samples were too small for immunostaining. The subjects selected for this study comprised 65 patients treated with 5-FUci and 66 treated with FP from whom sufficient amounts of pretreatment biopsy specimens had been obtained endoscopically. These patients met the eligibility criteria of JCOG9205 (11): (1) histological confirmation of gastric cancer, (2) measurable or assessable lesions, (3) ability to accept oral administration of UFT, (4) aged 75 years or younger, (5) a performance status of two or less on the ECOG scale, (6) no prior treatment except surgery, (7) fully functioning liver, kidney, and bone marrow, (8) life expectancy of eight weeks or longer, and (9) written informed consent. All the patients in the study received the protocol chemotherapy as the first line therapy.

Treatment Schedule

The treatment schedule for the 5-FUci group comprised a continuous infusion of 5-FU (800 mg/m^2 per day) on days 1–5. The FP schedule consisted of a drip infusion of CDDP (20 mg/m^2 per day) on days 1–5, together with the same dose of 5-FUci as the 5-FUci group. These two treatments were repeated every four weeks until the appearance of disease progression, unacceptable toxicity, or the patient's voluntary withdrawal from the study.

Immunohistochemistry

Biopsy samples from 180 patients were immunostained as described in our previous report (17). All immunohistochemical analyses were performed using tissue sections from formalin-fixed, paraffin-embedded biopsy material obtained endoscopically from primary tumors. Serial 3 μm thick slices were cut, deparaffinized in xylene, dehydrated with graded ethanol and then immersed in methanol containing 0.3% H_2O_2 for 20 min to inhibit endogenous peroxidase activity. Sections stained for p53 and TS were heated to 95°C by microwave irradiation for 10 min in phosphate buffered saline (PBS) or 10 mM citrate buffer, respectively. Sections stained for VEGF were treated with 0.05% pepsin in 0.01 N HCl for 20 min at room temperature. After blocking with 10% normal swine serum in PBS (blocking buffer) for 60 min, all sections were incubated overnight at room temperature with the primary antibodies diluted in blocking buffer to the following concentrations: anti-p53 antibody (Nichirei, Tokyo, Japan), 1:20000; anti-bcl-2 antibody (DAKO, Glostrup, Denmark), 1:40; anti-GST-π antibody (MBL, Nagoya, Japan), 1:24000; anti-TS antibody (TS106 (16)), 1:200; anti-VEGF antibody (Santa Cruz Biochemistry, CA, USA), 1:500. The sections were washed with PBS and then incubated for 1 h with biotinylated secondary antibody diluted 1:200. After washing with PBS, the sections were incubated with ABC reagent (Vector Laboratories, CA, USA) and the color reaction was

Table 1. Patient characteristics

		5-FUci ($n = 65$)	FP ($n = 66$)	P value
Age	Median (range)	63 (34–73)	63 (19–75)	0.6065
Gender	M/F	49/16	48/18	0.7257
Performance status (ECOG scale)	0/1/2/unknown	34/18/11/2	24/35/5/2	0.0242
Macroscopic type	Expansive/infiltrative/others	15/48/2	11/53/2	0.6520
Histological type	Intestinal/diffuse unknown	29/31/5	32/29/5	0.9019
Tumor extent	Locally advanced/ascites/metastatic	15/33/17	17/39/10	0.1196
Resection of the primary tumor	+/ −	17/48	16/50	0.8011

Source: A phase III study of the Japan Clinical Oncology Group (JCOG9205); continuous infusion of 5-fluorouracil (5-FUci) versus a combination of 5-FU and cisplatin (CDDP). Pre-treatment biopsies were available in 65 patients treated with 5-FUci and 66 patients with FP.

developed in Tris buffer containing 2% 3,3′-diaminobenzidine and 0.3% hydrogen peroxide. The sections were then counterstained with hematoxylin or methyl green.

All immunostained specimens from the 180 patients were assessed by an investigator (N.B.) who was not informed of clinical information such as treatment schedules and clinical outcomes. The intensity of staining for p53 and GST-π was graded as (+) when strong, as (±) when faint, and as (−) when no staining was visible. For bcl-2, the intensity of staining was graded as (++) when stronger than that of correspondingly stained lymphocytes, as (+) when equal to that of stained lymphocytes, and as (−) when weaker than that of stained lymphocytes. The staining of VEGF was graded as (++) when the intensity of staining in cancer cells was stronger than that in stromal cells, as (+) when equal to that of stromal cells, and as (−) when weaker than that of stromal cells. TS expression was graded as (++), (+), (±), or (−) based on the intensity of staining. For all markers, patients were defined as positive when more than 20% of the cancer cells in each section were (++) or (+). VEGF (+), p53 (−), bcl-2 (−), TS (−) and GST-p (−) were defined as favorable phenotypes for chemoresponse to FP on the basis of the results of our previous phase II study.

Anti-tumor Effects

The responses of measurable metastatic lesions and of primary lesions were evaluated according to the standard World Health Organization criteria (18) and evaluation criteria proposed by the Japanese Gastric Cancer Association (19). All patients were followed up for at least 1 year after registration for the study. Survival was calculated from the date of registration to the date of death from any cause or to the last confirmation of survival. TTP was estimated from the interval between the date of registration and the date of confirmation of disease progression by image and clinical diagnosis, or the date of death for patients for whom confirmation of disease progression was absent. All clinical information was obtained from the JCOG data center.

Statistical Analysis

Survival curves were constructed using the Kaplan–Meier method and compared using the Log-rank test. Patient characteristics and response rates were compared using a χ^2 test.

RESULTS

Patient Characteristics

Patient characteristics are shown in Table 1. The subjects constituted two thirds of all patients enrolled in JCOG9205 (11). The numbers of patients treated with 5-FUci and FP were similar. The two groups were well balanced in respect of age, sex, macroscopic type, histological type, and history of resection of primary lesions, but there were more patients with poor performance status in the FP group than in the 5-FUci group ($P = 0.0242$). Seventeen patients (26%) in the 5-FU group and 10 (15%) in the FP group had distant metastases ($P = 0.1196$).

Overall Survival and Time to Progression

Figure 1 shows the overall survival times of subjects treated with 5-FUci or FP. There was no significant difference in survival between patients treated with 5-FUci or with FP;

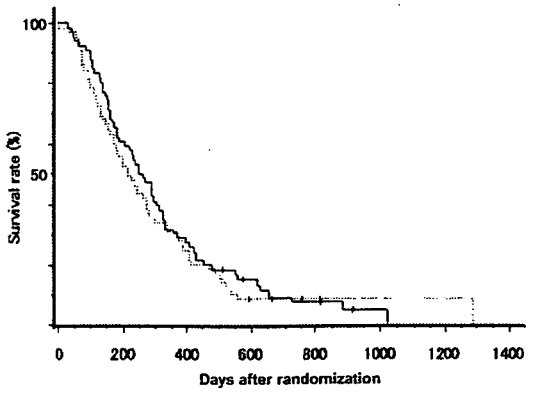

Figure 1. Overall survival of 66 patients treated with FP (solid line) and 65 patients treated with FUci (dotted line).

Table 2. Expression of biological markers in 5-FUci and FP

		5-FUci ($n = 65$)	FP ($n = 66$)
VEGF	(+)	30 (49)	32 (47)
	(−)	35 (51)	34 (53)
TS	(+)	37 (57)	21 (32)
	(−)	28 (43)	45 (68)
p53	(+)	28 (43)	28 (42)
	(−)	37 (57)	38 (58)
GST-π	(+)	38 (58)	41 (62)
	(−)	27 (42)	25 (38)
Bcl-2	(+)	7 (11)	11 (17)
	(−)	58 (89)	55 (83)

Expressions of vascular endothelial growth factor (VEGF), thymidylate synthase (TS), p53, gluthathione S-transferase π (GST-π) and bcl-2 were examined immunohistochemically. Values in parentheses are percentages.

Table 3. Expression of biological markers and response to 5-FUci and FP

		5-FUci ($n = 65$)	FP ($n = 66$)
VEGF	(+)	1/30 (3)	13/32 (41)
	(−)	7/35 (20)	16/34 (47)
TS	(+)	5/37 (14)	9/21 (43)
	(−)	3/28 (11)	20/45 (44)
p53	(+)	2/28 (7)	11/28 (39)
	(−)	6/37 (16)	18/38 (47)
GST-π	(+)	3/38 (8)	20/41 (49)
	(−)	5/27 (19)	9/25 (36)
Bcl-2	(+)	1/7 (14)	4/11 (36)
	(−)	7/58 (12)	25/55 (45)

Expressions of vascular endothelial growth factor (VEGF), thymidylate synthase (TS), p53, gluthathione S-transferase π (GST-π) and bcl-2 were examined immunohistochemically. The number of patients with complete or partial remission after treatment with 5-FUci and FP in all patients with positive or negative expression of each biological marker. Values in parentheses are percentages.

median survival times were 216 days for the 5-FUci group and 253 days for the FP group ($P = 0.6953$). TTP was longer after FP treatment than after 5-FUci treatment (median TTP: 111 days and 61 days, respectively; $P = 0.0477$).

Expression of Biological Markers and Response

The staining patterns and incidences of positive reactions for the biological markers were similar to those observed in our previous study (17) (Table 2). Table 3 shows the relationships between biological markers and chemoresponses. The overall response rates in the FP and 5-FUci groups were 44% (29/66) and 12% (8/65), respectively. While the response rates of the patients with VEGF (−) were higher than those with VEGF (+) in the 5-FUci group ($P = 0.0599$), there was no significant difference in response between patients with (+) and (−) types of VEGF, p53, bcl-2, TS or GST-π.

Eleven of the 20 patients (55%) with four or five favorable phenotypes and 18 of the 46 patients (39%) with three or less favorable phenotypes were responders ($P = 0.2326$) in the FP treatment group. The response rate of the 16 patients with four or five favorable phenotypes (13%) was similar to that (12%) of the other 49 patients with three or fewer favorable phenotypes ($P > 0.9999$) in the 5-FUci treatment group.

Number of Favorable Phenotypes, Survival and Time to Progression

In the FP treatment group, the 20 patients with four or five favorable phenotypes survived longer than the 46 patients with three or less favorable phenotypes (MST, 334 and 243 days, respectively; $P = 0.0463$) (Fig. 2A), whereas there was no difference between the two types of patient in the 5-FUci group (MST, 203 and 216 days, respectively; $P = 0.315$) (Fig. 2B). No significant differences were observed in TTP between patients with four or five favorable phenotypes and patients with three or less favorable phenotypes in the FP or 5-FUci groups (FP: favorable, 118 days; others, 102 days; $P = 0.2766$, and 5-FUci: favorable, 41 days; others, 61 days; $P = 0.6830$).

VEGF, Survival and Time to Progression

In the 5-FUci and FP groups, there were no significant differences in survival times between patients with (+) or (−) types of p53, bcl-2, TS or GST-π. The survival times of the 32 (49%) patients with VEGF (+) and the 34 (51%) patients with VEGF (−) were almost equal in the FP treatment group (MST: 269 and 253 days, respectively; $P = 0.6317$) (Fig. 3A), whereas the 30 patients with VEGF (+) had shorter survival times than the 35 with VEGF (−) in the 5-FUci treatment group (MST: 142 and 302 days, respectively; $P = 0.0043$) (Fig. 3B). In the FP group, there was no difference in TTP between patients with VEGF (+) and those with VEGF (−) (median TTP: 111 days and 123 days, respectively; $P = 0.3497$). However, the TTP of patients with VEGF (−) was significantly longer than that of patients with VEGF (+) in the 5-FUci group (median TTP: 101 days and 36 days, respectively; $P = 0.0046$).

DISCUSSION

The recruitment rates of patients from the phase III study (JCOG9205 (11)) into the present study were equal among the three regimens. Patient characteristics and rates of positive reactions for biological markers were well balanced. These data indicate that biopsy samples were collected without bias. The overall response rates, survival times and

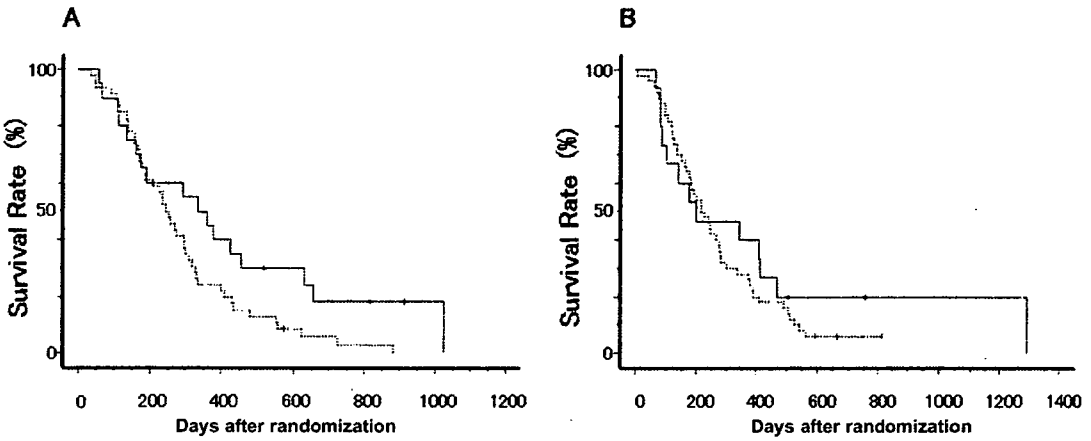

Figure 2. Overall survival of patients (solid line) with four or five favorable phenotypes out of VEGF (+), TS (−), p53 (−), bcl-2 (−), GST-π (−), and those (dotted line) with 3 or fewer, after treatment with FP (A) or 5-FUci (B).

TTPs were similar to those of patients enrolled in the phase III study (11). Although biopsy specimens were collected only from two-thirds of the patients enrolled in JCOG9205 (11), the subjects of this study were considered representative of those of the phase III study.

Biopsy samples can only be obtained from the superficial part of primary tumors and may not be representative of the biological behavior of the entire tumor. Because many patients destined to be treated with chemotherapy have unresectable tumors, only biopsy samples can be used to assess biological markers. Takiuchi (20) and our group (17) have shown that VEGF (+) is a predictive marker of chemoresponse in advanced gastric cancer patients treated with FP. Nagashima (21) reported that patients with VEGF (+) who were treated with a combination of irinotecan (CPT-11) and CDDP had a higher response rate than those with VEGF (−). These results suggest that assessment of biological markers using endoscopic biopsy samples can yield useful information and that the expression of VEGF in the biopsy samples of gastric cancer patients may be a predictor of chemotherapeutic effects in CDDP containing regimens.

The incidence of VEGF (+) was 47% (62/131), which recapitulated the result of our previous study (51%, 20/39). The incidences of other biological markers in the two studies were also similar. These results show that the method used for evaluating biological markers was reproducible.

In our previous study, expression of VEGF and the number of favorable phenotypes were significant predictors of chemoresponse to FP (17). In the present study, there was no relationship between the expression of VEGF and chemoresponse to FP. The response rate of patients with four or five favorable phenotypes was slightly but not significantly

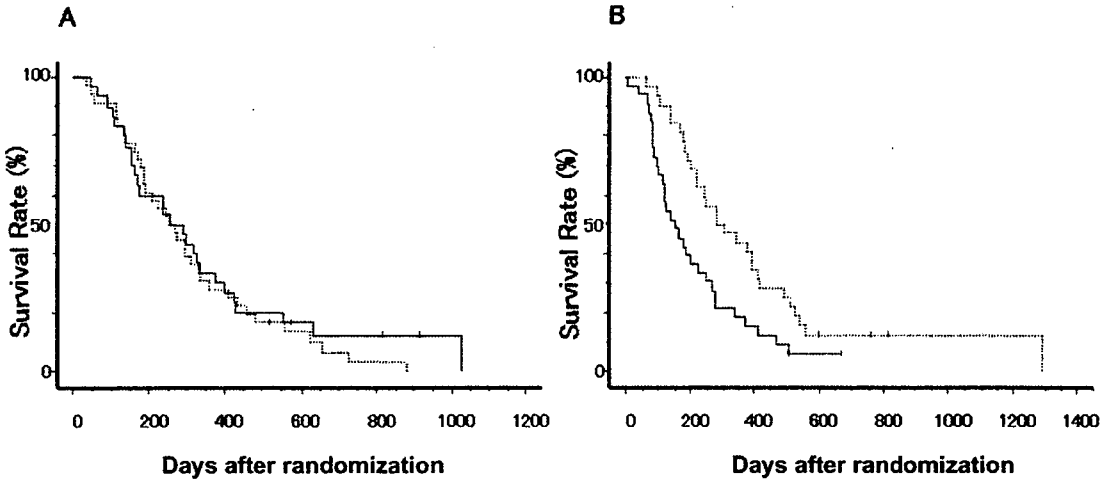

Figure 3. Overall survival of patients (solid line) with VEGF (+) and those (dotted line) with VEGF (−) after treatment with FP (A) or 5-FUci (B).

higher than that of patients with three or less favorable phenotypes. It could be argued that these discrepancies indicate that expression of VEGF and the number of favorable phenotypes are not good predictive markers of chemoresponse to FP. However, Takiuchi et al. (20) reported that immunohistochemical expression of VEGF can predict the response to FP in patients with gastric cancer. Nagashima et al. (21) reported that VEGF and the number of favorable phenotypes of similar biological markers were predictive markers of chemoresponse to irinotecan plus CDDP and of survival. Several reports have described differences in chemoresponse between phase II and III studies of the same chemotherapy regimens. It is possible that the discrepancies between our previous and present studies were caused by the difference in the method of evaluating responses between the phase II and III studies of FP.

In the FP group of this study, the 20 patients with four or five favorable phenotypes survived longer than the 46 patients with three or less favorable phenotypes. This result recapitulates our previous phase II findings on survival. In the 5-FUci treatment group, there was no difference in survival between these two phenotype groups. However, the differences in survival between the FP and 5-FUci treatments were small in patients with four or five favorable phenotypes and in those with three or less favorable phenotypes. Moreover, in the FP and 5-FUci treatment groups, there were no significant differences in TTP between patients with four or five favorable phenotypes and those with three or less favorable phenotypes. These results suggest that the presence of favorable phenotypes is a prognostic marker for patients treated with FP, but not a selective marker between FP and 5-FUci.

VEGF promotes angiogenesis and permeability of blood vessels and is associated with microvessel abundance and metastasis (22,23). It has been reported that VEGF is a marker of poor prognosis after surgical resection in various kinds of malignancies including gastric cancer (24–30). Our previous study showed no differences in survival between patients with VEGF (+) or (−) despite a higher response rate in those with VEGF (+). Similarly, in the present study, there were no differences in survival or TTP between patients with VEGF (+) or (−) after treatment with FP. However, in the 5-FUci group, patients with VEGF (+) had a significantly shorter survival time and TTP than those with VEGF (−). Thus, VEGF is considered to be a risk factor for a poor prognosis in patients treated with 5-FUci. It is suggested that CDDP in addition to 5-FUci may overcome the malignant potential of VEGF, although the relationship between VEGF and the chemoresponse to FP was not as clear in the present study as in our previous study (17).

In the phase III study (JCOG9205 (11)), FP treatment had no survival benefit over treatment with 5-FUci even though the response rate and TTP after FP treatment was significantly better than after 5-FUci treatment. This study showed that in the subset of patients with VEGF (−), 5-FUci treatment resulted in slightly longer survival times than FP treatment and the TTPs were almost equal (5-FUci 101 days, FP 123 days). In contrast, in the subset of patients with VEGF (+), survival and TTP of patients treated with FP were longer than those of patients treated with 5-FUci. From these results, it is speculated that patients with VEGF (+) may achieve longer survival and TTP after treatment with FP than with 5-FUci and that the status of VEGF expression might be a selective marker for treatment with 5-FUci versus FP.

In conclusion, the number of favorable phenotypes (≥ 4 versus ≤ 3) of markers VEGF (+), p53 (−), bcl-2 (−), TS (−), and GST-π (−) was prognostic for the outcome of advanced gastric cancer treatment with FP. Clinical outcomes such as TTP and survival differed between 5-FUci and FP treatment according to the status of VEGF expression. Although the methodology used to evaluate biological markers in this study might be considered less advanced than methods based on microarrays or proteomics, the results illustrate some important points: (i) multiple factors should be investigated to clarify prognostic markers of cytotoxic agents, (ii) confirmation of results is mandatory, (iii) comparison in a phase III study is necessary to clarify the utility of markers for selecting treatments.

Acknowledgments

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Conflict of interest statement

None declared.

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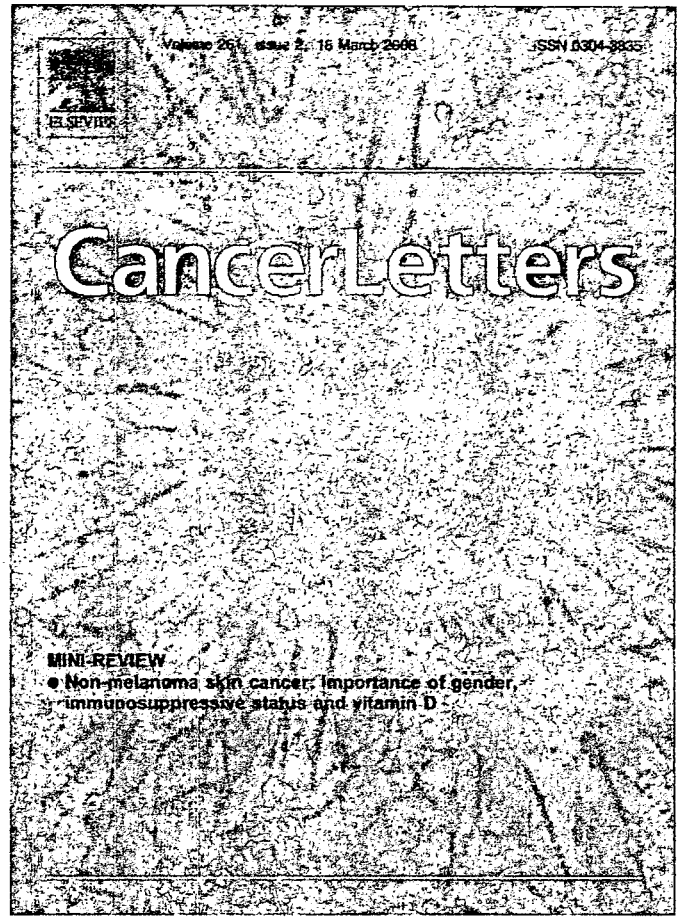

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Importance of *UDP-glucuronosyltransferase 1A1*6* for irinotecan toxicities in Japanese cancer patients

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Abstract

Recent pharmacogenetic studies on irinotecan have revealed the impact of *UDP glucuronosyltransferase (UGT) 1A1*28* on severe irinotecan toxicities. Although the clinical role of *UGT1A1*6*, which is specifically detected in East Asian patients, in irinotecan toxicities is suggested, clear evidence remains limited. To examine the impact of *6*, the association of *UGT1A1* genotypes with severe irinotecan toxicities was retrospectively investigated in Japanese cancer patients. A significant *6*-dependent increase in the incidence of grade 3 or 4 neutropenia was observed in 49 patients on irinotecan monotherapy ($p = 0.012$). This study further clarifies the clinical importance of *6* in irinotecan therapy in East Asians.

Keywords: UGT1A1; Pharmacogenetics; Irinotecan; SN-38

1. Introduction

Irinotecan, an anticancer prodrug, is widely applied for a broad range of carcinomas, including colorectal and lung cancers. The active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor, is generated by hydrolysis of the parent compound by carboxylesterases [1]. SN-38 is subsequently glucuronidated by uridine diphosphate glucuronosyltransferase 1As (UGT1As) such as 1A1, 1A7, 1A9 and 1A10, to form the inactive metabolite, SN-38 glucuronide (SN-38G) [2–5]. Among the UGT

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isoforms, UGT1A1 is thought to be a predominant contributor to SN-38G formation [2,6]. The dose-limiting toxicities in irinotecan therapy are severe diarrhea and leucopenia [7], and lowered UGT activity is well correlated with severe irinotecan toxicities [8]. Since Ando et al. first reported the significant relevance of *UGT1A1*28* – a repeat polymorphism in the TATA box (−40_−39insTA) – to severe neutropenia/diarrhea [9], a number of clinical studies, primarily conducted in Caucasian patients, have shown associations between *UGT1A1*28* and lowered SN-38G formation or severe neutropenia/diarrhea [10–13]. Based on these findings, the Food and Drug Administration (FDA) of the United States approved a revision of the label for Camptosar (irinotecan HCl) (NDA 20-571/S-024/S-027/S-028), recommending "a reduction in the starting dose by at least one level of irinotecan for the *UGT1A1*28* homozygous patients". Subsequently, the clinical application of *UGT1A1*28* testing was put into practice for irinotecan therapy in the United States.

To implement personalized irinotecan therapy in Asian countries, the racial differences in *UGT1A1* polymorphisms among Caucasians, African-Americans, and Asians must be taken into consideration [14]. For East Asians, the frequency of **28* is one third of that of Caucasians or African-Americans, and another low-activity allele **6* [211G>A(G71R)], which is not detected in Caucasians or African-Americans, shows the same frequency as the **28* allele. Clinical studies in Japanese cancer patients have demonstrated that significantly low area under concentration-time curve (AUC) ratios of SN-38G to SN-38 are observed in patients having **6* and/or **28* [15–17], suggesting the necessity of typing **6* in addition to **28*. A recent report on Korean lung cancer patients who received a combination therapy of irinotecan and cisplatin, showed a significant association of **6* homozygotes with severe neutropenia [18]. However, data on the role of **6* in irinotecan toxicities is still limited in terms of the various irinotecan-containing regimens. In the first study by Ando et al. on Japanese cancer patients, the association of **6* with irinotecan toxicities was not evident, but a possible enhancement of **28*-related toxicities by **6* was suggested [9]. Other studies in Japanese patients showed an additive effect of **6* on the lowered UGT activity by **28* [15–17]. A significant association of the genetic marker "**6 or *28*" with severe neutropenia was also shown in our previous study, but due to a lack of **6* homozygotes in our patient population, the effect of **6* alone was not confirmed [17].

In this study, to further demonstrate the clinical importance of **6* alone, *UGT1A1* genotypes were determined using DNA extracted from paraffin-embedded specimens (non-cancerous tissues) from 75 Japanese cancer patients by the pyrosequencing method [19,20], and the associations between *UGT1A1* genotype and severe irinotecan toxicities and serum total bilirubin levels were retrospectively analyzed.

2. Materials and methods

2.1. Patients and irinotecan treatment

In a post-marketing surveillance study conducted by Daiichi Pharmaceutical Co., Ltd. (currently Daiichi Sankyo Co., Ltd., Tokyo, Japan), irinotecan was prescribed to 297 patients with various types of cancers from 1995 to 2000 at the National Cancer Center Hospital. The patients were selected through standard clinical practice according to the drug label for indications and contraindications. Methanol-fixed, paraffin-embedded archival tissue specimens, which were necessary for high-quality extraction of DNA greater than 2 kb in size [21], were available for 75 of the 297 patients and were analyzed in this study. Irinotecan was administered by intravenous 30-min infusion as a single agent or in combination chemotherapy at a dose of 60 mg/m^2 (weekly or biweekly), 100 mg/m^2 (biweekly), or 150 mg/m^2 (biweekly). Profiles of the patients in this study, including cancer type, treatment history, and regimens, are summarized in Table 1. The pre-treatment levels of serum total bilirubin were determined by a kit (VL T-BIL, Azwell Inc., Osaka, Japan) according to an enzymatic method using bilirubin oxidase [22]. Toxicities were monitored during irinotecan therapy and graded according to the Common Toxicity Criteria version 2 of the National Cancer Institute.

Because the samples in this study were residual specimens remaining after histopathological diagnosis in the hospital and not collected specifically for research purposes, the samples and their clinical information were anonymized in an unlinkable fashion according to the Ethics Guidelines for Human Genome/Gene Analysis Research by the Ministry of Education, Culture, Sports, Science and Technology, Ministry of Health, Labour and Welfare, and Ministry of Economy, Trade and Industry of Japan. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences.

2.2. DNA extraction from paraffin-embedded tissue sections and genotyping of UGT1A1 polymorphisms

Three sections (20 μm of pathologically normal tissues around tumors) were deparaffinized twice by treat-