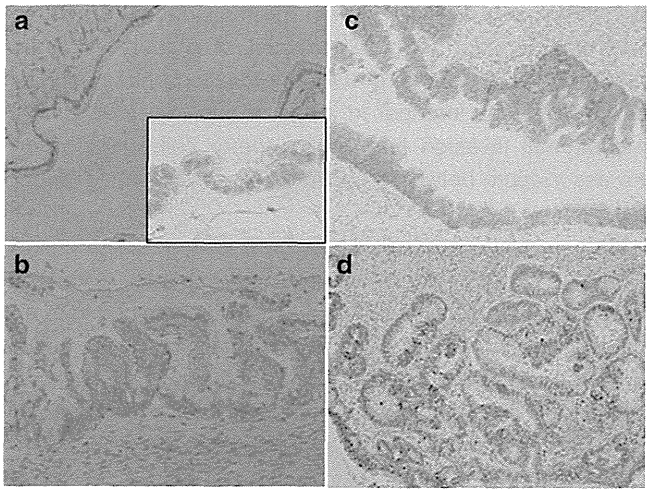

Figure 2 Immuohistocheical staining of ovarian cancer immunoreactive antigen domain containing 2 (OCIAD2) in mucinous ovarian tumors. Immunohistochemistry of OCIAD2 for adenoma (2a), borderline lesion (endocervical type) (2b), borderline lesion (intestinal type) (2c), and adenocarcinoma (2d). OCIAD2 is granularly positive in borderline tumor and carcinoma.

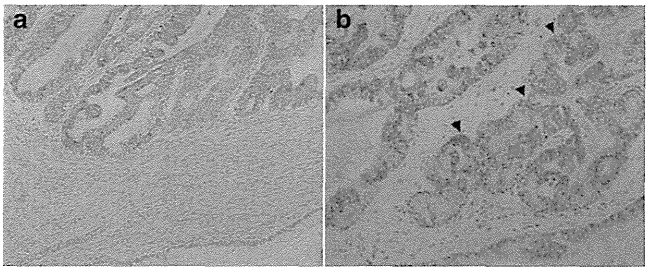

Figure 3 Histological staining pattern of ovarian cancer immunoreactive antigen domain containing 2 (OCIAD2) for OCIAD2 in mucinous borderline tumors and invasing mucinous adenocarcinoma. (a) Immunohistochemial staining of OCIAD2 for the case of mucinous borderline tumor. Areas of papillary proliferation showed more intense reactivity for OCIAD2 than flat lining tumor cells. (b) Immunohistochemical staining of OCIAD2 in a case of mucinous adenocarcinoma. Areas of stromal invasion (arrow heads) showed more intense reactivity for OCIAD 2 than flat lining tumor cells.

for OCIAD1 (Table 2, Fig. 4d). As was the case in adenocarcinoma, OCIAD1 showed a granular staining pattern that was separated from intracytoplasmic mucin. Stromal cells also showed positive background staining.

With regard to CEA, tumor cells showed a diffuse cytoplasmic staining pattern, as had been observed in colonic adenocarcinoma, and also positive staining for mucin. Among the 43 cases of mucinous adenoma, 0 (0%) were positive for CEA (Table 2, Fig. 5a). Background staining for intracystic and brush border secreted mucin was evident, but this was judged as negative. Among the 40 cases of mucinous borderline tumor, 10 (25%) including five among ten of the endocervical type (50%) were positive for CEA (Table 2, Fig. 5b, c). There was strongly positive background staining

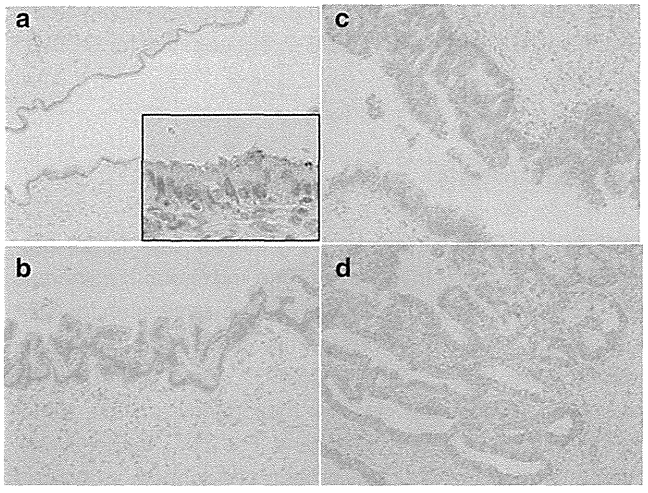

Figure 4 Immuohistocheical staining of ovarian cancer immunoreactive antigen domain containing 1 (OCIAD1) in mucinous ovarian tumors. Immunohistochemistry of OCIAD1 for adenoma (4a), borderline lesion (endocervical type) (4b), borderline lesion (intestinal type) (4c), and adenocarcinoma (4d). OCIAD1 is granularly positive in borderline tumor and carcinoma.

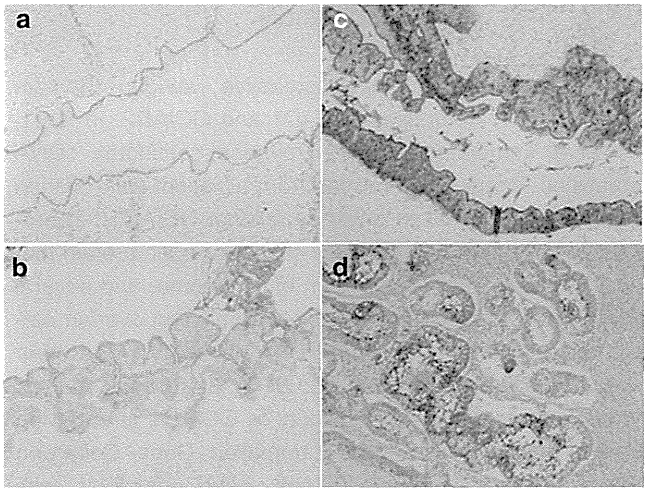

Figure 5 Immuohistocheical staining of CEA in mucinous ovarian tumors. Immunohistochemistry of CEA for adenoma (5a), borderline lesion (endocervical type) (5b), borderline lesion (intestinal type) (5c), and adenocarcinoma (5d). CEA is granularly positive in borderline tumor and carcinoma.

for intracystic and brush border secreted mucin. Non-specific positive staining was evident in the nuclei of tumor cells and in the tumor stroma. Among the 34 cases of mucinous carcinoma, 25 (71%) (5 of the infiltrating invasive type and 20 of the expansile invasive type) were positive for CEA (Table 2, Fig. 5d). Strongly positive background staining for intracystic and brush border secreted mucin was evident, similarly to adenoma and borderline lesions. Diffuse cytoplasmic staining was judged as positive. The tumor cell nucleoli and tumor stroma showed non-specific positive staining.

The one case of borderline tumor showing pseudomyxoma peritonei was stained with OCIAD2, but negative for OCIAD1 and CEA.

Statistical analysis

Positivity for OCIAD2 increased gradually with tumor progression, and more than 70% of the mucinous carcinomas were positive (Table 2). A similar tendency was seen for CEA and OCIAD1, more than 70% of the carcinomas also being positive. The expression of OCIAD2, CEA and OCIAD1 increased significantly as the malignancy of the tumor increased ($P < 0.01$, $P < 0.01$, and $P < 0.01$, respectively). Table 2 compares the positivity ratio of adenoma vs borderline lesion and adenocarcinoma. Among the cases of adenoma, 6 (14%), 21 (49%), and 0 (0%) were positive for OCIAD2, OCIAD1, and CEA, respectively, whereas among the cases of borderline lesion and adenocarcinoma, 51 (68%), 56 (75%), and 35 (47%) were positive, respectively.

DISCUSSION

The ovarian cancer immunoreactive antigen domain (OCIAD) family comprises OCIAD1 and OCIAD2. In 2001, Luo *et al.* immunoscreened an ovarian carcinoma cDNA expression library using ascites from a patient with ovarian cancer and detected an antibody against OCIAD1.[8] Therefore, OCIAD1 is thought to be a cancer-specific protein that could be applicable for detection of carcinoma, and several reports have stressed the association between ovarian carcinoma malignancy and OCIAD1 expression. On the other hand, OCIAD2 was identified in 2001 by Strausberg *et al.* on the basis of its sequence similarity to OCIAD1 through the National Institute of Health Mammalian Gene Collection project.[19] Although OCIAD2 has high homology with OCIAD1, to date, no reports have examined the relationship between its expression and carcinoma or autoantibody against human tissue. Ishiyama T. first reported the association between lung adenocarcinogenesis and OCIAD2 on the basis of a cDNA microarray study.[7] In the present study, we examined the expression of OCIAD2 in ovarian carcinoma, especially ovarian mucinous tumors, since the diagnosis of their malignancy is based on histology, and no biomarkers for these tumors have been characterized.

In order to examine the relationship between the OCIAD family and other immunohistochemical biomarkers, we also examined the expression of CEA. As Table 2 shows, OCIAD2 expression was detected in 74% of mucinous ovarian carcinomas and 63% of borderline tumors (i.e. 69% of ovarian mucinous tumors with malignancy), but in only 14% of mucinous adenomas (benign counterpart) ($P < 0.01$). Expression of OCIAD2 was associated with the malignancy of ovarian mucinous tumors. OCIAD2 showed a granular staining pattern in the cytoplasm of the tumor cells, but interestingly its positivity was stronger in areas of papillary proliferation and stromal invasion than in flat tumor cells, suggesting an association between OCIAD2 expression and tumor malignancy.

With regard to borderline tumors, it was of considerable interest that positivity for all of the markers examined (OCIAD2, OCIAD1, and CEA) had a tendency to be higher in the endocervical type than in the intestinal type. As the tumor cells of intestinal-type tumors contain much mucin in their cytoplasim, relative to those of endocervical-type tumors, it might be difficult to judge the staining positivity of intestinal-type tumors. On the other hand, in the carcinomas, the rates of positivity for the three proteins showed no significant difference between the infiltrating invasive type and the expansile invasive type.

The staining patterns of OCIAD1 and CEA were similar to that of OCIAD2, but several differences were evident. OCIAD1 was positive in 86% of carcinomas and 65% of borderline tumors (i.e. 75% of all ovarian mucinous tumors with malignancy), whereas 49% of adenomas were also positive for OCIAD1. These results indicated that positivity for OCIAD1 increased in the earlier stage of malignant progression. Although we are unable to verify whether the staining was non-specific, stromal cells also showed positive staining for OCIAD1. However, the results suggested that OCIAD2 is a marker more associated with malignancy of ovarian mucinous tumor cells than is OCIAD1.

CEA was immunopositive in 71% of carcinomas and 25% of borderline tumors (i.e. 47% of all ovarian mucinous tumors with malignancy). The specificity of CEA for diagnosis of malignancy in ovarian mucinous tumors was highest among the three markers we examined, thus confirming that CEA is a very useful biomarker for detection of ovarian tumors. The CEA positivity rate mainly increased during the course from borderline tumor to carcinoma. Therefore, CEA might be a marker of more advanced-stage mucinous tumors in comparison with OCIAD2.

The association between OCIAD2 stainability and tumor malignancy was of considerable interest; papillary proliferating and invasive tumor cells were positive, whereas flat lining tumor cells were negative. Six cases of adenoma gave a positive reaction with anti-OCIAD2 antibody. These tumors were histologically diagnosed as adenoma, but may have had the potential to progress to borderline tumors. As it has been suggested that OCIAD2 may be localized to the tumor cell membrane, OCIAD2 protein in exfoliated tumor cells or cell fragments in ascites or blood could be detectable and applicable as a new biomarker of ovarian mucinous tumors.

In summary, we have demonstrated specific expression of OCIAD2 in ovarian mucinous tumors. Immunohistochemi-

cally. OCIAD2 appeared to be more specific than OCIAD1 and more sensitive than CEA. Examination of OCIAD2 expression is thus expected to become a new immunohistochemical biomarker of the malignancy of ovarian mucinous tumors. OCIAD2 is a membrane-localized protein expressed in several malignant tumors including lung cancer and ovarian cancers, but its function is still unclear. As normal tissue is unreactive with a specific antibody against OCIAD2, it appears that OCIAD2 is not a basic protein required for the survival of human cells. However, the biological implications of OCIAD2 should be examined extensively and utilized for the detection or treatment of malignant tumors expressing it.

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● 特 集 ● 術前化学療法の新展開

卵巣癌

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Summary

The current standard treatment for advanced ovarian cancer is primary debulking surgery (PDS) followed by postsurgical chemotherapy. We can expect better prognosis in cases where optimal debulking (residual diseases < 1 cm) can be achieved. Neoadjuvant chemotherapy (NAC) has been recognized as an alternative treatment to primary surgical debulking for patients with poor performance status or apparently unresectable bulky tumors. Retrospective analyses and non-randomized comparative studies revealed that overall survival was comparable between patients treated with NAC followed by interval debulking surgery (IDS) and those treated with PDS, though the former group had more advanced disease and poorer performance status. Two reports of meta-analyses of these studies revealed that the NAC setting treatment does not compromise the treatment outcome of the patients with advanced ovarian cancer. Until now, at least four phase Ⅲ studies comparing NAC setting treatment with standard treatment for advanced müllerian cancer have been conducted. The results of the first study conducted by the European Organization for Research and Treatment of Cancer (EORTC) were published in 2010. They revealed a comparative outcome of NAC setting treatment with standard treatment (median survival 30 M vs 29 M) with less common surgery-related adverse effects. NAC setting treatment is now expected to become a standard treatment or one of the effective treatment options for advanced ovarian cancer in cases when other phase Ⅲ studies reproduce similar results. **Key words**: Neoadjuvant chemotherapy, Primary debulking surgery, Ovarian cancer, **Corresponding author**: Takashi Onda, Department of Gynecology, Kitasato University School of Medicine, 1-15-1 Kitasato, Minami-ku, Sagamihara, Kanagawa 252-0374, Japan

要旨 進行卵巣癌に対する現在の標準治療は初回腫瘍縮小手術（PDS）と術後化学療法であり，PDSで残存腫瘍1 cm未満のoptimal手術が達成できれば予後の改善が期待できる。術前化学療法（NAC）は，全身状態不良の症例や明らかに切除不能な腫瘍を有する症例に対して，標準治療の代替治療として行われてきた。その後方視的検討や無作為ではない比較試験により，NACおよび中間期手術（IDS）からなる治療は，進行例や全身状態不良例を対象としているにもかかわらず，標準治療施行例と遜色ない治療成績が示された。2本のメタアナライシスの報告でも，NAC療法は進行卵巣癌の治療成績を損なうものではないことが示された。現在までに，NAC療法と標準治療を比較する第Ⅲ相比較試験が少なくとも4本行われており，最初の試験であるEuropean Organization for Research and Treatment of Cancer（EORTC）の試験結果は2010年に発表された。結果，NAC療法では標準治療と遜色のない治療成績（生存期間中央値30か月と29か月）と手術関連の有害事象が低頻度であることが示された。今後，他の第Ⅲ相試験により同様の結果が再現されれば，NAC療法は進行卵巣癌の標準治療あるいは標準治療の一つの有効な選択肢となることが期待される。

はじめに

進行卵巣癌の予後向上をめざした治療法として，薬剤投与法（間隔）の変更（weekly投与），投与経路の変更（腹腔内投与），分子標的薬（bevacizumabなど）の併用とともに，術前化学療法（neoadjuvant chemotherapy: NAC）が注目されている。本誌の2007年11月号でも紹介したように，多くの後方視的検討と無作為ではない比較試験での標準治療との比較では，NAC療法［ここでは，NACで始まり，interval debulking surgery（IDS），術後化学療法からなる治療全体をNAC療法と呼ぶ］により，良好な成績が得られており，第Ⅲ相比較試験での

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表 1　NAC療法と標準治療の比較（腫瘍縮小手術における optimal surgery，生存率）

報告者（年） 治療法［症例数］	生存率の比較		腫瘍縮小手術	NAC群の選択
Jacob（1991）	MST		optimal（<2 cm）	NAC群，標準群とも他院で生検のみ施行。標準治療群は，進行期，組織型，分化度，年齢を match させた control。
標準治療［n=18］	18 M		39%（7/18）	
NAC療法［n=22］	16 M		77%（17/22）	
	NS		p=0.02	
Onnis（1996）	3 year	5 year	optimal（<2 cm）	胸水，肝転移の有無，試験開腹による切除可能性の評価により NAC 療法群を決定。NAC 療法群は，より進行した症例が多い。
標準治療［n=284］	31%	21%	29%（83/284）	
NAC療法［n=88］	27%	19%	42%（37/88）	
	NS	NS	NA	
Vergote（1998）	3 year			試験開腹，腹腔鏡による切除可能性の評価により NAC 療法群を決定。
NAC 導入前［n=112］	26%			
NAC 導入後［n=173］	42%			
	p=0.0001			
Kayikçioğlu（2001）	5 year	MST	optimal（=0）	胸水，肝転移，切除不能な多発転移の有無，全身状態により NAC 療法群を決定。NAC 療法群は有意に高齢（p=0.01），PS 不良（p<0.001）で，Ⅳ期症例が多い（p=0.03）。
標準治療［n=158］	24%	38 M	14%（22/158）	
NAC療法［n=45］	30%	34 M	49%（22/45）	
	NS	NS	p<0.001	
Kuhn（2001）	MST		optimal（<2 cm）	対象は，多量の腹水（>500 mL）を有する卵巣癌ⅢC期に限定。臨床試験に同意が得られなかった症例に標準治療。標準治療群と NAC 療法群の背景に有意差なし。
標準治療［n=32］	23 M		63%（20/32）	
NAC療法［n=31］	42 M		84%（26/31）	
	p=0.007		p=0.04	
Loizzy（2005）	MST	DFI	optimal（<1 cm）	多量の胸水，腹水，全身状態，CT による切除可能性の評価により NAC 療法群を決定。標準治療群は，組織型，進行期を match させた control。NAC 群は有意に高齢（p=0.03），有意に PS 不良（p=0.02）。
標準治療［n=30］	40 M	16 M	60%（18/30）	
NAC療法［n=30］	32 M	21 M	63%（19/30）	
	NS	NS	NS	
Lee（2006）	MST	DFI	optimal（<2 cm）	CT，MRI により切除可能性を評価し，NAC 群を決定。
標準治療［n=22］	55 M	17 M	46%（10/22）	
NAC療法［n=18］	53 M	15 M	78%（14/18）	
	NS	NS	p=0.04	
Everett（2006）	MST		optimal（<1 cm）	肝転移，大きな上腹部転移，広範なリンパ節転移，重篤な合併症などにより NAC 群を決定。NAC 群は有意にⅣ期（p=0.042），低分化（p=0.025）症例が多い。
標準治療［n=102］	42 M		54%（55/102）	
NAC療法［n=98］	33 M		86%（84/98）	
	NS		p<0.001	
Inciura（2006）	MST	DFI	optimal（<2 cm）	多量の腹水，大きな骨盤内または腹部腫瘍の存在により，NAC 療法群を決定。
標準治療［n=361］	25 M	15 M	67%（242/361）	
NAC療法［n=213］	24 M	13 M	63%（134/213）	
	NS	NS	NS	
Hou（2007）	MST	DFI	optimal（<1 cm）	重篤な合併症および画像診断で腹部を越えた進展，広範な腹腔内進展により NAC 群を決定。NAC 群で有意にⅣ期症例が多い（<0.05），NAC 群でより高齢，より低分化腫瘍であったが有意差はなし。
標準治療［n=109］	47 M	14 M	71（77/109）	
NAC療法［n=63］	46 M	16 M	95（60/63）	
	NS	NS	<0.001	

NA: not available, MST: median survival time, DFI: disease free interval

検討が開始されていた。ここでは NAC 療法に関する治療成績を再確認し，新たな解析結果や第Ⅲ相試験の経過について紹介する。

Ⅰ．進行卵巣癌に対する NAC 療法

卵巣癌に対する治療は，初めに原発臓器，組織型の診断，進行期の診断（staging laparotomy）を目的とした手術を行うのが通常である。進行例においては，optimal surgery（残存腫瘍が<1 cm）となるように，転移巣の可及的切除（maximum debulking）を行うことも目的とするため，この手術は primary debulking surgery（PDS）と呼ばれる。進行癌に対しては，術後に化学療法を 6～8 コース行うのが標準である。PDS で optimal surgery が達成できるか否かは，進行卵巣癌の重要な予後因子の一つであるため，全身状態不良で手術自体が困難な場合，画像診断や腹腔鏡診断で optimal 不能と診断された場合など，良好な予後が期待できない場合に標準治療の代替治療として，まず化学療法を行い全身状態の改善あるいは腫瘍の縮小が得られた後に腫瘍縮小手術を行い，さらに化学療法を追加する治療が行われてきた。

表 2 NAC 療法と標準治療の比較（手術合併症などの比較）

報告者（年） 治療法［症例数］	手術合併症などの比較				NAC 群の選択
Vergote（1998）	手術関連死亡率				試験開腹，腹腔鏡による切除可能性の評価により NAC 療法群を決定。
NAC 導入前［n=112］	6%				
NAC 導入後［n=173］	0%				
	NA				
Schwartz（1999）	出血量	ICU 滞在	入院期間		全身状態，合併症による手術可否の評価，CT による切除可能性の評価により NAC 療法群を決定。NAC 療法群は有意に高齢（<0.001），PS 不良（<0.001）であった。
標準治療［n=206］	1,000 mL	1.26 days	11 days		
NAC 療法［n=59］	600 mL	1.03 days	7 days		
	p=0.001	p=0.01	p<0.001		
Kayikçioğlu（2001）	結腸切除	脾摘			胸水，肝転移，切除不能な多発転移の有無，全身状態により NAC 療法群を決定。NAC 療法群は有意に高齢（p=0.01），PS 不良（p<0.001）で，Ⅳ期症例が多い（p=0.03）。
標準治療［n=158］	16%	11%			
NAC 療法［n=45］	2%	0%			
	p=0.01	p=0.02			
Morice（2003）	腸切	脾摘	重篤な合併症	輸血割合	試験開腹，腹腔鏡による切除可能性の評価により NAC 療法群を決定。
標準治療［n=28］	61%	7%	36%	39%	
NAC 療法［n=57］	19%	5%	7%	21%	
	p=0.01	NS	p=0.01	NS	
Hegazy（2005）	出血量	ICU 滞在	入院期間		試験開腹，腹腔鏡による切除可能性の評価により NAC 療法群を決定。NAC 群は有意に高齢（p=0.04）
標準治療［n=32］	735 mL	4.4 days	15.9 days		
NAC 療法［n=27］	420 mL	1.7 days	10.5 days		
	p=0.02	p=0.03	p<0.05		
Lee（2006）	出血量				CT，MRI により切除可能性を評価し NAC 群を決定。
標準治療［n=22］	1,061 mL				
NAC 療法［n=18］	620 mL				
	p=0.04				
Hou（2007）	出血量	手術時間	入院期間	輸血量	重篤な合併症および画像診断で腹部を越えた進展，広範な腹腔内進展により NAC 群を決定。NAC 群で有意にⅣ期症例が多い（<0.05），NAC 群でより高齢，より低分化腫瘍であったが有意差はなし。
標準治療［n=109］	1,033 mL	276 min	8.5 days	2.4 U	
NAC 療法［n=63］	546 mL	211 min	5.7 days	1.2 U	
	p<0.0001	p<0.0001	p<0.0001	p=0.03	

NA: not available

表 3 NAC 療法の利点と問題点

利点	問題点
1．化学療法の効果により全身状態を改善し，より安全な状態で腫瘍縮小手術を行い得る。	1．治療開始時に，staging laparotomy を兼ねた PDS を行わないため，対象疾患や進行期の診断が不正確となる可能性がある。
2．化学療法中の抗凝固療法により，あるいは腫瘍縮小により，卵巣癌に高頻度にみられる深部静脈血栓症の改善が期待できる。	2．化学療法の効果が得られなければ腫瘍縮小手術の機会を逸する，optimal surgery の達成を逸するなどの可能性がある。
3．腫瘍の縮小により，他臓器合併切除など術式を拡大しなくても optimal surgery の可能性が高くなる。	3．腫瘍縮小手術に際して，肉眼的な腫瘍および範囲の縮小により，術式を縮小しすぎて根治性を損なう可能性がある。
4．手術枠の確保，他科との連携などを要することなく，速やかに治療を開始することができる。	4．腫瘍量の多い状態で化学療法を行うため，血流不十分な細胞の存在により薬剤耐性の出現の可能性も高くなる。
5．腫瘍縮小のための手術は 1 回のみで済む。	5．1 回の手術で，すべての腫瘍を摘出するには手術が広範囲となる可能性がある。

予後不良例に対して行われた NAC 療法の成績を，標準治療を行い得た症例の成績と比較した検討により，治療成績に関して，NAC 群では条件が悪い対象症例でありながらも標準治療を行い得た症例と概して同等の治療成績が得られ，手術侵襲に関しては，多くの報告で，出血量の有意な減少，腸切，脾摘などの合併切除の有意な減少，重篤な合併症の有意な減少，ICU 滞在期間や入院期間の有意な短縮が示された。表 1 に治療成績，表 2 に手術侵襲について比較した報告のまとめを再掲する[1]。NAC 療法には，理論上いくつかの利点が考えられるが，逆にその利点とも関連した問題点も存在している。表 3 に NAC 療法の利点と問題点をまとめた。

Ⅱ．NAC 療法の meta-analysis による解析

Bristow ら[2]は，1989～2005 年に報告された，卵巣癌Ⅲ/Ⅳ期に対する NAC 療法の論文 21 本を meta-analy-

表 4　標準治療とNAC療法の第Ⅲ相無作為比較試験

試験グループ	EORTC	RCOG/CTU-MRC	All India Institute of Medical Sciences	JCOG
試験名	EORTC55971	CHORUS	ID I473	JCOG0602
中心国	Belgium	United Kingdom	India	Japan
研究代表者	Vergote IB	Kehoe S	Kumar L	Yoshikawa H
対象疾患	卵巣癌/卵管癌/腹膜癌	卵巣癌/卵管癌/腹膜癌	卵巣癌	卵巣癌/卵管癌/腹膜癌
進行期	ⅢC/Ⅳ期	Ⅲ/Ⅳ期	ⅢC/胸水Ⅳ期	Ⅲ/Ⅳ期
試験のタイプ	第Ⅲ相	第Ⅱ/Ⅲ相	第Ⅲ相	第Ⅲ相
悪性の確認方法	(登録前) 腹腔鏡生検あるいは針生検	画像診断/腫瘍マーカー (登録後) 腹腔鏡生検, 針生検, 穿刺細胞診	(登録前) 細胞診, 組織診	(登録前) 穿刺細胞診
化学療法種類	Platinum+Taxane	CBDCA を含む regimen	TC regimen	TC regimen
NAC群の化学療法回数	NAC 3+3コース	NAC 3+3コース	NAC 3+3コース	NAC 4+4コース
症例数	704	150 (第Ⅱ相)+400 (第Ⅲ相)	180	300
開始	1998.9.21	2004.3 (第Ⅲ相)	2001.11	2006.11.17
予定登録期間	4年間	4年間 (第Ⅲ相)	約5年間	3年+1.5年 (改訂)
登録状況	2006.12.6登録完了	2010.8登録完了	詳細不明	2011.10登録完了
試験デザイン	非劣性	(EORTCと併せて1,250例で) 非劣性	(恐らく) 非劣性	非劣性
臨床試験登録番号	NCT00003636	NCT00075712	NCT00715286	UMIN000000523
臨床試験登録日	1999.11.1	2004.1.9	2008.7.14	2006.11.17

EORTC: European Organization for Research and Treatment of Cancer, JCOG: Japan Clinical Oncology Group
RCOG: Royal College of Obstetricians and Gynaecologists, CTU-MRC: Medical Research Council Clinical Trials Unit
CHORUS: Chemotherapy or Upfront Surgery

sisにより解析した。生存期間中央値 (median survival time: MST) は24.5か月，optimal surgery (ここでは，残存腫瘍径≤2 cmと定義) 達成率は65%であった。NAC療法の治療成績は，論文の年代，paclitaxel (PTX) の使用割合，optimal症例の割合，Ⅳ期症例の割合，NACのコース数と有意に相関が認められた。optimal症例の割合は，10%増えるごとに1.9か月の予後の改善が認められたが，NACのコース数は1コース増えるごとに4.1か月の生存期間の短縮が認められた。著者らは，Gynecologic Oncology Group (GOG) による臨床試験で，PDSでoptimalが達成できなかった (suboptimalと呼ぶ) 症例のMSTが24か月であることと比較して，NAC療法のMST 24.5か月は，これらsuboptimal症例と同等であるとしてNAC療法に否定的な見解を示した。

一方，Kangら[3]は，1989～2008年に報告された21本の論文を用いて同様の解析を行った。3年間選択範囲が広がった以外は論文の選択基準はほぼ同様であるが，7本の論文が除外され，新しい年代の論文が7本追加され，Bristowらのsimple linear regression modelに対して，より高度なrandom effect modelという違う解析の手法が用いられた。MSTは27.5か月，optimal surgery達成率は70%であった。この論文では，治療成績と相関がみられたのは，論文の年代，PTXの使用割合，optimal症例の割合の3因子で，Ⅳ期症例の割合，NACのコース数とは相関を認めなかった。さらに，NAC療法を標準治療と比較した10論文を解析し，NAC療法により手術でsuboptimalとなるriskは有意に減少し，optimal達成率を上げることができるとNAC療法を評価している。解析に含まれる論文の違いや解析方法の違いにより解析の結果が異なっており，NAC療法の有用性に関して結論は出せないが，解析に含まれる報告の多くは前述のように全身状態不良やoptimal surgeryが期待できない症例に対する治療成績であり，少なくともNAC療法により治療成績が損なわれることはないと考えられる。

Ⅲ．NAC療法と標準治療の第Ⅲ相比較試験

NAC療法が，真に現在の標準治療である手術先行治療に勝ることを示すには，第Ⅲ相無作為比較試験による証明が必要である。米国の臨床試験のデータベース (ClinicalTrials.gov) によれば，日本以外の地域でこれまでに少なくとも三つの第Ⅲ相試験が行われており，日本では，Japan Clinical Oncology Group (JCOG) で第Ⅲ相比較試験が行われている。表4にこれら四つの試験の概要を示す。

そのうち，いち早く開始されたEuropean Organization for Research and Treatment of Cancer (EORTC) の試験は，2010年その結果が報告された[4]。試験開腹，

腹腔鏡，画像診断下の生検などで診断された718例のⅢC/Ⅳ期症例が登録され，標準治療群336例，NAC療法群334例，併せて670例について解析が行われ，生存期間中央値はそれぞれ29か月と30か月でほぼ同等であった。術後の有害事象に関しては統計的には検討されていないが，標準治療群で頻度が高い傾向を認めた。以上より，進行卵巣癌に対する治療の選択肢として，NAC療法は手術先行治療（標準治療）に劣るものではないと結論している。

また，イギリスのRoyal College of Obstetricians and Gynaecologists（RCOG）と，Medical Research Council Clinical Trials Unit（CTU-MRC）のグループでは，当初からEORTC試験の結果と共同解析を予定した第Ⅲ相試験であるChemotherapy or Upfront Surgery（CHORUS）試験を行い，2010年8月に登録を完了している。試験デザインはEORTCとほぼ同等であるが，登録前には細胞診，組織診は行わず，登録後に確認するデザインとなっている。

インドのグループは2006年，2007年にAmerican Society of Clinical Oncology（ASCO）で中間の解析を発表している。NAC群では，optimal surgeryが高率に達成できて手術による出血や合併症などの頻度が少なく，生存期間，無増悪生存期間はほぼ同等という結果であった。2008年7月以降，この試験に関してデータベースが更新されておらず，進捗状況などの詳細は不明である[NCT00715286 in ClinicalTrial. gov（http://clinicaltrial.gov/）]。

JCOGではNAC療法と標準治療との第Ⅲ相比較試験の前段階として，Ⅲ/Ⅳ期の卵巣癌，卵管癌，腹膜癌を対象として，2003年1月から第Ⅱ相feasibility試験（JCOG0206）を行った[5,6]。その結果，NAC療法は第Ⅲ相試験の試験治療とするに足る有望な治療であり（NAC療法では開腹手術を行わないため，診断が不正確になる懸念があるが），画像診断，腫瘍マーカー，穿刺細胞診の組み合わせによる臨床診断によりNAC療法の対象疾患を十分に診断可能であることが示された。

JCOG0206の結果を受けてJCOGでは，2006年11月に第Ⅲ相比較試験を開始し，2011年10月に登録を終了した（JCOG0602）[7]。現在，経過観察中である。この試験の特徴は，第Ⅱ相試験の結果より，試験開腹や腹腔鏡による診断を要せず臨床的な診断のみで登録を可能としたことであり，手術による治療開始の遅延をなくし，NAC療法の利点である早期治療開始を可能とし，実地臨床と同様の条件で比較可能とした点である。

Ⅳ．NAC療法の今後の課題

NAC療法を標準治療の代替治療ではなく標準治療として，あるいは標準治療の一つのオプションとして施行する場合，種々の問題点もあげられる。対象疾患はいかにして正確に診断するか，現在開腹所見により行っている臨床進行期をどのように適応していくか，至適な術前，術後化学療法の薬剤の種類，投与回数，投与経路は，IDSにおける摘出範囲や残存腫瘍径の目標はどのように設定するかなどについて，今後検討が必要と考えられる。

おわりに

これまでの後方視的検討あるいは無作為ではない比較試験の治療成績をまとめると，NAC療法では手術に関する侵襲が少なく，現在の標準治療である手術先行治療と遜色のない，ほぼ同等の治療成績が得られている。また，最初に結果が判明したEORTCの第Ⅲ相試験の結果では治療侵襲に関する解析は十分ではないが，治療成績はほぼ同等であることが示された。現在進行中の第Ⅲ相試験の結果が出そろい，NAC療法の非劣性が再現されれば進行卵巣癌に対する標準治療となることが期待される。

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Phase II Study of Oral Etoposide and Intravenous Irinotecan for Patients with Platinum-resistant and Taxane-pretreated Ovarian Cancer: Japan Clinical Oncology Group Study 0503

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A single-arm Phase II study evaluating combination chemotherapy utilizing oral etoposide and irinotecan for platinum-resistant and taxane-pretreated ovarian cancer has started. The aim of this study is to evaluate the efficacy and safety of this regimen as a test arm regimen in a subsequent Phase III trial. Patients with platinum-resistant and taxane-pretreated ovarian cancer are given etoposide at 50 mg/m^2 p.o. from days 1 to 21 and irinotecan 70 mg/m^2 i.v. at days 1 and 15, repeated every 28 days, up to six cycles. A total of 60 patients will be enrolled at 36 institutions. The primary endpoint is response rate. The secondary endpoints include adverse events and progression-free and overall survival.

Key words: Chemo-Gynecology – Gynecol-Med – clinical trials

INTRODUCTION

Ovarian cancer is one of the most lethal gynecologic cancers in Japan. The first-line standard chemotherapy regimen is carboplatin plus paclitaxel (1,2). Although first-line chemotherapy is very effective, more than 60% of the patients with an advanced stage will die of recurrent disease. After relapse, the choice of second line chemotherapy depends on 'platinum-free interval (PFI)', which is prognostic and predictive for the effect of repeating platinum agents. Usually, the cut-off point of PFI is regarded as 6 months. Patients recurred within 6 months after first-line chemotherapy are regarded as 'platinum-resistant' and receive second-line chemotherapy with single agent such as pegylated liposomal doxorubicin (3), topotecan (3) and gemcitabine (4) as the standard treatment. Many single cytotoxic agents have shown activity against recurrent ovarian cancer; however, response rates generally have been low, such as 6–12% (3,4), and of short duration because of emerging resistance to the monotherapy regimens. Combination chemotherapy may circumvent this resistance and halt progression of disease, because lower dose of two drugs with different mechanism may reduce the toxicity and enhance the efficacy (5).

Irinotecan, a semi-synthetic derivative of camptothecin, is a prodrug with little inherent topoisomerase inhibitory activity and is converted by carboxylesterases to its more active metabolite, SN-38 (7-ethyl-10-hydroxycamptothecin). *In vitro*, SN-38 is 250–1000 times more potent than irinotecan as an inhibitor of topoisomerase. For platinum-resistant patients, irinotecan has shown modest activity (6–8) as monotherapy in weekly, every 2-week and every 3-week schedules.

Etoposide is a semi-synthetic glycosidic derivative of podophyllotoxin (9). Intravenous dosing of etoposide has been tested in two Phase II studies and shown relatively low response rates (10,11) (0 and 8.3%). On the contrary, oral etoposide has shown better efficacy, whose response rate was 26.8% for patients with platinum-resistant relapse (12).

Topoisomerase-I treatment induces an increase in the S-phase cell population with an increase in topoisomerase-II mRNA expression. Thus, topoisomerase-I can modulate topoisomerase-II levels to enhance the effect of topoisomerase-II inhibitors (13,14).

Eder et al. (15) reported the result of the *in vivo* study. They showed that a combination of irinotecan and etoposide showed more than an additive effect by both the tumor excision assay and tumor growth delay assay.

A Phase I study of topotecan and oral etoposide revealed severe myelosuppression but promising efficacy for ovarian cancer (16).

The dose-limiting toxicity of irinotecan is diarrhea, different from that of topotecan (myelosuppression). Then, utilizing etoposide with irinotecan may improve the risk–benefit balance of dual inhibition of topoisomerase. The result of the Phase I study was reported in ASCO 2002 (17).

The recommended dose for further study was oral etoposide: 50 mg/m^2/days 1–21 and intravenous irinotecan: 60 mg/m^2/days 1 and 15, repeated every 4 weeks.

In this Phase I study, four objective responses [two complete responses and two partial responses (PRs)] were achieved among 24 patients, including one PR in clear cell.

Nishio et al. (18) reported the result of feasibility study run by selected hospitals in Tohoku and Kyushu districts in Japan. Response rate, time to progression and overall survival were 44%, 9 months and 17 months, respectively.

This very promising result lead us to conduct a nationwide Phase II study run by Japan Clinical Oncology Group (JCOG).

The protocol review committee of the JCOG approved this protocol in January 2009 and the study was initiated in April 2009. This trial was registered at UMIN-CTR as UMIN000001837 (http://www.umin.ac.jp/ctr/index.htm).

PROTOCOL DIGEST OF THE JCOG0503

Objectives

The aim of this study is to evaluate the safety and efficacy of oral etoposide and intravenous irinotecan for patients with platinum-resistant and taxane-pretreated ovarian, tubal and peritoneal cancer as the test arm regimen in a subsequent Phase III trial.

Study Setting

The study is a multi-institutional open-label two-stage design Phase II trial.

Resources

This study is supported by Grants-in-Aid for Cancer Research (20S-1 and 20S-6) and Health and Labor Sciences Research Grant for Clinical Cancer Research (18–6), from The Ministry of Health, Labor and Welfare of Japan.

Endpoints

The primary endpoint is response rate in all eligible patients. For patients with measurable lesion, response is evaluated according to the RECIST criteria (19). For patients with non-measurable lesion, response is evaluated according to the GCIG CA-125 criteria (20). The secondary endpoints are progression-free survival, overall survival and adverse events. Overall survival is defined as days from registration to death from any cause, and it is censored at the last follow-up day when the patient is alive. Progression-free survival is defined as days from registration to disease progression (either of radiological, CA-125, symptomatic) or death from any cause, and it is censored at the latest day when the patient is alive without any evidence of progression.

Eligibility Criteria

Inclusion Criteria

For inclusion in the study, patients are required to fulfill all of the following criteria:

(i) cytologically or histologically proven ovarian, tubal or peritoneal cancer
(ii) platinum-resistant disease
(iii) taxane-pretreated disease
(iv) age: 20–75 years old
(v) PS (performance status): 0–2
(vi) one of the followings, or both of them:
  (a) patients have measurable lesion
  (b) patients have assessable lesion with elevated CA-125 (more than 70 U/ml)
(vii) no prior treatment with irinotecan, topotecan or etoposide
(viii) no prior radiation to abdomen
(ix) oral intake without parenteral nutrition
(x) both of the followings:
  (a) no drainage to effusion or ascites within 28 days
  (b) no effusion or ascites to be drained at registration
(xi) both of the followings:
  (a) no chemotherapy or surgery within 28 days
  (b) no hormonal or biologic therapy within 14 days
(xii) patients without severe organ dysfunction
(xiii) written informed consent

Exclusion Criteria

Patients are excluded if they meet any of the following criteria:

(i) synchronous or metachronous (within 5 years) malignance other than carcinoma *in situ* or intramucosal cancer
(ii) mental disease or mental symptoms that would affect the participant's decision to participate
(iii) pregnant or lactating
(iv) continuous systemic steroid

(v) active bacterial or fungal infection with fever of 38.5°C or higher
(vi) uncontrollable hypertension
(vii) uncontrollable diabetes requires continuous insulin administration
(viii) history of myocardial infarction or heart failure within 6 months, or current unstable angina
(ix) bowel obstruction

Treatment Methods

Etoposide is orally administered once a day at 50 mg/m^2 from days 1 to 21, and irinotecan is infused at 70 mg/m^2 on days 1 and 15, repeated every 28 days. Protocol treatment is continued up to six cycles unless disease progression, unacceptable toxicity or patient refusal.

Follow-up

Enhanced abdominal computed tomography (CT)/magnetic resonance imaging, chest CT/X-rays and tumor marker (CA-125) are evaluated at least every 8 weeks during the protocol treatment. Adverse events are evaluated at least every 2 weeks during the protocol treatment using CTCAE ver. 3.0.

Study Design and Statistical Analysis

This study is a Phase II trial with two-stage design by Southwest Oncology Group (21) to evaluate this regimen as the test arm for a subsequent Phase III trial.

The sample size was determined as follows by the SWOG design. We assumed that the expected value for the primary endpoint of 35% and the threshold value of 20%. In this situation, the sample size ensuring at least 80% power with one-sided α of 5% is 55. Considering the likelihood of some ineligible patients being enrolled, the total number of patients was set at 60.

Interim Analysis and Monitoring

We plan interim analysis for futility after 30 patients enrolled. In house monitoring will be performed every 6 months by the JCOG Data Center to evaluate the study progress and to improve the study quality.

PARTICIPATING INSTITUTIONS

The participating institutions (from north to south) are as follows: Hokkaido University Hospital, Sapporo Medical University, Iwate Medical University, Tohoku University Hospital, Institute of Clinical Medicine, Tsukuba University, National Defense Medical College, Saitama Cancer Center, Saitama Medical Center, Saitama Medical School, National Cancer Center Hospital, Jikei Kashiwa Hospital, Tokyo Metropolitan and Infectious diseases Center Komagome Hospital, The University of Tokyo Hospital, Jikei University Hospital, Cancer Institute Hospital, Juntendo University School of Medicine, Kitasato University School of Medicine, Niigata Cancer Center Hospital, Shinshu University School of Medicine, Aichi Cancer Center Hospital, Kyoto University Hospital, Osaka city University Hospital, Kinki University School of Medicine, Osaka Medical Center for Cancer and Cardiovascular Disease, Osaka City General Hospital, Sakai Hospital, Kinki University School of Medicine, Hyogo Cancer Center, Tottori University, Kure Medical Center Chugoku Cancer Center, Shikoku Cancer Center, Kyushu Cancer Center, Kurume University School of Medicine, Kyushu University Hospital, Faculty of Medicine Saga University, Kumamoto University Medical School, Kagoshima City Hospital and University of the Ryukyu.

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Conflict of interest statement

None declared.

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ORIGINAL ARTICLE

Docetaxel/irinotecan combination chemotherapy in platinum/taxane-refractory and -resistant ovarian cancer: JGOG/WJGOG Intergroup Study

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Abstract
Background The aim of this phase II study was to evaluate the efficacy and toxicity of docetaxel and irinotecan combination chemotherapy in patients with ovarian cancer refractory and resistant to both platinum and taxan treatment.
Patients and methods Patients who had been treated with platinum and paclitaxel but whose ovarian cancer progressed or recurred within 6 months of treatment ($n = 41$) received docetaxel 60 mg/m^2 (day 1) and irinotecan 60 mg/m^2 (days 1, 8), repeated every 21 days [Japan Gynecologic Oncology Group (JGOG) study 3015] or every 28 days [West Japan Gynecologic Oncology Group (WJGOG) study 002] until disease progression was observed or unacceptable toxicity. Sixteen patients had platinum/paclitaxel-refractory disease, and 25 patients had platinum/paclitaxel-resistant disease.
Results Thirty-two patients were available for determination of the clinical response. The overall response rate [complete response (CR) + partial response (PR)] was 6.3%, and the disease control rate (CR + PR + stable disease) was 34.4%. Among the 23 patients with resistant tumor, the disease control rate was 47.8%. Ten patients with refractory tumor showed a 10% disease control rate. The median progression-free interval was 12.1 weeks and the median overall survival time was 45.3 weeks. The major toxic adverse effect was neutropenia (grade 4, 56.1%), but the incidence of neutropenic fever was less frequent (4.9%). Neurotoxicity and gastro-intestinal toxicity were mild.
Conclusion Among our patients, a combination of docetaxel and irinotecan was well tolerated. However, this combination may not be a beneficial option for patients with platinum-refractory and -resistant ovarian cancer in terms of response rate and survival.

Keywords Ovarian cancer · Recurrence · Platinum refractory · Platinum resistance · Docetaxel · Irinotecan

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Introduction

Cytoreductive surgery and adjuvant chemotherapy by paclitaxel/carboplatin is the standard of care for epithelial ovarian cancer (EOC). However, over 70% of patients with an advanced stage of EOC are reported to relapse. The

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therapeutic strategy involving chemotherapy for recurrent ovarian cancer is planned on the basis of the platinum-free interval. Markman et al. [1] found that patients with longer than a 24-month platinum-free interval showed a superior response to chemotherapy than those with an interval of between 5 and 12 months. This phenomenon has been observed by many researchers [2, 3]. Despite recent advances in the treatment of EOC, many phase II trials with a single agent have achieved only a 10–20% response rate in patients with platinum-refractory or -resistant disease [4–6].

Docetaxel is an alternative taxane which demonstrates a similar antitumor effect as paclitaxel but has a different toxicity profile [7]. Docetaxel has also shown some effect on platinum-resistant tumors. Two phase II studies showed a 35 and 40% response, respectively, in platinum-refractory ovarian cancer, with the accompanying adverse effect of rather severe toxicity [8, 9]. Irinotecan hydrochloride, one of the topoisomerase-1 inhibitors, achieved a 40% response rate in patients with refractory and recurrent ovarian cancer when used in combination with cisplatin [10].

The development of a new chemotherapeutic regimen for platinum/taxane-refractory or -resistant ovarian cancer is a matter of great urgency. Docetaxel and irinotecan each show promising antitumor effects in ovarian cancer. Moreover, the toxicity profile of these two drugs differs. As such, an investigation of the efficacy of these two drugs would provide valuable information. In this context, a phase II clinical trial was conducted to assess both the antitumor effect and the toxicity of the docetaxel/irinotecan combination for patients with platinum-refractory and -resistant ovarian cancer. This clinical trial was conducted in two groups [Japan Gynecologic Oncology Group (JGOG) and West Japan Gynecologic Oncology Group (WJGOG)] at the same time. Here, we have combined and analyzed the data from these two studies because both had the same eligibility criteria and used the same dosage of docetaxel and irinotecan.

Patients and methods

Eligibility

This phase II trial was conducted by the JGOG (study 3015) and the WJGOG (study 002). Patients were eligible if they satisfied the following criteria (note: throughout all subsequent text, the asterisk following a value presented in parenthesis indicates criteria/values for the WJGOG002 study only): (1) histologically confirmed EOC; (2) recurrent disease after previous treatment with a treatment-free interval of <6 months (resistant disease) or failure to respond to first-line chemotherapy with at least two cycles of platinum and/or taxane (refractory disease); (3) age >20 and <75 years; (4) an Eastern Cooperative Oncology Group (ECOG) performance status of <2; (5) >3 months life expectancy; (6) presence of a measurable target lesion; (7) adequate bone marrow, liver and kidney function, white blood cell (WBC) count $\geq$3000 ($\geq$4000*)/mm^3, neutrophil count $\geq$1500 ($\geq$2000*)/mm^3, platelet count $\geq$100 000, hemoglobin $\geq$9.5 g/dl/mm^3, serum creatinine level of $\leq$1.5 mg/dl, creatinine clearance $\geq$50 ($\geq$60*) ml/min, serum bilirubin $\leq$1.5 mg/dl, alanine aminotranferease/aspartate aminotransferase ratio $\leq$2 ($\leq$1.5*) times the upper limit of normal; (8) signed informed consent.

Patients were excluded from the study if any of the following applied: (1) active or uncontrolled infection; (2) other active malignancy; (3) life expectancy of $\leq$3 months; (4) clinically significant morbidity, such as history of myocardial infarction, congestive heart failure; (5) poor oral intake due to intestinal obstruction; (6) large amount of pleural effusion, pericardial fluid, or ascites requiring repeated drainage; (7) previous abdominal radiation therapy; (8) apparent pulmonary fibrosis or interstitial pneumonia; (9) interval of $\leq$3 weeks (JGOG) or 4 weeks (WJGOG) since any previous chemotherapy.

Treatment schedule

Irinotecan 60 mg/m^2 was administrated as a 90-min intravenous infusion on days 1 and 8, and docetaxel 60 mg/m^2 was administered as a 60-min intravenous infusion on day 1. The treatment cycles were repeated at 21-day (JGOG) or 28-day (WJGOG) intervals until there was evidence of disease progression or unacceptable toxicity. A 5HT3-antagonist was given before the administration of the anticancer agents. Granulocyte colony-stimulating factor (G-CSF) could be administered according to Japanese health insurance guidelines [neutrophil count $\leq$1000/mm^3 with fever ($\geq$38°C) or neutrophil count $\leq$500/mm^3 or neutrophil count $\leq$1000/mm^3 in patients with grade 4 neutropenia in the previous cycle]. Subsequent treatment was not started until patients had a neutrophil count of $\geq$1500/mm^3, platelet count $\geq$100 000, grade 0 diarrhea, and grade 1 neurotoxicity. The dose of irinotecan was reduced to 50 mg/m^2 and that of docetaxel reduced to 55 mg/m^2 (50 mg/m^2*) if grade 4 neutropenia persisted more than 5 days (3 days*) or grade 4 platelet count (level 1). If the patients had grade $\geq$2 diarrhea, only the dose of irinotecan was reduced, while if patients had grade $\geq$2 neurotoxicity, only the dose of docetaxel was reduced. If patients showed toxicity under level 1 dose reduction, further dose reduction was offered to the patients following the same protocol. The minimum dose of irinotecan an docetaxel was 40 and

50 mg/m^2, respectively. Patients were able to withdraw from the study at any time.

Study evaluation and endpoints

Antitumor effects were evaluated according to the Response Evaluation Criteria in Solid Tumors (RECIST) version 1.0. The CA-125 level was determined at the end of every treatment cycle and evaluated by Rustin's criteria [11]. Adverse effects were evaluated according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTC) version 2 (National Cancer Institute, National Institutes of Health, Bethesda, MD). Survival was calculated from the date of study treatment to the date of death, or time of last contact. Subsequent treatment after recurrence was not regulated.

The primary endpoint was the clinical response rate. The secondary endpoints were adverse effects, CA125 response, progression-free survival (PFS), and overall survival (OS). The protocol was reviewed and approved by the institutional review board of each participating institute.

Statistical method

According to Simon's minimax design, the expected response rate was 15% (JGOG), 20% (WJGOG), $\alpha = 0.05$, and $\beta = 0.20$. The estimated number of patients was 55 (JGOG) and 40 (WJGOG). Interim analysis was scheduled when more than half of the patients were evaluable. If the response rate was below the threshold (10%), the trial would be stopped. The Kaplan–Meier method was used in the analysis of the PFS and OS.

Results

Patients' characteristics and treatment summary

Between December 2001 and November 2003 (JGOG3015) and between December 2001 March 2005 (WJGOG002), 45 patients were registered for this study from 27 Japanese institutions. Among these patients, the background characteristics of 41 patients who were eligible for enrollment are shown in Table 1. The median age was 53.6 years (range 23–72). There were 33 patients with FIGO stage III and IV disease, two patients with mucinous histology, and five with clear cell histology. All patients had received paclitaxel and/or platinum treatment as a front-line chemotherapy, and responses had been assessed as refractory in 14 patients (34.1%) and resistant in 27 (65.9%). Sixteen patients had received more than two chemotherapy regimens. Toxicity evaluation was possible in all patients, and clinical response was evaluable in 32 patients. Overall, 159 courses of treatment were delivered to 41 patients, with 21 patients (51.2%) receiving more than four treatment cycles. Two patients stopped treatment after only one cycle because of disease progression. No patients discontinued the treatment because of toxicity.

Table 1 Patients' characteristics

Characteristics	WJGOG002	JGOG3015	Total
Mean age, years (range)	53 (23–72)	54.5 (36–72)	53.6 (23–72)
Number of patients (*n*)			
Eligible (toxicity)	22	19	41
Eligible (response)	17	16	33
FIGO stage (*n*)			
IA	2		2
IC	1	3	4
IIA	1		1
IIC	1		1
IIIA		1	1
IIIB	3	2	5
IIIC	8	11	19
IV	6	2	8
Histological type (*n*)			
Serous	16	12	28
Mucinous	0	2	2
Clear cell	2	3	5
Endometrioid	3	2	5
Undifferentiated	1		1
Eastern Cooperative Oncology Group (ECOG) performance status (*n*)			
0	12	13	25
1	7	6	13
2	3	0	3
Prior treatment (*n*)			
One regimen	15	16	31
Two regimens	7	3	10
Refractory or resistant (*n*)			
Refractory	11	5	16
Resistant	11	14	25
Number of cycles			
Mean	4.1	3.6	3.9
Four cycles completion rate (%)	9/22 (40.9)	12/19 (63.1)	21/41 (51.2)

JGOG Japan Gynecologic Oncology Group, *WJGOG* West Japan Gynecologic Oncology Group

Toxicity profiles

Toxicity data were available for all patients. The number and type of hematologic toxicity events are shown in

Table 2 Incidence of different grades of hematologic/non-hematologic toxicity events associated with the treatment

Hematologic/non-hematologic toxicity events	Toxicity profile			
	Grade 1 (%)	Grade 2 (%)	Grade 3 (%)	Grade 4 (%)
Hematologic toxicity				
Leukopenia	3 (7.3)	6 (14.6)	20 (48.8)	9 (22.0)
Neutropenia	1 (2.4)	1 (2.4)	12 (29.3)	23 (56.1)
Thrombocytopenia	4 (9.8)	0	1 (2.4)	0
Anemia	5 (12.2)	19 (46.3)	11 (26.8)	2 (4.9)
Liver dysfunction	6 (14.6)	4 (9.8)	0	0
Non-hematologic toxicity				
Nausea	20 (48.8)	11 (26.8)	1 (2.4)	0
Vomiting	10 (24.3)	5 (12.2)	3 (7.3)	0
Diarrhea	5 (12.2)	11 (26.8)	8 (19.5)	2 (4.9)
Constipation	6 (14.6)	1 (2.4)	1 (2.4)	0
Alopecia	14 (34.1)	11 (26.8)	N/A	N/A
Neutropenic fever	N/A	N/A	2 (4.9)	0
Edema	5 (12.2)	4.9	0	0
Neurotoxicity (sensory)	11 (26.8)	0	0	0

Data are presented as the number of patients, with the percentage of total study cohort ($n = 41$ patients) given in parenthesis

Table 2. The incidence of grade 4 leukopenia and neutropenia was 22.0 and 56.1%, respectively, among the patients. Grade 3 anemia was observed in 26.8% of patients, but grade 4 anemia was found in only 4.9% of the patients. Thrombocytopenia was rarely seen, and only one patient had grade 3 toxicity. Many patients required G-CSF support during the course of treatment and two patients developed neutropenic fever.

Table 2 also shows the incidence of non-hematologic toxicity events. The most frequent subjective adverse event was diarrhea, with 19.5 and 4.9% of patients experiencing grade 3 and grade 4 diarrhea, respectively. Nausea and vomiting were generally mild but did not occur in patients at grade 3 or 4 toxicity. Neurotoxicity was also mild. Only grade 1 sensory neuropathy was observed (11 patients; 26.8%). Grade 2 alopecia was seen in 11 patients (26.8%). Other non-hematologic toxicities, such as skin or mucosal toxicity, were not observed, with the exception of one grade 2 stomatitis. Dose reduction occurred in 46.3% (19/41) of patients, including 14 patients who required reduction of the docetaxel dose due to grade 4 neutropenia and 16 patients who required reduction of the irinotecan dose reduction due to grade 2 or 3 diarrhea. The dose intensity of docetaxel was 19.6 ± 3.6 mg/m^2/week in the JGOG patient group and 19.8 ± 3.5 mg/m^2/week in the WJGOG group, while that of irinotecan was 35.9 ± 5.5 mg/m^2/week (JGOG) and 39.0 ± 7.95 mg/m^2/week (WJGOG).

Response and survival

Among 41 patients, eight patients were not evaluable for response because they failed to complete more than two cycles of treatment or had no radiologically measurable lesions. Thus, 33 patients were assessed for clinical response (23 resistant, 10 refractory) (Table 3). Two patients showed a clinical response [1 complete response/remission (CR) and 1 partial response/remission (PR)], and another ten patients had stable disease (SD). The remaining patients showed progressive disease (PD). The overall objective response rate (CR + PR) was 6.1%, and the disease control rate [complete response/remission (CR) + PR + SD] was 36.4%. According to the stopping rule, this study was forced to discontinue at this stage.

Table 3 Tumor response and CA-125 response rate to treatment

	Number of patients assessed ($n = 33$)	Refractory ($n = 10$)	Resistant ($n = 23$)
Clinical response			
CR	1	0	1
PR	1	0	1
SD	10	1	9
PD	21	9	12
Response rate (%)	2/33 (6.1)	0/10 (0)	2/23 (8.7)
Disease CTL rate (%)	12/33 (36.4)	1/10 (10)	11/23 (47.8)
CA-125 response			
75% response	3	0	3
50% response	3	0	3

CR Complete response, *PR* partial response, *SD* stable disease, *PD* progressive disease, *CTL* control

Data are presented as the number of patients, with the percentage of each group given in parenthesis

The median PFS was 12.1 weeks (range 19–720 days) and the median OS was 45.3 weeks (range 90–1032 days)

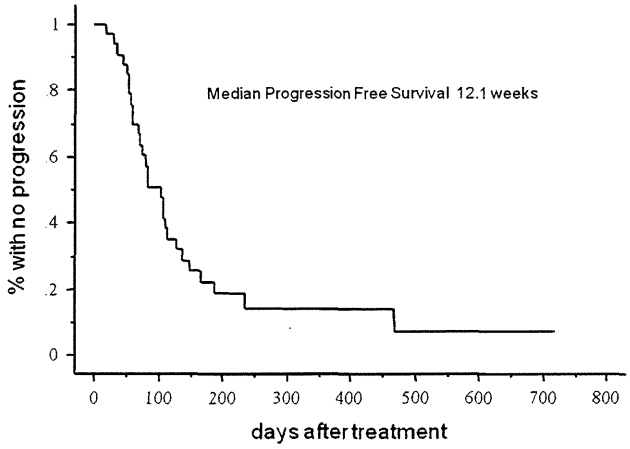

Fig. 1 Progression-free survival with docetaxel/irinotecan treatment

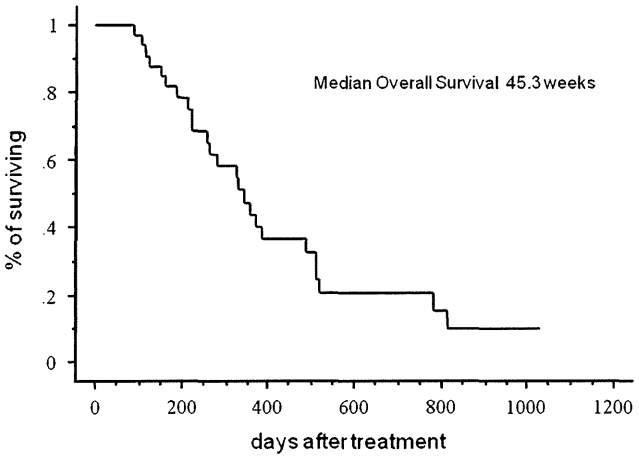

Fig. 2 Overall survival with docetaxel/irinotecan treatment

(Figs. 1, 2). CA-125 response data were available for 28 patients, of whom six were responders (21.4%), including three patients with a 75% decrease in CA-125 level and three patients with a 50% decrease. Among the 23 patients with platinum/paclitaxel-resistant tumor, the disease control rate was 47.8% (CR, PR, SD: 1, 1 and 9 patients, respectively). On the other hand, among the ten patients with platinum/paclitaxel-refractory tumor, the disease control rate was 10 % (SD, 1 patient). All CA-125 responders were patients with platinum/paclitaxel-resistant tumor (6/22). There was a significant difference in the disease control rate between resistant and refractory cases ($P < 0.05$).

Discussion

Recurrence is a leading cause of death in patients with ovarian cancer, with those patients with platinum-resistant or -refractory disease having less chance to obtain remission of disease. Therefore, the primary aim of any treatment for these patients is to control the disease with minimal toxicity. Previous studies have reported that the administration of docetaxel alone for platinum-refractory ovarian cancer achieved approximately a 35% response rate and 10 months of OS [8, 9]. However, the 100 mg/m^2 dose of docetaxel used in these studies gave rise to significant bone marrow toxicity, with grade 4 neutropenia occurring in up to 83–87% of patients and neutropenic fever occurring in 44%. In addition, 69% of the patients developed fluid retention, of whom 67% required diuretics and/or corticosteroids. The combination of a smaller dose of docetaxel (60 mg/m^2) in combination with irinotecan used in this study appeared to reduce the toxicity of docetaxel. In another study, weekly paclitaxel was expected to have anti-angiogenetic activity on platinum/taxane refractory ovarian cancer at the expense of severe neurotoxocity [12]. However, docetaxel did not show significant neurotoxicity in that study.

In our study, patients with platinum/paclitaxel-refractory or -resistant disease responded differently. Vershragen et al. [13] reported that docetaxel achieved only a 11% response rate in patients with absolute paclitaxel-refractory tumor, while a 45% response rate was observed among those with paclitaxel-resistant tumors. Therefore, in terms of treatment response, these patients should be analyzed separately. In our study, we found only a 10% disease control rate and no CA-125 responders among patients with platinum/paclitaxel-refractory tumor, as was expected. On the other hand, patients with platinum/paclitaxel-resistant tumor showed a 47.8% disease control rate and 27.3% CA-125 response.

In terms of the doectaxel/irinotecan combination therapy for platinum-resistant ovarian cancer, Polyzos et al. [14] reported that a combination with docetaxel 60 mg/m^2 and a single dose of irinotecan 200 mg/m^2 achieved a 20% response rate and 27% SD, a median PFS of 5 months, and 11 months of OS. Despite the prophylactic administration of G-CSF from days 2 to 6, 16% of patients on their regimen showed febrile neutropenia and one patient died of sepsis. The incidence of diarrhea was relatively low (13%), but two patients had grade 3 or 4 diarrhea. In comparison, in our study protocol, irinotecan 60 mg/m^2 was given on day 1 and day 8. Among our patients, 56% showed grade 4 neutropenia and 4.9% developed grade 4 diarrhea. The dose reduction of irinotecan was caused by bone marrow toxicity (neutropenia) rather than by gastrointestinal toxicity (diarrhea). Weekly administration of both drugs was studied by an Austrian study group, but the patients failed to show a good response or reduced toxicity [15].

Long-term disease control with less toxicity would be the most important aim when treating patients with platinum/taxane-resistant or -refractory EOC because the

disease at this stage is not curable. When this trial was planned, a number of new agents, such as topotecan, pegylated liposomal doxorubicin (PLD), and gemcitabine were not available for treating ovarian cancer in Japan. Since 2000, a number of phase II or phase III studies for platinum-resistant EOC using these agents have been published [4, 6, 16, 17], and Japanese health insurance currently covers the cost of these agents for recurrent EOC. Topotecan, PLD, and gemcitabine achieved a 44–66% disease control rate but a relatively short PFS (9–14 weeks). The PFS and disease control rates in these phase 2 studies of new agents were similar to those observed in our study for the patients with the platinum/paclitaxel-resistant disease, but they were significantly better than the response obtained in patients with platinum/paclitaxel-refractory disease. An agent which has different toxicity profiles from those used in first-line chemotherapy, such as PLD, might be a good candidate for second-line chemotherapy for platinum/paclitaxel-resistant tumors.

In conclusion, docetaxel and irinotecan combination chemotherapy was well tolerated but failed to show the expected tumor response in patients with platinum/patclitaxel- refractory and -resistant EOC. Therefore, we conclude that it was not the suitable treatment choice for the chosen population and that further study, including phase III trials, is not warranted.

Conflict of interest K. Tamura received honoraria from Kyowa Hakko Kirin, Astellas Pharma, Yakult Honsya, Ono Pharmaceutical, Takeda Pharmaceutical, Asahi Kasei Pharma, as well as research funding from Shionogi, Taisho Toyama Pharmaceutical, Kyowa Hakko Kirin, and Astellas Pharma. The other authors declare that they have no conflict of interest.

Appendix

The following institutions participated in this study:

JGOG3015: Gifu Prefectural Tajimi Hospital, Mie Prefectural General Medical Center, West Shizuoka Hamamatsu Medical Center, Niigata Prefectural Saiseikai Sanjyo Hospital, Kawasaki Municipal Kawasaki Hospital, Nagasaki Municipal Hospital, Jichi Medical University Omiya Medical Center, Keio University Hospital, Tottori University Hospital, Osaka Medical University Hospital, Jikei University Third hospital, Dokkyo Medical University Hospital, Niigata Prefectural Cancer Center Hospital, Yamada Red-Cross Hospital, Nantan General Hospital.

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High-throughput detection of aberrant imprint methylation in the ovarian cancer by the bisulphite PCR-Luminex method

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Abstract

Background: Aberrant DNA methylation leads to loss of heterozygosity (LOH) or loss of imprinting (LOI) as the first hit during human carcinogenesis. Recently we developed a new high-throughput, high-resolution DNA methylation analysis method, bisulphite PCR-Luminex (BPL), using sperm DNA and demonstrated the effectiveness of this novel approach in rapidly identifying methylation errors.

Results: In the current study, we applied the BPL method to the analysis of DNA methylation for identification of prognostic panels of DNA methylation cancer biomarkers of imprinted genes. We found that the BPL method precisely quantified the methylation status of specific DNA regions in somatic cells. We found a higher frequency of LOI than LOH. LOI at *IGF2*, *PEG1* and *H19* were frequent alterations, with a tendency to show a more hypermethylated state. We detected changes in DNA methylation as an early event in ovarian cancer. The degree of LOI (LOH) was associated with altered DNA methylation at *IGF2/H19* and *PEG1*.

Conclusions: The relative ease of BPL method provides a practical method for use within a clinical setting. We suggest that DNA methylation of *H19* and *PEG1* differentially methylated regions (DMRs) may provide novel biomarkers useful for screening, diagnosis and, potentially, for improving the clinical management of women with human ovarian cancer.

Keywords: Genomic imprinting, Ovarian cancer, DNA methylation, Bisulphite PCR-Luminex(BPL)method, LOI (loss of imprinting)

Background

Human ovarian cancer (HOC) is the leading cause of death from gynecological malignancies, primarily due to the lateness of detection when the cancer is already at an advanced stage. Effective screening protocols for early stages are not currently available. HOC is characterized by complex genetic and epigenetic alterations, including loss of heterozygosity (LOH) and loss of imprinting (LOI) [1,2]. Such alterations are presumed to represent the second hit, according to Knudson's two-hit hypothesis (OMIM #167000) [3]. However, alterations in DNA methylation can also occur as the first hit during human carcinogenesis [4].

For childhood cancers such as retinoblastoma (OMIM #180200), Wilms' tumor (OMIM #194070) and osteosarcoma (OMIM #259500), changes primarily occur on the paternal allele first, followed by a second hit on the maternal allele [5,6]. Complete hydatidiform moles, which are of androgenetic or paternal origin, are characterized by malignant transformation whereas ovarian teratomas, which are of parthenogenetic or maternal origin, are benign [7,8]. These observations suggest a role for altered genomic imprinting in the malignant transformation process.

Alterations in the expression of imprinted genes represent one of the most common changes seen in cancer [9,10]. Some imprinted genes, including *H19*

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[11], *GTL2* [12], *PEG1, PEG3* [13], *LIT1* (*KCNQ1OT1*) [14] and *ZAC* [15], are known to act, or strongly implicated to act, as tumor suppressor genes (TSGs). The monoallelic expression of imprinted genes is reliant on epigenetic mechanisms, most notably DNA methylation, which initiates the imprinting process in the male and female germlines at discrete locations termed differentially methylated regions (DMRs) [16]. Imprinted domains generally contain several genes displaying allele-specific expression and these DMRs, which can be located over the promoter of a protein coding gene or the promoter of a functional non-coding RNA or within intergenic regions, are known to control imprinted gene expression within the domain, acting as imprinting centers or imprint control regions [17]. We recently developed a new high-throughput, high-resolution DNA methylation analysis method called bisulphite PCR-Luminex (BPL) for the rapid analysis of DNA methylation [18]. In this study, we applied this method to 21 HOC cell lines and 74 HOC tissues to efficiently and accurately determine the methylation status of DMRs at eight imprinted loci, six of which contained TSGs. To determine whether abnormal methylation of these DMRs acts as an indicator for potential LOH and/or LOI, we also examined the association between abnormal hypermethylation and LOI or LOH. We found a higher frequency of LOI than LOH. LOI at *IGF2, PEG1* and *H19* was a frequent alteration, with a tendency to show a more hypermethylated status. The degrees of LOI and altered DNA methylation were similar among histology, progression and tumor grades. This suggests that DNA methylation of the *H19* and *PEG1* DMRs may provide novel biomarkers useful for screening, diagnosis and, potentially, for improving the clinical management of women with HOC.

Results

Frequencies of the 8 imprinted gene profiles in HOC

We first determined whether the ovarian malignancies showed LOH by comparing the restriction fragment length polymorphism (RFLP) patterns of normal lymphocyte DNA and 74 matching primary HOC DNA samples. Samples where RFLPs were present in the lymphocyte DNA sample but absent or with an altered ratio in the tumor sample were considered to exhibit LOH in the regions of 8 imprinted genes (*H19, IGF2, KCNQ1, LIT1, GTL2, PEG1, PEG3* and *NDN*). The average percentage of heterozygosity was 48.0% (16.2-58.5%). We found only 14 cases of LOH in the 8 imprinted genes in the 74 HOC samples we analysed (Table 1). The most frequent gene with LOH was *IGF2* (9.0%, 3/33), followed by *PEG1* (8.1%, 3/37) and *GTL2* (7.1%, 3/42). LOH of *NDN* and *LOT1* was not detected (0/31 and 0/12). The samples with LOH were not from the same cases (Additional file 1: Table 1).

We next performed RT-PCR and RFLP analysis to identify the samples of LOI without LOH. The frequency of LOI was higher than that of LOH for all 8 imprinted genes and we found a total of 46 cases of LOI (Table 1, Additional file 1: Table S1). The most frequent sites of LOI were *PEG1* (45.9%, 17/37), *IGF2* (45.4%, 15/33) and *H19* (29.2%, 12/41). *NDN* had the lowest frequency. In 19 of the 46 cases, the abnormal gene expression pattern was apparent at two or more imprinted loci. A normal imprinting pattern, maintenance of imprint (MOI), was most frequent in *NDN* (93.5%, 29/31). ND (not determined) means no amplification of RT-PCR at 3 times in several samples, perhaps indicating low expression of the genes. In 9 of the 14 LOH cases, LOI was also found in at least one gene. In HOC cell lines, LOI was found in 2 of 3 informative cases for *IGF2*, and 3 of 9 cases for *PEG1*. We did not find any LOH or LOI in 7 normal ovarian surface tissues and 4 normal cell lines. We compared patients' ages, progression, histology and tumor grades with imprinted gene expression pattern profiles. Patients with LOI had a tendency to be younger than patients with LOH (mean ages for LOH and LOI: 55.0 ± 7.4 and 47.7 ± 6.9, respectively), but the difference was not statistically significant by ANOVA, and no other correlations were apparent.

Analysis of the methylation status of DMRs in ovarian cancers by the BPL method

The proof-of principle experiment of the BPL method has been described in detail [18]. Briefly, bisulphite-DNA can be used to distinguish between methylation and non-methylation status in the genome, e.g. cytosine and uracil. The BPL method can determine one base substitution by specific hybridization and detect the ratio of methylation to non-methylation. We examined the quality of the BPL method in spermatic DNA, which should show 100% methylation of the paternally methylated DMRs: *ZDBF2, H19* and *GTL2*, whereas the maternally methylated DMRs: *PEG1, ZAC, SNRPN, PEG3* and *LIT1* are non-methylated. We applied the classic methylation assay COBRA technique and our recently devised BPL method to the DNA of 7 normal ovarian surface epithelium tissues, 4 primary cultures of normal human ovarian surface epithelium (OSE1-4) and 21 HOC cell lines, and performed statistical analysis with Spearman's and Pearson's rank correlations. For all 8 DMRs a good correlation was found between these two methods (Figure 1, Table 2, Additional file 2: Figure S1).

We next determined the methylation status of the 8 DMRs from the 74 samples of primary ovarian cancer tissue by the BPL method. Overall, we compared the average DNA methylation status of cancer and normal samples for each DMR and found that *PEG1* from ovarian cancers was