2 対　象

対象は開腹手術を行い，進行期II期～IV期と診断された上皮性卵巣癌，原発性腹膜癌，卵管癌患者で，十分な臓器機能を有した患者である。研究者のバイアスを排除する目的で，初回手術終了直前に術中ランダム化を行い，carboplatin の IV 群と IP 群に割り付ける。プライマリーエンドポイントは progression-free survival (PFS)，セカンダリーエンドポイントは overall survival (OS)，奏効率，QOL および医療経済評価とした。安全性評価は，NCI-CTC AE Ver 4.0 を用いて血液毒性および非血液毒性を評価することとした。

一方，paclitaxel 3 週ごと投与と carboplatin IP 併用療法の効果安全性に関する第II相試験の結果は存在しているが[2]，paclitaxel 毎週投与と carboplatin IP 併用療法の安全性情報が不足していたため，第II相で安全性評価を総合的に行うこととし，本試験はランダム化II/III相試験とした。目標症例数はII相部分を 120 例，II/III相部分を総合して 746 例とした。

本試験で用いる carboplatin の腹腔内投与と paclitaxel の毎週投与はいずれも保険償還されていないため，高度医療評価制度による混合診療を用いざるをえないと判断し，申請手続きを行った。

II 結　　果

高度医療評価制度に基づく臨床試験の申請用件を満たすための諸手続きは，コーディネーティングセンターである北里大学臨床薬理研究所臨床試験コーディネーティング部門，ならびに申請医療機関である埼玉医科大学国際医療センターを中心に行った。

1 高度医療評価制度申請から承認まで

2009 年 8 月，厚生労働省医政局研究開発振興課において事前相談を行った。その際最も重要な要望事項は，paclitaxel の毎週投与を通常の保険診療として認めてもらいたい，ということであった。卵巣癌における paclitaxel 毎週投与は，従来の 3 週ごと投与と比較して PFS，OS ともに有意に改善することが JGOG3016 試験で示されていること[3]，また乳癌では paclitaxel 毎週投与が保険承認されていること，学会や患者団体から paclitaxel の毎週投与に関する「未承認・適応外薬に係る要望」が提出されて「医療上の必要性の高い未承認薬・適応外薬検討会議」において，いわゆる公告申請に関する検討がなされていることの 3 点が，その理由であった。しかし，厚生労働省の見解は保険適応「不可」であった。したがって，paclitaxel 毎週 IV 投与と carboplatin IP の両方を高度医療の対象として申請することになった。

2009 年 9 月，埼玉医科大学国際医療センター倫理審査委員会（IRB）において，本試験の医学的・倫理的妥当性の検討および高度医療評価制度下での本試験遂行の可否について審議し，承認された。本試験は，後述するように試験薬剤の無償提供が必須であったため，その見通しがたった段階（2009 年 12 月）に，高度医療の正式申請を行った。

本試験は 2010 年 1 月 29 日に開催された高度医療評価会議において承認され，引き続き 4 月 16 日に開催された先進医療専門家会議において承認された。

2 薬剤無償提供の交渉と契約

本試験において，paclitaxel の毎週投与を自費診療で行った場合は 1 サイクルあたりの薬剤費は約 10 万円であり，6 サイクル行った場合には 60 万円となる。この費用は 746 例全症例分が必要となるため，paclitaxel の薬剤購入費のみで 4 億を超える。これを研究費として捻出することは不可能であると判断し，試験薬剤無償提供の要請をした結果，日本化薬(株)，沢井製薬(株)の 2 社との合意が成立した。一方，carboplatin の 1 サイクルあたりの薬剤費は約 5 万円であり，ランダム化により総登録症例数の半数が試験治療である IP 投与を受けると仮定して，おおよそ 1 億 2 千万円となる。IP 投与による carboplatin も保険償還されていないため，ブリストル・マイヤーズ(株)，サンド(株)の 2 社と無償提供について交渉し，協力を得ることができたため，試験実施が可能となった。各社の内部手続き後，医療用医薬品製造販売業公正取引協議会に諮られて承認を得た後に，研究責任者と試験薬剤提供企業の覚書を締結した。

3 薬剤保管配送業務の契約

無償提供された薬剤の取り扱いは，試験薬として，一般診療で用いる薬剤との区別を明確にする必要がある。試験実施医療機関での試験薬取り扱いは，開発治験薬に準じた厳正な管理が必要となる。加えて，

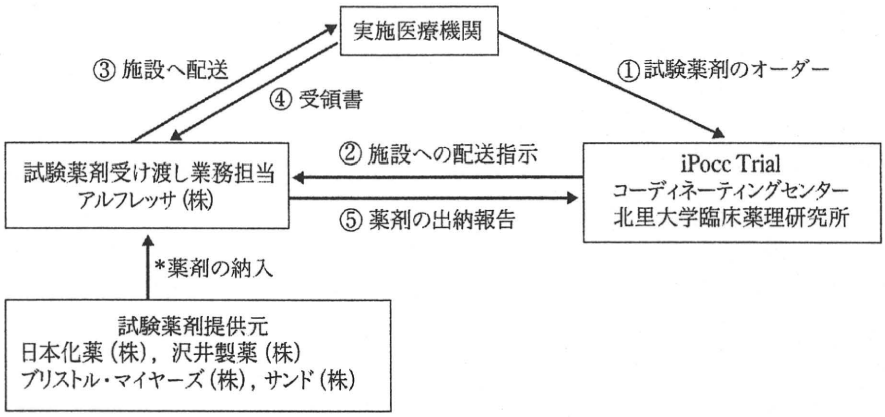

図 2　iPocc 試験における試験薬の流れ

表 1　高度医療評価制度の対象となる医療技術

(1) 薬事法（昭和 35 年法律第 145 号）第 14 条第 1 項に規定する承認又は同法第 19 条の 2 第 1 項に規定する認証（以下「承認又は認証」という。）を受けていない医薬品又は医療機器の使用を伴う医療技術
(2) 薬事法上の承認又は認証を受けて製造販売されている医薬品又は医療機器を，承認又は認証された事項に含まれない用量，用法，適応等による同一の又はほかの効能，効果等を目的とした使用を伴う医療技術

無償提供される 2 薬剤は毒薬であるため，薬事法上の毒薬に準じた管理も必要となる。また，試験薬の保管・運搬に関しても薬事法上定められた要件を遵守しなければならない。また，多施設共同試験であるため，約 60 施設への配送回数とコストについても考慮しなければならない。そのため本試験では，薬剤保管配送業者を慎重に選定し，アルフレッサ(株)と平成 22 年 2 月契約を締結した。本試験における試験薬の発注・受注ならびに薬剤管理業務は，試験実施医療機関，iPocc Trial コーディネーティングセンター（北里大学臨床薬理研究所），保管配送業者（アルフレッサ）の 3 者が協力して行うこととして，それぞれが標準業務手順書を作成した。試験薬の流れを**図 2** に示す。

III　考　　察

わが国において，本試験のように保険診療が認められていない薬剤や投与経路を用いて研究者主導臨床試験を合法的に行う方法は，治験を除いては高度医療評価制度を用いるしかない。

高度医療評価制度創設の目的は，「薬事法の承認等が得られていない医薬品・医療機器の使用を伴う先進的な医療技術については，一般的な治療法ではないなどの理由から原則として保険との併用が認められていないが，医学医療の高度化やこれらの医療技術を安全かつ低い負担で受けたいという患者のニーズ等に対応するため，今般，これらの医療技術のうち，一定の要件の下に行われるものについて，先進医療の一類型として保険診療との併用を認め，薬事法による申請等に繋がる科学的評価可能なデータ収集の迅速化を図る」とされている[4]。

その対象となる医療技術は，2 つに分類されている（**表 1**）が，本試験の対象となる医療技術は (2) に相当する。今回われわれが遂行する臨床試験は，卵巣癌に対する carboplatin IP 療法の有用性を検討するという，製薬メーカーにとっては新たな「治験」を行うメリットのない研究課題に取り組んだものである。さらに，JGOG3016 試験において paclitaxel の毎週投与が，通常の 3 週ごと投与と比較して有効であることが示されているが[3]，卵巣癌に対しては保険償還されていないため，混合診療として実施せざるをえない状況であったことを鑑みると，高度医療の承認が得られた意義は大きい。

しかしながら，本試験のように試験薬剤費が高額になると，企業からの無償提供はきわめて困難になり，研究費でまかなうこともできず，通常は全額患者負担とせざるをえない。この制度の運用は，第 II 相試験（単群試験または小規模比較試験デザイン）では成り立つが，大規模比較試験ではきわめて難しいと言える。すなわち，第 III 相比較試験における試験治療群に対する薬剤・技術費用を自費で徴収し，

表 2　高度医療に係る要件

次の要件をすべて満たす医療技術であること。

(1) 国内外の使用実績や有用性を示す文献等により，安全性及び有効性の確保が期待できる科学的な根拠を有する医療技術であること。

(2) 高度医療の試験計画が次の項目をすべて網羅する内容であること。

1 臨床研究に関する倫理指針に適合していること。

2 万が一不幸な転帰となった場合の責任と補償の内容，治療の内容，合併症や副作用の可能性及び費用等について，事前に患者やその家族に説明し文書により同意を得ること。

3 当該医療機関に所属する医師のうち，当該高度医療の実施に関し責任を有する医師を明示し，当該医師の下に，当該高度医療を実施する医師を管理していること。

4 安全性及び有効性が客観的に確認できることが期待でき，院内の倫理審査委員会等において認められた試験計画（試験期間，症例数，評価基準等に関する記載を含む）であること。

5 試験記録の保管や管理が適切に行われ，データの信頼性が一定程度確保されていること。

6 多施設共同研究の場合は，当該研究に協力する施設（以下「研究協力施設」という）との調整等を行う医療機関，研究協力施設及び各施設の実施責任医師が明示されていること。

(3) なお，臨床データの信頼性確保においては，次の体制の確保に努められたい。

1 データマネージメント体制が確保されていること。

2 多施設共同研究の場合は，試験実施を調整する医療機関及び多施設共同研究としての実施可能なモニタリング体制等が確保されていること。

いわゆる混合診療を認めるのみでは，標準治療群に割り当てられた患者負担と試験治療群に割り当てられた患者負担との間の差があまりにも大きくなるので，比較試験は成り立たないと考えられる。

この点を改善するためには，第II相試験などですでに有効性・安全性が証明されている医療技術で，大規模比較試験を行って標準治療に優ることを証明することが必要な場合，高度医療評価制度の審査・承認を経た臨床試験に対しては，高度医療に係る部分か否かにかかわらず，全面的に保険との併用を認める制度が望まれる。

また，試験薬剤が無償提供される場合であっても，高度医療評価制度の対象となる試験の準備は容易ではない。無償提供薬剤は通常の保険診療で用いる薬剤とは，研究組織および試験実施医療機関においてまったく別の管理を行わなくてはならない。この体制は新薬開発治験ならびに毒薬の薬剤管理に準じて行われるが，治験と異なり，その管理上の必要経費の請求先は患者となる。たとえば，高度医療（すなわち試験治療である paclitaxel 毎週 IV 投与ならびに carboplatin IP 投与）に直接関係する輸液セット等の代金や薬剤管理費は保険適応とならないため，患者に別途自費請求せざるをえない。医療機関における薬剤管理費は，薬剤師の時給換算等から医療機関ごとに設定して，患者に自費請求することができる。病院医務課では，この臨床試験に参加している患者にかぎり，保険診療分と患者自費診療分ならびに無償提供薬剤費分を区別して会計処理を行うように，会計システムの変更が必要となり，この作業は煩雑である。しかし，これらの手数料を試験に参加する全医療機関に配分できるほどの十分な研究費があるわけではない。したがって，試験薬の無償提供が実現したとしても，試験実施医療機関にとっては負担を伴う制度であるといわざるをえず，この観点からも，高度医療に係る部分か否かにかかわらず，試験治療を含めた全面的な保険適応が強く望まれるのである。この考え方は，わが国の健康保険法の根幹概念に関わる問題であろうが，議論を深める価値は十分にあると思われる。

表2に示したように，高度医療評価制度を用いて臨床試験を行う場合には，モニタリング体制の確保など，通常の研究者主導臨床試験以上のデータの品質管理体制の整備が明確に要求されている。大規模第III相がん臨床試験においてこの基準を満たすためには，独立したデータセンターは必須であり，その必要資金の確保が試験開始の大前提となる。幸い，本試験は厚生労働省科学研究に採択され，研究資金の確保が可能となった。しかし，厚生労働科学研究費交付決定を受けて，試験実施計画書を完成させて高度医療評価制度への申請を行ったところ，本試験が実際に開始できるまでの準備にほぼ1年を要した。このうち高度医療の初回事前相談から試験開始までの期間はおおよそ8ヵ月であった。厚生労働省の担当官の尽力をもってしても時間を要した理由としては，薬剤無償提供交渉ならびに薬剤保管・配送体制の整備に半年以上かかったこと，高度医療評価会議承認から次の先進医療専門家会議開催までに約3ヵ月を要したことなどがあげられる。試験実施

が遅れることは，厚生労働科学研究評価委員サイドからは重大な批判の対象となり，次年度の研究費にも影響する可能性がある。研究資金が確保できなければ，大規模比較試験を高度医療評価制度のもとに行うことは不可能であることを考えると，より迅速な審査体制の調整を望みたいところである。

高度医療評価制度で最も重要な点は，政府が研究者主導臨床試験を公式に審査・評価するわが国初のシステムであることである。わが国から発信されるエビデンスの国際的な信頼度をより高めるために，このような公的な審査・評価システムは非常に重要である。前述したような障害を取り除くことにより，わが国での臨床試験がより活性化することが期待される。

本試験では，4社からの試験薬剤無償提供が可能となったが，このうち3社の薬剤は後発品である。これは，諸外国では例をみない事例であり，わが国における後発品製薬メーカーの社会貢献という見地からきわめて意義深く，その貢献は大いに評価されるべきであると考える。

結　論

厚生労働省の新しいシステム，高度医療評価制度を用いた大規模第Ⅲ相がん臨床試験の申請を行い，承認を得て試験実施が可能となった。本制度は，これまでのわが国の臨床試験実施体制に一石を投じる重要なものと位置づけられる。しかし，一方では実践上いくつか改善が望まれる点もあり，そのひとつが高度医療として実施する際の保険診療の範囲にあることを述べた。引き続き，研究者の意見をふまえた本制度の運用見直しが望まれる。

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The Optimal Debulking after Neoadjuvant Chemotherapy in Ovarian Cancer: Proposal Based on Interval Look During Upfront Surgery Setting Treatment

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Objective: The optimal goal of interval debulking surgery (IDS) following neoadjuvant chemotherapy (NAC) remains undefined. The aim of this study was to determine the optimal goal of IDS following NAC on the basis of long-term survival by the disease status at the end of interval look surgery (ILS) or IDS during the treatment in the setting of upfront primary debulking surgery (PDS).

Methods: From January 1986 through December 2000, we performed treatment in the setting of upfront PDS in 128 patients with Stage III/IV epithelial ovarian cancer. Sixty-six patients with residual disease (RD) at PDS underwent interval surgery (IS) such as ILS or IDS; 4 patients after two cycles of chemotherapy and 62 after three or more cycles. We investigated how disease status at the end of IS was associated with overall survival (OS).

Results: The 5-year OS rates for no, minimal and gross RD were not available ($n = 0$), 67% ($n = 3$) and 0% ($n = 1$) after two cycles, and 47% ($n = 42$), 0% ($n = 18$) and 0% ($n = 2$) after three or more cycles, respectively. No visible tumors at the end of IS after three or more cycles of chemotherapy were necessary for 5-year survival.

Conclusions: If the optimal goal of IDS is defined as the surgery that is expected to result in long-term survival in the NAC setting treatment, our data on the assessment of peritoneal findings during the upfront PDS setting treatment suggest that only complete resection with no RD could be the optimal goal of IDS in the NAC setting treatment.

Key words: ovarian cancer – neoadjuvant therapy – gynecol-surg – chemo-gynecology

INTRODUCTION

Primary debulking surgery (PDS) followed by chemotherapy is a standard treatment for ovarian cancer. For patients with advanced ovarian cancer, the goal of PDS is optimal cytoreduction, usually defined as surgery with residual disease (RD) <1 or <2 cm in diameter. Proportion of patients who achieved optimal surgery or size of RD is one of the important prognostic factors for the patients with advanced ovarian cancer (1–4). Unfortunately, optimal cytoreduction for advanced ovarian cancer is achieved in only 30–60% of the patients at most institutions (5,6). One reason for this low rate is that patients with advanced ovarian cancer are often poor candidates for aggressive surgery because of low performance status (PS) caused by massive ascites, pleural effusion and large abdominal tumors. Another reason is that some patients have unresectable tumors at the time of primary surgery.

Thus, because of recent advances in chemotherapy, neoadjuvant chemotherapy (NAC) followed by interval debulking surgery (IDS) and further chemotherapy has become an alternative treatment for patients with low PS and those with apparently unresectable tumors evaluated with computed

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tomography (CT) or laparoscopy. Several retrospective studies revealed comparable results by the NAC setting treatments with standard treatment (7–9), and a few prospective Phase II (10) or feasibility study (11,12) revealed promising results by NAC setting treatment. Taking into account these favorable outcomes of NAC setting treatment, several prospective clinical trials are now under way to compare this treatment with the standard treatment for advanced ovarian cancer, not only in patients with low PS or unresectable tumors (13,14). Most previous studies have emphasized that the greatest advantage of the treatment in the setting of an NAC is a higher rate of optimal cytoreduction at IDS (7,9,10). These studies used the same definition of optimal cytoreduction at IDS as that at PDS. At the time of PDS, optimal cytoreduction indicates an optimal goal of surgery that lengthens survival. However, there is limited information on the survival of patients in relation to the size of RD after IDS. Thus, the appropriate definition of 'optimal cytoreduction' at the time of IDS in the setting of NAC is undetermined.

Since 1986, we have performed interval look surgery (ILS) for patients who have minimal RD (<2 cm in diameter) at PDS or IDS for patients who have gross RD (≥2 cm in diameter) at PDS after two to six cycles (mostly three or four cycles) of chemotherapy. We investigated how peritoneal findings at the end of interval surgery (IS) are associated with the overall survival (OS) of patients. These associations should help us to clarify the optimal goal of IDS in the setting of NAC for advanced ovarian cancer.

PATIENTS AND METHODS

Patients

From January 1986 through December 2000, we treated 230 patients with epithelial ovarian cancer, including 128 patients with Stage III–IV disease, at the Department of Obstetrics and Gynecology, University of Tokyo Hospital. According to the International Federation of Gynecology and Obstetrics (FIGO) staging, disease was classified as Stage IIIB in 14 patients, Stage IIIC in 89 patients and Stage IV in 25 patients. Histologic type was serous in 94 patients, clear cell in 18 patients, endometrioid in 6 patients, mucinous in 5 patients, transitional cell in 2 patients, mixed epithelial in 2 patients and undifferentiated in 1 patient. Median age at the time of PDS was 54 years, with a range of 29–78 years. Median follow-up period after PDS, excluding patients who died, was 94 months, with a range of 8–201 months. All but two surviving patients were followed up for >5 years.

Our standard surgical treatment for advanced ovarian cancer at the time of PDS consists of total abdominal hysterectomy, bilateral salpingo-oophorectomy, infracolic or total omentectomy, and debulking of peritoneal tumor masses with maximum efforts. Patients with no or minimal RD (<2 cm in diameter) also underwent systematic retroperitoneal lymphadenectomy, except for patients with severe medical complications, low PS or long operation time. Retroperitoneal lymphadenectomy included both the pelvic and aortic lymph nodes.

In principle, our primary management for ovarian cancer was performed as follows according to the outcome of PDS: (i) patients with no RD received six cycles of chemotherapy and underwent no additional surgery, (ii) patients with minimal RD (<2 cm in diameter) received three or four cycles of chemotherapy followed by ILS and two to four cycles of additional chemotherapy, (iii) patients with gross RD (≥2 cm in diameter) received two to four cycles of chemotherapy until a favorable response was obtained and underwent IDS followed by four to five cycles of additional chemotherapy.

Cisplatin-based regimens, such as CAP or TC, were used for post-operative chemotherapy. From 1986 through 1997, we used the CAP regimen, consisting of 400–600 mg/m^2 of cyclophosphamide, 30–40 mg/m^2 of doxorubicin and 50–75 mg/m^2 of cisplatin. Thereafter, we used the TC regimen consisting of paclitaxel (175 mg/m^2 infused over 3 h) and an area under the curve 6 of carboplatin.

Statistical Methods

OS was measured from the day of starting primary treatment. The survival curves were determined with the Kaplan–Meier product-limit method. Differences in survival were analyzed with the log-rank test and Cox proportional-hazard regression model using the SPSS program ver. 11.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Survival of all Patients in Relation to the Size of RD at PDS

In 128 patients with Stage III or IV ovarian cancer, complete resection of all visible tumors was achieved in 37 patients (28.9%), minimal RD remained in 52 patients (40.6%) and gross RD remained in 39 patients (30.5%). Figure 1 shows the OS of all 128 patients with Stage III/IV disease in relation to the largest size of RD at PDS. Median OSs and 5-year OS rates of the above three groups were 112 months and 65%, 50 months and 40%, and 22 months and 13%. The difference in OS among the three groups was statistically significant ($P < 0.0001$ with log-rank test). In particular, the difference in OS between patients with minimal RD and gross RD was more significant than that between patients with no RD and minimal RD ($P < 0001$ vs. $P = 0.02$). Hazard ratio and 95% confidence interval (CI) for patients with minimal RD and gross RD against patients with no RD were 1.92 (1.08–3.42) and 5.43 (2.98–9.89), respectively.

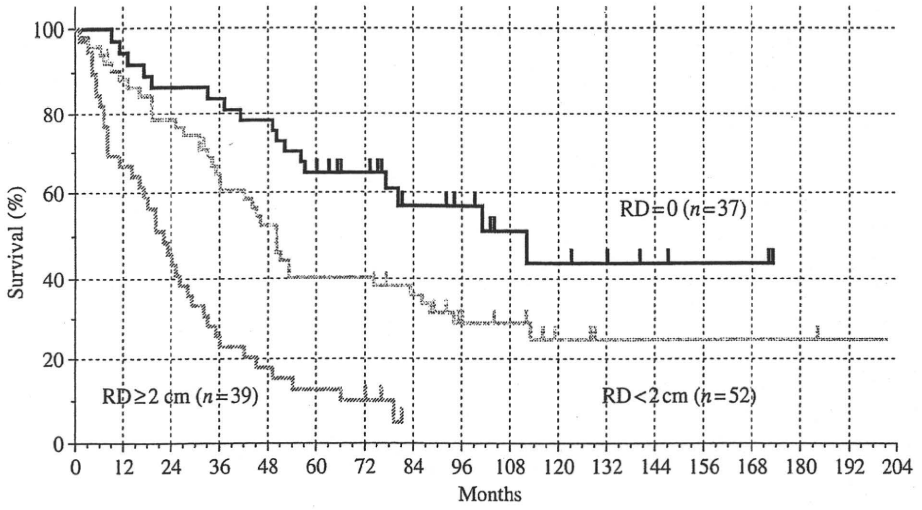

Figure 1. Overall survival of the patients with Stage III/IV ovarian cancer according to the size of largest RD at the time of PDS. RD, residual disease; PDS, primary debulking surgery.

Performance of IS

For Patients with Minimal RD at PDS

Of the 52 patients with minimal RD at PDS, 29 underwent ILS after three or four cycles of post-operative chemotherapy. Nine patients underwent ILS after five or six cycles of chemotherapy. The remaining 14 patients did not undergo ILS due to the following reasons: progressive disease in 2 patients, unfavorable response in 2 patients, entry to clinical trial in 4 patients, patient refusal in 1 patient, medical complications in 4 patients and unknown reason in 1 patient.

For Patients with Gross RD at PDS

Of 39 patients with gross RD at PDS, 28 underwent IDS after two to six cycles of post-operative chemotherapy. Four patients underwent IDS after two cycles of chemotherapy because of early partial responses, 20 patients underwent IDS after three or four cycles of chemotherapy and 4 patients underwent IDS after six cycles of chemotherapy. The remaining 11 patients did not undergo IDS because of progressive disease in 9 patients and medical complications in 2 patients.

RD at the end of IS and OS

IDS after Two Cycles of Chemotherapy

Four patients underwent IDS after two cycles of chemotherapy. Three patients had minimal RD and one patient had gross RD at the end of IDS. Median OSs and 5-year OS rates were 66 months and 67% in patients with minimal RD and 8 months and 0% in a patient with gross RD. The mean number of chemotherapy cycles after IDS was 5.3 (range, 3–6) for patients with minimal RD and 1 (range, 1–1) for a patient with gross RD. Two patients with minimal RD after IDS survived >5 years.

ILS and IDS after Three or More Cycles of Chemotherapy

Thirty-eight patients underwent ILS after three or more cycles of chemotherapy. At the end of ILS, 32 patients had no RD, 5 had minimal RD and 1 had gross RD. Median OSs and 5-year OS rates were 83 months and 55% in patients with no RD, 16 months and 0% in patients with minimal RD and 11 months and 0% in a patient with gross RD. The mean number of chemotherapy cycles after ILS was 2.8 (range, 0–5) for patients with no RD, 2.8 (range, 0–6) for patients with minimal RD and 2 (range, 2–2) for a patient with gross RD.

Twenty-four patients underwent IDS after three or more cycles of chemotherapy. At the end of IDS, 10 patients had no RD, 13 had minimal RD and 1 had gross RD. Median OSs and 5-year OS rates were 28 months and 20% in patients with no RD, 23 months and 0% in patients with minimal RD and 8 months and 0% in a patient with gross RD. The mean number of chemotherapy cycles after IDS was 3.4 (range, 0–5) for patients with no RD, 4.1 (range, 2–7) for patients with minimal RD and 1 (range, 1–1) for a patient with gross RD.

Overall, 42 patients had no RD, 18 had minimal RD and 2 had gross RD at the end of IS such as ILS and IDS after three or more cycles of chemotherapy. Median OSs and 5-year OS rates were 53 months and 47% in patients with no RD, 23 months and 0% in patients with minimal RD and 11 months and 0% in patients with gross RD. The difference in OS among the three groups was statistically significant ($P < 0.0001$ with the log-rank test, Fig. 2). The difference in OS between patients with no RD and minimal RD was much more significant than that between patients with minimal RD and gross RD ($P < 0.0001$ vs. $P = 0.04$). None of these patients with RD at the end of IS after three or more cycles of chemotherapy survived >5 years. Hazard ratio and 95% CI for patients with minimal RD and gross RD against

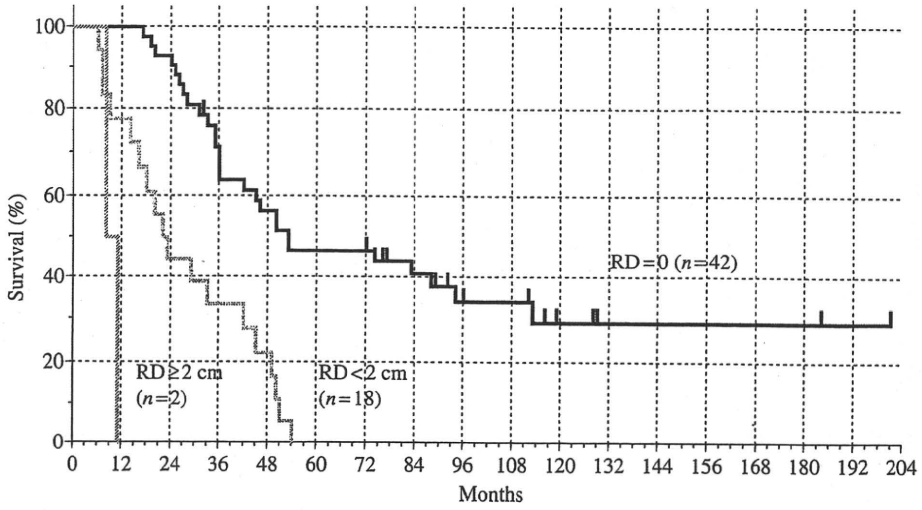

Figure 2. Overall survival of the patients who underwent IS after three or more cycles of chemotherapy according to the size of largest RD at the end of IS. IS, interval surgery.

patients with no RD were 3.99 (2.11–7.55) and 32.78 (5.67–189.55), respectively.

DISCUSSION

NAC setting treatment for advanced ovarian cancer has lately attracted much attention and randomized controlled trials are now under way comparing the outcome with the treatment in the setting of upfront PDS (13,14). However, because of the paucity of the data, optimal goal of IDS in the NAC setting treatment has not yet determined. For our management of advanced ovarian cancer, we performed ILS for patients with minimal RD to assess the peritoneal findings mainly after three to four cycles of chemotherapy separate from IDS for patients with gross RD. Although our data are not based on the treatment results of NAC setting treatment, we thought that the disease status at the time of IDS or ILS in patients who had good outcomes would be useful for determining the optimal goal of IDS following NAC from the standpoint of cell biology. Similar assessments may be possible by the data of two large Phase III studies of IDS after suboptimal PDS for advanced ovarian cancer (15,16). However, it is regrettable that these studied did not address the issue.

Patients with Stage III/IV disease in our series had relatively good outcomes: a median OS of 46 months and a 5-year OS rate of 39%. We used RD < 2 cm in diameter as the definition of optimal cytoreduction at PDS because our study is a retrospective analysis of patients treated from 1980s. Among these patients, those with no RD had good outcomes: a median OS of 112 and a 5-year OS rate of 65%, whereas patients with minimal RD also had good outcomes: a median OS of 50 months and a 5-year OS rate of 40%. However, patients with gross RD had much poorer outcomes: a median OS of 22 months and a 5-year OS rate of 13% (Fig. 1). Patients who underwent optimal debulking at PDS survived significantly longer than those who underwent suboptimal debulking at PDS (median OS of 74 vs. 22 months, 5-year OS rate of 51% vs. 13%, $P < 0.0001$ with the log-rank test). Hazard ratio of the patients with suboptimal debulking against optimal debulking was 3.65 (95% CI: 2.31–5.71). In agreement with previous reports, our present study confirmed that the optimal goal at PDS is cytoreduction with no or minimal RD.

To determine the optimal goal of IDS following NAC, OS in relation to the size of RD after surgery should be known. However, at present, we have little information on the relation between the outcome of IDS following NAC and long-term survival. A recent analysis of NAC and IDS by Le et al. (17) has found that progression-free survival was significantly improved in patients with complete resection at IDS and did not differ significantly among patients with various sizes of macroscopic RD (<1, 1–2 or >2 cm). However, Le et al. could not find significant improvement in OS of patients with complete resection, likely because of the small number of patients in each group and the short median follow-up time of 19 months. In the present study, we tried to determine the optimal goal of IDS following NAC using peritoneal findings at corresponding timing in patients undergoing treatment in the setting of upfront PDS and having fairly good outcomes. The optimal goal of IDS following NAC should be a favorable status that leads to good long-term survival. The present study suggests that no RD at the end of IS after three or more cycles of chemotherapy can lead to fairly good survival. Although the survivals are not identical following ILS or IDS, combined survival of the patients with no RD at ILS or IDS is comparable to that achieved with minimal RD at PDS in the setting of upfront PDS (median OS of 53 and 50 months and 5-year OS rate of 47% and 40%, Figs 2 and 1, respectively). The survival of the patients with no RD was much better than the patients with any RD, especially in 5-year OS rate (median OS of 53 vs. 22 months, 5-year OS rate of 47% vs. 0%, $P < 0.0001$

with the log-rank test). Hazard ratio of the patients with any RD against no RD was 4.26 (95% CI: 2.27–7.96). However, if IDS is performed after good response to two cycles of chemotherapy, even patients with minimal RD may be expected to obtain good long-term survival (median OS of 66 months and 5-year OS rate of 67%).

In the setting of upfront PDS, RD is chemo-naive and will be exposed to at least six cycles of post-operative chemotherapy. However, in the treatment of NAC and IDS, RD is not chemo-naive, and the number of chemotherapy cycles given after IDS is limited (usually three to four cycles), suggesting that residual cancer cells are less likely to disappear completely following IDS than following PDS. In our series, patients with minimal RD at the end of IS after three to six cycles of chemotherapy received, an average, 3.9 cycles of additional chemotherapy and a total of 8.0 cycles of chemotherapy, which are slightly more than those received by patients with no RD at the end of IS (2.9 and 7.1 cycles, respectively). Previous reports have shown that additional cycles of chemotherapy after six cycles do not improve survival (18,19). Thus, the OS might not improve with an increased number of chemotherapy cycles in patients with minimal RD at the end of IS.

Because of long study period and retrospective nature of the study, we used the definition of <2 cm as minimal RD at IDS. Thus, there may be a room to discuss about survival of patients with much smaller RD. However, our result showed that none of the 20 patients with any RD at the end of IS after three or more cycles of chemotherapy survived >5 years. Because we tried to define the optimal surgery mainly by the condition that leads patients to long-term survival, the results may be similar even if we could divide the patients at smaller RD such as <0.5 or <1 cm.

From our results, we believe that OS of patients with no RD after IDS in the setting of NAC is comparable to that of patients with minimal RD after PDS and is slightly inferior to that of patients with no RD after PDS in the setting of upfront PDS. Therefore, to obtain better OS by the NAC setting treatment compared with standard treatment, complete resection with no RD at IDS by the NAC setting treatment should be higher than the rate of cytoreduction with no or minimal RD at PDS by the upfront PDS setting treatment. Recent presentation of the results of Phase III study conducted by European Organization for Research and Treatment of Cancer (13) at the meeting of International Gynecologic Cancer Society (Bangkok, Thailand, October 2008) showed that OSs for patients treated with PDS or NAC setting treatment are similar (29 vs. 30 months), irrespective of much higher rate of achieving residual tumor <1 cm in IDS compared with PDS (83% vs. 48%). These results may support our result that definition of the optimal surgery for PDS and IDS should be different.

In conclusion, on the basis of long-term follow-up data in patients undergoing upfront PDS setting treatment and having assessment of peritoneal findings during chemotherapy, we propose that the optimal goal of the IDS following three or more cycles of NAC is only complete resection of all visible tumors. However, our study was a retrospective analysis and included only a small number of patients. The definition of optimal cytoreduction at PDS has been established on the basis of long-term clinical data. Similarly, accumulation of data regarding IDS outcomes and OSs in the setting of NAC may be necessary for wide spread acceptance of our proposal.

Conflict of interest statement

None declared.

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ORIGINAL REPORT

Outcomes of Fertility-Sparing Surgery for Stage I Epithelial Ovarian Cancer: A Proposal for Patient Selection

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ABSTRACT

Purpose
The objective of this study was to assess clinical outcomes and fertility in patients treated conservatively for unilateral stage I invasive epithelial ovarian cancer (EOC).

Patients and Methods
A multi-institutional retrospective investigation was undertaken to identify patients with unilateral stage I EOC treated with fertility-sparing surgery. Favorable histology was defined as grade 1 or grade 2 adenocarcinoma, excluding clear cell histology.

Results
A total of 211 patients (stage IA, n = 126; stage IC, n = 85) were identified from 30 institutions. Median duration of follow-up was 78 months. Five-year overall survival and recurrence-free survival were 100% and 97.8% for stage IA and favorable histology (n = 108), 100% and 100% for stage IA and clear cell histology (n = 15), 100% and 33.3% for stage IA and grade 3 (n = 3), 96.9% and 92.1% for stage IC and favorable histology (n = 67), 93.3% and 66.0% for stage IC and clear cell histology (n = 15), and 66.7% and 66.7% for stage IC and grade 3 (n = 3). Forty-five (53.6%) of 84 patients who were nulliparous at fertility-sparing surgery and married at the time of investigation gave birth to 56 healthy children.

Conclusion
Our data confirm that fertility-sparing surgery is a safe treatment for stage IA patients with favorable histology and suggest that stage IA patients with clear cell histology and stage IC patients with favorable histology can be candidates for fertility-sparing surgery followed by adjuvant chemotherapy.

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INTRODUCTION

The standard surgical treatment for early-stage epithelial ovarian cancer (EOC) is total hysterectomy plus bilateral salpingo-oophorectomy with peritoneal and lymph-node sampling. Fertility-sparing surgery that includes unilateral salpingo-oophorectomy and optimal surgical staging is an option available to young women with stage I EOC. However, the recommended indications for such treatment remain controversial.

Fertility-sparing surgery for reproductive-age patients with invasive EOC has been adopted for stage IA and non-clear cell histology grade 1 (G1)/grade 2 (G2) according to the 2007 guidelines of the American College of Obstetrics and Gynecology (ACOG)[1] and for unilateral stage I tumor without dense adhesions showing favorable histology (ie, non-clear cell histology G1/2) according to the 2008 guidelines of the European Society for Medical Oncology (ESMO).[2] In Japan, fertility-sparing surgery has been recommended for patients with stage IA tumor or unilateral stage IC tumor on the basis of intraoperative capsule rupture [IC(b)] and favorable histology, according to the 2004 guidelines[3] and the 2007 guidelines[4] of the Japan Society of Gynecologic Oncology (JSGO). EOC with clear cell or grade 3 (G3) histology and with bilateral ovarian involvement has been excluded from indications for fertility-sparing surgery in all three guidelines. The recommendations regarding fertility-sparing surgery for unilateral and stage IC EOC differ widely among these guidelines, although those for unilateral and stage IA EOC with favorable histology are common to all three guidelines.

The number of published studies concerning fertility-sparing surgery in young EOC patients who wish to preserve the possibility of pregnancy is

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limited,[4-14] and each study included fewer than 60 patients, too small a population to allow consensus regarding recommendations for patient selection for fertility-sparing surgery in stage I EOC. This study attempted to determine selection criteria for fertility-sparing surgery in stage I EOC patients on the basis of clinical outcomes for more than 200 stage I EOC patients who underwent fertility-sparing surgery.

PATIENTS AND METHODS

Patients

Between 1985 and 2004, patients with stage I invasive EOC who underwent fertility-sparing surgery in 30 institutions belonging to the Gynecologic Cancer Study Group of the Japan Clinical Oncology Group or who were referred to these hospitals immediately after fertility-sparing surgery performed elsewhere were enrolled onto this study. Patients were eligible if they had stage I, G1, G2, or G3 EOC; if they were treated using fertility-sparing surgery (conservation of the uterus and contralateral ovary and fallopian tube); and if they were ≤ 40 years of age at the time of fertility-sparing surgery. Four patients (stage IB, n = 2; stage IC, n = 2) who showed microscopic metastases in biopsy specimens from the opposite ovary were excluded from this study because of the small number of patients and the insufficient durations of follow-up.

Reassessment of histologic cell type and tumor differentiation was performed in each institution according the WHO criteria before enrollment onto the present study. Histologic differentiation was defined as G1, well differentiated; G2, moderately differentiated; or G3, poorly differentiated. Staging was determined according to the International Federation of Gynecology and Obstetrics (FIGO) classification (1987). In this study, stage IC patients were classified into three subgroups: stage IC(b), intraoperative capsule rupture with negative peritoneal cytology; IC(a), preoperative capsule rupture and/or tumor on ovarian surface with negative peritoneal cytology; and IC(1/2), malignant cells in ascites or peritoneal washings. Institutional review board approval was obtained from each institution before initiating this investigation.

Factors for Analysis

Mucinous, serous, endometrioid, and mixed epithelial adenocarcinoma were classified by histologic grade (G1, G2, or G3). Clear cell histology was not graded in this study. We defined G1/2 non-clear cell adenocarcinoma as showing favorable histology.

Stage IA or IC patients with unilateral ovarian involvement were divided into six subgroups to determine patient selection for fertility-sparing surgery, as follows: stage IA and favorable histology, stage IA and clear cell histology, stage IA and G3, stage IC and favorable histology, stage IC and clear cell histology, or stage IC and G3.

We defined lethal recurrence (LR) as recurrence showing lesions outside the remaining ovary, because a considerable number of previous reports[15] have suggested that patients with recurrence exclusively within the remaining ovary show much better prognosis following salvage surgery compared with patients displaying other patterns of recurrence. Outcomes for patients were analyzed using overall survival (OS), recurrence-free survival (RFS), and lethal recurrence–free survival (LRFS). We also investigated reproductive outcomes after fertility-sparing surgery in patients who provided the information.

Statistical Analysis

Statistical analysis of data was performed using the JMP Statistics package (SAS Institute, Cary, NC). Two-sided probability values were calculated throughout and considered to be significant at the level of $P < .05$. Survival estimates were generated using Kaplan-Meier methods. Differences between groups were tested using log-rank testing.

RESULTS

Patient Characteristics

A total of 211 patients with unilateral stage I EOC (stage IA, n = 126; stage IC, n = 85) were entered onto the study. Table 1 summarizes the main characteristics of patients and tumors. Mean patient age was 29 years (range, 14 to 40 years). Median duration of follow-up after excluding patients who died was 78 months from initial fertility-sparing surgery (range, 3 to 270 months).

Surgical Treatments

Of the 211 patients, 23 (10.9%) patients underwent restaging laparotomy because of inadequate staging or cytoreduction at initial surgery. Nine of the 23 patients underwent unilateral ovarian cystectomy at initial surgery (laparoscopy, n = 4; laparotomy, n = 5) and unilateral salpingo-oophorectomy at restaging laparotomy. As a result, 205 patients underwent unilateral salpingo-oophorectomy. The

Table 1. Patient Characteristics (N = 211)

Characteristic	No.	%
Age, years		
Median	29	
Range	14-40	
Parity		
Parous	26	12.3
Nulliparous	185	87.7
FIGO stage		
IA	126	59.7
IC	85	40.3
Substage		
IC(b)	55	26.1
IC(a)	18	8.5
IC(1/2)	12	5.7
Cell type		
Mucinous	126	59.7
Serous	27	12.8
Endometrioid	27	12.8
Clear cell	30	14.2
Mixed epithelial	1	0.5
Histologic differentiation		
Well (G1)	160	75.8
Moderate (G2)	15	7.1
Poor (G3)	6	2.8
Not classified (clear cell)	30	14.2
FIGO stage and histologic differentiation		
IA		
G1	95	47.3
G2	13	6.2
G3	3	1.4
Clear cell	15	7.1
IC		
G1	65	30.8
G2	2	0.9
G3	3	1.4
Clear cell	15	7.1

Abbreviations: G(1/2/3), non-clear cell histology grade (1/2/3); FIGO, International Federation of Gynecology and Obstetrics; IC(b), intraoperative capsule rupture with negative peritoneal cytology; IC(a), preoperative capsule ruptured and/or tumor on ovarian surface with negative peritoneal cytology; IC(1/2), malignant cells in ascites or peritoneal washings.

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Table 2. Types of Surgery in Initial Treatment

Surgery Type	No. of Patients
Unilateral salpingo-oophorectomy	205
Alone	64
BO	43
OM	16
RLND	5
BO + OM	27
BO + RLND	5
OM + RLND	18
BO + OM + RLND	26
Unknown	1
Unilateral ovarian cystectomy	6
BO	3
RLND	1
BO + OM	1
Unknown	1

Abbreviations: BO, biopsy from the opposite ovary; OM, partial omentectomy; RLND, retroperitoneal lymph node dissection or biopsy.

remaining six patients underwent unilateral ovarian cystectomy at initial laparotomy, not followed by restaging surgery. As for other surgeries, 105 patients underwent biopsy (wedge resection) of the opposite ovary, 88 patients underwent partial omentectomy, and 55 patients underwent retroperitoneal lymph node dissection or biopsies. Table 2 provides details of surgical treatments.

Surgical staging included careful inspection and palpation of peritoneal surfaces with biopsies of any suspect lesions and peritoneal washing cytology. No patients received endometrial curettage during surgery, although most patients had endometrial cytology or biopsy before surgery. If optimal surgical staging required at least omentectomy in addition to unilateral salpingo-oophorectomy, 87 (41.2%) of the 211 patients were optimally staged and 124 (58.8%) were nonoptimally staged. Only 74 (35.1%) patients were optimally staged in one-step surgery.

Adjuvant Chemotherapy

Platinum-based adjuvant chemotherapy was administered to 125 (59.2%) patients, with a mean number of four cycles (range, 1 to 12 cycles). The most common chemotherapy regimens were cisplatin + cyclophosphamide ± doxorubicin (57 of 125; 45.6%) and carboplatin + paclitaxel (46 of 125; 36.8%). Fifteen (7.1%) patients received adjuvant chemotherapy without platinum (including oral medication). The remaining 71 (33.6%) patients received no adjuvant treatment after initial surgery.

Clinical Outcomes

Recurrence was identified during the follow-up period for 18 (8.5%) of 211 patients. Of these 18 patients, five showed recurrence exclusively in the remaining ovary (non-LR; Table 3) and 13 had LR in sites other than the remaining ovary (Table 4). At the end of this investigation, eight patients were alive with no evidence of disease, five patients were alive with disease, and five patients had died of disease. All five patients with non-LR were treated with salvage surgery and showed no evidence of disease.

Stage IA and favorable histology. This subgroup included 108 stage IA patients with favorable histology. Of these, 44 (40.7%) patients received platinum-based adjuvant chemotherapy after surgery, and the 5-year OS, RFS, and LRFS were 100%, 97.8%, and 99.1%, respectively. Three patients with mucinous histology G1 developed LR at 14, 70, and 73 months after fertility-sparing surgery (Table 4). Median duration of follow-up for this group was 79 months.

Stage IA and clear cell histology. This subgroup included 15 stage IA patients with clear cell histology. Of those, nine (60%) patients were treated with platinum-based adjuvant chemotherapy. The 15 patients showed rates of 100% for 5-year OS, RFS, and LRFS. Median duration of follow-up for these patients was 78 months.

Stage IA and G3. One of the three stage IA patients with G3 received platinum-based adjuvant chemotherapy and was alive without recurrence 256 months after fertility-sparing surgery. Two patients without any adjuvant chemotherapy had LR at 25 and 31 months after fertility-sparing surgery (Table 4), although both were alive with disease at the end of this investigation (duration of follow-up, 65 and 90 months).

Stage IC and favorable histology. This subgroup included 67 stage IC patients with favorable histology. Platinum-based adjuvant chemotherapy was administered to 57 (85.1%) patients following surgery. The 5-year OS, RFS, and LRFS were 96.9%, 92.1%, and 95.4%, respectively. As for subgroups of stage IC [IC(b), n = 43; IC(a), n = 14; IC(1/2), n = 10], the 5-year RFS was 92.9%, 91.7%, and 90.0%, respectively. Three (4.5%) of 67 patients developed LR, with one stage IC(b) patient with endometrioid histology G1, one stage IC(b) patient with mucinous histology G1, and one IC(1/2) patient with serous histology G1 developing LR at 20, 8, and 3 months after fertility-sparing surgery, respectively (Table 4). Median duration of follow-up for this group was 76.5 months.

Table 3. Characteristics of Patients With Recurrence in the Residual Ovary Alone (non-lethal recurrence)

Patient No.	Age (years)	Stage	Histologic Type	Grade	Platinum-Based Chemotherapy	Time to Recurrence (months)	Follow-Up After Recurrence (months)	Status
1	18	IA	Mucinous	1	No	83	119	NED
2	26	IA	Serous	1	Yes	52	164	NED
3	26	IC(b)	Endometrioid	1	No	7	45	NED
4	36	IC(b)	Clear cell	Not graded	No	21	124	NED
5	26	IC(a)	Mucinous	1	Yes	43	16	NED

Abbreviations: NED, no evidence of disease; IC(b), intraoperative capsule rupture with negative peritoneal cytology; IC(a), preoperative capsule ruptured and/or tumor on ovarian surface with negative peritoneal cytology.

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Table 4. Characteristics of Patients Showing Recurrence With Lesions Outside the Residual Ovary (lethal recurrence)

Patient No.	Age (years)	Stage	Histologic Type	Grade	Platinum-Based Chemotherapy	Site of Recurrence	Time to Recurrence (months)	Follow-Up After Recurrence (months)	Status
1	19	IA	Mucinous	1	No	Peritoneum	70	149	NED
2	27	IA	Mucinous	1	No	Lung	73	34	DOD
3	29	IA	Mucinous	1	No	Abdominal wall	14	39	AWD
4	22	IA	Serous	3	No	Residual ovary, ascites	25	231	NED
5	40	IA	Endometrioid	3	No	Para-aortic lymph nodes	31	34	NED
6	15	IC(b)	Mucinous	1	Yes	Peritoneum	8	18	AWD
7	31	IC(b)	Endometrioid	1	Yes	Liver	20	6	DOD
8	29	IC(b)	Clear cell	Not graded	No	Para-aortic lymph nodes	15	86	AWD
9	29	IC(b)	Clear cell	Not graded	Yes	Residual ovary, ascites, peritoneum	11	19	DOD
10	36	IC(b)	Clear cell	Not graded	Yes	Liver	46	8	AWD
11	33	IC(a)	Endometrioid	3	Yes	Not recorded	1	5	DOD
12	26	IC(1/2)	Serous	1	Yes	Peritoneum	3	22	DOD
13	38	IC(1/2)	Clear cell	0	No	Residual ovary, pelvic lymph nodes, peritoneum	21	29	AWD

Abbreviations: NED, no evidence of disease; DOD, died of disease; AWD, alive with disease; IC(b), intraoperative capsule rupture with negative peritoneal cytology; IC(a), preoperative capsule ruptured and/or tumor on ovarian surface with negative peritoneal cytology; IC(1/2), malignant cells in ascites or peritoneal washings.

Stage IC and clear cell histology. This subgroup included 15 stage IC patients with clear cell histology. Eleven (73.3%) of these patients were treated with platinum-based adjuvant chemotherapy. LR occurred in two patients with and in two patients without platinum-based adjuvant chemotherapy (Table 4). These 15 patients showed rates of 93.3%, 66.0%, and 72.7% for 5-year OS, RFS, and LRFS. In particular, 5-year RFS of 11 stage IC(b) patients resembled that of the other four stage IC patients (63.6% *v* 75.0%, respectively). Median duration of follow-up for the 14 survivors was 64 months.

Stage IC and G3. All three stage IC patients with G3 were treated using platinum-based chemotherapy after surgery, but one patient developed LR and died of disease 6 months after fertility-sparing surgery. The remaining two patients were alive without recurrence 58 and 230 months after fertility-sparing surgery.

Comparison of Clinical Outcomes Among Subgroups

We compared OS and RFS among the four subgroups except for the two subgroups (stage IA and G3, or stage IC and G3) consisting of only three patients. In terms of OS, no significant differences were seen among the four subgroups. Significant differences in RFS were seen between the following three pairs of subgroups: stage IA favorable histology versus stage IC clear cell histology (97.8% *v* 66.0%; $P < .001$), stage IC favorable histology versus stage IC clear cell histology (92.1% *v* 66.0%; $P = .008$), and stage IA clear cell histology versus stage IC clear cell histology (100% *v* 66.0%; $P = .02$).

Figure 1 shows OS and RFS curves in those with good prognosis (group I: stage IA favorable histology [n = 108]), those with fairly good prognosis (group II: stage IA clear cell histology or stage IC favorable histology [n = 82]), and those with poor prognosis (group III: stage IA G3, stage IC clear cell histology, or stage IC G3 [n = 21]). No significant differences in OS were seen between groups I and II ($P = .21$) or between groups II and III ($P = .29$), whereas significant differences were identified between groups I and III ($P = .02$). No significant differences in RFS were apparent between groups I and II ($P = .65$), but significant differences were noted between groups I and III ($P < .001$) and between groups II and III ($P < .001$).

Reproductive Outcomes

After fertility-sparing surgery with or without adjuvant chemotherapy, 182 (96.8%) of 188 patients who gave information on menstruation had almost the same cycle of menstruation as before treatment. Six (5.0%) of 121 patients who received platinum-based adjuvant chemotherapy showed continued secondary amenorrhea for 6, 48, 66, 72, 172, and 224 months following two to six cycles of chemotherapy (median, four cycles).

Of the 195 patients who gave reproductive outcomes at the end of the investigation, 55 (28.5%) patients achieved 76 pregnancies and 53 gave birth to 66 healthy children after fertility-sparing surgery. Five (9.1%) of 55 patients had received some kind of infertility treatment before pregnancy. These patients and their babies showed no clinical problems during the perinatal period. Four (9.4%) of 53 patients who gave birth to children underwent completion surgery, including hysterectomy and contralateral salpingo-oophorectomy, after childbearing.

Forty-five (53.6%) of 84 patients who were nulliparous at fertility-sparing surgery and married at the end of the follow-up period had achieved 65 pregnancies, and 43 had given birth to 56 healthy children during follow-up (mean follow-up, 8.8 years). Of the 84 patients, the remaining 39 patients had not conceived during follow-up (mean follow-up, 7.2 years), and mean age was 37 years (range, 25 to 54 years) at the end of the investigation.

DISCUSSION

In this series, recurrence rate among the 211 stage I EOC patients after fertility-sparing surgery was 8.5% (18 of 211), falling within the 5.4% to 30.3% reported previously.[5,6,10,12,14] Of the 18 patients with recurrence, five (2.4%) patients showing recurrence exclusively in the residual ovary achieved no evidence of disease. According to data from five studies[5,6,10,12,14] that investigated relationships between sites of recurrence and clinical outcomes, eight of 10 patients with recurrence limited to the residual ovary achieved no evidence of disease following salvage therapy, whereas only three of 21 patients with recurrence at

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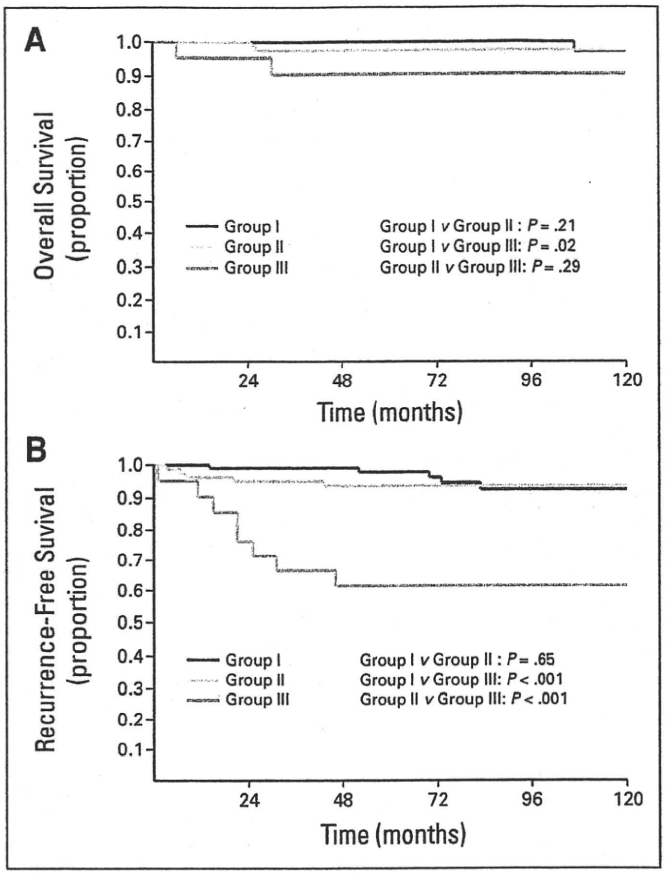

Fig 1. (A) Overall survival curves for patients with good prognosis (group I), fairly good prognosis (group II), and poor prognosis (group III). Group I: stage IA and favorable histology; group II: stage IA and clear cell histology, or stage IC and favorable histology; group III: stage IA and clear cell histology grade 3 (G3), stage IC and clear cell histology, or stage IC and G3. (B) Recurrence-free survival curves for groups I, II, and III.

extra-ovarian sites achieved no evidence of disease. We thus evaluated LRFS in addition to OS and RFS in this study.

The 108 stage IA patients with favorable histology showed a 5-year RFS of 97.8% and a 5-year LRFS of 99.1% (5-year recurrence rate, 2.2%; 5-year LR rate, 0.9%), although only 40.7% of these patients received platinum-based adjuvant chemotherapy after surgery. Stage IA patients with favorable histology were always included in selection criteria for fertility-sparing surgery in previous reports and in various guidelines.[1-14] The recurrence rate for stage IA patients with favorable histology in four previous reports[5,10,12,14] was 0% to 22.2% during follow-up. Our data confirm fertility-sparing surgery as a safe treatment option for stage IA patients with favorable histology, even when fertility-sparing surgery is not followed by adjuvant chemotherapy.

In this study, 15 stage IA patients with clear cell histology showed no recurrence, with lymph node biopsy or dissection performed in six (40%) patients and adjuvant platinum-based chemotherapy given to nine (60%) patients. Our data correspond with that in a recent report by Kajiyama et al[16] showing no recurrence in four stage IA patients with clear cell histology who had undergone fertility-sparing surgery. Other investigations,[10,12,14] however, have reported three recurrences among eight stage IA patients with clear cell histology after fertility-sparing surgery. These data suggest that stage IA patients with clear cell histology may be candidates for fertility-sparing surgery, including optimal staging followed by adjuvant chemotherapy.

In our series, only one of three stage IA patients with G3 survived for 5 years without recurrence. The recurrence rate for the 17 stage IA patients with G3 from six investigations[5,7,10-12,14] who underwent fertility-sparing surgery was 35.3% (6 of 17), although some reports classified clear cell histology into G3. These data suggest that fertility-sparing surgery cannot be recommended for stage IA patients with G3.

The 67 stage IC patients with favorable histology had a 5-year RFS of 92.1% and a 5-year LRFS of 95.5%. Outcomes seem to be better in our study compared with the recurrence rate of 12.8% (5 of 39) in previous studies.[7,10-12,14] Platinum-based adjuvant chemotherapy was more frequently given to this group compared with the stage IA and favorable histology group (85.1% *v* 40.7%; $P < .001$). In our series, no significant difference in 5-year RFS was seen among 43 IC(b) patients, 14 IC(a) patients, or 10 IC(1/2) patients with values of 92.9%, 91.7%, and 90.0%, respectively. Our data suggest that stage IC patients with favorable histology in the unilateral ovary can be candidates for fertility-sparing surgery, including optimal staging followed by adjuvant chemotherapy.

Our series included 15 stage IC patients with clear cell histology. These patients showed a 5-year RFS of 66.0% and a 5-year LRFS of 72.7%, even when 11 (73.3%) patients were treated with platinum-based adjuvant chemotherapy. Kajiyama[16] reported that one stage IC(2) patient among the six stage IC patients with clear cell histology experienced relapse and died of the disease. Five-year RFS was 63.6% for 11 IC(b) patients, 100% for two IC(a) patients, and 50% for two IC(1/2) patients. These data suggest that stage IC patients with clear cell histology cannot be candidates for fertility-sparing surgery.

Our series included three stage IC patients with G3. One patient developed LR and died of the disease 6 months after fertility-sparing surgery, although all three patients had been treated with platinum-based adjuvant chemotherapy. In previous reports,[10-14] four of nine stage IC patients with G3 who underwent fertility-sparing surgery displayed recurrence. These data suggest that fertility-sparing surgery cannot be recommended for stage IC patients with G3.

In addition to the study patients, during the study period, we managed four patients with unilateral stage I EOC treated with fertility-sparing surgery elsewhere, who were referred to these hospitals for treatment of lethal recurrent disease and died of the disease. These four patients included one stage IA patient with clear cell histology, one stage IA patient with G3, and two stage IC patients with G3. Clinical outcomes for these patients support our recommendations regarding fertility-sparing surgery for unilateral stage I EOC.

In our series, 5% of patients with platinum-based adjuvant chemotherapy developed secondary amenorrhea and infertility, suggesting that we should not administer adjuvant chemotherapy to patients with stage IA and favorable histology without serious consideration. As for the reproductive outcome, we confirmed that most married but nulliparous EOC patients undergoing fertility-sparing surgery can give birth to children within several years after fertility-sparing surgery.

In conclusion, this study confirmed that stage IA EOC patients with favorable histology can be safely treated with fertility-sparing surgery not followed by platinum-based adjuvant chemotherapy. We would thus propose that fertility-sparing surgery be considered

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Table 5. Recommendation for Fertility-Sparing Surgery in Young Patients With Unilateral Stage I Ovarian Cancer

Stage	Histology/Grade		
	FH	CCH	G3
1A	Offer FSS	Consider FSS + CT	No FSS
1C	Consider FSS + CT	No FSS	No FSS

Abbreviations: FH, favorable histology (mucinous, serous, endometrioid, or mixed histology and grade 1 or 2); CCH, clear cell histology; G3, clear cell histology grade 3; FSS, fertility-sparing surgery; CT, adjuvant chemotherapy.

for stage IA EOC patients with clear cell histology and for stage IC EOC patients with unilateral ovarian involvement and favorable histology, under conditions of performing complete staging surgery and platinum-based adjuvant chemotherapy (Table 5). Conversely, fertility-sparing surgery cannot be recommended for patients with stage IA with G3 histology or stage IC with clear cell or G3 histology. Theoretically, a randomized controlled trial may be needed to compare conservative surgery with radical surgery for young patients with EOC to achieve high-quality evidence. However, such trials may not be ethically feasible. Confirming the decision of patient criteria for selection in a phase II trial would be appropriate.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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Amplification of GNAS may be an independent, qualitative, and reproducible biomarker to predict progression-free survival in epithelial ovarian cancer

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ABSTRACT

Objectives. The purpose of this study was to identify genes that predict progression-free survival (PFS) in advanced epithelial ovarian cancer (aEOC) receiving standard therapy.

Methods. We performed microarray analysis on laser microdissected aEOC cells. All cases received staging laparotomy and adjuvant chemotherapy (carboplatin + paclitaxel) as primary therapy.

Results. Microarray analysis identified 50 genes differentially expressed between tumors of patients with no evidence of disease (NED) or evidence of disease (ED) ($p<0.001$). Six genes (13%) were located at 8q24, and 9 genes (19.6%), at 20q11–13. The ratio of selected gene set/analyzed gene set in chromosomes 8 and 20 are significantly higher than that in other chromosome regions (6/606 vs. 32/13656, $p=0.01$) and (12/383 vs. 32/13656, $p=1.3\times10^{-16}$). We speculate that the abnormal chromosomal distribution is due to genomic alteration and that these genes may play an important role in aEOC and choose GNAS (GNAS complex locus, NM_000516) on 20q13 based on the p value and fold change. Genomic PCR of aEOC cells also showed that amplification of GNAS was significantly correlated with unfavorable PFS ($p=0.011$). Real-time quantitative RT-PCR analysis of independent samples revealed that high mRNA expression levels of the GNAS genes, located at chromosome 20q13, was significantly unfavorable indicators of progression-free survival (PFS). Finally, GNAS amplification was an independent prognostic factor for PFS.

Conclusions. Our results suggest that GNAS gene amplification may be an independent, qualitative, and reproducible biomarker of PFS in aEOC.

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Introduction

Epithelial ovarian cancer (EOC) remains the most common cause of cancer death in women and the leading cause of death from gynecologic cancer. Early diagnosis of EOC is extremely difficult because most patients with early-stage disease are asymptomatic, so that 80% of patients present with advanced disease. Standard therapy includes surgical procedures (bilateral adnexectomy + hysterectomy + greater omentectomy) with staging laparotomy, debulking surgery, and postoperative chemotherapy using a combination of platinum and taxane. In 70% of advanced EOC (aEOC) patients, complete clinical responses are achieved; however, tumor recurs in most patients within 1 to 2 years after diagnosis and death is due to the development of chemotherapy resistance. In contrast, small numbers of patients with aEOC are cured by standard therapy. Although several clinical features are associated with poor prognosis, including poor performance status, suboptimal debulking surgery, clear cell or mucinous histology, high histologic grade, old age, or slow decrease in serum CA125 during adjuvant chemotherapy, reliable predictive biomarkers for aEOC are still lacking. If such markers could be established, patients who are likely to relapse and die of disease might be identified. These patients would be appropriate candidates for experimental approaches using novel anticancer drugs or new combination chemotherapy.

Recently, molecular diagnostic methods have been developed and BAX or BRCA1 have been reported to be predictive biomarker for aEOC [1,2]. We also previously reported that abnormalities of cell cycle regulators are predictive prognostic indicators for EOC [3]. Similarly, gene expression profiles or array comparative genomic hybridization (aCGH) has been reported to offer predictive/prognostic information for aEOC [4–7]. However, in order to identify useful predictive biomarkers for EOC, it is important that the markers should be tested in the context of standard therapy. In addition, the histology of the

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tumors should be considered because clear cell and mucinous types are usually more chemoresistant than other histologic types [8,9].

In this study, we used oligonucleotide microarrays combined with RNA isolated from microdissected tumor tissue to identify new prognostic biomarkers for aEOC patients receiving standard therapy. We excluded clear cell or mucinous tumors from our analysis based on the chemosensitivity.

Materials and methods

Patients and samples

Subjects eligible for this study were patients with histologically confirmed stage IIc–IV EOC (excluding mucinous and clear cell types) receiving standard therapy. Histologic grade was determined using WHO grading system. Additional inclusion criteria included an Eastern Cooperative Oncology Group performance status of 0 to 2. Exclusion criteria included a history of prior chemotherapy or major surgery. All patients received standard surgery and chemotherapy using carboplatin and paclitaxel. Standard surgery was bilateral adnexectomy, hysterectomy, and greater omentectomy with staging laparotomy and debulking surgery. Thirty-three aEOC patients were enrolled for microarray analysis, and an additional 107 patients were for real-time PCR analysis. The progression-free survival (PFS) was defined from the date of primary surgery to the date of the first occurrence of any of the following events: appearance of any new lesions, tumor progression, elevation of the CA125 level to at least two times the upper limit of normal or a nadir CA125 level, or death from any cause. The patients were determined to be no evidence of disease (NED) or evidence of disease (ED) at the disease progression or final visit. The study was approved by the Institutional Review Board of the Osaka City General Hospital and School of Medicine, Keio University, and written informed consent was obtained from all patients. Tumor specimens were obtained at operation and were immediately stored at −80 °C.

Study design

One hundred and forty aEOC samples were evaluated. The samples were divided between microarray analysis ($n=33$) and a real-time PCR analysis ($n=107$). Microarray analysis was performed using 33 samples, and candidate genes showing significant correlation with disease progression were identified. The GNAS gene was evaluated in an independent set of 107 samples, and PFS was predicted using the results of real-time PCR analyses of both mRNA and DNA.

Microdissection

Microdissection was performed as described previously. In brief, frozen sections (6 μm) prepared from tumor tissue specimens were affixed to glass slides and stained by Histogene LCM Frozen Section Staining Kit (Arcturus Engineering, Mountain View, CA). Stained sections were microdissected using a PixCell IIe LCM system (Arcturus Engineering, Mountain View, CA). Tumor cells and adjacent non-tumor stromal cells were visualized under the microscope and tumor cells selectively released by activation of the laser. Approximately 15,000 tumor cells were dissected in each case.

RNA and DNA extraction and amplification

Total RNA and DNA extractions were performed using the PicoPure RNA Isolation Kit and PicoPure DNA Extraction Kit according to the manufacturer's instructions (Arcturus Engineering, Mountain View, CA). RNA was amplified using a modified single-round T7 RNA amplification protocol. In brief, total RNA (600 ng) was first incubated with 1 μl of T7 primer(5′-GCATTAGCGGCCGCGAAATTAATACGACTCACTATAGGGAGATTTTTTTTTTTTTTTTTVN-3′, 200 ng/μl) in a total volume of 50 μl for 3 min at 70 °C. First-strand cDNA synthesis was then performed by incubating 5 μl of primer-annealed sample and 5 μl of first strand master mix containing 2 μl of 5× first-strand buffer, 1 μl of 0.1 M DTT, 0.5 μl of DEPC water, 0.5 μl 10 mM dNTP mix, 0.5 μl RNase inhibitor, and 0.5 μl of MMLV reverse transcriptase (200 U/μl) for 1 h and 15 min at 37 °C. Subsequently, second-strand cDNA synthesis was performed by incubating the 10 μl first-strand reaction with 65 μl of second master mix, which contained 46 μl DEPC water, 15 μl 5× second-strand buffer, 1.5 μl of 10 mM dNTP mix, 0.5 μl of *Escherichia coli* DNA ligase (10 U/μl), 1.5 μl E. coli DNA polymerase I (10 U/μl), and 0.5 μl *E. coli* RNase H (2 U/μl), for 2 h at 16 °C, and then for 15 min at 70 °C. The entire 75 μl cDNA sample was loaded onto a ChromaSpin TE-200 spin column (BD Biosciences, San Diego, CA), which was centrifuged for 5 min at 2900 rpm (700×g) in an Eppendorf centrifuge. Purified cDNA was collected, lyophilized, dissolved in 8 μl of RNase-free water, and incubated at 70 °C for 10 min. In vitro transcription was subsequently performed by incubating the 8 μl post-lyophilization cDNA product with 12.2 μl of master mix containing 2 μl of 10× T7 reaction buffer, 6 μl of 25 mM rNTP Mix, 2 μl of 100 mM DTT, 0.2 μl of RNase inhibitor (40 U/ml), and 2 μl of T7 RNA polymerase for 3 h at 37 °C. The amplified RNA was purified on an RNeasy mini column (Qiagen, Valencia, CA) as per the manufacturer's protocol. The purified amplified RNA was quantified using RiboGreen RNA Quantitation Reagent (Molecular Probes, Eugene, OR).

Oligonucleotide microarray analysis

The microarray procedure was performed according to Affymetrix protocols (Santa Clara, CA). In brief, total RNA extracted from tumor samples was checked for quality using an Agilent 2100 Bioanalyzer (Agilent Technologies, Waldbronn, Germany) and cRNA was synthesized using the GeneChip 3′-Amplification Reagents One-Cycle cDNA Synthesis Kit (Affymetrix). The labeled cRNAs were then purified and used for construction of probes. Hybridization was performed using the Affymetrix GeneChip HG-U133 Plus2.0 array for 16 h at 45 °C. Signal intensities were measured using a GeneChip Scanner3000 (Affymetrix) and converted to numerical data using the GeneChip Operating Software, Ver.1 (Affymetrix).

DNA copy number analysis

The method has been described previously [5,10]. From array data, we focused on 20q11–13 loci for further examination, because we thought that 20q11–13 loci are amplified in the ED group. We chose GNAS (GNAS complex locus, NM_000516) on 20q13 based on the p value and fold change. Results were normalized to the amount of RH78455 of chromosome 5q22.2 as genomic internal control locus. Regarding the internal DNA copy number control, we selected the genomic region of chromosome 5q, which is less frequently received the genomic alterations in ovarian cancers referred to previous report [11–13]. Next, we checked 10 primers (D5S818, D5S409, D5S349, D5S346, D5S519, D5S422, STSR33609, RH46186, RH78455, RH68508) of chromosome 5 region according to database of sequence tagged sites (STSs, http://www.ncbi.nlm.nih.gov/unists). Among them, RH78455 was most specific and reproducible primers, then we used it as internal DNA copy number control. The DNA was quantified using the Power SYBR Green PCR Master Mix (Applied Biosystems) and 7900HT Fast Real-time PCR system (Applied Biosystems) and reported relative to the control primer. The control DNA for standard DNA copy numbers was purchased from Invitrogen (Carlsbad, CA). The PCR conditions were as follows: one cycle of denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s and 60 °C for 60 s. If copy number was >1.5 relative to the chromosome control, we judged that there was amplification [5]. The primers used for estimating DNA copy numbers were as follows: GNAS: SHGC59923-FW: 5-GGG TGG GCT TTT GTT CTT TG-3, SHGC59923-RW: 5-AGG CAT AAA CGG GGG AGA TT-3, and

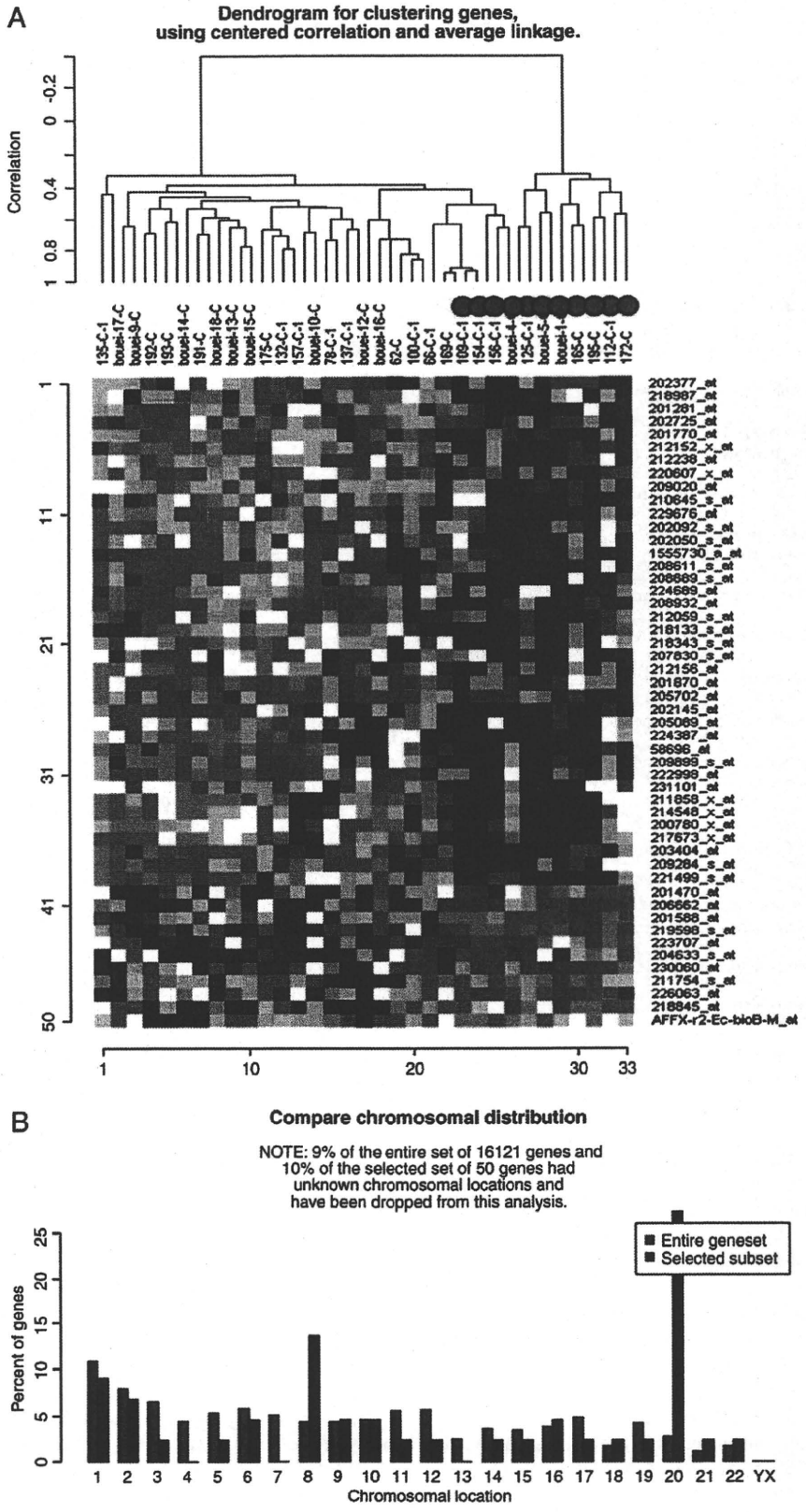

Fig. 1. (A) Fifty genes differentially expressed between tumors in ED and NED patients. Dendrogram for clustering genes using centered correlation and average linkage. Red circles indicate NED cases. (B) The chromosome distribution of the entire gene set and the selected subset of 50 genes. Among genes, which are located on chromosome 8 and 20, 50 genes are frequently selected in aEOC.

Table 1
Identification of 50 candidate PFS-related genes from microarray analysis.

No.	Name	Symbol	Cytoband	Fold Difference	Probe Set
1	Zinc finger, MYM-type 4	ZMYM4	Chr:1p32-p34	1.6	202050_s_at
2	Putative homeodomain transcription factor 1	PHTF1	Chr:1p13	1.5	205702_at
3	Protein phosphatase 1, regulatory (inhibitor) subunit 8	PPP1R8	Chr:1p35	1.8	207830_s_at
4	AT-rich interactive domain 1A (SWI-like)	ARID1A	Chr:1p35.3	1.8	212152_x_at
5	NIF3 NGG1 interacting factor 3-like 1 (S. pombe)	NIF3L1	Chr:2q33	1.6	218133_s_at
6	General transcription factor IIIC, polypeptide3, 102 kDa	GTF3C3	Chr:2q33.1	1.5	218343_s_at
7	Cell division cycle associated 7	CDCA7	Chr:2q31	0.4	230060_at
8	Chromosome 3 open reading frame 63	C3orf63	Chr:3p14.3	1.9	209284_s_at
9	Glutaredoxin (thioltransferase)	GLRX	Chr:5q14	0.3	206662_at
10	Dual specificity phosphatase 22	DUSP22	Chr:6p25.3	0.5	218845_at
11	RWD domain containing 1	RWDD1	Chr:6q13-q22.33	0.5	219598_s_at
12	Lymphocyte antigen 6 complex, locus E	LY6E	Chr:8q24.3	3	202145_at
13	Zinc finger protein 7	ZNF7	Chr:8q24	1.6	205089_at
14	Fuse-binding protein-interacting repressor	SIAHBP1	Chr:8q24.2-qter	1.8	209899_s_at
15	MAF1 homolog (S. cerevisiae)	MAF1	Chr:8q24.3	1.8	222998_at
16	COMM domain containing 5///COMM domain containing 5	COMMD5	Chr:8q24-qter	1.8	224387_at
17	Exosome component 4	EXOSC4	Chr:8q24.3	1.7	58696_at
18	Spectrin, alpha, non-erythrocytic 1 (alpha-fodrin)	SPTAN1	Chr:9q33-q34	2	208611_s_at
19	vav 2 oncogene	VAV2	Chr:9q34.1	0.6	226063_at
20	Glutathione S-transferase omega 1	GSTO1	Chr:10q25.1	0.6	201470_at
21	PAP-associated domain containing 1	PAPD1	Chr:10p11.23	1.8	229676_at
22	Cofilin 1 (non-muscle)	CFL1	Chr:11q13	2.4	1555730_a_at
23	Activating transcription factor 7 interacting protein	ATF7IP	Chr:12p13.1	1.6	218987_at
24	Ribosomal protein S6 kinase, 90 kDa, polypeptide 5	RPS6KA5	Chr:14q31-q32.1	0.5	204633_s_at
25	Vacuolar protein sorting 39 (yeast)	VPS39	Chr:15q15.1	1.6	212156_at
26	ADP-ribosylation factor-like 2 binding protein	ARL2BP	Chr:16q13	1.5	202092_s_at
27	Protein phosphatase 4 (formerly X), catalytic subunit	PPP4C	Chr:16p12-16p11	1.9	208932_at
28	Polymerase (RNA) II (DNA-directed) polypeptide A, 220 kDa	POLR2A	Chr:17p13.1	1.8	202725_at
29	Thioredoxin-like 1	TXNL1	Chr:18q21.31	0.6	201588_at
30	Small nuclear ribonucleoprotein polypeptide A	SNRPA	Chr:19q13.1	1.7	201770_at
31	GNAS complex locus	GNAS	Chr:20q13.3	1.4	200780_x_at
32	Adhesion regulating molecule 1	ADRM1	Chr:20q13.33	1.6	201281_at
33	Ribophorin II	RPN2	Chr:20q12-q13.1	1.9	208689_s_at
34	Chromosome 20 open reading frame 111	C20orf111	Chr:20q13.11	1.5	209020_at
35	GNAS complex locus	GNAS	Chr:20q13.3	1.9	211858_x_at
36	Transient receptor potential cation channel, subfamily C, member 4-associated protein	TRPC4AP	Chr:20q11.22	1.7	212059_s_at
37	Additional sex combs like 1 (Drosophila)	ASXL1	Chr:20q11.1	1.7	212238_at
38	GNAS complex locus	GNAS	Chr:20q13.3	1.8	214548_x_at
39	GNAS complex locus	GNAS	Chr:20q13.3	1.6	217673_x_at
40	TH1-like (Drosophila)	TH1L	Chr:20q13	1.7	220607_x_at
41	Ayntaxin 16	STX16	Chr:20q13.32	1.7	221499_s_at
42	Mannosidase, beta A, lysosomal-like	MANBAL	Chr:20q11.23-q12	1.5	224689_at
43	Tetratricopeptide repeat domain 3	TTC3	Chr:21q22.2	1.5	210645_s_at
44	Solute carrier family 25 (mitochondrial carrier; peroxisomal membrane protein, 34 kDa), member 17	SLC25A17	Chr:22q13.2	0.6	211754_s_at
45	Translocase of outer mitochondrial membrane 34	TOMM34		1.6	201870_at
46				1.7	202377_at
47	Hypothetical protein MGC10850	MGC10850		0.5	223707_at
48	Transcribed locus			1.6	231101_at
49	Armadillo repeat containing, X-linked 2	ARMCX2	Chr:Xq21.33-q22.2	2.8	203404_at
50				0.7	AFFX-r2-Ec-bioB-M_at

chromosome 5q22.2: RH78455-FW: 5-TCC TGC AAA CAT TTA AAC TCC A-3, RH78455-RW: 5-AAC AGC AAC TGT TTT TTC CCC-3. Finally, for PCR, 1.5-fold was used as the cutoff for amplification, respectively [5].

Real-time quantitative RT-PCR for mRNA expression

In addition, mRNA expression levels were validated for GNAS (GNAS complex locus, NM_000516) on chromosome 20q13. All results were normalized to the amount of glyceraldehyde 3 phosphate dehydrogenase (GAPD, NM_002046). RNA was converted to cDNA using a GeneAmp RNA PCR Core kit (Applied Biosystems, Foster City, CA). The cDNAs were quantified using the Power SYBR Green PCR Master Mix (Applied Biosystems) and 7900HT Fast Real-time PCR system (Applied Biosystems) and reported relative to the GAPD expression levels. The PCR conditions were as follows: one cycle of denaturation at 95 °C for 10 min, followed by 40 cycles at 95 °C for 15 s, and 60 °C for 60 s. To amplify the target genes, all of the primers used for real-time RT-PCR were purchased from Takara (Yotsukaichi, Japan), which is the major company of molecular biology in Japan. We are frequently using their primers [14–16], and we consider that the primers are reliable one. GNAS-FW: 5-TGT ACA AGC AGT TAA TCA CCC ACC A-3, RW: 5-TCT GTA GGC CGC CTT AAG CTT TC-3, GAPD-FW: 5-GCA CCG TCA AGG CTG AGA AC-3, RW: 5-ATG GTG GTG AAG ACG CCA GT-3. Finally, we determined the case as overexpression when the relative mRNA expression is larger than median relative mRNA expression in all cases.

Statistical analysis

The microarray analysis was performed using the BRB Array Tools software ver. 3.3.0 (http://www.linus.nci.nih.gov/BRB-ArrayTools.html) developed by Dr. Richard Simon and Dr. Amy Peng. In brief, a log base 2 transformation was applied to the raw microarray data, and global normalization was used to calculate the median over the entire array. Genes were excluded if the percentage of data missing or filtered out exceeded 20% or if less than 20 % of expression data had at least a 1.5-fold change in either direction from the median value.