# Strategies for the Management of Non-epithelial Ovarian Tumors

10

Satoru Kyo

**Abstract**

The rarity of non-epithelial ovarian tumors provides many challenging aspects for the clinician, with most general gynecologists only seeing a patient every several years. The first barrier to the management of these tumors is the difficulty of pathological diagnosis, and specialists in pathology must therefore be involved in the diagnostic process. The second barrier is a lack of clinical practice guidelines, due to the paucity of reliable clinical studies resulting from the rarity of such patients. A more advanced information base can be found in the field of testicular cancer, and some treatment strategies have thus been based on clinical studies of testicular tumors. Fortunately, the prognosis of patients with non-epithelial ovarian tumors is not poor in the early clinical stages, and fertility-sparing operations can be selected although there are some unresolved issues concerning the indication of this type of surgery. Furthermore, established chemotherapies have been associated with a favorable prognosis. Recent advances in molecular biology have identified a variety of genetic alterations in these tumors, some of which can be useful as biomarkers. Further basic research to dissect the molecular mechanisms of carcinogenesis of these tumors is now necessary to develop novel molecular-targeting approaches that can be combined with existing chemotherapeutic regimens, such as BEP (bleomycin, etoposide, and cisplatin), that have been shown to be effective in this type of tumors.

**Keywords**

Granulosa cell tumor • Germ cell tumor • Fertility-sparing surgery • BEP

S. Kyo, M.D., Ph.D.
Department of Obstetrics and Gynecology, Shimane University Faculty of Medicine,
89-1, Enya-Cho, Izumo, Shimane 693-8501, Japan
e-mail: satoruky@med.shimane-u.ac.jp



H. Katabuchi (ed.), *Frontiers in Ovarian Cancer Science*, Comprehensive Gynecology and Obstetrics, DOI 10.1007/978-981-10-4160-0_10

## 10.1 Introduction

Malignant non-epithelial ovarian tumors are relatively rare, but account for approximately 10% of ovarian malignancies. Although there are few reliable clinical trials on the treatment of these tumors, surgical procedures and appropriate chemotherapy regimens have now been established. Each of these tumors has characteristic clinical features that are helpful for proper preoperative diagnosis. In the latest World Health Organization (WHO) classification guidelines for ovarian cancer [1], non-epithelial tumors encompass a large variety of types, including mesenchymal tumors (low- and high-grade endometrioid stromal tumors), mixed epithelial and stromal tumors (adenosarcomas and carcinosarcomas), pure stromal tumors (e.g., fibromas and thecomas), pure sex cord-stromal tumors (SCSTs, e.g., adult granulosa cell tumors or AGCTs and juvenile granulosa cell tumors or JGCTs), mixed SCSTs (e.g., Sertoli-Leydig cell tumors), and germ cell tumors (see Table 6.1 in Chap. 6). Considering relatively high prevalence of SCSTs and malignant ovarian germ cell tumors (MOGCTs) in malignant ovarian tumors, this chapter focuses on the management strategies for these tumors, with a discussion on the molecular aspects of each.

## 10.2 Ovarian Sex Cord-Stromal Tumors (SCSTs)

### 10.2.1 Clinical Features of Ovarian SCSTs

In Japanese population, the SCSTs account for 0.3–0.5% of malignant ovarian neoplasia [2–4]. Among the various SCSTs, two types of pure sex cord tumor, namely, AGCTs and JGCTs, are representative. They are usually characterized by age at diagnosis, with the former commonly arising in perimenopausal and early postmenopausal women and the latter in younger patients (most often 10–30 years of age). Although patient age is informative, clinical symptoms are variable in SCSTs, and a definitive diagnosis can only be made by pathological examination of the dissected tumors. Approximately 50% of patients with granulosa cell tumor (GCT) exhibit estrogen-related symptoms, such as atypical bleeding and menstrual disorders, and may have abdominal symptoms, including distension and pain. Elevation of serum estradiol (E2) levels is representative of this disease, but is only observed in 70% of patients [5], meaning that it has limitations as a diagnostic marker and that a diagnosis of GCT cannot therefore be ruled out simply by the absence of elevated serum E2.

Some differences in clinical behavior are observed between AGCTs and JGCTs, with JGCTs appearing to have more favorable clinical outcome with less likelihood of recurrence and metastasis. However, when recurrence occurs in JGCT, it is typically early (within a few years), while AGCTs are likely to have late onset of recurrence [6]. About 80–90% of SCSTs are diagnosed as Stage I, and 95% are unilateral. The SEER (Surveillance, Epidemiology, and End Results) Program of the National Cancer Institute (NCI) has demonstrated that 5-year

survival of Stage I and II patients is excellent (95%), but is poorer in Stage III and IV patients (59%), suggesting that surgical staging may be as important in GCTs as it is in epithelial ovarian cancer [7]. Of additional clinical relevance is the accompaniment of endometrial disorders alongside GCTs caused by tumor-produced estrogen, with 50% of patients having endometrial hyperplasia and up to 10% having endometrial cancer. This is an important issue because the presence of such disorders, particularly endometrial cancer, may affect operative procedures such as the addition of pelvic and para-aortic lymphadenectomy. Preoperative and postoperative evaluation of the endometrium is therefore required to detect endometrial neoplasms.

Although Sertoli-Leydig cell tumors are representative of the mixed type of SCSTs, they are rare and account for <0.5% of ovarian neoplasms, in which moderately and poorly differentiated forms are more common [1]. Sertoli-Leydig cell tumors have been reported in patients with a wide range of ages, but with a mean age of 25 years [8]. Between 40% and 60% of patients are virilized, while occasional patients have estrogenic manifestations [9]. Androgenic manifestations include amenorrhea, hirsutism, breast atrophy, clitoral hypertrophy, and hoarseness [1]. Patients typically present with abdominal pain, ascites, or tumor rupture. About 2–3% of tumors are found to have spread beyond the ovary at presentation, but lymph node metastases are rare [1]. The prognosis of Sertoli-Leydig cell tumors is favorable overall, but this depends significantly on the particular grade. Well-differentiated tumors are associated with close to 100% survival, while tumors with moderate differentiation are clinically malignant in about 10% of cases. Poorly differentiated tumors behave in a malignant fashion, with recurrence usually within 2 years and occurring in the peritoneal cavity [1].

### 10.2.2 Molecular Aspects of Ovarian SCSTs

No reports exist regarding genetic susceptibility to AGCT and in families with multiple AGCTs. There are few somatic molecular abnormalities in AGCTs, but recent molecular analyses have identified a frequent somatic mutation in approximately 95% of AGCTs in the *FOXL2* (forkhead box protein L2) gene, which encodes a nuclear transcription factor expressed mainly in the adult ovary and which is critically important for the development of granulosa cells [9]. The reported somatic mutation in *FOXL2* is a recurrent missense mutation in codon C134W (402C>G). Of particular interest is that this mutation is rare in other types of SCST, suggesting that it is specific to AGCTs. It may therefore be useful as a molecular marker for the differential diagnosis of SCSTs, especially in cases with equivocal clinical features.

In contrast to *FOXL2* mutations, FOXL2 expression itself is specific to most SCSTs, and immunostaining for this protein can therefore be used as a marker for these tumors. FOXL2 immunostaining has shown higher sensitivity for the diagnosis of SCSTs compared to α-inhibin and calretinin, the two traditional

immunomarkers for SCSTs, and FOXL2 staining is typically more intense in positive cases than either [10]. In SCSTs that are negative for FOXL2 expression, α-inhibin and/or calretinin immunostaining has been shown to yield positive results [9]. Thus, FOXL2 is a sensitive and specific marker for SCSTs. Although most AGCTs carry a somatic mutation in the *FOXL2* gene, the mutation does not affect expression of the protein, and positive immunostaining has thus also been confirmed in AGCTs. In summary, FOXL2 staining is detectable in nearly all SCST cases, even those with a FOXL2 mutation, and that together with α-inhibin and calretinin, forms part of an immunomarker panel that results in positive staining with at least one marker in essentially all cases of SCST.

In contrast to AGCTs, JGCTs arise in the context of a variety of genetic syndromes, including Ollier's disease (a rare bone disease characterized by multiple enchondromatosis) and Maffucci's syndrome (enchondromatosis with hemangiomas) [6, 11, 12]. In Ollier's disease and Maffucci's syndrome, somatic mutations in *IDH1* (isocitrate dehydrogenase 1) and *IDH2* (isocitrate dehydrogenase 2) have been frequently reported, suggesting that mutation of these genes plays a key role in the pathogenesis of these diseases [13]. Somatic *DICER1* (a gene encoding an RNase III endonuclease involved with the processing of microRNA) mutations have occasionally been reported in JGCTs, with one study describing low-frequency (1 out of 14 patients) "hotspot" mutations in the gene [14]. In contrast, mutations in *DICER1* are found in 60% of Sertoli-Leydig cell tumors [14]. Germline mutations are also seen in familiar multinodular goiter with Sertoli-Leydig cell tumors, and tumor susceptibility includes pleuropulmonary blastoma in childhood [1]. Sertoli-Leydig cell tumors have been associated with cervical embryonal rhabdomyosarcoma in four cases [1].

In conclusion, the characteristic genetic difference between AGCTs and JGCTs is the status of *FOXL2* gene. The former tumors have very frequent mutations in *FOXL2,* while the latter tumors rarely have them, suggesting that AGCTs and JGCTs arise in different molecular pathways. Sertoli-Leydig cell tumors frequently have mutations in *DICER1*.

### 10.2.3 Treatment Strategy of Ovarian SCSTs

The key to success in the treatment is surgery. Considering the relatively worse 5-year survival of advanced cases (59% in Stages III and IV) [7], primary surgery should have the basic aim of tumor debulking, including the complete dissection of peritoneal disseminations, as well as strict surgical staging [15]. Retrospective studies have reported that retroperitoneal lymph node metastasis is very rare in SCSTs [16] and that lymphadenectomy can therefore be omitted [15]. One important issue is that preoperative and intraoperative differential diagnoses of GCTs from epithelial ovarian cancers are occasionally difficult. It is essential, therefore, not to delay radical surgeries, including lymphadenectomy and staging laparotomy, in such situations [15].

Fertility-sparing surgery for SCSTs has been accepted due to the rarity of bilateral occurrence (especially in Stage I disease) and because of the excellent prognosis for these patients, with the 5-year survival of Stage I–II patients being reported as 95% [4]. In particular, most patients with JGCTs are candidates for fertility-sparing surgery, considering the age of the patients. However, while radical surgery and adjuvant chemotherapy are recommended by some clinicians for better prognosis, quality of life and long-term morbidity should be considered for such young patients. Although Stage IA disease appears to be an appropriate indication for fertility-sparing surgery, it remains unclear whether this approach should be recommended for patients with Stage IC or more advanced disease, with the indication for Stage IC disease being particularly controversial.

Adjuvant chemotherapy is not recommended for GCTs with Stage I disease, because most cases can be cured by surgery alone, without recurrence. This concept is based on the biology of such indolent tumors, in that they usually have slow growth rates and are less effective to chemotherapy compared with faster-growing tumors. Furthermore, slow growth generates longer disease-free intervals, even without chemotherapy. Nevertheless, some researchers recommend chemotherapy for Stage IC disease in the presence of poor prognostic factors, such as nuclear atypia, high mitotic index, aneuploidy, or age >40 years [17]. Adjuvant therapy may be considered for patients with more advanced stages, residual tumor burden, or risk factors for recurrence, although there is no strong evidence to support prognostic improvement, and considerable caution is required given that adjuvant chemotherapy for young patients is likely to significantly affect long-term morbidity and quality of life. The risk factors for Stage I disease have been found to be a rupture of the membranes, a tumor diameter more than 10–15 cm, poorly differentiated Sertoli-Leydig tumors, and moderately differentiated Sertoli-Leydig tumors with heterologous elements [15].

In adjuvant therapy, combination chemotherapies with cisplatin, vinblastine, and bleomycin (PVB) or with bleomycin, etoposide, and cisplatin (BEP) have been used, with an EORTC (European Organization for Research and Treatment of Cancer) study using PVB in 38 AGCT patients (7 primary and 31 recurrent cases) exhibiting a 61% response rate [18], while a Gynecologic Oncology Group (GOG) study using BEP in 57 SCST patients (16 primary and 41 recurrent cases) had a 37% response rate [19]. Taxanes in conjunction with cisplatin have also been used for GCTs, with relatively high response rates (54%) observed [20]. However, there have been no randomized control trials (RCTs) comparing BEP and taxane-based chemotherapies, and for the time being, BEP appears to be the standard regimen for the treatment of GCTs.

The Japan Society of Gynecologic Oncology published the guidelines for the treatment of ovarian tumors [21], and the flow chart for the treatment of SCSTs is shown in Fig. 10.1.

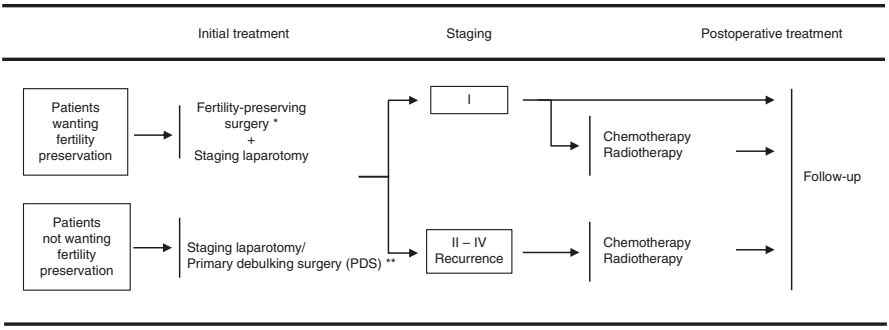



**Fig. 10.1** Treatment of malignant sex cord-stromal tumors. *Fertility-preserving surgery—affected-side salpingo-oophorectomy + omentectomy + peritoneal cytology + detailed intra-abdominal examination. **Lymph node dissection (biopsy) can be omitted. Reprint with permission from ref. 21

## 10.3 Malignant Ovarian Germ Cell Tumors (MOGCTs)

### 10.3.1 Clinical Features of MOGCTs

In Japanese population, the MOGCTs account for 3–4% of malignant ovarian neoplasia [2–4] and have very characteristic clinical features. Firstly, they represent 80% of preadolescent ovarian malignancies. Secondly, they have excellent sensitivity to chemotherapy. Thirdly, most cases show unilateral occurrence. These features permit the possibility of fertility-sparing treatment in such patients.

Malignant transformation in ovarian mature cystic teratoma is the most frequent type of MOGCT, accounting for 38% of MOGCT patients in Japan, while yolk sac tumors, dysgerminomas, and immature teratomas account for 23%, 17%, and 11%, respectively. Grading of immature teratoma is a recent important issue, with these tumors having been graded from 1 to 3, depending on the amount of immature neuroectodermal component in tissue specimens [22]. Recently, however, a two-tiered (low- and high-grade) system has been more commonly used [23]. In this new system, Grade 1 is categorized as low grade, while Grades 2 and 3 are classified as high grade. The latter is considered as an indication for chemotherapy irrespective of clinical staging, but chemotherapy can be omitted in low-grade (Grade 1) tumors. The recurrence rates of immature teratoma are 18%, 37%, and 70%, in Grade 1, 2, and 3 tumors, respectively [22], with 3-year disease-free survivals after fertility-sparing surgery being 100%, 70%, and 66% [24], respectively. While most MOGCTs have extremely high sensitivity to chemotherapy, dysgerminomas have high sensitivity to irradiation as well, and this can therefore be a potent tool for local control of such tumors. Yolk sac tumors, embryonal carcinomas, and non-gestational choriocarcinomas are rare and sometimes have mixed components of each histology type. Since tumor diameter and histological type are considered as important prognostic factors in these mixed germ cell tumors, careful pathological examination is required, with a sufficient number of histological sections [20, 24]. Large tumors of

high-grade immature teratoma, or those composed of yolk sac or choriocarcinoma components in over one third of histological specimens, have a worse prognosis, while tumors with <10 cm diameter have good overall prognosis irrespective of the histological composition [25].

The initial symptoms and signs of MOGCTs include subacute pain or palpation of the pelvic mass, which are observed in 80–90% of patients [26]. Some present as acute abdominal cases due to rupture of the membranes, bleeding from tumors, or torsion. It should be noted that it is not uncommon to find that patients being treated for appendicitis or other abdominal conditions, especially those that are young or preadolescent, are occasionally diagnosed during surgery as having these tumors.

Elevation of specific tumor markers is one of the characteristics of MOGCTs, in particular AFP (alpha-fetoprotein) for yolk sac tumors, hCG (human chorionic gonadotropin) for choriocarcinomas, LDH (lactate dehydrogenase) for dysgerminomas, and SCC (squamous cell carcinoma antigen) for malignant transformation of mature cystic teratomas. However, there are a considerable number of patients without significant elevation of these markers, meaning that their diagnostic value is limited. Nevertheless, their expression can be useful to monitor residual postoperative tumor burden, as well as treatment efficacy and recurrence during follow-up.

The clinical stage of MOGCTs is determined according to the guidelines established for epithelial ovarian cancers. Extraovarian lesions of MOGCTs mainly consist of retroperitoneal lymph node metastases and peritoneal dissemination. A SEER study of 760 cases of MOGCT reported that 76% of cases were Stages I and II, while 24% were Stages III and IV [27]. The prognostic factors for MOGCT have been studied by multivariate analysis, with clinical stage and preoperative levels of tumor marker (AFP and hCG) found to be independent prognostic factors for survival in one report [28], while SEER has reported that patient age at diagnosis, clinical stage, and histological type (i.e., yolk sac tumor) were independent prognostic factors [27]. SEER also reported that patients with retroperitoneal metastasis have significantly worse 5-year survival compared to those without retroperitoneal metastasis (83% vs. 96%) and that retroperitoneal metastasis is another independent prognostic factor [29].

### 10.3.2 Molecular Aspects of Ovarian Germ Cell Tumors

A wide variety of molecular studies, including genome sequencing and transcriptome profiling, have characterized the biological features of MOGCTs and their potential biomarkers. The characteristic features reported for the main histological subtypes of MOGCTs are summarized in Fig. 10.2, with pure dysgerminoma and yolk sac tumors having been found to be mainly non-diploid (i.e., tetraploid, polyploid, or aneuploid), while only 8% of immature teratomas are thought to be non-diploid [30]. DNA copy number analyses have revealed that part or whole gains of chromosomal arm 12p are frequent among both MOGCTs and testicular germ cell tumors [31]. A transcriptome profiling study comparing dysgerminoma and yolk

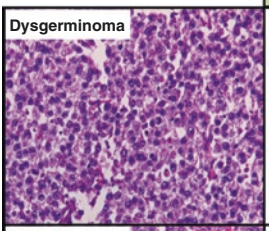
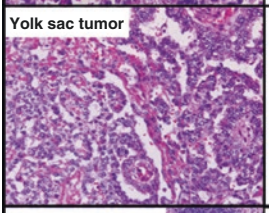
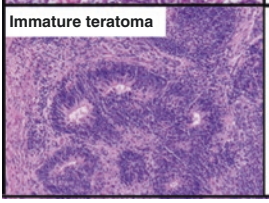
| | Ploidy | CGH | miRNA | mRNA | Protein |
|---|---|---|---|---|---|
|  Dysgerminoma | Non-diploid (91%) | Gain 1q<br>7q<br>8<br>12<br>19<br>21<br>Loss 13 | miR-146b-5p<br>miR-155<br>miR-182 | CASP8<br>CDH3<br>CXCL10<br>IL6R<br>NANOG<br>PDPN<br>PLBD1<br>POU5F1 | GATA4<br>KIT<br>KRT8<br>LIN28A<br>NANOG<br>PAD14<br>PDPN<br>POU5F1<br>SMAD3<br>TFAP2C |
|  Yolk sac tumor | Non-diploid (86%) | Gain 1q<br>12p<br>20q<br>Loss 1p | miR-122<br>miR-200b<br>miR-200c<br>miR-302a<br>miR-302c<br>miR-375<br>miR-638 | BMP1<br>TGFB2 | AE1/AE3<br>AFP<br>FUT4<br>GATA4<br>GATA6<br>GGT1<br>GPC3<br>LIN28A<br>PAD14 |
|  Immature teratoma | Diploid (92%) | No Characteristic alterations | No specific miRNAs | Non-studied | LIN28A<br>PAD14<br>SOX2 |

**Fig. 10.2** Representative molecular characteristics of the main histological subtypes of MOGCTs. Hematoxylin- and eosin-stained sections of the subtypes are illustrated to the left; dysgerminoma (growing with sheets or nests of polygonal cells with round vesicular nuclei, abundant clear cytoplasm with glycogen, and well-defined cell membranes), yolk sac tumor (commonly with reticular (*left side*) and endodermal sinus (*right side*) growth patterns) and immature teratoma (with variable amounts of immature embryonal-type tissues, mostly in the form of neuroectodermal tubules and rosettes (as shown)). The typical diploid or non-diploid, copy number alterations reported in ≥30% of the subtypes and aberrantly expressed miRNAs, mRNAs, and proteins are also listed. Adapted with alterations from Endocr Rev. 2013; 34: 339–376

sac tumors revealed that a subset of eight WNT/β-catenin signaling components is sufficient to distinguish between the two histological subtypes [32]. Immunohistochemical analysis from the same study indicated that cytoplasmic β-catenin is expressed in all histological subtypes, but with only weak focal staining in dysgerminoma, and that β-catenin nuclear accumulation is observed only in yolk sac tumors and teratomas [32]. Other work has indicated that the IL6R (interleukin 6 receptor) and C-X-C motif chemokine 10 (CXCL10), known to be involved in cytokine signaling and immune responses, are overexpressed in dysgerminomas [30]. Upregulation of IL6R expression prevents premature entry into meiosis and maintains an immature germ cell population in the human fetal ovary [33]. On the other hand, the expression of CXCL10 and its receptor CXCR3 can lead to tumor recruitment of T-lymphocytes [34], which is in keeping with the observation of infiltration of T-lymphocytes in dysgerminoma, although the biological function and significance of this phenomenon remains unclear.

The pluripotency genes, NANOG (nanog homeobox), POU5F1 (POU domain, class 5, transcription factor 1), POU5F1B (POU domain, class 5, transcription factor 1B), and PDPN (podoplanin), have also been found to be overexpressed in dysgerminoma [35] and seminoma [36]. The fact that the expression pattern for these genes is similar between dysgerminoma and seminoma indicates that common tumorigenic pathways exist for a subgroup of ovarian and testicular germ cell tumors and/or that such expression patterns represent the remnant traits of their mutual precursor, i.e., the primordial germ cell. Other groups have reported that the cell signaling genes *BMP1* (bone morphogenetic protein 1) and *TGFB2* (transforming growth factor-beta 2) are overexpressed in yolk sac tumors [32, 37]. The TGF-β/BMP signaling pathway regulates embryonic development, and its biological relevance is underlined by the fact that mutations in the BMP receptor Alk6b (activin receptor-like kinase 6b) impairs germ cell differentiation and initiates germ cell tumors in zebra fish [38].

Several microRNA (miRNA) expression profiling studies have identified that two miRNA clusters, namely, miR-302-367 and miR-371-373, are overexpressed in MOGCTs when compared with nonmalignant control tissues [35, 37, 39, 40]. The coordinate overexpression of these miRNAs appears to be specific for MOGCTs, with no similar findings having been reported for other malignancies or diseases to date. Gene ontology analysis has shown that the downregulated mRNA targets for miR-302-367 and miR-371-373 mediate cellular processes important in oncogenesis and malignant progression, supporting the functional significance of these miRNA clusters in the biology of MOGCTs [35]. On the other hand, the most significantly overexpressed miRNA in yolk sac tumors has been reported to be miR-375 [37, 40]. Dysregulation of miR-375 has been observed for various tumor types, including head and neck, esophageal, lung, and gastric cancers [30]. Signaling pathway analyses of miR-375-regulated genes have indicated the involvement of cell cycle regulation, focal adhesion, MAPK (mitogen-activated protein kinase), TGF-β, WNT, and VEGF (vascular endothelial growth factor) pathways [41]. In dysgerminoma, three other miRNAs have been identified as being highly expressed, namely, miR-0146b-5p, miR-155, and miR-182 [37, 40]. Although the specific functions of these miRNAs remain unclear, they are known to be overexpressed in other tumor types, including breast, lung, cervix, and colon cancers, and interactions with *BRCA1* (breast cancer associated gene 1) have been reported [30].

In regard to potential biomarkers for MOGCTs, protein expression analyses have indicated that pluripotency/developmental factors and histology-specific markers may be the two most important functional categories. POU5F1 and NANOG are significantly expressed more often in dysgerminoma, for example, supporting their application as biomarkers for this subtype [42, 43]. The pluripotency factor SOX2 (sex-determining region Y-box 2), on the other hand, has been shown to be more significantly expressed in immature teratoma and to be very specific to this subtype [30, 43]. Primordial germ cells do not express SOX2 and remain capable of proliferation, and thus the absence of SOX2 expression in dysgerminoma underlines their strong resemblance to this progenitor cell type.

In addition to POU5F1, PDNP has also been proposed as a diagnostic marker of dysgerminoma [30]. In contrast, the differential diagnosis of yolk sac tumor is difficult due to its complex and varied histological appearance, especially between yolk sac tumor and clear cell carcinoma of the ovary, and good markers for this tumor type are limited. Mixed tumors exhibit further complexity, with small components of yolk sac tumor growing in close proximity to other subtypes such as immature teratoma. In the past, AFP has been a famous tumor marker for yolk sac tumors [44], but the diagnostic use of AFP immunohistochemistry has low sensitivity and specificity [45]. Alternatively, the transcription factor GATA6 (GATA-binding factor 6) has been shown to be more frequently expressed in yolk sac tumors than dysgerminomas, with GATA4 (GATA-binding factor 4) being expressed in dysgerminoma, yolk sac tumors, and immature teratomas [46]. The differential expression pattern of GATA4 and GATA6 may thus be used as a marker to distinguish between yolk sac tumors and dysgerminomas.

### 10.3.3 Treatment Strategy of Ovarian Germ Cell Tumor

Surgery is the primary treatment of MOGCTs. Since most patients with MOGCTs are of preadolescent or reproductive ages and have unilateral tumors, fertility-sparing surgery should be considered, especially considering the fact that patients with MOGCTs are extremely sensitive to chemotherapy. Unilateral salpingo-oophorectomy of the affected side with omentectomy and peritoneal cytology are the basic procedures in operation for MOGCTs. A routine biopsy of the contralateral ovary should be avoided to preserve ovarian function, unless macroscopic findings are detected [47]. However, since dysgerminoma occasionally (in 10–15% of cases) occurs bilaterally, careful examination of the contralateral ovary is necessary [48]. Stage III and IV patients who desire fertility-sparing surgery can be permitted this option, with a focus on tumor debulking [47, 49], based on the evidence that fertility-sparing surgery does not adversely affect prognosis [26, 27, 50–52].

Intraoperative frozen section analysis is necessary irrespective of the type of surgery undertaken (fertility-sparing or otherwise). However, the diagnostic accuracy of such an analysis is of limited value, and it is recommended to avoid overtreatment during the operation. In the event that a differential diagnosis is required to distinguish the tumor from types that do not permit fertility-sparing surgery, it may be appropriate to initially perform fertility-sparing surgery without overtreatment and then reoperate if necessary after postoperative pathological examination.

When patients do not require fertility-sparing surgery, standard operative procedures for epithelial ovarian malignancies should be performed, with the addition of pelvic and para-aortic lymphadenectomy, although the prognostic impact of retroperitoneal lymphadenectomy is not proven. A recent

retrospective study of 1083 patients with MOGCTs that were deemed to be at clinical Stage I at the time of surgery reported no significant difference in the 5-year survival between patients with and without retroperitoneal lymphadenectomy, including patients who were upstaged to FIGO (International Federation of Gynecology and Obstetrics) Stage IIIC after lymphadenectomy [53]. On multivariate analysis, lymphadenectomy was not an independent predictor of survival when controlling for age, histology, and race. Moreover, the presence of lymph node metastasis had no significant effect on survival [54]. Thus, neither lymphadenectomy nor lymph node metastasis was an independent predictor of survival in patients with MOGCTs confined to the ovary. This probably reflects the highly chemosensitive nature of these tumors, and retroperitoneal lymphadenectomy can thus be omitted [54].

There are issues about the selection of surgical procedures and postoperative treatments in each tumor type. It remains unresolved whether patients with Stage I (Grade III) immature teratoma, pathologically diagnosed after ovarian cystectomy for mature cystic teratoma, require the addition of adnexectomy [55]. It has been accepted, however, that there is no need for chemotherapy in patients with Stage IA dysgerminoma or Stage IA (Grade I) immature teratoma [47]. Furthermore, in patients with Stage IA dysgerminoma that undergo operation with incomplete surgical staging, chemotherapy can be delayed until there is evidence of relapse, since these tumors have been shown to respond well to chemotherapy upon recurrence [56].

The current standard chemotherapy regimen for MOGCTs is BEP (bleomycin, etoposide, and cisplatin), based on the clinical trial results for testicular germ cell tumors, as well as the excellent cure rates achieved in early-stage patients (almost 100%) and even in advanced patients (at least 75%) [57]. Despite the lack of Phase III trials, BEP is strongly recommended as standard chemotherapy regimen for MOGCTs, although special attention should be paid to guarantee the best outcomes with this approach. Firstly, drug doses should be maintained, without reckless reduction. Only in the case of pyrogenic neutropenia, or thrombocytopenia with bleeding, can a 20% decrease in etoposide be permitted [58]. Secondly, the drugs should not be substituted for alternatives. In testicular tumors, the attempt to omit bleomycin in favor of decreasing pulmonary toxicity has been shown to fail, worsening the prognosis of the patient [59]. Furthermore, a change from cisplatin to carboplatin has also been reported to adversely affect prognosis [60]. Thirdly, treatment schedule compliance is strictly important. Even with the presence of neutropenia, the next cycle of chemotherapy must commence at day 22 [61], and although the presence of severe bone marrow suppression, such as neutropenia <500 per $mm^3$ or thrombocytopenia $<10^5$ per $mm^3$, may permit delay of the next cycle of chemotherapy, it should only do so for a maximum of 3 days [62]. This compliance requirement is thus quite different from more common epithelial tumors of the ovary. Finally, and as mentioned above, postoperative adjuvant chemotherapy with BEP can be omitted in patients with Stage IA dysgerminoma and Stage I (Grade 1)

immature teratoma [44] and is in fact recommended to be omitted in young patients (<15 years old) with immature teratoma [63, 64].

One of the critical issues in chemotherapy for MOGCTs is how many cycles should be performed, since there have been no RCTs to assess the optimal number. Based on GOG78 (in which one arm of the trial performed three cycles of BEP for early-stage MOGCTs), the NCCN (National Comprehensive Cancer Network) guidelines now recommend three cycles of BEP [55, 57]. In the BEP protocol, however, accumulative pulmonary toxicity caused by bleomycin and secondary neoplasms induced by etoposide should be a concern. The rate of occurrence of pulmonary toxicity from bleomycin is 0–2% over three cycles of BEP and is 6–18% over four or more cycles. A pulmonary function test performed during bleomycin therapy is unfortunately not a good predictor of toxicity, since it has been shown to have a relatively low sensitivity and specificity [65, 66]. Secondary neoplasms triggered by etoposide are also accumulative, and the rate of occurrence is very low (0.4%) with a total dose of less than 2000 mg/m$^2$, but increases at doses over 2000 mg/m$^2$ [67]. The threshold for etoposide to induce neoplasms is thus thought to be 2000 mg/m$^2$ [68]. Prognosis of secondary leukemias caused by etoposide is poor, with most cases arising 2–3 years after initial chemotherapy, and it is thus important to monitor closely for occurrence of secondary leukemia when >2000 mg/m$^2$ of etoposide is used [69].

There are unfortunately no RCTs comparing different regimens of chemotherapy for MOGCTs. In testicular tumors, the BEP regimen was compared with etoposide, ifosfamide, and cisplatin (VIP therapy), with no significant difference in long-term prognosis reported, although bone marrow suppression was found to be more prominent in the former [70].

Following postoperative BEP chemotherapy, the failure of ovarian function due to toxicity, as well as secondary neoplasms induced by etoposide, should be cared for in particular. Failure of ovarian function is most frequently observed when cyclophosphamide is used in treatment regimens, but BEP has shown a relatively rare rate of failure for ovarian function. Amenorrhea is frequently (62%) observed during BEP chemotherapy, but 91% of patients undergoing this regimen appear to recover menstruation [71]. In general, 80–90% of patients receiving chemotherapy for MOGCTs eventually recover menstruation following treatment [72]. It has been reported that the incidence of infertility, congenital malformation, and spontaneous abortion do not increase after MOGCT chemotherapy [37, 72–75], and there are several reports suggesting that pretreatment with GnRH (gonadotropin-releasing hormone) analogues or oral contraceptives may protect ovarian function during chemotherapy [76–78]. A randomized trial in breast cancer has reported the preservation of ovarian function by GnRH analogues during chemotherapy [79], but there is no consensus regarding the utility of such protection.

The Japan Society of Gynecologic Oncology published the guidelines for the treatment of ovarian tumors [21], and the flow chart of the treatment of MOGCTs is shown in Fig. 10.3.

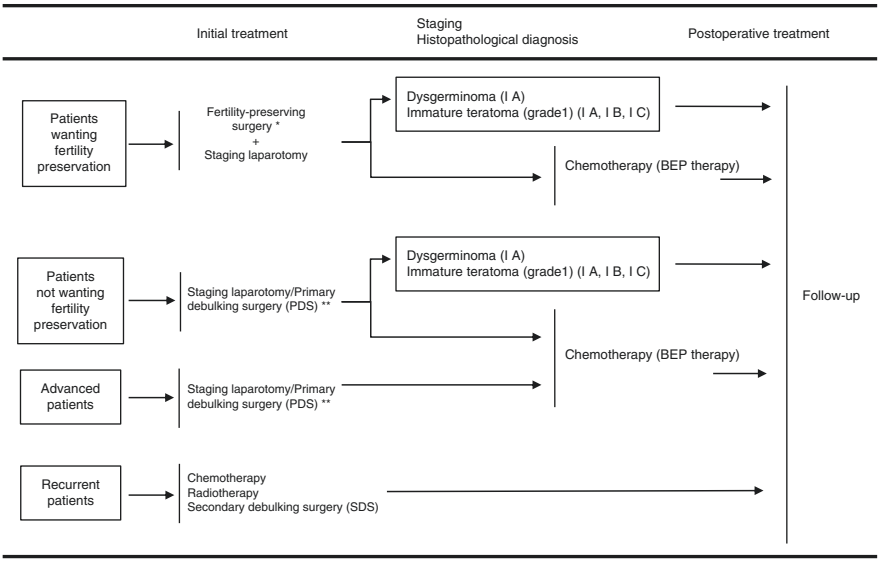



**Fig. 10.3** Treatment of malignant ovarian germ cell tumors. *Fertility-preserving surgery—affected-side salpingo-oophorectomy + omentectomy + peritoneal cytology + detailed intra-abdominal examination. **Lymph node dissection (biopsy) can be omitted. Reprint with permission from ref. 21

## 10.4 Conclusions and Future Directions

Non-epithelial ovarian tumors are rare, and there are few RCTs for the treatment of these tumors. We therefore have limited information in regard to the most appropriate management strategy for these cancers. However, considerable efforts have been made to apply fertility-sparing surgeries for young patients, an approach that has proven to be relatively safe in early-stage tumors. Although pathological diagnosis is occasionally difficult, especially in intraoperative cases, and thus it is not always easy to judge where fertility-sparing surgery may be indicated, it is important that radical surgery be avoided in patients with difficult intraoperative diagnoses. In such cases, fertility-sparing surgery should be performed first, with radical surgery conducted subsequently only if postoperative pathological assessment indicates necessity.

The establishment of BEP chemotherapy has greatly improved outcomes in patients with ovarian germ cell tumors. However, most of the evidence regarding the indications for chemotherapy, as well as the composition of these regimens, has been derived from experience with testicular germ cell tumors, and further evidence from ovarian germ cell tumors is required in the future. Moreover, some issues remain concerning the indication for chemotherapy. Although it is currently standard practice that adjuvant chemotherapy be omitted in patients with Stage IA dysgerminoma and Stage I (Grade 1) immature teratoma, we do not still have conclusive

evidence to support this, and the omission of chemotherapy may be extended to more advanced cases. An additional issue is the need for strict compliance of MOGCT chemotherapy regimens to achieve optimal efficacy, something that is completely different from the situation in epithelial ovarian tumors.

An emerging number of molecular studies have revealed some useful biomarkers for MOGCT tumors, but specific biomarkers for each tumor type are limited. The molecular mechanisms through which these tumors arise remain unclear, and we have no information regarding their cells of origin. It is hoped that future progress in these studies will identify the molecular pathways through which these tumors arise and grow, something that is essential for the development of molecularly targeted therapies. Ultimately, it is hoped that such novel molecular approaches can then be combined with effective conventional chemotherapies such as BEP, an approach that has successfully been applied to the treatment of epithelia ovarian tumors.

## References

1. Kurman RL, Carcangiu ML, Simon Herrington C, Young RH, editors. WHO classification of tumours of female reproductive organs. 4th ed. Lyon: International Agency for Research on Cancer; 2014.
2. Yamagami W, Aoki D. Annual report of the committee on gynecologic oncology, the Japan society of obstetrics and gynecology. J Obstet Gynaecol Res. 2015;41:1861–9.
3. Saito T, Katabuchi H. Annual report of the committee on gynecologic oncology, Japan society of obstetrics and gynecology: patient annual report for 2013 and treatment annual report for 2008. J Obstet Gynaecol Res. 2016;42:1069–79.
4. Yamagami W, Nagase S, Takahashi F, Ino K, Hachisuga T, Aoki D, Katabuchi H. Clinical statistics of gynecologic cancers in Japan. J Gynecol Oncol. 2017;28:e32. Epub 2017 Feb 10.
5. Colombo N, Parma G, Zanagnolo V, Insinga A. Management of ovarian stromal cell tumors. J Clin Oncol. 2007;25:2944–51.
6. Young RH, Dickersin GR, Scully RE. Juvenile granulosa cell tumor of the ovary. A clinicopathological analysis of 125 cases. Am J Surg Pathol. 1984;8:575–96.
7. Zhang M, Cheung MK, Shin JY, Kapp DS, Husain A, Teng NN, et al. Prognostic factors responsible for survival in sex cord stromal tumors of the ovary–an analysis of 376 women. Gynecol Oncol. 2007;104:396–400.
8. Young RH, Scully RE. Ovarian sertoli-leydig cell tumors. A clinicopathological analysis of 207 cases. Am J Surg Pathol. 1985;9:543–69.
9. Shah SP, Köbel M, Senz J, Morin RD, Clarke BA, Wiegand KC, et al. Mutation of FOXL2 in granulosa-cell tumors of the ovary. N Engl J Med. 2009;360:2719–29.
10. Al-Agha OM, Huwait HF, Chow C, Yang W, Senz J, Kalloger SE, et al. FOXL2 is a sensitive and specific marker for sex cord-stromal tumors of the ovary. Am J Surg Pathol. 2011;35:484–94.
11. Vaz RM, Turner C. Ollier disease (enchondromatosis) associated with ovarian juvenile granulosa cell tumor and precocious pseudopuberty. J Pediatr. 1986;108:945–7.
12. Tanaka Y, Sasaki Y, Nishihira H, Izawa T, Nishi T. Ovarian juvenile granulosa cell tumor associated with Maffucci's syndrome. Am J Clin Pathol. 1992;97:523–7.
13. Amary MF, Damato S, Halai D, Eskandarpour M, Berisha F, Bonar F, et al. Ollier disease and Maffucci syndrome are caused by somatic mosaic mutations of IDH1 and IDH2. Nat Genet. 2011;43:1262–5.
14. Heravi-Moussavi A, Anglesio MS, Cheng SW, Senz J, Yang W, Prentice L, et al. Recurrent somatic DICER1 mutations in nonepithelial ovarian cancers. N Engl J Med. 2012;366:234–42.
15. Ovarian Cancer Guideline (Version 1. 2014). NCCN Clinical Practice Guidelines in Oncology.

16. Brown J, Sood AK, Deavers MT, Milojevic L, Gershenson DM. Patterns of metastasis in sex cord-stromal tumors of the ovary: can routine staging lymphadenectomy be omitted? Gynecol Oncol. 2009;113:86–90.
17. Foulkes WD, Gore M, McCluggage WG. Rare non-epithelial ovarian neoplasms: pathology, genetics and treatment. Gynecol Oncol. 2016;142:190–8.
18. Pecorelli S, Wagenaar HC, Vergote IB, Curran D, Beex LV, Wiltshaw E, et al. Cisplatin (P), vinblastine (V) and bleomycin (B) combination chemotherapy in recurrent or advanced granulosa(−theca) cell tumours of the ovary. An EORTC gynaecological cancer cooperative group study. Eur J Cancer. 1999;35:1331–7.
19. Homesley HD, Bundy BN, Hurteau JA, Roth LM. Bleomycin, etoposide, and cisplatin combination therapy of ovarian granulosa cell tumors and other stromal malignancies: a gynecologic oncology group study. Gynecol Oncol. 1999;72:131–7.
20. Brown J, Shvartsman HS, Deavers MT, Ramondetta LM, Burke TW, Munsell MF, et al. The activity of taxanes compared with bleomycin, etoposide, and cisplatin in the treatment of sex cord-stromal ovarian tumors. Gynecol Oncol. 2005;97:489–96.
21. Komiyama S, Katabuchi H, Mikami M, Nagase S, Okamoto A, Ito K, et al. Japan society of gynecologic oncology guidelines 2015 for the treatment of ovarian cancer including primary peritoneal cancer and fallopian tube cancer. Int J Clin Oncol. 2016;21:435–46.
22. Norris HJ, Zirkin HJ, Benson WL. Immature (malignant) teratoma of the ovary: a clinical and pathologic study of 58 cases. Cancer. 1976;37:2359–72.
23. O'Connor DM, Norris HJ. The influence of grade on the outcome of stage I ovarian immature (malignant) teratomas and the reproducibility of grading. Int J Gynecol Pathol. 1994;13:283–9.
24. Mangili G, Scarfone G, Gadducci A, Sigismondi C, Ferrandina G, Scibilia G, et al. Is adjuvant chemotherapy indicated in stage I pure immature ovarian teratoma (IT)? A multicentre Italian trial in ovarian cancer (MITO-9). Gynecol Oncol. 2010;119:48–52.
25. Kurman RJ, Norris HJ. Malignant germ cell tumors of the ovary. Hum Pathol. 1977;8:551–64.
26. Gershenson DM. Management of ovarian germ cell tumors. J Clin Oncol. 2007;25: 2938–43.
27. Chan JK, Tewari KS, Waller S, Cheung MK, Shin JY, Osann K, et al. The influence of conservative surgical practices for malignant ovarian germ cell tumors. J Surg Oncol. 2008;98:111–6.
28. Murugaesu N, Schmid P, Dancey G, Agarwal R, Holden L, McNeish I, et al. Malignant ovarian germ cell tumors: identification of novel prognostic markers and long-term outcome after multimodality treatment. J Clin Oncol. 2006;24:4862–6.
29. Kumar S, Shah JP, Bryant CS, Imudia AN, Cote ML, Ali-Fehmi R, et al. The prevalence and prognostic impact of lymph node metastasis in malignant germ cell tumors of the ovary. Gynecol Oncol. 2008;110:125–32.
30. Kraggerud SM, Hoei-Hansen CE, Alagaratnam S, Skotheim RI, Abeler VM, Rajpert-De Meyts E, et al. Molecular characteristics of malignant ovarian germ cell tumors and comparison with testicular counterparts: implications for pathogenesis. Endocr Rev. 2013;34:339–76.
31. Atkin NB, Baker MC. I(12p): specific chromosomal marker in seminoma and malignant teratoma of the testis? Cancer Genet Cytogenet. 1983;10:199–204.
32. Fritsch MK, Schneider DT, Schuster AE, Murdoch FE, Perlman EJ. Activation of Wnt/β--catenin signaling in distinct histologic subtypes of human germ cell tumors. Pediatr Dev Pathol. 2006;9:115–31.
33. Eddie SL, Childs AJ, Jabbour HN, Anderson RA. Developmentally regulated IL6-type cytokines signal to germ cells in the human fetal ovary. Mol Hum Reprod. 2012;18:88–95.
34. Schweyer S, Soruri A, Baumhoer D, Peters J, Cattaruzza M, Radzun HJ, et al. Expression of CXC chemokine IP-10 in testicular germ cell tumours. J Pathol. 2002;197:89–97.
35. Palmer RD, Barbosa-Morais NL, Gooding EL, Muralidhar B, Thornton CM, Pett MR, et al. Children's cancer and Leukaemia group. Pediatric malignant germ cell tumors show characteristic transcriptome profiles. Cancer Res. 2008;68:4239–47.
36. Looijenga LH, Hersmus R, Gillis AJ, Pfundt R, Stoop HJ, van Gurp RJ, et al. Genomic and expression profiling of human spermatocytic seminomas: primary spermatocyte as tumorigenic precursor and *DMRT1* as candidate chromosome 9 gene. Cancer Res. 2006;66: 290–302.

37. Fustino N, Rakheja D, Ateek CS, Neumann JC, Amatruda JF. Bone morphogenetic protein signalling activity distinguishes histological subsets of paediatric germ cell tumours. Int J Androl. 2011;34:e218–33.
38. Neumann JC, Chandler GL, Damoulis VA, Fustino NJ, Lillard K, Looijenga L, et al. Mutation in the type IB bone morphogenetic protein receptor *alk6b* impairs germ-cell differentiation and causes germ-cell tumors in zebrafish. Proc Natl Acad Sci U S A. 2011;108:13153–8.
39. Gillis AJ, Stoop HJ, Hersmus R, Oosterhuis JW, Sun Y, Chen C. High-throughput microRNAome analysis in human germ cell tumours. J Pathol. 2007;213:319–28.
40. Murray M, Saini H, van Dongen S, Palmer RD, Muralidhar B, Pett MR, et al. The two most common histological subtypes of malignant germ cell tumour are distinguished by global microRNA profiles, associated with differential transcription factor expression. Mol Cancer. 2010;9:290.
41. Zhang X, Yan Z, Zhang J, Gong L, Li W, Cui J, et al. Combination of hsa-miR-375 and hsa-miR-142–5p as a predictor for recurrence risk in gastric cancer patients following surgical resection. Ann Oncol. 2011;22:2257–66.
42. Hoei-Hansen CE, Kraggerud SM, Abeler VM, Kaern J, Rajpert-De Meyts E, Lothe RA. Ovarian dysgerminomas are characterised by frequent KIT mutations and abundant expression of pluripotency markers. Mol Cancer. 2007;6:12.
43. Chang MC, Vargas SO, Hornick JL, Hirsch MS, Crum CP, Nucci MR. Embryonic stem cell transcription factors and D2–40 (podoplanin) as diagnostic immunohistochemical markers in ovarian germ cell tumors. Int J Gynecol Pathol. 2009;28:347–55.
44. Palmer PE, Wolfe HJ. Immunocytochemical localization of oncodevelopmental proteins in human germ cell and hepatic tumors. J Histochem Cytochem. 1978;26:523–31.
45. Esheba GE, Pate LL, Longacre TA. Oncofetal protein glypican-3 distinguishes yolk sac tumor from clear cell carcinoma of the ovary. Am J Surg Pathol. 2008;32:600–7.
46. Mannisto S, Butzow R, Salonen J, Leminen A, Heikinheimo O, Heikinheimo M. Transcription factors GATA-4 and GATA-6, and their potential downstream effectors in ovarian germ cell tumors. Tumor Biol. 2005;26:265–73.
47. NCI Ovarian Germ Cell Tumors treatment (PDQ) Health Professional Version.
48. Pectasides D, Pectasides E, Kassanos D. Germ cell tumors of the ovary. Cancer Treat Rev. 2008;34:427–41.
49. Williams SD, Blessing JA, DiSaia PJ, Major FJ, Ball HG 3rd, Liao SY. Second-look laparotomy in ovarian germ cell tumors: the gynecologic oncology group experience. Gynecol Oncol. 1994;52:287–91.
50. Gershenson DM. Management of early ovarian cancer: germ cell and sex cord-stromal tumors. Gynecol Oncol. 1994;55:S62–72.
51. Gershenson DM, Miller AM, Champion VL, Monahan PO, Zhao Q, Cella D, et al. Reproductive and sexual function after platinum-based chemotherapy in long-term ovarian germ cell tumor survivors: a gynecologic oncology group study. J Clin Oncol. 2007;25:2792–7.
52. Peccatori F, Bonazzi C, Chiari S, Landoni F, Colombo N, Mangioni C. Surgical management of malignant ovarian germ-cell tumors: 10 years' experience of 129 patients. Obstet Gynecol. 1995;86:367–72.
53. Mahdi H, Swensen RE, Hanna R, Kumar S, Ali-Fehmi R, Semaan A, et al. Prognostic impact of lymphadenectomy in clinically early stage malignant germ cell tumour of the ovary. Br J Cancer. 2011;105:493–7.
54. Beiner ME, Gotlieb WH, Korach Y, Shrim A, Stockheim D, Segal Y, et al. Cystectomy for immature teratoma of the ovary. Gynecol Oncol. 2004;93:381–4.
55. Ovarian Cancer Guideline (Version 1. 2014). NCCM Clinical Practice Guidelines in Oncoloft.
56. Mangili G, Sigismondi C, Lorusso D, Cormio G, Scollo P, Viganò R, et al. Is surgical restaging indicated in apparent stage IA pure ovarian dysgerminoma? The MITO group retrospective experience. Gynecol Oncol. 2011;121:280–4.
57. Williams S, Blessing JA, Liao SY, Ball H, Hanjani P. Adjuvant therapy of ovarian germ cell tumors with cisplatin, etoposide, and bleomycin: a trial of the gynecologic oncology group. J Clin Oncol. 1994;12:701–6.

58. Nichols CR, Catalano PJ, Crawford ED, Vogelzang NJ, Einhorn LH, Loehrer PJ. Randomized comparison of cisplatin and etoposide and either bleomycin or ifosfamide in treatment of advanced disseminated germ cell tumors: an eastern cooperative oncology group, southwest oncology group, and cancer and leukemia group B study. J Clin Oncol. 1998;16:1287–93.
59. Loehrer PJ Sr, Johnson D, Elson P, Einhorn LH, Trump D. Importance of bleomycin in favorable-prognosis disseminated germ cell tumors: an eastern cooperative oncology group trial. J Clin Oncol. 1995;13:470–6.
60. Bajorin DF, Sarosdy MF, Pfister DG, Mazumdar M, Motzer RJ, Scher HI, et al. Randomized trial of etoposide and cisplatin versus etoposide and carboplatin in patients with good-risk germ cell tumors: a multiinstitutional study. J Clin Oncol. 1993;11:598–606.
61. Einhorn LH, Williams SD, Loehrer PJ, Birch R, Drasga R, Omura G, et al. Evaluation of optimal duration of chemotherapy in favorable-prognosis disseminated germ cell tumors: a southeastern cancer study group protocol. J Clin Oncol. 1989;7:387–91.
62. Krege S, Beyer J, Souchon R, Albers P, Albrecht W, Algaba F, et al. European consensus conference on diagnosis and treatment of germ cell cancer: a report of the second meeting of the European germ cell cancer consensus group (EGCCCG): part II. Eur Urol. 2008;53:497–513.
63. Marina NM, Cushing B, Giller R, Cohen L, Lauer SJ, Ablin A, et al. Complete surgical excision is effective treatment for children with immature teratomas with or without malignant elements: a pediatric oncology group/children's cancer group intergroup study. J Clin Oncol. 1999;17:2137–43.
64. Cushing B, Giller R, Ablin A, Cohen L, Cullen J, Hawkins E, et al. Surgical resection alone is effective treatment for ovarian immature teratoma in children and adolescents: a report of the pediatric oncology group and the children's cancer group. Am J Obstet Gynecol. 1999;181:353–8.
65. McKeage MJ, Evans BD, Atkinson C, Perez D, Forgeson GV, Dady PJ. Carbon monoxide diffusing capacity is a poor predictor of clinically significant bleomycin lung. New Zealand clinical oncology group. J Clin Oncol. 1990;8:779–83.
66. Lewis BM, Izbicki R. Routine pulmonary function tests during bleomycin therapy. Tests may be ineffective and potentially misleading. JAMA. 1980;243:347–51.
67. Kollmannsberger C, Hartmann JT, Kanz L, Bokemeyer C. Therapy-related malignancies following treatment of germ cell cancer. Int J Cancer. 1999;83:860–3.
68. Pedersen-Bjergaard J, Daugaard G, Hansen SW, Philip P, Larsen SO, Rørth M. Increased risk of myelodysplasia and leukaemia after etoposide, cisplatin, and bleomycin for germ-cell tumours. Lancet. 1991;338:359–63.
69. Ezoe S. Secondary leukemia associated with the anti-cancer agent, etoposide, a topoisomerase II inhibitor. Int J Environ Res Public Health. 2012;9:2444–53.
70. Hinton S, Catalano PJ, Einhorn LH, Nichols CR, David Crawford E, Vogelzang N, et al. Cisplatin, etoposide and either bleomycin or ifosfamide in the treatment of disseminated germ cell tumors: final analysis of an intergroup trial. Cancer. 2003;97:1869–75.
71. Nicosia SV, Matus-Ridley M, Meadows AT. Gonadal effects of cancer therapy in girls. Cancer. 1985;55:2364–72.
72. Low JJ, Perrin LC, Crandon AJ, Hacker NF. Conservative surgery to preserve ovarian function in patients with malignant ovarian germ cell tumors. A review of 74 cases. J Gynecol Oncol. 2014;25:174–82.
73. Gershenson DM. Menstrual and reproductive function after treatment with combination chemotherapy for malignant ovarian germ cell tumors. J Clin Oncol. 1988;6:270–5.
74. Brewer M, Gershenson DM, Herzog CE, Mitchell MF, Silva EG, Wharton JT. Outcome and reproductive function after chemotherapy for ovarian dysgerminoma. J Clin Oncol. 1999;17:2670–5.
75. Segelov E, Campbell J, Ng M, Tattersall M, Rome R, Free K, et al. Cisplatin-based chemotherapy for ovarian germ cell malignancies: the Australian experience. J Clin Oncol. 1994;12:378–84.
76. Meistrich ML, Shetty G. Hormonal suppression for fertility preservation in males and females. Reproduction. 2008;136:691–701.

77. Blumenfeld Z. GnRH-agonists in fertility preservation. Curr Opin Endocrinol Diabetes Obes. 2008;15:523–8.
78. Weinberg LE, Lurain JR, Singh DK, Schink JC. Survival and reproductive outcomes in women treated for malignant ovarian germ cell tumors. Gynecol Oncol. 2011;121:285–9.
79. McLaren JF, Bates GW. Fertility preservation in women of reproductive age with cancer. Am J Obstet Gynecol. 2012;207:455–62.