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Assessment of Ovarian Function Following Preparative Chemotherapy and Total Body Radiation for Adoptive Cell Therapy

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Abstract

To analyze treatment induced gonadal damage and premature ovarian failure after adoptive cell therapy (ACT) following a cytotoxic lymphodepleting preparative regimen. Records of 66 consecutive females who received adoptive cell therapy at the Surgery Branch, National Cancer Institute, NIH (Bethesda, Maryland) were reviewed. Patients received a conditioning regimen of high dose cyclophosphamide (60mg/kg $\times$ 2 doses) and fludarabine (25mg/m² $\times$ 5 doses). Some patients also received total body radiation (TBI) at 200 or 600 cGy. Assessment of ovarian function was determined by analysis of monthly FSH levels, menstrual history and symptoms. Among patients with serum available and normal pre-treatment ovarian function, 21 had a preparative regimen with chemotherapy alone and 5 patients had received chemotherapy with total body radiation. 9 (43%) patients in the chemotherapy cohort and all five patients in the chemotherapy plus TBI cohort had persistently elevated FSH levels and were given the diagnosis of premature ovarian failure. 12 (57%) patients had normal FSH levels at six months post treatment. Median age of all patients at treatment was 34 years. Median age of women retaining normal ovarian function was 30 (range, 19 to 45) vs. 41 years (range, 30 to 49) for those who did not regain function. The conditioning regimen of two doses of cyclophosphamide (60mg/kg) and five doses of fludarabine (25mg/m²) may induce gonadal damage and premature ovarian failure. Younger age at treatment was associated with a higher frequency of normal ovarian function post-treatment, whereas adding total body radiation was associated with a high risk of ovarian failure.

Keywords

Ovarian toxicity; Follicle Stimulating Hormone; Ovarian Recovery; Premature Ovarian Failure; Cyclophosphamide

Introduction

Adoptive cell therapy (ACT) infusing cultured tumor-reactive T-cells following a preparative immunosuppressive regimen has emerged as an effective and potentially curative therapy for patients with melanoma. However, due to the cytotoxic lymphodepleting preparative regimen, premenopausal female patients are at risk of

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developing iatrogenic ovarian failure. Loss of ovarian function results from the inability of female germ cells to regenerate following injury. In women patients of reproductive age questions arise regarding issues of premature ovarian failure, sexual dysfunction, fertility and pregnancy. Therefore the main aim of our study was to analyze the data in a hypothesis generating fashion in order to further other studies.

All alkylating agents harbor the potential for gonadal toxicity as they are not cell cycle specific drugs.¹ This is best documented for high dose cyclophosphamide, which is commonly used in preparation for a stem-cell transplant or ACT. High dose therapy damages oocytes and the supporting granulosa cells of both proliferating and resting follicles in a dose dependant manner.^{2,3} It is thought that cyclophosphamide leads to follicular death by damaging rapidly dividing granulosa cells, which produce estrogen and progesterone to support developing follicles.⁴ Decreased production of gonadal steroids also stimulates pituitary gonadotropin production, which further enhances recruitment of follicles into the pool of maturing follicles susceptible to cyclophosphamide.⁴ This cycle therefore results in hypergonadotropic hypogonadism and accelerated depletion of ovarian oocytes.⁴ Following treatment with high doses of cyclophosphamide, ovarian biopsies have confirmed a complete absence of primordial follicles in some patients.^{2,3}

While return of ovarian function following the use of alkylating agents has been reported, there is limited data estimating the probability of premature ovarian failure (elevated FSH, loss of menses). In order to counsel patients of a reproductive age on their risk of experiencing premature ovarian failure and possible premature menopause, information that would facilitate family planning and timing of future pregnancies with accurate risk estimates is needed.^{5,6,7} Limitations of these prior studies include small sample size, lack of detailed information on treatment exposures and failure to distinguish between ovarian and pituitary etiologies.^{5,7} We therefore attempted to assess the rate of recovery of ovarian function in women following ACT using a preparative regimen of cyclophosphamide and fludarabine with or without TBI and identify variables that predict its recovery. Keeping in mind the lethality of the diseases being studied and the lack of long term survivors we stress our results are hypothesis generating.

Materials and Methods

A retrospective review of a prospectively maintained database at the Surgery Branch, National Cancer Institute, NIH (Bethesda, Maryland) identified female patients who received ACT including a T-cell transfer combined with high dose bolus Interleukin-2 (0.72 million IU/kg/dose) following a nonmyeloablating lymphodepleting preparative regimen encompassing two consecutive daily doses of high dose cyclophosphamide at 60mg/kg each and five doses of fludarabine at 25mg/m² (Cy-Flu) between 1999 and 2009. All patients had non-gynecologic metastatic cancers, an Eastern Cooperative Oncology Group (ECOG) performance score of 0 or 1 and were enrolled on a protocol approved by the National Cancer Institute, NIH Investigational Review Board. Inclusion criteria were age less than 50, no previous Cy-Flu treatments, post-treatment serum samples obtained beyond three months and a normal FSH level (defined as FSH $\leq$ to 11 U/L during the follicular stage or FSH $\leq$ 21 midcycle) within four weeks prior to treatment. Childbirth histories were obtained to elaborate upon pre-treatment fertility. Patients alive at the time of this study were contacted and questioned regarding their pre-treatment and post-treatment menstrual history and menopausal symptoms. Of the 66 women identified, 40 were excluded; 33 did not have serum samples beyond three months post-treatment and 7 had abnormal baseline FSH levels. Of the remaining cohort, 24 patients had metastatic melanoma, one patient had metastatic renal cell carcinoma and one patient had metastatic synovial cell carcinoma. These 26 patients were followed for a median of 6 months (range, 3 – 43 months) post-treatment. Pre-

treatment contraceptive histories were not obtained. No patients received hormonal therapy in the post-treatment period.

Post-treatment normal ovarian function was defined as FSH levels persistently within normal limits and regular menses without hormonal therapy.

Serum samples were obtained at baseline and every month following treatment and cryopreserved. FSH levels were analyzed in the same assay using a solid phase, two-site chemiluminescent immunometric assay (Immulite 2500, Diagnostic Products Corp., Los Angeles, CA, USA). Premature ovarian failure was defined as a persistently elevated FSH level > 21 U/L accompanied by a failure to resume menstruation post-treatment. Fisher's exact test was used to analyze the impact of age and TBI on loss of ovarian function.

Results

Patient Characteristics

26 patients met all inclusion criteria. 21 received only Cy-Flu as a conditioning regimen and 5 received Cy-Flu accompanied by TBI at either 200 or 600 cGy. Table 1 lists details of demographics, prior treatment and patient characteristics.

In the Cy-Flu group (n=21) 19 patients had metastatic melanoma, 1 patient had metastatic renal cell carcinoma, 1 patient had metastatic synovial cell carcinoma, thirteen patients had given birth prior to treatment.

Nine out of the 21 patients (43%) had persistently elevated FSH levels greater than 21 U/L (range, 38 – 138 U/L) and were diagnosed with iatrogenic premature ovarian failure (Fig. 1). Median age was 41 years (range, 30 – 49). The median length of post-treatment follow up was 5 months (range, 3 – 15 months). Two patients in this group had a previous history of chemotherapy. Three patients who had persistently high FSH levels also lost menses. Menstrual histories were not obtained in five patients due to death and one patient was lost to follow up.

Seven patients had transient elevations in FSH levels post-treatment which normalized (range 2 – 20 U/L) by 6 months post-treatment (Fig. 2). Five patients reported a normal menstrual history at the time FSH levels normalized, two were not alive at time of study and menstrual history was not obtained. Median age was 31 years (range, 19 – 45). Median time to recovery for these patients was three months post-treatment (range, 3 – 6 months). Median length of follow up was 6 months (range, 3 – 43 months). Four patients had a previous history of chemotherapy.

Figure 3 depicts five patients who maintained continuously normal FSH levels following treatment (range, 2 – 20 U/L). Median age was 25 (range, 21 – 45). Median length of follow up was 9 months (range, 5 – 13 months.) Three reported normal menstrual histories and two were not alive at time of study. No patients received prior chemotherapy.

In the patients given Cy-Flu plus TBI group (n=5), the median age was 37 years, (range, 25 – 49 years), all patients had metastatic melanoma and 4 patients had given birth prior to treatment. All patients who received Cy-Flu plus TBI at either 200 cGy or 600 cGy had persistently elevated FSH levels ranging from 39 – 210 U/L and cessation of menses (Fig. 4). Median follow up was 6 months, (range 3 – 8 months). One patient had a history of previous chemotherapy. No patient in this group recovered normal menses post-treatment.

Age and Loss of Ovarian Function in the Cy-Flu group

We attempted to assess the impact of age on post treatment ovarian function in patients who received Cy-Flu only as their preparative regimen ($n = 21$). Median age at treatment of the twelve patients who retained normal ovarian function was 30 years (range, 19 – 45) versus 41 years, (range, 30 – 49 years) for the nine patients where ovarian function did not return. Nine of 11 patients (82%) below the median age of 34 years had persistently normal ovarian function, in contrast to only three of 10 patients (30%) above this median age. A statistical significance was seen in the risk of ovarian failure in patients beyond 34 years of age ($p = 0.03$).

Previous History of Chemotherapy

Previous chemotherapy did not significantly impact on the risk of ovarian failure in these patients. A total of seven patients had previous chemotherapy, six patients in the Cy-Flu group and one patient in the Cy-Flu with TBI group. Two out of the six (Cy-Flu) had post-treatment ovarian failure. Prior chemotherapy agents and patient outcomes are depicted in Table 2.

Treatment Results

The solitary sarcoma patient was a partial responder by RECIST criteria. In the 24 patients with metastatic melanoma who underwent ACT, seven (29%) reached a partial response and four (16%) were complete responders in this group as categorized by RECIST criteria.

Discussion

Treatment induced premature ovarian failure can have detrimental psychological as well as physiological effects on premenopausal female patients. One third of these women have reported anxieties about sterility, femininity, physical appearance and strain on relationships due to vaginal dryness and sexual dysfunction.² Medical consequences also include an increased risk of developing cardiovascular disease and osteoporosis.^{2,5} Better data on the risk of ovarian failure after therapy would improve the ability to counsel, anticipate and address these problems. Taking into consideration the fact that many of these patients will not see long term survival.

In general, data assessing ovarian toxicity of various chemotherapy regimens is incomplete. In 10 female patients with cervical cancer receiving cisplatin 40mg/m² days 1–4 and bleomycin 15mg/m² on days 1–8 every 21 days, 5 patients had postmenopausal FSH levels, defined as >40mIU/mL after course 1. In three of these five patients, FSH levels dropped below 40mIU/mL after 7–9 weeks.⁸ In female patients receiving ifosfamide for sarcoma treatment, there was no significant difference in FSH levels between the treatment group and an observational control group. The median age of the treatment group was 12.1 years, and the median dose of ifosfamide was 59.4g/m².⁹ In 26 female patients with Hodgkin's lymphoma, 13 patients received 6 courses of ABVD (adriamycin 25mg/m² plus bleomycin 10mg/m² plus vinblastine 6mg/m² and dacarbazine 375mg/m²: day 1 and 15) and 13 patients received six courses of ABVD alternating with COPP (cyclophosphamide 600mg/m² plus vincristine 1.4 mg/m² day 1 and day 8, plus procarbazine 100mg/m²/day and prednisone 1mg/kg/day days 1–8). FSH levels were collected on the 3rd day of each patient's menstrual cycle. Regardless of the chemotherapy regimen, women who were less than four years out from their chemotherapy treatment had normal FSH levels (mean±SD: 6.15±4.99mIU/mL), while women who were more than four years out from their chemotherapy treatment had FSH levels of 20.61±23.94mIU/mL.¹⁰ This suggests that ABVD/MOPP has little early gonadal toxicity, but may result in early menopause in this patient population. There have been case reports associating thalidomide with ovarian

failure (measured by FSH, LH and estradiol levels), which were temporally related to the initiation and/or discontinuation of thalidomide therapy in patients with aphthosis.¹¹ In summary, it is difficult to assess the ovarian toxicity of individual agents, since they are usually given in combination.

Chemotherapy regimens that include alkylating agents such as cyclophosphamide, carry the greatest risk for inducing premature ovarian failure.¹⁻⁹ Koyama et al published one of the earliest studies to suggest gonadal toxicity induced by cyclophosphamide. They found that the average cyclophosphamide dose given prior to the onset of ovarian failure is 5.2g for patients in their 40's and 9.3g for those in their 30's.³ Our preparative regimen gives a 60kg female a total of 7.2g of cyclophosphamide and is similar to these values.

Non-myeloablative regimens using high dose cyclophosphamide and fludarabine are now being used not only for patients undergoing allotransplantation, but also to dramatically enhance the survival and efficacy of adoptively transferred T-cells given to treat cancer^{12,13}. Because these therapies are often applied to younger patients and may be curative, their impact on fertility is of increasing importance. In our study, 57% of females who received conditioning with chemotherapy only (Cy-Flu) had normal ovarian function post-treatment, with 81% of females less than or equal to 34 years of age maintaining normal ovarian function. These results are comparable to the bone marrow transplant literature. Schimmer et al published a 79% rate of ovarian recovery in female patients younger than 25 years of age and in the large study performed by Sanders et al 84% of female patient's recovered ovarian function. The high rate of function of patients in the Sanders study may have been due to their younger age and the absence of TBI.^{2,13} In addition to the bone marrow literature a retrospective study in patients with systemic lupus erythematosus showed that age and cumulative cyclophosphamide dose are predictors of ovarian failure with 32 years or older marking the difference.⁴

Median follow up of our study was 6 months (range 3 – 43), largely due to deaths from disease or patients with progression who sought care elsewhere. In the literature, there are rare published cases of late ovarian recovery after chemotherapy induced ovarian failure. In the study presented by Schimmer *et al.* a small group of patients who initially lost ovarian function recovered function at a median of 24 months post-treatment.² This indicates that recovery may not occur for many years following immunotherapy.⁷ Also, it should be mentioned that despite the presence of high FSH levels and amenorrhea after cancer treatment, pregnancies in childhood cancer survivors have been reported.¹² Therefore our results may represent a worst case analysis of acute ovarian failure but may not detect an acceleration of normal menopause.

Patients of reproductive age should consider counseling regarding pregnancy and fertility preservation prior to the initiation of conditioning regimens which include cyclophosphamide and or TBI.¹⁻⁹ For those patients interested in preserving fertility, options may be considered before starting therapy.⁶ Currently the choices for maintaining fertility include in vitro fertilization using superovulation, embryo cryopreservation, oocyte/ovarian tissue cryopreservation and the use of gonadotropin-releasing hormone (GnRH) agonists to preserve ovarian function.^{14,15} Gonadotropin-releasing hormone agonists (GnRH-a) such as leuprolide are thought to be efficacious in ovarian preservation by downregulating GnRH receptors in the pituitary, suppressing gonadal steroid secretion.^{4,15} Suppression of FSH and LH is thought to prevent accelerated recruitment and then depletion of ovarian follicles by cyclophosphamide and therefore protect against premature ovarian failure.⁴ Note, all of the techniques listed above may require delaying treating for approximately one month and may be impractical based on the clinical situation. In reality, options may be limited.

For control of post-menopausal symptoms, hormonal replacement therapy has successfully been used.² However in our immunotherapy population who largely have metastatic melanoma, it is not clear if this option is risk free. Up to 30% of melanoma's will express estrogen receptors and the biological significance of this is unclear. Therefore, hormone replacement can not be recommended without reservations.

This study has clear limitations such as the small sample size, short term post-treatment follow up and the use of FSH levels rather than more sensitive Anti-Mullerian Hormone (AMH) levels.⁶ The advantages of this study include full information on treatment exposure and previous chemotherapy history, the use of menstrual history as well FSH levels, the broad age range and exclusion of patients who had normal menses but baseline FSH levels out of the normal range.

In our study, younger age at the time of ACT in the Cy-Flu group was an independent predictor of maintaining or recovering normal ovarian function as assessed by the Fisher's exact test, but we recognize the limitations of this analysis in view of the small sample size. Also, a trend was observed for TBI to predict loss of ovarian function however this statement should be interpreted cautiously because of the small sample size and short follow up. Previous use of chemotherapy was not predictive of loss of ovarian function, again subject to the constraints of the sample size.

Adoptive Cell Therapy following conventional lymphodepleting regimens including chemotherapy with or without irradiation has emerged as an effective and potentially curative therapy in patients with metastatic disease. However, due to the conditioning regimen including cyclophosphamide and/or TBI female patients are at risk of premature ovarian failure. As women age, oocytes are lost. This suggests older women are less likely to recover ovarian function after insult to gonadal tissue via chemotherapy or radiation.² Therefore, younger women have a higher probability of restoration of ovarian function and this point is corroborated by our data. Premenopausal women may seek pre and post treatment counseling with an obstetrician gynecologist and or reproductive endocrinologist for antral follicle counts and family planning/counseling if considering such therapy. The results of this study enable more accurate risk assessment and provide a frame work for evaluating future therapeutic interventions. That being said, we respect the lethality of the diseases for which we are treating and recognize the majority of patients will not have long term survival. Therefore, we stress that our results should be interpreted as a hypothesis generating study.

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Figure 1. Serum FSH levels as a function of time after Cy-Flu conditioning in patients experiencing durable ovarian failure. Upper limit of normal is 21 U/L.

Figure 2. Serum FSH levels as a function of time after Cy-Flu conditioning in patients with transient ovarian failure. Upper limit of normal is 21 U/L.

Figure 3. Serum FSH levels as a function of time after Cy-Flu conditioning in patients maintaining continuously normal FSH levels following treatment. Upper limit of normal is 21 U/L.

Figure 4. Serum FSH levels as a function of time in five patients conditioned with Cy-Flu plus TBI at either 200 cGy or 600 cGy. Upper limit of normal is 21 U/L.

Table 1

Demographics, prior treatment, patient characteristics and outcomes of patients treated with ACT following conditioning with either Cy-Flu or Cy-Flu plus TBI.

Patient	Diagnosis	Age	Birth History	Previous Chemotherapy	Preparative Regimen	Post-Treatment FSH level	Outcome	Final Serum sample	Status
1	Renal Cell	33	Yes	None	Cy/Flu	Elevated	PD	3 months	DOD
2	Melanoma	21	No	None	Cy/Flu	Normal	PD	11 months	AWD
3	Melanoma	45	Unk.	None	Cy/Flu	Normal	PD	3 months	AWD
4	Melanoma	34	No	Cisplatin, Dacarbazine, Vinblastine	Cy/Flu	Normal	PD	6 months	DOD
5	Melanoma	43	Yes	None	Cy/Flu	Elevated	PD	3 months	AWD
6	Melanoma	19	No	None	Cy/Flu	Normal	PR	5 months	AWD
7	Melanoma	25	No	None	Cy/Flu	Normal	PD	6 months	DOD
8	Melanoma	21	No	None	Cy/Flu	Normal	PD	5 months	DOD
9	Melanoma	41	Yes	None	Cy/Flu	Elevated	CR	12 months	NED
10	Melanoma	44	Yes	None	Cy/Flu	Elevated	PD	4 months	DOD
11	Melanoma	31	Yes	None	Cy/Flu	Normal	PR	9 months	AWD
12	Melanoma	30	Yes	None	Cy/Flu	Elevated	PD	5 months	DOD
13	Melanoma	20	Yes	Velban, Dacarbazine, Temodar	Cy/Flu	Normal	PR	43 months	AWD
14	Melanoma	38	Unk.	Temodar, Thalidomide	Cy/Flu	Elevated	PD	5 months	DOD
15	Melanoma	49	Yes	Dacarbazine	Cy/Flu	Elevated	PD	8 months	DOD
16	Melanoma	45	Yes	None	Cy/Flu	Normal	CR	13 months	NED
17	Melanoma	35	Unk.	None	Cy/Flu	Elevated	PD	3 months	DOD
18	Melanoma	33	Yes	None	Cy/Flu	Normal	PD	20 months	AWD
19	Melanoma	29	Yes	Dacarbazine, Carmustine, Cisplatin	Cy/Flu	Normal	PD	9 months	DOD
20	Melanoma	44	Yes	None	Cy/Flu	Elevated	PR	15 months	AWD
21	Sarcoma	37	Yes	Ifosfamide, Adriamycin, Epirubicin	Cy/Flu	Normal	PR	6 months	AWD
22	Melanoma	49	Yes	None	Cy/Flu + 600 cGy	Elevated	CR	6 months	NED
23	Melanoma	37	Yes	None	Cy/Flu + 600 cGy	Elevated	PR	6 months	AWD
24	Melanoma	25	No	None	Cy/Flu + 600 cGy	Elevated	PR	6 months	AWD
25	Melanoma	34	Yes	Temodar	Cy/Flu + 200 cGy	Elevated	PD	3 months	DOD
26	Melanoma	47	Yes	None	Cy/Flu + 200 cGy	Elevated	CR	8 months	NED

Key: Unk = unknown; Cy = Cyclophosphamide, Flu = Fludarabine, PD = progressive disease, PR = partial response; CR = complete response by RECIST criteria; DOD = dead of disease, AWD = alive with disease, NED = no evidence of disease, N/A = not available

Table 2

Previous chemotherapies administered, their potential ovarian toxicity and post treatment ovarian function in the 21 patients who received Cy-Flu as a conditioning regimen.

Previous Chemotherapy History (Cy-Flu)				
Number of Patients	Age	Chemotherapy Agent	Agents with Ovarian Toxicity	Post Treatment Ovarian Function
1	34	Cisplatin, DTIC, Vinblastine	Cisplatin	abnormal → normal
1	37	Ifosfamide, Adriamycin, Epirubicin	-	abnormal → normal
1	20	Velban, Dacarbazine, Temodar	-	abnormal → normal
1	29	Dacarbazine, Carmustine, Cisplatin	Cisplatin	abnormal → normal
1	49	Dacarbazine	-	abnormal
1	38	Temodar, Thalidomide	Thalidomide	abnormal
15		None		