Ovarian tissue autotransplantation in acute leukemia: balancing the risk of relapse and the hope of parenthood

Myeloablative conditioning regimens (MAC) used in hematopoietic stem cell transplantation (HSCT) often result in premature ovarian insufficiency (POI),[1] adversely affecting quality of life by compromising parenthood. To mitigate this risk, ovarian tissue cryopreservation (OTC) can be proposed as a prevention strategy. The therapeutic emergency in acute leukemia (AL) generally precludes oocyte preservation, making OTC the only option for fertility preservation. OTC followed by ovarian tissue autotransplantation (OTT) has proven effective in restoring ovarian function.[2,3] In the largest series of women who underwent OTT, 106 out of 285 (38%) conceived, and 75 (26%) gave birth.[3]

For patients treated for AL, concerns persist regarding the risk of reintroducing leukemic cells through OTT, limiting its use. To evaluate this risk, minimal residual disease (MRD) assessment using multiparameter flow cytometry (MFC) or molecular techniques has been performed on cryopreserved ovarian fragments, representing the most sensitive technique for detecting leukemic cells.[4-6] The likelihood of detectable MRD in ovarian fragments is reduced when OTC is performed after achieving complete remission (CR) of AL, particularly when bone marrow MRD is undetectable. However, discrepancies exist[6] and MRD assessment is constrained by sensitivity threshold and is not feasible on all fragments prior to OTT. Consequently, an undetectable MRD result does not fully exclude residual leukemic cells in some ovarian fragments. The first case of OTT in a woman with AL was reported in 2018 following leukemic cell screening by histology, immunohistochemistry, FISH, next-generation sequencing (NGS) and xenotransplantation.[7] Since then, only a limited number of OTT cases for AL have been reported.[3,8-11] While these reports are encouraging, with no relapse, data remain scarce. The absence of validated tools to ensure the complete safety of OTT in leukemic patients underscores the need for larger studies with extended follow-up.

We report the largest series of OTT in women in CR of AL at the time of OTC. The women included were referred to three fertility centers collaborating with the French Research and Study Group on Ovarian and Testicular Preservation (GRECOT). They were identified through the GRECOT national database, which records OTT performed in France. Inclusion criteria were: 1) women transplanted for AL; 2) OTC performed before HSCT as part of a fertility preservation program; 3) development of POI; and 4) OTT for fertility restoration between January 2012 and May 2024, without pregnancy contraindication. Local ethical committees approved the study (CLEA-2024 n. 397). After signing informed consent, all women underwent laparoscopic removal of one ovary. After transport to the reproductive biology laboratory, ovarian cortex was separated from the medulla, fragmented, slowly frozen according to specific protocols, and stored in liquid nitrogen.[12] When the patient expressed a desire to conceive, OTT was proposed if she experienced POI and remained in CR of AL. Prior to OTT, whenever possible, leukemic infiltration was assessed through MRD analysis using flow cytometry and/or molecular biology techniques, following the local protocol, in one or two ovarian fragments. OTT was performed laparoscopically either in an orthotopic or heterotopic position. Patient characteristics and clinical data were collected from medical records.

Thirteen women were included: 7 with acute lymphoblastic leukemia (ALL) and 6 with acute myeloid leukemia (AML). Patient characteristics are summarized in Table 1. The median age at OTC was 19.8 years (range: 15.6-36.1 years) and at OTT was 32.9 years (range: 27.7-39.3 years). Three patients had extramedullary leukemic localization at diagnosis or relapse. The indication for OTC was HSCT after a MAC for all women, with 9 having received total body irradiation (TBI) as part of the conditioning regimen. The main results of OTC and OTT are summarized in Table 2.

Ovarian tissue cryopreservation was performed between June 2003 and May 2018 after achieving CR in all patients: 10 were in first CR and 3 were in second CR. At the time of their request for OTT, all patients were in persistent CR and experienced POI. Eleven patients received hormone replacement therapy, while this was declined in 2 patients for personal reasons.

Before OTT, an evaluation of leukemic infiltration in cryopreserved ovarian cortex by MRD assessment was carried out for 9/13 patients. MRD analysis methods included quantitative polymerase chain reaction targeting clonal rearrangements of immunoglobulin or T-cell receptor genes (IG/TR) in 5 cases, *NPM1* mutation in 2 cases, NGS *RUNX1* somatic mutation detection in one case, and genomic breakpoint of *KMT2A::AFDN* (*MLL::AF6*) in one case. A second MRD assessment by MFC was conducted for 2 patients. All 9 patients tested had undetectable MRD in the cryopreserved ovarian fragments tested with a sensitivity threshold ranging from 10^{-5} to 5.10^{-3}. Two patients had suboptimal MRD sensitivity (5.10^{-3}) due either to the detection method used (NGS-based RUNX1 mutation detection, patient n. 2) or to limited DNA yield from ovarian tissue (patient n. 4). In our series, as well as in previous case reports,[10] MRD assessment was not systematically performed, even when a suitable marker was available, underlying the heterogeneity of clinical practices. The 4

Table 1. Hematologic characteristics of the patients and minimal residual disease results.

Patient ID N	Type of AL	Extramedullar disease	Oncogenetics	Remission status at OTC	Cumulative cyclophophamide dose at OTC, g/m²	TBI in conditioning regimen	MRD marker	Threshold of sensitivity
1	AML	No	*KMT2A-r*	CR1	0	No	*KMT2A::AFDN*	10^{-4}
2	AML	No	Normal karyotype, *RUNX1* mut	CR1	0	Yes	*RUNX1*	5.10^{-3}
3	B-cell ALL	No	Low Hypodiploidy	CR1	3	Yes	IG/TR and MFC	10^{-5} and 5.10^{-5}
4	B-cell ALL	Yes (CNS)	NA	CR2	NR	Yes	IG/TR	5.10^{-3}
5	AML	No	Normal karyotype, *NMP1* mut, *FLT-ITD*, *IDH1*	CR1	0	No	*NPM1*	10^{-4}
6	B-cell ALL	Yes (mammary)	46, XX with t(8;14)	CR2	3	Yes	IG/TR	10^{-4}
7	B-cell ALL	No	NA	CR1	2.5	Yes	IG/TR and MFC	10^{-5} and 10^{-4}
8	AML	No	Normal karyotype	CR1	0	Yes	Not assessed	NA
9	T-cell ALL	No	NA	CR1	NR	Yes	Not assessed	NA
10	AML	No	Normal karyotype	CR1	0	Yes	Not assessed	NA
11	B-cell ALL	Yes (CNS)	NA	CR1	2.5	Yes	Not assessed	NA
12	B-cell ALL	No	*KMT2A-r*	CR2	6	Yes	IG/TR	10^{-5}
13	AML	No	45,XX,-22,add(22)(p11)[8]/ 46,XX,add(22)(p11) x2[11]/46,XX[1]; *NPM1* mut, *FLT3-TKD*, *NRAS* mut	CR1	0	No	*NPM1*	10^{-4}

AL: acute leukemia; ALL: acute lymphoblastic leukemia; AML: acute myeloid leukemia; CNS: central nervous system; CR1: first complete remission; CR2: second complete remission; *FLT3-ITD*: Fms-like tyrosine kinase 3 internal tandem duplication; *FLT3-TKD*: Fms-like tyrosine kinase domain; IDH1: isocitrate dehydrogenase 1; IG/TR: immunoglobulin T-cell receptor; *KMT2A-r*: lysine methyltransferase 2A gene rearrangement; MFC: multiparameter flow cytometry; MRD: minimal residual disease; mut: mutation; N: number; NA: not applicable; *NPM1*: nucleophosmin1; NR: not reported in medical record; *NRAS*: neuroblastoma rat sarcoma virus; OTC: ovarian tissue cryopreservation; *RUNX1*: Runt-related transcription factor 1; TBI: total body irradiation.

patients who did not undergo MRD assessment in ovarian fragments were managed at the same center, with OTT performed between 2012 and 2018. Since the publication of national guidelines[13] recommending MRD assessment prior to OTT, practices at this center have aligned with these recommendations.

Ovarian tissue autotransplantation was performed with a median time of 10.5 years (range: 3.2-18.6 years) from HSCT in an orthotopic position for 12 of the 13 patients. Five patients underwent more than one OTT: 3 had 2 OTT and 2 had 3 OTT to prolong ovarian function. Ten women (77%) recovered ovarian endocrine function defined by resumption of menstruation with a median time of 4.4 months (range: 1.7-8.0 months) after OTT, which is slightly lower than the 88.7% previously reported by Dolmans *et al.* in the largest OTT series to date.[3] Advanced age at OTC or prior exposure to gonadotoxic chemotherapy may negatively influence OTT outcomes.[2,3] Among the 3 patients who did not resume menstruation, patients n. 6 and n. 12 had undergone OTC after a second CR of AL. Patient n. 6 received 3 g/m² of cyclophosphamide at the age of 19 years and had a follicle density of 10/mm². Patient n. 12 received 6 g/m² of cyclophosphamide at the age of 36 years (a likely gonadotoxic dose at that age) and had a follicle density of 0/mm², supporting the early referral of leukemia patients to fertility specialists prior to gonadotoxic treatment. Patient n. 8 had a follicle density of 0/mm², despite having experienced no alkylating agent exposure. However, follicle density was assessed from a single fragment, which may not reflect the heterogeneous distribution of primordial follicles in the adult ovary.

Although the majority of patients experienced ovarian function restoration, only 3 became pregnant with a total of 4 pregnancies after natural conception. Three patients experienced a first trimester miscarriage and one delivered a healthy child. Interestingly, the limited cases in the literature

Table 2. Ovarian tissue transplantation outcomes.

Patient ID N	Age at OTC in years	Primordial follicle density /mm^2	Time between HSCT and first OTT in years	N of OTT; site	Resumption of menstrual cycle (time since OTT in months)	Pregnancy after OTT	Relapse after OTT (time since OTT; ovarian MRD result if relapse)	Follow-up since first OTT in years	Menstrual cycle at last follow-up
1	19	7.2	8.7	2; orthotopic	Yes (6.7)	No	No	7	Yes
2	26.5	3.1	10.5	3; orthotopic	Yes (4.5)	No	No	6.8	Yes
3	16.2	12.5	16.9	1; orthotopic	Yes (3.4)	No	Therapy-related AML (25 months)	4.2	No
4	18.4	0	16.9	1; orthotopic	Yes (6.1)	No	No	2.7	Yes
5	16.4	0	14	1; orthotopic	Yes (4.5)	Yes : 1 miscarriage	No	2.1	Yes
6	19.8	10	8.5	1; orthotopic	No	No	No	1.5	No
7	31.8	0.8	5.2	1; orthotopic	Yes (1.8)	Yes : 1 miscarriage	Relapse (13.8 months; undetectable <10^{-5})	4.5	No
8	24.3	0	18.6	2; orthotopic	No	No	No	0.8	No
9	17.3	0.9	11.8	1; orthotopic	Yes (3)	Yes: 1 live birth and 1 miscarriage	No	11.8	Yes
10	22.4	0.6	6.4	3; heterotopic	Yes (8)	No	No	10.5	Yes
11	23	4	6.7	2; orthotopic	Yes (4.4)	No	No	4.1	Yes
12	36.1	0	3.2	1; orthotopic	No	No	No	2.5	No
13	15.6	0.7	17.2	1; orthotopic	Yes (2.9)	No	No	0.8	Yes

AL: acute leukemia; HSCT: hematopoietic stem cell transplantation; MRD: minimal residual disease; OTC: ovarian tissue cryopreservation; OTT: ovarian tissue transplantation.

showed a high number of pregnancies and births with 8 out of 12 women giving birth.[7-11] This discrepancy might suggest a selection bias in previously reported cases. Reporting OTT cases on a national level provides a more comprehensive understanding of outcomes in this population.

Several hypotheses may explain the low birth rate observed in our cohort. First, one patient underwent heterotopic OTT, a technique associated with poorer outcomes. Oktay *et al*. recently reported significantly lower fertilization rates and fewer embryos generated per retrieval in heterotopic OTT than in orthotopic OTT.[14] Additionally, uterine function may have been compromised, especially in women exposed to TBI, potentially leading to higher miscarriage rates and negative effects on OTT outcomes. Sanders *et al.* reported that the incidence of spontaneous abortion was significantly higher in TBI recipients compared to the chemotherapy group in HSCT survivors.[1] Notably, in our series, the woman who gave birth, and 2 of the 3 women who miscarried, had received TBI. Moreover, in our cohort, psychosocial factors such as separation from a partner (patients n. 1 and n. 4) or a lack of a persistent desire for pregnancy (patient n. 2) have influenced outcomes. Finally, at the last follow-up, 8 of the 10 women who recovered ovarian function maintained menstrual cycles at a median time of 5.4 years (range: 0.8-11.8 years) since OTT, while 2 experienced amenorrhea in the context of relapse and therapy-related AML, raising hopes for improved outcomes with extended follow-up.

A major concern is whether OTT could trigger AL relapse. With a median follow-up of 4.07 years (range: 0.8-11.8 years) after OTT, 11 of the 13 remained in CR of AL. Patient n. 3 transplanted for B-cell ALL developed therapy-related AML 25 months after OTT and 19 years post HSCT in a context of Li Fraumeni syndrome. Patient n. 7 experienced a medullary relapse of B-cell ALL 13.7 months post OTT and six years post HSCT; this case has previously been reported by Fontczak *et al*. The same IG/TR rearrangement was found at diagnosis and relapse.[15] For this patient, MRD was tested before OTT in two ovarian fragments using two different techniques with sensitivity thresholds of 10^{-5} and 10^{-4}, respectively. Late relapses occurring more than two years after HSCT are rare in ALL.[16] Considering the potential homing of leukemic cells from the transplanted ovarian tissue to the bone marrow, the role of OTT in this relapse cannot be definitively excluded.[17] Moreover, this case highlights

the fact that undetectable MRD in cryopreserved ovarian cortex may not ensure the absolute safety of OTT.
For many years, OTC has been the technique of choice for fertility preservation before HSCT in women with AL. Although alternatives like artificial ovaries and *in vitro* folliculogenesis hold promise, none have yet resulted in live births. Consequently, demand for OTT is likely to increase. In the absence of other fertility restoration options for women with AL, our results provide critical information for counseling patients before OTC and when considering OTT.

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No conflicts of interest to disclose.

Contributions

CP conceived and designed research. CR identified patients through the GRECOT national database. EL, RI, GS, GL, JHD, RPdL, BS, CR and CP provided patients. FC, EL and CP collected data. EC, HL and PB analyzed and interpreted minimal residual disease data. FC and CP wrote the paper. FC, EL, EC, HL, PB, BBL, RI, GS, JHD, ND, NB, RPdL, CR and CP reviewed the paper. All authors gave final approval to the manuscript.

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Data-sharing statement

The data are available upon request from the corresponding author.

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