Footnotes

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TO THE EDITOR:

Minimal residual disease quantification in ovarian tissue collected from patients in complete remission of acute leukemia

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Acute leukemia (AL) is the most common cancer occurring during childhood and adolescence. Allogeneic hematopoietic stem cell transplantation (HSCT) is recommended in patients with very high-risk AL and usually at relapse. Myeloablative conditioning regimens result in premature ovarian failure in most women.[1] Ovarian tissue cryopreservation (OTC) may allow fertility restoration, with more than 130 live births reported in 2017 after ovarian tissue transplantation (OTT),[2] mainly in women

Table 1. MRD results in ovarian samples and bone marrow

No.	Type of ALL	Molecular marker	Ovarian cortex MRD	Ovarian medulla MRD	Bone marrow MRD	Relapse Y/N	Follow-up (d)
1	BCP-ALL	Ig/TCR	Positive $<10^{-4}$	Positive $<10^{-4}$	Undetectable	N	1368
2	BCP-ALL	Ig/TCR	Undetectable	Positive $<10^{-4}$	Undetectable	N	1647
3	BCP-ALL	Ig/TCR	Positive $<10^{-4}$	3.10^{-4}	10^{-3}	N	84
4	BCP-ALL	Ig/TCR	NA	Undetectable	Undetectable	N	995
5	BCP-ALL	Ig/TCR	Undetectable	Positive $<10^{-4}$	Positive $<10^{-4}$	N	1126
6	BCP-ALL	Ig/TCR	Undetectable	NA	Undetectable	N	987
7	BCP-ALL	Ig/TCR	Undetectable	Undetectable	Undetectable	N	1096
8	BCP-ALL	Ig/TCR	Undetectable	Undetectable	Undetectable	N	49
9	BCP-ALL	Ig/TCR	Undetectable	Undetectable	Undetectable	N	26
10	BCP-ALL	Ig/TCR	Undetectable	Undetectable	Undetectable	N	686
11	BCP-ALL	Ig/TCR	Undetectable	Undetectable	Undetectable	Y	477
12	BCP-ALL	Ig/TCR	NA	Undetectable	Undetectable	N	577
13	BCP-ALL	Ig/TCR	Undetectable	Undetectable	Positive $<10^{-4}$	N	344
14	BCP-ALL	Ig/TCR	Undetectable	NA	Undetectable	N	2136
15	BCP-ALL	Ig/TCR	Undetectable	Undetectable	NA	N	400
16	BCP-ALL	KMT2A-AFF1	Undetectable	Undetectable	Positive $<10^{-4}$	Y	520
17	BCP-ALL	KMT2A-AFF1	Undetectable	Undetectable	Undetectable	N	245
18	BCP-ALL	KMT2A-USP2	NA	Positive $<10^{-4}$	Undetectable	N	1105
19	Ph^+ ALL	BCR-ABL1 Ig/TCR	Undetectable*	NA	0.0011% Undetectable	N	1862
20	Ph^+ ALL	BCR-ABL1 Ig/TCR	Undetectable*	Undetectable Positive $<10^{-4}$	0.00076% Undetectable	N	408
21	Ph^+ ALL	BCR-ABL1 Ig/TCR	Undetectable*	Undetectable*	0.11% Undetectable	N	9
22	Ph^+ ALL	BCR-ABL1 Ig/TCR	Undetectable*	Undetectable*	0.85% NA	N	427
23	Ph^+ ALL	Ig/TCR	Undetectable	Undetectable	Undetectable	Y	793
24	Ph^+ ALL	BCR-ABL1	0.01%	0.005%	Undetectable	N	922
25	T-ALL	Ig/TCR	Undetectable	Undetectable	Undetectable	Y	548
26	T-ALL	Ig/TCR	Undetectable	Undetectable	2.10^{-4}	N	1513
27	T-ALL	Ig/TCR	Undetectable	Undetectable	Undetectable	N	610
28	MPAL	Ig/TCR	3.10^{-5}	Positive $<10^{-4}$	Positive $<10^{-5}$	Y	635
29	MPAL	KMT2A-AFF1	Undetectable	Undetectable	Undetectable	Y	692
30	AUL	Ig/TCR	Undetectable	Positive $<10^{-4}$	Positive $<10^{-4}$	Y	678

Undetectable MRD means negative result obtained with a sensitivity of 10^{-4} or 10^{-5}. BCR-ABL1 MRD results are expressed as BCR-ABL1/ABL1 transcripts ratio.

AUL, acute undifferentiated leukemia; BCP-ALL, B-cell precursor ALL; MPAL, mixed-phenotype acute leukemia; NA, not available; N, no; Ph^+ ALL, ALL with Philadelphia chromosome; Y, yes.

*MRD was undetectable in both BCR-ABL1 and Ig/TCR.

Table 2. Ovarian MRD results according to disease characteristics

Disease characteristics	Detectable ovarian MRD	Undetectable ovarian MRD	*P* (Fisher exact test)
Total	9/30	21/30	
Age			
>18 y	7/9	9/21	.12
Type of AL			.99*
BCP-ALL	5/9	13/21	
Ph$^+$ ALL	2/9	3/21	
T-ALL	0/9	4/21	
Other	2/9	1/21	
High WBC† at diagnosis	3/9	6/21	.99
Extramedullary disease (excluding CNS)	4/9	6/21	.43
CNS involvement	2/9	6/21	.99
Time between diagnosis/relapse and OTC‡			
>4.8 mo	4/9	11/21	.99
Disease status at transplant			.10
First CR	8/9	11/21	
Second or third CR	1/9	10/21	

Patient with undifferentiated acute leukemia had normal WBC at diagnosis.

CNS, central nervous system; T-ALL, T-cell acute lymphoblastic leukemia; WBC, white blood cell count.

*BCP-ALL vs others.

†High WBC was defined as >30 G/L in BCP-ALL and >100 G/L in T-ALL.

‡Median time between diagnosis or last relapse and OTC was 4.8 mo (range, 1.7-7.2).

treated for lymphoma. In AL, concerns have been expressed on the risk of reintroducing leukemic cells after OTT. Evaluation of minimal residual disease (MRD) by molecular techniques showed infiltration of ovarian samples by leukemic cells, not detectable by histology or immunohistochemistry.[3-5] In this report, we assessed MRD by sensitive molecular techniques in cryopreserved ovarian samples harvested in patients in complete remission (CR) of AL a few days before HSCT when patients reached the lowest bone marrow (BM) MRD.

This study was conducted from August 2014 to July 2019 in 3 centers. Inclusion criteria were: (1) to be a woman transplanted for acute lymphoblastic leukemia (ALL), undifferentiated AL, or mixed phenotype AL in CR; (2) to have a molecular marker validated to MRD assessment[6]; and (3) to undergo OTC before HSCT as part of a preservation fertility program. All patients or their guardians provided written informed consent. The specific local ethical committees approved the study (n.6.7.16). OTC was performed after CR achievement, with a median of 19 days before HSCT (range, 11-120 days). Ovarian cortex was isolated from the medulla, cut into fragments, and cryopreserved according to a slow-freezing protocol.[7] One ovarian cortex fragment was dedicated to MRD assessment as well as ovarian medulla, usually not preserved. MRD evaluation was performed by quantitative polymerase chain reaction of clonal rearrangements of immunoglobulin or T-cell receptor genes (Ig/TCR) or oncogenic fusion genes, according to EuroMRD or European Against Cancer guidelines.[6] KMT2A/MLL fusions were quantified using a genomic breakpoint sequence. BCR-ABL1 fusion transcripts were quantified and normalized on ABL1 transcripts (BCR-ABL1/ABL1%). MRD was considered positive if detectable in at least 1 tested ovarian fragment by at least 1 technique when several were used. The sensitivity threshold was between 10^{-4} and 10^{-5}. MRD was compared in ovarian cortex, medulla, and BM samples collected at the same time point. Methods are detailed in the supplemental Appendix (available on the *Blood* Web site).

Thirty patients were included. Twenty-four patients had B-cell precursor ALL, 3 had T-cell ALL, 2 had mixed phenotype AL, and 1 had undifferentiated AL. Median age at transplant was 18 years (range, 0.8-35.2). Indication for HSCT was: previous relapse (n = 12), slow MRD response after initial treatment (n = 11), Philadelphia positive ALL (n = 6), and undifferentiated AL with complex karyotype (n = 1). MRD markers were Ig/TCR rearrangements in 22 cases (74%), genomic KMT2A-AFF1 in 3 cases (10%), genomic KMT2A-USP2 in 1 case (3%), BCR-ABL1 transcript in 1 case (3%), and both Ig/TCR rearrangements and BCR-ABL1 transcript in 3 cases (10%). At the time of OTC, 18 patients (60%) had MRD undetectable in BM. At the last follow-up, no patient underwent OTT.

MRD was assessed in 1 fragment from both medulla and cortex in 24 patients. Twenty-one of 30 (70%) patients had no detectable MRD, 8 (27%) had at least low positivity below 10^{-4} in 1 tested sample, and 1 (3%) had positivity between 10^{-3} and 10^{-4} (Table 1). No disease characteristics were statistically associated with MRD results in ovarian samples (Table 2). Data were concordant between medulla and cortex in 20 of the 24 patients tested, whereas MRD was detectable at low levels in medulla but

not in cortex in 4 patients. Although these apparently discordant results may simply reflect the random distribution of very few leukemic cells in ovarian samples, it may also point to heterogeneity in leukemic infiltration within the ovary. These data underline the need to analyze more samples of cortex and medulla from the same patient to address this point.

In 29 patients, we have compared ovarian and BM MRD harvested at the time of OTC: in 20 patients, data were concordant with undetectable (n = 15) or detectable (n = 5) MRD in both samples. From the remaining 9 patients, 5 had positive MRD in BM but undetectable in ovary and 4 had undetectable MRD in BM while positive in ovary. Although this apparent discrepancy between undetectable and low positive MRD should be interpreted with caution, it could reveal a preferential persistence of leukemic cells in ovary in some cases. With a median follow-up of survivors of 24 months (range, 11-70) after HSCT, 7 patients relapsed, including 2 patients with detectable MRD in ovarian samples (Table 1).

With a high rate of negative MRD in ovarian samples, our results differ from published data in which ovarian samples were usually harvested at diagnosis of leukemia or early after the start of chemotherapy.[3,4,8] Interestingly, Jahnukainen et al showed dramatic decrease of ovarian MRD after CR achievement and strong concordance between BM and ovarian MRD.[8] However, some groups still recommend performing OTC before chemotherapy exposure to preserve ovarian function.[9] We recently reported that OTC performed after the start of chemotherapy was efficient, suggesting that prior chemotherapy does not alter the function of cryopreserved ovarian tissue.[10] We thus recommend performing OTC after patients reached CR with low or undetectable MRD in BM to decrease the risk of ovarian leukemic infiltration.

The major issue of OTT in patients with AL is the risk of reintroducing leukemic cells. The absence of detectable disease in 70% of patients may be reassuring and a sufficient criterion to consider OTT. However, one should bear in mind that MRD negativity is defined for a given sensitivity threshold that depends on tissue sample size and assay sensitivity. The potential leukemia recurrence from such low levels of leukemic cells in cryopreserved ovarian fragments is currently unknown. Using xenograft experiments in severe combined immune deficient (SCID) mice, Dolmans et al demonstrated the viability and malignant potential of leukemic cells contained in human ovarian samples harvested at diagnosis.[4] We can hypothesize that the number of leukemic cells in these fragments was higher than in those harvested from patients in CR. Moreover, severe combined immune deficient mice xenograft experiments do not account for the allogeneic graft versus leukemia effect that may succeed in containing leukemia development from low tumor load.

In summary, our results underline the importance of reaching the best disease control before performing OTC. We recommend systematically assessing MRD in ovarian samples even if MRD is undetectable in BM because we cannot rule out preferential persistence of leukemic cells in ovary in some patients. The discordant results between cortex, medulla, and BM require testing the most ovarian samples available with sensitive techniques before considering OTT. The lack of validated tool to ensure the complete safety of OTT in leukemic patients requires conducting prospective studies with a long period of follow-up after OTT.

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Authorship

Contribution: C.P. and N.D. conceived and designed research; F.C. performed research; F.C., J.H.D., R.P.d.L., M.U., N.B., C.P., and N.D. provided patients; F.C., E.C., C.A., J.-M.C., R.K., A.C.-E., C.A., M.A., M.D., and V.M. analyzed and interpreted the data; F.C., C.P., and N.D. wrote the paper; F.C., E.C., C.A., J.-M.C., J.H.D., R.K., A.C.-E., C.C., C.A., V.D., R.P.d.L., M.A., M.U., M.D., V.M., H.D., N.B., C.P., and N.D. reviewed the paper; and all authors gave final approval to the manuscript.

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