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It takes two to tango: ctDNA MRD in large B-cell lymphoma nears the Finnish line

Running Head: Two to tango: ctDNA MRD nears the Finnish line

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In this issue of Haematologica, Meriranta and colleagues from Helsinki investigate measurable residual disease (MRD) in high-risk large B-cell lymphoma (LBCL) using circulating tumor DNA (ctDNA) from a prospective Nordic trial (1). At the low concentrations encountered after treatment, technical sequencing errors can obscure tumor DNA. Recent studies have overcome this with phased-variant sequencing, which requires two tumor mutations on the same DNA fragment (2-5). Meriranta and colleagues instead use duplex sequencing, which counts a variant only when it appears on both strands of the original molecule, since errors rarely affect both. Either way, it takes two to tango: two pieces of evidence are required before a molecule is counted as tumor-derived (**Figure 1**). Their findings strengthen a growing and consistent body of evidence that ctDNA MRD is nearing clinical readiness in LBCL.

Imaging leaves considerable uncertainty. Some patients with negative end-of-treatment (EoT) PET-CT relapse, while many positive scans do not indicate viable lymphoma (3, 4). Three recent phased-variant studies showed that EoT ctDNA predicts relapse more reliably (3-5). Meriranta and colleagues provide essential, independent validation using a different assay.

The investigators analyzed 447 plasma samples from 119 patients aged 18–64 years enrolled in the Nordic LBC-06 trial (6), with median follow-up of five years. Patients received intensive chemoimmunotherapy with CNS prophylaxis. A median of 252 tumor-specific mutations per patient was tracked by duplex sequencing after cycles two and four, at EoT, and during surveillance. Uniform trial treatment, serial sampling and long follow-up are major strengths.

After two cycles, 67% of patients had cleared ctDNA (negative predictive value 91%). The positive predictive value was only 35%, and about half of those still positive cleared by cycle four, suggesting that early persistence may not justify intensification. Persistent positivity after cycle four identified patients with poor outcomes.

At EoT, 11 of 99 patients were MRD-positive, with two-year progression-free survival of 18% versus 94% for MRD-negative patients (hazard ratio [HR] 18.7). ctDNA also helped interpret PET abnormalities: both biopsies confirming lymphoma were MRD-positive, while four of five benign biopsies were MRD-negative. During surveillance, ctDNA preceded clinical relapse in 11 of 12 patients by a median of six months,

including patients negative at EoT. Isolated CNS relapses had shorter plasma lead times, suggesting a potential limitation of ctDNA. In one case, cerebrospinal fluid ctDNA detected recurrence earlier.

Phased-variant studies reported HRs of approximately 10–30 for detectable EoT ctDNA (3-5). Meriranta and colleagues reach a strikingly similar conclusion (HR 18.7) with a different error-suppression strategy, population and treatment, showing that the prognostic value of EoT ctDNA is reproducible across technologies. Their cycle-4 and surveillance results extend monitoring to additional time points. Their findings raise questions for MRD-adapted trials. Could early clearance justify de-escalation? Should persistent MRD prompt consolidation? Could ctDNA guide management of equivocal PET findings, or replace surveillance imaging?

Duplex and phased-variant sequencing address the same challenge, distinguishing rare tumor-derived DNA fragments from a sea of technical noise, but make different compromises (7). Phased-variant sequencing exploits mutation clusters generated by somatic hypermutation (2). Two tumor mutations on the same fragment provide strong evidence of tumor origin even when only one DNA strand is recovered, but isolated mutations cannot be tracked. Duplex sequencing can track isolated or clustered mutations, but requires recovery of both original strands, discarding potentially informative molecules when only one strand is recovered. Sensitivity ultimately depends on the number of informative molecules, which each approach maximizes differently (8, 9).

Duplex sequencing establishes that a mutation is genuine, but not that it derives from the lymphoma. Clonal hematopoiesis can contribute real mutations. Phasing provides additional protection against this, although mutations shared with ancestral or indolent clones remain a potential confounder. Duplex sequencing may be advantageous in lymphomas that lack clustered hypermutation, including mantle cell or T-cell lymphomas (10). The approaches are not mutually exclusive: by our analysis of the supplementary data (1), 90% of duplex markers lie within 150 base pairs of another, so the existing libraries offer an opportunity to compare or combine the two.

Earlier comparisons favored phased variants at very low tumor concentrations (2). Meriranta and colleagues compensated by tracking many mutations and sequencing

deeply, yet their median limit of detection was 17 parts per million (ppm), and no EoT sample achieved the 1 ppm limit of detection cited in NCCN guidance for residual PET-positive disease when biopsy is infeasible. Clinical discrimination was nonetheless comparable (**Table 1**), cautioning against judging assays on a single technical specification.

PET-avid disease may shed readily detectable ctDNA, so greater sensitivity may matter most in PET-negative patients and for predicting late relapse. With five-year follow-up, the negative predictive value of EoT MRD fell from 94% at two years to 88% overall; nine of 12 late relapses followed an MRD-negative EoT result. Whether greater sensitivity would have detected these is uncertain. Even sub-ppm phasing missed some late relapses (4). Poor shedding from isolated CNS disease and evolution from precursor clones may also explain false negatives.

Despite these caveats, the message is encouraging. Across four studies, three assays and two error-suppression strategies, ctDNA MRD consistently distinguishes patients with sharply different relapse risks, creating a real opportunity to adapt the intensity and duration of therapy to each patient's molecular response. The next step is not simply more prognostic validation, but randomized trials to establish whether MRD-guided de-escalation or consolidation benefits patients. Pooled trial data might also support MRD as a surrogate endpoint with potential to accelerate drug development.

A priority now is harmonization. Ultrasensitive ctDNA assays are emerging from academic, diagnostic and commercial laboratories, each differing in the mutations tracked, error correction and analytical methods. Standardizing these assays is neither necessary nor feasible, but ensuring they reach the same clinical conclusions is critical. This will require agreed thresholds and reporting standards, including criteria for non-evaluable results, together with reference materials and interlaboratory comparisons to establish concordance.

By providing independent validation, Meriranta and colleagues have brought ctDNA MRD closer to the Finnish line. Treatment-adapted trials, harmonization and accessible testing must cover the remaining distance.

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Table 1. Technical characteristics and clinical performance of EoT ctDNA-MRD in first-line LBCL

Study	Method	N (MRD+)	2-year PFS (MRD− vs MRD+)	HR MRD	HR Radiology	Median follow-up	Median LoD95 (ppm)	Best LoD95 (ppm)	Median IR
Meriranta et al. 2026 (LBC-06)	Duplex sequencing	95(11)	94.3% vs 18.2%	18.7[a]	4.42[a]	60.4 mo	16.81	2.08	181,267
Krupka et al. 2026 (DIRECT)	Phased variants (academic assay)	111(27)	95.1% vs 39.2%[b]	14.51[b]	5.49[b]	24.5 mo	4.54	0.53	557,516
Wang et al. 2026 (HOVON-902)	Phased variants (Foresight Diagnostics)	131(22)	88.9% vs 31.8%[c]	9.3[c]	6.2[c]	33.2 mo	NR[e]	NR[e]	NR[e]
Roschewski et al. 2025	Phased variants (Foresight Diagnostics)	125(28)[d]	97% vs 29.5%[d]	28.3[d]	3.6[d]	36.6 mo	NR[e]	NR[e]	NR[e]

a. 2-year PFS not reported for the MRD/PET-matched subset (n=90, Table S3B). PET+: DS 4–5.

b. DIRECT Cohort 2 (full-dose anthracycline), MRD/PET-matched (Fig 3D). PET+: DS 4–5.

c. MRD/PET-matched; 2-year PFS inferred from Fig 3C. PET+: non-CMR.

d. MRD/PET-matched; 2-year PFS inferred from Fig 5B. PET+: DS 4–5.

e. Patient-level LoD95 not reported but assay LoD cited to be 0.7 ppm in optimal conditions.

Abbreviations: CMR, complete metabolic response; DS, Deauville score; HR, hazard ratio for PFS; IR, informative reads; LoD95, 95% limit of detection; NR, not reported; ppm, parts per million.

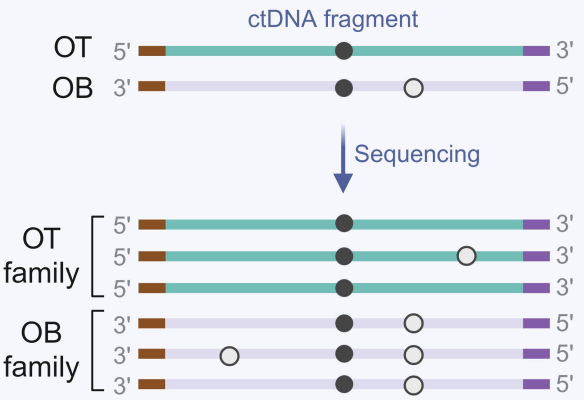
Figure Legends

Figure 1. Error suppression by duplex UMI and phased-variant sequencing. Left, duplex sequencing. Unique molecular identifiers (UMIs) distinguish the original top (OT) and bottom (OB) strands of each cfDNA fragment, and reads are grouped into OT and OB families. A tumor-specific variant is counted only if present in the consensus of both strands. Errors confined to one strand, and molecules whose complementary strand is not recovered, are discarded. **Right, phased-variant sequencing.** A molecule is counted when two or more tumor-specific variants occur together on the same read; the strand of origin need not be resolved (gray). Because an error would have to occur at both positions, background is approximately the product of the per-site error rates.

Tumour reporters

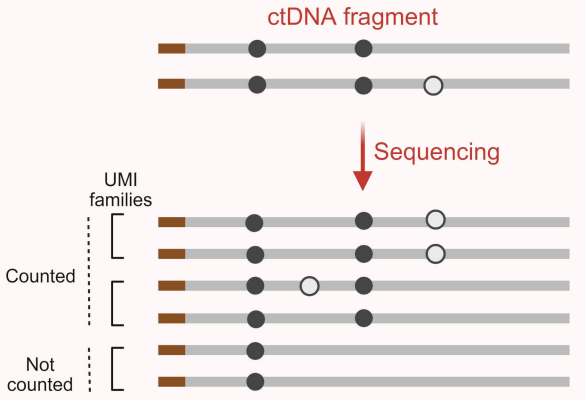
Duplex UMI SNVs

Double in *trans*: 1 SNV, 2 strands



Phased variants

Double in *cis*: 2 SNV, any strand



Candidate tumour markers
- True SNV
- Error

ctDNA fragment
- Original top (OT)
- Original bottom (OB)
- Unknown

UMI layout
- Duplex
- Single