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Real-world clinical experience with NGS-based chimerism analyses in haematopoietic stem cell transplant patients

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Summary

Next-generation sequencing (NGS) has improved the sensitivity of chimerism assays beyond the limitations of conventional short tandem repeat (STR) methods, enabling the detection of minimal recipient haematopoiesis after haematopoietic stem cell transplantation (HSCT). We evaluated the clinical utility of CASAL, an NGS-based chimerism assay, in routine practice. We retrospectively analysed 310 patients who underwent STR or CASAL chimerism testing between April 2021 and September 2023. CASAL provided significantly more informative markers than STR (median 18 vs. 6; $p < 0.001$). Among 260 CASAL samples with paired molecular minimal residual disease (MRD) data, concordance at the 10^{-4} threshold was ~84%. Low-level mixed chimerism (2%–5%) detected beyond 1 month post-HSCT was associated with impending relapse. In survival analyses, patients with both MC and MRD positivity (MC/MRD$^+$) had the highest relapse risk across both platforms. Multivariable Cox regression confirmed MC/MRD$^+$ as an independent predictor of relapse (hazard ratio 5.87, 95% CI: 1.17–29.57). CASAL enables sensitive chimerism monitoring and shows a strong correlation with molecular MRD and clinical outcomes. These findings support its clinical utility for individualized post-HSCT surveillance, especially in patients lacking leukaemia-specific molecular targets.

KEYWORDS
chimerism analysis, HSC transplantation, minimal residual disease, next generation sequencing

INTRODUCTION

Allogeneic haematopoietic cell transplantation (HSCT) is a cornerstone treatment for a range of malignant and non-malignant haematological disorders.[1] Following HSCT, quantitative chimerism analysis serves as a critical tool for evaluating the proportion of donor- versus recipient-derived haematopoietic cells, thereby providing critical insight into engraftment kinetics, graft sustainability and risk of relapse.

Complete chimerism (CC) indicates the absence of recipient haematopoiesis, whereas mixed chimerism (MC) reflects recipient haematopoiesis, which may suggest suboptimal engraftment or disease persistence.[2] Even low levels of MC (<1%) have been linked to relapse in haematological malignancies.[3,4] In non-malignant disorders such as aplastic anaemia or immunodeficiencies, chimerism monitoring serves to assess the long-term durability of donor-derived haematopoiesis and engraftment outcomes.[2,5]

Traditional chimerism monitoring has relied on short tandem repeat (STR)-based PCR methods. However, the limited number of informative loci and lower sensitivity (typically 1%–5%) restricts its ability to detect early or subtle MC.[6,7]

Jin Ju Kim and Soon Sung Kwon contributed equally to this work.

Recent advances in next-generation sequencing (NGS)–based methods have significantly improved the resolution of chimerism analysis by expanding marker panels and enabling the quantification of recipient DNA at levels below 1%.

While the feasibility and technical validity of NGS-based chimerism analysis have been demonstrated in multiple studies,[8–11] its clinical application in large patient cohorts has yet to be comprehensively reported. Our group previously developed an NGS-based chimerism assay using single nucleotide polymorphisms (SNPs) adjacent to regions of linkage disequilibrium, termed CASAL, to achieve high sensitivity and accuracy.[12] Implemented at our institution in July 2022, we retrospectively evaluated the performance of NGS-based chimerism monitoring in a substantial cohort of post-HSCT patients, utilizing data derived from routine clinical practice at a single tertiary centre.

METHODS

Study design and data collection

We retrospectively reviewed 310 patients who underwent chimerism testing at Yonsei university Severance Hospital between April 2021 and September 2023. To reflect the timeline of technology adoption, STR-based chimerism was performed exclusively from April 2021 to June 2022. CASAL, an NGS-based assay, was introduced into clinical routine in July 2022 and replaced STR as the standard method thereafter.

Patients were categorized into three groups based on the chimerism testing platform(s) used during follow-up within the study timeframe: STR-only ($n = 96$), CASAL-only ($n = 100$) and both ($n = 114$). In the both STR and CASAL groups, all patients initially underwent STR testing before July 2022, followed by CASAL-based monitoring thereafter; thus, the two platforms were applied sequentially, not concurrently. Baseline clinical and transplant-related variables—including age, diagnosis and donor type—were broadly compared among the three groups (Table 1). A study flow diagram outlining patient inclusion, grouping (STR only, CASAL only, both) and outcome stratification is shown in Figure 1.

A total of 1240 chimerism tests were performed across 310 patients during the study period, comprising 971 peripheral blood (PB) and 269 bone marrow (BM) samples. All samples were collected in EDTA tubes and processed according to institutional protocols.

TABLE 1 Baseline clinical and transplant characteristics of the study cohort.

Variable	STR ($N = 96$)	STR and CASAL ($N = 114$)	CASAL ($N = 100$)	p-value
Patient demographics and diagnosis				
Gender (M/F)	54/42	67/47	44/56	0.075
Median age, range	43.5 (2–69)	34.5 (0–70)	39.7 (0–70)	0.092
Diagnosis—AML	37 (38.5%)	48 (42.1%)	43 (43.0%)	0.764
B-ALL	24 (25.0%)	29 (25.4%)	15 (15.0%)	0.136
MDS	10 (10.4%)	19 (16.7%)	12 (12.0%)	0.550
Aplastic anaemia	4 (4.2%)	3 (2.6%)	10 (10.0%)	0.036
TLL	5 (5.2%)	2 (1.8%)	0 (0.0%)	0.044
Acute undifferentiated leukaemia	0 (0.0%)	3 (2.6%)	0 (0.0%)	0.074
MPAL	1 (1.0%)	1 (0.9%)	2 (2.0%)	0.743
Other haematological malignancies[a]	11 (11.5%)	5 (4.4%)	10 (10.0%)	0.143
Non-malignant disorders[b]	4 (4.2%)	4 (3.5%)	2 (2.0%)	0.676
Transplant characteristics				
Donor type (related/unrelated/both)	62/32/2	67/44/3	58/41/1	0.741
Graft source (BM/PB/both)	4/91/1	8/105/1	2/97/0	0.532
Number of transplantations (1/2/3)	89/6/1	102/11/1	98/2/0	0.166
Outcomes and follow-up				
Relapse	24 (25.0%)	23 (20.2%)	4 (4.0%)	0.002
Survive	64 (66.7%)	107 (93.9%)	93 (93.0%)	<0.001
Follow-up from diagnosis (months), median [range]	48.0 (6–186)	36.5 (12–152)	15.0 (4–69)	<0.001

Abbreviations: AML, acute myeloid leukaemia; B-ALL, B-lymphoblastic leukaemia; BM, bone marrow; MDS, myelodysplastic syndrome; MPAL, Mixed phenotypic acute leukaemia; PB, peripheral blood; T-ALL, T-lymphoblastic leukaemia.

[a]Other haematological malignancies included: chronic myelomonocytic leukaemia (CMML), chronic myeloid leukaemia (CML), pure red cell anaemia (PRCA), myeloproliferative neoplasms (essential thrombocythaemia (ET), primary myelofibrosis (PMF)), mature lymphoid neoplasms (e.g. diffuse large B cell lymphoma (DLBCL), T-cell lymphoma, anaplastic large cell lymphoma, extranodal NK/T cell lymphoma) and histiocytic/dendritic disorders.

[b]Non-malignant disorders included: IL10RA deficiency, severe combined immunodeficiency (SCID), chronic granulomatous disease (CGD), *GATA2* deficiency, VEXAS syndrome, adrenoleukodystrophy, Pelizaeus–Merzbacher disease and activated PI3K delta syndrome.

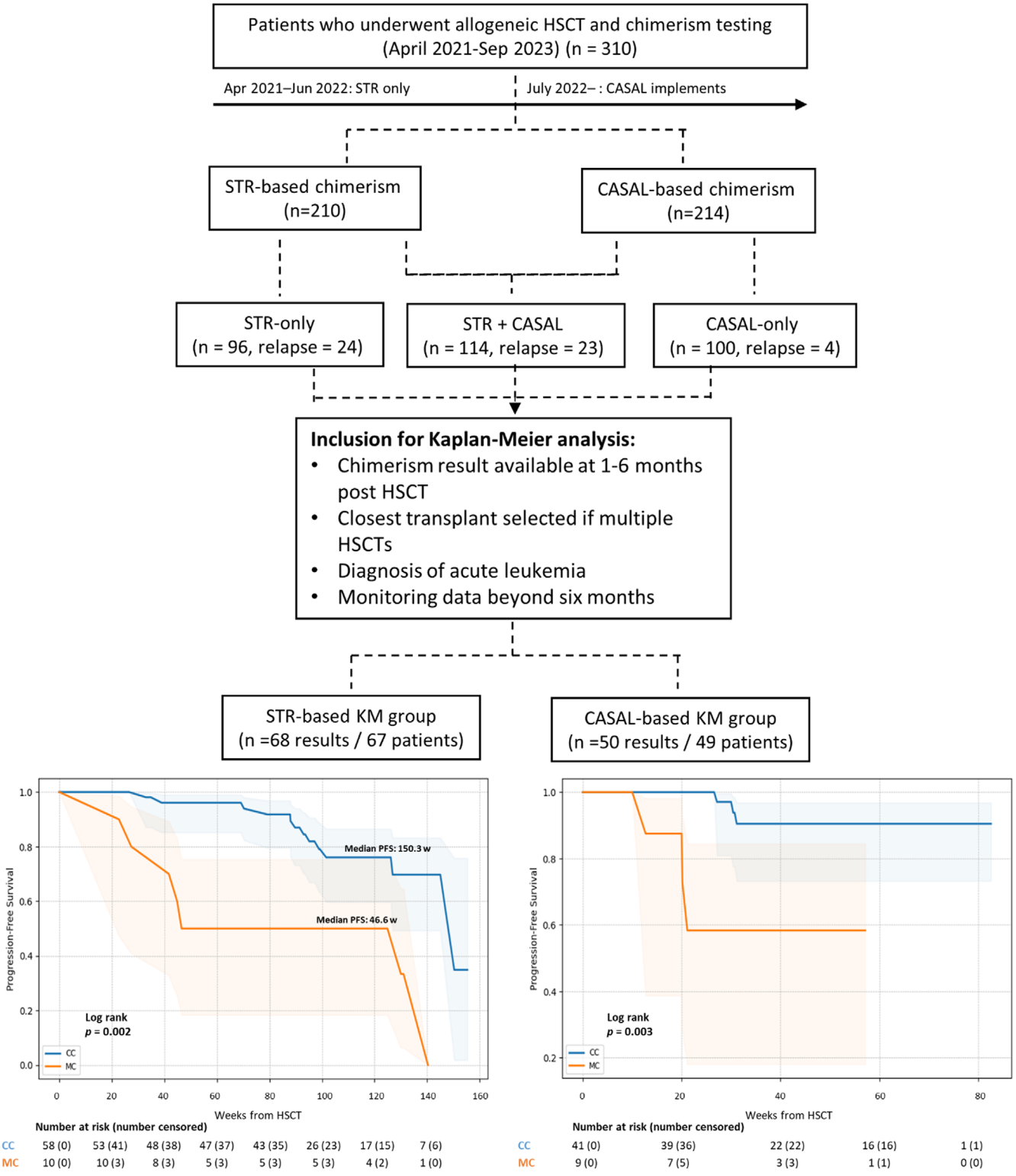


FIGURE 1 Study inclusion diagram and survival cohort segmentation. Study flow and Kaplan–Meier analysis of chimerism monitoring strategies. Patients who underwent allogeneic HSCT between April 2021 and September 2023 were grouped based on the timing and method of chimerism testing (STR vs. CASAL). A timeline above illustrates the institutional shift from STR-based to NGS-based (CASAL) chimerism analysis in July 2022. Patients were selected for Kaplan–Meier analysis if they had (1) a chimerism result between 1 and 6 months post-HSCT, (2) a diagnosis of acute leukaemia and (3) monitoring data available beyond 6 months. Kaplan–Meier curves show progression-free survival stratified by chimerism status (complete vs. mixed) in each group. Log-rank *p*-values are shown.

PB was the preferred specimen for routine post-transplant monitoring, owing to its accessibility and greater patient compliance. However, when BM aspiration was clinically indicated—such as for disease assessment, minimal residual disease (MRD) evaluation or suspicion of relapse—the concurrently obtained BM sample was used for chimerism analysis as well. This strategy reflects standard institutional practice, prioritizing PB for surveillance and BM only when already being collected for other diagnostic purposes. Sample type selection was guided by clinical context rather than by conditioning regimen.

Chimerism analysis

Genomic DNA was extracted using a QIAmp blood mini kit (Qiagen, Hilden, Germany). STR-based fragment analysis employed the AmpFlSTR® Identifiler® PCR amplification kit (Thermo Fisher Scientific, Carlsbad, CA, USA) on an ABI 3730xL genetic analyser (Thermo Fisher Scientific), with data interpretation through GeneMapper 5.0 software (Thermo Fisher Scientific). CASAL, a targeted NGS assay using 84 SNP loci (DxSeqTM personal ID kit, Dxome, Korea), was sequenced on a NextSeq 550Dx instrument (Illumina).

CC was defined as:

- STR: ≤5% recipient DNA[6,11]
- CASAL: ≤2% recipient DNA (based on previous validation[12]).

MC was defined as any result exceeding these thresholds. Low-level MC (LL-MC) was further defined as CASAL-detected values between 2% and 5%—a range typically classified as CC by STR due to its higher detection limit. This category was used to explore CASAL's potential for early relapse prediction.

Minimal residual disease (MRD) testing

MRD testing was performed exclusively using molecular assays, based on the availability of disease-specific genetic markers identified at diagnosis. Methods included quantitative RT-PCR (*NPM1, BCR::ABL1, CBFB::MYH11, PML::RARA, RUNX1::RUNX1T1*), qualitative RT-PCR (*ETV6::RUNX1*), fragment analysis (*FLT3*-ITD) and various NGS assays (immunoglobulin gene clonality or customized MRD panels) (Supporting Information).

Statistical analysis

Statistical analyses were conducted using R (v 4.2.1) and Analyse-it. Normality was assessed via the Shapiro–Wilk test. Continuous variables were compared using Mann–Whitney *U* or Kruskal–Wallis tests, and categorical variables with chi-squared or Fisher's exact test. Paired STR and CASAL results were evaluated using the Wilcoxon signed-rank test. ROC analysis was used to evaluate CASAL's MRD predictiveness (threshold 10^{-4}). Kaplan–Meier and log-rank tests evaluated survival. Progression-free survival (PFS) was defined as the interval between the date of chimerism testing and either documented relapse or the last follow-up date. Overall survival (OS) was calculated from the date of the most recent allogeneic HSCT preceding the selected chimerism result to the last follow-up. $p < 0.05$ was considered statistically significant.

RESULTS

Marker informativeness and platform comparison

Because having sufficient informative markers is crucial for an accurate quantitative analysis, we compared the number of informative markers between CASAL and STR in 102 patients with single HSCT and had results from both methods. CASAL yielded significantly more informative markers compared to STR (median 18.0 vs. 6.0; IQR 15–28 vs. 5–8; $p < 0.001$, Wilcoxon signed-rank test), supporting its superior resolution (Figure S1A). This trend persisted after multiple transplants: for first HSCTs, CASAL yielded a median of 19 markers (range 6–40, $n = 205$), compared with 7.5 (range 1–14, $n = 210$) for STR ($p < 0.001$); after second HSCT, marker counts declined in both platforms; however, CASAL still showed a significantly higher median count (14.5; range 7–29, $n = 14$) compared to STR (4.5; range 4–7, $n = 6$) ($p < 0.001$).

Marker yield varied by donor type and transplant number. In first HSCTs, CASAL demonstrated substantially more markers in unrelated than related donors (median 28 vs. 16; $p < 0.001$), reflecting its larger marker pool. Although overall marker counts decreased after the second transplant across all platforms, CASAL maintained a clear advantage over STR (median 14.5 vs. 4.5; $p < 0.001$), providing more than threefold higher marker count. These findings underscore the platform's robustness in complex transplant settings. Full stratified comparisons are shown in Figure S1.

Correlation of CASAL with molecular MRD testing

Molecular MRD data from the same samples analyzed by CASAL were available for 260 of the 689 results from 122 patients and were included. The primary objective was to evaluate the correlation between CASAL-based chimerism results and molecular MRD status. Patients without a disease-defining genetic aberration at diagnosis—such as *NPM1* mutation or fusion genes—were excluded, as molecular MRD monitoring was not feasible. These patients were clinically followed but were not suitable for direct CASAL-MRD comparison.

In the BM samples (Figure 2A), most CC results were MRD-negative (56/74), while 18 were positive—6 with MRD

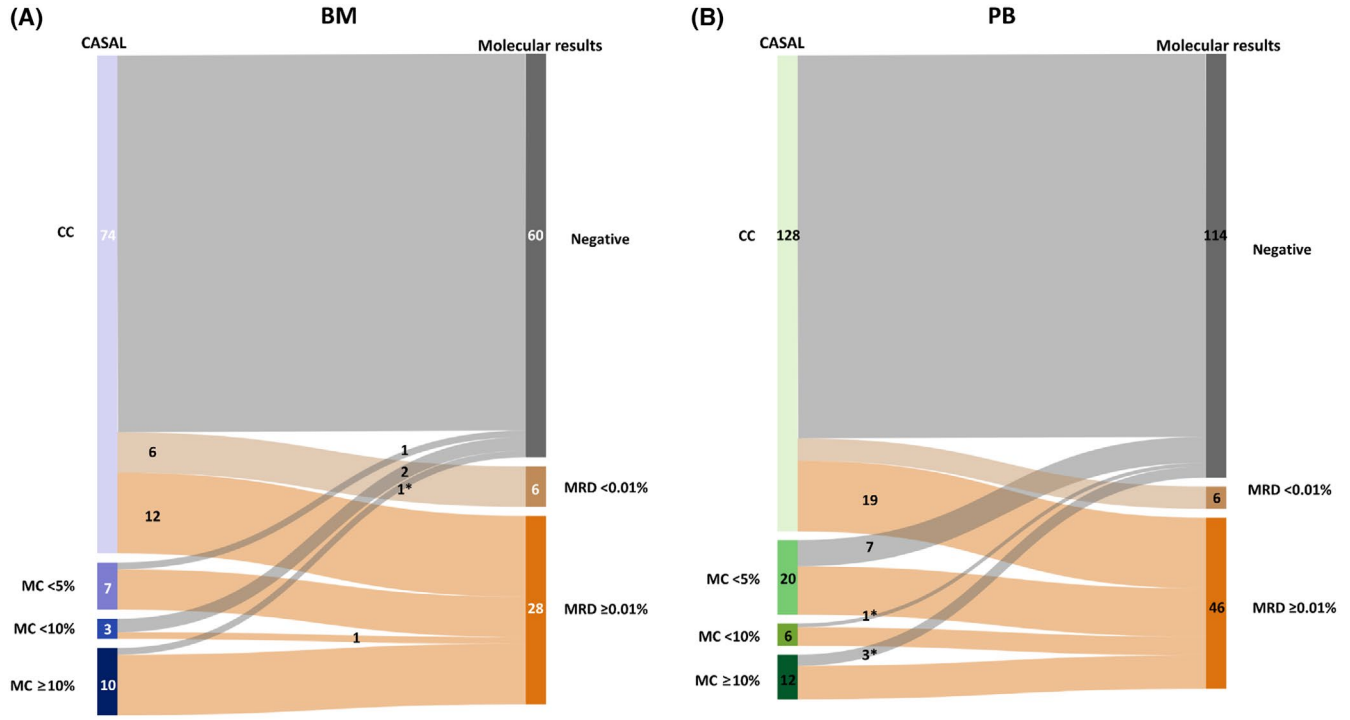


FIGURE 2 Concordance between CASAL chimerism results and molecular MRD findings. (A) and (B) compare CASAL results with molecular MRD (minimal residual disease) results in bone marrow (BM, $n = 94$) and peripheral blood (PB, $n = 166$) samples respectively. Samples were categorized based on their MRD levels as negative, MRD <0.01% and MRD ≥0.01%. The high concordance rates indicate the reliability of CASAL in assessing MRD across various thresholds. CC, complete chimerism, and MC denotes mixed chimerism at different levels. * indicates data from a single AML patient with *RUNX1::RUNX1T1* who demonstrated persistent high recipient chimerism without relapse.

<0.01% and 12 with MRD ≥0.01%. In contrast, MC results showed a stronger correlation with MRD positivity: Nine of 10 with ≥10% chimerism and six of seven with <5% chimerism were MRD ≥0.01%. Four MC cases had discordant molecular results.

PB samples (Figure 2B) showed similar trends. Of 128 CC results, 103 were MRD-negative. Among MC samples, 14 of 18 cases with ≥5% chimerism were MRD ≥0.01%, supporting the link between higher recipient chimerism and molecular disease burden.

Notably, five of seven discordant samples with ≥5% MC but negative molecular MRD originated from one AML patient with *RUNX1::RUNX1T1*. This patient showed persistent MC (9.8%–41.2%) over 9 months but remained relapse-free and MRD-negative throughout follow-up. This discordance may be explained by a germline *FANCG* mutation and the use of reduced-intensity chemotherapy. Given that most discordant samples clustered within this unique clinical context, the overall discordance rate between CASAL and molecular MRD may be overestimated.

Excluding this patient results, CASAL—molecular MRD concordance (threshold 10^{-4}) was 83–84% across both specimen types, with PPV 79%–84% and NPV 83%–85% (Table S1). ROC analysis further supported these findings, with AUC of 0.69 (BM) and 0.78 (PB) (Figure S2), indicating acceptable discriminative performance. The optimal thresholds for molecular MRD positivity by Youden's Index were 0.995% (BM) and 0.624% (PB), consistent with those from our previous study[12] (BM: 1.97%, PB: 1.32%), reinforcing the reproducibility and clinical validity of CASAL-based monitoring.

Low-level mixed chimerism and relapse prediction

CASAL's enhanced analytical sensitivity enables the detection of MC below the 5% threshold, a range conventionally classified as CC by STR-based methods. A total of 36 LL-MC samples (8 BM, 28 PB) were identified from 25 patients (median chimerism: 3.25%; range: 2.05–4.81%). After excluding nine samples from patients with non-malignant conditions (e.g. aplastic anaemia, severe combined immunodeficiency), 27 samples from 19 patients with haematological malignancies remained, including both de novo acute leukaemias and secondary leukaemic transformations from other haematological malignancies (e.g. myelodysplastic syndrome, myelofibrosis).

Eleven samples from 10 patients were collected within the first month post-HSCT, and the majority (9/11) were MRD-negative. Clinical trajectories of 18 patients with LL-MC are shown in Figure 3 and Figure S3 (one excluded due to lack of follow-up).

Among nine patients with LL-MC only within the first month, all maintained CC and remained relapse-free (Figure 3A). In contrast, eight patients had LL-MC beyond

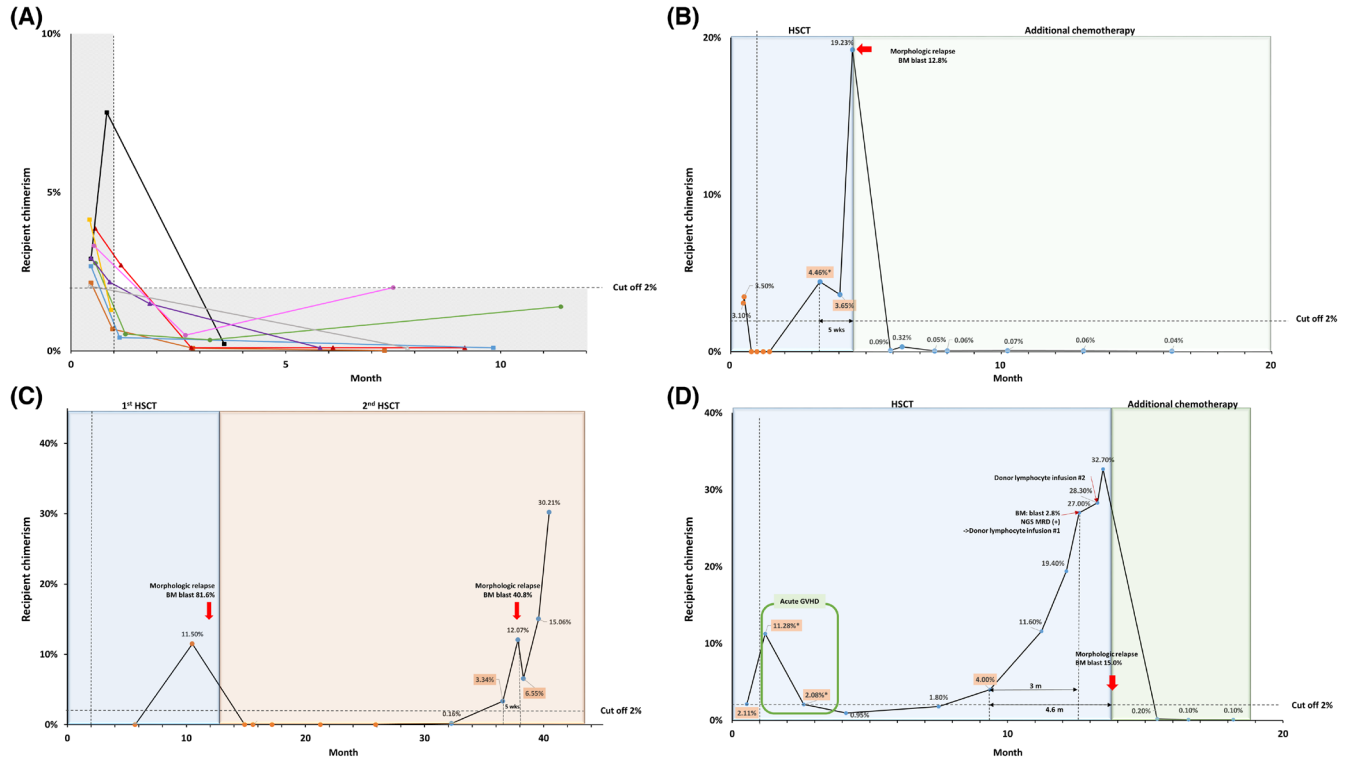


FIGURE 3 Clinical course of patients with low-level mixed chimerism detected by CASAL. The clinical progression of haematological malignancy patients with low-level mixed chimerism (2%–5%) detected by CASAL during the study. Low mixed-donor chimerism results are highlighted in orange, and results with minimal residual disease (MRD) validated by other molecular results are presented with an asterisk. (A) Patients with low-level mixed chimerism identified within the first month post-HSCT who subsequently achieved and maintained complete chimerism without relapse. (B–D) Patients with low-level mixed chimerism detected beyond 1 month after HSCT, which is associated with an elevated risk of relapse. Orange dots: STR results, Blue dots: CASAL results.

the first month; seven subsequently developed morphologic relapse within 3 weeks to 6.3 months (Figure 3B–D; Figure S3A–D), and one showed molecular relapse 6 months later (Figure S3E). One additional LL-MC (3.89%) case beyond 1 month was collected after salvage chemotherapy, complicating interpretation (Figure S3F).

Taken together, these findings suggest LL-MC observed within the first month post-HSCT is likely transient and clinically insignificant, whereas delayed or persistent LL-MC may indicate early relapse—supporting CASAL's utility in subclinical detection.

Prognostic impact of chimerism and MRD status

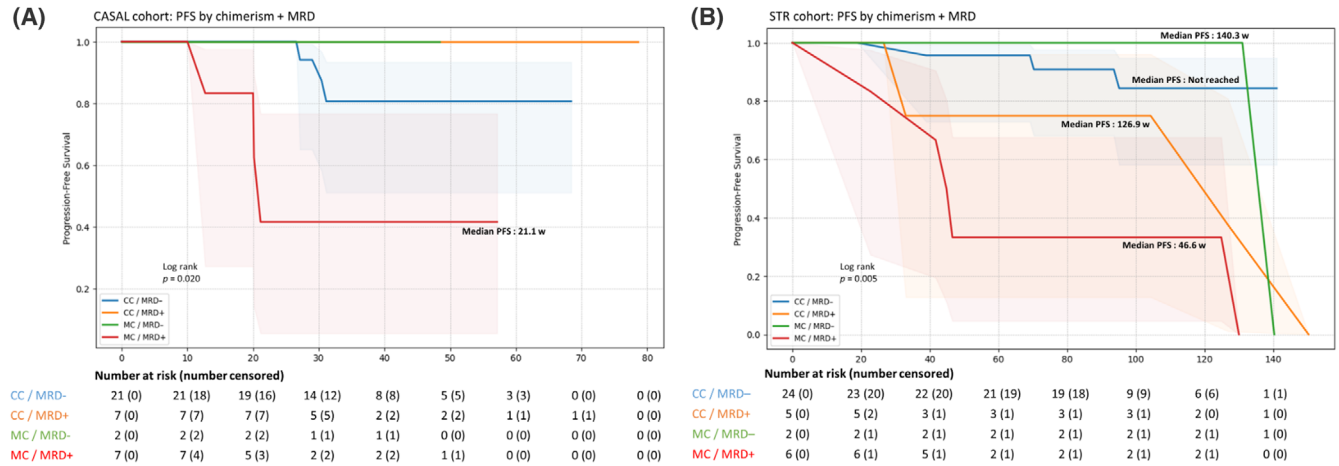
To evaluate relapse risk associated with chimerism and MRD status, we conducted stratified Kaplan–Meier and Cox regression analyses within each platform. Patients with acute leukaemia were included if they had chimerism results 1–6 months post-HSCT; for multiple transplants, the corresponding transplant was selected. If multiple eligible results existed, we prioritized the earliest MC or latest CC result. PFS was measured from the chimerism testing to relapse or last follow-up (Figure 1).

Among 107 patients (118 test results), nine were in both cohorts due to multiple transplants or discordant platform results within the same post-transplant window. Median PFS was not reached in the CASAL cohort but remained significantly different between CC and MC groups (log-rank $p = 0.003$). In the STR group, median PFS was 150.3 weeks for CC versus 46.6 weeks for MC ($p = 0.002$).

Chimerism-based subgroup analysis was further refined by incorporating molecular MRD status assessed on the same day. MRD positivity was defined as a detectable target at or above 10^{-4}. This analysis included only patients with molecular MRD data ($n = 37$ each for CASAL and STR).

In CASAL, only patients with both MC and MRD positivity (MC/MRD+) demonstrated significantly inferior PFS (median: 21.1 weeks; log-rank $p = 0.020$) (Figure 4A). Similarly, the STR-based MC/MRD+ subgroup had the poorest outcome (median: 46.6 weeks; $p = 0.005$) (Figure 4B). Other subgroups, including CC/MRD+ and MC/MRD–, show no statistically significant differences (Table S2).

To explore prognostic implications further, we performed univariable Cox regression within each platform. In the STR group, the MC group had a significantly inferior outcome compared to the CC group (HR = 3.97, 95% CI: 1.53–10.30, $p < 0.005$). A similar trend was observed in the CASAL



(C)

Platform	Group	HR	95%CI	p-value
STR	**MC vs CC**	**3.97**	**1.53 - 10.30**	**<0.005**
	CC / MRD- vs MC / MRD+	**9.03**	**2.11 - 38.60**	**<0.005**
	CC / MRD- vs CC / MRD+	2.67	0.41 - 17.15	0.30
	CC / MRD- vs MC / MRD-	1.54	0.14 - 16.42	0.72
CASAL	**MC vs CC**	**8.07**	**1.61 - 40.56**	**0.01**
	CC / MRD- vs MC / MRD+	**5.87**	**1.17 - 29.57**	**0.03**
	CC / MRD- vs Other groups	--	low N	--

FIGURE 4 Kaplan–Meier analysis of progression-free survival (PFS) based on combined chimerism status and MRD results. (A) CASAL cohort: Patients with complete chimerism and MRD negativity (CC/MRD–) showed significantly prolonged PFS compared to those with mixed chimerism and MRD positivity (MC/MRD+). (B) STR cohort: A similar trend was observed, with CC/MRD– patients demonstrating superior PFS. (C) Analysis of prognostic factors based on chimerism status and MRD results. Median PFS values and log-rank *p*-values are shown for each group. These findings highlight the additive prognostic value of integrating molecular MRD with chimerism assessment in post-HSCT monitoring.

cohort (HR = 8.07, 95% CI: 1.61–40.56, $p = 0.01$). Notably, the CASAL group had a shorter observation period (median OS: 277 days; range, 70–578) than STR (686 days; range, 67–1087), reflecting later clinical adoption. This may have limited its capacity to capture late relapse, potentially underestimating its prognostic utility. To address this temporal imbalance, we performed multivariable Cox regression adjusting for OS. When stratified by MRD status, MC/MRD+ status associated with relapse in both platforms: HR = 9.03 (95% CI: 2.11–38.60, $p < 0.005$) for STR and HR = 5.87 (95% CI: 1.17–29.57, $p = 0.03$) for CASAL. Other comparisons (e.g. CC/MRD– vs. CC/MRD+ or MC/MRD–) were not of statistical significance, likely due to small sample sizes (Figure 4C).

To assess the timing of relapse, we investigated the temporal relationship between chimerism status and clinical relapse (Table S2). MC/MRD+ patients showed relapse rates up to 83.3% (STR) and 42.9% (CASAL). Across both platforms, the median time from MC detection to relapse was 0 days, indicating that MC often emerges near the time of clinical relapse. Since chimerism testing is often performed alongside bone marrow examinations prompted by clinical suspicion, this likely reflects reactive testing rather than routine surveillance. In this context, MC may reflect existing disease rather than early progression.

In contrast, relapse cases classified as CC demonstrated longer test-to-relapse intervals (CASAL: median 163 days; STR: 129 days), suggesting biological latency or underdetection. Notably, several STR-monitored patients maintained CC despite molecular MRD positivity for over 80 days, raising concerns regarding the limited sensitivity of STR in capturing early disease progression.

These findings highlight the importance of test timing and clinical context in interpreting chimerism results. While MC status strongly correlates with relapse risk, its role as a predictive versus concurrent biomarker may depend on whether monitoring is performed proactively or in response to clinical suspicion.

DISCUSSION

Our study underscores the clinical utility of NGS-based chimerism analysis in HSCT, particularly through the CASAL assay. Compared to conventional STR-based methods, CASAL offers greater analytical sensitivity and marker informativeness, especially in multiple transplant settings and related donor pairs. While NGS-based assays like CASAL require more resources and batch sequencing workflow,[13] these are offset by superior sensitivity and broader marker coverage. This enhanced resolution enables more granular detection of MC, including LL-MC that would be undetectable by STR.

Chimerism status was prognostically relevant across both platforms. MC patients had significantly inferior PFS compared to CC patients, and integrating molecular MRD status further refined relapse risk stratification. MC/MRD+ status consistently experienced the worst outcome across both platforms, underscoring the value of integrated monitoring.

CASAL's higher marker resolution enabled the detection of LL-MC in samples that would be classified as CC by STR.[13] Although transient LL-MC observed within the first month post-HSCT may not always correlate with imminent relapse, persistent LL-MC beyond this period significantly increases relapse risk. This finding highlights CASAL's role in detecting relapse-predictive LL-MC, which aligns with previous studies highlighting the importance of post-transplant chimerism dynamics in relapse prediction.[14,15] Still, interpretations must remain contextual. For instance, in our cohort, a B-ALL patient had 6.41% donor chimerism by CASAL within 1-month post-HSCT, remained relapse-free and was molecular MRD-negative despite elevated recipient fraction. Clinically, the patient exhibited signs of acute graft-versus-host disease (GVHD) at the time of testing.

This suggests the recipient chimerism exceeding the CASAL-specific threshold of 2% of CC may not always indicate relapse—particularly within the first month post-HSCT or in the setting of GVHD. Immune activation during GVHD could transiently elevate recipient fractions, leading MC unrelated to malignant relapse.[16] Therefore, chimerism results—especially those observed early post-HSCT or during GVHD—should be interpreted cautiously with molecular MRD findings and clinical context.

Furthermore, CASAL and molecular MRD testing showed high concordance (≈84%) at the 10^{-4} threshold, supporting the clinical relevance of this sensitivity level. All samples with MC ≥10% were MRD-positive, reinforcing that high-level recipient chimerism likely reflects residual disease. Notably, molecular MRD negativity at $<10^{-4}$ has been associated with improved PFS and OS in AML[17–20] and ALL,[21–23] and our data suggest that CASAL's 2% MC threshold may align well with this prognostic boundary. Although chimerism analysis does not directly assess leukaemic clones,[24] the correlation between MC defined by CASAL and molecular MRD supports its potential role as a surrogate marker. This finding is particularly valuable in cases lacking disease-specific molecular targets, where CASAL-based chimerism monitoring may offer indirect yet clinically meaningful insight into residual disease burden and relapse risk.

This study has several strengths, including a large real-world cohort, integrated MRD-chimerism analysis and platform-stratified outcome evaluation. However, several limitations remain. First, its retrospective design and the platform transition during the study period limited direct intra-sample comparisons. Second, molecular MRD results were not available for all cases, which may have reduced the statistical power of subgroup analyses. Third, follow-up duration was significantly shorter in the CASAL group than STR, given the later implementation of the assay. This temporal limitation may have influenced prognosis analysis. Although transplant-related variables such as conditioning regimen and remission status were not included in our primary analysis, future prospective studies could explore their potential influence on chimerism dynamics and clinical outcomes.

Multiparameter flow cytometry (MFC) was also not included, as it is not routinely employed for MRD monitoring in adult AML at our centre. In rare ALL cases where MFC was performed, molecular assays were concurrently applied, limiting its standalone contribution. Future prospective studies could explore comparative designs incorporating both molecular and flow-based strategies—for example, a 'molecular-only' versus 'integrated' monitoring arm—to evaluate the additive value of multiparameter approaches, particularly in patients lacking disease-specific markers or showing ambiguous chimerism patterns.

Despite these limitations, CASAL offers distinct clinical advantages. Unlike patient-specific MRD assays, it provides a universal, multi-sample strategy applicable across diagnoses, without the need for individualized primers. Compared with other high-sensitivity assays, NGS-based chimerism analysis allows for scalable batch processing across multiple patients, streamlining workflows and enhancing feasibility in clinical laboratories.[2,12,13]

Our findings support integrating CASAL into routine post-HSCT surveillance workflow in the clinical field, especially in a setting with limited access to MFC-based MRD testing. By enhancing early relapse detection and complementing molecular testing, CASAL holds strong potential for improving post-HSCT risk stratification and individualized care.

AUTHOR CONTRIBUTIONS

Jin Ju Kim and Soon Sung Kwon managed specimen collection and data acquisition. Yu Jeong Choi and Yehyun Kang contributed to the data analysis. Yu Jin Park, Saeam Shin, Seung-Tae Lee and Jong Rak Choi supervised the project. Jin Ju Kim wrote the manuscript, and all authors reviewed and approved the final version.

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CONFLICT OF INTEREST STATEMENT

Authors Seung-Tae Lee and Jong Rak Choi were employed by Dxome. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

DATA AVAILABILITY STATEMENT

The dataset supporting the conclusion of this article is available upon reasonable request from the corresponding author, Saeam Shin (saeam0304@yuhs.ac).

ETHICS STATEMENT

This study protocol was approved by the institutional review board (IRB) of Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea (IRB 4–2024-0989).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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