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# Real-world outcomes of acute lymphoblastic leukaemia in AYA and older adults: impact of molecular MRD on risk-adapted therapy and allogeneic transplantation

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# Abstract

Real-world evidence supporting measurable residual disease (MRD) guided management of ALL is limited. We conducted a multi-centre retrospective cohort study of 121 Philadelphia-negative ALL patients, referred for allele-specific oligonucleotide real-time quantitative PCR (ASO-RQPCR) MRD testing outside a clinical trial. 5-year overall survival (OS) was inferior in older adults (≥40 years) at 47.9% versus 77.9% in adolescents and young adults (AYA, p<0.001). Among older adults, achievement of MRD negativity ($MRD^{neg}$) at TP2 (median 98 days) was associated with superior leukaemia-free survival (LFS 68.2% vs 12.5%, p=0.0076); 18 of 22 $MRD^{neg}$ avoided allogeneic transplant (SCT) in first complete remission (CR1). In AYA, TP2 MRD status did not show significant difference in outcomes, reflecting real-world use of CR1 transplant mitigating the impact of persistent MRD. 19.5% of TP2 $MRD^{neg}$ AYA patients relapsed, highlighting the need for broader access to genomic profiling to refine risk stratification. Pre-SCT MRD by ASO-RQPCR defined three prognostic cohorts: $MRD^{neg}$, low-positive (≤1x10-4) and high-positive (>1x10-4) with 3-year OS of 84.1%, 53% and 16.7% respectively (p=0.0009). These real-world data highlight the value of molecular MRD in ALL management, including to guide treatment intensification, omission of CR1 SCT in selected older adults achieving $MRD^{neg}$, and support efforts to achieve $MRD^{neg}$ pre-SCT.


# Introduction

In recent years there have been improvements in outcome for adult acute lymphoblastic leukaemia (ALL) patients. Several trials have demonstrated that paediatric-inspired regimens can be delivered and are effective in the adolescent and young adult (AYA) population[1,2]. In addition, immunotherapies, including bispecific and conjugate antibodies, have seen impressive responses in both the de novo and relapsed/refractory setting[3,4]. Despite these advances, there remains a significant proportion of adult patients with poor outcomes, particularly in older adults[5].

Multiple trials have demonstrated that measurable residual disease (MRD) response is strongly predictive of overall outcome[1,6,7]. However, real-world data guiding the use of MRD outside clinical trials is lacking. Indeed, trials to date report differing methods of MRD evaluation with varying assay sensitivity, as well as differing prognostic MRD thresholds and timepoints. Some trials have utilised flow cytometry MRD with rapid turnaround. A large proportion of trials however, have used molecular MRD determined using allele-specific oligonucleotide real-time quantitative PCR (ASO-RQPCR), with more complex methodology, but increased sensitivity[6,8]. More recently, MRD assessment using next-generation sequencing (NGS) has been shown to be highly sensitive, prognostically informative and highly correlated to ASO-RQPCR results[9,10]. However, unlike PCR standardised according to EuroMRD guidelines, NGS-based MRD remains insufficiently standardised, with NGS prognostic thresholds awaiting longer term clinical trial data[11,12].

As MRD assessment has become increasingly integrated into ALL management, it has been used to guide therapy in clinical trials. In the UKALL2003 study, treatment intensity was reduced for MRD low-risk patients[13]. Conversely, other studies have used persistent MRD as indication for allogeneic stem cell transplant (SCT)[1,14]. Overall, the bulk of evidence supporting MRD-directed approaches has been derived from studies in paediatric and AYA ALL, with clear MRD-directed protocols less well established for those aged > 40 years.

Herein, we report multicentre real-world outcomes in Philadelphia chromosome-negative (Ph-) B- (B-ALL) and T-lineage (T-ALL) ALL, confirming the clinical value of ASO-RQPCR MRD outside the clinical trial setting, across both AYA and older adults. We demonstrate that molecular MRD is valuable in guiding therapy including the use of SCT, with molecular MRD pre-SCT strongly predictive of outcome, and identifying three risk strata. Finally, we demonstrate that as therapy continues to improve, prognostic tools beyond MRD are required to further tailor therapy and improve outcomes.

## Methods

We performed a longitudinal retrospective study in patients with Ph- ALL referred for ASO-RQPCR MRD testing to the EuroMRD-accredited Children's Cancer Institute (CCI) MRD Laboratory, Randwick, Australia. Eligible patients were aged ≥ 16 years, diagnosed between 2004 and 2018, not enrolled in a clinical trial and treated at 12 participating sites across Australia. Treatment approaches, MRD timing and interpretation were determined independently by clinicians at each centre. The main objective was to evaluate the role of MRD for both AYA and older adults in a real-world setting. At the time of MRD sample collection, all patients were consented for future research, and retrospective data collection was conducted with ethics approval (HREC/17/SCHN/92). Patients with MRD samples were identified and eligible for analysis if sufficient clinical data was available.

ASO-RQPCR methodology

MRD assessment was performed using ASO-RQPCR. Screening multiplex PCR at diagnosis with a primer set, followed by heteroduplex analysis, was used to identify Immunoglobulin or T-cell receptor clonal rearrangements, with Sanger sequencing then performed. Patient-specific primers were designed and used for real-time quantitative PCR in subsequent samples for MRD monitoring[15,16]. MRD assessment and interpretation were performed in accordance with contemporary EuroMRD guidelines[17].

Patient diagnostic samples were also screened for IKZF1 deletion of exons 4–7 by RQPCR, and where present, utilised as a further MRD marker as described by Venn et al (2012)[18].

## Statistical analysis

Survival analysis was performed for de novo patients from date of diagnosis. A separate analysis was then performed to assess outcomes in patients referred for SCT, which included patients only referred

for MRD testing at relapse.

In the de novo cohort, leukaemia-free survival (LFS) was defined as time from first morphological remission until relapse or death, and overall survival (OS) was calculated from diagnosis. In the SCT cohort, LFS and OS were calculated from date of SCT. Kaplan-Meier analysis was performed to determine LFS and OS, and compared using Mantel-Cox log rank test. Multivariate survival analysis was performed by logistic regression using Wald statistic. Patients who were refractory and achieved CR1 post salvage, were grouped with CR2 for survival analysis[19]. MRD negativity ($MRD^{neg}$) was defined as no amplifiable product. MRD positivity ($MRD^{pos}$) was defined as any level of MRD that was detectable, including positive below quantifiable range (PBQR). High-positive MRD was defined as $>1x10^{-4}$ ($MRD^{hi}$) and low-positive MRD as $\leq 1x10^{-4}$ ($MRD^{lo}$), similar to previous studies, as this cut-off tends to represent threshold for MRD detection by flow cytometry[1,8]. AYA was defined as 16–39 years old, whereas older adults were those ≥ 40 years old. Karyotype and FISH results were reviewed to assign disease subtype with high-risk genetic features defined according to the 2024 World Health Organisation classification and Yeung et al[20,21].

# Results

121 patients fulfilling enrolment criteria were identified for analysis. Of these 121 patients, 111 had MRD testing from diagnosis with demographics, disease characteristics and outcomes outlined in Table 1.

Table 1
Patient characteristics, events and survival

| Characteristics | N | Events | | | LFS at 5y | OS at 5y | SCT |
|---|---|---|---|---|---|---|---|
| | | Relapse/Ref | Ref | Deaths | | | |
| Total | 111 | 31 (27.9%) | 4 | 34 (30.6%) | 63.1% | 67.5% | 49 (44.1%) |
| Age | | | | | | | |
| AYA16-24 | 40 | 10 (25.0%) | 1 | 9 (22.5%) | 73.3% | 75.8% | 17 (42.5%) |
| 25–39 | 33 | 8 (24.2%) | 2 | 6 (18.2%) | 70.7% | 81.5% | 18 (54.5%) |
| Older adults ≥ 40 (40–68) | 38 | 13 (34.2%) | 1 | 19 (50.0%) | 45.9% | 47.9% | 14 (36.8%) |
| 40–49 | 11 | 0 (0%) | 0 | 4 (36.4%) | 63.6% | 63.6% | 5 (45.5%) |
| ≥ 50 | 27 | 13 (48.1%) | 1 | 15 (55.6%) | 38.4% | 41.3% | 9 (33.3%) |
| ALL lineage | | | | | | | |
| B-ALL | 95 | 27 (28.4%) | 3 | 28 (29.5%) | 63.4% | 68.7% | 42 (44.2%) |
| T-ALL | 16 | 4 (25.0%) | 1 | 6 (37.5%) | 61.4% | 60.9% | 7 (43.8%) |
| Subtype | | | | | | | |
| B-ALL NOS | 65 | 17 (26.2%) | 1 | 17 (26.2%) | 73.8% | 73.8% | 28 (43.1%) |
| B-ALL Hyperdiploidy | 6 | 2 (33.3%) | 0 | 2 (33.3%) | 66.7% | 66.7% | 1 (16.7%) |
| B-ALL Hypodiploidy | 5 | 3 (60.0%) | 1 | 4 (80.0%) | 20.0% | 20.0% | 1 (20.0%) |
| B-ALL ETV6::RUNX1 | 4 | 0 (0%) | 0 | 0 (0%) | 100% | 100% | 0 (0%) |
| B-ALL TCF3::PBX1 | 2 | 1 (50.0%) | 0 | 1 (50.0%) | 50.0% | 50.0% | 1 (50.0%) |
| B-ALL KMT2A-r | 7 | 0 (0%) | 0 | 1 (14.3%) | 85.7% | 85.7% | 7 (100%) |
| B-ALL Ph like | 3 | 3 (100%) | 1 | 2 (66.7%) | 0% | 33.3% | 2 (66.7%) |
| B-ALL with iAMP21 | 3 | 1 (33.3%) | 0 | 1 (33.3%) | 66.7% | 66.7% | 2 (66.7%) |
| T-ALL NOS | 11 | 3 (27.3%) | 1 | 4 (36.4%) | 63.6% | 63.6% | 4 (36.4%) |
| T-ALL KMT2A-r | 1 | 0 (0%) | 0 | 1 (100%) | 0% | 0% | 1 (100%) |
| T-ALL CDKN2A del | 2 | 1 (50.0%) | 0 | 1 (50.0%) | 50.0% | 50% | 1 (50.0%) |
| ETP-ALL | 2 | 0 (0%) | 0 | 0 (0%) | 100% | 100% | 1 (50.0%) |

| Characteristics | N | Events | | | LFS at 5y | OS at 5y | SCT |
|---|---|---|---|---|---|---|---|
| | | Relapse/Ref | Ref | Deaths | | | |
| IKZF1 del | | | | | | | |
| present | 21 | 5 (23.8%) | 0 | 7 (33.3%) | 69.1% | 63.8% | 14 (66.7%) |
| absent | 87 | 25 (28.7%) | 4 | 26 (29.9%) | 61.9% | 69.1% | 34 (39.1%) |

Ref: Refractory; LFS and OS at 5 years

The median age of the cohort was 30 years (range, 16–68). There were a total of 31 relapses and 34 deaths, with a median follow up time of 4.9 years (range, 0.9–13.7). Overall LFS and OS at 5 years was 63.1% and 67.5% respectively. In the AYA group, 91.7% of patients received paediatric-inspired therapy. Among older adults, 18.4% received a paediatric-inspired protocol, including 6 of 11 patients between 40–49 years old, and 1 patient at 53 years old. The majority of older adults received adult protocols such as HyperCVAD (Supplementary appendix).

The median age in the AYA group was 24 years (range, 16–39) vs 54 years (range, 40–68) in older adults. Older adults had significantly lower LFS and OS at 5 years, compared with AYA (45.8% and 47.9% vs 72.2% and 77.9% respectively, p = 0.0012 and p = 0.00019, Fig. 1). Within the AYA group, there was no significant difference in 5-year LFS and OS, between those aged 16–24 and those 25–39 years old (73.3% and 75.8% vs 70.9% and 81.5% respectively, p = 0.55 and p = 0.93). In older adults, 7/19 (36.8%) deaths were due to treatment-related mortality (TRM), including 5 deaths secondary to sepsis and 3 deaths related to post-SCT complications. TRM was particularly notable in those aged 40–49, where it accounted for all mortality with no relapses and 4 TRM deaths (2 post SCT, 2 in induction/consolidation). Relapse rates were significantly higher in older adults ≥ 50 years old at 48.1% vs 0% in those 40–49 years old ($\chi^2$ = 8.05, df 1, n = 37, p = 0.005). Of the 49 patients proceeding to SCT, 37 (75.5%) were in CR1 and 12 (24.5%) were in CR2 post salvage therapy.

Genomic subtypes are outlined in Table 1. B-ALL with hypodiploidy had generally poor outcomes, along with Ph-like, including 2 patients with CRLF2 rearrangement, and one with JAK2 rearrangement. T-ALL had similar LFS and OS compared with B-ALL (61.4% and 60.9% vs 63.4% and 68.7% respectively, p = 0.95 and p = 0.50). Amongst 8 patients with KMT2A rearrangement (KMT2A-r), there were no relapses and 2 TRM deaths post-SCT, with all 8 undergoing SCT in CR1. However, of 5 hypodiploidy patients, there were 3 relapses and 4 deaths, with only 1 patient receiving SCT in CR1. There were no relapses or deaths in 2 ETP-ALL patients. Presence of IKZF1 deletion did not significantly affect survival, with 5-year LFS and OS of 69.1% and 63.8% vs 61.8% LFS and 69.1% OS in those without detectable IKZF1 deletion (p = 0.52 and p = 0.99 respectively).

MRD in AYA and older adults

A consort diagram outlining MRD time points in the study cohort is depicted in Fig. 2. Of the 111 patients with MRD screening performed at diagnosis, no MRD marker was identified in 4 patients (3.6%: 3 AYA, 1 older adult). Among the remaining 107 patients, 70 were AYA and 37 aged ≥ 40 years (older adults) with 92 having B- and 15, T-phenotype disease.

In older adults, median times of clinician-chosen MRD timepoints 1 and 2 (TP1 and TP2) were at 34 days (range, 25–83) and 98 days (range, 58–126) respectively. Among the 37 older adults with assessable MRD, there were 3 deaths prior to TP2: 1 refractory at TP1, and 2 early TRM deaths. Of the remaining 34 patients, 15 had MRD tested at both TP1 and TP2, with 32 patients tested at one or both timepoints. Of these 32 older adults, 22 (68.8%) achieved $MRD^{neg}$ at either TP1 or TP2. Among older adults, achievement of $MRD^{neg}$ was associated with significantly superior 3-year LFS (68.2% vs 12.5%; p = 0.0076) and OS (67.9% vs 36.0%; p = 0.017) compared with persistent $MRD^{pos}$ by TP2. Median OS was not reached in the $MRD^{neg}$ group versus 20.4 months in the $MRD^{pos}$ group (Fig. 3). TP1 MRD results were available for 28 of 34 patients. Those achieving TP1 $MRD^{neg}$ had superior 3-year LFS and OS compared to TP1 $MRD^{pos}$ patients (73.3% vs 26.9%, p = 0.029 and 72.7% vs 44%, p = 0.037 respectively). Of the 13 TP1 $MRD^{pos}$ patients, 4 achieved TP2 $MRD^{neg}$, 3 of whom remained alive in CR at 4 years. Of the 22 older patients achieving TP2 $MRD^{neg}$ only 4 proceeded to SCT in CR1 versus 7 of 10 with persistent $MRD^{pos}$.

In the AYA cohort, MRD was assessed at a median of 37 (range, 27–56) days at TP1 and 94 days (range, 71–173) at TP2. Among the 60 patients evaluable at TP1, 3-year LFS and OS did not differ significantly between $MRD^{hi}$ and $MRD^{lo}$ groups (LFS, 81.2% vs 76.3%, p = 0.73; OS, 84.4% vs 88.1%, p = 0.49; Supplementary appendix).

Of 67 AYA patients with evaluable MRD at TP2, 41 (61.2%) achieved $MRD^{neg}$. Patients who were $MRD^{neg}$ at TP2 had 3-year LFS of 79.6% vs 67.5% in the $MRD^{pos}$ cohort (HR 0.52, 95% CI: 0.21–1.28, p = 0.14), and 3-year OS of 89.6% vs 76.3%, (HR 0.45, 95% CI: 0.15–1.29, p = 0.13, Fig. 4). Notably, 16 of 26 (61.5%) TP2 $MRD^{pos}$ patients underwent SCT in CR1, compared with only 7 of 41 (17.1%) TP2 $MRD^{neg}$ patients. When survival analysis was censored for CR1 SCT, 3-year LFS in the TP2 $MRD^{neg}$ cohort was 78.5% vs 55.9% in the $MRD^{pos}$ cohort (HR 0.40, 95% CI: 0.13–1.25, p = 0.10) with corresponding 3-year OS of 90.6% versus 82.9% (HR 0.37, 95% CI: 0.10–1.43, p = 0.14, Supplementary appendix).

Three-year LFS and OS were not significantly different between TP2 $MRD^{pos}$ and TP2 $MRD^{neg}$ patients who underwent SCT in CR1 and TP2 $MRD^{neg}$ who did not undergo SCT in CR1 (LFS, 80.2%, 85.7%, and 78.4%; OS, 80.2%, 85.7%, 90.6% respectively). In contrast, TP2 $MRD^{pos}$ patients not undergoing CR1 SCT had numerically inferior outcomes with 3-year LFS of 46.7% and OS of 70.0% (p = 0.24 and p = 0.28).

Of 10 TP2 $MRD^{pos}$ patients who did not receive SCT in CR1, 4 had quantifiable MRD. Two received blinatumomab with one subsequently relapsing and receiving CAR-T therapy, while the remaining 2 were initially refractory and later proceeded to SCT. The remaining 6 patients had PBQR results. Among these 6 patients, 1 died from early TRM, 1 relapsed and underwent SCT in CR2 and 1 was lost to follow up at 1

year. The remaining 3 PBQR patients remained alive in CR without proceeding to SCT (Supplementary appendix).

Among the 41 TP2 $MRD^{neg}$ patients, 8 relapsed and 6 died, including 3 deaths from progressive disease. Of the 8 patients who relapsed, 2 had identifiable high-risk genetics; 1 with presumed Ph-like ALL with JAK2 rearrangement and 1 likely PAX5 deletion with del9p13. The remaining 6 patients were not classified as high-risk by karyotype or FISH, including 1 T-ALL NOS, 1 hyperdiploidy and 4 with ≤ 3 karyotype abnormalities. Of these 8 patients, 2 were refractory to salvage therapy, including blinatumomab, and died. The remaining 6 proceeded to SCT in CR2 following salvage. 3 of these 6 patients died post SCT with 2 from TRM and 1 from relapse.

Allogeneic stem cell transplant outcomes

Among the 121 patients, 59 underwent SCT. One patient was excluded because only 1 month of follow up was available, leaving 58 patients for analysis: 42 AYA and 16 older adults. The median age of the SCT cohort was 30 years; 24 years in the AYA SCT group (range, 16–39) and 54 years (range, 41–68) in the older adult SCT group. Median follow up post-SCT was 4.4 years (range, 0.8–10.6). Donor source included 18 matched siblings, 27 matched unrelated, 3 umbilical cord, 7 haploidentical and 3 not recorded.

Of the 16 older adults, 12 had SCT in CR1 including 7 patients $MRD^{pos}$ by TP2, and 1 without MRD assessed by TP2. 4 patients received CR1 SCT despite $MRD^{neg}$ by TP2, including one with KMT2A-r, while the remaining 3 did not have high-risk genetics as assessed by karyotype and FISH. Of the 12 patients undergoing SCT in CR1, there were 2 post-SCT relapses and 5 deaths including 4 TRM. 4 patients received SCT in CR2, with all 4 deceased: 2 due to relapse and 2 TRM. Among older adults aged < 55 years, 4/9 (44.4%) died (2 TRM and 2 relapses), and in those ≥ 55 years, 5/7 (71.4%) died (4 TRM and 1 relapse).

Of the 42 AYA SCT patients, 25 had SCT in CR1, including 15 with TP2 $MRD^{pos}$. Of the 7 patients who underwent CR1 SCT despite TP2 $MRD^{neg}$, 6 had high-risk genetics: 2 KMT2A-r, 1 iAMP21, 2 CKDN2A loss, 1 PAX5 deletion, 1 Ph-like, 4 with complex karyotype. The remaining $MRD^{neg}$ patient had an IKZF1 deletion, but no identified high-risk genetics. Three additional CR1 SCT recipients had no available MRD results at TP2, but had other high-risk features including 1 with high WCC at presentation, 1 ETP-ALL, and 1 with KMT2A-r. In the CR1 SCT cohort there were 4 relapses and 5 deaths, including 2 from TRM. The remaining 17 AYA patients underwent SCT in CR2; 8 relapsed and 10 died including 3 deaths from TRM.

Within the SCT cohort, older adults had significantly inferior 3-year LFS (29.8% vs 64.4%, p = 0.031) and OS (36.0% vs 70.1%, p = 0.038) compared with AYA patients (Fig. 5). Post-SCT TRM was also significantly higher among older adults (37.5% vs 11.9%; $\chi^2 = 4.94$, df 1, n = 58, p = 0.026), and accounted for the majority of deaths in this group. Patients undergoing SCT in CR2 had inferior 3-year OS versus

those offered SCT in CR1 (39.9% vs 74.2%, p = 0.0051). 3 patients with refractory disease achieving CR post salvage were included in the CR2 group.

Among the 58 SCT patients, 38 had MRD available pre-SCT. 21 of the 38 patients (55.3%) were $MRD^{neg}$ pre-SCT, 11 were $MRD^{lo}$ and 6 were $MRD^{hi}$. 9 of 11 $MRD^{lo}$ results were PBQR. Increased MRD level pre-SCT was associated with poorer survival outcomes, with 3-year LFS of 79.8%, 53.0% and 16.7% (p = 0.001), and OS of 84.1%, 53.0% and 16.7% for the $MRD^{neg}$, $MRD^{lo}$ and $MRD^{hi}$ groups respectively (p = 0.0009, Fig. 5). Pairwise analysis with Hochberg adjustment showed significantly poorer LFS and trend towards poorer OS for $MRD^{lo}$ vs $MRD^{neg}$ pre-SCT (p = 0.0483 and p = 0.06 respectively).

Pre-SCT remission and MRD status significantly impacted LFS and OS. Patients proceeding to SCT in CR1 with $MRD^{neg}$ had the most favourable outcomes, whereas $MRD^{pos}$ patients transplanted in CR2 experienced frequent early events and the poorest survival (LFS HR 17.31, 95% CI: 3.39–88.37, p = 0.001; OS HR 14.50, 95% CI: 2.86–73.41, p = 0.001). Within the CR1 SCT cohort, $MRD^{neg}$ patients had numerically higher 3-year LFS (85.6% vs 58.3%, HR 0.20, 95% CI: 0.04–1.03, p = 0.055) and OS (85.6% vs 58.3%, HR 0.25, 95% CI: 0.05–1.38, p = 0.11) than those with pre-SCT $MRD^{pos}$. Within the CR2 SCT cohort, pre-SCT $MRD^{neg}$ was associated with a trend towards superior 3-year LFS (60.0% vs 14.3%, HR 0.20, 95% CI: 0.04–1.04, p = 0.056) and a significantly improved OS (80.0% vs 14.3%, HR 0.11, 95% CI: 0.01–0.90, p = 0.04) versus $MRD^{pos}$.

In univariate analysis, SCT in CR1 and pre-SCT $MRD^{neg}$ were associated with significantly superior LFS and OS, while age < 40 years was associated with superior OS and a trend toward improved LFS (Table 2). SCT outcomes did not differ between patients with standard or adverse risk genetic features. Multivariate analysis demonstrated that age < 40 years, SCT in CR1 and pre-SCT $MRD^{neg}$ were each independently associated with superior post-SCT LFS and OS (Fig. 6).

Table 2
**Univariate and multivariate survival analysis for SCT patients** *Factors used in multivariable analysis. ^SR – Standard risk, AR – adverse risk included patients with ETP-ALL, T-ALL with CDKN2A del, KMT2A-r, iAMP21, Ph-like, 9p del, hypodiploidy and complex karyotype.

| **Univariate Analysis** | **LFS** | | | **OS** | | |
|---|---|---|---|---|---|---|
| | **HR** | **95% CI** | **p** | **HR** | **95% CI** | **p** |
| Age < 40 vs ≥ 40* | 0.45 | 0.2–1.01 | 0.053 | 0.65 | 0.42–0.99 | 0.045 |
| CR1 vs CR2* | 0.301 | 0.14–0.65 | 0.002 | 0.56 | 0.38–0.86 | 0.008 |
| Pre SCT MRD neg vs pos* | 0.19 | 0.06–0.61 | 0.005 | 0.16 | 0.05–0.60 | 0.006 |
| SR vs AR^ genetics | 1.18 | 0.51–2.70 | 0.708 | 1.09 | 0.46–2.56 | 0.846 |

*Factors used in multivariable analysis. ^SR – Standard risk, AR – adverse risk included patients with ETP-ALL, T-ALL with CDKN2A del, KMT2A-r, iAMP21, Ph-like, 9p del, hypodiploidy and complex karyotype.

| Multivariate Analysis | LFS | | | OS | | |
|---|---|---|---|---|---|---|
| | HR | 95% CI | p | HR | 95% CI | p |
| Age < 40 vs > 40 | 0.03 | (0.005–0.173) | < 0.001 | 0.019 | (0.003–0.136) | < 0.001 |
| CR1 vs CR2 | 0.12 | (0.034–0.423) | < 0.001 | 0.145 | (0.039–0.544) | 0.004 |
| pre SCT MRD neg vs pos | 0.052 | (0.011–0.238) | < 0.001 | 0.025 | (0.004–0.179) | < 0.001 |

# Discussion

To date, evidence supporting the prognostic utility of MRD in ALL management has been derived predominantly from prospective clinical trials, with relatively limited real-world data[22,23]. This multi-centre retrospective study provides the first real-world assessment of MRD in guiding therapeutic decisions, including referral and timing of SCT for both AYA and older adults, in the absence of protocol-mandated treatment. Importantly, MRD testing was performed at a single EuroMRD-accredited reference laboratory, ensuring standardised methodology.

Outcomes in our AYA cohort were comparable to those reported in the Australian ALL06 study, despite treatment occurring outside of a clinical trial[1]. Older adult outcomes were superior to those seen in the HyperCVAD trial with 5-year LFS and OS of 63.9% and 47.2% vs 38% and 38% respectively[24]. Despite access to MRD assessment, older adults continued to experience inferior outcomes, likely reflecting adverse disease biology and intolerance of intensive therapy. TRM accounted for over one third of deaths in older adults and was particularly high in patients aged > 50 years. Most patients aged 40–49 years received paediatric-inspired therapy and experienced no relapses, but TRM remained high, emphasising the need to balance improved disease control against toxicity in this age group. In the AYA cohort, patients aged < 25 and 25–39 years had similar outcomes, supporting the continued use of paediatric-inspired regimens up to 40 years of age.

MRD assessment in real-world practice was performed at variable time points. However, among older adults, achievement of $MRD^{neg}$ by TP2 (median 98 days) identified patients with significantly superior LFS and OS, with median OS not reached. Although $MRD^{neg}$ at TP1 was associated with favourable outcomes, achievement of $MRD^{neg}$ by TP2 provided greater prognostic discrimination. Notably, 81.9% of older adults achieving molecular remission by TP2 did not undergo SCT in CR1 but still appeared to have favourable survival. Together with the inferior SCT outcomes seen in older adults in our cohort, these findings suggest avoiding SCT in CR1 in older patients who achieve TP2 $MRD^{neg}$ in the absence of other high-risk features.

In contrast, TP2 MRD status did not significantly discriminate outcomes in our AYA cohort. This differs from prospective clinical trials where post-consolidation MRD is among the strongest predictors of relapse[1,6,25]. These findings likely reflect adoption of effective MRD-directed treatment in routine

practice, informed by contemporary risk-adapted clinical trial protocols, with TP2 MRD$^{pos}$ patients significantly more likely to proceed to SCT in CR1. Furthermore, the majority of TP2 MRD$^{pos}$ patients who did not proceed to CR1 SCT had PBQR results with 50% experiencing long term LFS.

Conversely, most pre-SCT MRD$^{lo}$ results were also PBQR, but were associated with inferior LFS post-SCT. In comparison, this was predominantly a high-risk group with other risk factors present in 91.4% of patients referred for transplant (i.e. TP2 MRD$^{pos}$, high-risk genetics or relapsed disease). This suggests that the interpretation of PBQR MRD may depend on clinical context. Such discrepant results might also suggest that PBQR results by ASO-RQPCR may lack sufficient specificity to distinguish patients at high risk of relapse. This is consistent with previous studies suggesting that PBQR results obtained using ASO-RQPCR have lower specificity than NGS, supporting the use of NGS to better discriminate PBQR results and assist in refining management decisions[10,26–28].

In the TP2 MRD$^{neg}$ cohort 19.5% relapsed, with most lacking high-risk abnormalities detected by conventional karyotyping or FISH. Conventional cytogenetics identifies only a subset of high-risk lesions, whereas recent studies have shown that RNA sequencing and multiplex ligation-dependent probe amplification can identify additional lesions with increased risk of relapse despite achieving MRD negativity[20]. Interestingly, all patients with KMT2A-r received SCT in CR1, resulting in excellent outcomes. In contrast, only 20% of patients with hypodiploidy underwent CR1 SCT despite 5-year LFS of just 20%, suggesting the impact of this high-risk group was under-recognised in our cohort. Interpretation of these findings is limited by variable use of FISH across participating centres and limited availability of comprehensive genomic testing in this real-world cohort. Finally, our results support emerging evidence that not all IKZF1 deletions confer equivalent risk, with patients having dominant-negative exon 4–7 deletions showing outcomes comparable to those without[29,30]. Together, these findings demonstrate that MRD assessment alone is insufficient to identify all patients at risk of relapse and highlight the need to integrate comprehensive genomic profiling into the standard diagnostic workup in ALL[20]. Together with MRD assessment, this may improve the identification of high-risk subgroups that may benefit from early treatment intensification.

Our study also provides important insights into the contemporary role of SCT in AYA and older adults. Previous studies have suggested equivalent SCT outcomes based on age, using a threshold of 55 years[31,32]. In our cohort, SCT in those aged 40–54 was associated with inferior outcomes, which were particularly poor in those aged > 55 years, largely due to TRM. Most CR1 transplants were performed for TP2 MRD$^{pos}$ or adverse genetics. In the AYA group, this strategy achieved similar survival to TP2 MRD$^{neg}$ patients not receiving CR1 SCT, which strongly validates this risk-adapted approach. Multivariate analysis confirmed that age < 40 years, SCT in CR1 vs CR2, and pre-SCT MRD$^{neg}$ were all independently associated with improved survival, affirming that in the contemporary era, patient age and pre-SCT disease status remain key determinants of SCT outcome.

This study confirmed the impact of pre-SCT ASO-RQPCR-based MRD in AYA and older adults[33]. Patients achieving pre-SCT $MRD^{neg}$ experienced improved SCT outcomes, with pre-SCT $MRD^{lo}$ and $MRD^{hi}$ showing progressively poorer post-SCT outcomes. These findings extend those of Liang et al. with our study demonstrating significant difference between MRD levels for both LFS and OS[34]. Our data highlight the value of highly sensitive molecular MRD rather than MRD as assessed by flow cytometry, which generally lacks sufficient sensitivity to detect MRD < $10^{-4.8}$ Importantly, the survival advantage associated with $MRD^{neg}$ was observed for SCT performed in both CR1 and CR2, suggesting that every effort be made to minimise MRD prior to SCT irrespective of remission status. With increasing access to bispecific antibodies, antibody-drug conjugates and CAR-T cell therapy, the achievement of $MRD^{neg}$ prior to SCT is an increasingly realistic therapeutic objective[22].

This study has several limitations. The retrospective nature of the study and variable duration of follow up may have introduced selection bias. Some subgroup analyses are limited by small numbers. In addition, most patients were treated before the widespread availability of novel immunotherapies which may impact the contemporary application of our findings.

# Conclusion

While the prognostic value of MRD is well established, our study provides the first real-world evidence that incorporation of molecular MRD assessment by ASO-RQPCR into routine ALL management enables effective risk-adapted treatment, with results comparable or superior to contemporary clinical trials. The use of ASO-RQPCR MRD remained highly prognostic, particularly in older adults, where achievement of $MRD^{neg}$ by TP2 was associated with excellent survival and may support the omission of CR1 SCT in selected patients. In AYA patients, comparable outcomes between TP2 $MRD^{pos}$ patients undergoing CR1 SCT and $MRD^{neg}$ patients without CR1 SCT, suggest that MRD-directed treatment intensification can mitigate the impact of persistent MRD. However, relapse despite $MRD^{neg}$ highlights the need to integrate comprehensive genomic profiling into future risk stratification, while correlative NGS may improve interpretation of PBQR results. Finally, our data indicates ASO-RQPCR–based MRD pre-SCT can be highly prognostic with three risk strata, demonstrating increasing survival benefit with MRD reduction. This affirms efforts to eliminate residual MRD pre-SCT and maximise outcome, including through novel immunotherapies. Together these results help establish the utility of molecular MRD in contemporary ALL management for AYA and older adults in the real-world setting.

# Declarations

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Author Contributions

RP wrote the manuscript. RP and RS curated data and performed analysis. AE, SF, JK, MH, CB, JD, PW contributed to data collection. NC, TL and MH conducted MRD assessment and analysis. TT, JM, MO, AA, DW, MH, AE, WS and RS reviewed the manuscript. TT, RS and MG conceptualised study design. MG reviewed and supervised both analysis and manuscript.

Competing Interests

M.O. has served on advisory boards for Amgen. M.G. has served on advisory boards for Amgen, Pfizer, Servier, and Jazz Pharmaceuticals and has received trial and research support from Amgen and Servier. A.E. has served on advisory boards for Amgen, Pfizer, Servier, Taiho and Jazz Pharmaceuticals, has been a consultant for RACE Oncology and received research funding from Novartis.

Data Availability Statement

Raw data supporting the findings of this paper can be made available upon reasonable request to the corresponding author.

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# Figures

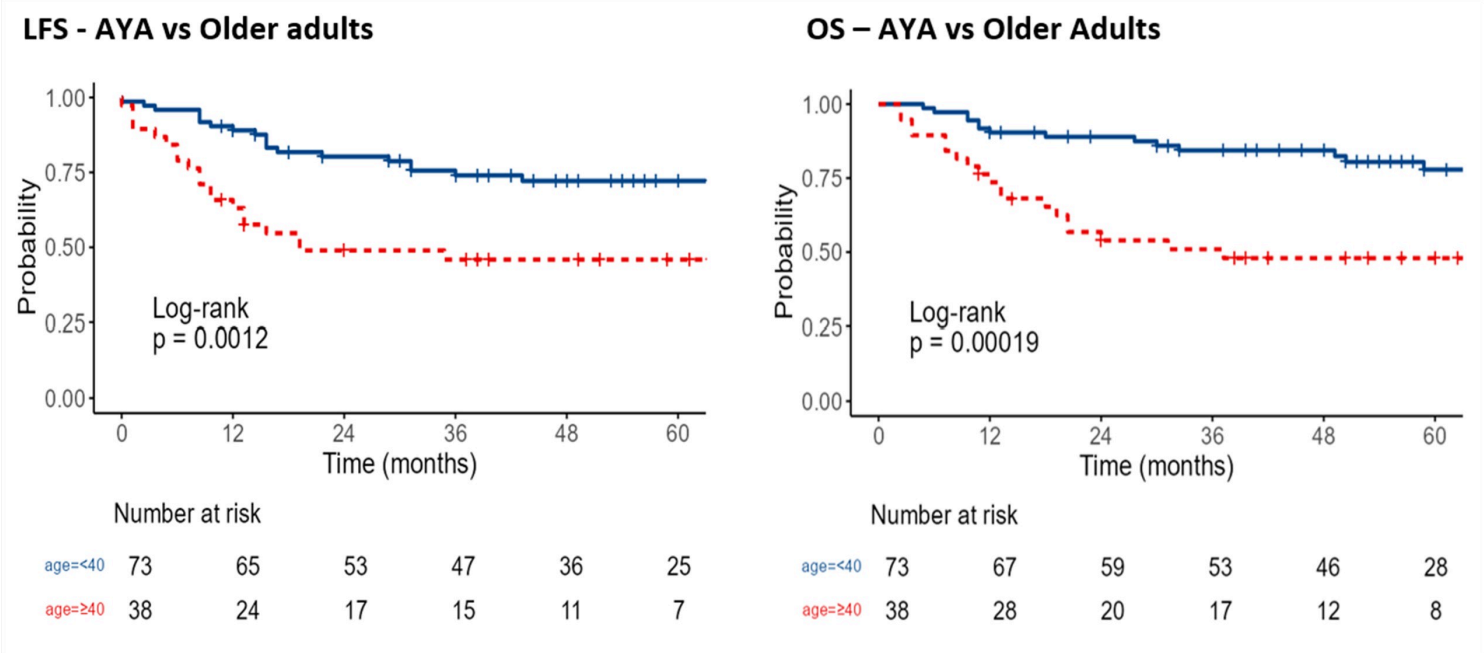



**Figure 1**

**LFS and OS in AYA versus Older adults.** 5-year LFS in AYA was 72.2% vs 45.8% in older adults (p=0.0012). 5-year OS in AYA was 77.9% vs 47.9% in older adults (p=0.00019).

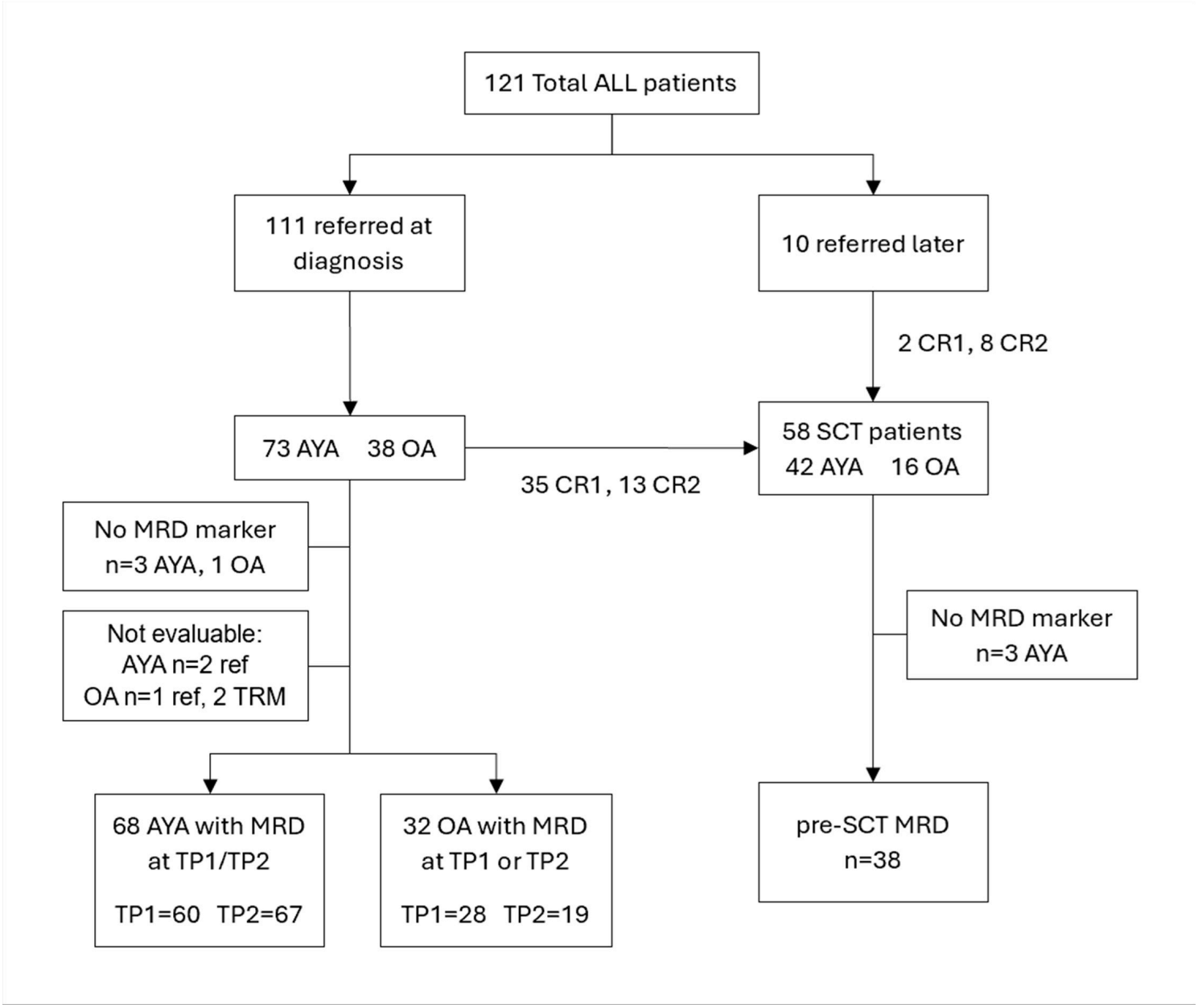



**Figure 2**

**Consort Diagram outlining MRD time points for analysis.** OA – Older adults, ref – refractory and not in morphological remission, TRM – treatment related mortality prior to TP2.

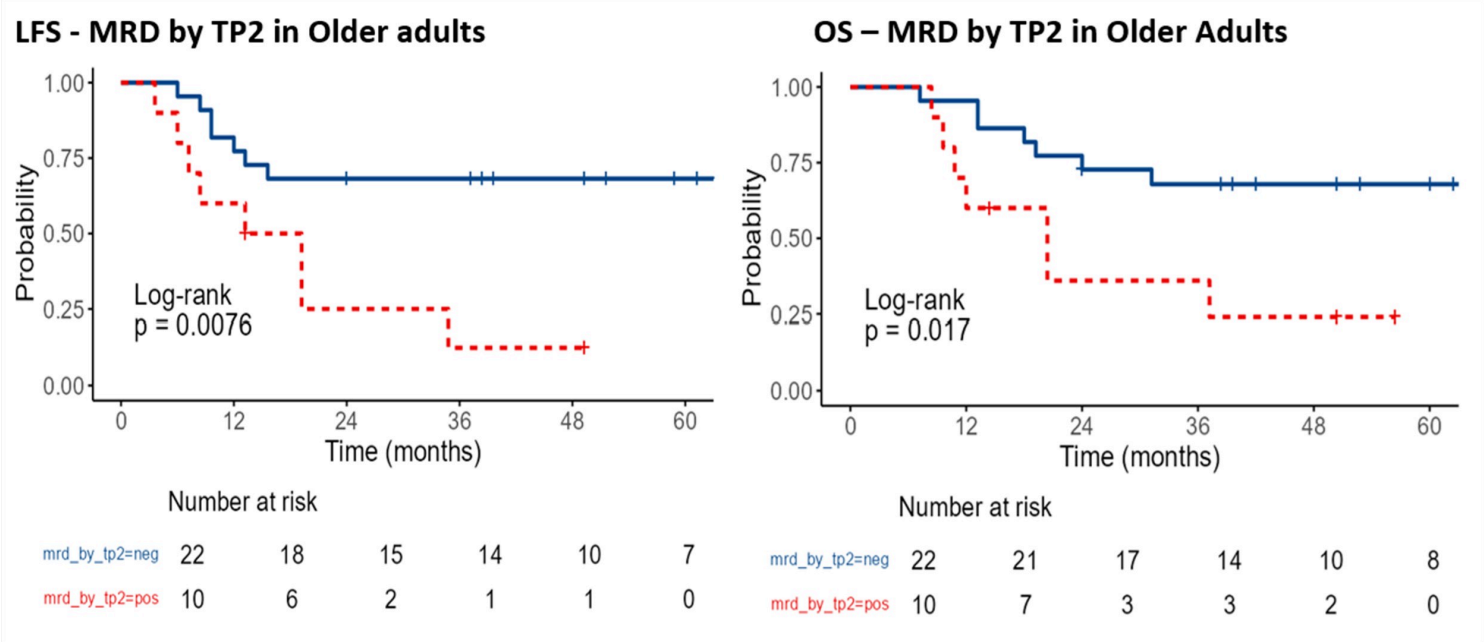



**Figure 3**

**LFS and OS by TP2 MRD in Older Adults.** 3-year LFS for those $MRD^{neg}$ by TP2 was 68.2% vs 12.5% in $MRD^{pos}$ (p=0.0076). 3-year OS for those $MRD^{neg}$ by TP2 was 67.9% vs 36.0% in $MRD^{pos}$ (p=0.017). Median OS was not reached in $MRD^{neg}$ group, vs 20.4 months in $MRD^{pos}$

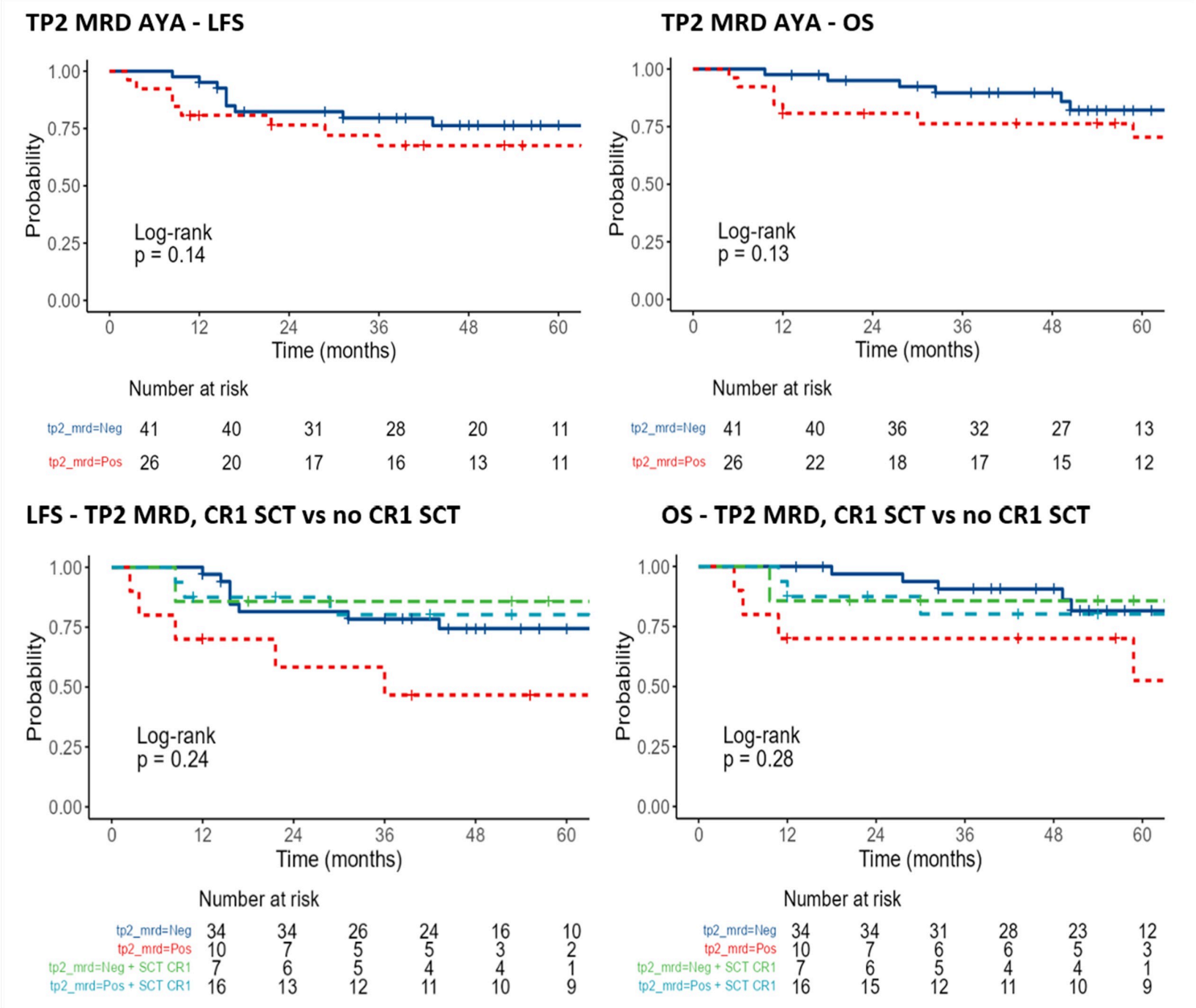
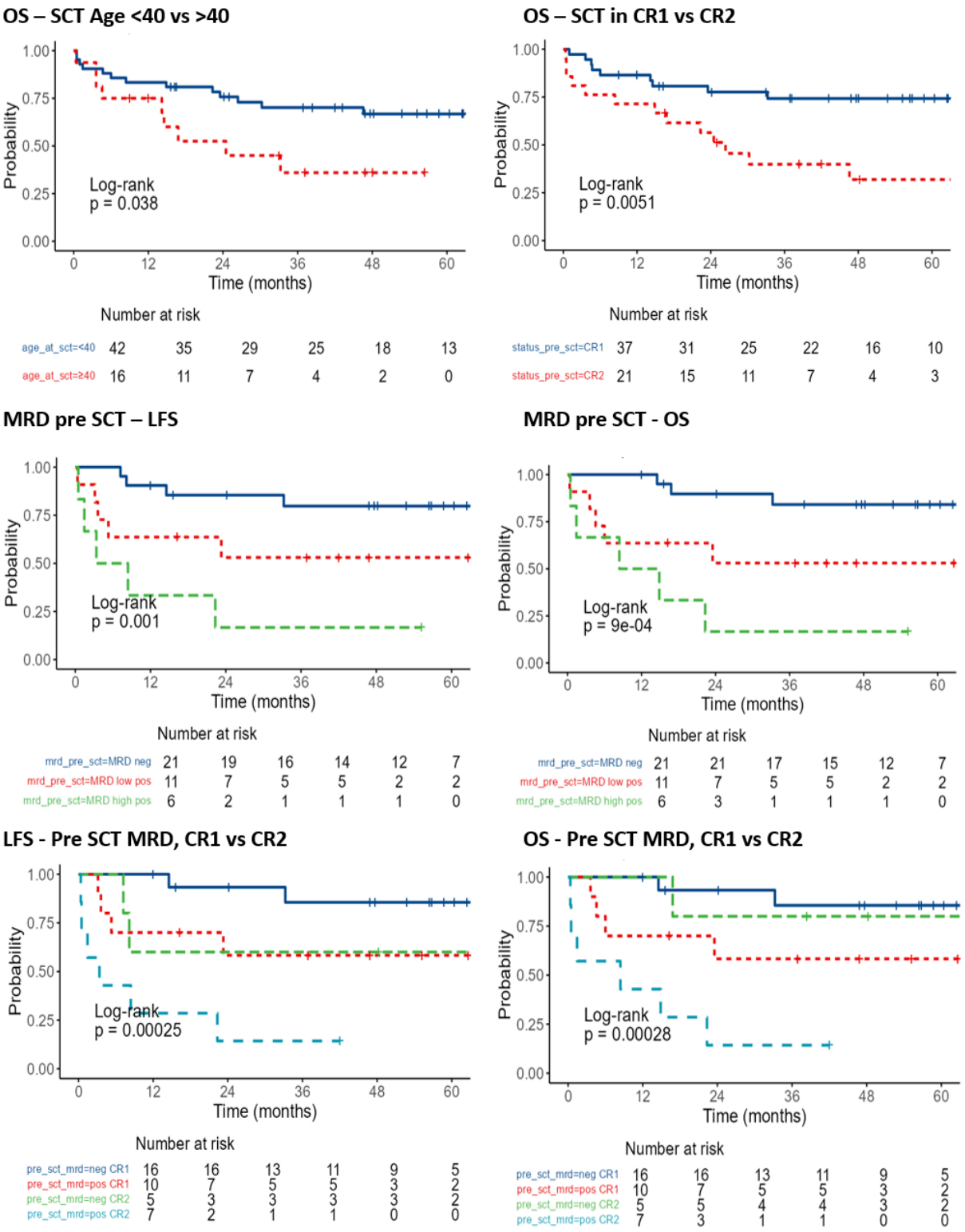



**Figure 4**

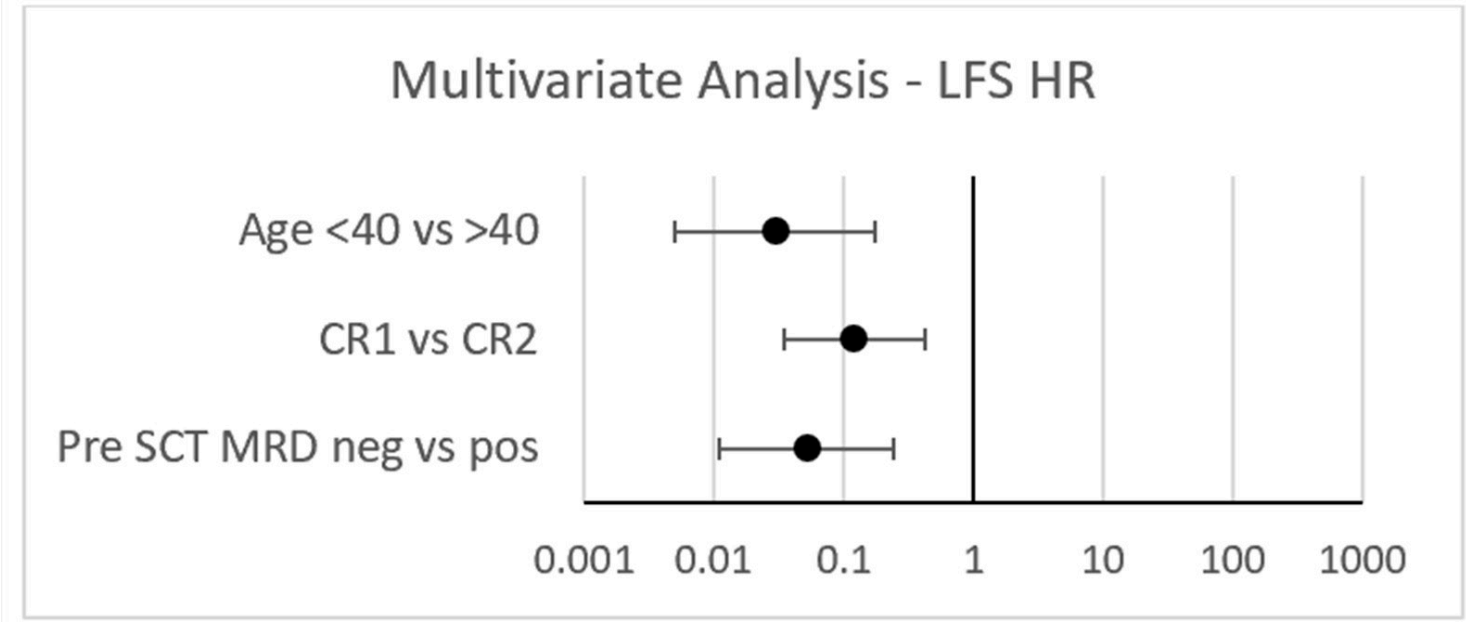
**Survival outcomes by TP2 MRD in AYA patients.** LFS and OS for AYA by TP2 MRD; by TP2 MRD with CR1 SCT vs no CR1 SCT.




## Figure 5

**Survival outcomes in transplanted patients.** AYA vs older adults: 3-year OS 70.1% vs 36.0% (p=0.038); SCT in CR1 vs CR2: 3-year OS 74.2% vs 39.9% (p=0.0051); LFS and OS by pre-SCT $MRD^{neg}$, $MRD^{lo}$ (≤$1x10^{-4}$), $MRD^{hi}$ (>$1x10^{-4}$) – 3-year LFS: 79.8%, 53.0%, 16.7% respectively (p=0.001), 3-year OS: 84.1%, 53.0%, 16.7% respectively (p=<0.001); LFS and OS by pre-SCT $MRD^{neg}$ CR1, $MRD^{pos}$ CR1, $MRD^{neg}$ CR2 and

MRD$^{pos}$ CR2 – 3-year LFS: 85.6%, 58.3%, 60.0%, 14.3% respectively (p=<0.001), 3-year OS: 85.6%, 58.3%, 80.0%, 14.3% respectively (p<0.001).



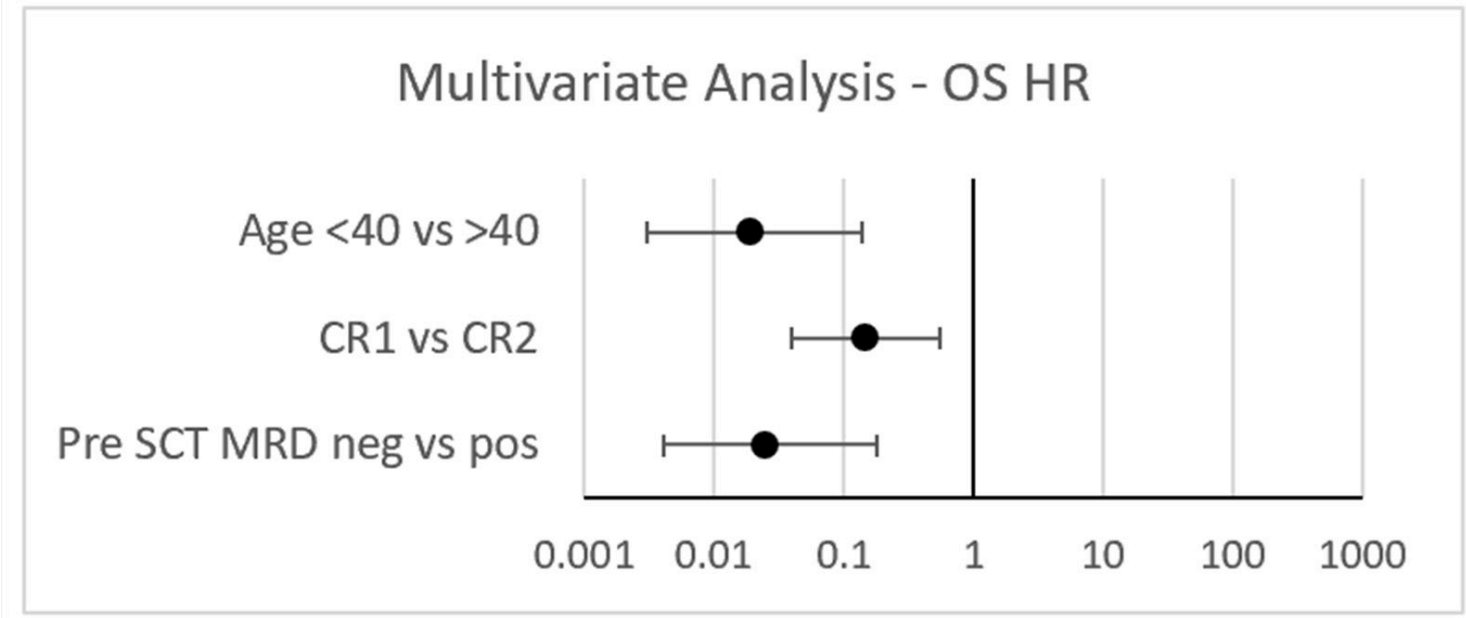




**Figure 6**

Multivariate LFS and OS survival analysis for SCT patients.

## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- supplementaryappendix.pdf