# *MRD for ALL*

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MEDICAL COLLEGE OF WISCONSIN AND NMDP

# ALL MRD on Pre-TED Oct 2025

185. Specify method(s) that was used to assess measurable residual disease status *(check all that apply) (at infusion)*

- ☐ FISH – ***Go to question 186***
- ☐ Karyotyping – ***Go to question 187***
- ☐ Flow cytometry – ***Go to question 188***
- ☐ PCR – ***Go to question 192***
- ☐ NGS – ***Go to question 193***
- ☐ ClonoSEQ – ***Go to question 194***
- ☐ Not assessed – ***Go to end of form***

186. Was measurable residual disease detected by FISH? *(at infusion)*

- ☐ Yes
- ☐ No

187. Was measurable residual disease detected by karyotyping assay? *(at infusion)*

- ☐ Yes
- ☐ No

188. Was measurable residual disease detected by flow cytometry? *(at infusion)*

- ☐ Yes
- ☐ No

189. Which leukemia immunophenotype was used for measurable residual disease detection? *(check all that apply) (at infusion)*

- ☐ Original leukemia immunophenotype – ***Go to question 190***
- ☐ Aberrant phenotype – ***Go to question 191***

190. Lower limit of detection: *(for the original leukemia immunophenotype)* __ __ __ __. __

191. Lower limit of detection: *(for the aberrant phenotype)* __ __ __ __. __

192. Was measurable residual disease detected by PCR? *(at infusion)*

- ☐ Yes
- ☐ No

193. Was measurable residual disease detected by NGS? *(at infusion)*

- ☐ Yes
- ☐ No

194. Was measurable residual disease detected by ClonoSEQ? *(at infusion)*

- ☐ Yes
- ☐ No

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# *What is the need?*

- Expand MRD data for better interpretation and predictive value
- Establish a minimum standard representing “high sensitivity” testing
  - Most important for “negative” MRD status
- Capture discrete data where possible
- Maintain balance between feasibility for data professionals and value for use in studies and CSA risk adjustment

# *Possible changes for MFC*

- Continue to collect
- Include value in %

Was measurable residual disease detected using a multiparameter flow cytometry? *(at infusion)*

☐ Yes

☐ No

If yes, the level of detection was reported in __ %

What was the source of tissue assayed?

Blood

Bone Marrow

What was the level of sensitivity reported for the multiparameter flow cytometry assay

Sensitivity of less than 1 x $10^{-4}$ (eg 1 x $10^{-3}$, 1 x $10^{-2}$)

Sensitivity of at least 1 x $10^{-4}$ or greater (eg 1 x$10^{-5}$, 1 x $10^{-6}$)

Unknown

# *Possible changes for NGS*

Was measurable residual disease detected by NGS specific for Immunoglobulin/T-cell receptor (IG/TCR) gene re-arrangements? *(at infusion)*

- ☐ Yes
- ☐ No
- ☐ Indeterminant/assessed below the level of detection

If yes report the level of residual sequences detected ___ clonal cells/mil and submit the redacted report

Specify assay used for assessment

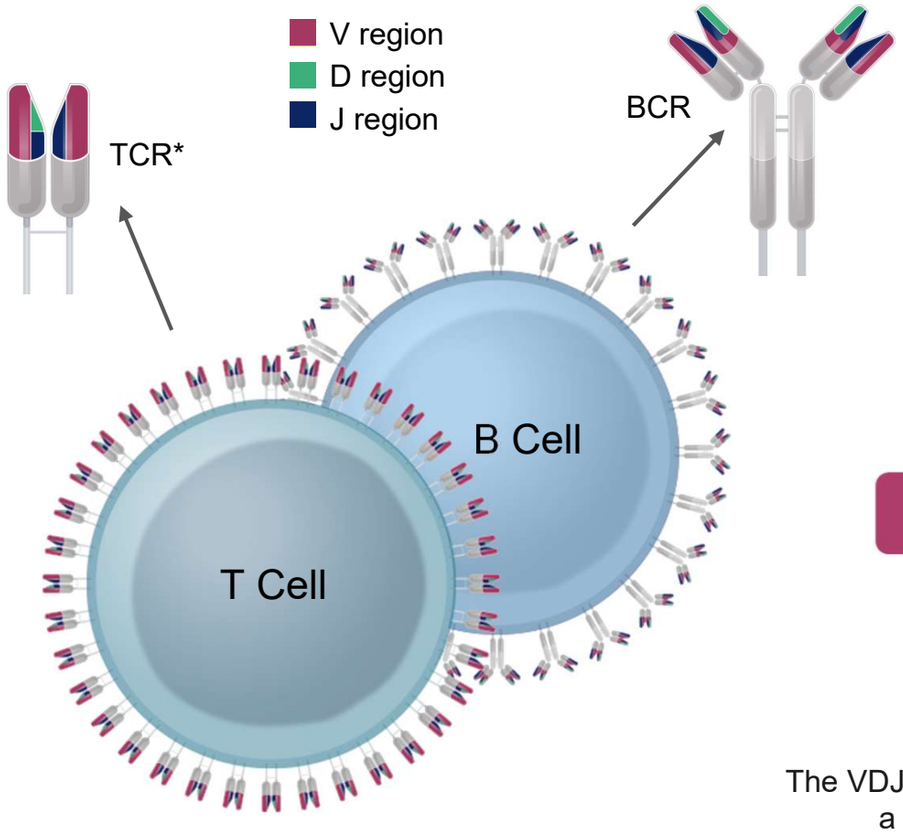
- Clonoseq
- Invivoscribe
- Other assay specify….

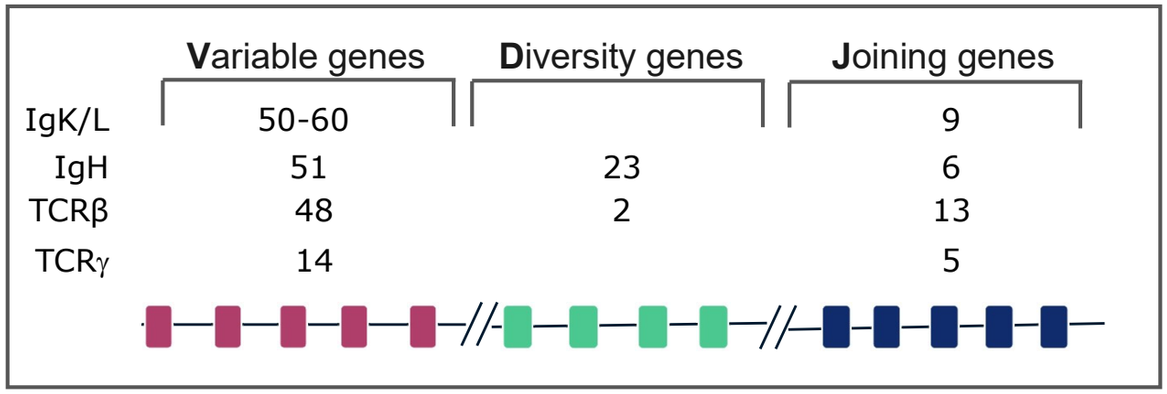
Specify source of tissue assayed ? *(at infusion)*

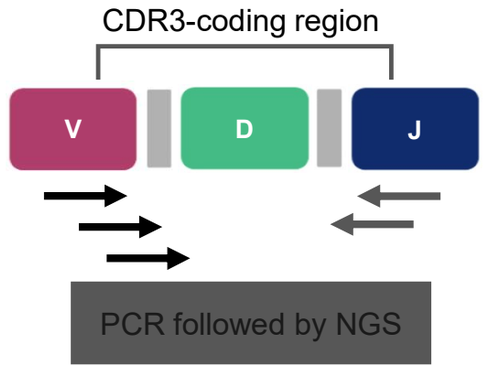
- ☐ BM
- ☐ PB

# What Is NGS-MRD? Clonoseq Test Principles

Immunosequencing of Receptor Genes Enables Precise and Sensitive MRD Measurement[1,2]




The VDJ region of the receptor gene represents a quantifiable molecular "barcode"

GCTAGTGGCAGTCGTTCGTAGTACGAGCATGCA
CGTAGCAGATGGATGGCATCGTACGAGCATTAG
GTAGCGAGTCAGTACAGGCAGTGCAGGTAGTAA
GTCAGCGATCAGTCGGTACGATCGATATAGGAG
ATGCTCAGTCACGTAGCGAATCTATAGGCATGC

This "barcode":

- Identifies all malignant cells associated with the tumor
- Is retained over time even as the clone evolves
- Is a stable, trackable marker of disease
- Directly measures cancer cell burden at any given time throughout clinical management

* T-cell testing is available as a CLIA-validated LDT and has not been cleared or approved by the FDA.

BCR, B-cell receptor; CDR, complementarity-determining region; Ig, immunoglobulin; MRD, measurable (minimal) residual disease; NGS, next-generation sequencing; PCR, polymerase chain reaction; TCR, T-cell receptor.

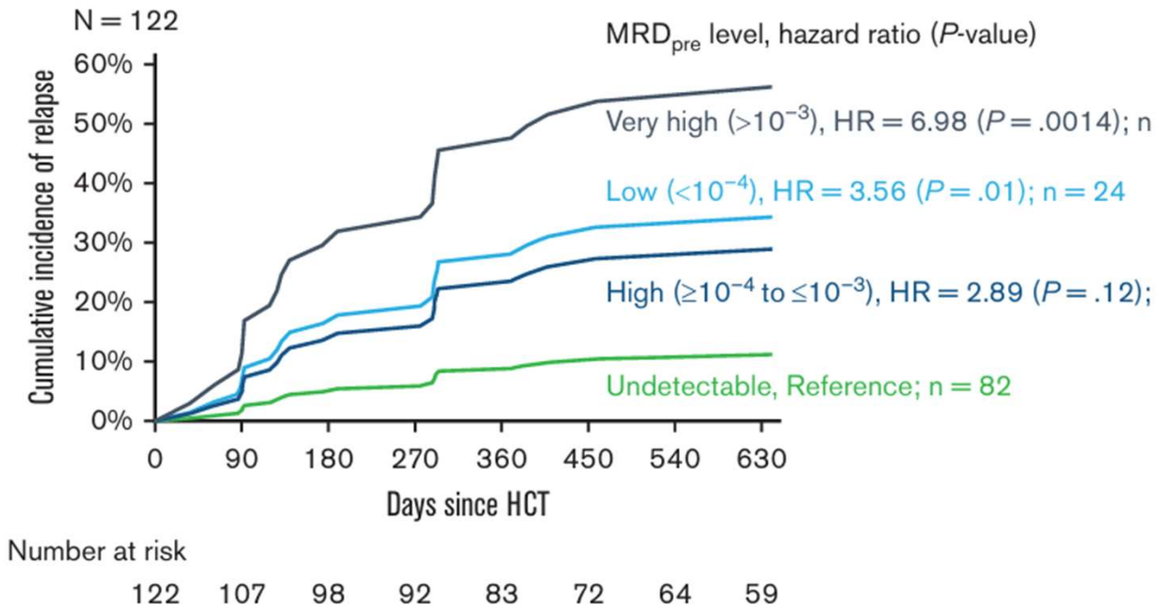
1. Abbas AK, et al. *Cellular and Molecular Immunology*. 8th ed. 2. Carlson CS, et al. *Nat Commun*. 2013;4:2680. https://doi.org/10.1038/ncomms3680

**Theoretical limit of detection of ~$10^{-7}$, limit of quantitation $10^{-6}$.**

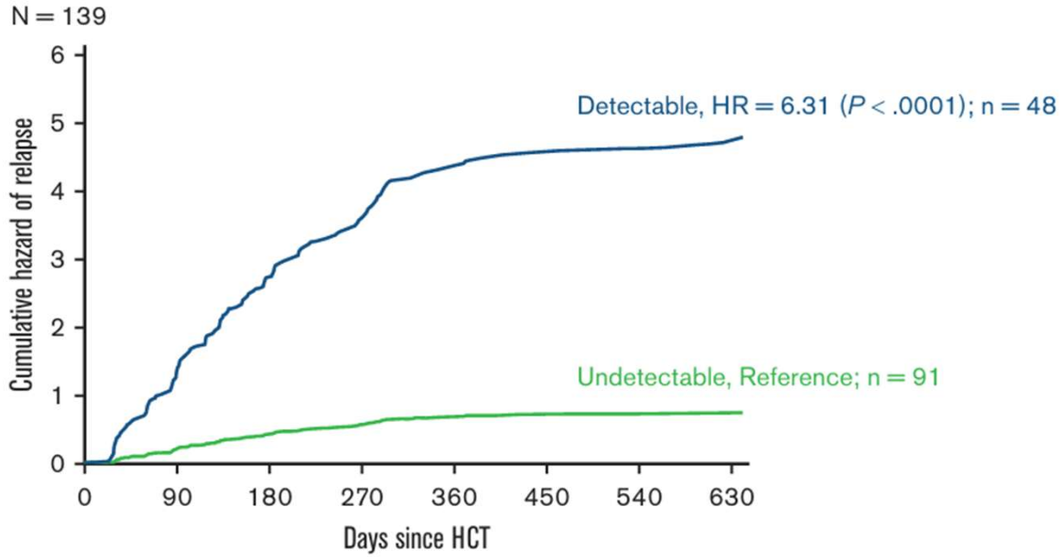
# *Pre and Post HCT Ig/TCR NGS MRD Predicts for Relapse in ALL*

Retrospective multi-center analysis of pre and post HCT NGS MRD in adult ALL

## Pre-HCT NGS MRD at any value increases incidence of post-HCT relapse




## Post-HCT NGS MRD at any value increases hazard of post-HCT relapse

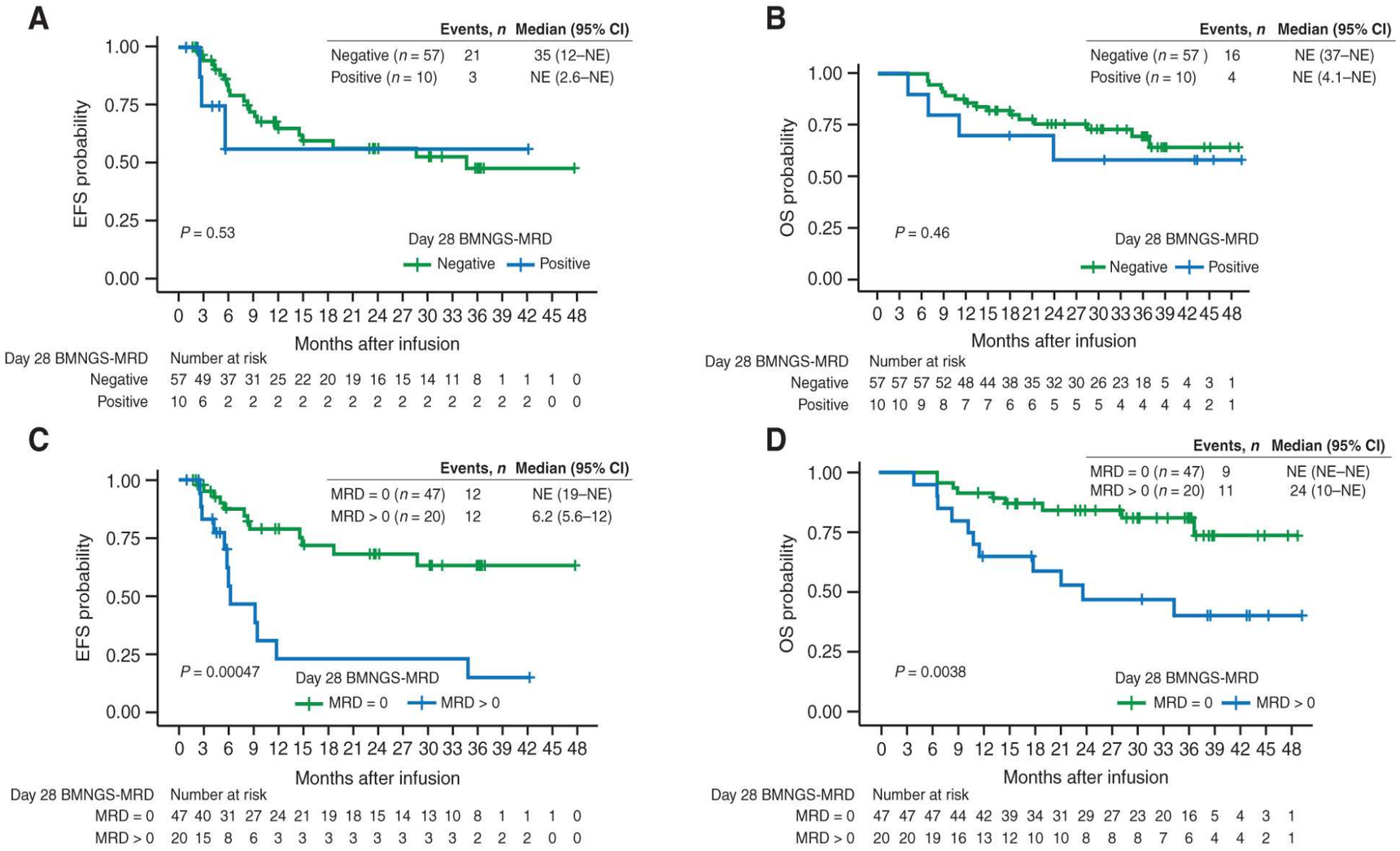



Liang et al Blood Adv. 2023 Jul 25;7(14):3395-3402.

# Low-level MRD positivity predicts relapse after Tisagenlecleucel

MRD threshold at $10^{-6}$

Including detectable but not quantifiable MRD ($<10^{-6}$)

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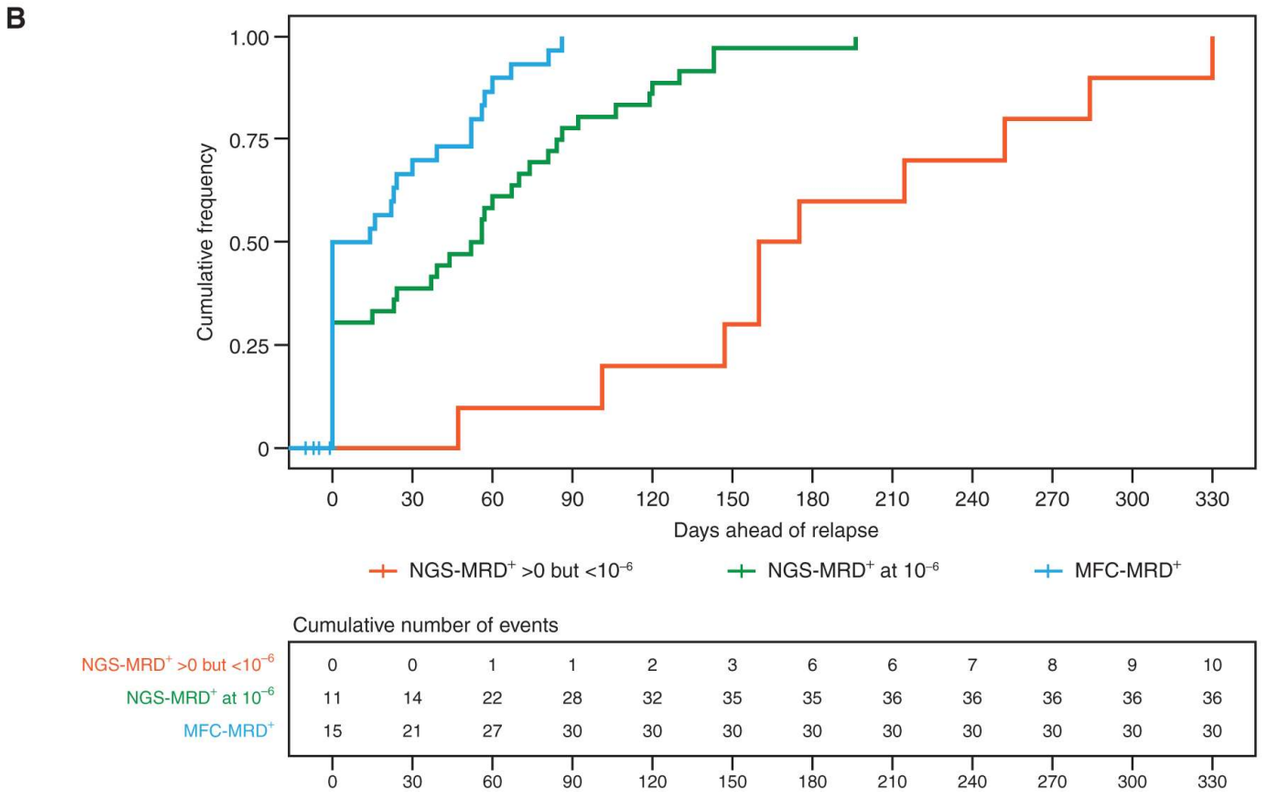
# *Low-level MRD positivity precedes overt relapse by several weeks*




**<u>Consecutive testing:</u>**
Of 26 patients with consecutive MRD-NGS>0:

--19 relapsed
--6 were censored for HCT
--1 lost to follow-up

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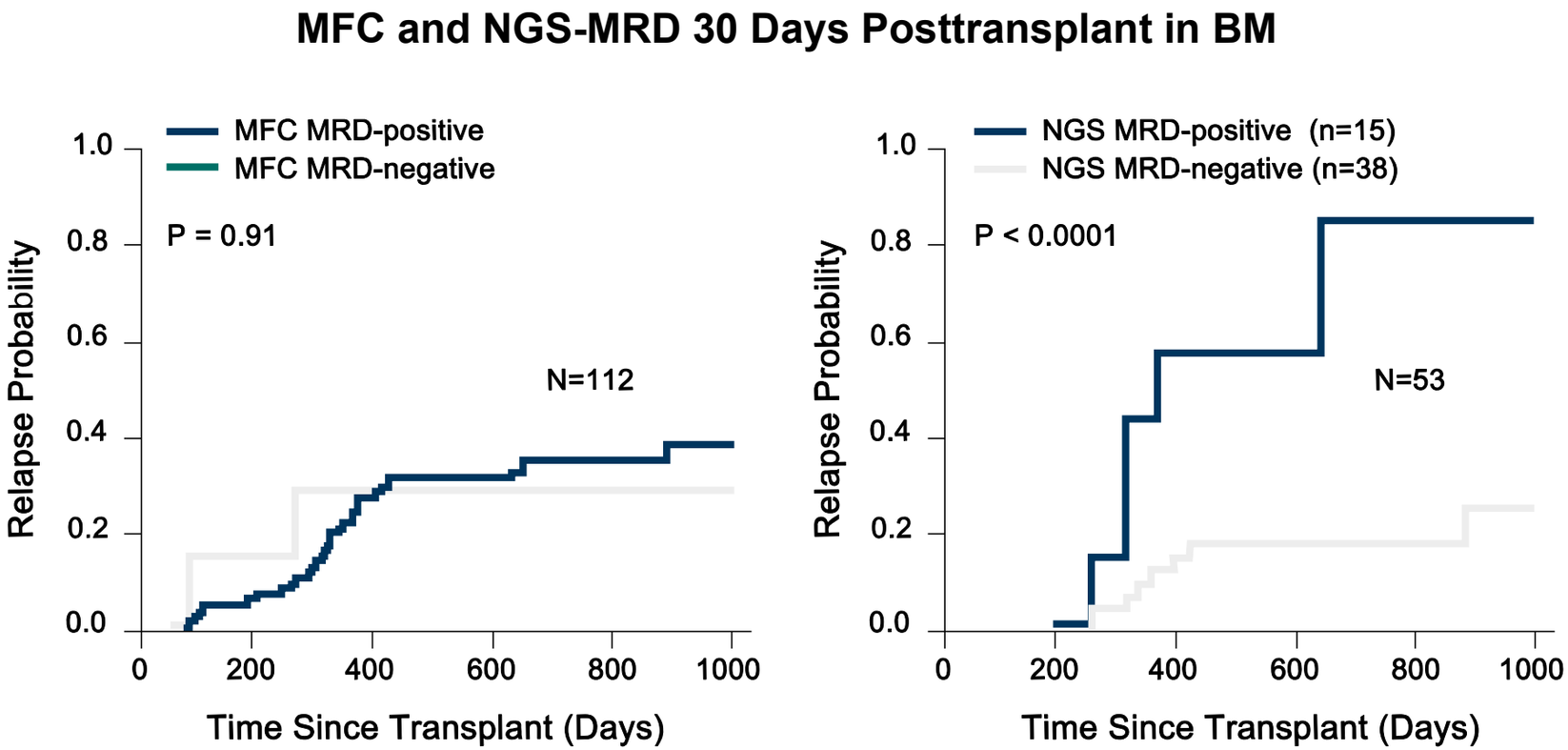
*Blood Cancer Discov* (2022) 3 (1): 66–81.

# *NGS-MRD in BM predicted relapse in pediatric patients post-transplant*




Flow cytometry in BM At $10^{-4}$. NGS in BM At $10^{-6}$.

BM, bone marrow; MFC, multiparametric flow cytometry; MRD, measurable (minimal) residual disease; NGS, next-generation sequencing.

Pulsipher M et al. *Blood*. 2015;125(22):3501-3508.



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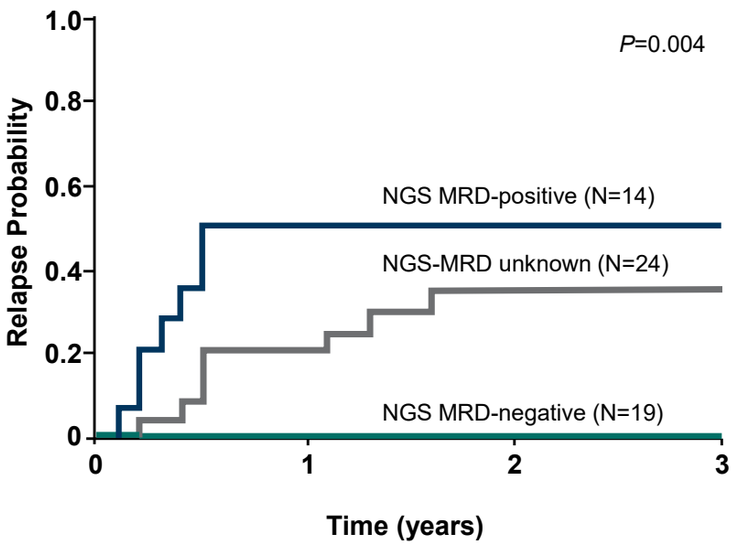
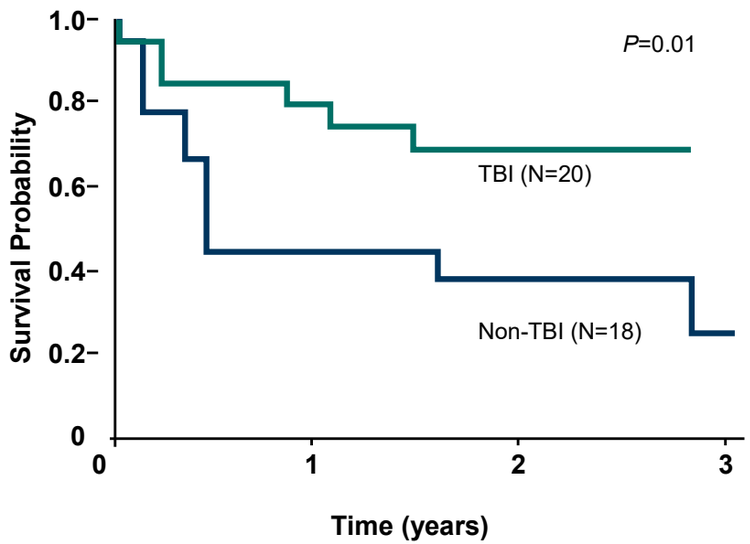
# *Pretransplant NGS-MRD Predicted Relapse in Children/Young Adults Independent of Conditioning Regimen*

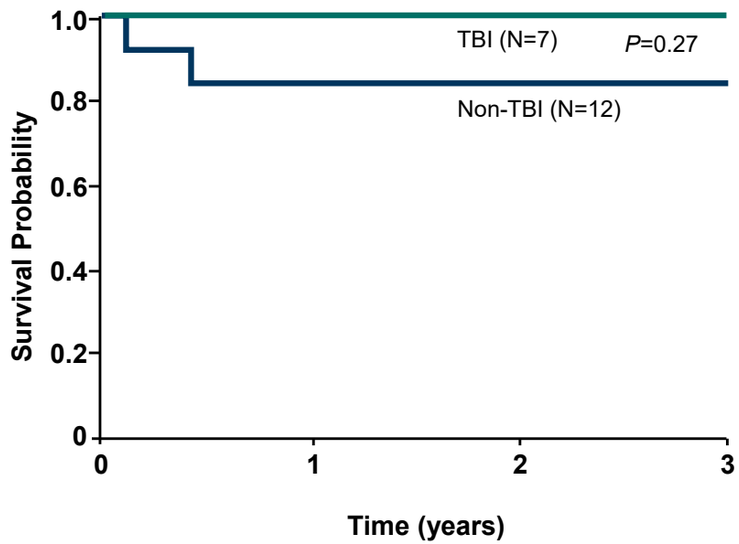
**Cumulative Incidence of Relapse by Pretransplant NGS-MRD Status**




**EFS by Conditioning Regimen in Patients Who Were NGS MRD-Positive or Unknown**




**EFS by Conditioning Regimen in Patients Who Were NGS MRD-Negative**




NGS-MRD in PB or BM.

BM, bone marrow; MRD, measurable (minimal) residual disease; NGS, next-generation sequencing; PB, peripheral blood; TB
body irradiation.

Friend BD et al. *Pediatr Blood Cancer*. 2020;67(2):e28079.



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# *Adult ALL MRD Data*

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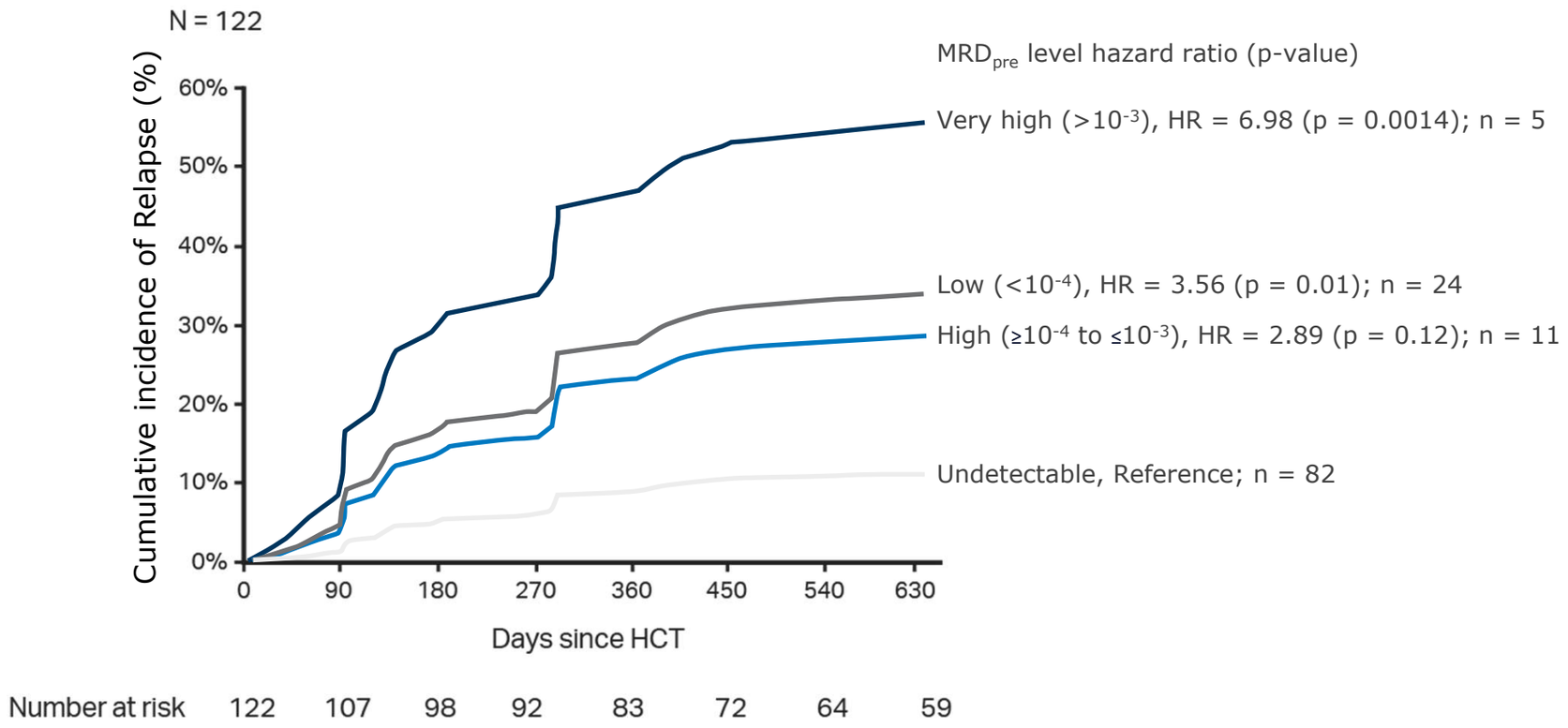
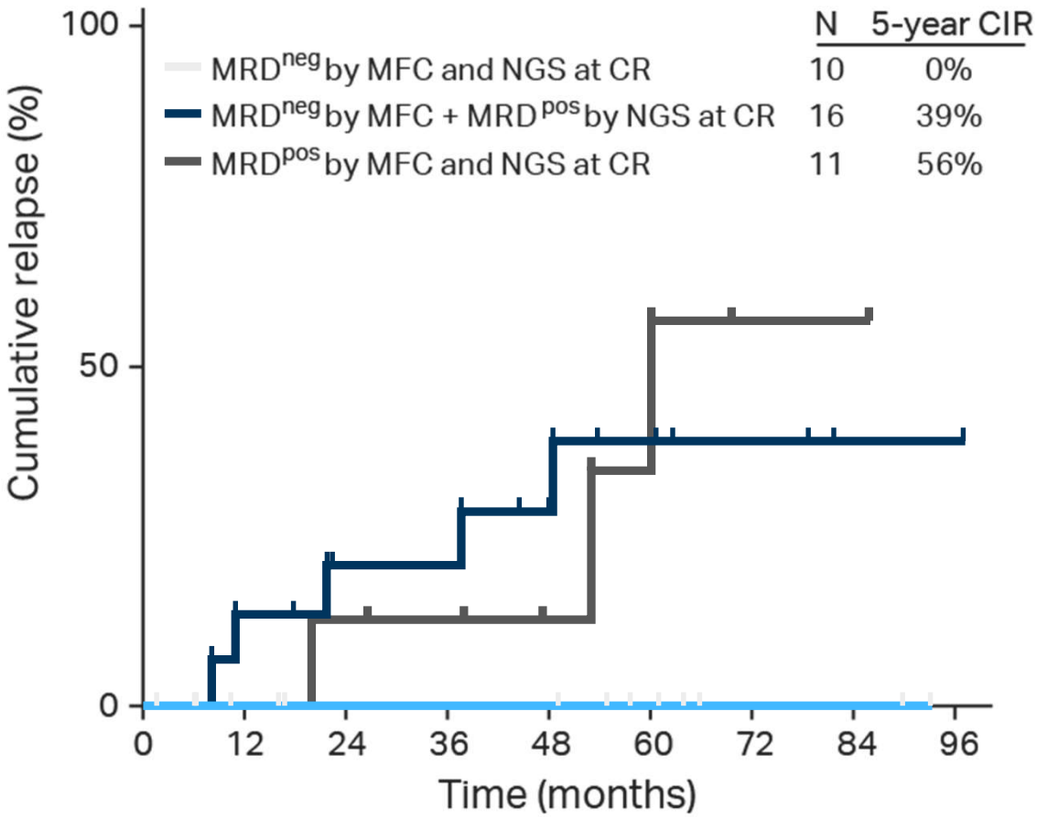
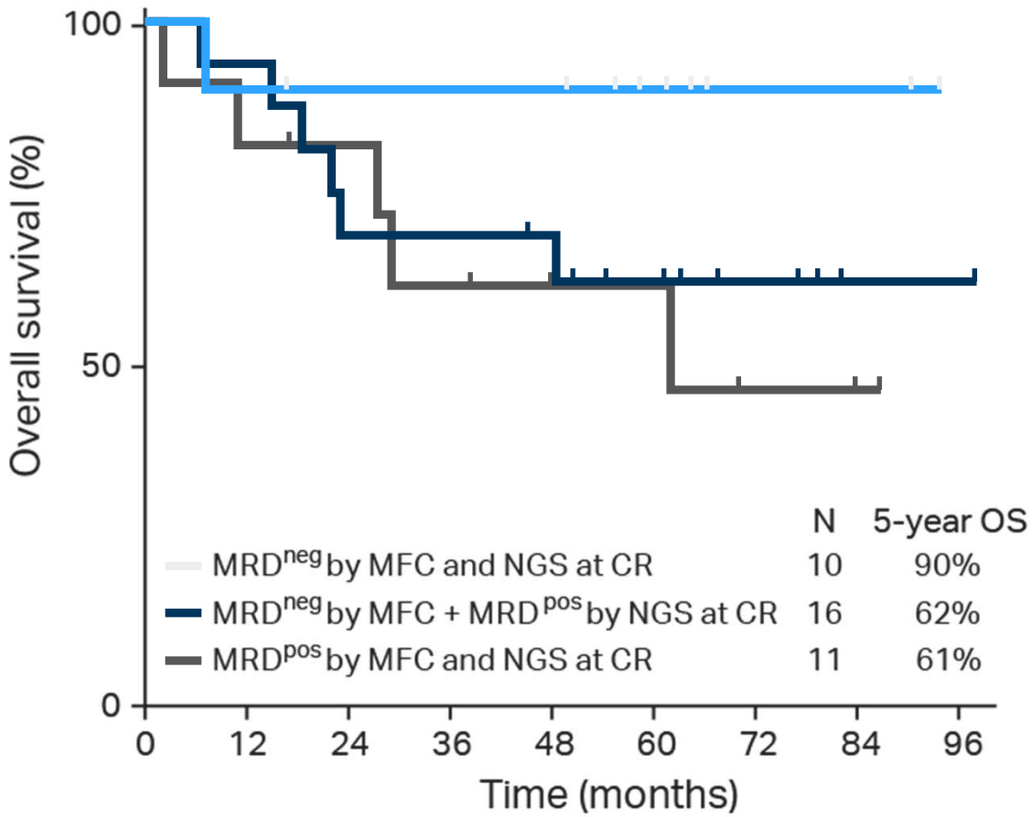
# *RWE: Pre-Transplant NGS MRD is Prognostic for Risk of Relapse in Adults with ALL*




Flow cytometry in BM At $10^{-4}$. NGS in BM At $10^{-6}$.
BM, bone marrow; MFC, multiparametric flow cytometry; MRD, measurable (minimal) residual disease; NGS, next-generation sequencing.
Liang EC. et al. *Blood Adv*. 2023.

# *NGS MRD Further Risk Stratifies Patients who are MRD-Negative by MFC*



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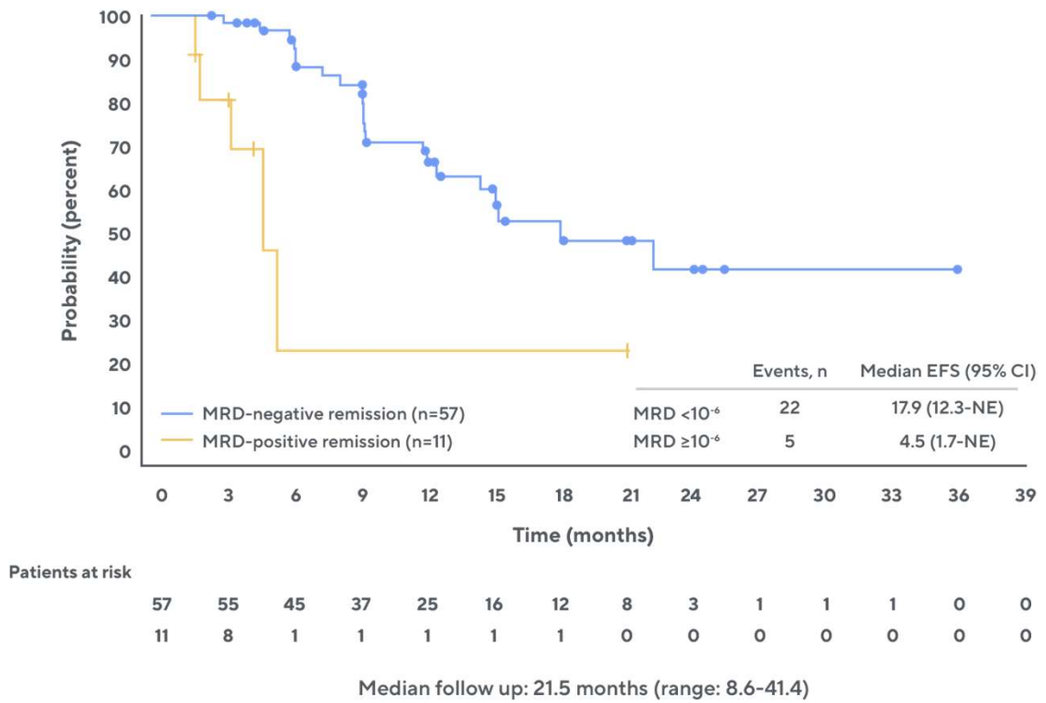
Short NJ, et al. *Blood Adv*. 2022;6(13):4006-14.

# *FELIX: Depth of MRD response ($10^{-6}$) to CAR-T therapy correlates with prolonged survival*

## Event-free survival by MRD status

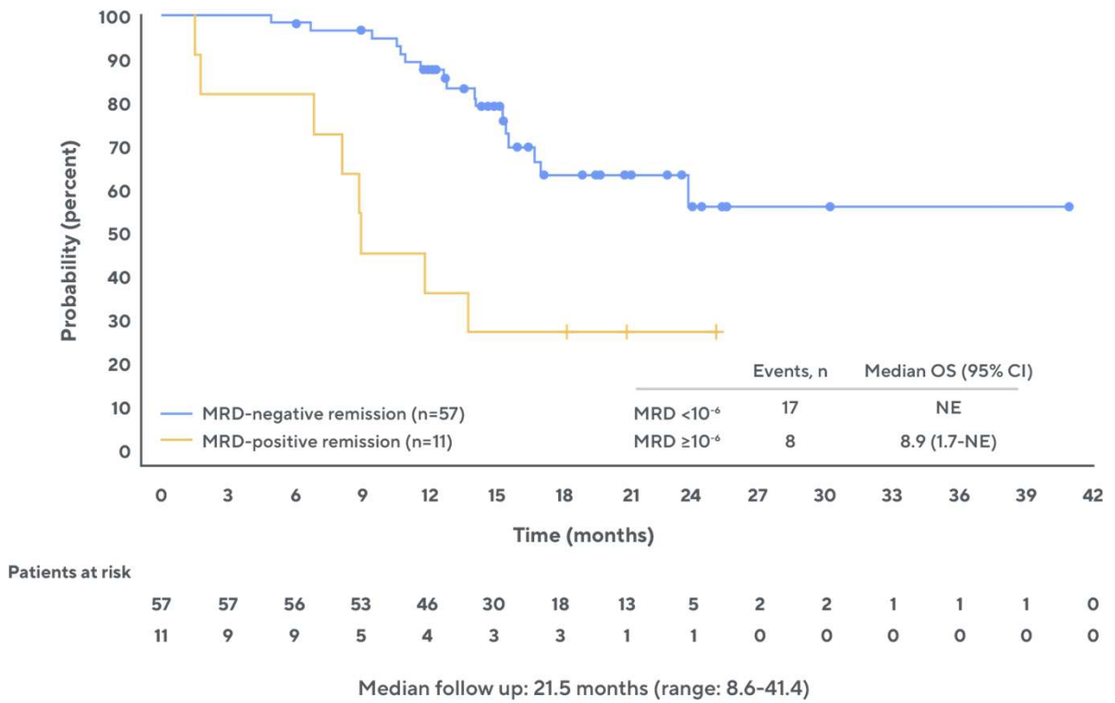
Patient with MRD-negative remission had longer EFS




Median follow up: 21.5 months (range: 8.6-41.4)

## Overall survival by MRD status

70% of patients in MRD-negative remission are alive




Median follow up: 21.5 months (range: 8.6-41.4)

CI: confidence interval; EFS: event-free survival; MRD: minimal residual disease; OS: overall survival

Jabbour E, et al. *Blood*. 2024;144(Suppl 1):963



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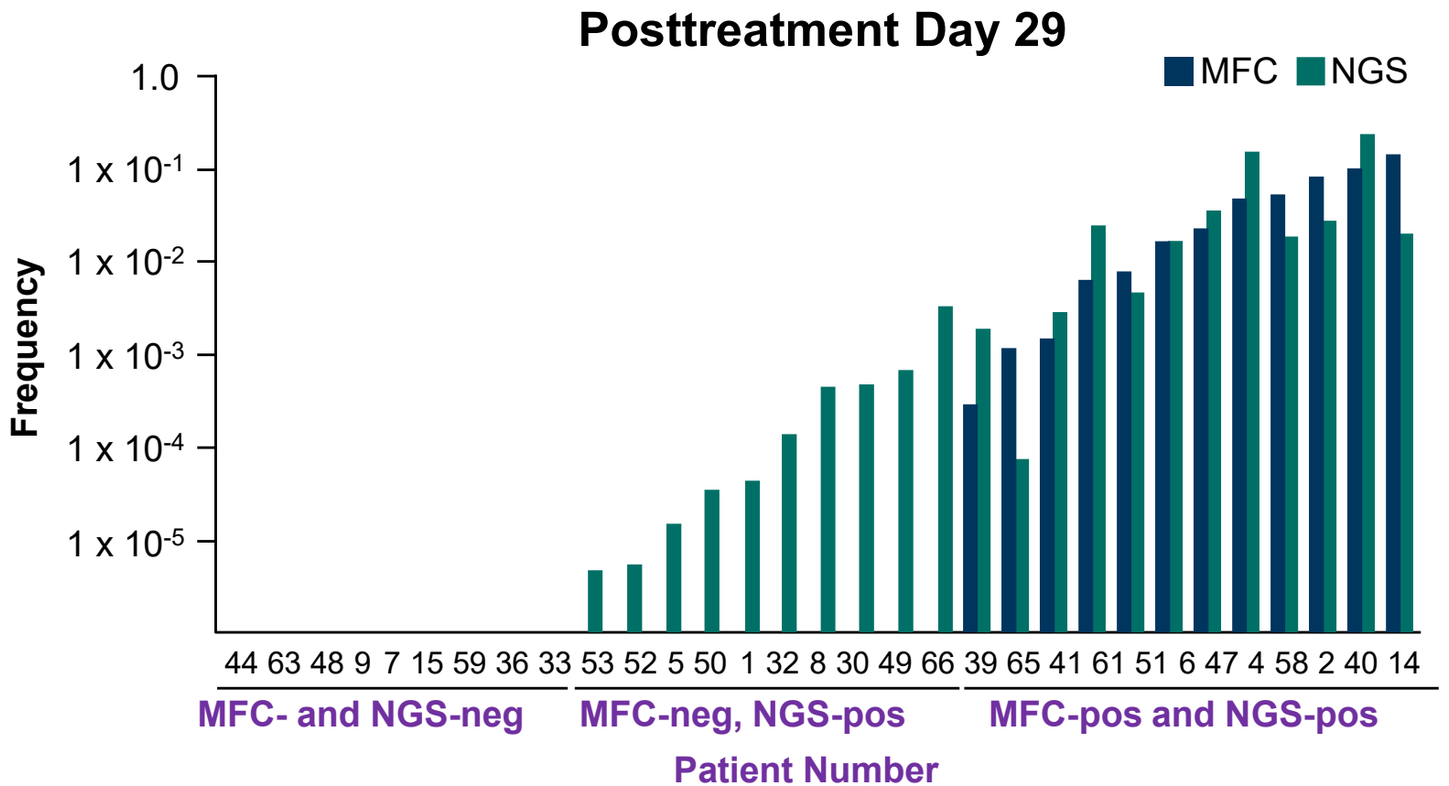
# NGS-MRD Identified Patients With T-Cell* ALL by TCRB Rearrangements

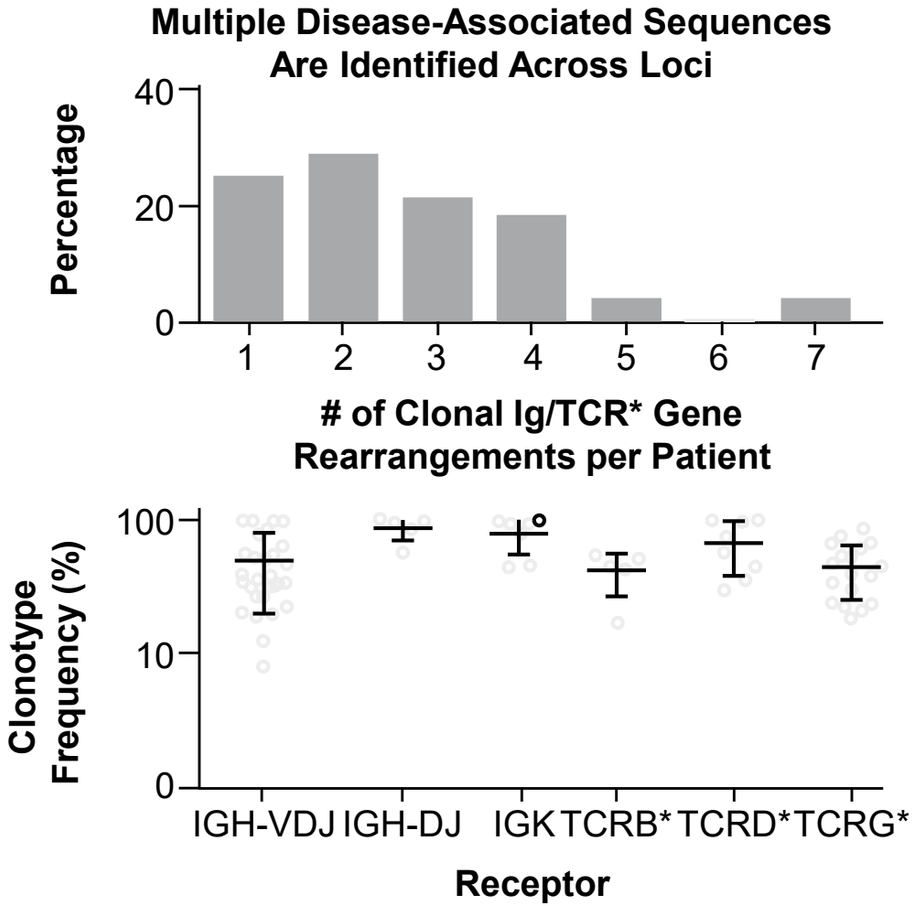


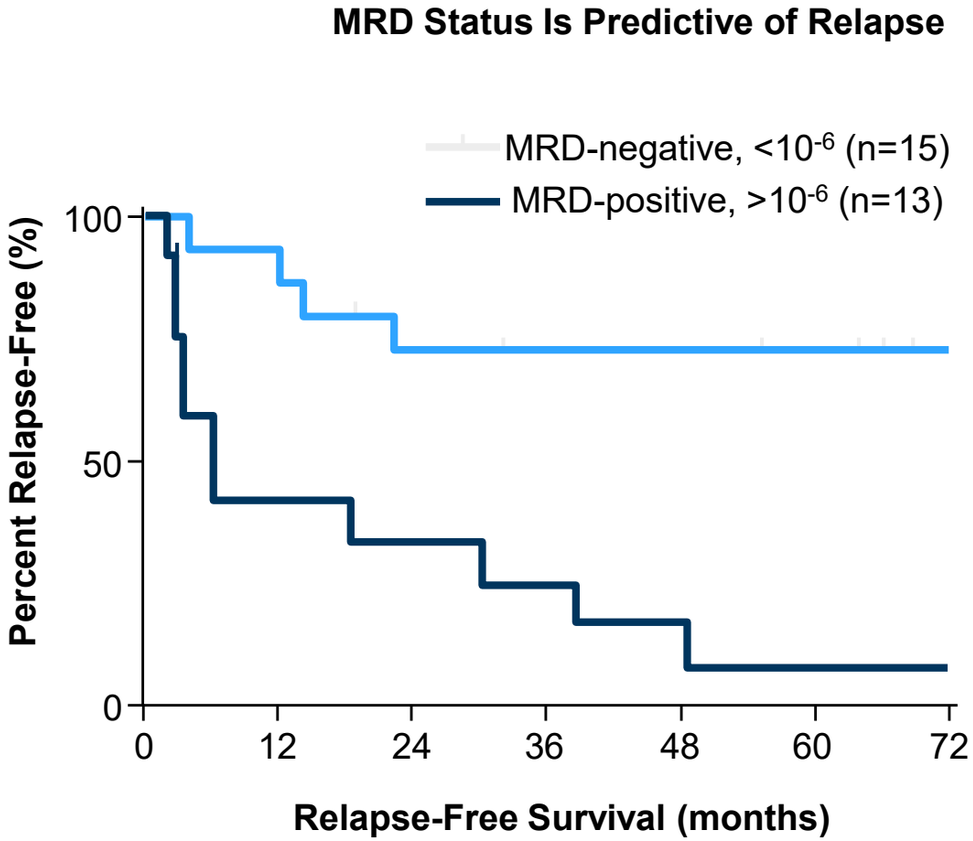

* T-cell testing is available as a CLIA-validated LDT and has not been cleared or approved by the FDA.

ALL, acute lymphoblastic leukemia; MFC, multiparametric flow cytometry; MRD, measurable (minimal) residual disease; neg, negative; NGS, next-generation sequencing; pos, positive; TCRB, T-cell receptor beta.

Wu D et al. *Sci Transl Med*. 2012;4(134):134ra63.

# NGS-MRD Identified Leukemia-associated Receptor Rearrangements and Track These Sequences to Predict RFS




* T-cell testing is available as a CLIA-validated LDT and has not been cleared or approved by the FDA.
NGS-MRD in PB at $10^{-6}$.
MFC, multiparametric flow cytometry; Ig, immunoglobulin; MRD, measurable (minimal) residual disease; NGS, next-generation sequencing; PB, peripheral blood; RFS, relapse-free survival; TCR, T-cell receptor.
Mannis GN et al. *Biol Blood Marrow Transplant*. 2016;22(6):1030-1036. https://doi.org/10.1016/j.bbmt.2016.02.004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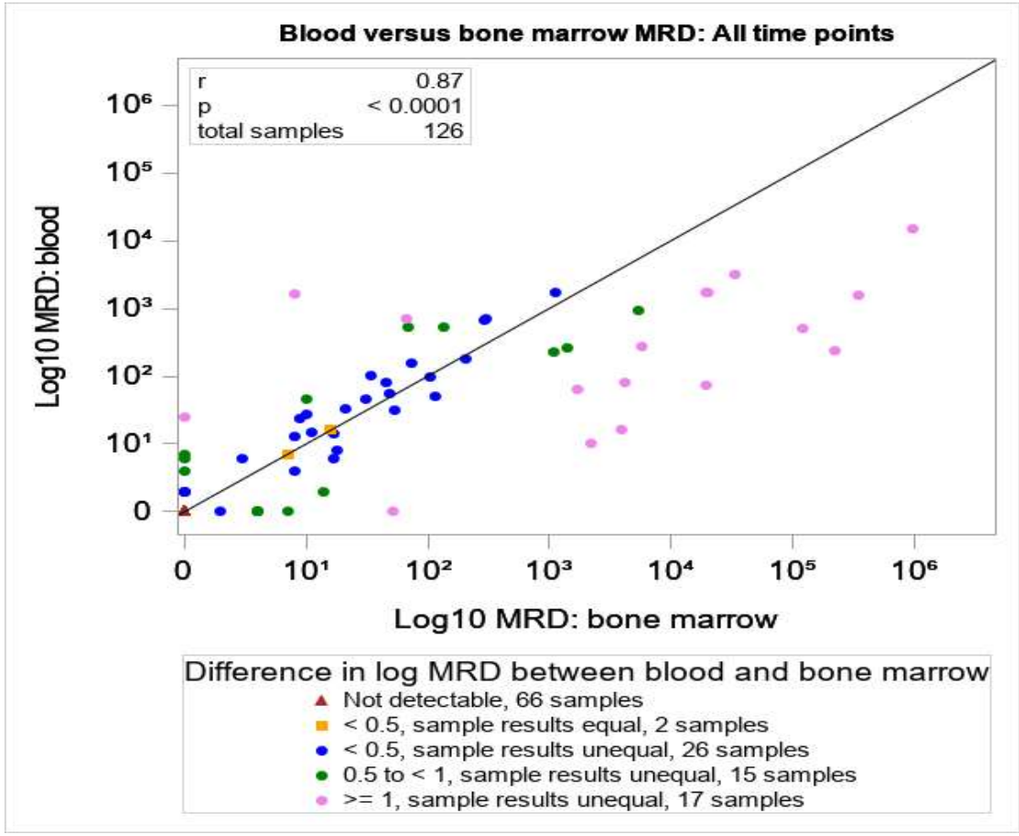
# *Evidence for Blood-Based Monitoring of Post-HCT Ig/TCR NGS MRD in ALL*

Prospective observational study comparing blood and marrow-based NGS MRD in A

126 paired marrow/blood samples among 69 adult ALL patients undergoing HCT



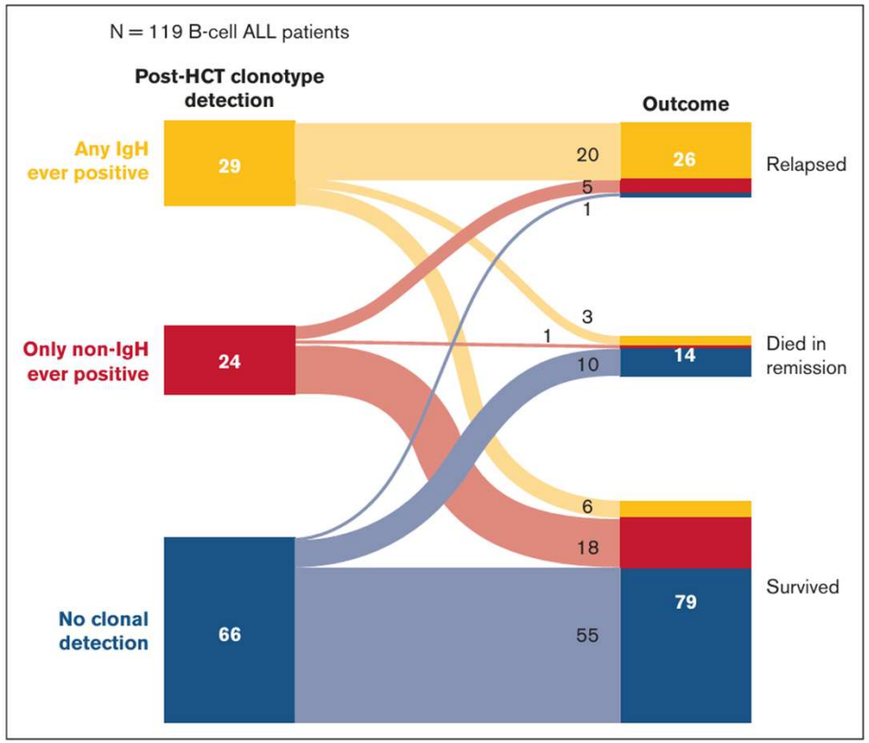

- r= 0.87
- PPV 87%; NPV 90%
- 14 discordant PB/BM pairs:
  - 7 PB+/BM-
  - 7 PB-/BM+
- Median 90 days - first MRD$^{+}$ to clinical relapse

Muffly et al Blood Adv. 2021 Aug 24;5(16):3147-3151

# *Additional Insights and Tips re Ig/TCR NGS MRD in ALL*

Retrospective multi-center analysis of pre and post HCT NGS MRD in adult ALL

## Clonotype is important for interpretation




## Sequence LOD is important for interpretation

**Residual Sequence Detected**

ESTIMATED MRD VALUE:

Assessed below LOD: Range 0 - <1 residual clonal cells per million nucleated cells **

*** Sequence detected below Limit of Detection; frequency too low to enable consistent MRD determination across samples.*

*Total nucleated cells evaluated from this sample: 2,055,018*

| | SPECIMEN TYPE | ESTIMATED SEQUENCE ABUNDANCE (RESIDUAL CLONAL CELLS PER MILLION NUCLEATED CELLS) | 95% CONFIDENCE INTERVAL |
|---|---|---|---|
| **IGH - Sequence F** | Blood | *not detected* | 0 - <1 |
| **IGK - Sequence G** | Blood | 10†† | 4 - 17 |

**SUPPLEMENTAL SEQUENCE INFORMATION**

| SEQUENCE | LIMIT OF DETECTION (PER MILLION CELLS) [5] | LIMIT OF QUANTITATION (PER MILLION CELLS) [6] |
|---|---|---|
| IGH - Sequence F | <1 | 1 |
| IGK - Sequence G | 93 | 116 |

Liang et al Blood Adv. 2023 Jul 25;7(14):3395-3402.

# *Possible changes quant PCR*

- Continue to collect
- Include value in %

Was measurable residual disease detected using a quantitative PCR -based assay? *(at infusion) (need to have instructions for handling more than one category in this section)*

☐ Yes

☐ No

bcr/abl transcripts (applies only to Ph+/bcr-abl positive ALL)

1. Yes
2. No

If yes, report the level at which bcr/abl1/control ratio is detected __%

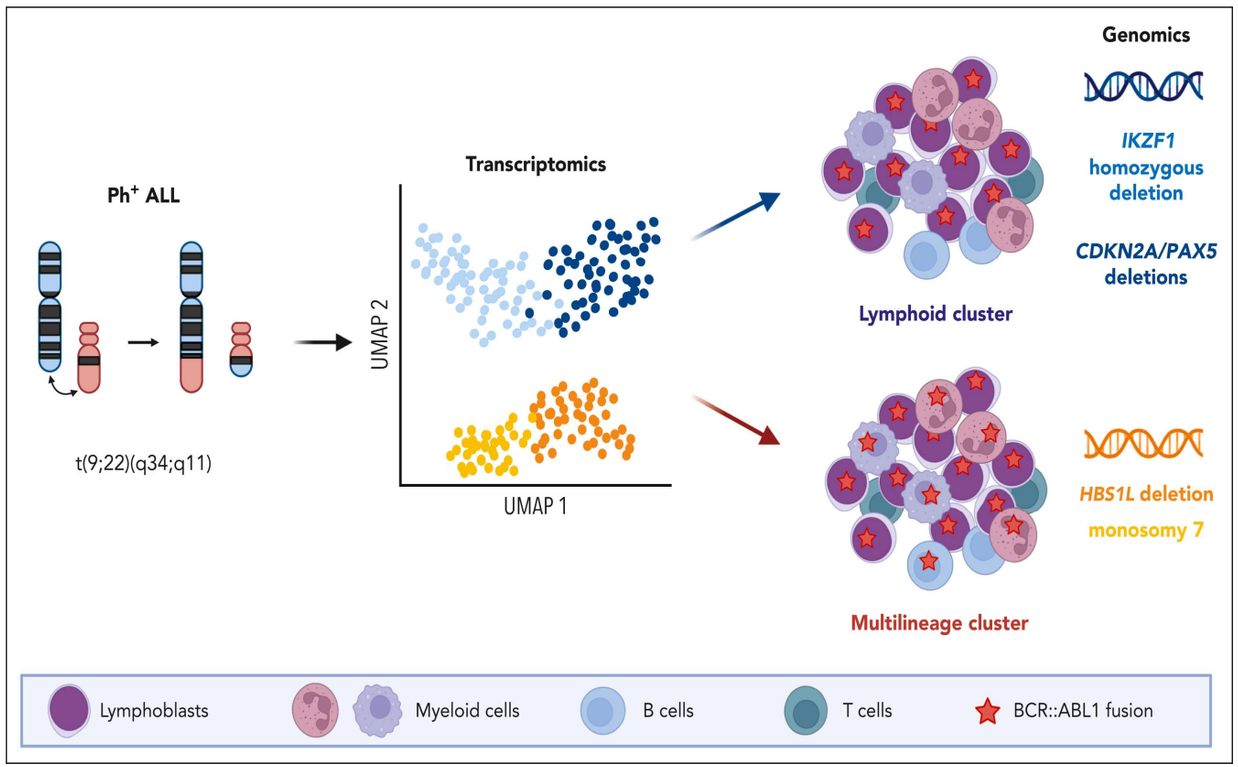
What was the source of tissue assayed?

Blood

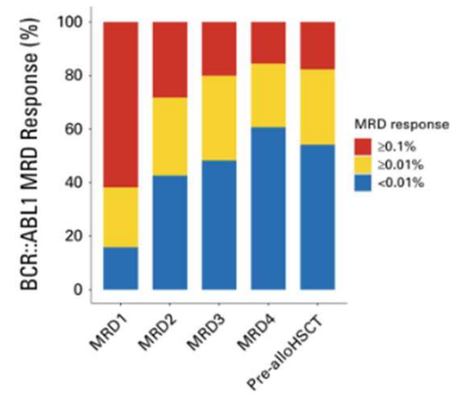
Bone Marrow

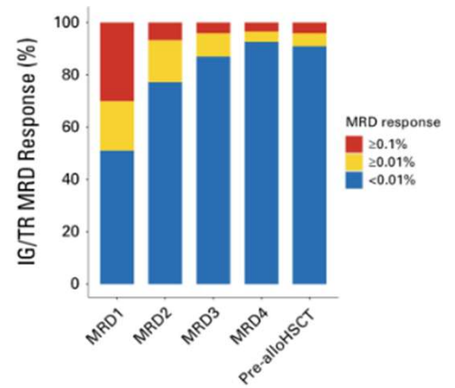
# *Ig/TCR NGS vs BCR::ABL1 RTPCR MRD in ALL*

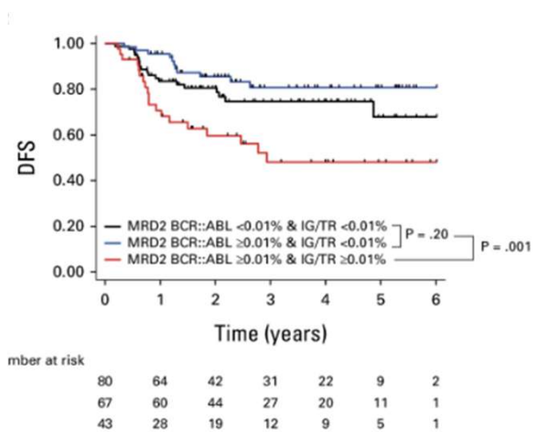
## Multilineage vs lymphoid-only Ph+ ALL

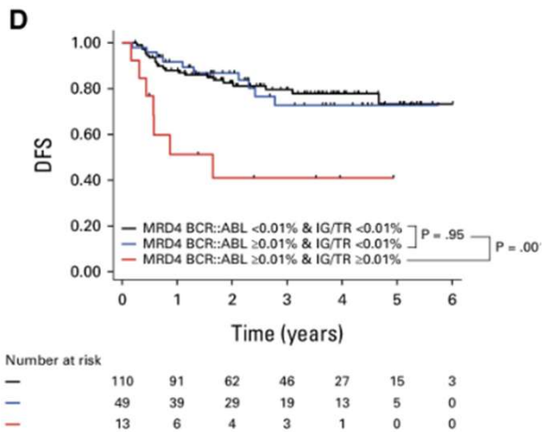



## Ig/TCR tracking more specific and progn




Kim D, Gardner E. Blood, 2024, Apr 4;143(14):1322-1323

Kim R et al *JCO* 42, 3140-3150(2024).