TO THE EDITOR:

Hybrid immunity in immunocompromised patients with CLL after SARS-CoV-2 infection followed by booster mRNA vaccination

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Patients with chronic lymphocytic leukemia (CLL) are immunocompromised.[1] They are at high risk for developing severe COVID-19[2-4] and mount suboptimal immunity after messenger RNA (mRNA) vaccination,[5,6] but they have slightly conflicting results regarding cellular immunity.[7,8] In a "hybrid immunity" setting, immune protection is influenced by prior infection status and vaccination. Here, we studied humoral and cellular hybrid immunity in a CLL cohort of 29 patients with a history of COVID-19[2] and 3 consecutive mRNA vaccinations against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (2 patients received 2 doses).

The study was approved by Swedish Ethical Review Authority (www.etikprovningsmyndigheten.se). Written informed consent was obtained before sampling. Serum and saliva antibodies were measured prevaccination, after dose 2, and before and after dose 3 vaccinations. SARS-CoV-2 immunoglobulin G (IgG) antibodies in serum were analyzed using Roche Elecsys qualitative anti-SARS-CoV-2 and quantitative anti-SARS-CoV-2 immunoassays as described elsewhere.[2,5] Saliva was collected using a self-sampling technique, and SARS-CoV-2 IgG antibodies were analyzed as described elsewhere.[9-11] T-cell responses were analyzed by activation-induced marker assay as described elsewhere[12] before vaccination and before and after dose 3. We also applied the interferon gamma (IFN-γ) ELISpot as described elsewhere.[2,7] Experimental methods are described in the supplemental Methods, available on the *Blood* website.

Twenty-nine patients from a previous COVID-19 study were included.[2] Patient characteristics and mRNA vaccinations are summarized in Table 1 and supplemental Table 1. Twenty-one patients were untreated, 6 had received CD20 monoclonal antibody–containing treatment >12 months before, and 2 received BTK inhibitor (BTKi) therapy. The variability in time since recovery from COVID-19 and between vaccinations and tests is depicted in Table 1. Breakthrough infections were not observed during the study (until 21 December 2021). Immune responses are shown in Figure 1 (entire cohort) and supplemental Figure 1 (patients without missing data points). Serology results are shown in Figure 1A. Ninety-three percent of patients (26/28) were seropositive before the first vaccination with titer levels available in 25 patients (Figure 1A). The median spike-specific antibody titer was 47 U/mL (range <0.8-911), including 3 seronegative patients. After 2 doses, 96% (24/25) were seropositive and the median antibody titer had increased to 17 208 U/mL (range <0.8 to >25 000, interquartile range [IQR] 2793 to >25 000) ($P < .0001$). This titer fell to a median of 6825 U/mL (range <0.8 to >25 000, IQR 2532 to >25 000) prior to dose 3 and then increased to a median of 24 956 U/mL following dose 3 (range <0.8 to >25 000 U/mL, IQR 4219 to >25 000) ($P < .0001$) (upper detection limit 25 000 U/mL). Nucleocapsid-specific antibody levels tended to decline over time (Figure 1A).

Next, we analyzed salivary IgG, and 95% of patients (19/20) had spike-specific IgG prior to dose 3 (Figure 1B), with a moderate correlation between spike antibodies in serum and saliva ($r = 0.4622$, $P < .0006$) (supplemental Figure 2A). Salivary spike IgG levels appeared with slower kinetics and increased before dose 3 ($P < .05$) (Figure 1B). The average level before and after dose 3 was comparable to naive healthy controls after 2 vaccine doses.[10] Nucleocapsid reactivities were stable with a transient rise before dose 3 ($P < .05$) (Figure 1B).

Thereafter, we analyzed T-cell responses against wild-type Wu-Hu1 and Omicron (BA.1) as described elsewhere.[12] Thirteen patients were included, with memory responses shown in Figure 1C. After dose 3, the spike-specific $CD4^+$ T cells ($P < .05$) (Figure 1D) and $CD8^+$ T cells ($P < .01$) increased (Figure 1E) with a similar magnitude for Wu-Hu1 (wild-type) and Omicron (BA.1).

IFN-γ ELISpot analysis showed no significant differences following dose 3 (Figure 1F), although spike-specific responses tended to increase and non-spike-specific membrane, envelope, and nucleocapsid responses declined over time.

Additionally, we also made paired analyses of results in patients without missing data points (supplemental Figure 1). Almost identical and statistically significant changes over time were observed in this group compared with the entire cohort for spike antibodies in serum (n = 19, supplemental Figure 1A) and saliva (n = 14, supplemental Figure 1B) and for $CD8^+$ T cells (n = 7, supplemental Figure 1D). The change in $CD4^+$ T cells

Table 1. Clinical characteristics at start of mRNA vaccination against SARS-CoV-2 as well as timepoints of vaccination and tests in patients with CLL (n = 29) with a prior history of COVID-19 infection

All patients	
Median age, y (range)	65 (47-83)
Male/female	20/9
CLL treatment status	
Never treated	72% (21/29)
Previously treated*	21% (6/29)
Time (mo) since last treatment	45.5 (15-70)
Ongoing therapy†	7% (2/29)
Ongoing Ig supplement	10% (3/29)
CLL stage (by Rai)	
0	83% (24/29)
I-II	17% (5/29)
III-IV	0% (0/29)
CLL remission status (by iwCLL)	
SD	0% (0/29)
PD	0% (0/29)
PR/CR	34% (10/29)
Not applicable (never treated, early-stage)	62% (18/29)
Not applicable (never treated, progressive disease)	3% (1/29)
Time (mo) since COVID-19 diagnosis to vaccination, median (range)	
Dose 1 (n = 29)	5.75 (1.75-13.75)
Interquartile range	4.00-11.25
Dose 3 (n = 27)‡	12 (7.75-19.75)
Interquartile range	9.75-17.75
Time (mo) since pretest to vaccination, median (range)	
Dose 1 (n = 28)‡	0.25 (0-4.25)
Interquartile range	0-1.00
Dose 3 (n = 25)‡	1.25 (0.25-2.75)
Interquartile range	1.00-1.75
Time (mo) since vaccination to test, median (range)	
Dose 2 (n = 25)‡	0.75 (0.25-2.5)
Interquartile range	0.51-1.00
Dose 3 (n = 27)‡	0.75 (0.5-1.5)
Interquartile range	0.75-1.00
Time (mo) between vaccine doses, median (range)	
Dose 1-2 (n = 29)	1.5 (0.75-2.0)
Interquartile range	1.37-1.50
Dose 2-3 (n = 27)	5 (2.75-8.25)
Interquartile range	4.25-5.75

Table 1 (continued)

All patients	
Type of vaccine§	
Dose 1 (n = 29) C = 25, S = 4	
Dose 2 (n = 29) C = 25, S = 4	
Dose 3 (n = 27) C = 25, S = 2	
COVID-19 management‖	
Hospital admission	66% (19/29)
ICU admission	7% (2/29)
Supplemental oxygen	48% (14/29)
Corticosteroids	31% (9/29)
Antiviral therapy (Remdesivir)	14% (4/29)
Anticoagulation	59% (17/29)
IvIg	3% (1/29)
Convalescent plasma	0% (0/29)
BTKi	3% (1/29)
Tocilizumab	0% (0/29)
Hydroxychloroquine	3% (1/29)

C, Comirnaty; ICU, intensive care unit; IvIg, intravenous immunoglobulin; iwCLL, International Workshop on Chronic Lymphocytic Leukemia; PD, progressive disease; PR/CR, partial remission/complete remission; S, Spikevax; SD, stable disease.

*With no current treatment. All with anti-CD20 monoclonal antibody–containing immunochemotherapy (bendamustine-rituximab/fludarabine-cyclophosphamide-rituximab) and all >12 months prior to vaccination.

†Both with BTKi (ibrutinib). One was previously treated with immunochemotherapy >12 months ago and stopped ibrutinib therapy shortly after the second vaccine dose.

‡Number of patients at each time point is shown in Figure 1 and supplemental Figure 1.

§Comirnaty (BNT162b2, Pfizer BioNTech), Spikevax (mRNA-1273, Moderna). Two patients did not receive dose 3.

‖March 2020 to March 2021.

(n = 7, supplemental Figure 1C) no longer reached statistical significance. In contrast, the change in nucleocapsid antibody levels was more pronounced than in the entire cohort, decreasing in serum (n = 13, supplemental Figure 1A) and increasing in saliva (n = 14, supplemental Figure 1B).

Patients with CLL have shown low anti-spike titers following mRNA vaccination against SARS-CoV-2[5,6] even after 3 doses.[13] Primary COVID-19 infection resulted in higher titers and T-cell responses,[2] although, as shown here, at low levels at the time of first vaccination. The present results mimic those observed of hybrid immunity in otherwise healthy individuals that a combined effect of infection and vaccination results in robust humoral and cellular anti-SARS-CoV-2 immunity.[14]

To the best of our knowledge, the present study is the first to report on hybrid immunity in patients with CLL. The median serology titers of 17 208 to 24 956 U/mL after 2 and 3 doses were not affected by the analytical range of the assay even though several patients had serology titers above the upper level 25.000 U/mL (Figure 1A). Titers are markedly higher than the median of <100 U/mL that were found after 2 vaccine doses in non-COVID patients with CLL who participated in an earlier prospective vaccine trial.[5] This includes abundant local immunity in saliva.[5] The plateau at dose 3 in the present study is in

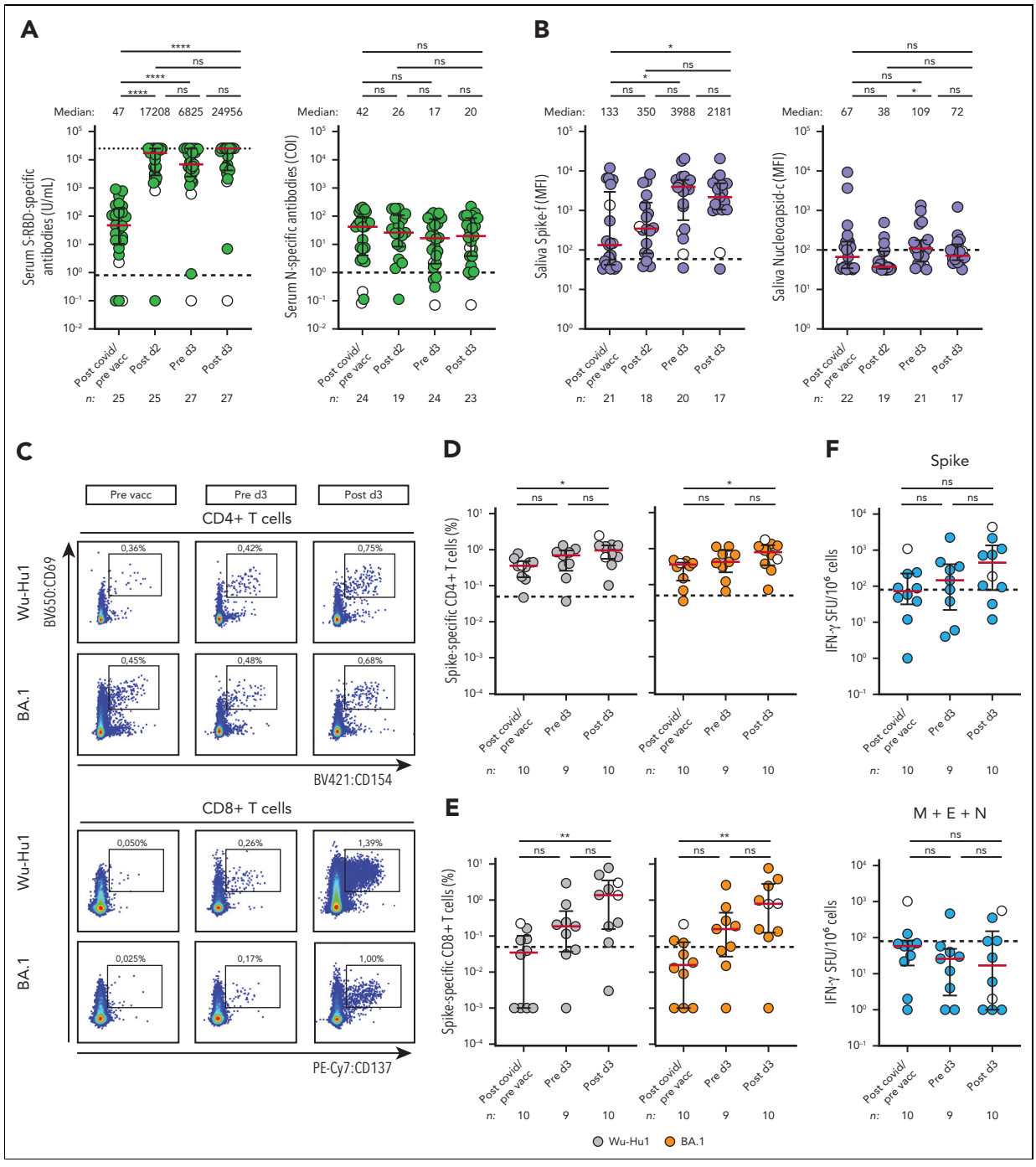

Figure 1. Time kinetics of humoral and cellular responses against spike and nonspike epitopes of SARS-CoV-2 in CLL patients after COVID-19 infection followed by 3 mRNA vaccination doses. (A) Serum and (B) salivary levels of S (spike)-receptor-binding domain (RBD) and N (nucleocapsid)-specific antibody responses in patients post–COVID-19 and pre- (pre vacc) and postvaccination (dose 2 [d2]) and pre- (dose 3) and postvaccination (dose 3) with indicated median values. (C) Spike-specific $CD4^+$ ($CD69^+CD154^+$) and $CD8^+$ ($CD69^+CD137^+$) T cells were detected by flow cytometry (activation-induced marker assay). Spike-specific $CD4^+$ (D) and $CD8^+$ (E) T-cell response against Wu-Hu1 (wild-type) and Omicron (BA.1) post–COVID-19 and prevaccination, prevaccination (dose 3), and postvaccination (dose 3). (F) ELISpot IFN-γ-specific T-cell responses to spike and membrane, envelope, and nucleocapsid (M + E + N) peptide pools post–COVID-19 and prevaccination, prevaccination (dose 3), and postvaccination (dose 3). White dots represent patients on BTKi treatment in the respective analysis. Number (*n*) of patients tested at each time point is indicated below graph. Assay upper limit of detection of 25 000 U/mL is shown as dotted line (A). Dashed line represents positive threshold for each assay: (A) 0.8 U/mL and 1 cutoff index (COI) respectively; (B) median fluorescence intensity (MFI) of 59 and 100 for S and N, respectively; (D-E) 0.05% and (F) 80 spot-forming units (SFU)/10^6 cells. Error bars represent the median (red line) and interquartile range. Kruskal-Wallis test with Dunn multiple comparison correction was used. *P < .05, **P < .01, ****P < .0001. ns, not significant.

line with 4 vaccine doses in healthy individuals.[15] Saliva antibodies followed a slower time kinetics compared with serum, not being significantly increased until predose 3. Whether this is due to rebound infection limited to the oral cavity[16] is unknown but partly supported by the increase in nucleocapsid-specific IgG. Robust spike-specific $CD8^+$ T-cell responses occurred after vaccine dose 3, in line with a report on patients with multiple sclerosis receiving 3 vaccine doses.[17]

T-cell immunity was also assessed against Omicron (BA.1), with higher $CD8^+$ T-cell magnitudes after 3 doses in the CLL cohort than in healthy non-COVID-19 individuals after 2 mRNA vaccine doses.[12]

There are limitations with the present study. The cohort is limited, most patients had early-stage CLL, and few had ongoing therapy, which may affect immune responses favorably. The variation in time between COVID-19 and the start of vaccination and between vaccinations may affect the magnitude of the immune responses. Neutralizing antibodies were not measured even though we found a strong correlation between serum and saliva IgG levels with neutralization in the same patients earlier[2] and confirmed by others.[18] Finally, there was no control group tested in parallel with this real-world cohort, and the groups studied (healthy donors and CLL patients) were not COVID-19 convalescents and received only 2 vaccine doses at the time of reporting.[5,7,10,12]

Hybrid immunity was recently reported to confer long-lasting protection from severe disease in healthy persons[19-21] even though not preventive against Omicron.[22] The serial measurement of systemic B- and T-cell responses were spike restricted. Nucleocapsid-directed immune responses were relatively stable over time, albeit with a slow decline, 9 to 20 months after COVID-19. Also, nucleocapsid antibody levels were stable or showed a slight increase in saliva, which may serve as a first-level defense barrier against reinfection by ancestral SARS-CoV-2 strains.[16,23] In conclusion, we demonstrate robust hybrid immunity in serum, saliva, and the T-cell compartment in patients with CLL who received 3 doses of mRNA vaccine following COVID-19 infection. The results are encouraging in the context of immunocompromised patients who have recovered after COVID-19 and need continuous protection against new SARS-CoV-2 variants of concern. To obtain protection, patients who remain seronegative shall be offered available anti-SARS-CoV-2 preventive therapies.

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Authorship

Contribution: L.B., L.H., A.Ö., G.B., M.S.C., H.-G.L., and M.B. contributed to the conceptualization, funding acquisition, and discussion of data; Y.G., K.H., S.M., and D.W. performed experiments and analyzed data; L.B., H.M.I.-S., C.K., L.H., and A.Ö. recruited study participants, conducted the management of participants during the study, and analyzed data; P.N. and E.P. helped perform saliva analyses; A.G. and A.S. provided peptide pools to measure the spike-specific T-cell responses; L.B., D.W., A.Ö., L.H., H.-G.L., and M.B. wrote the original draft of the manuscript; and all authors reviewed and edited revisions of the manuscript and had final responsibility for the decision to submit for publication.

Conflict-of-interest disclosure: M.B. is a consultant for Oxford Immunotech. A.S. is a consultant for Gritstone Bio, Flow Pharma, Arcturus Therapeutics, ImmunoScape, CellCarta, Avalia, Moderna, Fortress, and Repertoire. La Jolla Institute has filed for patent protection for various aspects of T-cell epitope and vaccine design work. The remaining authors declare no competing financial interests.

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Footnotes

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Data are available on request from the corresponding author, Lotta Hansson (lotta.hansson@regionstockholm.se).

The online version of this article contains a data supplement.

REFERENCES

1. Palma M, Gentilcore G, Heimersson K, et al. T cells in chronic lymphocytic leukemia display dysregulated expression of immune checkpoints and activation markers. *Haematologica*. 2017;102(3):562-572.
2. Blixt L, Bogdanovic G, Buggert M, et al. Covid-19 in patients with chronic lymphocytic leukemia: clinical outcome and B- and T-cell immunity during 13 months in consecutive patients. *Leukemia*. 2022;36(2):476-481.
3. Chatzikonstantinou T, Kapetanakis A, Scarfo L, et al. COVID-19 severity and mortality in patients with CLL: an update of the international ERIC and Campus CLL study. *Leukemia*. 2021;35(12):3444-3454.
4. Mato AR, Roeker LE, Lamanna N, et al. Outcomes of COVID-19 in patients with CLL: a multicenter international experience. *Blood*. 2020;136(10):1134-1143.
5. Bergman P, Blennow O, Hansson L, et al. Safety and efficacy of the mRNA BNT162b2 vaccine against SARS-CoV-2 in five groups of immunocompromised patients and healthy controls in a prospective open-label clinical trial. *EBioMedicine*. 2021;74:103705.
6. Herishanu Y, Avivi I, Aharon A, et al. Efficacy of the BNT162b2 mRNA COVID-19 vaccine in patients with chronic lymphocytic leukemia. *Blood*. 2021;137(23):3165-3173.
7. Blixt L, Wullimann D, Aleman S, et al. T-cell immune responses following vaccination with mRNA BNT162b2 against SARS-CoV-2 in patients with chronic lymphocytic leukemia: results from a prospective open-label clinical trial. *Haematologica*. 2022;107(4):1000-1003.
8. Parry H, Bruton R, Roberts T, et al. COVID-19 vaccines elicit robust cellular immunity and clinical protection in chronic lymphocytic leukemia. *Cancer Cell*. 2022;40(6):584-586.
9. Alkharaan H, Bayati S, Hellstrom C, et al. Persisting salivary IgG against SARS-CoV-2 at 9 months after mild COVID-19: a complementary approach to population surveys. *J Infect Dis*. 2021;224(3):407-414.
10. Healy K, Pin E, Chen P, et al. Salivary IgG to SARS-CoV-2 indicates seroconversion and correlates to serum neutralization in mRNA-vaccinated immunocompromised individuals. *Med (N Y)*. 2022;3(2):137-153 e133.

11. Hober S, Hellstrom C, Olofsson J, et al. Systematic evaluation of SARS-CoV-2 antigens enables a highly specific and sensitive multiplex serological COVID-19 assay. *Clin Transl Immunology*. 2021;10(7):e1312.

12. Gao Y, Cai C, Grifoni A, et al. Ancestral SARS-CoV-2-specific T cells cross-recognize the Omicron variant. *Nat Med*. 2022;28(3):472-476.

13. Herishanu Y, Rahav G, Levi S, et al. Efficacy of a third BNT162b2 mRNA COVID-19 vaccine dose in patients with CLL who failed standard 2-dose vaccination. *Blood*. 2022;139(5):678-685.

14. Primorac D, Brlek P, Matisic V, et al. Cellular immunity-the key to long-term protection in individuals recovered from SARS-CoV-2 and after vaccination. *Vaccines (Basel)*. 2022;10(3):442.

15. Regev-Yochay G, Gonen T, Gilboa M, et al. Efficacy of a fourth dose of Covid-19 mRNA vaccine against omicron. *N Engl J Med*. 2022;386(14):1377-1380.

16. Huang N, Perez P, Kato T, et al. SARS-CoV-2 infection of the oral cavity and saliva. *Nat Med*. 2021;27(5):892-903.

17. Madelon N, Heikkila N, Sabater Royo I, et al. Omicron-specific cytotoxic T-cell responses after a third dose of mRNA COVID-19 vaccine among patients with multiple sclerosis treated with ocrelizumab. *JAMA Neurol*. 2022;79(4):399-404.

18. Ujjani C, Shadman M, Lynch RC, et al. The impact of B-cell-directed therapy on SARS-CoV-2 vaccine efficacy in chronic lymphocytic leukaemia. *Br J Haematol*. 2022;197(3):306-309.

19. Cerqueira-Silva T, Andrews JR, Boaventura VS, et al. Effectiveness of CoronaVac, ChAdOx1 nCoV-19, BNT162b2, and Ad26.COV2.S among individuals with previous SARS-CoV-2 infection in Brazil: a test-negative, case-control study. *Lancet Infect Dis*. 2022;22(6):791-801.

20. Hall V, Foulkes S, Insalata F, et al. Protection against SARS-CoV-2 after Covid-19 vaccination and previous infection. *N Engl J Med*. 2022;386(13):1207-1220.

21. Nordstrom P, Ballin M, Nordstrom A. Risk of SARS-CoV-2 reinfection and COVID-19 hospitalisation in individuals with natural and hybrid immunity: a retrospective, total population cohort study in Sweden. *Lancet Infect Dis*. 2022;22(6):781-790.

22. Medigeshi GR, Batra G, Murugesan DR, et al. Sub-optimal neutralisation of omicron (B.1.1.529) variant by antibodies induced by vaccine alone or SARS-CoV-2 Infection plus vaccine (hybrid immunity) post 6-months. *EBioMedicine*. 2022;78:103938.

23. Koerber N, Priller A, Yazici S, et al. Dynamics of spike-and nucleocapsid specific immunity during long-term follow-up and vaccination of SARS-CoV-2 convalescents. *Nat Commun*. 2022;13(1):153.

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TO THE EDITOR:

Pretransplant *FLT3*-ITD MRD assessed by high-sensitivity PCR-NGS determines posttransplant clinical outcome

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FMS-like tyrosine kinase-3 internal tandem duplication (*FLT3*-ITD) is identified in ~25% of patients with acute myeloid leukemia (AML), making it one of the most common variants identified in this disease.[1] Although frontline incorporation of the FLT3 inhibitor midostaurin has been shown to improve survival of patients with *FLT3* mutant AML, it is broadly accepted that allogeneic hematopoietic cell transplant (allo-HCT), performed in approximately 25% to 30% of patients in first remission, is a key contributor to enhanced outcomes.[2] Two studies have shown that detection of *FLT3*-ITD before transplant in morphologic remission using conventional fluorescence-based polymerase chain reaction (PCR) techniques with 1% to 5% sensitivity was associated with higher post-HCT relapse risk (39%-59%), compared with patients "negative" for *FLT3*-ITD (relapse risk 23%-41%).[3,4] Assessment of *FLT3*-ITD by capillary electrophoresis (CE)-based approaches, however, has low sensitivity (~1%), in contrast to high-coverage PCR and next-generation sequencing (NGS), which enables detection of *FLT3*-ITD with 100- to 1000-fold greater sensitivity.[5-7] By using NGS to assess *FLT3*-ITD with a limit of detection of 10^{-4} to 10^{-5}, the proportion of patients whose measurable residual disease (MRD) was negative after 2 cycles of intensive chemotherapy combined with midostaurin was 67%.[8] Currently, it is not known whether detection of *FLT3*-ITD clones below the sensitivity of CE-based methods has clinical relevance in forecasting post-HCT relapse risk, especially if myeloablative conditioning (MAC) is administered. One study incorporating peripheral blood (PB) detection of *FLT3*-ITD before MAC or reduced-intensity conditioning HCT found that 9 of 10 patients positive for *FLT3*-ITD MRD (variant allele frequency [VAF], 0.03%-3.97%) relapsed, compared with 1 of 7 patients relapsing if *FLT3*-ITD MRD was negative pre-HCT.[9]

We sought to evaluate the prognostic impact of MRD assessment by PCR-NGS to detect *FLT3*-ITD with high sensitivity prior to allo-HCT and to determine the added value of this approach compared with CE. The study was approved by Alfred Health, Peter MacCallum Cancer Centre, Royal Melbourne Hospital (181/21), and All-Wales (08/MRE09/29) ethics committees. All