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The U.S. Food and Drug Administration (FDA) encourages submission of suspected adverse events for drug and biologic products to the FDA Adverse Event Reporting System (FAERS) through MedWatch (www.fda.gov/medwatch). Due to reference limitations, the authors were unable to cite all literature reports submitted to FAERS describing red cell morphology abnormalities and/or hemolytic anemia following exposure to alectinib.

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COVID-19 vaccine response in patients with hematologic malignancy: A systematic review and meta-analysis

To the editor:

Hematologic malignancies encompass a group of heterogenous diseases with variable effects on immune function, and the degree of immune dysfunction may be further exacerbated by therapies used to treat specific diseases. Emerging real-world data suggest that patients with hematologic malignancy, particularly B-cell malignancies and those receiving B-cell depleting therapies, likely do not elicit robust immunologic responses following COVID-19 vaccination; however, existing studies include modest sample sizes and uncertainty remains. Thus, we performed a systematic review and meta-analysis aggregating data on anti-SARS-CoV-2 IgG antibody seroresponse (SR) to COVID-19 vaccination in patients with hematologic malignancy.

PubMed and EMBASE were searched from January 1, 2021 to November 4, 2021, to identify studies of vaccine immunogenicity following COVID-19 vaccination in patients with hematologic malignancy (Supplementary Methods; Tables S1 and S2; Figure S1). The primary outcomes were pooled SR in all studies, and pooled relative benefit ratio (RB) compared to controls in studies with a comparator group. Secondary outcomes were pooled SR and pooled RB by hematologic malignancy subtype and treatment status and type. Pooled estimates, RBs, along with 95% confidence intervals (CIs) were calculated using a random-effects model using MetaXL and Review Manager 5.4. Further details on data extraction and synthesis are provided in the Supplementary Methods.

We identified 2205 unique publications, of which 64 studies met inclusion criteria, comprising 8546 adult patients with hematological malignancy (Table S3). Full results are provided in Supplementary Results. Figure 1 provides a visual depiction of pooled SR and RB for outcomes. Pooled SR of all included patients was 59% (95% CI 55%–64%, with considerable heterogeneity I^2 95%; Figure S2). RB when compared to controls (either health care workers, healthy volunteers, or age-matched cancer-free controls) in available reports was 0.61 (95% CI 0.55–0.66, I^2 91%; Figure S3). SR varied according to hematologic malignancy subtype with better responses seen in myeloid malignancies (SR for myeloproliferative neoplasms [MPN] 81%, 95% CI 72%–89%; SR for myelodysplastic syndrome [MDS] 63%, 95% CI 47%–78%; SR for acute leukemias 83%, 95% CI 77%–89%), and lower responses observed in lymphoid malignancies (SR for chronic lymphocytic leukemia [CLL] 44%, 95% CI 35%–53%; SR for lymphoid malignancies excluding CLL 52%, 95% CI 44%–61%; SR for plasma cell dyscrasias [PCD] 72%, 95% CI 64%–79%) (Figures S9-S14). Patients with history of stem cell transplant (SCT) had good SR of 79% (95% CI 75–82), irrespective of allogeneic (78%) or autologous (88%) SCT (Figures S15). In contrast, patients with a history of chimeric antigen receptor T-cell (CAR-T) therapy had poor SR of 33% (95% CI 18–49) (Figures S16). RB from studies with comparators showed similar findings (Figures S17–S19).

Serologic responses were abrogated by cancer treatment, with SR in patients receiving treatment 47% (95% CI 36%–58%) compared to untreated SR 83% (95% CI 75%–90%), and RB of 0.38 (95% CI 0.25–0.58; Figure S4). This was particularly notable for prior anti-CD20 therapy with RB 0.16 (95% CI 0.08–0.30) when comparing receipt of anti-CD20 therapy <9–12 months of vaccination to >9–12 months after vaccination (Table S4 and Figure S5). Similarly, poor SR was observed in patients receiving novel targeted therapies Bruton tyrosine kinase inhibitor (BTKi) or venetoclax (37%, 95% CI 22%–54%) and anti-CD38 therapy (48%, 95% CI 27%–68%) (Figures S20 and S21).

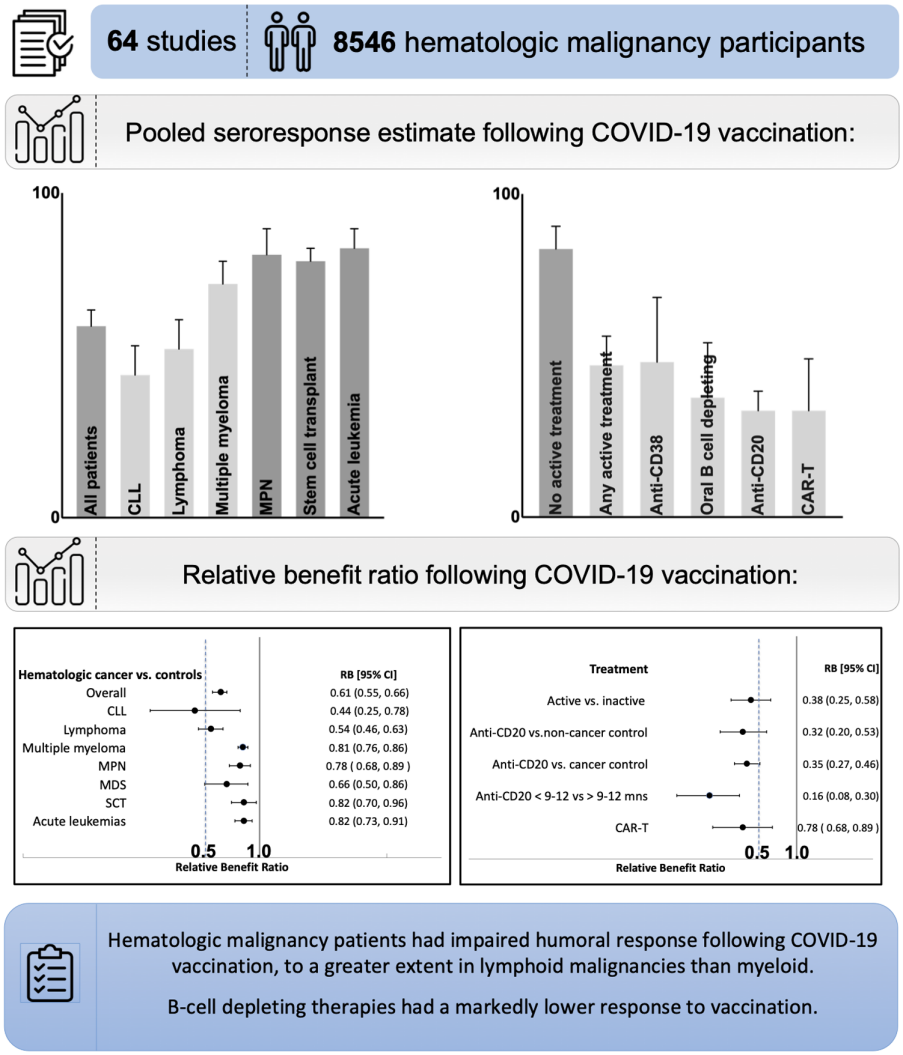

FIGURE 1 Visual depiction of pooled seroresponse (95% confidence intervals shown are error bars) and relative benefit ratios when compared to controls following COVID-19 vaccination in hematologic malignancy patients. Data are presented by hematologic malignancy subtype on the left and by treatment status and type on the right for both pooled response and relative benefit ratios. CLL, chronic lymphocytic leukemia; CAR-T, chimeric antigen receptor T cell therapy; MDS, myelodysplastic syndrome; MPN, myeloproliferative neoplasm; SCT, stem cell transplantation

There were 10 studies reporting on T-cell responses and conflicting results were observed. While some studies demonstrated concordance between reduced T-cell response and low antibody response, particularly in anti-CD20 treatment patients, others demonstrated the presence of T-cell responses in patients without antibody response (Table S5).

We identified three studies reporting on SR following booster (third) dose in hematologic malignancy patients. These studies demonstrated that a booster dose could achieve SR up to 55% of patients who were seronegative following initial vaccination series.[1]

Overall, our data support the rapidly emerging evidence demonstrating impaired humoral response following vaccination in hematologic malignancy patients. Patients with B-cell malignancies, particularly CLL and lymphoma, had the lowest SR. This likely reflects the disease-specific biology which causes underlying immune dysfunction in many patients as well as therapies used to treat lymphoid malignancies, including B-cell depleting therapies such as anti-CD20 and BTK inhibitors. Indeed, patients receiving treatment with B-cell depleting therapy had markedly impaired antibody responses compared to noncancer controls and disease patients, with reported SR ranging from 0% to 25% for those who received anti-CD20 within 3 months of vaccination. Response rates following COVID-19 vaccination improved with the passage of time, with higher serologic responses observed in those who received vaccination more than 9–12 months following anti-CD20 therapy. These findings are in keeping with prior studies suggesting that B-cell reconstitution following anti-CD20 antibody treatment requires 9–12 months.[2] Similarly, studies reporting on oral B-cell depleting therapies also observed impaired antibody response, with reported SR ranging from 14% to 57%. Importantly, seroconversion from seronegative to seropositive following booster vaccinations was evaluated in several studies, support the role of this strategy in achieving seroconversion. Furthermore, a study evaluating the kinetics of antibody titers demonstrated a rapid decline in titers from 36 days onward, and resulted in conversion from seropositive to seronegative in patients with hematologic malignancy while SR was conserved in patients with solid malignancies.[3] Collectively, these data support the use of booster doses to achieve optimal serologic response in patients with hematologic malignancy.

Although PCD can also suppress the immune system and affect normal B-cell function, patients with PCD had higher responses compared to lymphoma and CLL. However, studies showed conflicting results in terms of effect of antimyeloma therapy on serologic response. Several reports observed impaired antibody response with anti-CD38 therapy, anti-B-cell maturation antigen therapy, along with number of lines of therapy, while

other studies did not demonstrate a significant difference in SR when comparing treated versus untreated patients. We postulate that these conflicting results may be due to difficulty discerning specific treatment regimen effects on serological response in MM patients given that anti-myeloma therapy are usually given in combination incorporating several drug classes (immunomodulators, proteasome inhibitors, alkylating agents, steroids, and anti-CD38 therapy).

Patients with myeloid malignancies and their associated treatment such as tyrosine kinase inhibitors, did not have blunted SR. SCT recipients generally attained moderate to higher SR ranging from 50% to 89%, although studies suggest reduced SR within 1 year of transplantation (SR ranged between 20%–54% within 1 year of SCT vs. 80%–91% ≥1 year of SCT as reported from two studies included in this review). Albeit limited by sample size, CAR-T therapy was associated with very poor serologic response. For these patients, ASH and the American Society of Transplantation and Cellular Therapy advised that COVID-19 vaccines should be offered to patients 3 months or later following SCT and CART-T therapy.

There are several limitations to consider when interpreting results of this study. We observed significant heterogeneity in reported outcomes. This is likely due to several disease- and treatment-related factors including heterogeneous disease biology impacting on humoral and cellular immune system, disease status, and type of therapy received, particularly B-cell depleting therapies. To explore the heterogeneity, we conducted subgroup analysis based on hematologic malignancy subtype; heterogeneity was reduced for acute leukemia and SCT but remained high for other subtypes. Similarly, when analyzing data based on treatment status, heterogeneity only slightly reduced. As such, our pooled SR estimates should be interpreted with caution and highlight the need for larger and more robust studies. Another limitation is that most studies included in this systematic review measured SR by using anti-SARS-CoV-2 spike protein IgG, with only a small number of studies measuring neutralizing antibody response. Although neutralizing antibody response is the gold standard for humoral response, with higher levels inferring protection, recent studies demonstrate a high degree of correlation between neutralizing antibody titers and IgG antibodies in both convalescent and vaccinated individuals.[4] Furthermore, among fully vaccinated healthcare workers, breakthrough infections correlate with lower levels of both anti-spike IgG antibodies and neutralizing antibodies, compared to matched uninfected controls,[5] supporting the importance of serologic response in protective immunity against COVID-19. As outlined above, studies correlating humoral responses with T-cell responses showed conflicting results with some studies demonstrating concordance (double negativity) while others demonstrated presence of T-cell responses in patients without humoral response. As such, additional studies are needed to evaluate the relative importance of antibody and cellular responses to COVID-19 infection protection, and whether T-cell responses are sufficient to decrease severity of COVID-19 disease in those without humoral response.

In summary, in this meta-analysis aggregating SR following COVID-19 vaccination in patients with hematologic malignancy, the lowest response was observed in lymphoid malignancies, particularly those treated with anti-CD20 therapy, and other B-cell depleting therapies. Emerging data correlating neutralizing antibody response to anti-Sars-CoV-2 antibody levels and infection risk suggest that hematologic malignancy patients without adequate antibody levels remain at high risk of COVID-19 infection. Additional studies are urgently needed to determine whether immunologic response can be improved with tailored dosing and booster vaccination doses. Furthermore, therapies such as anti-COVID-19 monoclonal antibodies and convalescent serum should be evaluated in hematologic malignancy patients as prophylactic and treatment modalities, particularly for those unable to mount an immunologic response to vaccination.[6]

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CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Inna Y. Gong performed literature search, article selection, analysis, and manuscript writing; Abi Vijenthira performed article selection, and manuscript review and revision; Stephen D. Betschel reviewed and revised the manuscript; Lisa K. Hicks and Matthew C. Cheung conceived of the study, assisted with analysis, and reviewed and revised the manuscript.

DATA AVAILABILITY STATEMENT

All data are reported in the paper in figures or supplemental figures. Data tables are available from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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Sequential next generation sequencing analysis in homogeneously treated low risk NPM1-mutated acute myeloid leukemia with an adverse clinical outcome

To the Editor:

NPM1 is the most frequently mutated gene in adult acute myeloid leukemia (AML), defining a distinct leukemia entity of the 2016 World Health Organization classification. In the absence of *FLT3-ITD* or in the case of co-occurrent *FLT3-ITD* with a low allelic ratio (AR <0.5), de novo *NPM1*^{mut} AML is classified as a low-risk AML by the European Leukemia Net (ELN) guidelines.

However, a significant fraction of these patients eventually experiences a poor outcome, with a documented relapse rate up to 50%.[1] This heterogeneity of clinical outcome highlights the unmet clinical need of identifying patients at high risk of relapse and then defining personalized treatment strategy at diagnosis.

Aim of this study was to assess the presence of co-occurring mutations and their clonal evolution at relapse in de novo low-risk *NPM1*^{mut} and *FLT3-ITD*^{neg} AML, in order to gain insights into the molecular pathogenesis of relapse mechanisms, through the application of a deep targeted NGS protocol.

We performed the analysis at sequential time points in a cohort of intensively treated adult patients who experienced *NPM1*^{mut} relapse after the achievement of a complete response (CR). We focused on *FLT3-ITD*^{neg} because several reports pointed out the peculiar behavior of *NPM1* and *FLT3-ITD* mutated AML regardless of the ITD AR.[2]

Patients with a de novo low-risk *NPM1*^{mut} and *FLT3-ITD*^{wt} AML diagnosed and treated at Division of Hematology, Fondazione IRCCS Policlinico San Matteo, Pavia, Italy, were included in the study. This study was approved by the local Ethics Committee. Between 2010 and 2020, 54 de novo low-risk *NPM1*^{mut} *FLT3-ITD*^{neg} AML patients have been diagnosed at our institution (Figure S1). Clinical and biological characteristics of the cohort and disease outcome are summarized in Table S1. All patients received intensive chemotherapy with 3+7 regimen as per institutional standard. Response criteria defined by the ELN recommendations were applied. We analyzed paired samples at diagnosis, CR with molecular minimal residual disease (mMRD) persistence, CR without mMRD (CR_{MRD-}) and molecular or hematological relapse.

Bone marrow DNA of diagnosis and hematological relapse was analyzed by conventional NGS analysis with a custom TruSight Myeloid Sequencing Panel of 54 genes (Illumina, San Diego, CA) sequenced on a MiSeq instrument, as previously published (see Supplementary Methods) The average depth of coverage was 4.300×.

Bone marrow DNA of CR_{MRD-} and CR with mMRD and molecular relapse was analyzed by deep sequencing with the same gene panel run on a HiSeq2500 instrument. The average depth of coverage was 63.470× (median coverage of *NPM1* exon 11 was 34.000×, range 18.220–61.721×).

NPM1 mutation status was also tested in all samples by a standardized RNA-based assay (Muta*Quant*™ Kit, Ipsogen). According to manufacture's statements, the analytical (limit of detection) was determined on known low positive samples and found to be equal to 10.7 NPM1-A copies ($n = 52$ measures), 15.7 NPM1-B copies ($n = 36$ measures), and 10.2 NPM1-D copies ($n = 36$ measures), respectively, corresponding to approximately 0.009 NPM1-A copies, 0.008 NPM1-B copies, and 0.008 NPM1-D copies normalized to 100 ABL copies. A sample was considered MRD positive when 2 of 3 replicates had Ct <40.

For mutation profiling details and statistical analysis see Supplementary Material and Table S2.

After a median follow-up of 34 months (interquartile range [IQR] 9–60), the median overall survival was 43.4 months (IQR 10–not reached) and the median relapse-free survival was 22 months (IQR 8–90). A total of 24 patients (50%) eventually relapsed (20 hematological and 4 molecular relapses), the observed relapse incidence is higher