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Letter

Efficacy of booster doses in augmenting waning immune responses to COVID-19 vaccine in patients with cancer

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We and others have shown the significant impact of COVID-19 infection among patients with a cancer diagnosis, with increased morbidity and mortality with advanced age, co-morbidities, and hematologic malignancies receiving highly immunosuppressive therapies (Mehta et al., 2020). Subsequently, several studies have demonstrated that, following standard COVID-19 vaccinations, most patients with solid tumors develop robust anti-viral immunity as measured by anti-spike IgG antibodies (Addeo et al., 2021; Thakkar et al., 2021). Approximately 20% of patients with hematologic malignancies, however, develop lower seroconversion rates, furthermore patients who had received anti-CD20 antibodies, CAR T cell therapy, and stem cell transplantation (SCT) had the lowest rates post-vaccination (Greenberger et al., 2021a; Thakkar et al., 2021). Since our initial report, there has been increasing interest in waning immunity (Levin et al., 2021), as evident from decreasing IgG levels as well as with reports of breakthrough infections (Mittelman et al., 2021). In August 2021, the FDA authorized "booster" shots (now preferentially called third or additional vaccine doses) for patients who are immunosuppressed. A randomized study demonstrated significant efficacy of such booster vaccinations in solid organ transplant patients (Hall et al., 2021). In addition, population datasets from Israel, where booster dosing has been made available early, highlight both the serological and clinical impact of booster vaccinations for the overall population (Barda et al., 2021). Of particular concern for the vulnerable cancer patient population, a recent small observational study revealed that some patients with hematologic malignancies who were seronegative after a full course of vaccination may benefit from booster vaccination (Greenberger et al., 2021b).

In our current study, we provide follow-up on our original vaccinated cohort with patients who consented for further assessment of anti-COVID-19 immunity (follow-up immunity cohort) and also present data from a single-arm clinical trial where we assessed anti-COVID-19 immunity before and after a "booster" vaccine in patients with a cancer diagnosis (booster vaccine cohort). The primary endpoint was to assess the rate of booster-induced seroconversion among patients who remained seronegative at least 28 days following the standard set of FDA-authorized COVID-19 vaccinations.

Patients who were included in our COVID-19 vaccine studies (Thakkar et al., 2021; Shapiro et al., 2021), who were seen in follow-up during the current study period, were offered a follow-up SARS-CoV-2 spike IgG level 4–6 months after the completion of their primary vaccine series. One hundred and twenty-three patients underwent repeat anti-SARS-CoV-2 spike antibody (S) testing. Of these, 24 patients that were not part of the initial analysis were excluded, and 99 patients were analyzed (Table S1A). Table S1B summarizes the baseline characteristics of this follow-up cohort. No breakthrough COVID-19 infections were reported between completion of the primary vaccine series and follow-up testing. Overall, the initial median anti-S IgG titer was 5,162 AU/mL (mean 14,634, range 50–50,000) after completion of vaccine series and 724.6 AU/mL (mean 6,220, range 50–50,000) at 4–6 months of follow-up ($p < 0.001$). We observed that the majority of patients (34/36, 94% hematologic malignancies; and 55/55, 100% with solid tumors) maintained detectable anti-S IgG titers >50 AU/mL at 4–6 months (Figure S1A). Two patients with hematologic malignancies (multiple myeloma and AL amyloidosis) did not have detectable antibodies at 4–6 month follow-up. Albeit small numbers in the adenoviral vaccine cohort, we observed that patients that had received mRNA vaccination series had a steeper decline in antibody titers compared to those that had received the adenoviral vaccine ($p = 0.03099$) (Figure S1B). The mean change (Δ) for BNT162b2 vaccine was −7,496 AU/mL, whereas the Δ for mRNA-1273 and Ad26.CoV2.S were −11,639 AU/mL and −3,326 AU/mL, respectively.

One hundred and eighty-nine patients with a cancer diagnosis were assessed for enrollment to receive a booster COVID-19 vaccine after at least 28 days following completion of a standard COVID-19 vaccination series. While our study initially offered the BNT126b2 vaccine on study, following FDA/CDC authorization of booster dosing for immunocompromised patients, patients were also permitted to receive the mRNA-1273 vaccine. One hundred and thirty-one patients

met inclusion criteria (Table S1A) and were enrolled in the study via informed-consent process. A cohort of 88 patients underwent anti-S IgG testing pre- and at 4 weeks post-booster vaccination by our analysis cutoff date and are included in the efficacy analysis. The key cohort of seronegative patients also had anti-SARS-CoV-2 T cell response testing pre- and post-booster. The median age of our cohort was 69 years (range 30–91). Fifty-seven patients (65%) had a hematologic malignancy, while 31 patients (35%) had a solid tumor diagnosis (Table S1B). Sixty-four patients (73%) were on active cancer treatment at the time of booster vaccination. Sixty-two patients (70%) received BNT162b2, 22 patients (25%) mRNA-1273, and 4 patients (5%) AD26.COV2.S vaccination prior to booster vaccination, with a median time since last vaccination of 177 days. All patients received a booster vaccination with the vaccine type received at baseline except 8 patients (2 patients received a heterologous BNT162b2, 2 patients a heterologous mRNA-1273, and all 4 AD26.COV2.S patients received heterologous BNT162b2 booster vaccinations).

Among the total 88 patients who received booster vaccinations, 56 patients (64%) were seropositive prior to booster vaccination, and 32 patients (36%) seronegative. Of the 32 seronegative patients, all had hematologic malignancies except for one patient (Table S1B). Our study met its primary endpoint with 18/32 (56%) seronegative patients seroconverting anti-S IgG titers after booster vaccination ($p = 0.000062$) with 14 patients (44%) remaining seronegative. In our cohort, the overall immunogenicity of booster vaccination was affected by disease type with hematologic malignancies having both a statistically significant lower pre-booster antibody response as well as a smaller change in anti-S IgG mean titers post-booster as compared to solid tumors (10,034 versus 22,686 AU/mL, $p = 0.00263$) (Figure S1C). Despite the majority of patients (73%) being on active therapy at the time of booster, even those patients who received therapy within 30 days of booster vaccination had a statistically significant chance for seroconversion ($p = 0.02$). Prior therapy with either a Bruton Tyrosine Kinase inhibitor (BTKi) or anti-CD20 therapy (or both) was also statistically significant for a decrease in both pre- and post-booster antibody seroconversion ($p = 0.01333$) and titer ($p = 0.0000575$). Those patients who received anti-CD20 therapy within 6 months of booster vaccination (Figure S1D) were especially at high risk for reduced seroconversion ($p = 0.04566$).

As most patients in our cohort received BNT162b2 boosters, we were not powered to uncover significant differences in post-vaccination titers between vaccine types, although surprisingly there appeared to be quantitatively higher mean titers after booster vaccination with initial mRNA-1273 or Ad26.CoV2.S vaccination (25,523 and 23,141 AU/mL) as compared to BNT162b2 vaccination (14,829 AU/mL) as well as higher mean titers after mRNA-1273 booster as compared to BNT162b2 (23,948 versus 15,858 AU/mL) (Figure S1E).

Our study cohort also included a subset of patients with known prior COVID-19 infection ($n = 7$). These patients, as anticipated, showed more robust vaccine responses both after standard and booster vaccinations (Figure S1F). Lastly, our study included a unique cohort of patients ($n = 28$) who were tested for anti-S IgG titers at post-initial vaccination, pre-booster and post-booster time points. This representative cohort highlights significant waning of anti-COVID-19 immunity 4–6 months post-vaccination that can be rescued to above pre-vaccination titers after booster vaccination (Figure S1G), suggesting benefit to booster vaccination in the majority of patients with cancer.

Of the patients remaining seronegative after the booster, all had B cell malignancies: 6 patients had chronic lymphocytic leukemia (CLL), 3 patients had Waldenstrom's macroglobulinemia (WM), 2 patients had multiple myeloma (MM), 1 patient had diffuse large B cell lymphoma (DLBCL), and 1 patient each had Mantle Cell and Marginal Zone lymphoma (Table S1C). Of the 32 seronegative patients prior to booster vaccination, 27 patients (84%) had evaluable anti-SARS-CoV-2 T cell response assays at baseline. Of these 27 patients, 20 (63%) had detectable anti-SARS-CoV-2 T cell responses prior to booster vaccination despite a negative antibody response (median 577 mIU/mL, range 133 to >1,800) (Figure S1H). Of the 14 patients who remained seronegative post-booster vaccination, 10 (71%) had evaluable anti-SARS-CoV-2 T cell responses post-vaccination, with 8 patients (80%) having detectable anti-SARS-CoV-2 T cell responses (median 1,146 mIU/mL, range 1,193 to >1,800), only one of which had no baseline detectable T cell response. For those that remained seronegative after booster vaccination, 57% (8/14) were on active therapy at time of booster, with one CLL patient never having received prior therapy. Within the seronegative cohort alone, significantly lower seroconversion rates were seen in those patients treated with prior or current anti-CD20 therapies ($p = 0.042$), with a median time since last anti-CD20 therapy of 3.9 months. Other common prior or current therapies received included cytotoxic chemotherapy (10/14), BTKi (6/14), CAR T therapy (2/14), and autologous SCT (2/14), although sample size likely limits further conclusions.

In conclusion, our results suggest excellent potentiation of anti-COVID-19 immunity with additional dosing of COVID-19 vaccine in patients with cancer.

Even more importantly, our results clearly show a high, more than 50% seroconversion rate along with corresponding stimulation of measurable anti-SARS-CoV-2 T cell activity among the most vulnerable patient cohort, i.e., patients with no detectable immunity following standard vaccinations, calling for broad efforts to provide third vaccinations to such patients. However, our results also demonstrate that some patients will not have a serological immune response to a third mRNA vaccine dose, highlighting the need for continued efforts to develop valid laboratory correlates of anti-COVID-19 immunity and specific studies assessing the potential benefit of subsequent homologous vaccine doses, heterologous vaccinations, passive immunizations, and other unique approaches for these patients.

In addition, our study finds significant waning of anti-COVID-19 immunity over 4–6 months post-standard vaccination as measured by SARS-CoV-2 spike IgG titers among patients with a cancer diagnosis.

While waning antibody titers are not necessarily associated with risk or severity of breakthrough infections, population-based studies in the case of COVID-19 do suggest such association.

Of particular concern is our novel finding of complete loss of detectable immunity in some patients, in particular patients with lymphoid malignancies and especially those on anti-CD20 and BTKi therapies. These data provide further impetus for additional dosing alongside passive immunization strategies as well as other research efforts for this vulnerable cohort.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.ccell.2021.11.006.

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AUTHOR CONTRIBUTIONS

L.C.S., A.T., A.V., and B.H. conceived and managed the study; J.D.G.-L., R.Q., M.M., R.A.S., M.G., S.G., J.D.A.M., D.L., A.F., A.P.S., C.S., G.A., and L.G. participated in patient recruitment; L.C.S., A.T., J.D.G.-L., R.Q., C.S., and G.A participated in data curation; T.D.B. and G.S.C. participated in laboratory investigations; K.P. oversaw data analyses; A.R. contributed to project administration; S.C., S.F., and L.W. oversaw investigations. All authors contributed to writing the manuscript.

DECLARATION OF INTERESTS

R.A.S. serves as a consultant with Morphosys and Miragen and is on the faculty at Physicians' Education Research. A.V. has received research funding from GlaxoSmithKline, BMS, Janssen, Incyte, MedPacto, Celgene, Novartis, Curis, Prelude, and Eli Lilly and Company; has received compensation as a scientific advisor to Novartis, Stelexis Therapeutics, Acceleron Pharma, and Celgene; and has equity ownership in Stelexis Therapeutics. All other authors declare no competing interests.

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Supplemental information

Efficacy of booster doses

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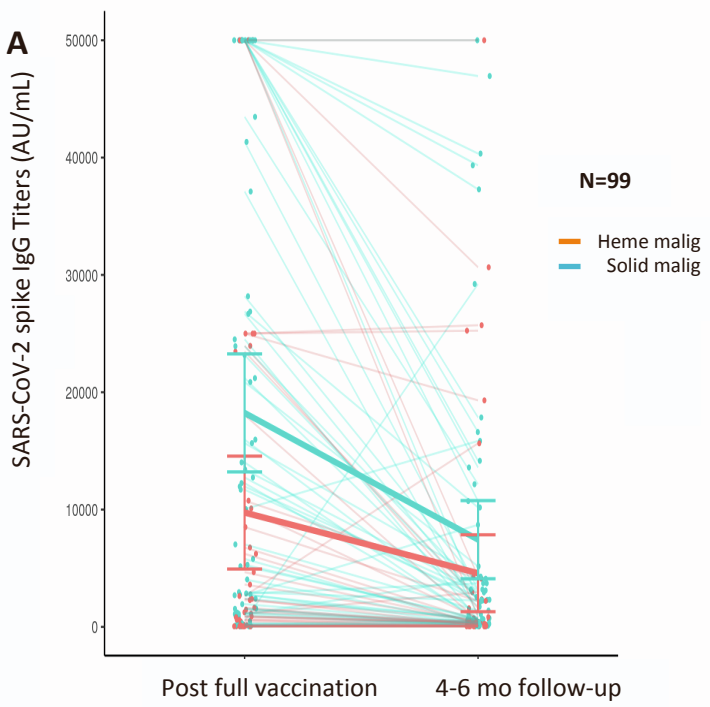
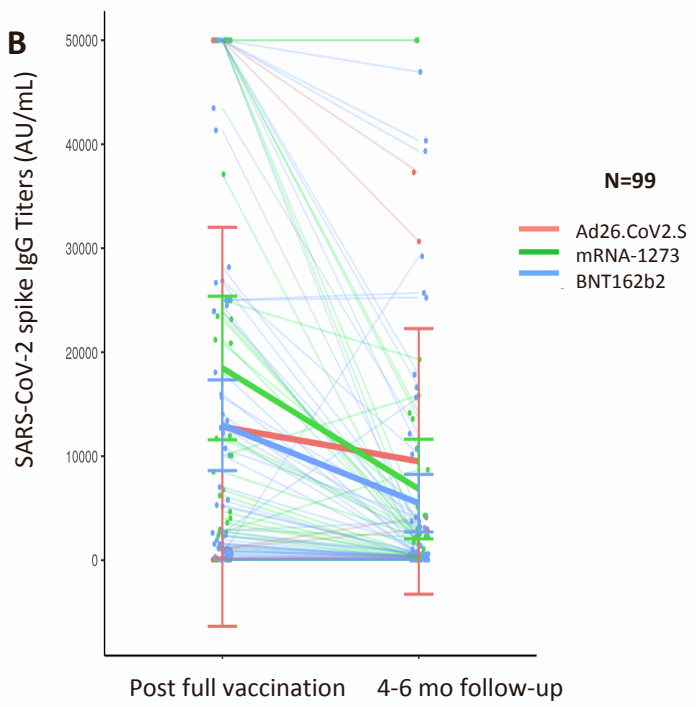
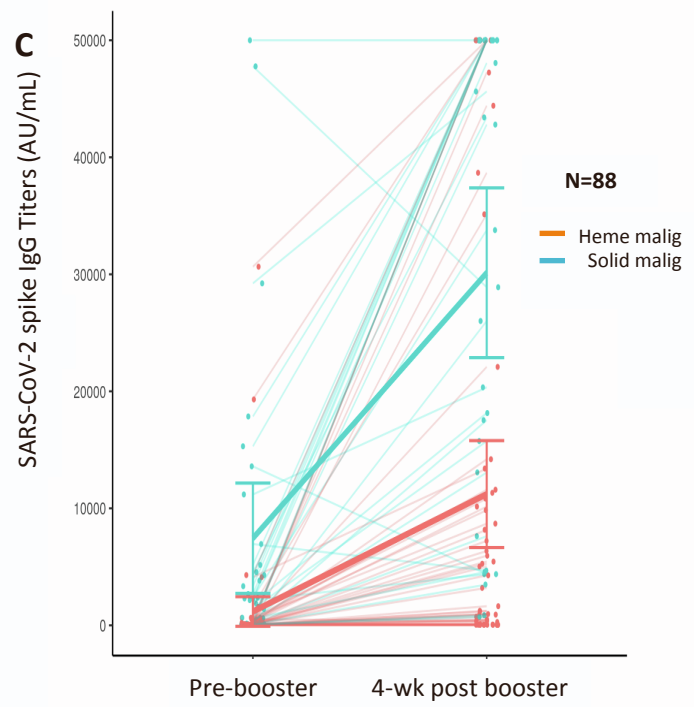

A
SARS-CoV-2 spike IgG Titers (AU/mL)
50000
40000
30000
20000
10000
0
N=99
Heme malig
Solid malig
Post full vaccination
4-6 mo follow-up


B
SARS-CoV-2 spike IgG Titers (AU/mL)
50000
40000
30000
20000
10000
0
N=99
Ad26.CoV2.S
mRNA-1273
BNT162b2
Post full vaccination
4-6 mo follow-up


C
SARS-CoV-2 spike IgG Titers (AU/mL)
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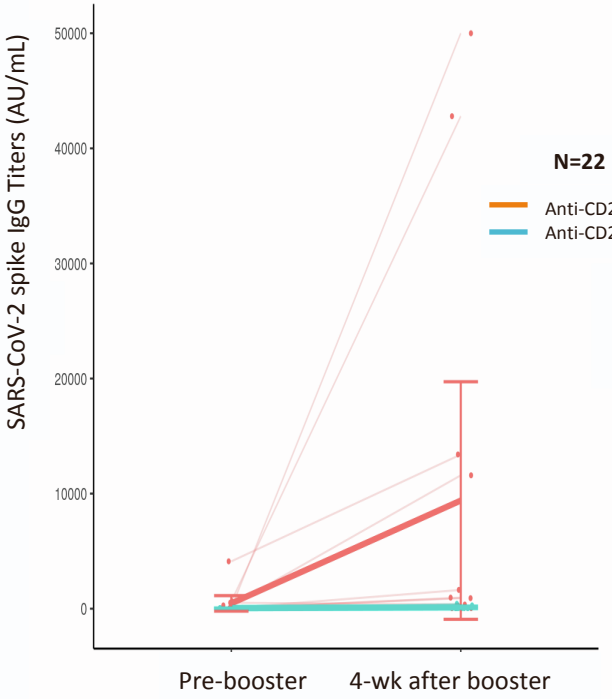
40000
30000
20000
10000
0
N=88
Heme malig
Solid malig
Pre-booster
4-wk post booster


D
SARS-CoV-2 spike IgG Titers (AU/mL)
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30000
20000
10000
0
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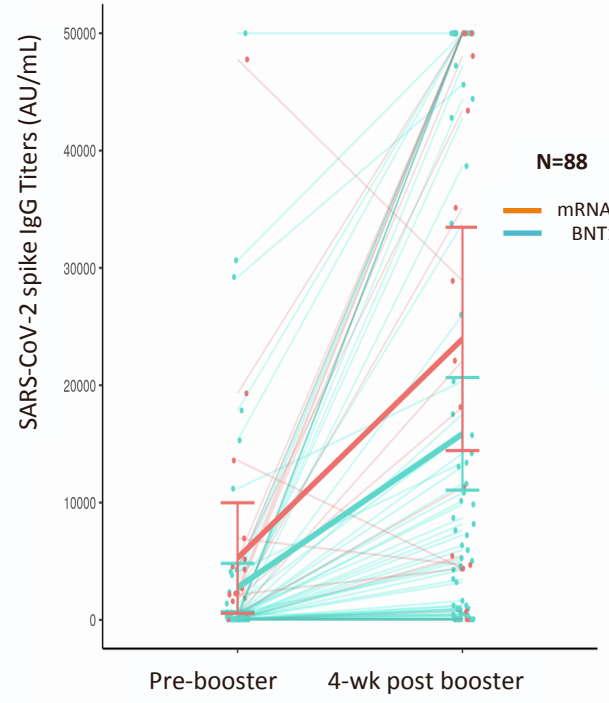
Anti-CD20 >6 mo
Anti-CD20 <6 mo
Pre-booster
4-wk after booster


E
SARS-CoV-2 spike IgG Titers (AU/mL)
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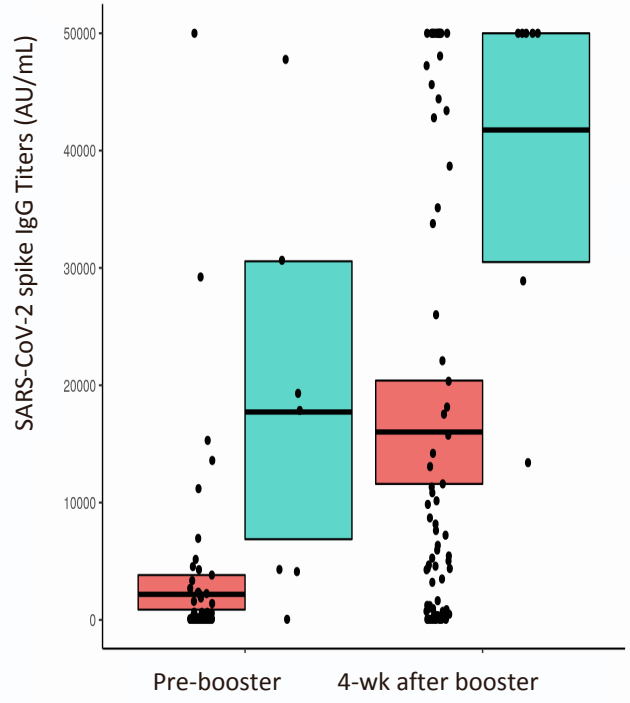
40000
30000
20000
10000
0
N=88
mRNA-1273
BNT162b2
Pre-booster
4-wk post booster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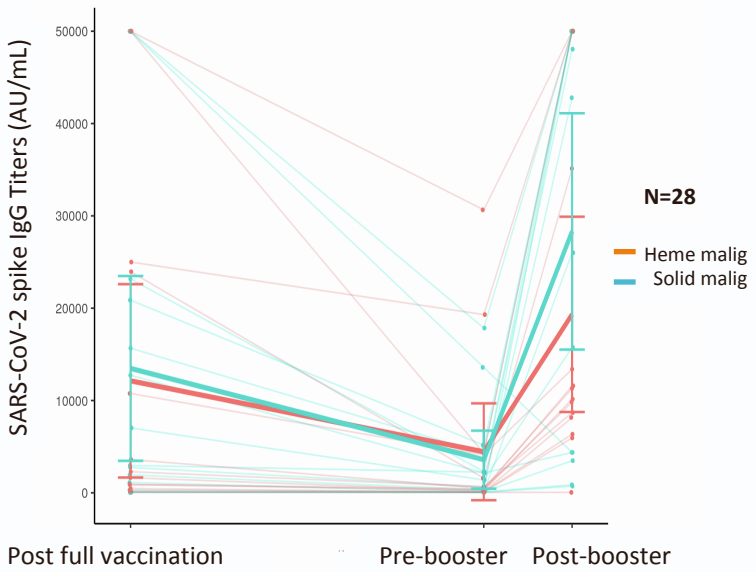
No COVID
Prior COVID
Pre-booster
4-wk after booster


G
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Heme malig
Solid malig
Post full vaccination
Pre-booster
Post-booster

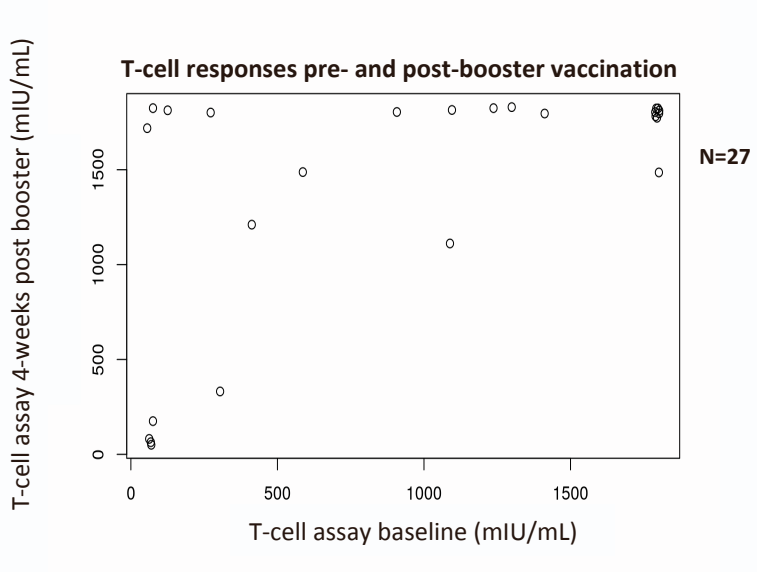
H
T-cell responses pre- and post-booster vaccination
T-cell assay 4-weeks post booster (mIU/mL)
1500
1000
500
0
N=27
0
500
1000
1500
T-cell assay baseline (mIU/mL)

Fig S1. Dynamics of immune responses against SARS-CoV-2 after standard and booster vaccination in patients with cancer.

A: Plot showing waning immunity over time in 99 patients with solid (mean after standard vaccination = 18239 AU/mL, 13210-23269; mean after 4-6 month follow-up = 7434 AU/mL, 4104-10763) and hematologic cancer (mean after standard vaccination = 9742 AU/mL, 4929-14554; mean after 4-6 month follow-up = 4573 AU/mL, 1295-7851). Mean values are denoted with solid thicker lines with error bars denoting 95% confidence interval (p=0.009151, Kruskal Wallis test).

B: Plot showing waning immunity over time in 99 patients with mean values for types of vaccines denoted with solid thicker lines with error bars denoting 95% confidence interval. Mean for BNT162b2 after standard vaccination = 12979 AU/mL, 95% CI 8617-17340; mean for BNT162b2 after 4-6 month follow-up = 5483 AU/mL, 95% CI 2713-8252. Mean for mRNA-1273 after standard vaccination = 18483 AU/mL, 95% CI 11577-25389; mean for mRNA-1273 after 4-6 month follow-up = 6844 AU/mL, 95% CI 2053-11365. Mean for Ad26.CoV2.S after standard vaccination = 12828 AU/mL, 95% CI -6356-32013; mean for Ad26.CoV2.S after 4-6 month follow-up = 9503, 95% CI -3269-22274 (p=0.03099, Kruskal Wallis test).

C: Plot showing increase in anti-SARS-CoV-2 spike (S) antibody titers at 4 weeks after the booster vaccine in 88 patients with solid (mean prior to booster vaccination = 7446 AU/mL, 95% CI 2732-12159; mean after booster vaccination = 30132 AU/mL, 95% CI 22877-37386) and hematologic cancer (mean prior to booster vaccination = 1183 AU/mL, 95% CI -81.83-2448; mean after booster vaccination = 11217 AU/mL, 95% CI 6649-15786). Mean values are denoted with solid thicker lines with error bars denoting 95% confidence interval (p=0.00263, Kruskal Wallis test).

D: Plot showing increase in anti-S antibody titers at 4 weeks after the booster vaccine. Mean values for hematologic malignancy patients who received anti-CD20 antibodies within 6 months (mean prior to booster vaccination = 51.84 AU/mL, 95% CI 47.59-56.1; mean after booster vaccination = 118.4, 95% CI 12.82-223.9) of booster vaccine or not (mean prior to booster vaccination = 462.6 AU/mL, 95% CI -211.8-1137; mean after booster vaccination = 9401 AU/mL, 95% CI -920.6–19723) are denoted with solid thicker lines with error bars denoting 95% confidence interval (p=0.04566, Kruskal Wallis test).

E: Plot showing increase in anti-S antibody titers at 4 weeks after the booster vaccine. Mean values for type of booster vaccine, BNT162b2 (mean prior to booster vaccination = 2762 AU/mL, 95% CI 707.4-4816; mean after booster vaccination = 15858 AU/mL, 95% CI 11060-20656) and mRNA-1273 (mean prior to booster vaccination = 5272 AU/mL, 95% CI 561.2-9983; mean after booster vaccination = 23948 AU/mL, 95% CI 14429-33468) are denoted with solid thicker lines with error bars denoting 95% confidence interval (p=0.6, Kruskal Wallis test).

F: Box plot showing increase in anti-S antibody titers at 4 weeks after the booster vaccine with mean values in patients that had prior SARS-CoV-2 infection (mean prior to booster vaccination = 17719 AU/mL, 95% CI 1923-33515; mean after booster vaccination = 41757 AU/mL, 95% CI 28095-55418) or not (mean prior to booster vaccination = 2177 AU/mL, 95% CI 654.7-3700; mean after booster vaccination = 16014 AU/mL, 11705-20323) denoted with solid thicker lines with error bars denoting 95% confidence interval (p=0.14, Kruskal Wallis test).

G: Plot showing trend in titers for patients (n=28) that had anti-S antibody titers at 3 time-points: 1) Post full vaccination 2) 4-6 month follow-up and 3) 4-weeks post-booster vaccine. Mean values are denoted with solid thicker lines with error bars denoting 95% confidence interval.

H: Plot showing T cell assay results at baseline and 4 weeks after booster vaccination in evaluable pre-booster seronegative cohort.

Abbreviations: 4-wk: 4 week, malig: malignancy, mo: months

Supplemental Table S1. Consort Diagram and Patient Characteristics Prior to SARS-CoV-2 Booster Vaccination.

Supplemental Methods

Anti-SARS-CoV-2 spike (S) IgG assay

The AdviseDx SARS-CoV-2 IgG II assay was used for the assessment of anti-S IgG antibody. AdviseDx is an automated, two-step chemiluminescent immunoassay performed on the Abbott i1000SR instrument. The assay is designed to detect IgG antibodies directed against the receptor binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2. The RBD is a portion of the S1 subunit of the viral spike protein and has a high affinity for the angiotensin converting enzyme 2 (ACE2) receptor on the cellular membrane (Pillay, 2020 ;Yang et al., 2020). The procedure, in brief, is as follows. Patient serum containing IgG antibodies directed against the RBD is bound to microparticles coated with SARS-CoV-2 antigen. The mixture is then washed of unbound IgG and anti-human IgG, acridinium-labeled, secondary antibody is added and incubated. Following another wash, sodium hydroxide is added and the acridinium undergoes an oxidative reaction, which releases light energy which is detected by the instrument and expressed as relative light units (RLU). There is a direct relationship between the amount of anti-spike IgG antibody and the RLU detected by the system optics. The RLU values are fit to a logistic curve which was used to calibrate the instrument and expresses results as a concentration in AU/mL (arbitrary units/milliliter). This assay recently has shown high sensitivity (100%) and positive percent agreement with other platforms including a surrogate neutralization assay (Bradley et al., 2021) and also demonstrated high specificity both in the post COVID-19 infection and post vaccination settings. The cutoff value for this assay is 50 AU/mL with <50 AU/ml values reported as negative and the maximum value is 50000 AU/mL.

SARS-CoV-2 Interferon Gamma Release Assay

The Euroimmun SARS-COV-2 Interferon Gamma Release Assay (Quan-T-Cell SARS-CoV-2) was used for the assessment of patient's T-cell response to SARS-CoV-2 antigens before and after the third vaccine dose, through analysis of the production of interferon gamma by patient T-cells after exposure to SARS-CoV-2 specific proteins. The assay does not differentiate between vaccine- or infection-induced T-cell responses. The SARS-CoV-2 IGRA assay is performed in two steps as per manufacturer instructions, and a brief protocol follows. First, patient samples from lithium heparin vacutainers are aliquoted into three separate tubes each. These tubes contain either nothing ('blank'), general T-cell activating proteins ('mitogen'), or components of the S1 domain of SARS-CoV-2 ('SARS-CoV-2 activated'). These samples were incubated at 37 degrees for 24 hours before being centrifuged and the plasma separated and frozen at -80 degrees for later analysis. Samples were then batched to be run as a full 96 well plate along with calibrators and controls. Paired samples from a single patient before and after a booster dose were run together on a single plate in order to lower possible analytical variability between plates. Plasma samples were unfrozen and added to an ELISA plate, which was prepared with monoclonal interferon-gamma binding antibodies, along with calibrators and controls. After incubation at room temperature the plate was washed and biotin-labeled anti-interferon gamma antibody was added to bind the patient interferon gamma bound to the plate. The plate was again incubated before being washed of excess antibody and a streptavidin-bound horseradish peroxidase (HRP) enzyme added, which binds strongly to the biotin-labeled antibodies present. This was again incubated and then washed of excess enzyme before a solution of H_2O_2 and TMB (3,3', 5,5;-tetramethylbenzidine, a peroxide-reactive chromogen) is added and allowed to react in the dark for 20 minutes. The reaction is then stopped through the addition of sulfuric acid and the results read at 450 nM with background subtraction at 650 nM. Results

for controls and samples were quantified by the calibration curve generated on the same plate, and results were interpreted as long as controls were within the pre-specified range. Blank results for each specimen set were subtracted from each tube in the set and the mIU/mL for both the mitogen and SARS-CoV-2 activated samples were determined with the calibration curve. Samples with mitogen results below 400 mIU/mL were considered 'invalid', as the overall T-cell activity for that set was too low, and excluded from analysis. All other sample sets were interpreted as per manufacturer's instructions based on the SARS-CoV-2 activated sample results: less than 100 mIU/mL were denoted as negative, and greater than or equal to 100 mIU/mL were denoted as positive.

Statistical Analysis

The primary endpoint of this study was to assess the rate of booster induced seroconversion amongst patients who remained seronegative at least 28 days following standard set of FDA authorized COVID-19 vaccinations. We hypothesized that booster dosing would convert at least 30% of the enrolled seronegative patients to seropositive as defined by our institutional CLIA certified SARS-CoV-2 spike IgG assay (as compared to 10% as our null hypothesis). In a pre-specified analysis, at least 26 evaluable seronegative patients were required to have sufficient power to be able to reach this assessment. A McNemar's test was used to determine the equality of marginal frequencies for paired nominal data with the aid of a homogeneity of stratum effects (HSE ref1) test to check if the effect was the same across all levels of a stratifying variable (Zhao et al., 2014). A Wilcox test was used to determine if titers of two paired observations have changed over time subsequently using a Kruskal Wallis test to determine if this difference is associated with another variable. An alpha < 0.05 was considered statistically significant. All analysis was performed in R (version 3.6.2). This study was approved by The Albert Einstein College of Medicine Institutional Board Review.

Supplemental References

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