Humoral immunogenicity of the seasonal influenza vaccine before and after CAR-T-cell therapy

Running head: Vaccination before and after CAR-T-cells

Authors: Carla S. Walti, MD[1], Andrea N. Loes, PhD[2,3], Kiel Shuey, MS[4], Elizabeth M. Krantz, MS[1], Jim Boonyaratanakornkit, MD, PhD[1,4], Jacob Keane-Candib, BS[1],Tillie Loeffelholz, BS[1], Caitlin R. Wolf, BS[4], Justin J. Taylor, PhD[1], Rebecca A. Gardner, MD[5,6,7], Damian J. Green, MD[4,5], Andrew J. Cowan, MD[4,5], David G. Maloney, MD, PhD[4,5], Cameron J. Turtle, MBBS, PhD[4,5], Steven A. Pergam, MD, MPH[1,4,5], Helen Y. Chu, MD, MPH[4], Jesse D. Bloom, PhD[2,3,8,9], and Joshua A. Hill, MD[1,4,5]

Affiliations:
[1]Vaccine and Infectious Disease Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.
[2]Basic Science Division and Computational Biology Program, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.
[3]Howard Hughes Medical Institute, Seattle, WA, USA.
[4]Department of Medicine, University of Washington, Seattle, WA, USA.
[5]Clinical Research Division and Immunotherapy Integrated Research Center, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.
[6]Seattle Children's Hospital, Seattle, WA, USA.
[7]Department of Pediatrics, University of Washington, Seattle, WA, USA.
[8]Public Health Science Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA.
[9]Department of Genome Sciences, University of Washington, Seattle, WA, USA.

Contact information for corresponding author
Joshua A. Hill, MD
Fred Hutchinson Cancer Research Center
1100 Fairview Ave N. E4-100, Seattle, WA 98109-1024, USA
Email: jahill3@fredhutch.org

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Key Points

- Influenza vaccination was immunogenic pre- and post-CAR-T-cell therapy, despite hypogammaglobulinemia and B-cell aplasia.
- Vaccination with inactivated vaccines can be considered before CAR-T-cell therapy and in individuals with remission after therapy.

ABSTRACT

Recipients of chimeric antigen receptor-modified T (CAR-T) cell therapies for B-cell malignancies are immunocompromised and at risk for serious infections. Vaccine immunogenicity is unknown in this population. We conducted a prospective observational study of the humoral immunogenicity of 2019-2020 inactivated influenza vaccines (IIV) in children and adults immediately prior to (n=7) or 13-57 months after (n=15) CD19-, CD20-, or BCMA-targeted CAR-T-cell therapy, as well as controls (n=8). Individuals post-CAR-T-cell therapy were in remission. We tested for antibodies to 4 vaccine strains at baseline and ≥1 time point after IIV using neutralization and hemagglutination inhibition assays. An antibody response was defined as a ≥4-fold titer increase from baseline at the first post-vaccine time point. Baseline A(H1N1) titers in the CAR-T cohorts were significantly lower compared to controls. Antibody responses to ≥1 vaccine strain occurred in 2 (29%) individuals before CAR-T-cell therapy; one individual maintained a response for >3 months post-CAR-T-cell therapy. Antibody responses to ≥1 vaccine strain occurred in 6 (40%) individuals vaccinated after CAR-T-cell therapy. An additional 2 (29%) and 6 (40%) individuals had ≥2-fold increases (at any time) in the pre- and post-CAR-T cohorts, respectively. There were no identified clinical or immunologic predictors of antibody responses. Neither severe hypogammaglobulinemia nor B-cell aplasia precluded antibody responses. These data support consideration for vaccination before and after CAR-T-cell therapy for influenza and other relevant pathogens such as SARS-CoV-2, irrespective of hypogammaglobulinemia or B-cell aplasia. Larger studies are needed to determine correlates of vaccine immunogenicity and durability in CAR-T-cell therapy recipients.

INTRODUCTION

The development and approval of chimeric antigen receptor-modified T (CAR-T) cell therapies for lymphoma, leukemia, and multiple myeloma (MM) is leading to wider-scale use in children and adults.[1–3] These individuals are profoundly immunocompromised from their underlying malignancy and prior anti-tumor treatments, in addition to CAR-T-cell therapy related factors including lymphodepleting chemotherapy and cytokine release syndrome (CRS).[4] Severe and often persistent cytopenias occur in part due to “on-target/off-tumor” depletion of non-malignant B-lineage cells expressing the CAR-T-cell targets.[1–7]

Strategies to prevent infections after CAR-T-cell therapy are not well established. Many patients are treated with prophylactic immunoglobulin replacement therapy (IGRT), which consists of pooled immunoglobulin G (IgG) isolated from blood from over 1,000 donors.[8] However, there is limited evidence to support the efficacy of prophylactic IGRT in this context, and IGRT is primarily beneficial for prevention of only serious bacterial infections.[9] Vaccination is a potentially more cost-effective and durable approach to infection prevention for some pathogens, but there are no published data regarding vaccine immunogenicity in CAR-T-cell therapy recipients. Vaccine immunogenicity, while often lower in immunocompromised patients compared to healthy individuals, is often nonetheless beneficial. For example, influenza vaccination in immunocompromised patients may be associated with lower rates of influenza infection and lower respiratory tract disease, a reduction in hospitalization, and lower mortality.[10,11]

Understanding vaccine immunogenicity in the context of CAR-T cell therapy is critically important to guide infection prevention strategies. These data are particularly relevant given the availability of vaccines for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Vaccination before treatment, as is preferred in solid organ transplant recipients,[12] may be particularly important as the B-cell depletion that results from CAR-T-cell therapy may further

abrogate immunogenicity. Vaccination starting 3-6 months after CAR-T-cell therapy is advocated by current guidelines,[13,14] but the recommendation is extrapolated from other patient populations and treatments.[15–20]

Respiratory tract infections, particularly with viruses, are the most common infectious complication after CAR-T-cell therapy, and influenza has been reported as a cause of death.[20–23] Among patients with cancer and hematopoietic cell transplant (HCT) recipients, influenza causes substantial morbidity and mortality with death occurring in 11% to 33% of affected individuals.[11] Thus, there is an urgent need to understand the specific utility of influenza vaccination prior to and after CAR-T cell therapy, and to inform the broader question of vaccine immunogenicity in this patient population.

We report the results of a prospective observational study of the humoral immunogenicity of the seasonal inactivated influenza vaccine (IIV) among CD19-, CD20-, and BCMA-targeted CAR-T-cell therapy recipients vaccinated before or after CAR-T-cell therapy compared to controls.

METHODS

Study design and participants

We enrolled 3 distinct cohorts in the fall and winter of 2019-2020. We approached all children and adults planning to receive an IIV (1) prior to CD19-, CD20- or BCMA-CAR-T-cell therapy (pre-CAR-T cohort; IIV administered after leukapheresis and ≥2 weeks prior to CAR-T-cell therapy per institutional practice) at Fred Hutchinson Cancer Center (Fred Hutch) or Seattle Children's Hospital (SCH), and (2) in remission after CAR-T-cell therapy without initiating new anti-neoplastic therapies (post-CAR-T cohort). The third cohort included Fred Hutch employees between 18 and 64 years of age who received an IIV through occupational health, were not immunocompromised, and volunteered to participate in the study (control cohort). Individuals who received IGRT within 2 months prior to enrollment were excluded. This study was approved by the Fred Hutch Institutional Review Board; all participants provided informed consent in accordance with the Declaration of Helsinki.

Inactivated influenza vaccines

Individuals received a commercially available trivalent or quadrivalent 2019-2020 Northern Hemisphere IIV. Vaccines in the CAR-T cohorts are detailed in **Table 1**. All controls received a quadrivalent IIV (Flucelvax, Seqirus). The World Health Organization (WHO) recommended strains were: A/Brisbane/02/2018 (H1N1)pdm09-like virus, A/Kansas/14/2017 (H3N2)-like virus, and B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage) for the trivalent IIV, with the addition of a B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage) for the quadrivalent IIV.[24]

Data and blood collection

For the CAR-T-cell cohorts, data were abstracted from medical records. IGRT within 4 months (≥4 half-lives of circulating IgG) before any study sample collection was documented because of the potential for influencing measured antibodies.[25,26] For the control cohort, date of birth, sex, and information about influenza vaccination in the prior year were collected.

In the pre-CAR-T cohort, blood samples were obtained before vaccination (baseline), before lymphodepleting chemotherapy, and approximately 30 and 90 days after CAR-T-cell therapy (**Figure 1**). In the post-CAR-T cohort, samples were collected at baseline and once approximately 30-90 days after vaccination. No samples were collected after relapse or start of new anti-tumor therapies. In the control cohort, samples were obtained at baseline and approximately 30, 60, and 90 days after vaccination. Serum and peripheral blood mononuclear cells (PBMCs) were isolated and stored (**Supplementary Methods**). Laboratory work was blinded to clinical characteristics.

Laboratory testing

Hemagglutination inhibition (HAI) assay

Influenza hemagglutinin (HA) is the main target of neutralizing antibodies, and quantitation of HA-specific antibodies is the gold standard for measuring humoral immunity to influenza. We performed HAI assays on all serum samples and tested, in replicate serial 2-fold dilutions, for antibodies to all 4 vaccine strains as detailed in the **Supplementary Methods** and elsewhere.[27] The highest dilution of serum that caused complete inhibition of hemagglutination was considered the titration end point. We reported the reciprocal of this dilution as the HAI titer. The lower and upper limits of detection (LOD) were 10 and 1280, respectively.

Neutralization assay

We also tested all serum samples with a fluorescent-based neutralization assay for antibodies against H1 of the A(H1N1) vaccine strain as previously described[28–30] and detailed in the **Supplementary Methods**. This assay often correlates with the HAI assay and may be more sensitive. Additionally, it specifically measures neutralizing antibody activity, which might provide a better estimate of protection against infection.[31–33] Two replicate dilution columns were used for each sample to calculate average infectivity. Neutralization was measured against virus containing the H1 sequence derived from the A/Brisbane/2/2018 H1N1pdm09 virus strain that carried a gene for the green fluorescent protein (GFP) in the PB1 segment. The assays were performed in MDCK-SIAT1-CMV-PB1 cells.[34] Curves of fluorescence intensity were plotted and half maximal inhibitory concentrations (IC50s) were calculated using the *neutcurve* Python package. The IC50 is defined as the dilution of serum needed to inhibit infectivity of virus by 50% of its maximum infectivity as measured when no antibodies are present. We reported the reciprocal of IC50 as the neutralization titer. The lower and upper LOD ranged from 12.5-25 and 2680-5369, respectively.

Flow cytometry for B- and T-cells

We immunophenotyped B-cells and T-cells from PBMCs as detailed in the **Supplementary Methods**.

Total immunoglobulins

In addition to influenza-specific antibodies, we measured total serum IgG, IgM, and IgA using turbidometry (University of Washington Immunology Laboratory, Seattle, WA). In

individuals with IgG MM, total functional IgG was estimated by subtracting the monoclonal component from the gamma region of serum protein electrophoresis.

Outcomes

The primary outcome of interest for humoral immunogenicity from the IIV was an antibody response to the respective vaccine strains at the first post-vaccine time point. For the neutralization assay, we defined an antibody response as a ≥4-fold neutralization titer increase from baseline. For the HAI assay, we defined an antibody response as a titer of ≥40 if the baseline titer was <10 or a ≥4-fold rise from a baseline titer ≥10 ('seroconversion' as per the Food and Drug Administration [FDA][35]). We separately reported the proportion of individuals with HAI antibody titers ≥40, a threshold often considered to correlate with seroprotection.[36]

Analyses

We depicted absolute antibody titers at all time points in line and dot plots. A value of half of the lower LOD was assigned for values below the LOD. For each assay and vaccine strain, we compared baseline titers between cohorts using Kruskal-Wallis tests. If those tests were significant, Dunn's test was conducted for pairwise comparisons using the Holm stepwise procedure to account for multiple comparisons. We calculated geometric mean titers (GMT) as summary measures. We described the proportion of individuals with an antibody response to each tested strain as defined above and with HAI titers ≥40. We also computed the proportion of individuals with an antibody response to ≥1 vaccine strain with Wilson 95% confidence intervals (CI). Post-vaccine HAI results for the B(Yamagata) strain were excluded for individuals without confirmed receipt of a quadrivalent vaccine. We used Spearman's correlation to determine the correlation between the neutralization and the HAI assays for the H1N1 vaccine strain. We

described differences in the primary outcomes by clinical and immunological characteristics. Two-tailed P values are reported. *P*<.05 was considered significant. All analyses were conducted using Stata (16.0).

Data sharing

For original data, contact the corresponding author.

RESULTS

Baseline characteristics

We enrolled 30 children and adults: 7 in the pre-CAR-T cohort, 15 in the post-CAR-T cohort, and 8 in the control cohort. All received the IIV between September 2019 and March 2020. The most frequent vaccine type was the standard dose quadrivalent IIV. Clinical characteristics, baseline immunologic results, and vaccine information are detailed in **Table 1 and Tables S1 and S2**. The pre-CAR-T cohort included 7 adults with relapsed or refractory acute lymphoblastic leukemia (ALL; n=1), non-Hodgkin lymphoma (NHL, n=3), and MM (n=3). Four (57%) had a prior autologous HCT and 5 (71%) received a B-cell lineage targeted monoclonal antibody (mAb) therapy in the preceding 6 months. The post-CAR-T cohort included 2 adolescents and 13 adults who achieved a remission after receiving CAR-T-cell therapy a median of 21 months before IIV administration (range, 13-57 months). These individuals were treated for ALL (n=5), chronic lymphocytic leukemia (CLL; n=3), NHL (n=6), and MM (n=1). The majority of individuals in both CAR-T cohorts had hypogammaglobulinemia in addition to low absolute $CD19^+$ B-cells and $CD4^+$ T-cell counts. Controls were adults 25-62 years of age. The IIV was administered in the prior year to 13 (86%) individuals in the post-CAR-T cohort and all (100%) individuals in the control cohort; data were not reliably available for individuals in the pre-CAR-T cohort.

Baseline influenza antibody titers

Baseline antibody titers in each cohort are depicted in **Figure 2** and summarized in **Table 2**. At baseline, neutralizing antibody titers to A(H1N1) were similar in the pre- and post-CAR-T-cell cohorts (GMT 26.5 vs. 45.4, *P*=.23) but were significantly higher in the control cohort (GMT 228.8; *P*=.01 compared to pre-CAR-T cohort, *P*=.02 compared to post-CAR-T cohort). These findings were similar using the HAI assay to A(H1N1), which demonstrated that

antibodies at baseline were detectable in only 2 (29%) individuals in the pre- and 3 (20%) individuals in the post-CAR-T cohort compared to 7 (88%) in the control cohort. Correlation between the neutralization and HAI assay was high, but the neutralization assay was more sensitive (**Supplemental Results**). Baseline titers to A(H3N2) were low among all cohorts. Baseline titers to B(Victoria) or B(Yamagata) did not differ significantly between cohorts but tended to be slightly lower in the CAR-T-cell cohorts. Correspondingly, baseline HAI titers ≥40 to A(H1N1), B(Victoria), and B(Yamagata), but not to A(H3N2), were less frequent among CAR-T-cell therapy recipients than controls (**Table 2**).

IIV immunogenicity and kinetics of influenza antibody responses

Pre-CAR-T cohort

In the pre-CAR-T cohort (n=7), the IIV was administered a median of 35 days (range, 30-112) after the last dose of antineoplastic treatment, within a day after leukapheresis, a median of 0 days after baseline sample collection (range, 0-8), and 26 days (range, 14-50) before CAR-T-cell therapy. Two (29%) individuals received bridging antineoplastic therapy between leukapheresis and CAR-T-cell therapy. Five (70%) received treatment for immune related adverse events after CAR-T-cell therapy. By day 90 after CAR-T-cell therapy, 4 (57%) individuals achieved complete or very good partial responses of the underlying malignancy, 1 (14%) had persistent disease, and 2 (29%) died with progressive disease.

Plots of antibody titers over time for each strain are depicted in **Figure 3A**. At the first post-vaccine time point, a median of 15 days (range, 13-35) after IIV and before CAR-T-cell therapy, 2 (29%; 95% CI, 8%-64%) individuals demonstrated antibody responses to ≥1 vaccine strain (study ID, ‘pre-6’ and ‘pre-7’). Both had a response based on the neutralization assay to A(H1N1). These 2 individuals also had increased antibody titers based on the HAI assay to A(H1N1) and to other strains, but only ‘pre-7’ met the HAI antibody response definition. After

CAR-T-cell therapy, their titers decreased over time, but both still had a neutralization titer to A(H1N1) above baseline at ~30 days after CAR-T-cell therapy, and 'pre-7' maintained a ≥4-fold increased titer for over 3 months. Both received immunosuppressive therapy for cytokine release syndrome and immune effector cell-associated neurotoxicity syndrome following CAR-T-cell therapy. Two other individuals (29%; 'pre-3' and 'pre-5') had ≥2-fold increases in antibody titers to two strains each at the first time point after CAR-T-cell therapy. Among non-responders, some had stable and some decreasing antibody titers over time. One individual ('pre-4') received IGRT after the first post-vaccine time point but had stable antibody titers at the next time point.

Post-CAR-T cohort

In the post-CAR-T cohort (n=15), the IIV was administered a median of 21 months (range, 13-57) after CAR-T-cell therapy (**Table 1**). All individuals were in ongoing remission. IgG was <400 mg/dL in 10 (66%) individuals; median $CD19^+$ B-cell and $CD4^+$ T-cell counts were 2.5 cells/μL and 392 cells/μL, respectively. The median time between the baseline sample collection and vaccination was 7 days (range, 0-82) and between vaccination and post-vaccine sample collection was 48 days (range, 20-104).

Plots of antibody titers over time for each strain are depicted in **Figure 3B**. Antibody responses to ≥1 vaccine strain occurred in 6 (40%; 95% CI, 20%-64%) individuals. Three (20%) individuals had an antibody response based on the neutralization assay to A(H1N1) and one of them also based on the HAI assay to A(H1N1). Three (20%) additional individuals had an antibody response to A(H3N2) or B(Yamagata). Six (40%) individuals did not meet response criteria but had a ≥2-fold increase to ≥1 strain each. Four (27%) individuals received IGRT within 62-95 days prior to the baseline sample, 3 of whom had subsequent IGRT within 23-71 days prior to the post-vaccine sample. Baseline titers in these individuals were similar to those who did not receive IGRT. One of three individuals who received IGRT between vaccination and

post-vaccine time point had an antibody response which could have been affected by this measurement.

Control cohort

In the control cohort (n=8), the first post-vaccine time point was a median of 29 days from vaccination (range, 27-37 days). Plots of antibody titers over time for each strain are depicted in **Figure 3C**. All 3 (38%) individuals with an antibody response had increased titers for A(H3N2) only. Peak titers were observed at the first post-vaccine time point, and the responses were maintained through 90 days after vaccination. One of these individuals had a late ≥4-fold antibody titer increase to B(Victoria) at the 90-day time point. Three (38%) additional individuals had a ≥2-fold increase to ≥1 strain each.

Kinetics of influenza antibodies and GMTs for each cohort over time

Summary plots showing longitudinal antibody titers with GMTs for each cohort are depicted in **Figure 4**. This plot highlights a number of observations across the cohorts. Among both CAR-T cohorts, there was a modest increase in the GMT at the first post-vaccine time point. The pre-CAR-T cohort had a relatively rapid decrease in the GMT over time to a level below the baseline by the 90-day time point. Some individuals in the post-CAR-T cohort generated antibody titers as high or higher than the controls. The IIV for the 2019-2020 season had relatively low immunogenicity in the controls aside from strain A(H3N2), the strain to which no controls had a pre-vaccine HAI titer ≥40. Post-vaccine HAI titers ≥40 were more frequent in controls than in either CAR-T cohort.

Correlates of IIV immunogenicity

To explore possible correlates of IIV immunogenicity, we determined the clinical and immunologic characteristics of individuals who did and did not generate antibody responses. We depicted the fold-changes in neutralizing and HAI antibody titers for the pre- and post-CAR-T cohort in **Figure 5**, stratified for key baseline clinical and immunologic characteristics. Overall, there were no apparent correlates of antibody responses in either CAR-T-cell therapy cohort with evidence of immunogenicity across most categories of clinical and immunologic characteristics. Although only individuals with MM in the pre-CAR-T cohort had responses, and neither of the 2 individuals with $CD4^+$ T-cell counts <200 cells/μL had responses, these observations are limited by small numbers. Importantly, in the post-CAR-T cohort, antibody responses were observed in individuals with very low peripheral $CD19^+$ B-cells (including one individual with no detectable $CD19^+$ B-cells at baseline) and individuals with severe hypogammaglobulinemia (IgG <400 mg/dL). All or most individuals with an antibody response had IgA and IgM levels below the lower limit of normal, respectively (**Table S1**). Additional clinical characteristics, baseline immunologic results, and IIV information of responders and non-responders are described in the **Supplement**, **Table 1**, **Table S1 and S2**.

DISCUSSION

Development of humoral immunity in response to vaccination plays an important role in protection against infection and severe disease[37] as recently underscored by the SARS-CoV-2 pandemic.[38,39] CAR-T-cell therapy recipients are highly immunocompromised prior to and for months following therapy, rendering them high-risk for infections.[4,7,23,40,41] Vaccination may be an effective strategy to prevent the acquisition and severity of infections,[13] but there are no reported data about vaccine immunogenicity, or predictors of responses to vaccines, in this patient population. Nonetheless, certain factors, such as hypogammaglobulinemia or low B-cell counts, are often considered when deciding upon the utility of vaccination. In this study of the IIV administered either shortly before CAR-T-cell therapy or in prior CAR-T-cell therapy recipients in remission, we demonstrated that 60-80% of individuals in both cohorts developed robust or partial antibody increases to ≥1 vaccine strain despite substantial humoral and cellular immunodeficiency. These findings support consideration for administration of relevant vaccines before CAR-T-cell therapy and for (re)vaccination, as indicated, of individuals in long-term remission, irrespective of serum IgG level and total B-cell count.

Immunity to influenza at baseline, prior to vaccination, reflects an individual's history of prior exposure to vaccines and natural infection and can exhibit (cross-)reactivity to current vaccine strains. The 2019/2020 H1N1 vaccine strain only differed by a few amino acids compared to the 2018/2019 formulation.[24] This may explain the high baseline antibody titers to A(H1N1) in the controls, all of whom were vaccinated in the prior year.[42–45] In contrast, we demonstrated a high proportion of undetectable baseline titers to A(H1N1) in individuals pre-CAR-T-cell therapy, which may be due to lack of vaccination in the prior year, poor responses to prior vaccination, or loss of pre-existing immunity related to their malignancy and its treatment.[15,16] Among individuals in remission after CAR-T-cell therapy, baseline titers to A(H1N1) were also significantly lower than in controls despite a similarly high frequency of prior

year vaccination, suggesting either poor responses and/or rapid waning due to inability to establish long-lived antibody-secreting plasma cells.[46,47] Baseline antibody titers to the A(H3N2) vaccine strain were low among all cohorts, likely due to a new A(H3N2) strain in the 2019/2020 vaccine formulation.[24] Both 2019/2020 influenza B vaccine strains were unchanged from the previous year formulation and there was a trend towards lower baseline titers in the CAR-T-cell cohorts. Overall, a higher proportion of controls had HAI titers ≥40 for most strains, demonstrating that the CAR-T-cell cohorts may have higher risk for morbidity from influenza infection.[37,48]

After receiving the IIV, 29% of the pre-CAR-T cohort, 40% of the post-CAR-T cohort, and 38% of the controls had ≥4-fold increases in antibody titers for ≥1 vaccine strain, most of whom also developed a post-vaccine HAI titer ≥40. Sixty to 80% of individuals in all groups had a ≥2-fold increase, and smaller increases in antibody titers may nonetheless be clinically relevant and provide some protection from infection or disease.[37,48] The relatively limited responses to some strains could be reflective of the known phenomena that individuals with higher baseline titers and vaccination in the prior year have lower responses to subsequent vaccination against the same strain, as evidenced by the absent responses to A(H1N1) and the most pronounced responses to A(H3N2) in the controls.[42–45,49] Among responders, peak titers generally occurred at the first post-vaccine time point. In the pre-CAR-T-cell cohort, this was prior to CAR-T-cell therapy, and we observed a relatively rapid antibody decay after CAR-T-cell therapy. Given that the 2 individuals with antibody responses received plasma cell targeted BCMA-CAR-T-cells for MM, this observation may be related to destruction of newly generated influenza-specific antibody-secreting plasma cells by the expanding CAR-T-cells.[50,51] However, in both responders, antibody titers generally persisted above baseline for at least 30 days and up to 4 months after CAR-T-cell therapy, which may provide at least some immunity during the period of highest immunosuppression and infection risk.[4,23,37,40,41,48] As patients are often hospitalized during this period, the finding might be particularly relevant for respiratory virus outbreaks with nosocomial

transmission like influenza and SARS-CoV-2.[52,53] Overall, these findings are consistent with observations of relatively impaired vaccine immunogenicity in individuals being treated for hematologic malignancies or who received a HCT, with influenza vaccine response rates between 0%-60%,[11,15,16,54–57] but still indicate sufficient immunogenicity to support vaccination.

Our study cohorts had heterogeneity in clinical characteristics and CAR-T cell products, but there were no clear correlations between clinical or immunologic characteristics and antibody responses. Key observations included demonstration of vaccine immunogenicity in individuals with low peripheral $CD19^{+}$ B-cell counts (<20 cells/μL) and serum IgG (<400mg/dL), as well as low IgA and IgM levels. Although some guidelines and clinical heuristics would suggest not vaccinating the majority of individuals in our CAR-T-cohorts, we nonetheless demonstrate clinically relevant immunogenicity of the IIV.[15,16] The observations in this study were consistent with our hypotheses that antibody responses to vaccines before or after CAR-T-cell therapy are biologically plausible based on studies demonstrating responses post-rituximab, even without measurable peripheral blood B-cells.[58–60] This could be due to the presence of B-cells below the limit of detection in blood and the possibility of persistence or recovery of B-cells in lymphoid tissue or the bone marrow. Whether responses originated from de novo naïve B-cells or boosted memory B-cells is unclear.

This study has several strengths. To the authors' knowledge, it is the first study of vaccine immunogenicity prior to or post-CAR-T-cell therapy. We performed a prospective study using neutralization and gold-standard HAI assays of longitudinally collected samples to demonstrate IIV immunogenicity in a diverse cohort of CAR-T-cell therapy recipients, in addition to controls. Our data support consideration for administration of non-live vaccines before CAR-T-cell therapy for influenza, and by extrapolation, to other relevant pathogens in this clinical context (e.g., SARS-CoV-2 and pneumococcus). Additionally, vaccinations should be offered to patients in remission after CAR-T-cell therapy as previously suggested.[13] The primary limitation

is the relatively small sample size, but these data set the foundation for larger trials of both immunogenicity and efficacy. Other limitations include that IIV was at the discretion of clinical providers, and vaccine types and timing of sample collection were variable based on clinical follow up. It is possible that undocumented influenza infection in between blood draws confounded measurements, but this is unlikely. None of the vaccinations occurred within the first year after CAR-T-cell therapy, and additional data are needed to determine immunogenicity in these earlier time periods. Although HAI titers ≥40 generally correspond to a 50% reduction in the incidence of infection,[31] this is not established in immunocompromised individuals. Cellular responses are another critical component of immunity to influenza and other infections;[61] T-cell responses were not studied in this analysis but may demonstrate additional utility of IIV in this population with impaired B-cell immunity.

In summary, these data support consideration for vaccination for influenza and other pathogens, such as SARS-CoV-2, before and after CAR-T-cell therapy, irrespective of hypogammaglobulinemia or B-cell aplasia. Larger studies are needed to determine predictors of vaccine immunogenicity and durability in CAR-T-cell therapy recipients. Additional strategies to prevent infections, like vaccination of close contacts and standard precautions, should remain the backbone of infection prevention in these high-risk individuals.

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Conflict-of-interest disclosure:

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C.J.T. received research funding from Juno Therapeutics, Nektar Therapeutics, AstraZeneca, TCR^2 Therapeutics; is a member of scientific advisory boards for Precision Biosciences, Eureka Therapeutics, Caribou Biosciences, T-CURX, Myeloid Therapeutics, ArsenalBio, and Century Therapeutics; has served on ad hoc advisory boards for Nektar Therapeutics, Allogene, Asher Biotherapeutics, PACT Pharma, Astra Zeneca; has stock options for Precision Biosciences,

Eureka Therapeutics, Caribou Biosciences, Myeloid Therapeutics, ArsenalBio; and has patents licensed or optioned to Juno Therapeutics.

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H.Y.C. reported consulting with Ellume, Pfizer, Glaxo Smith Kline, and Merck. She has received research funding outside of the submitted work from Gates Ventures, Sanofi Pasteur, the Bill and Melinda Gates Foundation, and support and reagents from Ellume and Cepheid outside of the submitted work.

J.D.B. is on the scientific advisory board of Oncorus and has performed consulting for Moderna.

J.A.H. received consulting fees from Gilead Sciences, Amplyx, Allovir, Allogene therapeutics, CRISPR therapeutics, and Takeda and research funding from Takeda, Allovir, Karius, and Gilead Sciences.

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FIGURES

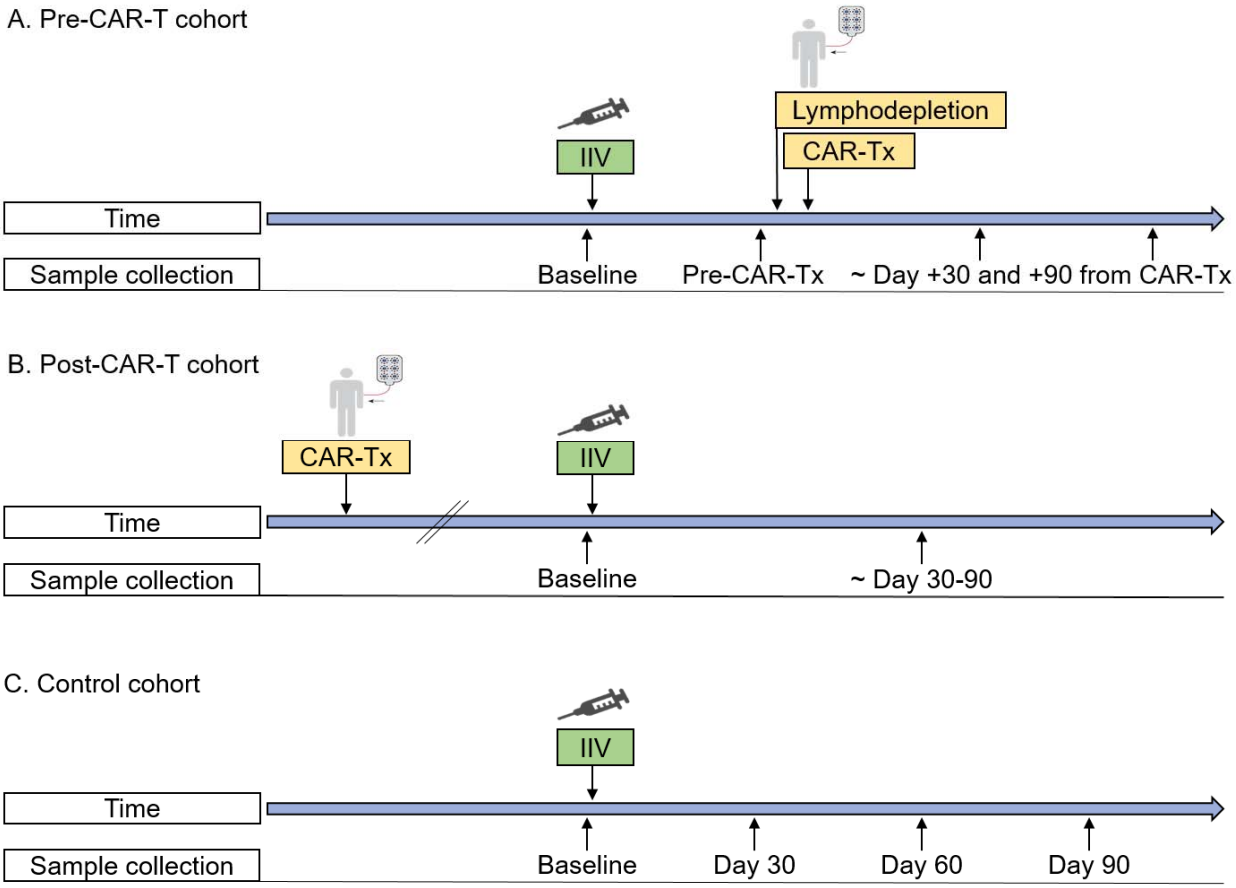

Figure 1. **Inactivated influenza vaccine administration and sample collection timelines.**

Timelines demonstrating blood sample collection, inactivated influenza vaccine (IIV) administration, and CAR-T-cell therapy (CAR-Tx) for the (**A**) pre-CAR-T cohort (**B**), post-CAR-T cohort, and (**C**) control cohort. Lymphodepletion indicates lymphodepleting chemotherapy. In all cohorts, exact days of sample collection varied as detailed in the text and tables and depicted in figures.

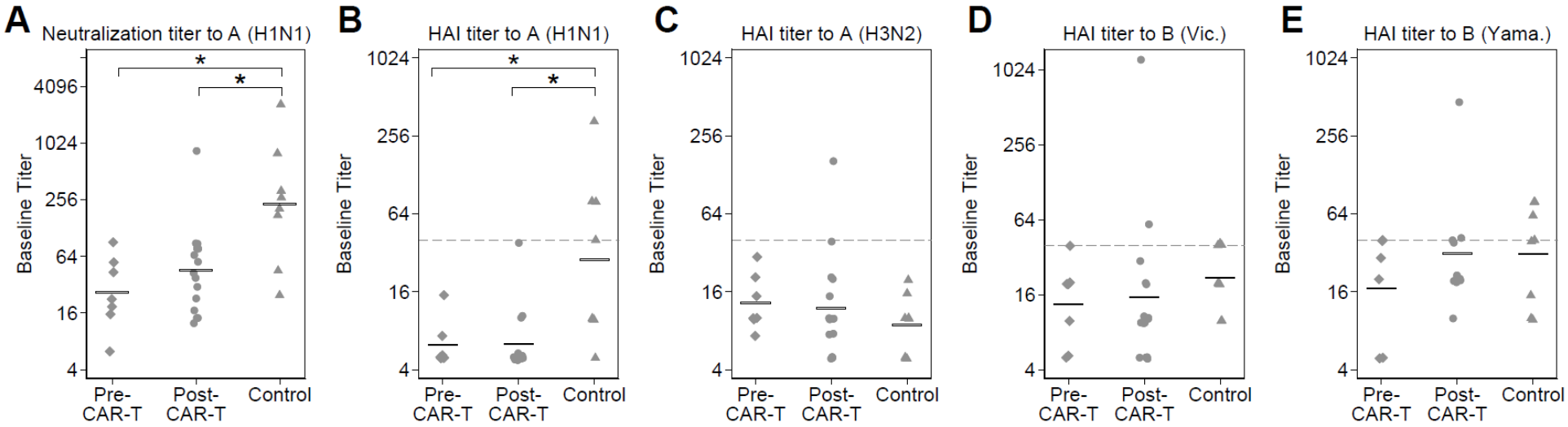

Figure 2. Baseline influenza antibody titers.

Individual baseline titers are plotted by cohort for (**A**) the neutralization assay to A(H1N1), and the hemagglutination inhibition (HAI) assay to (**B**) A(H1N1), (**C**) A(H3N2), (**D**) B(Victoria), and (**E**) B(Yamagata). Data have been jittered to allow viewing of overlapping values. Horizontal bars represent geometric mean titers (GMTs). Points on or above the dashed horizontal lines represent baseline HAI titers ≥40. For both assays, titers to A(H1N1) were significantly lower in the CAR-T cohorts when compared to the control cohort as indicated with a * (neutralization assay: pre-CAR-T vs controls, *P*=.01; post-CAR-T vs controls, *P*=.02. HAI assay: pre-CAR-T vs controls, *P*=.009; post-CAR-T vs controls, *P*=.001; based on Dunn's test with the Holm stepwise procedure for multiple comparisons). There were no significant differences between cohorts based on the HAI assay to A(H3N2), B(Victoria), or B(Yamagata) (Kruskal-Wallis, *P*=.46, *P*=.21 and *P*=.40, respectively), although in general, a higher proportion of individuals in the control cohort had HAI titers ≥40. GMTs and the proportion of individuals with an HAI titer ≥40 are detailed in **Table 2**.

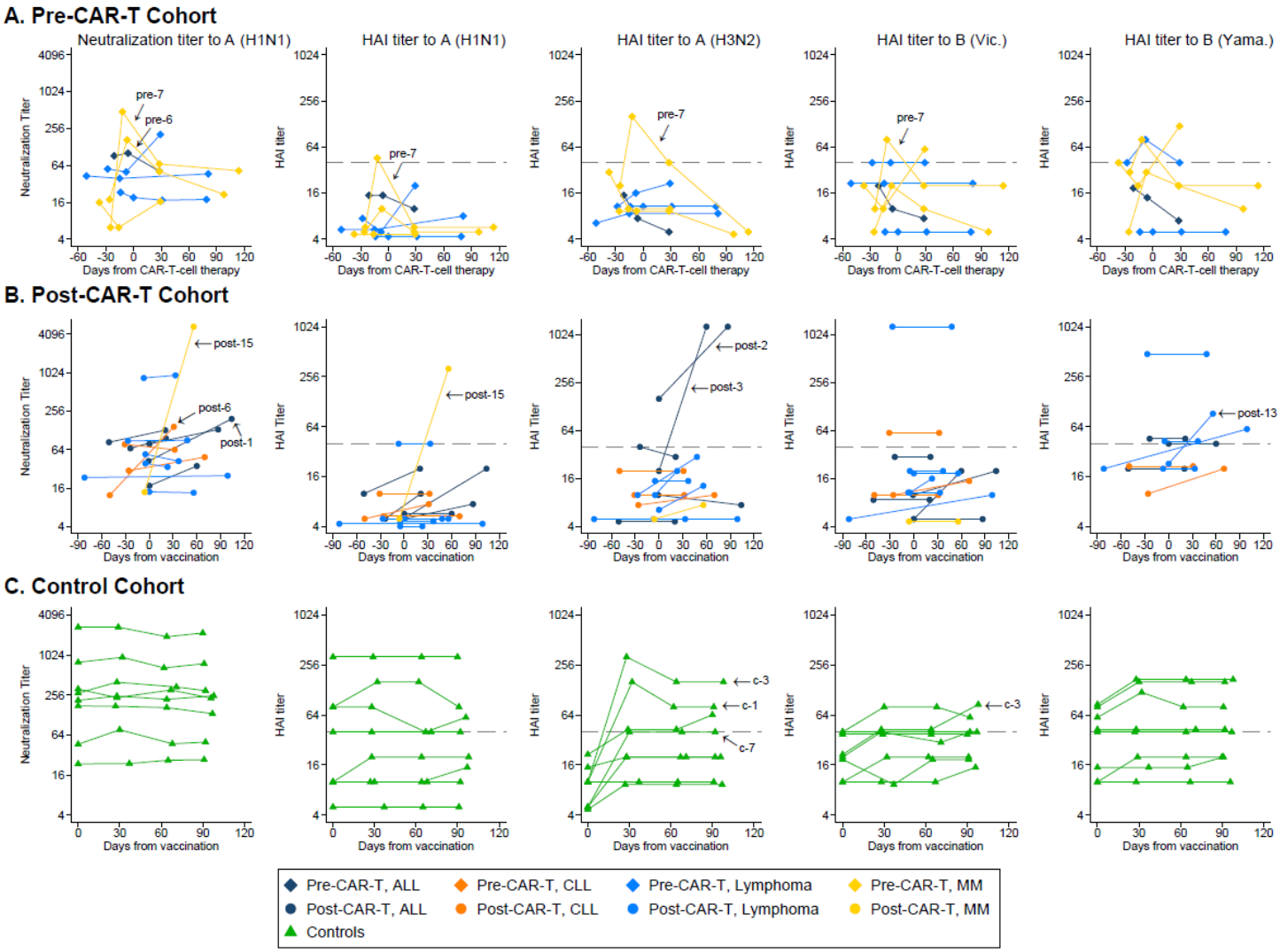

Figure 3. Kinetics of influenza antibody responses by individual.

Line plots demonstrating neutralization titers to A(H1N1) and hemagglutination inhibition (HAI) titers to A(H1N1), A(H3N2), B(Victoria), and B(Yamagata) for (**A**) the pre-CAR-T cohort, (**B**) the post-CAR-T cohort, and (**C**) the control cohort. Each line connects results from one individual over time. Individuals with antibody responses per definition are indicated with an arrow and their study ID (**Table 1**). Symbols on or above the dashed horizontal line represent HAI titers ≥40. For the pre-CAR-T cohort, day 0 was set at the day of CAR-T-cell therapy, vaccines were administered between 0 and 8 days after baseline sample collection (median, 0), and time between vaccine and sample collection prior to CAR-T-cell therapy ranged from 13 to 35 days (median, 15). For the post-CAR-T cohort and the control cohort, day 0 was set at the day of vaccination. Individuals without confirmed receipt of a quadrivalent vaccine are excluded from the plots showing HAI titers to B (Yamagata).

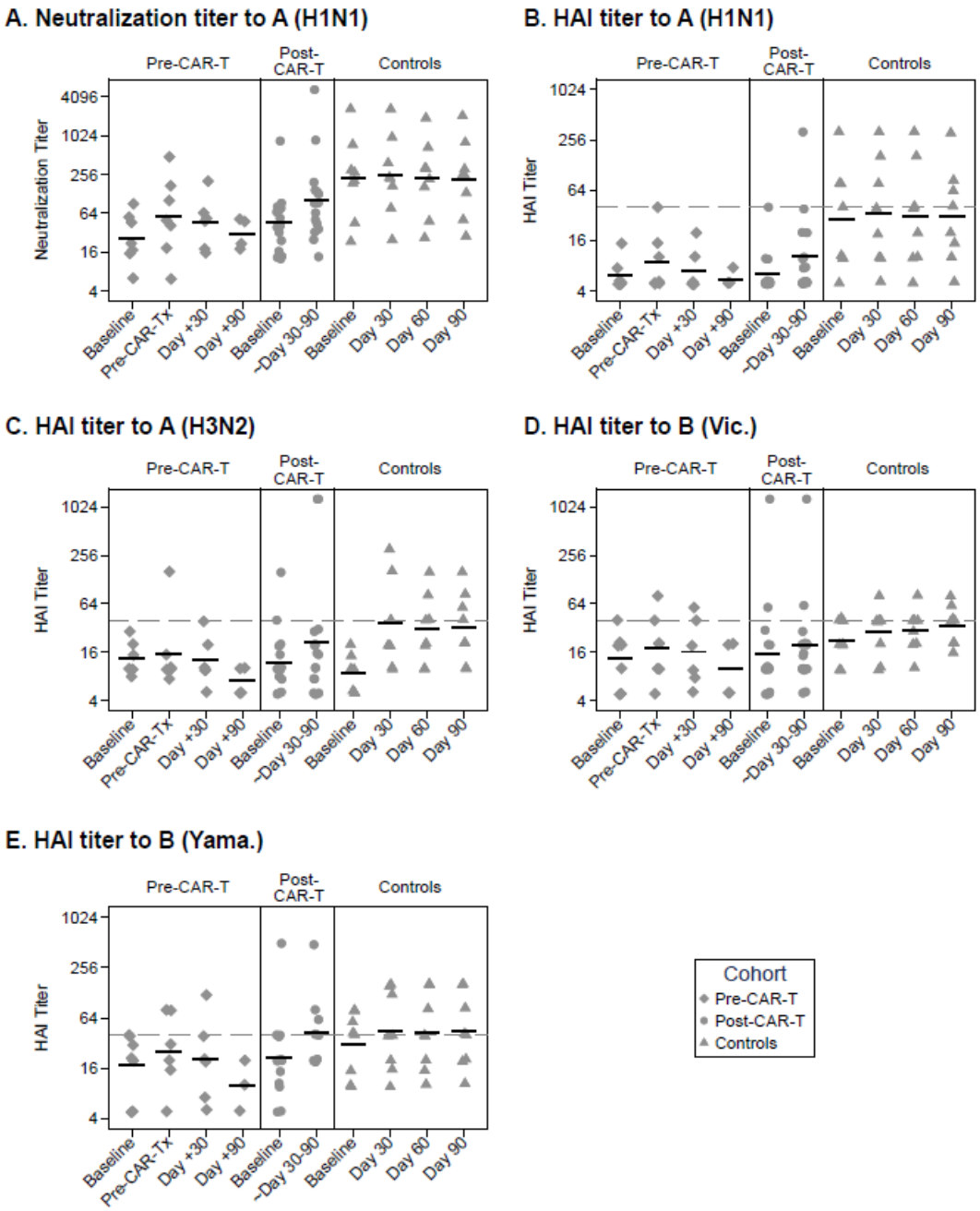

Figure 4. Summary of longitudinal influenza antibody kinetics and geometric mean titers for each cohort.

Individual titer results are plotted per sample collection time points for the pre-CAR-T, post-CAR-T, and control cohorts (from left to right in each panel). (**A**) Neutralization titers to A(H1N1) and (**B**) hemagglutination inhibition (HAI) titers to A(H1N1), (**C**) A(H3N2), (**D**) B(Victoria), and (**E**) B(Yamagata) are shown. Data have been jittered to allow viewing of overlapping values. Horizontal bars represent geometric mean titers (GMT). Symbols on or above the dashed horizontal line represent HAI titers ≥40.

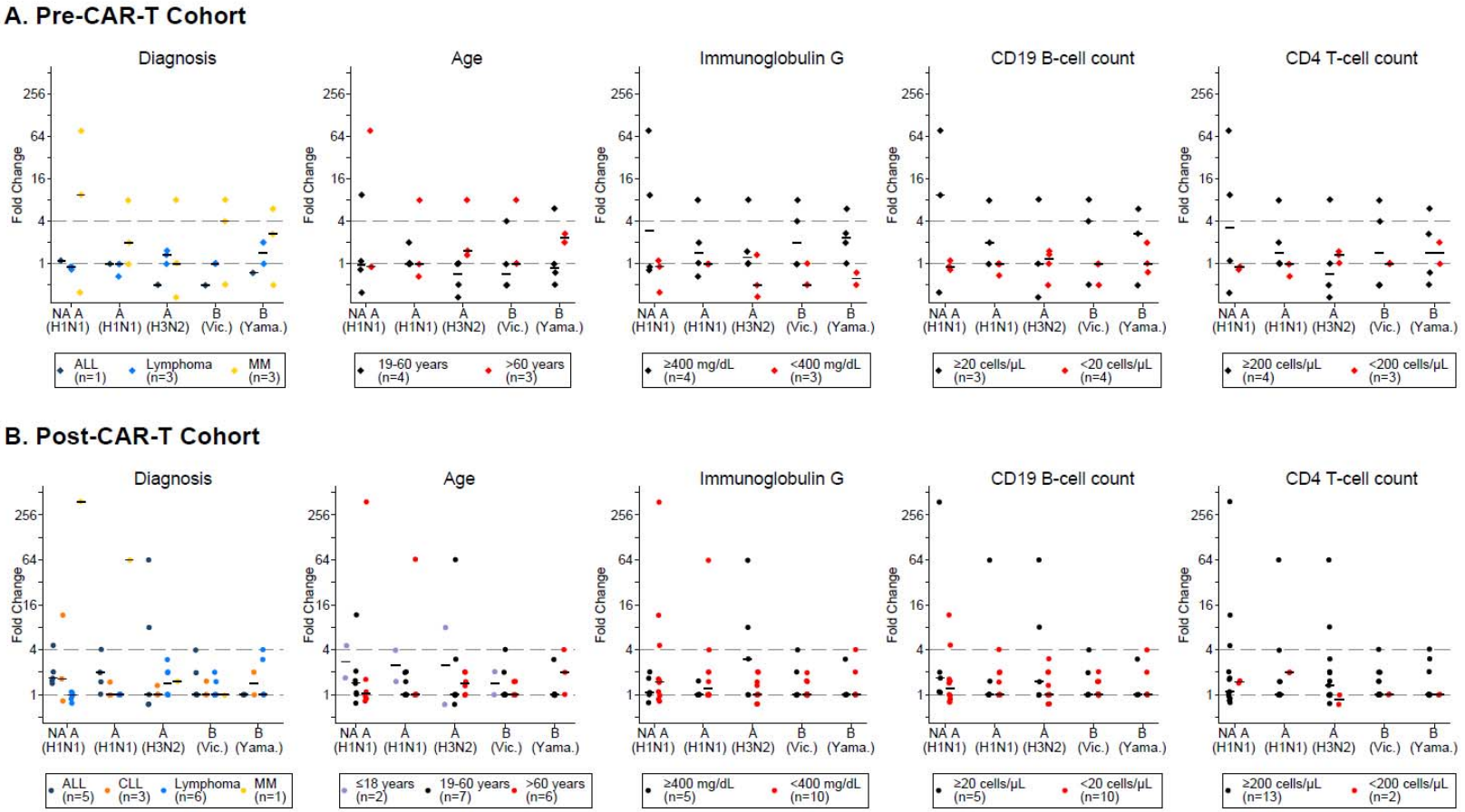

Figure 5. Antibody titer fold-changes by baseline clinical characteristics and immunologic findings.

Antibody titer fold-changes from baseline to the first post-vaccine time point are depicted for each vaccine strain in (**A**) the pre-CAR-T cohort and (**B**) the post-CAR-T cohort. Each panel is stratified by a baseline characteristic (diagnosis, age, immunoglobulin G, $CD19^+$ B-cell count, and $CD4^+$ T-cell count). Characteristics are specified in the figure legends and indicated with different symbol colors. NA indicates neutralization assay; other results are based on hemagglutination inhibition (HAI) assays to A(H1N1), A(H3N2), B(Victoria), and B(Yamagata). A fold-change of 1 (lower dashed horizontal line) indicates no change in antibody titer from baseline. Horizontal bars represent median fold-changes. Symbols on or above the upper dashed horizontal line represent ≥4 fold-changes.

TABLES

Table 1. Baseline clinical characteristics and immunologic findings of the pre- and post-CAR-T-cell therapy cohorts

Demographics		Diagnosis and prior treatments			CAR-T-cell therapy and vaccine			Baseline immunologic findings[7]		
Study ID[1]	Age group (years)	Underlying diagnosis[2]	Time HCT to vaccine (months)[3]	mAb in 6 months before vaccine[4]	CAR-Tx target[5]	Approximate time from CAR-Tx to vaccine (years)	Vaccine type[6]	IgG, mg/dL[8]	$CD19^+$ B-cells /µL	$CD4^+$ T-cells /µL
Pre-CAR-T cohort										
Pre-1	19-60	ALL		yes	CD19		IIV4	376	5	236
Pre-2	19-60	NHL	≤24		CD19		IIV4	552	<1	103
Pre-3	61-75	NHL	≤24	yes	CD19		IIV4	592	<1	123
Pre-4	61-75	NHL		yes	CD19		IIV3-HD	202	<1	113
Pre-5	19-60	MM	>24		BCMA		IIV4	46	47	433
Pre-6*	61-75	MM		yes	BCMA		IIV4	21	21	470
Pre-7*	61-75	MM	>24	yes	BCMA		IIV4	591	87	436
Post-CAR-T cohort										
Post-1*	10-18	ALL	>24		CD19	1-2	NK	264	2	1078
Post-2*	10-18	ALL			CD19	>2	NK	653	387	742
Post-3*	19-60	ALL	>24		CD19	1-2	IIV4	823	401	392
Post-4	19-60	ALL	>24		CD19	1-2	ccIIV4	371	0	176
Post-5	19-60	ALL	>24		CD19	>2	IIV4	310	<1	152
Post-6*	19-60	CLL			CD19	>2	ccIIV4	334	14	488
Post-7	61-75	CLL			CD19	>2	IIV4	217	<1	480
Post-8	61-75	CLL			CD19	1-2	IIV3-HD	286	3	332
Post-9	19-60	NHL			CD19	1-2	IIV4	527	<1	394
Post-10	19-60	NHL			CD19	1-2	IIV4	416	1	353
Post-11	19-60	NHL	>24		CD19	>2	IIV4	447	95	504
Post-12	61-75	NHL			CD19	1-2	IIV4	364	207	303
Post-13*	61-75	NHL			CD19	>2	ccIIV4	189	0	501
Post-14	61-75	NHL			CD19	1-2	IIV	324	<1	304
Post-15*	61-75	MM	>24		BCMA	1-2	aIIV3	290	165	317

For de-identification, certain continuous variables were grouped and other variables are provided as summary measures in the manuscript text. All but two participants were white. Additional information is provided in **Tables S1 and S2**. Blank fields indicate not applicable. NK indicates not known. Baseline is defined as the day of the baseline blood sample prior to vaccination.

ALL indicates acute lymphoblastic leukemia; CAR-Tx, CAR-T-cell therapy; CLL, chronic lymphocytic leukemia; HCT, hematopoietic cell transplant; mAb, B-cell lineage targeted monoclonal antibody; MM, multiple myeloma; NHL, non-Hodgkin lymphoma; NK, not known.

Vaccine abbreviations: IIV3, trivalent inactivated influenza vaccine; IIV4, quadrivalent inactivated influenza vaccine; HD, high dose; cc, cell culture based; a, adjuvant.

[1]Individuals with an antibody response to at least one vaccine strain are indicated with a *.

[2]All individuals in the pre-CAR-T cohort had relapsed/refractory disease at baseline. All individuals in the post-CAR-T cohort had complete remission or very good partial remission at baseline.

[3]Autologous HCT in 4 individuals in the pre-CAR-T cohort and in 1 individual in the post-CAR-T cohort. Allogeneic HCT in 4 individuals in the post-CAR-T cohort.

[4]Monoclonal antibodies were: blinatumomab, rituximab/polatuzumab or daratumumab.

[5]One individual with NHL received a CD20 targeted CAR-T-cell therapy but is indicated with CD19 for confidentiality.

[6]Vaccine strains were A/Brisbane/02/2018 (H1N1)pdm09-like virus, A/Kansas/14/2017 (H3N2)-like virus, B/Colorado/06/2017-like virus (B/Victoria/2/87 lineage) for IIV3, with the addition of a B/Phuket/3073/2013-like virus (B/Yamagata/16/88 lineage) for IIV4.

[7]Lower limit of normal; IgG, 610 mg/dL; $CD19^+$ B-cells, 100 cells/μL; $CD4^+$ T-cells, 500 cells/μL.

[8]For IgG MM, total IgG was estimated by subtracting the monoclonal component from the gamma region of serum protein electrophoresis. Four individuals received IGRT 3-4 months prior to baseline (**Table S2**) and had IgG levels between 25 and 450 mg/dL.

Table 2. Antibody titers and antibody responses at baseline and at the first post-vaccine time point

Antigen		Pre-CAR-T cohort (N = 7)	Post-CAR-T cohort (N = 15)	Control cohort (N = 8)
	Days from vaccination to first post-vaccine time point, median (range)	15 (13 – 35)	48 (20 – 104)	29 (27 – 37)
Neutralization assay				
A(H1N1)	Baseline GMT (range)	26.5 (6.3-92.0)	45.4 (12.5 – 847.5)	228.8 (23.5 – 2680.2)
	Antibody response[1], n (%)	2 (29)	3 (20)	0
	Titer fold change, median (range)	0.9 (0.4 – 76.9)	1.4 (0.8 – 385.4)	1.1 (0.7 – 1.7)
Hemagglutination inhibition assay				
A(H1N1)	Baseline GMT (range)	6.2 (5 – 15)	6.3 (5 – 40)	28.3 (5 – 320)
	Antibody response[1], n (%)	1 (14)	1 (7)	0
	Baseline titer ≥40[2], n (%)	0	1 (7)	4 (50)
	Post-vaccine titer ≥40, n (%)	1 (14)	2 (13)	4 (50)
A(H3N2)	Baseline GMT (range)	13.1 (7.5 - 30)	11.9 (5 – 160)	8.8 (5 – 20)
	Antibody response, n (%)	1 (14)	2 (13)	3 (38)
	Baseline titer ≥40, n (%)	0	2 (13)	0
	Post-vaccine titer ≥40, n (%)	1 (14)	2 (13)	4 (50)
B(Victoria)	Baseline GMT (range)	13.5 (5 - 40)	15.3 (5 – 1280)	21.8 (10 – 40)
	Antibody response, n (%)	1 (14)	0	0
	Baseline titer ≥40, n (%)	1 (14)	2 (13)	3 (38)
	Post-titer ≥40, n (%)	2 (29)	2 (13)	5 (63)
B(Yamagata)[3]	Baseline GMT (range)	17.4 (5 – 40)	21.1 (5 – 480)	31.3 (10 – 80)
	Antibody response, n (%)	0	1/10 (10)	0
	Baseline titer ≥40, n (%)	2 (29)	4 (27)	5 (63)
	Post-vaccine titer ≥40, n (%)	2 (33)	6 (60)	5 (63)

GMT indicates geometric mean titer.
[1]Antibody response is defined as a four-fold rise in neutralization or hemagglutination inhibition (HAI) titer or a HAI titer of ≥40 post-vaccine if the baseline HAI titer was <10.
[2]An HAI antibody titer ≥40 is often considered to be 'seroprotective'.
[3]B/Phuket/3073/2013 (Yamagata) is included in quadrivalent vaccines only; post-vaccine results from individuals without confirmed quadrivalent vaccine were excluded from post-vaccine summaries; remaining N were 6 in the pre-CAR-T cohort, 10 in the post-CAR-T cohort, and 8 in the control cohort.