

1 **Immunogenicity of Pfizer mRNA COVID-19 vaccination followed by J&J adenovirus**
2 **COVID-19 vaccination in two CLL patients**

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21 **Funding:** This work was funded in part by National Institute of Allergy and Infectious
22 Diseases NIAID 1R01AI145835 (WBM), by US National Institute of Health grant P51
23 OD011092 (MKS), and endowed funds from the Knight Cancer Institute's Scholar Award for
24 Leukemia and Lymphoma Research (SES).

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26 **Conflict of interest statement** (SES): Consulting: Velos Bio, Karyopharm, Genentech; Janssen,
27 Pharmacyclitics; Research: Acerta Pharma, Astrazeneca, Beigene, Bristol Myers Squibb,
28 Genentech, Gilead Sciences, Ionis, Janssen

29
30 All other authors, have no conflict of interest.

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37 Chronic Lymphocytic Leukemia (CLL) is characterized by monoclonal proliferation of
38 dysfunctional B-cells, leading to a broad range of immune defects. CLL patients face significant
39 risk of morbidity and mortality from infections (1), including from SARS-CoV-2, the causative
40 agent of COVID-19 (2). Vaccines can be instrumental in mitigating the risk of infections in
41 CLL; however, responses to vaccination is highly variable and significantly influenced by CLL
42 disease status, baseline characteristics, types of vaccine and active CLL therapy (3).
43 Although current COVID-19 vaccines elicit robust immunity in immunocompetent hosts (4), the
44 antibody response in CLL patients is highly variable (5, 6) and particularly poor in patients with
45 low total immunoglobulin levels, those that have had anti-CD20 monoclonal antibodies within
46 the past year, or are undergoing active therapy with agents such as Bruton’s Tyrosine Kinase
47 inhibitors (BTKi). The best responses to date have been in CLL patients who are in remission
48 and/or years out from active treatment.
49 Given decreased vaccine efficacy in CLL, an additional dose of vaccine may be beneficial in
50 CLL patients, especially given rise of variants of concern (VoCs). Initial data from solid organ
51 transplant recipients on immunosuppression showed a role for additional vaccination (7), leading
52 the FDA to extend the EUA for Pfizer-BioNTech and Moderna mRNA vaccines to include
53 additional doses in immunocompromised patients. However, the results in solid organ transplant
54 patients may not be generalizable to CLL, and additional studies are needed to better define
55 vaccine responses in the CLL patient population, including the role of mixing mRNA
56 vaccination with other vaccine formulations, such as the adenovirus vectored vaccine
57 Ad26COV2.s, commonly known as Johnson and Johnson (J&J) vaccine.
58 Here we describe two CLL patients who “self-referred” to outside pharmacies for an additional
59 vaccination with J&J COVID-19 vaccine following 2 doses of the BNT162b2 vaccine (Pfizer-

60 BioNTech). Both patients had previously enrolled as study subjects in an IRB approved
61 observational study, (OHSU IRB# 21230) to investigate immune response following COVID-19
62 vaccination. The additional J&J dose was subsequently self-reported to the study team. On
63 initial enrollment, demographics, CLL disease characteristics, and treatment details were
64 collected (Table 1), and baseline laboratory values were obtained, included semi-quantitative
65 SARS-CoV-2 spike antibody titer, serum IgG, a complete blood count, and multicolor flow
66 cytometry measuring immune cell populations (Table 1). Whole blood was collected for
67 additional serologic and cellular studies.

68 SARS-CoV-2 spike receptor binding domain (RBD)-specific antibody levels were tested by
69 ELISA and endpoint titers were calculated as previously described (8). In addition, baseline
70 PBMC samples were functionally tested for the presence of SARS-CoV-2 spike RBD-specific
71 memory B-cells (MBCs) by limiting dilution assay (9, 10) and CD4⁺ and CD8⁺ T-cells were
72 functionally assessed for the presence of IFN γ and TNF α secretion following spike protein
73 derived peptide stimulation.

74 Neither subject had pre-vaccination B-cell responses as measured by RBD-specific antibodies or
75 MBCs. Neither had a virus-specific CD8⁺ response at baseline. While Subject 2 had spike
76 peptide-reactive CD4⁺ T-cells at baseline these cells were unresponsive and did not expand
77 following vaccination. In contrast, CD8⁺ responses were observed after mRNA vaccination in
78 both subjects (Fig. 1). It has previously been reported that SARS-CoV-2 naïve individuals may
79 have preexisting cross-reactivity to SARS-COV-2 peptides through prior infection by common
80 cold coronaviruses: SARS-CoV-2 specific CD4⁺ T-cells have been identified in 20-50% of
81 people without SARS-CoV-2 exposure or vaccination (11).

82 Approximately four weeks after initial vaccination neither subject had detectable RBD-specific
83 SARS-CoV-2 antibodies or MBCs. Both had measurable vaccine-induced CD8+ T-cell
84 responses following mRNA vaccination, although CD4+ responses did not appear to increase
85 above baseline (Fig. 1).

86 Subject 1 received the J&J vaccine 104 days and Subject 2- 81 days after completion of the
87 BNT162b2 vaccine series. Following J&J vaccination additional samples were obtained, Subject
88 1, 30 days after third vaccine, and Subject 2, 27 days following third vaccine. Interestingly,
89 Subject 1 had undetectable RBD-specific antibodies, RBD-specific MBCs, and virus-specific
90 CD4+ T-cells after initial vaccination series. However, following an additional vaccination, all
91 three measures increased above the limit of detection, RBD ELISA titer of 625, RBD-specific
92 MBC frequency of $3.6 / 10^6$ B-cells, and 166 spike-specific CD4+ T-cells / 10^6 , and a spike-
93 specific CD8+ T-cell response that remained stable and did not boost appreciably following 3rd
94 vaccination (Fig. 1). Subject 2 did not seroconvert or have detectable virus specific MBCs after
95 their primary mRNA vaccination series however they had a spike-specific CD8+ T-cell response
96 that further boosted after a 3rd dose and a virus-specific CD4+ response that didn't change
97 following original vaccine series or 3rd dose of J&J.

98 Other than subject age (60s vs 80s), the most notable difference between the subjects' baseline
99 characteristics (table 1) is that Subject 1 was treatment naive, while Subject 2 had undergone
100 previous treatment (6 years ago) with obinutuzumab an anti-CD20 mAb and is currently on
101 active treatment with Ibrutinib, since 2017. Both had baseline B-cell frequencies outside of the
102 normal range, with Subject 1 exhibiting a low percentage of naïve B-cells (0.092) and a high
103 percentage of MBCs (59.1), while Subject 2 had a low percentage of naïve B-cells (11.37) and
104 MBCs (0.45). Although Subject 2 had mild hypogammaglobulinemia, neither had a history of

105 recurring infections or need for IgG supplementation. Levels of baseline CD4+ and CD8+ T-
106 cells (absolute values) were also normal, in each subject prior to vaccination (data not shown).
107 Both had very low percentages of naïve B cells which could explain the initial poor response to
108 vaccination. The significance of the increased percentage of MBCs in Subject 1 is unclear but
109 does suggest some broader preservation of normal B cell maturation and immune function.
110 Although Subject 1 did have an immune response, antibody levels were relatively low as
111 compared to some of the levels observed in immunocompetent post-vaccine populations (12) and
112 certain CLL populations (5). The clinical significance of specific antibody levels remains
113 unknown.

114 Active treatment with Bruton's Tyrosine Kinase (BTK) inhibitors like ibrutinib may have a
115 profound impact on B-cell survival, differentiation, and production of antibodies as the absence
116 of intact BTK-dependent B-cell receptor mediated signaling prevents B-cells from
117 differentiating into mature peripheral B-cells. Immune response following vaccination or natural
118 infection is limited in these patients (13). Recall to antigens encountered prior to treatment
119 appears to remain largely intact, however response to novel antigens encountered during
120 treatment seems to be abrogated. Subject 2 has been on ibrutinib for over four years. The impact
121 of prolonged treatment vs. shorter-term BTK inhibition on immune responses is unknown.
122 However, clinical data (14) suggest some improvement in humoral immunity with prolonged (>
123 6 months) treatment. T-cells are also disrupted in individuals with CLL and even further
124 disrupted with BTK treatment (15). In the cases presented here both subjects did have an
125 increase in virus specific CD8+ T-cells however the significance is unclear in terms of protection
126 as neutralizing antibodies are often viewed as the correlate of protection against COVID-19.

127 The results of this study, however small, provide initial evidence that a 3rd vaccination against
128 COVID-19 with the heterotypic vaccine Ad26COV2.s results in an immune response that was
129 not observed following the recommended 2-dose mRNA vaccination series. This is especially
130 promising news to subjects who are treatment naïve, not currently in active treatment, or who
131 may consider vaccination before beginning active treatment.

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133 **Acknowledgements**

134 The authors would like to thank the subjects for participating in this research study.

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200 **Figure legends**

201 Table 1. Baseline Characteristics and demographics for subjects included in the study. Normal
202 ranges for each of the B-cell subset are in parenthesis under each B-cell type.

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204 Figure 1. Immune response to COVID-19 vaccination in CLL subjects. RBD-specific antibody
205 (Ab) titer. Subjects without a detectable Ab titer ($< 1:50$ serum dilution) were assigned a value of
206 49. Frequency of RBD- specific MBCs per 10^6 CD19+ B-cells following ex vivo stimulation.
207 Subjects who did not have a detectable response were assigned a value of 5×10^{-6} . SARS-CoV-2
208 spike peptide-reactive CD4 and CD8 T-cells are defined as double positive for IFN γ and TNF α
209 cytokine secretion. Patients who did not have a detectable T-cell response were assigned an
210 arbitrary number between less than 2. Visit 1 (pre) blood draw was taken 21 and 40 days prior
211 Pfizer vaccine series (2-doses). Visit 2 (V2) blood draw was taken 33 and 24 days post
212 vaccination, and visit 3 (V3) was drawn 30 and 27 days after 3rd vaccination with J&J.

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Table 1.

Subject ID	Age	Gender	Year of Diagnosis	Current Treatment	Prior Treatment	IgG mg/dL	Absolute Lymphocyte Count K/mm ³	B-cells (CD19+) %	Naïve B-cells (IgD+CD27-) %	Memory B-cells (IgD-CD27+) %	B1 B-cells (CD5+CD19+) %
						(768 - 1632)	(1.00 - 4.80)	(4-17)	(50-80)	(5-21)	(<6)
1	60's	F	2014	None	None	834	21.09	76	0.092	59.1	76.18
2	80's	F	2014	Ibrutinib	Obinutuzumab	510	5.93	61	11.37	0.45	59.96

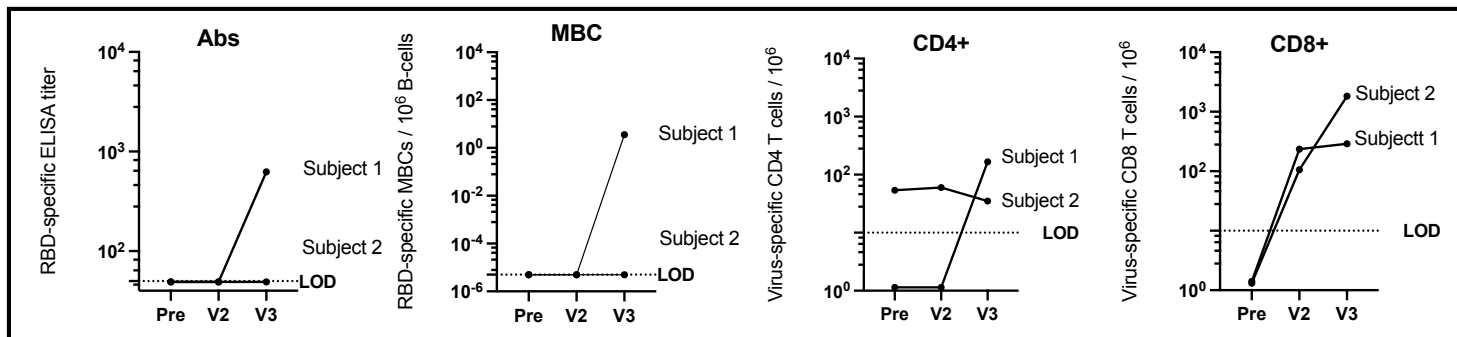


Fig. 1 Immune response to COVID-19 vaccination in CLL subjects. RBD-specific antibody (Ab) titer. Subjects without a detectable Ab titer (< 1:50 serum dilution) were assigned a value of 49. Frequency of RBD- specific MBCs per 10⁶ CD19+ B-cells following ex vivo stimulation. Subjects who did not have a detectable response were assigned a value of 5X10⁻⁶. SARS-CoV-2 spike peptide-reactive CD4 and CD8 T-cells are defined as double positive for IFN γ and TNF α cytokine secretion. Patients who did not have a detectable T-cell response were assigned an arbitrary number between less than 2. Visit 1 (pre) blood draw was taken 21 and 40 days prior to Pfizer vaccine series (2-doses). Visit 2 (V2) blood draw was taken 33 and 24 days post-vaccination, and visit 3 (V3) was drawn 30 and 27 days after 3rd vaccination with J&J.