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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Feld JJ, Kandel C, Biondi MJ, et al. Peginterferon lambda for the treatment of outpatients with COVID-19: a phase 2, placebo-controlled randomised trial. *Lancet Respir Med* 2021; published online Feb 5. http://dx.doi.org/10.1016/S2213-2600(20)30566-X.

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Feld JJ, Kandel C, Biondi MJ, *et al.* Peginterferon-lambda for the treatment of COVID-19 in outpatients: A phase 2, placebo-controlled randomized trial

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Supplemental Methods

SARS-CoV-2 Viral Load Testing

A self-collected mid-turbinate swab was obtained. Briefly, 400 µL of viral transport media was taken from the mid-turbinate swab inactivated and viral nucleic acid was extracted using the NucliSENS EasyMAG platform (bioMerieux, Marcy-l'Etoile, France), and one-step Reverse Transcriptase quantitative PCR was performed on the Rotorgene Q as previously described (Qiagen, Hilden, Germany) using the primers and probes for the SARS-CoV-2 E gene(1, 2). To generate standard curves, dilutions of a synthetic plasmid containing a segment of the E-gene were used (GenScript, USA). Ct values were obtained for both the plasmid-derived standards and samples. The number of viral copies per uL (then converted to mL as established for viral nucleic acid quantification) was determined based on the known initial concentration and then dilutions of the plasmid stock. Interpolation of the samples was carried out using GraphPad Prism. Copies/mL is an established measurement in traditional assays for viral quantification and has been also used in the context of SARS-CoV-2 quantification(3-6). Samples with either no Ct value or a value that was below the level of quantification were counted as negative. The lower limit of detection of 20 copies/mL was established using repeated testing of titred SARS-CoV-2 stocks. For the purposes of calculation, values below the limit of quantification (~20 copies/mL) were given an arbitrary value of 10 copies/mL.

Figure S1. Time to viral clearance in those with baseline viral load ≥ 10E6 copies/mL. The time to undetectable SARS-CoV-2 RNA is shown for the peginterferon-lambda and the placebo group for participants with a baseline viral load above 10E6 copies/mL. The curves are compared using the log-rank test and the median time to clearance with 95% CI is shown for each group.

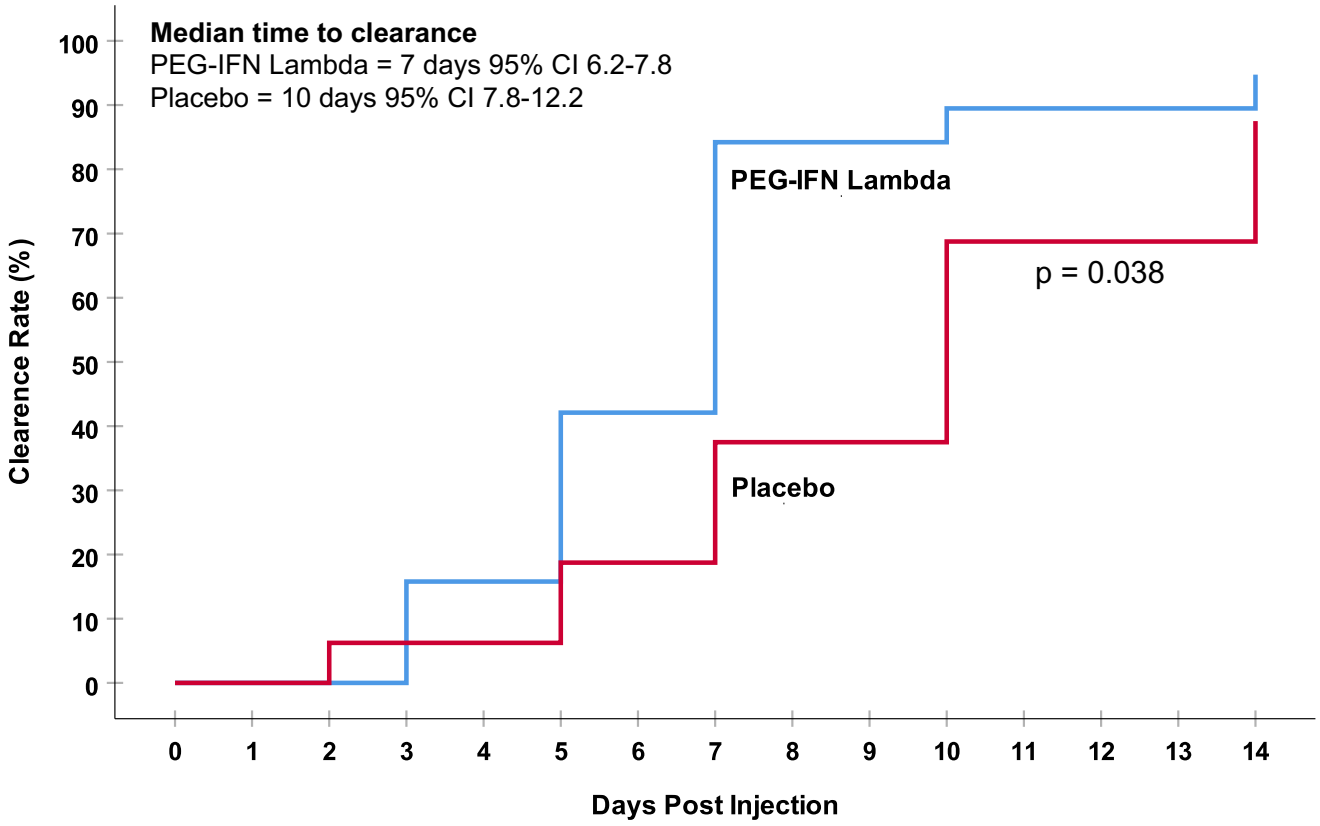

Figure S2. Proportion negative for SARS-CoV-2 RNA over time and mean absolute and change in SARS-CoV-2 viral load over time in those with detectable SARS-CoV-2 at baseline. The proportion of patients negative for SARS-CoV-2 RNA per day post-injection in those with a detectable viral load at baseline (Panel a) with the mean SARS-CoV-2 RNA viral load (Panel b) and log decline from baseline over time (Panel c) in participants with a detectable baseline viral load in the peginterferon-lambda treatment (n=25) and placebo groups (n=20). *Panel b: Difference at Day 7 p=0.042. *Panel c: Difference at Day 5 p=0.036 and Day 7 p=0.006.

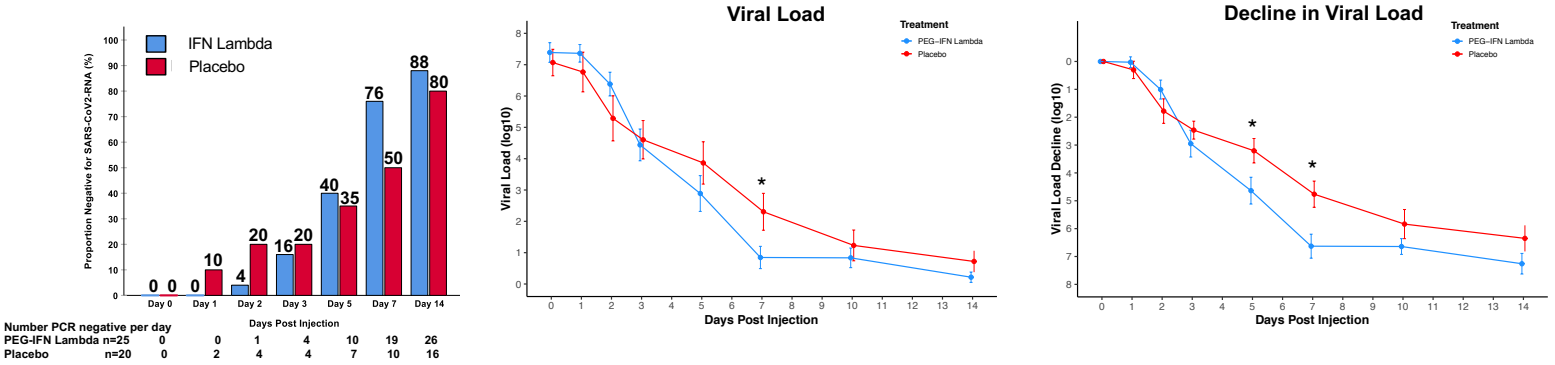

Figure S3. Odds ratio for clearance by Day 7 with peginterferon-lambda vs placebo, controlled for individual baseline covariates, in those with baseline viral load ≥ 10E6 copies/mL. Forest plot showing odds ratio with 95% CI for the odds of clearance by Day 7 with peginterferon lambda treatment compared to placebo, controlled for each individual (bivariate) covariate.

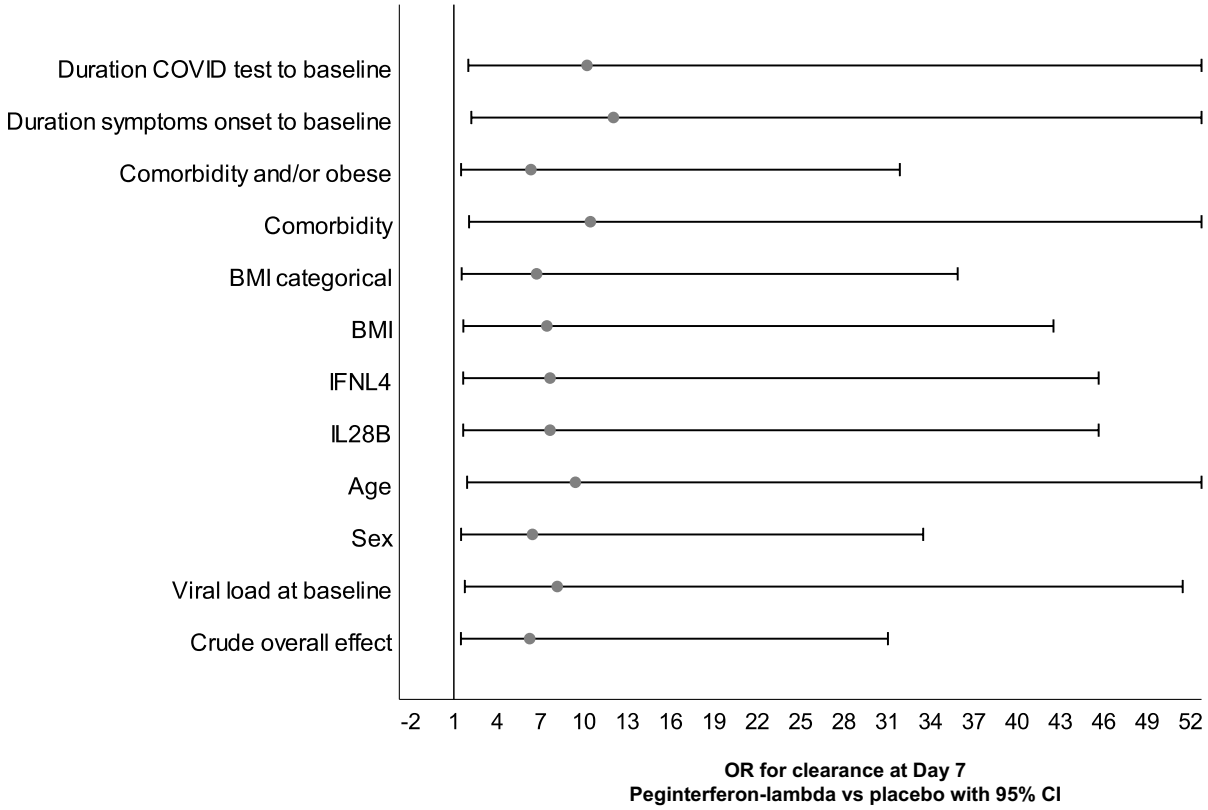

Figure S4. Presence of SARS-CoV-2 antibodies over time stratified by baseline viral load. The proportion of individuals with positive anti-SARS-CoV-2 S protein IgG antibodies per day post-injection, stratified by baseline viral load above or below 10E6 copies/mL.

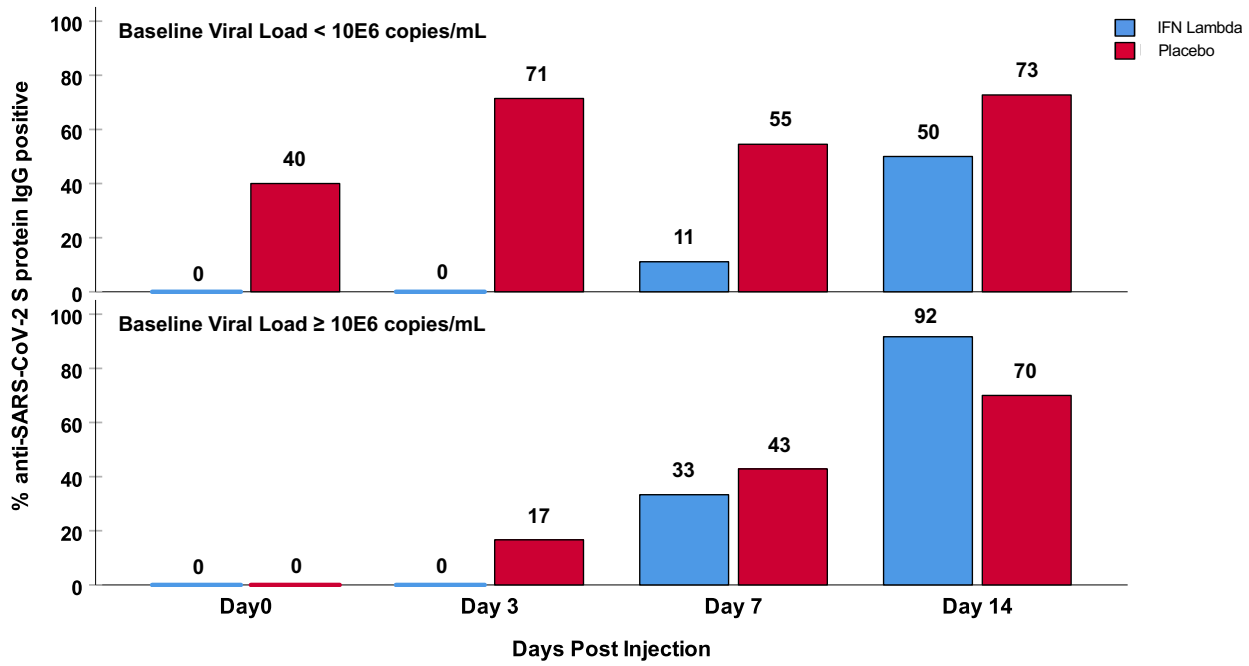

Figure S5. Self-collected oral temperature measurements. Self-collected oral temperature measurements on days 0, 2, 3, 4, 5, 6, 7, 10, 12 and 14 separated into <38° Celsius, 38-39° Celsius and above 39° Celsius stratified by group.

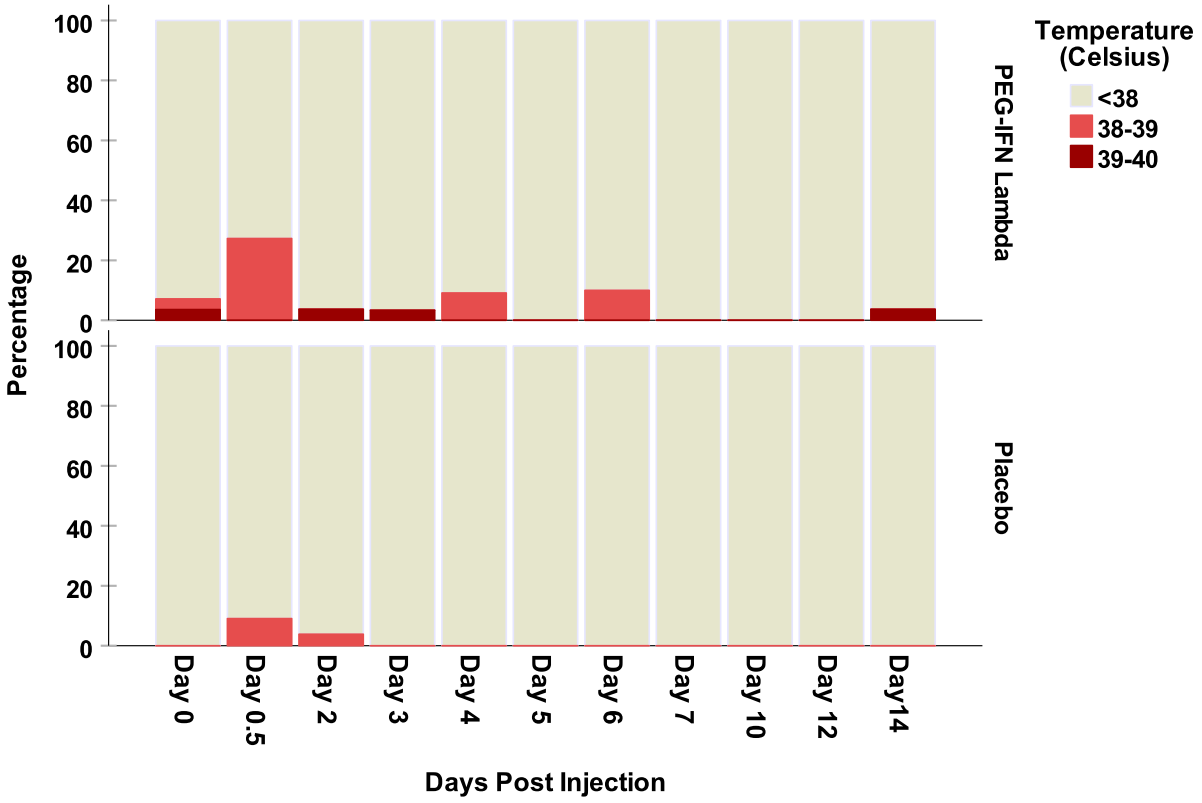

Table S1. Residual SARS-CoV-2 RNA levels at Day 7 post-treatment in those with baseline viral loads above and below 10E6 copies/mL

Baseline Viral Load	Residual Baseline Viral Load at Day 7 in Copies/mL	
	Peginterferon-Lambda	**Placebo**
≥ 10E6 copies/mL	n=4/19 (21%) 2.74E3 4.59E3 7.95E3 8.58E5	n=10/16 (62.5%) 1.61E1 3.10E2 5.10E3 4.28E4 1.46E5 1.66E5 2.62E5 4.17E5 6.49E5 6.40E6
< 10E6 copies/mL	n=2/11 (18%) 2.41E3 Missing	n=1/14 (7%) 1.82E3

Table S2. Categorization of symptoms that were assessed daily into whether they were likely attributed to COVID-19, peginterferon-lambda or either/both.

Symptom Category	Symptom	COVID-19	Peginterferon-Lambda
Systemic	Fever	X	X
	Chills	X	X
	Rigors	X	X
	Fatigue	X	X
Respiratory	Cough	X	
	Sore Throat	X	
	Shortness of breath	X	
	Chest pain	X	
	Runny nose	X	
	Conjunctivitis	X	
Gastrointestinal	Abdominal pain	X	
	Nausea	X	
	Vomiting	X	
	Diarrhea	X	
Musculoskeletal	Muscle Pain	X	X
Skin	Rash		X
	Itch		X
	Injection Site Reaction		X
Mood	Depressed Mood	X	X
Neurologic and Vascular	Loss of smell	X	
	Loss of taste	X	
	Change in color of fingers/toes	X	

Table S3. Association between peginterferon-lambda treatment vs placebo with specific symptom categories and with symptom evolution over time. Results shown for all participants and stratified by baseline viral load.

Symptom Category	Change in severity (day)	All samples		Baseline Viral Load ≥10E6 copies/mL		Baseline Viral Load <10E6 copies/mL	
		OR (95% CI)	p-value	OR (95% CI)	p-value	OR (95% CI)	p-value
All Symptoms							
	Peginterferon-lambda	0.71 (0.61, 0.82)	<.0001	0.74 (0.62, 0.88)	0.0007	0.68 (0.53, 0.87)	0.0024
	Placebo	0.64 (0.55, 0.75)	<.0001	0.60 (0.48, 0.74)	<.0001	0.76 (0.60, 0.96)	0.019
	*Difference in decline**	3.15 (0.77, 12.9)	0.11	2.39 (0.48-11.8)	0.28	1.64 (0.15, 17.5)	0.68
Fever/Systemic							
	Peginterferon-lambda	0.72 (0.62, 0.84)	<.0001	0.77 (0.67, 0.90)	0.0008	0.64 (0.53, 0.77)	<.0001
	Placebo	0.60 (0.49, 0.73)	<.0001	0.64 (0.52, 0.77)	<.0001	0.55 (0.43, 0.69)	<.0001
	Difference in decline	1.69 (0.42, 6.81)	0.46	1.24 (0.30. 5.23)	0.76	4.83 (0.11, 205)	0.41
Respiratory							
	Peginterferon-lambda	0.64 (0.51, 0.80)	<.0001	0.70 (0.55, 0.88)	0.002	0.48 (0.26, 0.91)	0.024
	Placebo	0.52 (0.39, 0.69)	<.0001	0.58 (0.42, 0.78)	0.0004	0.41 (0.20, 0.83)	0.013
	Difference in decline	4.67 (0.91, 23.9)	0.064	5.88 (0.81, 42.4)	0.076	3.67 (0.19, 70.1)	0.39
Gastrointestinal							
	Peginterferon-lambda	0.92 (0.75, 1.15)*	0.47	0.92 (0.83, 1.01)*	0.091	0.98 (0.74, 1.3)*	0.90
	Placebo	0.57 (0.42, 0.77)	0.0003	0.53 (0.41, 0.69)	<.0001	0.61 (0.42, 0.9)	0.013
	Difference in decline	0.48 (0.086, 2.62)	0.39	0.58 (0.093, 3.60)	0.55	0.40 (0.001, 123)	0.75
Musculoskeletal							
	Peginterferon-lambda	0.54 (0.35, 0.83)	0.005	0.51 (0.33, 0.8)	0.003	0.63 (0.46, 0.85)	0.003
	Placebo	0.50 (0.32, 0.77)	0.002	0.48 (0.31, 0.75)	0.001	0.48 (0.20, 1.17)	0.11
	Difference in decline	1.78 (0.44, 7.26)	0.41	1.19 (0.35, 4.08)	0.78	2.55 (0.05. 129)	0.64
Skin							
	Peginterferon-lambda	0.89 (0.57, 1.38)	0.59	0.79 (0.37, 1.67)	0.53	0.40 (0.02, 9.51)	0.37
	Placebo	0.88 (0.57, 1.35)	0.56	0.50 (0.16, 1.60)	0.24	0.91 (0.73, 1.13)	0.22
	Difference in decline	0.76 (0.097, 5.92)	0.79	1.90 (0.075, 48.1)	0.69		0.15
Neurologic/Vascular							
	Peginterferon-lambda	0.68 (0.47, 0.98)	0.037	0.72 (0.53, 0.98)	0.034	0.63 (0.48, 0.83)	0.001
	Placebo	0.60 (0.36, 0.98)	0.043	0.63 (0.43, 0.93)	0.019	0.51 (0.34, 0.76)	0.001
	Difference in decline	5.07 (0.24, 108)	0.30	2.61 (0.074, 92.8)	0.60	1.60 (0.01, 266)	0.86
Mood							
	Peginterferon-lambda	0.59 (0.29, 1.20)	0.14	0.64 (0.35, 1.19)	0.16	0.56 (0.30, 1.07)	0.077
	Placebo	0.34 (0.12, 0.98)	0.046	0.38 (0.13, 1.10)	0.074	0.52 (0.29, 0.93)	0.029
	Difference in decline	0.90 (0.097, 8.4)	0.93	1.29 (0.065, 25.6)	0.87	0.32 (0.02, 4.71)	0.40

***** Difference in decline indicates the odds ratio for the difference in rate of improvement between peginterferon-lambda and placebo. Values greater than 1 indicate more rapid improvement with peginterferon-lambda treatment than with placebo.

Table S4. The specific severe symptoms reported by participants in each treatment group.

PegIFN Lambda	ID 3	ID 18	ID 9	ID 15	ID 31	ID 47	ID 52
Day 0	throat			smell	smell	smell	smell
Day 0.5	throat					smell	
Day 1					smell	smell	
Day 2	fever				smell	smell	
Day 3	cough					smell	
Day 4							
Day 5			smell				
Day 6		cough, SOB	smell				
Day 7							
Day 10			smell				
Day 12			smell				
Day 14		cough, SOB					

Placebo	ID 5	ID 11	ID 16	ID 27	ID 33	ID 51	ID 60
Day 0	chills		fever, smell, nausea	fatigue	cough, fever, chills, nausea, vomit	smell	
Day 0.5					cough, throat	fatigue, headache	
Day 1					cough, fever, chills, throat, myalgia, abdo, vomit	smell	
Day 2			fatigue, nausea			fatigue, smell	
Day 3			fatigue, nausea			fatigue, smell	
Day 4		fatigue	fatigue				
Day 5		fatigue					cough
Day 6					diarrhea		
Day 7							
Day 10							
Day 12							
Day 14							

Smell – loss of sense of smell/taste, SOB – shortness of breath; abdo – abdominal pain

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