

**Supplementary Material.**

**Temporary methotrexate discontinuation for two weeks improves immunogenicity of seasonal influenza vaccination in patients with rheumatoid arthritis: a randomized clinical trial.**

Jin Kyun Park, Yun Jong Lee, Kichul Shin, Eun-Ha Kang, You-Jung Ha, Yun Jong Lee, Eun Young Lee, Yeong Wook Song, Yunhee Choi, Kevin L Winthrop, Eun Bong Lee.

**Supplementary table 1.** Antibody titers, fold-changes and vaccine response before and after vaccination with patients according to seroprotection.

**Supplementary table 2.** Antibody titers, fold-changes and vaccine response before and after vaccination to individual vaccine strains in Per-Protocol population.

**Supplementary figure 1.** Study design.

**Supplementary figure 2.** Satisfactory response in Per-Protocol population

**Supplementary table 1.** Antibody titers, fold-changes and vaccine response before and after vaccination with patients according to baseline seroprotection status in MITT population.

	Seroprotection negative			Seroprotection positive		
	Group 1	Group 2	p	Group 1	Group 2	P
<b>H1N1</b>	N= 118	N= 114		N=38	N=46	
Pre-vacc titer, GMT	9.5 (8.5 - 10.6)	9.8 (8.7 – 11.0)	0.692	59.5 (51.1 - 69.3)	56.6 (48.7 - 65.7)	0.637
Post-vacc titer, GMT	57.4 (45.7 - 72.1)	100.0 (79.2 - 126.4)	<b>0.001</b>	118.1 (92.4 - 150.9)	132.2 (104.3 - 167.6)	0.508
Fold increase, GM	6.1 (4.7 - 7.8)	10.2 (7.9 - 13.3)	<b>0.005</b>	2.0 (1.6 - 2.4)	2.3 (1.9 - 2.9)	0.291
Response, n (%)	71 (60.2)	83 (72.8)	0.052	8 (21.1)	17 (37.0)	0.151
Pre-vacc SP, n (%)	0 (0)	0 (0)		38 (100)	46 (100)	1.000
Post-vacc SP, n (%)	81 (68.6)	92 (80.7)	<b>0.049</b>	37 (97.4)	46 (100)	0.268
<b>H3N2</b>	N=135	N=139		N=21	N=21	
Pre-vacc titer, GMT	7.5 (6.9 - 8.2)	8.0 (7.2 - 8.8)	0.403	69.2 (50.0 - 95.7)	69.5 (50.2 - 96.3)	0.980
Post-vacc titer, GMT	37.6 (30.4 - 46.4)	75.9 (61.4 - 93.8)	<b>&lt;0.001</b>	119.5 (89.7 - 159.4)	168.6 (112.3 - 253.2)	0.158
Fold increase, GM	5.0 (4.0 - 6.2)	9.5 (7.6 - 11.9)	<b>&lt;0.001</b>	1.7 (1.3 - 2.3)	2.4 (1.4 - 4.1)	0.235
Response, n (%)	81 (60.0)	108 (77.7)	<b>0.002</b>	4 (19.0)	6 (28.6)	0.719
Pre-vacc SP, n (%)	0 (0)	0 (0)	0	21 (100)	21 (100)	1.000
Post-vacc SP, n (%)	76 (56.3)	105 (75.5)	<b>0.001</b>	21 (100)	20 (95.2)	0.311
<b>B-Yamagata</b>	N=96	N=109		N=60	N=51	
Pre-vacc titer, GMT	10.7 (9.4 - 12.1)	12.6 (11.3 – 14.0)	0.045	73.4 (62.2 - 86.6)	60.9 (52.8 - 70.3)	0.096
Post-vacc titer, GMT	44.7 (34.5 - 57.8)	90.4 (72.7 - 112.4)	<b>&lt;0.001</b>	145.7 (118.0 – 180.0)	195.8 (155.7 - 246.2)	0.060

Fold increase, GM	4.2 (3.2 - 5.5)	7.2 (5.8 - 8.9)	<b>0.002</b>	2.0 (1.6 - 2.4)	3.2 (2.6 - 4.0)	<b>0.001</b>
Response, n (%)	49 (51.0)	76 (69.7)	<b>0.007</b>	17 (28.3)	28 (54.9)	<b>0.006</b>
Pre-vacc SP, n (%)	96 (100)	109 (100)	1.000	60 (100)	51 (100)	1.000
Post-vacc SP, n (%)	57 (59.4)	90 (82.6)	<b>&lt;0.001</b>	59 (98.3)	51 (100)	0.354
<b>B-Victoria</b>	N=123	N=139		N=33	N=21	
Pre-vacc titer, GMT	9.9 (8.9 - 10.9)	9.4 (8.5 - 10.3)	0.479	49.1 (43.0 - 56.1)	49.7 (41.0 - 60.3)	0.910
Post-vacc titer, GMT	33.3 (27.4 - 40.5)	57.8 (49.3 - 67.8)	<b>&lt;0.001</b>	74.7 (57.2 - 97.6)	165.0 (118.0 - 230.5)	<b>&lt;0.001</b>
Fold increase, GM	3.4 (2.8 - 4.1)	6.2 (5.2 - 7.3)	<b>&lt;0.001</b>	1.5 (1.2 - 2)	3.3 (2.5 - 4.4)	<b>&lt;0.001</b>
Response, n (%)	60 (48.8)	104 (74.8)	<b>&lt;0.001</b>	4 (12.1)	14 (66.7)	<b>&lt;0.001</b>
Pre-vacc SP, n (%)	0 (0)	0 (0)	1.000	33 (100)	21 (100)	1.000
Post-vacc SP, n (%)	64 (52.0)	100 (71.9)	<b>&lt;0.001</b>	31 (93.9)	21 (100)	0.250

Data are expressed in n (%) or value (95% CI). Antibody titers and fold increase are in geometric mean titer (GMT). Satisfactory vaccine response (i.e. response = seroconversion) was defined as a  $\geq 4$ -fold improvement in titers relative to baseline. Seroprotection was defined as titers of  $\geq 1:40$ . P values were generated by independent t-test for continuous variables and chi-square test for categorical variables.

CI, confidential interval; GM, geometric mean; GMT, geometric mean titer; n, number; Pre-SP, prevaccination seroprotection rate; Post-SP, postvaccination seroprotection rate.

**Supplementary table 2.** Antibody titers, fold-changes and vaccine response before and after vaccination to individual vaccine strains in Per-Protocol population.

	<b>MTX continue</b>	<b>MTX hold</b>	<b>P</b>
	<b>(n=154)</b>	<b>(n=157)</b>	
<b>H1N1</b>			
Pre-vacc titer, GMT (95% CI)	14.8 (12.7 - 17.3)	16.2 (13.9 - 18.9)	0.329
Post-vacc titer, GMT (95% CI)	68.4 (56.8 - 82.4)	108.4 (90.7 - 129.5)	<b>0.001</b>
Fold increase, GM (95% CI)	4.6 (3.7 - 5.7)	6.7 (5.4 - 8.3)	<b>0.035</b>
Response, n (%)	78 (50.6)	97 (61.8)	<b>0.048</b>
Pre-vacc SP, n (%)	37 (24.0)	46 (29.3)	0.293
Post-vacc SP, n (%)	116 (75.3)	135 (86.0)	<b>0.017</b>
<b>H3N2</b>			
Pre-vacc titer, GMT (95% CI)	10.2 (8.8 - 11.8)	10.6 (9.1 - 12.3)	0.755
Post-vacc titer, GMT (95% CI)	43.9 (36.1 - 53.4)	84.3 (69.3 - 102.4)	<b>&lt;0.001</b>
Fold increase, GM (95% CI)	4.3 (3.5 - 5.3)	8 (6.4 - 9.9)	<b>&lt;0.001</b>
Response, n (%)	83 (53.9)	111 (70.7)	<b>0.002</b>
Pre-vacc SP, n (%)	21 (13.6)	20 (12.7)	0.815
Post-vacc SP, n (%)	97 (63.0)	123 (78.3)	<b>0.003</b>
<b>B-Yamagata</b>			
Pre-vacc titer, GMT (95% CI)	22.4 (18.7 - 26.7)	20.8 (18.1 - 24)	0.720
Post-vacc titer, GMT (95% CI)	70.4 (57.8 - 85.7)	115.6 (97.4 - 137.3)	<b>&lt;0.001</b>
Fold increase, GM (95% CI)	3.1 (2.6 - 3.8)	5.6 (4.7 - 6.6)	<b>&lt;0.001</b>
Response, n (%)	66 (42.9)	101 (64.3)	<b>&lt;0.001</b>
Pre-vacc SP, n (%)	59 (38.3)	51 (32.5)	0.283
Post-vacc SP, n (%)	115 (74.7)	139 (88.5)	<b>0.002</b>

**B-Victoria**

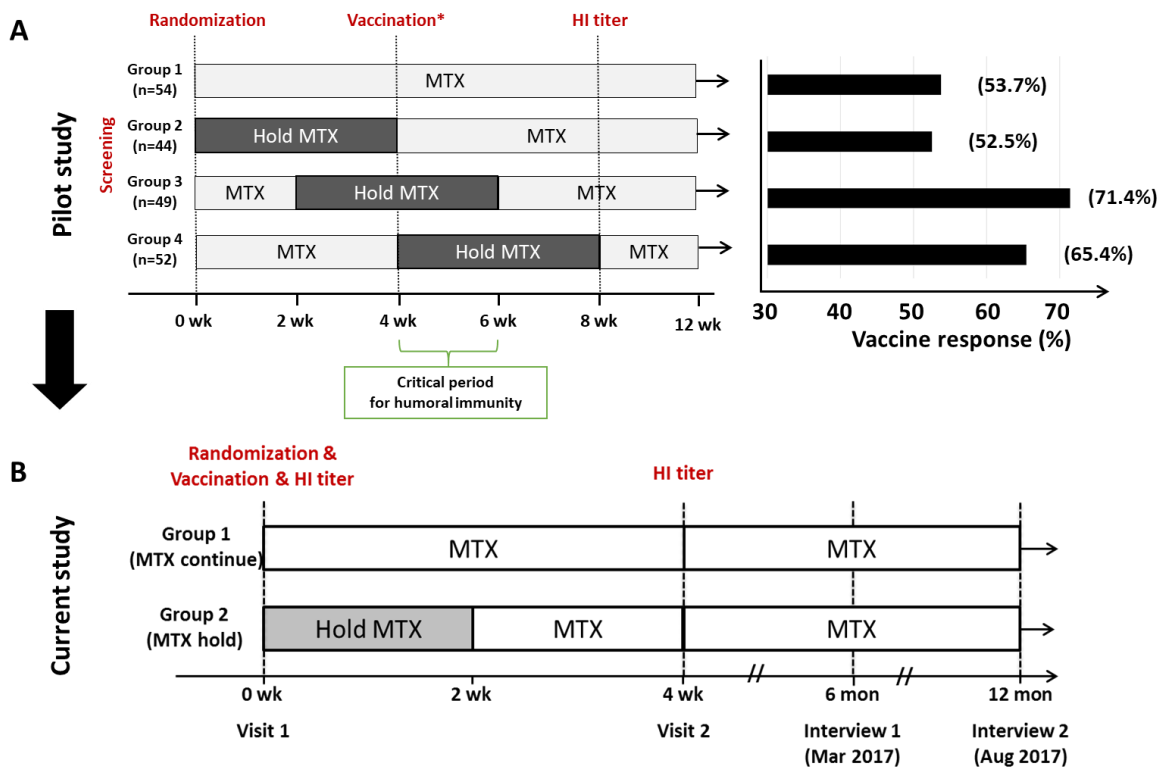
Pre-vacc titer, GMT (95% CI)	13.8 (12.1 - 15.8)	11.7 (10.3 - 13.2)	0.083
Post-vacc titer, GMT (95% CI)	39.5 (33.3 - 46.9)	66.3 (56.8 - 77.4)	<b>&lt;0.001</b>
Fold increase, GM (95% CI)	2.9 (2.4 - 3.4)	5.7 (4.9 - 6.7)	<b>&lt;0.001</b>
Response, n (%)	64 (41.6)	115 (73.2)	<b>&lt;0.001</b>
Pre-vacc SP, n (%)	32 (20.8)	21 (13.4)	0.083
Post-vacc SP, n (%)	94 (61.0)	119 (75.8)	<b>0.005</b>

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Data are expressed in n (%) or value (95% CI). Antibody titers and fold increase are in geometric mean titer (GMT). Satisfactory vaccine response (i.e. response = seroconversion) was defined as a  $\geq 4$ -fold improvement in titers relative to baseline. Seroprotection was defined as titers of  $\geq 1:40$ . P values were generated by independent t-test for continuous variables and chi-square test for categorical variables.

CI, confidential interval; GM, geometric mean; GMT, geometric mean titer; n, number; Pre-SP, prevaccination seroprotection rate; Post-SP, postvaccination seroprotection rate.

**Supplementary figure 1. Study rationale and design.** (A) A pilot study was conducted to estimate the efficacy and time of temporary MTX discontinuation to improve vaccine response. Group 2, where MTX was discontinued before vaccination, did not improve in vaccination response, suggesting that MTX discontinuation before the vaccination did not affect vaccine response. Groups 3 and 4, where MTX was stopped after vaccination, did show higher vaccine response than the reference group 1 (MTX continue group). Therefore, the “overlapping” 2 weeks after vaccination in Groups 3 and 4 is the critical period for vaccine response. (B) In this trial, patients were randomized to continue or hold MTX for 2 weeks after vaccination. HI=hemagglutination inhibition; MTX=methotrexate; mon=months; wk=week.



Supplementary figure 2. Satisfactory response in Per-Protocol population

