

1 ONLINE REPOSITORY:

2 ***Lolium perenne* Peptides for the Treatment of Grass Pollen Allergy: A Randomized**
3 **Double Blind Placebo-Controlled Clinical Trial**

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Table E1. Demographic characteristics of blood sample donors for *ex vivo* study

Variable	Non allergic N=12	Allergic N=12
Age (y), mean $\pm$ SD	29.6 $\pm$ 5.8	28.9 $\pm$ 8.2
Gender (Male:Female)	9:3	5:7
Skin Prick Test to grass pollen (mm), median and IQR	0.00 (0.0 - 0.0)	5.50 (4.3 - 6.8)

Abbreviations: IQR, interquartile range.

Table E2A. Allergenicity of LP Proteins, LPP, DnaK and LPP/DnaK in allergic subjects

Concentration (ng/ μ L)	mean $\pm$ SEM			
	LP Proteins	LPP	DnaK	LPP/DnaK
0	5.222 $\pm$ 2.257	5.222 $\pm$ 2.257	5.222 $\pm$ 2.257	5.222 $\pm$ 2.257
1	5.023 $\pm$ 1.346	3.177 $\pm$ 0.7998	2.079 $\pm$ 0.4251	1.858 $\pm$ 0.4536
10	17.84 $\pm$ 7.584	2.358 $\pm$ 0.5004	1.683 $\pm$ 0.2736	1.588 $\pm$ 0.3409
100	47.28 $\pm$ 8.597**	7.284 $\pm$ 2.753	2.076 $\pm$ 0.429	2.733 $\pm$ 0.8959
1000	52.75 $\pm$ 7.557**	20.3 $\pm$ 6.755	2.569 $\pm$ 0.7748	12.95 $\pm$ 6.122

*signifies comparison with 0; ** $P < 0.01$

Table E2B. Allergenicity of LPP, DnaK and LPP/DnaK compared to LP Proteins in allergic subjects

Concentration (ng/ μ L)	mean $\pm$ SEM			
	LP Proteins	LPP	DnaK	LPP/DnaK
0	5.222 $\pm$ 2.257	5.222 $\pm$ 2.257	5.222 $\pm$ 2.257	5.222 $\pm$ 2.257
1	5.023 $\pm$ 1.346	3.177 $\pm$ 0.7998	2.079 $\pm$ 0.4251	1.858 $\pm$ 0.4536 ^o
10	17.84 $\pm$ 7.584	2.358 $\pm$ 0.5004 ^o	1.683 $\pm$ 0.2736 ^{oo}	1.588 $\pm$ 0.3409 ^{oo}
100	47.28 $\pm$ 8.597	7.284 $\pm$ 2.753 ^{ooo}	2.076 $\pm$ 0.429 ^{ooo}	2.733 $\pm$ 0.8959 ^{ooo}
1000	52.75 $\pm$ 7.557	20.3 $\pm$ 6.755 ^{ooo}	2.569 $\pm$ 0.7748 ^{ooo}	12.95 $\pm$ 6.122 ^{ooo}

^osignifies comparison with LP Proteins; ^o $P < 0.05$, ^{oo} $P < 0.01$, ^{ooo} $P < 0.001$

90 Table E3A. Immunogenicity of LP Proteins, LPP, DnaK and LPP/DnaK in allergics and non-allergics

		mean ± SEM						
Concentration (ng/mL)	Allergics				Non-Allergics			
	LP Proteins	LPP	DnaK	LPP/DnaK	LP Proteins	LPP	DnaK	LPP/DnaK
<i>Proliferation (SI)</i>								
0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
3	5.482 ±	1.509 ±	1.109 ±	2.1 ±	3.075 ±	1.15 ± 0.1525	1.042 ±	1.475 ±
	1.557***	0.2855	0.09091	0.4016**	0.6256**		0.1209	0.1907*
10	9.242 ±	2.083 ±	1.208 ±	2.858 ±	8.4 ±	1.15 ± 0.1555	1.225 ±	1.525 ±
	1.355***	0.5337**	0.1111	0.5159***	1.367***		0.1737	0.3451
30	12.25 ±	2.683 ±	1.6 ± 0.1927*	4.592 ±	8.75 ±	1.158 ±	1.625 ±	2.858 ±
	1.918***	0.7799**		1.016***	1.013***	0.1485	0.2837	0.6103***
<i>IL-5 (pg/mL)</i>								
0	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307
3	40.55 ±	16.91 ±	9.957 ±	15.85 ±	9.548 ±	11.43 ±	15.92 ±	8.765 ±
	14.61**	3.329**	2.114*	5.097**	1.007*	1.765***	2.139***	1.081*
10	22.64 ±	21.13 ± 7.331	5.686 ±	30.28 ± 12.6	9.1 ± 2.285	6.267 ±	6.074 ±	6.092 ±
	6.798*		0.9235			0.9554	0.8506	0.7137
30	17.36 ± 5.903	27.02 ± 12.32	5.328 ± 1.079	25.28 ± 12	7.745 ± 1.19	4.337 ±	7.029 ±	6.708 ±
						0.3504	0.7648	0.7701
<i>IFN-g (pg/mL)</i>								
0	3.167 ±	3.167 ±	3.167 ±	3.167 ±	3.167 ±	3.167 ±	3.167 ±	3.167 ±
	0.6368	0.6368	0.6368	0.6368	0.6368	0.6368	0.6368	0.6368
3	103.9 ±	10.29 ±	10.87 ±	15.4 ±	90.19 ±	5.628 ± 1.823	10.08 ± 6.278	12.65 ±
	32.94***	3.011***	4.652*	5.667**	30.55***			6.428*
10	238.3 ±	8.135 ± 3.578	6.385 ±	18.62 ±	244.4 ±	4.648 ± 1.59	15.83 ± 12.64	13.27 ± 5.148
	24.96***		1.701*	8.648**	29.59***			
30	293.5 ±	21.77 ±	31.98 ±	41.74 ±	298.4 ±	14.77 ± 9.746	22.9 ±	36.01 ±
	6.468***	12.28***	13.04**	21.37***	1.594***		10.53**	24.28**

91 *signifies comparison with 0 per group; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

92 Table E3B. Immunogenicity of LP Proteins, LPP, DnaK and LPP/DnaK in non-allergics compared to allergics

Concentration (ng/mL)	mean ± SEM							
	Allergics				Non Allergics			
	LP Proteins	LPP	DnaK	LPP/DnaK	LP Proteins	LPP	DnaK	LPP/DnaK
<i>Proliferation (SI)</i>								
0	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00	1.00 ± 0.00
3	5.482 ± 1.557	1.509 ± 0.2855	1.109 ± 0.09091	2.1 ± 0.4016	3.075 ± 0.6256	1.15 ± 0.1525	1.042 ± 0.1209	1.475 ± 0.1907
10	9.242 ± 1.355	2.083 ± 0.5337	1.208 ± 0.1111	2.858 ± 0.5159	8.4 ± 1.367	1.15 ± 0.1555 ^ω	1.225 ± 0.1737	1.525 ± 0.3451 ^ω
30	12.25 ± 1.918	2.683 ± 0.7799	1.6 ± 0.1927	4.592 ± 1.016	8.75 ± 1.013	1.158 ± 0.1485 ^ω	1.625 ± 0.2837	2.858 ± 0.6103
<i>IL-5 (pg/mL)</i>								
0	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307	4.5 ± 1.307
3	40.55 ± 14.61	16.91 ± 3.329	9.957 ± 2.114	15.85 ± 5.097	9.548 ± 1.007	11.43 ± 1.765	15.92 ± 2.139	8.765 ± 1.081
10	22.64 ± 6.798	21.13 ± 7.331	5.686 ± 0.9235	30.28 ± 12.6	9.1 ± 2.285	6.267 ± 0.9554	6.074 ± 0.8506	6.092 ± 0.7137
30	17.36 ± 5.903	27.02 ± 12.32	5.328 ± 1.079	25.28 ± 12	7.745 ± 1.19	4.337 ± 0.3504	7.029 ± 0.7648 ^{ωω}	6.708 ± 0.7701
<i>IFN-g (pg/mL)</i>								
0	3.167 ± 0.6368	3.167 ± 0.6368	3.167 ± 0.6368	3.167 ± 0.6368	3.167 ± 0.6368	3.167 ± 0.6368	3.167 ± 0.6368	3.167 ± 0.6368
3	103.9 ± 32.94	10.29 ± 3.011	10.87 ± 4.652	15.4 ± 5.667	90.19 ± 30.55	5.628 ± 1.823 ^ω	10.08 ± 6.278	12.65 ± 6.428
10	238.3 ± 24.96	8.135 ± 3.578	6.385 ± 1.701	18.62 ± 8.648	244.4 ± 29.59	4.648 ± 1.59	15.83 ± 12.64	13.27 ± 5.148
30	293.5 ± 6.468	21.77 ± 12.28	31.98 ± 13.04	41.74 ± 21.37	298.4 ± 1.594	14.77 ± 9.746	22.9 ± 10.53	36.01 ± 24.28

93 ^ωsignifies comparison with Allergics; ^ω*P* < 0.05, ^{ωω}*P* < 0.01, ^{ωωω}*P* < 0.001

Table E4. Demographic characteristics of Phase I study

Characteristic	Placebo N=9	LPP N=9	LPP/DnaK N=9
Age (y), mean $\pm$ SD	25.6 $\pm$ 6.8	32.2 $\pm$ 11.4	36.1 $\pm$ 7.7*
Gender, n (%)			
Male	3 (33.3)	4 (44.4)	2 (22.2)
Female	6 (66.7)	5 (55.6)	7 (77.8)
Ethnicity, n (%)			
Caucasian/White	9 (100.0)	9 (100.0)	8 (88.9)
Black	0 (0.0)	0 (0.0)	1 (11.1)
Height (cm), mean $\pm$ SD	172.7 $\pm$ 7.3	176.0 $\pm$ 5.9	172.2 $\pm$ 10.1
Weight (kg), mean $\pm$ SD	63.56 $\pm$ 8.05	71.28 $\pm$ 11.51	72.06 $\pm$ 16.10
Grass pollen specific IgE (kU/L), median and IQR)	15.2 (8.4 – 47.3)	16.1 (4.7 – 43.7)	12.0 (3.9 – 22.7)
Skin Prick Test to grass pollen (mm), median and IQR	10.0 (9.5 – 12.5)	10.5 (9.0 – 12.5)	10.0 (8.0 – 12.5)
Other immune system disorders			
Allergic respiratory symptoms	1 (11.1%)	0	0
Allergy to animal	1 (11.1%)	0	3 (33.3%)
Allergy to plants	1 (11.1%)	0	0
Dermatitis allergic	0	1 (11.1%)	0
Dermatitis contact	0	0	1 (11.1%)
Drug hypersensitivity	1 (11.1%)	0	0
Food allergy	1 (11.1%)	0	2 (22.2%)
House dust mite allergy	1 (11.1%)	0	1 (11.1%)
Oral allergy syndrome	1 (11.1%)	0	0
Other disease			
Depression	0	0	2 (22.2%)
Asthma	2 (22.2%)	0	1 (11.1%)
Exercise-induced asthma	1 (11.1%)	0	0
Dyspnoea	1 (11.1%)	0	0
Eczema	2 (22.2%)	1 (11.1%)	3 (33.3%)
Hypertension	0	1 (11.1%)	0

Values are for the full analysis set. Abbreviations: SD, standard deviation; IQR, interquartile range. * $P = 0.005$. Mann Whitney U test.

106 **Table E5. Adverse Events related to Treatment**
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Event	Placebo N=9	LPP N=9	LPP/DnaK N=9
Nber of unsolicited AEs, n AE	2	16	12
Nber of patient reporting unsolicited AE, n patient (%)	1 (11.1)	7 (77.8)	5 (55.6)
Leukopenia	0 (0.0)	1 (11.1)	0 (0.0)
Palpitations	1 (11.1)	0 (0.0)	0 (0.0)
Injection site pruritus	0 (0.0)	5 (55.6)	3 (33.3)
Allergic cough	0 (0.0)	0 (0.0)	1 (11.1)
Anaphylactic reaction	0 (0.0)	1 (11.1)	0 (0.0)
Seasonal allergy	0 (0.0)	1 (11.1)	0 (0.0)
Neutrophil count decreased	0 (0.0)	1 (11.1)	0 (0.0)
Hypoaesthesia	1 (11.1)	0 (0.0)	1 (11.1)
Cough	0 (0.0)	1 (11.1)	0 (0.0)
Pruritus	0 (0.0)	2 (22.2)	1 (11.1)
AEs of special interest, n patient (%)	3 (33.3)	4 (44.4)	7 (77.8)
Headache	1 (11.1)	1 (11.1)	4 (44.4)
Muscle pain	0 (0.0)	0 (0.0)	2 (22.2)
Fatigue	0 (0.0)	3 (33.3)	2 (22.2)
Diarrhea	1 (11.1)	0 (0.0)	0 (0.0)
Difficulty breathing	1 (11.1)	0 (0.0)	0 (0.0)
Urticaria	0 (0.0)	1 (11.1)	1 (11.1)
AEs leading to study discontinuation, n patient (%)	0 (0.0)	0 (0.0)	0 (0.0)
Deaths, n (%)	0 (0.0)	0 (0.0)	0 (0.0)

108 Values are for the full analysis set (identical to Safety Set)
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119 Table E6. Safety parameters

	Placebo (mean ± SD) N=9		LPP (mean ± SD) N=9		LPP / DnaK (mean ± SD) N=9	
	Visit 1	Visit 6	Visit 1	Visit 6	Visit 1	Visit 6
<i>Display of Haematology data</i>						
Haematology - Values over Time - Safety Population						
Hemoglobin (g/dL)	137.7 ± 15.3	135.8 ± 15.7	145.8 ± 13.9	143.2 ± 15.1	138.2 ± 14.1	137.8 ± 12.5
Hematocrit (Fraction of 1)	0.4086 ± 0.0408	0.4002 ± 0.0413	0.4316 ± 0.0307	0.4266 ± 0.0347	0.4094 ± 0.0369	0.4053 ± 0.0331
Erythrocytes (10**12/L)	4.591 ± 0.478	4.513 ± 0.380	4.980 ± 0.455	4.889 ± 0.435	4.731 ± 0.459	4.673 ± 0.375
Leukocytes (10**9/L)	5.72 ± 0.90	6.11 ± 1.14	5.86 ± 2.04	5.87 ± 1.89	6.13 ± 1.41	6.24 ± 1.23
Basophils (%)	0.444 ± 0.194	0.500 ± 0.308	0.656 ± 0.354	0.700 ± 0.604	0.733 ± 0.527	0.589 ± 0.232
Eosinophils (%)	3.111 ± 2.103	4.800 ± 3.744*	2.267 ± 1.531	3.922 ± 3.124	3.878 ± 3.068	3.956 ± 2.470
Monocytes (%)	7.256 ± 1.700	7.600 ± 1.904	7.589 ± 2.322	6.378 ± 1.690	6.111 ± 1.043	6.678 ± 1.516
Lymphocytes (%)	32.133 ± 7.498	28.911 ± 5.216	27.967 ± 5.554	29.556 ± 7.560	32.033 ± 9.244	32.978 ± 8.266
Neutrophils (%)	57.056 ± 8.883	58.189 ± 7.241	61.522 ± 5.746	59.444 ± 10.488	57.356 ± 10.565	55.800 ± 7.770
Platelets (10**9/L)	253.1 ± 66.2	258.1 ± 54.2	236.0 ± 74.9	248.8 ± 74.2	271.1 ± 37.3	281.6 ± 59.4
<i>Display of Biochemistry data</i>						
Biochemistry - Values over Time - Safety Population						
Glucose (mmol/L)	5.186 ± 0.892	4.724 ± 0.570	5.081 ± 0.583	5.100 ± 0.559	5.828 ± 1.344	5.063 ± 0.710
Creatinine (umol/L)	72.783 ± 10.772	72.193 ± 11.424	74.747 ± 15.061	74.551 ± 13.738	71.899 ± 11.138	68.854 ± 8.227
Urea (mmol/L)	11.11 ± 3.23	9.68 ± 2.80	10.43 ± 1.96	10.79 ± 2.99	10.75 ± 2.44	10.12 ± 1.10
Cholesterol (mmol/L)	4.849 ± 0.710	4.616 ± 0.772	4.607 ± 0.659	4.504 ± 0.556	4.748 ± 0.654	4.812 ± 0.820
Triglycerides (mmol/L)	1.141 ± 0.372	1.115 ± 0.346	1.225 ± 0.929	1.084 ± 0.425	1.224 ± 0.518	1.130 ± 0.723
Sodium (mmol/L)	141.90 ± 1.29	140.24 ± 1.11	142.52 ± 1.94	141.61 ± 1.78	142.14 ± 1.78	140.32 ± 1.75
Potassium (mmol/L)	4.050 ± 0.340	3.970 ± 0.351	3.890 ± 0.245	4.177 ± 0.256	3.907 ± 0.156	4.223 ± 0.268
Chloride (mmol/L)	105.41 ± 2.09	104.61 ± 2.04	105.47 ± 1.22	105.27 ± 1.38	105.50 ± 1.50	105.29 ± 2.53
Calcium (mmol/L)	2.320 ± 0.061	2.280 ± 0.072	2.349 ± 0.057	2.309 ± 0.079	2.324 ± 0.035	2.290 ± 0.054
Total protein (g/L)	74.9 ± 3.1	71.8 ± 3.6	75.7 ± 2.0	73.1 ± 3.1	75.0 ± 3.4	73.9 ± 2.8
Albumin (g/L)	49.46 ± 3.14	47.27 ± 3.40	48.30 ± 2.44	48.22 ± 3.99	47.89 ± 2.48	47.72 ± 2.31
Aspartate Aminotransferase (IU/L)	21.6 ± 5.3	21.0 ± 6.1	20.2 ± 4.8	19.9 ± 4.1	17.9 ± 2.4	19.9 ± 2.8
Alanine Aminotransferase (IU/L)	19.1 ± 7.9	17.4 ± 7.8	18.6 ± 9.8	16.2 ± 7.3	15.9 ± 3.8	17.0 ± 6.2
Gamma Glutamyl Transferase (IU/L)	17.0 ± 6.2	14.9 ± 6.9	17.3 ± 4.6	16.0 ± 4.5	15.3 ± 4.0	15.7 ± 4.9

Total bilirubin (μmol/L)	7.429 ± 4.205	6.726 ± 3.440	12.027 ± 8.920	11.742 ± 10.715	10.469 ± 4.979	12.065 ± 8.548
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Display of Immunological parameter data

Immunological parameters - Total IgE and Total IgG - Values over Time - Safety Population

Total IgE (kU/L)	159.6 ± 128.9	162.0 ± 140.1	182.6 ± 160.2	192.4 ± 166.8	197.8 ± 349.3	170.4 ± 242.1
Total IgG (g/L)	11.888 ± 2.821	11.083 ± 2.747	11.411 ± 1.885	11.076 ± 2.428	12.153 ± 2.711	11.494 ± 2.239

Display of Inflammatory parameter data

Inflammatory parameters - Values over Time - Safety Population

Erythrocyte sedimentation rate (mm/hour)	5.1 ± 4.1	5.2 ± 3.4	10.6 ± 10.3	7.7 ± 6.6	7.7 ± 4.1	8.6 ± 4.9
C-reactive protein (mg/L)	2.42 ± 3.21	1.93 ± 1.88	2.88 ± 3.24	2.11 ± 3.05	1.90 ± 2.20	2.04 ± 1.58

**P* < 0.05 compared to Visit 1 (paired T test)

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Table E7. Clinical assessments over the grass pollen season

Measure	Placebo N=9	LPP N=6	LPP/DnaK N=6
Average combined score (ACS)			
Mean $\pm$ SD	0.479 $\pm$ 0.298	0.312 $\pm$ 0.284	0.747 $\pm$ 0.511
Relative change vs. placebo		-34.8%	55.8%
P-value		0.316	0.376
Rhinoconjunctivitis score (RTSS)			
Mean $\pm$ SD	3.49 $\pm$ 1.95	2.46 $\pm$ 2.19	5.45 $\pm$ 4.13
Relative change vs. placebo		-29.7%	56.1%
P-value		0.331	0.458
Rescue medication score (RMS)			
Mean $\pm$ SD	0.377 $\pm$ 0.330	0.215 $\pm$ 0.213	0.585 $\pm$ 0.414
Relative change vs. placebo		-42.8%	55.4%
P-value		0.464	0.365
Total symptom score (TSS)			
Mean $\pm$ SD	3.89 $\pm$ 2.22	2.63 $\pm$ 2.22	6.45 $\pm$ 5.98
Relative change vs. placebo		-32.4%	65.6%
P-value		0.234	0.659
Well days			
Mean $\pm$ SD	14.67 $\pm$ 9.79	20.83 $\pm$ 12.67	8.33 $\pm$ 11.36
Relative change vs. placebo		42.1%	-43.2%
P-value		0.414	0.220

Values are for the per-protocol analysis set. Abbreviation: SD, standard deviation.