FINAL REPORT: Clinical and Safety

A phase 2, single-centre, double blind, randomised, placebo-controlled, study testing the primary prevention of persistent asthma in high risk children by protection against acute respiratory infections during early childhood using OM-85

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Principal Investigators:

Peter D Sly AO, MBBS, MD, FRACP, DSc

Patrick G Holt, PhD, DSc

Background

Asthma imposes a huge social and economic burden on our community, accounting for approximately 1.4% of the total expenditure on health in Australia. Overall, approximately 25% of total asthma expenditure is for children and almost half of hospital expenditure for asthma is on children. Children with early onset asthma in the preschool years show the highest risk for persistence into later childhood and adulthood (1). The major risk factors for persistent asthma are: a family history of asthma and allergies (genetic predisposition); allergic sensitization to aeroallergens in early life; and recurrent severe symptomatic lower respiratory infections (sLRI), i.e. those associated with fever and/or wheeze in the first 1-3 years of life (2-6). The long-term solution to the burden of disease imposed by asthma is to prevent asthma from progressing to the persistent form; yet current treatment options provide only symptomatic relief, require continuous use and do not have any truly disease modifying effects.

Systematic testing of the hypothesis that preventing sLRI will prevent persistent asthma is limited by the availability of anti-viral therapies with proven efficacy and safety in young children. However an emerging class of therapeutics which is showing significant promise in this regard is microbial-derived "immunomodulators" which act via stimulation of systemic T-regulatory functions that can dampen inflammation in peripheral tissues. A previous Cochrane review (7) using data from 35 placebo-controlled trials including 4060 participants below the age of 18 years in which various types of "immunostimulants" were used to reduce acute respiratory infections (ARI), involving either upper or lower airways concluded that immunostimulants reduced the incidence of ARI by 40% on average in susceptible children, but that trial quality was generally poor and a "high level of statistical heterogeneity was evident". The orally-delivered bacterial-derived immunomodulator OM-85 has a long history of successful use in Europe in prevention of pathogen-triggered wheezing episodes in infants and children. However this agent has not as yet been trialled in the subgroup at high risk of development of persistent asthma, notably infants with positive atopic family history who are exhibiting early atopy manifestations. In addition, the main effect documented has been on the frequency and rates of ARI and effects on reducing the severity of ARIs is less clear.

Published clinical trials have reported OM-85 to be well tolerated in children. Discontinuation of drug with an adverse event has been reported in three studies; two children from two separate studies discontinued drug due to rash or diarrhoea, and a study in adults with chronic obstructive pulmonary disease or chronic bronchitis reported 5 participants in the active group (n=142) and 9 participants in the placebo group (n=131) discontinuing investigational product. In most clinical trials the number of participants experiencing adverse events were no different between the active (OM-85) and placebo groups (7). In administration of OM-85 to children under 12 years of age, the most common adverse events reported (as total of all studies referenced) in the literature were gastrointestinal (1.6%: including diarrhoea, abdominal pain, gastritis, gastroenteritis, melena and vomiting); skin and subcutaneous disorders (0.9%: including papular rash, rash and eczema); and general disorders and administration site conditions (0.7%: including fatigue, fever, allergic reaction, hernia, asthenia and

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adynamia) (7). In the same groups respiratory and thoracic mediastinal disorders (0.3%) and renal and urinary disorders (0.3%) were uncommon (7). Two studies reported no adverse events in either active or placebo, and one study only reported adverse events in the placebo group. In older children and adults, the most common reported adverse events were gastrointestinal, including nausea and diarrhoea. Six studies reported no adverse events.

Study population
The study population included children aged three to nine months old at inclusion whose biological mother, father, or sibling has a well-documented history of asthma and/or atopy (defined as skin prick test (SPT) reactivity to one or more allergen, food allergy, atopic dermatitis and/or allergic rhinitis). Power calculations (see below) determined that 60 children (30 per group) would be recruited and randomised.

Participants who meet ***all*** of the following criteria are ***eligible*** for enrolment:
1. Children of either sex, aged 3-9 months old whose biological mother, father, or sibling has a history of asthma and/or atopy (defined as SPT reactivity to one or more allergen, food allergy, atopic dermatitis, allergic rhinitis),
2. Participants who, in the opinion of the investigator, are able to comply with the protocol for its duration,
3. Written informed consent signed and dated by parent/legal guardian according to local regulations.

Participants who meet ***any*** of these criteria are ***not*** eligible for enrolment:
1. Children born less than 36 weeks gestation,
2. Children who have been diagnosed with asthma,
3. Children who have chronic pulmonary disease or other chronic disease (other than atopic dermatitis, food allergy, or chronic rhinitis) requiring therapy,
4. Participation in another randomized controlled trial within the 3 months preceding inclusion in this study,
5. Children who have previously received OM-85 or other immunostimulant or immunosuppressive drugs including cyclosporine.

Participants who withdrew consent or who discontinued the study for any reason were not replaced.

Study objectives
The primary objective of the study was to determine whether OM-85 protected high risk children against sLRI in early postnatal life.

The secondary objectives of this study were to assess whether treating children at high risk of developing asthma with OM-85:
- Decreased the frequency of sLRI in early life,
- Prevented the development of asthma during early life,
- Improved lung function in early life.

Mechanistic objectives of this study were to determine if OM-85 modulates:

- Microbial colonization of the nose,
- Sensitisation to perennial aeroallergens,
- The number and/or function capacity of circulating lung-homing T regulatory cells.

The safety objective of this study was to determine whether administration of OM-85 to infants is safe.

Study Design
This study was conducted as a single-centre, double-blind, randomized, placebo-controlled study testing the efficacy of OM-85 treatment during first two winter seasons for the reduction of sLRIs in high risk children. Eligible children were randomized into two groups. The "Active" group will

receive OM-85 during the first two winter viral seasons (April – August) of their life. The "Control" group will receive matched placebo.

Participants were randomised into one of the following two groups on a 1:1 ratio with 30 participants per group: Active Group: OM-85 3.5mg given as five blocks of 10 days treatment plus 20 days no treatment during the first 2 winter viral seasons of child's life; and Control Group: Matched placebo given as five blocks of 10 days treatment plus 20 days no treatment during the first 2 winter viral seasons of child's life. Capsules were opened by a parent and the contents dissolved in a small amount of liquid (breast milk, water or formula).

Efficacy Endpoints

Primary efficacy endpoint
The primary endpoint is the frequency of sLRI (fever +/- wheeze) over the first two winter viral seasons of the child's life

Secondary endpoints included:
- The time to first sLRI,
- The frequency of sLRI during each winter of the study period,
- The cumulative frequency of sLRI (fever +/- wheeze),
- The proportion of children with a doctor diagnosis of asthma during the first 3 years of life,
- The proportion of children requiring hospitalization for sLRI during the first 3 years of life,
- Incidence of hospitalization/Accident and Emergency department (A&E) visits for a sLRI,
- Number of days hospitalized for sLRI,
- Body mass index at 3 years of age,
- Lung function measures at 3 years of age.

Safety endpoints
- Treatment-related adverse events.

Statistics

Sample size calculations
There were no data in the literature on use of OM-85 for the primary prevention of sLRI, especially in infants. Previous studies in children have demonstrated a reduction in the frequency of wheezing attacks in pre-school age children, a reduction of respiratory infection in children with previous recurrent infections and a reduction in recurrent otitis media in older children. Razi et al demonstrated a group means reduction of 30-40% in wheezing episodes in preschool children with intermittent treatment with OM-85(3.57±1.61 OM-85 vs 5.75±2.71 placebo). Using this reduction in cumulative wheezing frequency over 12 months (-2.18) and the standard deviation of the placebo group (2.71); 26 children per group completing the trial will give 80% to detect a 38% difference in sLRI frequency between the groups. To allow for drop outs (12-15%) 30 children per group, 60 in total will be recruited.

The following groups of participants were predefined for endpoint analysis:
- Intention to Treat (ITT) sample – All participants randomised and who had evaluable data for the endpoint under investigation. Participants were analysed in the group to which they were randomised, regardless of compliance to their allocated treatment. The number of participants who had evaluable data differed for each endpoint being investigated. Parametric or non-parametric tests were used depending on the data distribution.
- Safety sample - All participants who took at least one dose of the investigational product were included in the safety analyses. All AEs reported during the study were included. The frequencies and incidence rates were calculated on a per patient basis.

Statistical analyses
Comparisons of proportions between groups were undertaken using the Fisher Exact test or Chi square for multiple groups. Grouped data were tested for normality using the Shapiro Wilk test. Group comparisons were undertaken using t-tests, Mann-Whitney Rank Sum Test, Kruskal-Wallis one-way

ANOVA on ranks, or Friedman Repeated Measures Analysis of Variance on Ranks, as appropriate. Time to first event analyses were performed using Kaplan-Meier survival analysis with Gehan-Breslow test.

Results

Fifty nine children were recruited and randomized, 29 to OM85 and 30 to placebo. Randomization produced groups with equivalent demographic profiles (table 1).

Table 1: Demographics

	Active (n=29)	**Control (n=30)**	**p**
Age at visit 1 (y), mean ± SD	0.43±0.17	0.49±0.16	0.68[1]
Sex (M:F)	12:17	17:13	0.30[2]
Height at visit 1 (cm), mean ± SD	66.1±4.41	66.6±3.86	0.61[1]
Weight at visit 1 (kg), mean ± SD	7.3±1.52	7.7±1.35	0.36[1]
Prior URTI (n, %)	2 (6.8%)	4 (13.3%)	0.67[2]
Prior LRTI (n, %)	3 (10.3%)	3 (10.0%)	1.00[2]
Prior sLRI (n, %)	2 (6.8%)	0	0.27[2]
First born child (n, %)	14 (48.3%)	19 (63.3%)	0.30[2]
Tobacco smoke exposure (n, %)	4 (13.8%)	3 (10.0%)	0.71[2]
Any pets (n, %)	19 (65.5%)	20 (66.7%)	1.0[2]
Furry pets (n, %)	16 55.2%)	19 (63.3%)	0.50[2]

[1]t-test; [2]Fisher Exact test

Fifty seven children (28 Active, 29 Control) received at least one dose of study treatment and 41 (23 Active, 18 Control) completed the three years of the study. Table 2 shows the number of participants with evaluable data at each time point.

Table 2: Number of participants with evaluable data at each time point.

Randomized (n)	29	30	p[1]
At least one dose of study treatment (n)	28	29	1.0
Evaluable data 1st winter (n)	25	27	1.0
Evaluable data 1st year (n)	25	27	1.0
Evaluable data 2nd winter (n)	24	22	0.53
Evaluable data 2nd year (n)	24	22	0.53
Evaluable data 3rd year (n)	23	18	0.16

[1]Fisher Exact test

Primary outcome

There was no difference in the frequency of sLRI over the first two winters between the groups. Within the Active group 17/24 (70.8%) recorded 37 sLRI [median 1.0 (25%-75% 0.0,2.0)] and 14/22 (63.6%) recorded 47 sLRI [median 1.0 (25%-75% 0.0,4.0)] (p=0.84 Mann-Whitney).

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Secondary outcomes
Time to first sLRI

The time to the first sLRI was significantly longer for participants in the Active group than for participants in the Control group [median 442.0 (25%, 75% >853.0, 124.0) days vs median 85.0 (25%, 75% 386.0, 54.0) days, p=0.006 Kaplan-Meier survival analysis with Gehan-Breslow test] (Figure 1). Children who did not experience a sLRI during the study were censored on the date that they left the study (withdrew or competed).

Figure 1: Time to 1st severe Lower Respiratory Illness (sLRI). Active group shown in black, Control group shown in red.
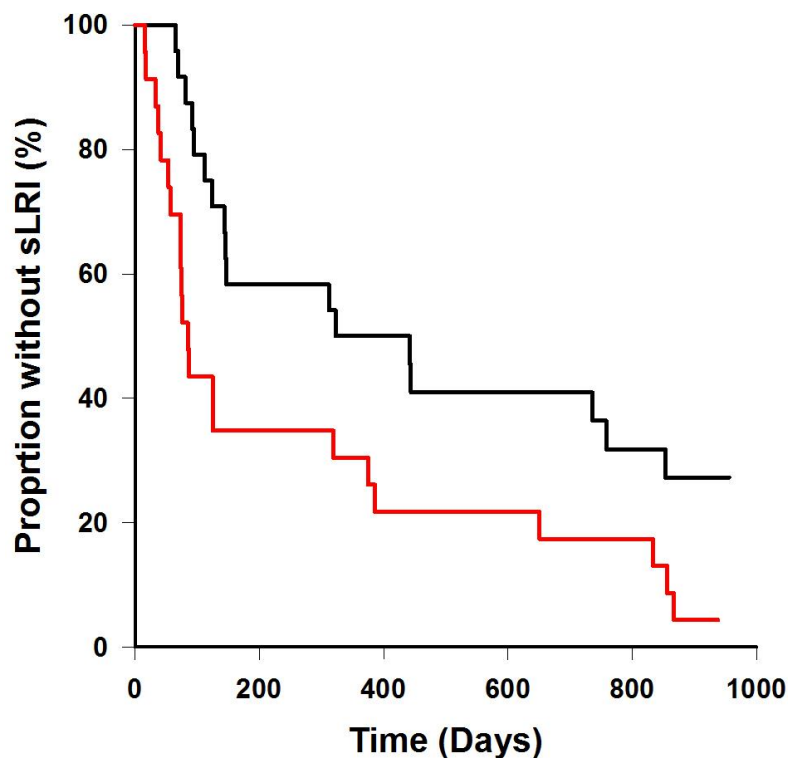
sLRI are LRI accompanied by fever (fLRI) and/or wheeze (wLRI). Examining the individual components showed similar effects of OM85 at increasing the time to first LRI (figure 2), number of LRI and the proportion of children experiencing them. However, lack of study power meant that most comparisons did not reach statistical significance. The longer time to first event was also see for sLRI with wheeze [median >758 (25%, 75% >758, 312) days vs median 856 (25%, 75% >860, 87) days, p=0.15] and sLRI with fever [median 795 (25%, 75% >853, 303) vs median 346 (25%, 75% 856, 87) days, p=0.12 Kaplan-Meier survival analysis with Gehan-Breslow test], but neither reached statistical significance.

Figure 2: Time to first sLRI with wheeze (A) and sLRI with fever (B). Active group shown in black, Control group shown in red.
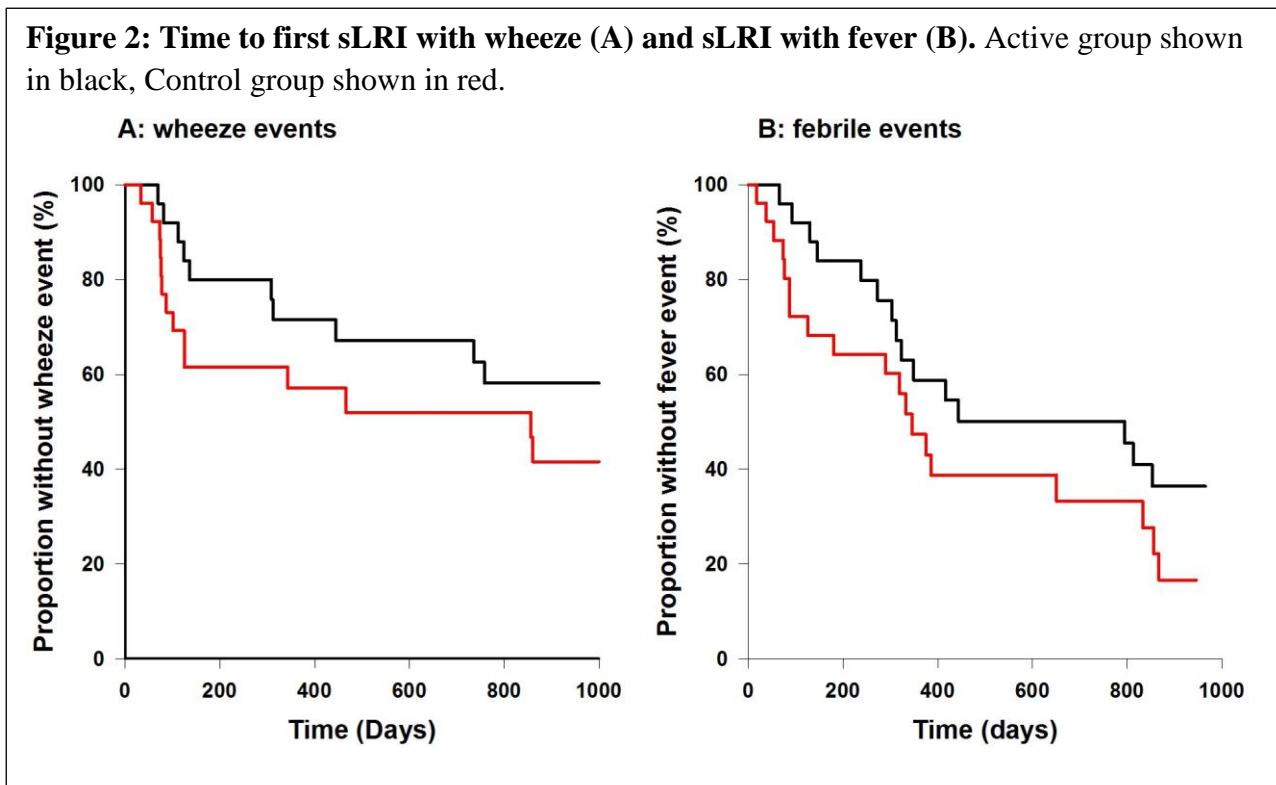
First winter season

OM85 appeared to be more effective at preventing sLRI in the first winter. Fewer children in the Active group had a sLRI than those in the Control group [6/25 (24.0%) vs 14/27 (51.9%), p=0.05 Fisher Exact). However, the study was underpowered to detect a true difference. Similarly, children in the Active group had fewer sLRI [7 infections, median 0.0 (25%, 75% 0.0, 0.75)] than those in the Control group [18 infections, median 1.0 (25%, 75% 0.0, 1.0)] but this did not reach statistical significance (p=0.052 Mann Whitney). For those children who did have sLRI, there was no difference in duration for those in the active and control groups [median 10.3 (25%, 75% 8.3, 25.8) days vs median 20.0 (25%, 75% 7.0, 23.3) days, p=0.61 Mann Whitney].

There did not appear to be any carry over protection for the rest of the first year of the study once children stopped taking OM85, in fact there appeared to be a rebound increase in sLRI in the Active group. This is not explained by a seasonal effect as it was not seen in the Control group (table 3). Note: as study treatment was started one month (April) before the anticipated winter viral season (May, June, July, August) the data for the winter season and while on treatment are not identical.

Table 3: Proportion of children with sLRI during the first year of the study on and off study treatment.

sLRI	On treatment	Off treatment	p*
Active	6/25 (24.0%)	11/25 (44.4%)	0.046
Placebo	12/27 (44.4%)	4/27 (14.8%)	

* Chi square

There was also a trend to an increase in the number of sLRI in the Active group off treatment that was not seen in the Placebo group (Table 4).

Table 4: Number of sLRI occurring during the first year of the study on and off study treatment.

sLRI	On treatment	Off treatment	p*
Active: number of infections, group median (25%, 75%)	6, 0.00 (0.00, 0.75)	11, 0.50 (0.00, 1.00)	0.081

| Placebo: number of infections, group median (25%, 75%) | 16, 0.00(0.00, 1.00) | 7, 0.00 (0.00,0.00) | |

* Kruskal-Wallis one-way ANOVA on ranks

There was a trend to a reduction in the proportion of children experiencing fLRI in the Active group [4/25 (16.30%) vs 8/27 (29.6%), p=0.33] and wLRI [3/25 (12.0%) vs 8/27 (29.6%), p=0.18] in the first winter and a reduction in the number of fLRI and wLRI in the 1st winter (Table 5) and a reduction while on treatment (Table 6).

Table 5: Febrile LRI (fLRI) and wheeze-associated LRI (wLRI) occurring in the first winter

	Active	Control	p*
fLRI: number of infections, group median (25%, 75%)	5, 0.00 (0.00, 0.00)	9, 0.00 (0.00, 1.00)	0.31
wLRI: number of infections, group median (25%, 75%)	3, 0.00 (0.00, 0.00)	11, 0.00(0.00, 1.00)	0.045

*Mann-Whitney

Table 6: Number of fLRI and wLRI occurring during the first year of the study on and off study treatment.

fLRI	On treatment	Off treatment	p*
Active: number of infections, group median (25%, 75%)	4, 0.00 (0.00, 0.00)	7, 0.00(0.00, 0.00)	0.37
Placebo: number of infections, group median (25%, 75%)	6, 0.00(0.00, 0.25)	3, 0.00(0.00,0.00)	
wLRI			
Active: number of infections, group median (25%, 75%)	2, 0.00(0.00, 0.00)	7, 0.00(0.00, 0.00)	0.035
Placebo: number of infections, group median (25%, 75%)	11, 0.00(000, 1.00)	6, 0.00(0.00, 0.00)	

* Kruskal-Wallis one-way ANOVA on ranks

Fewer children in the Active group had an URI in the first winter season than in the Control group (45.8% v 88.5%, p=0.002 Fisher Exact). The number of URI was also less in the Active group than in the Control group [median 0.00 (25%, 75% 0.00, 1.00) vs median 2.00 (25%, 75% 1.00, 3.00) p=0.002 Mann Whitney). There was no difference in the time to first URI [median 28.0 (25%, 75% 55.0, 13.0) days vs median 22.0 (25%, 75% 44.0, 11.0) days, p=0.69 Kaplan-Meier survival analysis with Gehan-Breslow test]. There was no evidence of a rebound increase in URI when off treatment.

Second winter season
A tendency for fewer children (21.2% v 45.5%, p=0.12 Fisher Exact) in the Active group having fewer sLRI was seen [median 0.00 (25%, 75% 0.00, 0.00) v median 0.00 (25%, 75% 0.00, 1.00), p=0.25 Mann Whitney] but did not reach statistical significance. A similar trend was seen for wLRI (13.0% v 36.4%, p=0.091 Fisher exact), [median 0.00 (25%, 75% 0.00, 0.00) v median 0.00 (25%, 75% 0.00, 0.00), p=0.58 Mann Whitney]. There was no tendency for a reduction in URI in the second winter season (87.0% v 90.0%, p=0.88 t-test), [median 2.00 (25%, 75% 1.00, 3.00) v median 2.50 (25%, 75% 1.00, 3.00), p=1.00 Mann Whitney].

Third winter season
There were no differences between the groups in the third winter for sLRI [Active 12 in 7 children, median 0.00 (25%, 75% 0.00,1.00) v Control 6 in 6 children, median 0.00 (25%, 75% 0.00, 0.00), p=0.84 Mann Whitney], fLRI [9 in 6 children, median 0.00 (25%, 75% 0.00, 0.00) v 4 in 4 children,

median 0.00 (25%, 75% 0.00, 0.00), p=0.85 MannWhitney], wLRI [10 in 5 children, median 0.00 (25%, 75% 0.00, 0.00) v 5 in 5 children, median 0.00 (25%, 75% 0.00, 0.00), p=0.52 MannWhitney] or URI [42 in 18 children, median 2.00 (25%, 75% 1.00, 2.25) v 47 in 18 children, median 2.00 (25%, 75% 2.00, 3.00), p=0.23 t-test].

Multiple sLRIs

Most children did not have a sLRI during the study period, of those who did, most had only one episode. Table 7 shows the number of sLRI experienced by children in each group. Note: this indicates number of children in each period; these are not necessarily the same children in each period.

Table 7: Number of sLRI reported by children in each time period.

Period	Group	Number of sLRI		
		0	**1**	**≥2**
1st Winter	Active	18	5	1
	Control	13	11	3
1st year	Active	13	7	4
	Control	12	9	6
2nd Winter	Active	18	5	1
	Control	14	5	3
2nd year	Active	10	9	4
	Control	11	3	8
3rd Winter	Active	16	5	2
	Control	14	6	0
3rd year	Active	14	6	3
	Control	9	5	6

Cumulative frequency of sLRI

The cumulative frequency of sLRI was determined by summing sLRI occurring in 3 month periods (Tables 8&9). Throughout the study period more sLRI occurred in children in the placebo group than those randomized to OM85 (figure 3). This difference was statistically significant [one way repeated measures analysis of variance p<0.001]

Figure 3: Cumulative frequency of sLRI. Active group shown in black, Control group shown in red.
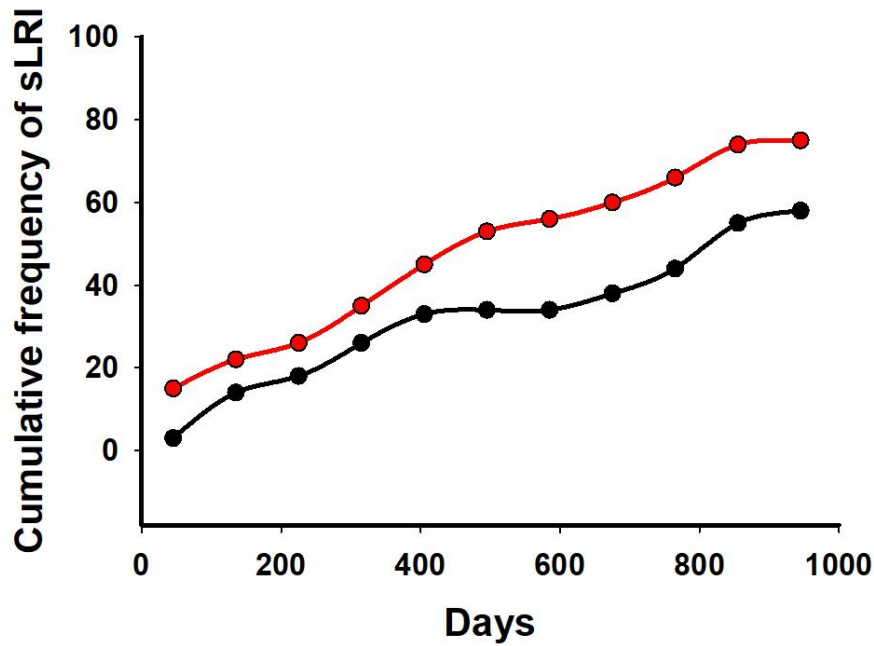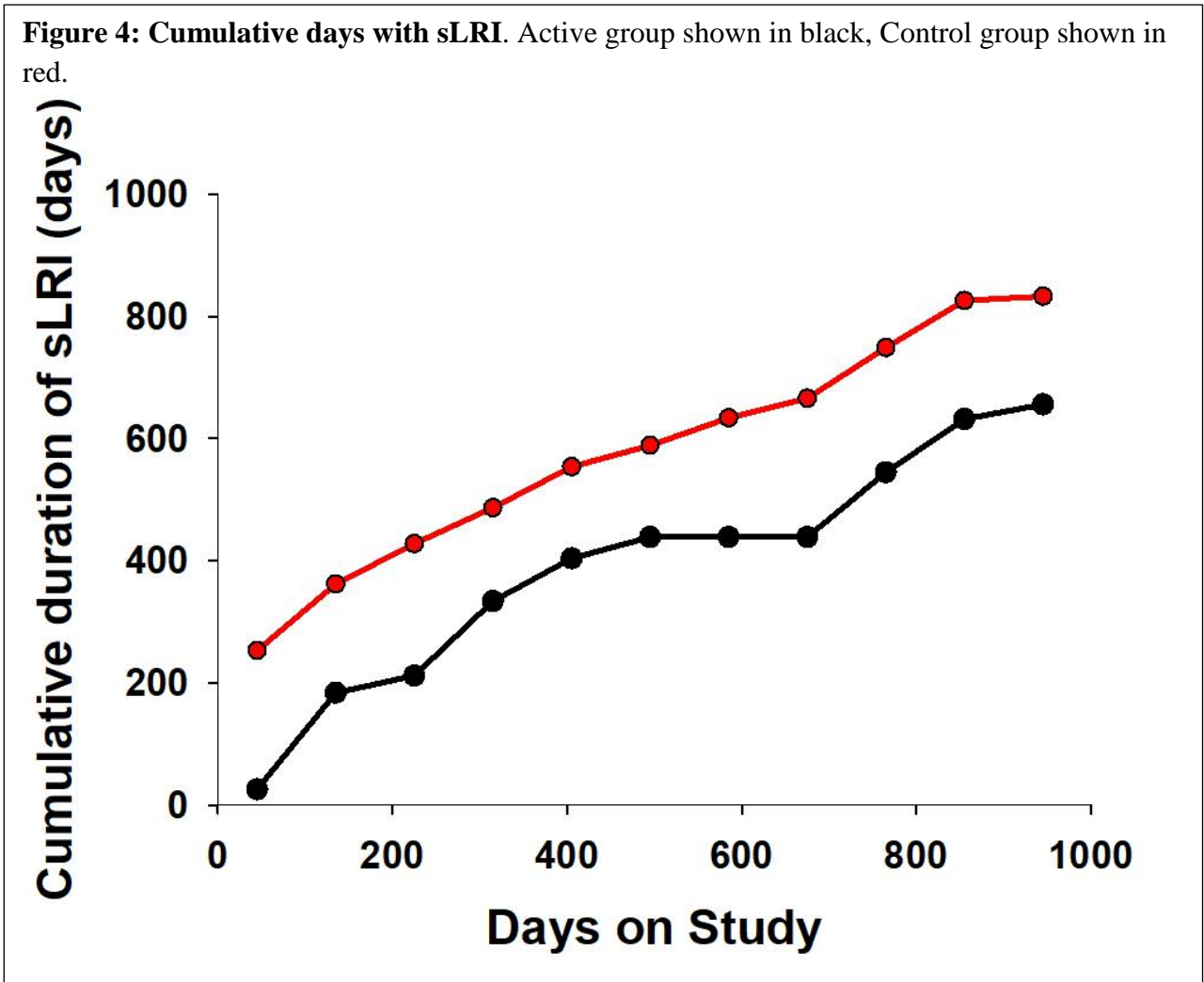

Table 8: Cumulative frequency of sLRI: Control group individual and grouped data

ID	Days on study										
	0-90	91-180	181-270	271-360	361-450	451-540	541-630	631-720	721-810	811-900	>900
2	1	1	1	1	1	1	1	1	1	1	1
3											
6	1	1	1	1	1	1	1	1	1	1	1
7	1	1	1	2	2	3	3	3	3	4	4
9	1	1	1	1	1	1	1	1	1	1	1
12	2	4	7	8	10	13	14	14	16	16	16
13											
14	0	0	0	0	1	1	1	1	2	3	3
18											
20	2	2	2	2	2	2	2	2	2	2	2
22	1	2	2	3	3	4	4	4	4	4	5
23	1	1	1	2	3	4	4	4	4	5	5
26	0	0	0	0	0	0	0	0	0	1	1
27	1	1	1	3	3	3	3	3	4	5	5
30	0	0	0	0	0	0	0	0	0	0	0
32											
33	0	0	0	0	0	0	0	0	0	1	1
36	0	1	1	1	2	2	2	2	2	2	2
37	0	0	0	0	0	0	0	1	1	1	1
39	1	1	1	1	1	1	1	1	1	1	1
41	0	0	0	2	3	3	5	7	7	7	7
44	0	0	0	0	0	0	0	0	0	0	0
46	1	1	1	1	1	1	1	1	1	1	1
48	0	0	0	0	0	0	0	0	0	0	0

51	1	2	2	2	3	4	4	4	4	5	5
52	1	1	1	1	1	1	1	1	1	1	1
54											
56	0	0	0	0	1	1	1	1	1	1	1
57	0	2	3	4	6	7	7	8	10	10	10
58	0	0	0	0	0	0	0	0	0	1	1
sum	15	22	26	35	45	53	56	60	66	74	75
Mean	0.58	0.85	1.00	1.35	1.73	2.04	2.15	2.15	2.31	2.85	2.80
SD	0.64	0.97	1.47	1.77	2.22	2.84	3.04	3.04	3.19	3.63	3.65
median	0.05	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
25%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00	1.00
75%	1.00	1.00	1.00	2.00	2.00	3.00	3.25	3.25	3.25	4.25	5.00

Table 9: Cumulative frequency of sLRI: Active group individual and grouped data

	Days on study										
ID	days 0-90	91-180	181-270	271-360	361-450	451-540	541-630	631-720	721-810	811-900	901-990
1	0	0	0	0	1	1	1	1	1	4	4
4	0	1	1	1	2	2	2	2	3	3	3
5	0	2	2	3	3	3	3	3	4	4	4
8	0	1	1	1	1	1	1	1	3	3	3
10	0	1	1	2	2	2	2	2	2	2	2
11	0	0	0	0	0	0	0	0	0	0	0
15	0	1	1	2	2	2	2	2	2	2	2
16	1	2	2	2	2	2	2	2	2	2	2
17	0	0	0	0	0	0	0	1	1	1	2
19											
21											
24	0	0	0	0	0	0	0	0	0	1	1
25	0	1	1	1	1	2	2	2	2	3	3
28											
29	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	2	2	3	3
34	1	3	6	8	10	10	10	10	13	17	17
35	0	1	1	2	2	2	2	2	2	2	2
38	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	1	2	2	2	2	2	2	2
43	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	1	1	1	1	1	1	3
47	1	1	2	2	3	3	3	4	4	4	4
49	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	1	1	1	1	1	1	1	1
53											
55	0	0	0	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0
sum	3	14	18	26	33	34	34	38	45	55	58
Mean	0.12	0.56	0.72	1.04	1.32	1.36	1.36	1.36	1.52	2.20	2.30
SD	0.33	0.82	1.31	1.72	2.08	2.08	2.08	2.08	2.08	3.39	3.39

median	0.00	.000	0.00	0.00	1.00	1.00	1.00	1.00	1.00	2.00	2.00
25%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
75%	1.00	1.00	1.00	2.00	2.00	2.00	2.00	2.00	2.00	3.00	3.00

Cumulative days with sLRI

The cumulative days with sLRI was determined by summing the duration of sLRI occurring in 3 month periods (Tables 10&11). Throughout the study period children in the Control group had more days with sLRI than those randomized to OM85 [group median placebo 589 (25%, 75% 428, 749) days v group median OM85 439 (25%, 75% 212, 545), p<0.001 one way repeated measures analysis of variance] (figure 4).

Figure 4: Cumulative days with sLRI. Active group shown in black, Control group shown in red.

Table 10: Cumulative days with sLRI: Active group individual and grouped data

ID	Days on study										
	0-90	91-180	181-270	271-360	361-450	451-540	541-630	631-720	721-810	811-900	>900
1	0	0	0	0	21	21	21	21	21	57	57
4	0	1	1	1	1	17	17	17	29	29	29
5	0	11	11	29	29	29	29	29	38	38	38
8	0	1	1	1	1	1	1	1	9	9	9
10	0	23	23	52	52	52	52	52	52	52	52
11	0	0	0	0	0	0	0	0	0	0	0
15	0	6	6	12	12	12	12	12	12	12	12

11

16	6	13	13	13	13	13	13	13	13	13	13
17	0	0	0	0	0	0	0	0	31	31	35
19											
21											
24	0	0	0	0	0	0	0	0	0	9	9
25	0	70	70	70	70	89	89	89	89	112	112
28											
29	0	0	0	0	0	0	0	0	0	0	0
31	0	0	0	0	0	0	0	0	14	15	15
34	11	27	48	70	106	106	106	106	131	149	155
35	0	16	16	21	21	21	21	21	21	21	21
38	0	0	0	0	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0	0	0	0
42	0	0	0	14	14	14	14	14	14	14	14
43	0	0	0	0	0	0	0	0	0	0	0
45	0	0	0	0	13	13	13	13	13	13	27
47	9	16	23	37	37	37	37	37	44	44	44
49	0	0	0	0	0	0	0	0	0	0	0
50	0	0	0	14	14	14	14	14	14	14	14
53											
55	0	0	0	0	0	0	0	0	0	0	0
59	0	0	0	0	0	0	0	0	0	0	0
sum	26	184	212	234	404	439	439	439	545	632	656
Mean	1.0	7.4	8.4	13.4	16.2	17.6	17.6	17.6	21.8	25.3	26.2
SD	3.0	15.3	17.1	21.8	26.0	27.7	27.7	27.7	31.0	36.3	37.1
median	0.00	0.00	0.00	0.00	1.00	12.00	12.00	12.00	13.00	13.00	14.00
25%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
75%	0.00	12.00	12.00	17.50	21.00	21.00	21.00	21.00	31.00	34.50	36.30

Table 12: Cumulative days with sLRI: Control group individual and grouped data

ID	Days on study										
	0-90	91-180	181-270	371-360	361-450	541-540	541-630	631-720	721-810	811-900	>900
2	29	29	29	29	29	29	29	29	29	29	29
3											
6	21	21	21	21	21	21	21	21	21	36	36
7	46	46	46	56	56	56	56	56	71	71	71
9	21	21	21	21	21	21	21	21	21	21	21
12	15	60	60	60	60	60	60	67	67	67	67
13											
14	0	0	0	12	12	12	12	60	107	114	114
18											
20	38	38	38	38	41	41	41	41	41	41	41
22	3	28	28	28	35	35	35		0	0	6
23	5	24	24	37	37	37	37	37	43	49	50
26	0	0	0	0	0	0	0	0	3	6	6

27	7	7	7	7	7	7	7	13	17	21	21
30	0	0	0	0	0	0	0	0	0	0	0
32											
33	0	0	0	0	0	0	0	0	0	23	23
36	36	43	43	53	53	53	61	61	61	61	61
37	0	0	0	0	0	0	0	0	0	0	0
39	4	4	4	4	4	4	4	4	4	4	4
41	0	0	0	0	6	17	54	54	54	54	54
44	0	0	0	0	0	0	0	0	0	0	0
46	0	0	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0	0	0
51	27	38	38	48	87	87	87	87	87	106	106
52	1	1	1	1	1	1	1	1	1	1	1
54											
56	0	0	0	3	3	3	3	3	3	3	3
57	0	0	0	0	1	1	1	1	1	1	1
58	0	6	72	73	84	108	108	114	122	122	122
Sum	253	362	428	487	554	589	634	666	749	826	838
Mean	10.1	15.1	17.8	20.3	23.1	24.5	26.4	29.0	31.2	34.4	34.7
SD	14.5	18.8	22.0	23.6	27.7	30.1	31.0	33.1	32.4	38.7	38.6
Median	1.00	3.50	4.00	9.50	9.50	14.50	16.50	21.00	19.00	22.00	22.00
25%	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.25	1.00
75%	21.00	28.75	35.75	37.75	40.00	40.00	50.25	56.00	59.25	59.25	59.25

Hospitalisation for respiratory disease

Six children in the Active group had 15 admissions for respiratory conditions, whereas 9 children in the Control group had 16 admission for respiratory conditions during the study period. Four children, all from the om85 group had 8 admissions for acute viral bronchiolitis.

Doctor-diagnosed asthma

We were unable to collect reliable data on doctor-diagnosed asthma during the study period. Australia general practitioners are reluctant to "label" children as having asthma by 3 years of age.

End of study assessment

Table 10 shows the number of subject completing the 3 years study and their demographic and clinical characteristics.

Table 10: Demographic and clinical characteristics of children completing the study

	Active (n=23)	Control (n=18)	p
Age at 3y assessment (y), mean ± SD	3.05±0.20	3.04±0.20	0.80[1]
Ht at 3y assessment (cm), mean ± SD	94.9±4.56	96.24±3.96	0.33[1]
Wt at 3y assessment (kg), mean ± SD	14.7±2.18	15.07±1.31	0.24[1]
BMI at 3y assessment (kg.cm^{-2}), mean ± SD	16.3±1.12	16.2±0.94	0.70[1]
Lung function at 3y, R10 Z-score median (25%-75%)	N =20 -0.15 (-0.98, 0.46)	N=14 0.18 (-0.52, 0.67)	0.16[2]
Lung function at 3y, X10 pre Z-score	N=20	N=14	0.62[2]

median (25%-75%)	-0.14 (-0.50, 0.74)	-0.10 (-0.68, 0.61)	
Lung function at 3y, ΔR median (25%-75%)	N=18 0.78 (0.48, 1.60)	n-14 1.52 (1.16, 2.15)	0.03[2]
Lung function at 3y, ΔX median (25%-75%)	N=18 0.72 (0.05, 1.16)	N=14 -0.14 (-0.50, 0.74)	0.05[2]

[1]t-test; [2]Mann Whitney

Despite the small numbers completing lung function testing, children in the Active group had better lung function at 3 years of age, with less tendency for airway obstruction on grouped data (p=0.03) and a trend for fewer children to have ΔR >1.42, our previously published cut-off for airway obstruction in preschool aged children (8) [Active 5/18 (27.8%) vs Control 8/14 (57.1%), p=0.15].

Safety
Drop-outs
Five children randomized to OM85 failed to complete the study, together with 11 children randomized to placebo. The reasons for withdrawing are shown in table 9.

Reason for withdrawal	Active (n)	Control (n)
Loss to follow-up (failed to return for visit, did not respond to contact)	1	6
Too busy (change in family/work circumstances)	4	2
Moving away from study area	0	2
Child refused medication	0	1

Adverse events
The most commonly reported adverse events were gastrointestinal disorders, skin conditions, ear infections and general disorders. There we no adverse events that occurred more commonly in the Active than Control group (Table 11).

Table 11: Adverse events in categories.

Category	Active (n=28)		Control (n=29)		p
	Events (n)	Subjects (%)	Events (n)	Subjects (%)	
Skin and subcutaneous disorders (total)	123	19 (67.9%)	117	14(48.3%)	0.22
• Eczema	41	5 (17.9%)	37	4 (13.8%)	0.73
• Diaper dermatitis	13	1 (3.6%)	6	2 (6.9%)	1.00
• Hand-foot-mouth disease	10	7 (25.0%)	40	3 (10.3%)	0.18
• Viral rash	13	1 (3.6%)	0	0	1.00
Injury, poisoning and procedural complications (total)	34	9 (32.1%)	15	9 (31.0%)	1.00
Infection and infestation (total)	28	8(28.6%)	34	9 (31.0%)	0.77
• viral infection	4	2 (7.1%)	13	3 (10.3%)	1.00
Immune system disorders (total)	18	7 (25.0%)	25	10 (34.4%)	0.57
• Hypersensitivity	6	2 (7.1%)	8	4 (13.8%)	0.67
• Urticaria	6	7 (25.0%)	10	3 (10.3%)	0.18
General disorders and administration site conditions (total)	285	23 (82.1%)	244	24 (82.8%)	1.00
• Irritability	8	1 (3.6%)	0	0	1.00
• Pyrexia	269	24 (85.7%)	244	23 79.3%)	1.00
Eye disorders (total)	22	9 (32.1%)	35	12 (41.4%)	0.59
• Conjunctivitis	21	8 (28.6%)	35	12 (41.4%)	0.41
Ear and labyrinth disorders (total)	72	13 (46.4%)	79	16 (55.2%)	0.60
• Ear infection	42	8 (28.6%)	58	12 (41.4%)	0.41
• Otitis media	10	2 (7.1%)	9	1 (3.4%)	1.00
• Tympanic membrane perforation	3	1 (3.36%)	7	2 (6.9%)	1.00
Gastrointestinal disorders (total)	133	21 (75.0%)	142	21(72.4%)	1.00
• Constipation	5	1 (3.6%)	15	2 (6.9%)	1.00
• Diarrhoea	10	2 (7.1%)	16	2 (6.9%)	1.00
• Gastroenteritis	0	0	16	2 (6.9%)	1.00
• Teething	78	14 (50.0%)	90	13 (44.8%)	0.80
• Vomiting	41	6 (21.4%)	24	5 (17.2%)	0.75

Note: adverse events affecting <1% of participants have not been listed

Discussion

The study did not achieve its primary outcome, in that there was no difference in the frequency of sLRI over the first 2 winters between children receiving OM85 and those in the placebo (control) group (p=0.84). An a priori decision was taken to undertake secondary analyses regardless of the primary outcome.

The time to first sLRI, a secondary outcome in this study, was significantly longer in the Active group than in the Control group (p=0.006). sLRI during infancy, i.e. those associated with fever and/or wheeze, increase the likelihood of subsequent asthma in at-risk subjects(3-6, 9). Moreover, the cumulative frequency of sLRI, and the number of days with sLRI symptoms, were also significantly lower in those receiving OM85, suggesting a reduction in the overall inflammatory burden in the lower airways, during this crucial period of early lung growth. Significant interest exists in primary prevention of asthma and we have previously postulated that this may be achievable by protection against sLRI during infancy(2).

The effects of OM85 were strongest in the first winter season, with a trend for fewer children in the Active group to have sLRI (p=0.05), fLRI (p=0.33), wLRI (0.18) and URI (p=0.002). There did not appear to be any carry over protection for the rest of the first year of the study once children stopped taking OM85 in the first study year. The lack of carry-over effect differs from the findings of Razi et al. (10). However, they studied older children, not infants. The maturational stage of immune development may play an influence here. This suggests that infants and young children may require treatment all year round to maintain the early benefit of OM85, as seen in the present study.

Aspects of the trial conduct warrant discussion. No previous study has used OM85 in primary prevention of sLRI in at-risk infants. As such, we had no guide for powering the study. We used the data published by Razi et al. (10) to determine the sample size required to show a similar effect size, i.e. a 30-40% reduction in wLRI in the Active group. Fifty one percent of the control group had a sLRI in the first winter, as did 45% in the second winter. We also found a reduction in the cumulative frequency of sLRI over the entire study period, as was shown by Razi et al. (10). Examination of our figure 3 suggests that the main effect occurred in the first winter, with the increase in sLRI occurrence occur at essentially the same rate for the Active and Control groups.

We lost a number of children to follow-up with 25 children in the Active group having evaluable data at the end of the 1st and 2nd winter seasons. More in the Control group were lost to follow-up, with 27 and 22 having evaluable data at the end of the 1st and 2nd winters, respectively. In this regard, the decision not to replace children who withdrew or were lost to follow-up was a mistake, albeit one dictated by available resources.

Conclusions
Despite the study being underpowered, some important lessons have been learned.
1) OM85 is safe and can be given to infants as young as 3 months of age by opnng the capsue and dissolving the contents in a small amount of fluid (breast milk, water or formula).
2) OM85 can be used for primary prevention of sLRI in the high-vulnerability period of early infancy.
3) The treatment regimen giving OM85 for the first 10 days of the winter months may not be adequate to provide sufficient protection of at-risk infants against sLRI occurring while off treatment. In this regard, animal studies (11) with OM85 suggest that protection against respiratory viral infection is maximal if treatment is ongoing during the infection period.

Further studies, with greater power, and possibly employing alternative treatment regimens, are warranted to determine whether OM85 can prevent asthma in at-risk infants and young children.

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