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# Frequently Asked Questions (FAQ's) Booklet

# Polyvalent Mechanical Bacterial Lysate (PMBL)

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# INDEX

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## FORMULATION

### 1. What is PMBL tablet, what does it contain?

PMBL means Polyvalent Mechanical Bacterial Lysate. PMBL is a mixture of antigens derived from 13 strains of inactivated pathogenic bacteria, which is the reason why it is Polyvalent. The 13 strains used in PMBL are typically the most commonly occurring pathogens of the upper and lower respiratory tract and are the following:

- *Staphylococcus aureus*
- *Streptococcus pyogenes*
- *Streptococcus (viridians) oralis*
- *Klebsiella pneumoniae*
- *Klebsiella ozaenae*
- *Haemophilus influenzae*
- *Neisseria catarrhalis*
- *Streptococcus (Diplococcus) pneumoniae* (Type 1, Type 2, Type 3, Type 5, Type 8, Type 47)

PMBL, active ingredient is made of 7mg of the bacterial fraction (each strain is concentrated at 6 billion) and 43 mg of Glycine as support of freeze-drying, thus the concentration of the active ingredient is 50mg.

### 2. Does PMBL tablet contain any excipients?

The excipients contained in PMBL tablets have been selected to ensure a regular dissolution under the tongue. Additionally PMBL tablet is mint natural flavored to let a fresh and sweet sensation, which is of importance when you have cold or respiratory disorders.

The excipients are:

- Colloidal hydrated silica
- Cellulose microcrystalline
- Calcium hydrogen phosphate dihydrate
- Magnesium stearate
- Ammonium glycyrrhizate
- Essence of mint-powder

### 3. Are the PMBL strains relevant to clinical practice? Why?

As it has been explained previously, the 13 inactivated strains used in PMBL are one of the most commonly pathogens found in respiratory tract infections. It is known that most of the upper respiratory tract infections are due to viruses, nevertheless epiglotitis and laryngotracheitis are often due to *Haemophilus influenzae* and most of bacterial pharyngitis is due to *Streptococcus pyogenes*. (1). Even if bronchitis and bronchiolitis are often of viral origin, supra-infections and pneumonia are

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of bacterial etiology with predominant pathogens such as: *Streptococcus pneumoniae*, *Mycoplasma pneumoniae*, *Chlamydiae*, *Legionella* and Coxiella *burnetti*.

It is a fact that bacterial strains have some common antigenic patterns. So, even if the preparation contains 13 strains, the formulation confers as well protection to similar strains. We can also cite the example of interaction between viruses and bacteria. The review of G.Palmieri demonstrated that viruses and bacteria act in synergy. Bacterial infections can enhance virus infectiveness through haemoagglutinin proteolysis, while viruses facilitate the adhesion and infections of target cells by bacteria. PMBL are able to act both on innate and adaptive immunity and thanks to this mode of action, the rationale to use as well PMBL during airways infections of viral etiology is convincing. (2)Several reviews on bacterial lysates have been published over the last 5 years. Most of them have highlighted the importance of having several strains and as well of the way to produce the antigens.

In his review on bacterial lysates, Pr F.Braido (3) clearly demonstrated that bacterial lysates have a protective effect in respiratory tract infections.

The review of Pr M Cazzola (4) assessed that PMBL are able to exert therapeutic and preventing effect in acute and recurrent respiratory infections thanks to their ability to activate and enhance both IgM memory B cells and IL-2 receptor-expressing lymphocytes (T lymphocytes).

Additionally, several studies have also demonstrated that the acute exacerbations in COPD patients are clearly associated with some bacterial species such as *Streptococcus pneumoniae*, *Neisseria catarrhalis* and *Haemophilus influenzae*. PMBL have shown their potential in the prevention of acute exacerbation in COPD. In a multicenter study (5), comprising 178 patients who were randomly assigned in PMBL or placebo group, PMBL led to a highly significant reduction in the frequency and duration of exacerbations, as well as in the antibiotic consumption and hospitalization time.

## INDICATION

### 1. *What is the indication of PMBL?*

The indication of PMBL is prevention of acute, sub-acute, recurrent or chronic infections of the upper and lower airways and of the bronchopulmonary tree.

### 2. *Since which age PMBL could be prescribed?*

A recent expert report from Pr Giovanni Melioli (6) has demonstrated that PMBL can be prescribed to children from 3 years old. *Why*? Because at this age, the immune system is considered as mature. *At what dosage*? There is no rationale to have a different dosage with PMBL for children from 3 years old and adults, because « *no evidence obtained by clinical studies as well as by pharmacological surveillance of PMBL in the last 15 years support the idea that a dose reduction is required in pediatric patients* ». We can take as example the flu vaccine, for which the dosage is the same for children and adults.

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### 3. Is PMBL recommended for prevention or treatment?

Actually PMBL is recommended for prevention as the clinical evidences are based in the use of PMBL in the prophylaxis of respiratory infections, BUT as the drug can be combined with antibiotics, mucolytic agents, inhaled corticosteroids and Long-Acting Beta-2 Agonist (LABA), PMBL can be used as well during conventional treatment of various respiratory infections. In that way PMBL will be able to strengthen the immune system to fight against the infection. As we will develop later in the mode of action section, PMBL is able to increase all the subsets of Natural killer cells which play an important role in the destruction of cells infected by viruses, as well as PMBL is able to induce the production of opsonizing immunoglobulins which in turn will allow a potent destruction of pathogenic bacteria through granulocytes.

As example we can cite the study of professor Mario Cazzola (7) which demonstrated in 63 patients with COPD and under treatment with salmeterol/fluticasone (LABA/inhaled corticosteroid), that in the group of patients who received PMBL, the rate of exacerbations (per patient, per year) was reduced from 0.67 to 0.54, as well as the rate of hospitalizations from 0.13 to 0.09 and antibiotics use was as well reduced.

Another study from Pr Boris (8), has shown that in population of 360 patients with positive tuberculin test and under treatment with isoniazid + pyridoxine, that only 2.8% patients who received as well PMBL developed tubercular disease while 6.1% who received the placebo developed tubercular disease. In conclusion PMBL allowed decreasing by more than half the development of tuberculosis in patients with conventional treatment as compared to those with conventional treatment plus placebo.

### 4. To whom PMBL is recommended to be prescribed?

PMBL is recommended to children over 3 years and adults to prevent upper and lower respiratory tract infections. In that way, the population targeted with PMBL is each and everybody. Nevertheless, it must be noted that children and elderly people are more prone to respiratory infections, as well as smokers (COPD risk), patients with latent tuberculosis or post-tuberculosis infection, people living in highly polluted area and/or in social promiscuity.

### 5. Can PMBL be used to prevent recurrent otitis?

PMBL can be used in the prophylaxis of upper respiratory tract infections; in that way PMBL can be used in the prevention of recurrent otitis as this disease is part of the upper respiratory tract infections. The most common bacterial pathogens isolated from children with otitis are *Streptococcus pneumoniae*, non typeable *Haemophilus influenzae* and *Moraxella catarrhalis. Streptococcus pneumoniae and Haemophilus influenzae are part of the formulation of PMBL thus conferring its prophylactic effect. (9)*

In fact a specific analysis has been done during the clinical study performed by Pr Davide Tricarico (10). A total of 47 patients with recurrent upper respiratory tract infections have been divided into 2 groups. The first group received PMBL while the second one received placebo. In the group receiving

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PMBL it has been demonstrated that after 3 months treatment, the symptoms of otalgia has dropped of 80% while in the placebo group the decrease was only 28.6%. In general all the clinical studies performed with PMBL in the prevention of upper respiratory tract infections have shown that PMBL was significantly efficient.

### 6. *Can PMBL be used in addition to current management of Tuberculosis to prevent development of the disease?*

As explained in the point 4, 2 clinical studies have been performed on patients with a positive tuberculin test. In both studies from Boris (8, 11), it has been demonstrated that PMBL allowed to reduce the number of concomitant and bronchial infections as compared to placebo and that PMBL in combination with isoniazid/pyridoxine was able to overcome the development of tuberculosis as compared to the conventional treatment plus placebo.

A recent article from Pr Ermanno Pux has concluded that PMBL efficacy as immunomodulators support the rationale for their use in the setting of tuberculosis. Additionally, several epidemiological studies have demonstrated a large overlap between the epidemics of COPD and Tuberculosis. Thus PMBL should be considered as therapeutic option in post tubercular patients and COPD patients to reduce exacerbation rate and lower the risk of the development of active forms of tuberculosis (12).

## MODE OF ADMINSITRATION (DOSAGE/USE)

### 1. *How long can PMBL tablet be used?*

PMBL tablet is recommended to be used in a course of 3 months treatment (10 days per month during 3 months) to offer an efficient protection during the winter period. However, 2 courses of 3 months treatment can be as well recommended to offer a full year of protection against respiratory infections especially in patients at risk (eg: COPD patients, elderly people, refer to patients at risk INDICATION, point 4).

In fact in all the clinical studies performed with PMBL, it has been demonstrated that PMBL allows offering protection during the 3 months treatment period and also during the 3 months follow-up.

### 2. *Can PMBL tablet be used for life long?*

Pharmacovigilance for PMBL tablets never showed either adverse events or serious adverse events regarding a long-term use with PMBL.

Additionally, the effect of PMBL tablet will not be during the lifelong, as the immunoglobulins have a very short half-life between 6-21 days and as well the activated immune cells have a limited life from several days to several months in the cases of phagocytes.

So to date and to our current knowledge, there is no rationale that PMBL could not be used during the lifelong.

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### 3. What if the tablet is swallowed?

The sublingual route allows direct contact of the antigens contained in PMBL with the mucosal immunocytes of the mouth and the pharynx, inducing a loco-regional activation of the immune system. PMBL tablet allows the production of secretory IgA at the application site resulting in a mucosal protection against bacterial infections (13).

If PMBL tablet is swallowed, then the mode of action will be then the same as other oral bacterial lysates, which means that PMBL shall act on the gastrointestinal mucosa through the GALT and thanks to the systemic circulation of immunoglobulins and cytokines PMBL shall have effect on the respiratory tract. However the immune answer shall be lesser as the circulating memory cells preferentially return to the area of first encounter with the antigen.

In any case, if patient swallows tablet of PMBL, never take one additional dose.

### 4. Can PMBL be given with other medicines?

Of course PMBL can be given with other medicines, such as antibiotics, mucolytic agents, Inhaled corticosteroids and LABA. Several clinical studies have been performed with PMBL and such medicines and no drug interactions have been reported.

The study from Pr M.Cazzola (7) with concomitant administration of fluticasone/salmeterol (inhaled corticosteroid/LABA) with PMBL did not demonstrate any drug interactions.

As well as the study from Boris (8) with concomitant administration of isoniazid/pyridoxine (antibiotic/VitB6) with PMBL did not demonstrate any drug interactions.

### 5. How long does it take for PMBL tablets to dissolve under the tongue?

The concept of PMBL is a sublingual administration so not a fast dissolving formulation. In that way, PMBL sublingual tablets will stay in contact with the oral mucosa to allow a loco-regional action of the antigens contained in the PMBL with the immunocytes. The usual time of complete disintegration of PMBL tablets in the oral cavity is around 1.5 minutes.

### 6. What is the effect or consequence to lose one or two intakes?

A loose of one or two intakes will not affect so much the immune answer as it has been demonstrated that PMBL still offers protection for an additional 3 months after the end of the treatment.

However it is always recommended to follow the posology scheme as prescribed, and even if you lose one or two intakes, never exceed the recommended dosage.

### 7. Should the 10 days recommended in PMBL tablet posology be consecutive?

The 10 days should be consecutive as per all the designs in PMBL clinical studies. Additionally, all the immunological studies proved that this schedule of treatment is the best possible one in order to obtain a raise of biomarkers.

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### 8. *Why it is recommended to take PMBL tablet before eating and what happens if the tablet is taken after eating?*

For a question of compliance it is recommended to take PMBL tablet before eating, however if the tablet is taken after eating there is no risk of a decrease of PMBL activity.

## MODE OF ACTION

### 1. *How long does the PMBL therapy offer protection?*

It has been demonstrated in several clinical studies (10, 11, 14) that PMBL starts to offer protection since the first week of intake and provides protection for an additional 3 months after the end of the 3 months treatment.

### 2. *Does PMBL offer protection against organisms not included as lysates in the product?*

As general rule PMBL offers protection against upper and lower respiratory tract infections. In our example we can cite tuberculosis case; the organisms responsible for tuberculosis are *Mycobacterium tuberculosis.* The study performed by Boris (8) on 360 patients having a positive tuberculin test, has demonstrated the interest of PMBL as adjuvant to the conventional treatment (isoniazid) to overcome the development of the disease.

Additional example is one of the flu, here we can cite the expert report we have from Pr M.Cazzola (15) demonstrating that PMBL could be used as adjuvant to antiviral viral medication as it has been demonstrated that PMBL can induce the activation of dendritic cells and in general can promote the activation of innate immune response and specific ones. The study carried out by Tuvim (16) in vivo on mices has demonstrated as well that aerosolized bacterial lysates can be beneficial to trigger an effective immune answer against influenza virus.

*Why PMBL offers such protection against organisms not included in the formulation*? In fact as explained previously there are some similar patterns between different strains and furthermore bacteria and viruses act in synergy. As PMBL is able to increase all the subset of Natural Killer cells and to activate the maturation of dendritic cells, so to stimulate the innate immune answer, this explains why PMBL offers protection against all the pathogens involved in upper and lower respiratory tract infections.

### 3. *How explain that PMBL can act both on viral and bacterial respiratory infections?*

As it has been explained before, PMBL is able to act both on innate and adaptive immunity. Especially it has been demonstrated by B.Morandi (17) that PMBL is able to activate both circulating dendritic cells and plasmacytoid dendritic cells. This latest subset of DC plays an important role in the recognition of viral components. Additionally, F.Braido (18, 19) demonstrated that PMBL was able to activate as well the NK cells which play an important role in the destruction of cells infected by viruses.

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### 4. Can PMBL effect on immune system be measured? How? What are the results?

Of course PMBL effect on the immune system can be measured. There are several immunological data existing on PMBL. The first studies demonstrated that PMBL were able to activate dendritic cells which act as sentinel and sample the antigens from the lumen into the mucosa and as well increase the secretion of efficient salivary IgA. (20, 2, 21, 22) To measure the activation of the dendritic cells, markers like CD80, CD83 and CD86 markers are evaluated. At that time studies performed by Pr G.Lanzilli demonstrated as well that PMBL was able to stimulate both T and B lymphocytes (23, 24).

The latest results obtained on PMBL have demonstrated that they can induce the differentiation and activation of T helper CD4+ subset, to increase the reservoir of naive B cells and early memory B cells which will allow the immune system to be ready to fight infections, to recruit a large number of precursor B cells to be differentiated in plasma cells, to increase specific and opsonizing immunoglobulins, and to increase the total number of NK cells (17, 18, 19).

### 5. Does PMBL provide a long life effect?

As explained in point 1 PMBL offers protection during the 3 months treatment and as well during the 3 months follow-up. For a complete protection all over the year, 2 courses of 3 months PMBL treatment are then recommended.

## CLINICAL RESULTS

### 1. Are there any Randomized Clinical Trial on PMBL in immunocompromised patients?

There are no specific clinical studies with PMBL in immunocompromised patients; however a clinical trial has been conducted with bacterial lysates in HIV positive patients which proved to be beneficial with no safety concern.

In fact Pr B.Petrunov from Bulgaria demonstrated on 100 patients that bacterial lysates can be beneficial in HIV patients. The bacterial lysates were able to significantly stimulate the different immune competent cells and to preserve in 50-60% of the patients an adequate level of monocytes and neutrophil granulocytes to activate the phagocytosis. The IgAs were as well increased in 76% of patients. (25)

### 2. Are there any studies demonstrating the immunological changes in the body response to PMBL?

Several clinical studies performed with PMBL have also evaluated the immunological parameters.

The first study we can cite is the study performed by Pr Tricarico(10), in which 47 patients received either PMBL or placebo and for the both groups immunological parameters, serum immunoglobulins (IgA, IgG and IgM) as well as salivary IgA have been measured before and after treatment. The results obtained have shown a significant increase of all the immunological parameters measured in the PMBL group: IgG (+35%), IgM (+86%), IgA (+80%) and salivary IgA (+110%).

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The second study which evaluated as well immunological parameters is the one from Boris (26). This study was performed on 300 patients at high risk of respiratory infections. One month after the end of the treatment with PMBL; the lymphocytes, monocytes, IgA and IgM have been significantly increased as compared to baseline. Another study from Boris (8) can be cited as well. This randomized, double blind, placebo controlled study evaluated the effect of PMBL on 360 patients with latent tuberculosis. The result was a significant increase of the activity of peripheral blood mononuclear cell (PBMC) in PMBL group ($p<0.05$) as compared to placebo, after both stimulation with purified protein derivative (PPD) *M.tuberculosis* and phytohemagglutinin (PHA). Additionally, it has been demonstrated in the PMBL group a significant increase as compared to baseline of the specific antibodies IgG against *S.pneumoniae (+36%)*, *S.pyogenes (+49%)* and *S.aureus (+71.5%).*

### 3. Has PMBL been evaluated for efficacy in other systemic infection (like UTI) apart for respiratory tract infection?

The indication of PMBL is in the respiratory tract infections in that way, PMBL has not been evaluated for efficacy in other systemic infections. But theorically, PMBL have very significant immunological effects and thanks to their benefits, they should be used as adjuvant for other therapies, including immunological therapies, allergies or cancer immunotherapy.

But of course the evidence of a benefit for PMBL has been demonstrated in respiratory tract infections, so additional clinical studies need to be performed to check the potential activity of PMBL in other therapeutic areas.

### 4. Are there any PMBL studies with large cohort?

Of course as already described all the studies from Boris (8, 11, 26) have been performed on cohort of equal or more than 300 patient population. The study from Aksic (27) has been performed on 180 school-aged children. If we pool all the studies performed on PMBL by indication we obtain the following:

- Prevention of URTI, 5 clinical studies, global population studied 650 patients
- Prevention of LRTI, 4 clinical studies, global population studied 1029 patients
- Prevention of exacerbations in COPD, 3 clinical studies, global population studied 298 patients
- Prevention of RRTI in children, 3 clinical studies, global population studied 389 patients

Furthermore, Lallemand Pharma (owner and in charge of the research and Development of PMBL) is running a clinical trial (AIACE study) on 288 patients to demonstrate the benefit of PMBL to prevent acute exacerbations in mild to very severe COPD patients.

In parallel, a meta-analysis has been run on all the clinical trials performed with PMBL. The final results have demonstrated the interest to use PMBL in the prevention of upper and lower respiratory tract infections as it allows reducing the number of episodes of infections.

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### 5. Are there any Randomized, double blind control trial in PMBL?

As already described there are several randomized double blind control trials, we can cite the study from Tricarico which demonstrated on 47 patients that PMBL prevents URTI as compared to placebo (10) ; the study from Boris (8) on 360 patients and which demonstrated that PMBL prevent the development of Tubercular disease and concomitant respiratory infections as compared to placebo, the study from Aksic (27) on 180 patients and which demonstrated that PMBL prevents RRTI in children population as compared to placebo ; the study from Cazzola (5) on 178 patients with moderate to very severe COPD and which demonstrated that PMBL prevents acute exacerbations as compared to placebo.

Additionally several clinical studies on PMBL have been evaluated not against placebo but against chemical lysates. We can cite as example the study from Macchi (14) on 114 patients and which demonstrated that PMBL is superior to prevent URTI as compared to chemical lysates and control.

### 6. Are there any clinical studies on going on PMBL?

As mentioned previously there is one clinical study on going with PMBL. AIACE study evaluates the effect of sublingual administration of PMBL in patients with moderate to severe and very severe COPD according to GOLD classification in term of reduction of acute exacerbations. This study is a multicenter, double blind, randomized controlled study. The design plans the enrolment of 288 patients, age >40 years with documented moderate to very severe COPD. Actually an interim analysis is performed on 170 patients, the first results will be known at the end of October 2011.

Additionally, a meta-analysis of all the clinical studies performed with PMBL has been carried out this summer, the results of the meta-analysis will be known by the end of October 2011.

## CONTRAINDICATION

### 1. What are the contraindications of PMBL tablet?

The contraindications of PMBL are:

- Hypersensitivity to the active substance or to any of the excipients
- Use of PMBL during the first 3 months of ascertained or presumed pregnancy, even though no toxic effects were observed in animals and no control study existing in pregnant woman
- Precaution must be taken during lactation as no specific studies have been conducted during lactation

### 2. Can PMBL tablet be used in pregnant women or lactating women?

The answers are above. Regarding lactation there is no specific contra-indication, but it must be highlighted that to date no specific studies have been conducted during lactation.

### 3. Can PMBL tablet be beneficial to Intensive Care Unit patients?

There are no specific clinical studies conducted with bacterial lysates in ICU patients.

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It is important to highlight the recent study on the community acquired polymicrobial (CAP) pneumonia (28), which evaluated the prevalence, clinical characteristics and outcomes of severe CAP in ICU patients. The results obtained have shown that polymicrobial infection was identified in 20% of the ICU patients studied with defined etiology. The highest prevalence was obtained with *Streptococcus pneumoniae* representing 72% of the CAP, followed by respiratory viruses (39%) and *Pseudomonas aeruginosa* (21%). At sight of such results, a particular attention should be drawn on the interest of PMBL in ICU patients.

However, the AIACE study has been run as well on moderate, severe and very severe COPD patients. The latest subgroup experienced a greater benefit from PMBL than the other groups. In that way, PMBL is beneficial in such very severe stage of patients.

### 4. Can PMBL be used in immunocompromised patients?

There are no specific clinical studies with PMBL in immunocompromised patients; however a clinical trial has been conducted with bacterial lysates in HIV positive patients which proved to be beneficial with no safety concern. (25)

### 5. Can PMBL be used in patients with autoimmune disease?

PMBL shall not be used in patients with autoimmune disease as there is no existing data about it.

## SAFETY

### 1. Are there any long term side effects?

Pharmacovigilance never showed any SAE directly linked to PMBL tablet, except a case of laryngitis. Regarding the possible side effects, the reference is the SPC and in very rare cases the following undesirable effects have been reported: respiratory, thoracic and mediastinal disorders, skin and subcutaneous tissue disorders

### 2. Can PMBL increase the disease symptoms?

All the clinical studies on PMBL have reported on the contrary a decrease of the intensity of the symptoms when PMBL is taken in prevention. Regarding respiratory infections in general, the study from Tricarico (10) demonstrated that PMBL group has shown a decrease of 79% of the symptoms as compared to baseline while in the chemical lysates group only a decrease of 25% was observed.

Furthermore we can also cite the study from Pr Cogo (29) which evaluated in a population of 57 patients with medical history of chronic obstructive bronchitis, the efficacy of PMBL. The results here also have been significant showing a reduction of the gravity of exacerbations during PMBL treatment period (247 exacerbations) as compared to baseline (496 exacerbations).

However, we must say that one case of laryngitis linked to the intake of PMBL has been recorded in our pharmacovigilance.

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### 3. *Are there any reported side effects for PMBL?*

Regarding the possible side effects, the reference is the SPC, and in very rare cases the following side effects have been reported:

- Respiratory, thoracic and mediastinal disorders
- Skin and subcutaneous tissue disorders
- General disorders and administration site conditions
- Gastrointestinal disorders

In case of any disorders, the treatment should be discontinued.

## CHILDREN

### 1. *What is the recommended dosage in Pediatric patients?*

Based on the expert's report written by Pr G.Melioli, and as already explained in the « Indication », point 2, there is no rationale to have a different dosage with PMBL for children from 3 years old and adults.

Additionally, if we look at the dosage of the flu vaccine, there is no different dosage between adults and children.

So the recommended dosage of PMBL in pediatric patients is the same as in adults.

### 2. *How it is recommended to give PMBL sublingual tablets to children between 3-5 years old?*

To avoid risk of aspiration, for children less than 6 years it is recommended to crush and melt the tablet with marmalade, compote or yoghurt, and to keep the mixture some seconds before swallowing.

### 3. *Are there any PMBL studies with children population?*

There are 3 clinical studies which have evaluated the efficacy of PMBL in respiratory tract infections in children.

The first one is the study from Pr Rosachino (30) (which evaluated in 89 children from 10 months to 10 years with recurrent respiratory infections, the preventive effect of PMBL. The patients were divided in 2 groups, 24 patients in the control group and 65 patients in the PMBL group. The patients were followed during autumn and winter of 3 consecutive years (2001-2002-2003).

The results obtained were significant for PMBL treated group and a decrease from 7.84 infections/patient (baseline, same group previous winter) and from 6.78 infections/patients (control group, same winter period) to 4, 78 infections/patient in the PMBL group was observed. It has also

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been demonstrated that PMBL induced a significantly increase in antibody titer even after one month treatment. The following figure shows the impact of PMBL on antibodies titer.

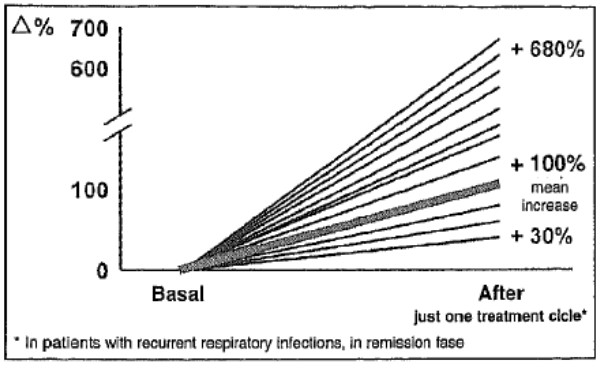


Figure 2. Significant variation in antibody titre using a polyvalent mechanical bacterial lysates (PMBL)

The second study that we already cite is the one from Aksic (27) on 180 children school-aged (5-10 years old). The children were divided in 2 groups: PMBL and placebo. The 2 groups received the treatment as recommended in the posology. The results have shown that there was a significant reduction of the mean number of infectious episodes in the PMBL group (1.9 infections/patient) as compared to placebo group (4.1 infections/patient), so a decrease in fact of 54%, $p<0.01$. This study also demonstrated that PMBL allowed to reduce the school absenteeism (8.1 days) as compared to placebo (16.1 days), so a decrease of almost 50%, $p<0.01$ ; additionally a significant reduction of the antibiotic use of 48% has been also demonstrated in the PMBL group as compared to placebo. No adverse events have been recorded.

And finally the third study was performed by Pr La Mantia (31) on 120 children, aged 4-9 years old, with URRI. The children were divided in 3 groups, the first group received PMBL, and the second group received chemical lysates, while the third group was control. The results obtained were significant for PMBL as regards to children with no infections during the treatment, $p<0.016$. Effectively, 67.5% of children who received PMBL did not develop any URI during the period, as compared to only 37.5% in the chemical lysates group and 22.5% in the placebo group. The secondary endpoint has also shown that PMBL allowed reducing the use of additional treatment (antibiotics, antipyretics, antiphlogistics). The duration of the episodes as well the school absenteeism were significantly reduced in the PMBL group as compared to chemical lysates group and control.

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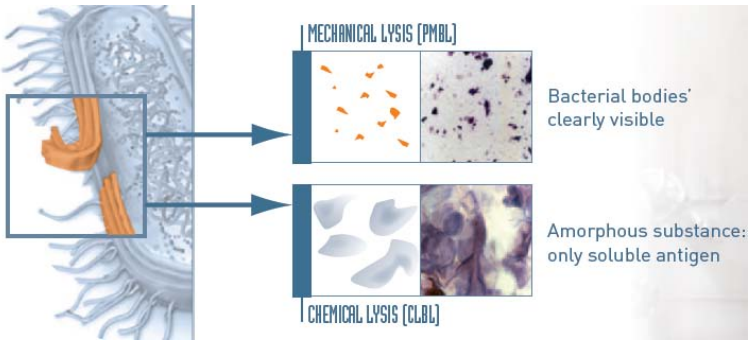
## MECHANICAL LYSIS

### 1. What is the difference between a Mechanical Lysis and a Chemical Lysis?

A mechanical lysis is performed by increasing the pressure on the inactivated bacteria's walls which is the specific process of Lallemand. This allows obtaining the particulate antigens from the inactivated bacteria (17).

On the contrary a chemical lysis is performed with the use of chemical alkalines on the inactivated bacteria. This process is much more aggressive for the antigens and may denature them (32). In fact such lysis allows obtaining only soluble antigens.

Under optical microscope, it is possible to clearly observe the differences between a chemical lysate and a mechanical lysate.




### 2. What are the differences of Mode of action between PMBL and Chemical lysates?

As explained above the drugs based on chemical lysates contain only soluble antigens. The latest immunological study from Pr. G.Melioli has demonstrated that chemical lysates were much less powerful regarding the activation of dendritic cells. The study has demonstrated that PMBL (particulate antigens) are 10 to 100 times more efficient than soluble antigens to induce the maturation of dendritic cells (17).

It has been demonstrated as well that PMBL can act on all the actors of the immune cascade: efficient maturation of DC, activation of NK cells, CD4+ T helper cells, increase of the reservoir of naïve B cells and early memory B cells to allow the immune system to be ready to fight infections, increase of the secretion of specific and opsonizing antibodies, induction of an efficient opsonization (33)

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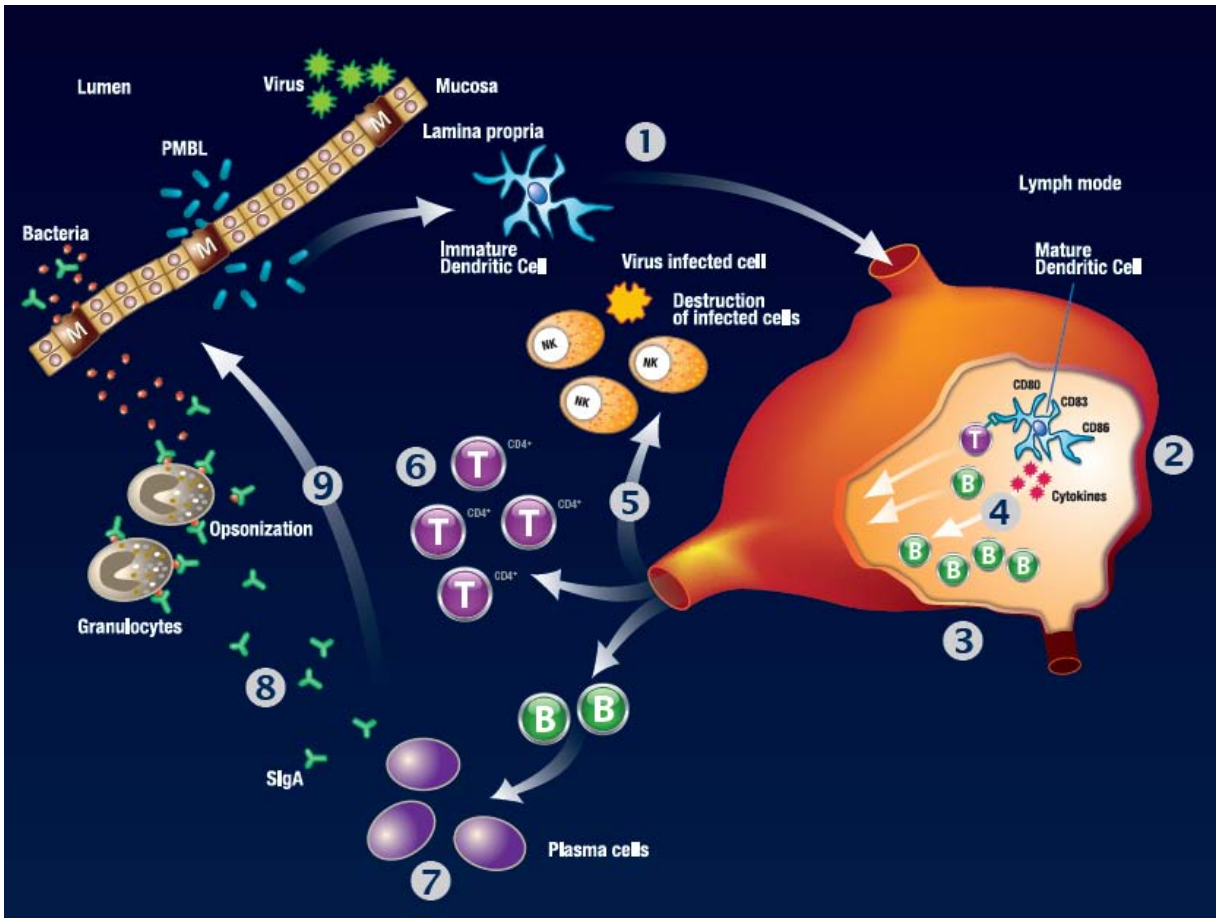


### 3. *Are there any clinical differences between PMBL and Chemical lysates?*

Of course as we have seen before that PMBL are really more efficient from an immunological point of view, this as well has been demonstrated in clinical studies.

The study from Macchi (14) performed on 114 patients with at least 4 episodes of acute URTI in the 12 previous months, demonstrated the superiority of PMBL as compared to chemical lysates. The results were a significant lower mean number of URTI in the PMBL group as compared to chemical lysates group and control group during the treatment period and the 3 months follow-up. Regarding the secondary end-points, it has been demonstrated that PMBL were superior in reducing the work absenteeism, the duration of symptoms and no antibiotic use was needed in the PMBL group.

The study from Rossi (34), performed on 69 patients with recurrent respiratory tract infections (3 or more acute episodes in the last 12 months) has shown that PMBL administered based on the standard posology scheme, that it significantly reduced the mean number of infections/patient as compared to chemical lysates and control and as well the number of patients with at least one LRTI, only 21.7% patients in PMBL group as compared to 69.6% in the chemical lysates group and 95.7% in

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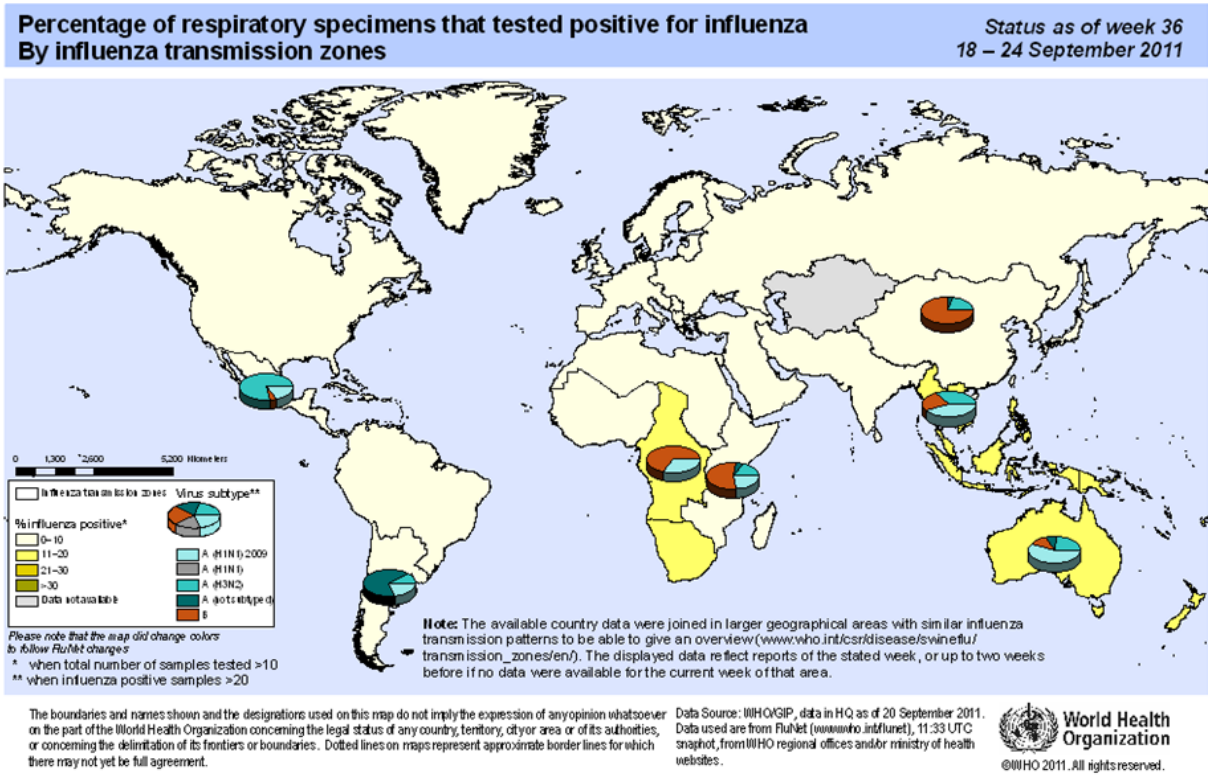
the control group. These results were correlated with the reduction of patients with LRTI using antibiotics in PMBL group (17.4%) as compared to 60.9% in the chemical lysates group and 91.3% in the control group.

The study performed by La Mantia (31) and described in the Children part of this booklet has also demonstrated the superiority of PMBL as compared to chemical lysates to prevent upper and lower RTI.

## ADVANTAGES

### 1. *What are the advantages of PMBL as compared to other vaccines (flu vaccine, Pneumococcal vaccine...)?*

As previously described PMBL is a Polyvalent Mechanical Bacterial Lysate, its mode of action is based on the activation and maturation at the level of oral mucosa of the dendritic cells. This maturation is really important as it allows an efficient recognition of the pathogens to activate all the actors of the immune system. By this action, PMBL is able to stimulate both innate and adaptive immune system. If we take the example of flu vaccine it can act only at the level of the adaptive immunity and can prevent infections by influenza viruses. Most of the seasonal flu vaccine are tri-valent vaccines containing the 3 types A/H1N1, A/H3N2 and B, and can prevent the flu caused by these 3 types which are the most involved in the seasonal flu (source WHO).



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The pneumococcal vaccine allows preventing infections from *Streptococcus pneumoniae*. Three vaccines are existing: Prevnar (Wyeth), Synflorix (GSK), Prevnar 13 (Pfizer). Here also these vaccines allow stimulation of the adaptive immune system with the production of specific immunoglobulins against *Streptococcus pneumoniae*.

The advantages of PMBL are its specific formulation containing 13 different inactivated strains from the most occurring pathogens of the upper and lower respiratory tract infections. In that way PMBL is a polyvalent vaccine which allows preventing a broad spectrum of respiratory infections. Its action on both innate and adaptive immunity confers also efficiency against either bacteria or viruses infections.

### 2. *What are the advantages of PMBL as compared to other bacterial lysates?*

As described previously PMBL is the only one polyvalent bacterial lysate to be obtained by mechanical lysis for which it is documented. It has been demonstrated the importance of the process of lysis as this later will affect the immunological power of the bacterial lysates. By obtaining particulate antigens with mechanical lysis, the immunological efficacy is multiplied by 10-100 times.

The following table shows the different formulation available on the market.

| Brand name | Process | Formulation | Administration | Dosage |
|---|---|---|---|---|
| **Ribomunyl, Immucytal, Ribovac** | ARN extraction, ribosomal fractions | **4 strains** : Klebsiella pneumoniae Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae | Oral | 1 unit for 4 days per week during 3 weeks, then for 4 days/month during the cold season |
| **Pulmonarom** | No documentation available | **9 strains** : *Haemophilus influenzae, Staphylococcus aureus, Moraxella catarrhalis, Klebsiella pneumoniae,Diplococcus pneumoniae, Streptococcus pyogenes, streptococcus agalactiae, Streptococcus dysgalactiae, Streptococcus anginosus* | Oral | 1 vial of 3 ml per day, 10 days a month, for 2 months |
| **Bronchovaxom, Ommunal, Bronchomunal** | Chemical lysis, soluble antigens | **8 strains** : *Haemophilus influenzae, Diplococcus pneumoniae, Klebsiella* | Oral | 1 capsule/Day 10 days a month, |

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| | | | | |
|---|---|---|---|---|
| | | *pneumoniae, Klebsiella ozaenae, Staphylococcus aureus, Streptococcus pyogenes, Streptococcus viridans, Neiserria catarrhalis* | | during 3 months |
| **Luivac, Paspat** | No documentation available | **7 strains :** *Staphylococcus aureus, Streptococcus mitis (viridans), Streptococcus pyogenes, Streptococcus pneumoniae, Klebsiella penumoniae, Branhamella(Neiserria) catarrhalis et Haemophilus influenzae.* | Oral | 1 tablet/day for one month (28D), stop one month and another course of 1 tablet/day for one month (28D) |
| **Ismigen, Immubron, Respibron, Pulmigen, PIR-05, Provax** | Mechanical lysis documented, particulate antigens | **13 strains :** *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus oralis (formerly viridans) , Klebsiella pneumoniae, Klebsiella ozaenae, Haemophilus influenzae, Neisseria (formerly Moraxella) catarrhalis, Streptococcus (formerly Diplococcus) pneumoniae (Type 1, Type 2, Type 3, Type 5, Type 8, Type 47)* | Sublingual | 1 tablet/day, 10 days a month, during 3 months |

Additionally a meta-analysis published in 2005 in Mexico (PMBL were not included in this meta-analysis as they were not already available on the Mexican Market) demonstrated that only Bronchovaxom and Ribovac had significant effect in the prevention of acute respiratory infections in children, the other formulations evaluated (Luivac, Biostim, Pidotimod) showed only a trend, while no control studies were available on Pulmonarom (35).

Saying that PMBL tablet has demonstrated its superiority to chemical lysates, even in children age group (31), it seems legitimate that PMBL tablet is the new generation of bacterial lysates and its advantages are the following:

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- Sublingual route of administration allows a direct contact of the antigens with the mucosal immunocytes of the mouth and the pharynx, inducing a loco-regional activation of the immune system.
- Mechanical lysis ensures to obtain particulate antigens which in turn are proved to be 10-100 times more immunogenic than soluble antigens. This superiority has been also demonstrated in clinical studies.
- PMBL tablet is proved to act on all the immunological cascade to stimulate both innate and adaptive immunity

### 3. *Why vaccination, and especially PMBL prophylactic is better than natural immunity?*

In fact during vaccination and especially PMBL prophylactic, the immune system is in contact with billion of inactivated bacteria which is not the case of the natural immunity when you are in contact with some pathogens. So the immune answer in case of natural immunity is longer to be put in place and is not sufficiently efficient. Additionally, PMBL is a prophylactic treatment able to efficiently activate the immune system in order to avoid the infections by such pathogens. Additional example can be made regarding flu vaccine, for which you need to be vaccinated every year.

### 4. *What are the advantages of the sublingual route?*

The advantages of the sublingual route is to allow direct contact of the antigens contained in the PMBL tablet with the immunocytes of the mouth and the pharynx, inducing a loco-regional activation of the immune system. The sublingual route offers also better surveillance along the oral mucosal barrier, since circulating memory cells preferentially return to the area of first encounter with the antigen (32).

### 5. *What are the advantages to use PMBL in addition with antibiotics?*

The advantages to use PMBL in addition with antibiotics are linked to their benefit to induce potent immune answer. Thanks to this action, PMBL is able to reduce the number of episodes of infections and thus to reduce the use of antibiotics. Knowing that the increase of antibiotics resistance is a worldwide health concern, the reduction of antibiotic use and the prevention approach are the best ways to counter this health issue.

## BUSINESS

### 1. *Where PMBL are distributed?*

PMBL tablet is distributed worldwide. The following map shows the presence and registration ongoing of PMBL, as well as their brand names.

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### 2. What is the standard public price of PMBL?

The public price of PMBL tablet varies depending on the region it is distributed and the value chain of the regional channels. The standard unit public price of PMBL tablet is comprised between 0, 60 Euros to 1 Euro in the Euro Zone. In LATAM, the price is comprised between 3, 10 to 3, 50 USD.

### 3. What are the sales data of PMBL tablets?

To give some examples of sales data of Ismigen:

- In Italy Ismigen is sold since 10 years and is the N°1 on the bacterial lysates market with 18,8% market share in units, which represents 14 millions tablets a year
- In Poland Ismigen is number 1 only after 6 months of launch, with 36,6% market share in value, which is planned to be 3 millions tablets a year
- In Ukraine, Respibron is second on the market after 18 months launch, with 19, 4% market share in units, which represent 3 millions tablets a year.
- In Peru, Ismigen is the number 1 after one year launch, with 34% market share in units which represents 100,000 tablets a year.

### 4. What is the sale model of PMBL tablet?

Except in Ukraine where PMBL tablet is an OTC drug, usually it is sold under prescription. PMBL is usually not reimbursed, except in Korea where it gained the reimbursement status in 2011 and in

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Argentina where it is reimbursed since 2009. The best way to promote PMBL tablet to increase the renown of the brand is to focus the medical visit on prevention of respiratory infections among general practitioners, pediatricians and ENT, and to increase the bacterial lysates market is to focus the medical visit on prevention of AECOPD among pulmonologists and GP's. In case of OTC product the information to pharmacists on why and how to recommend PMBL is necessary.

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