Immunotherapeutic vaccines and Microbicides for reproductive health

- A unique immunotherapeutic vaccine for leprosy approved by DCGI and USFDA, its new found utility in tuberculosis and cancers

- Two microbicides for cure and prevention of reproductive tract and sexually transmitted infections
Importance of Immunity in Leprosy

- Most ($\geq 99\%$) do not contract the disease on exposure to M. leprae.

- Those who do, fall in a spectrum between polar TT to polar LL depending on the degree of Immune deficit.
Nature of defect is the inability to mount CMI response of the Th1 type to M. leprae

Macrophages unable to kill and eliminate M. leprae

Normalcy of reactivity to other bacteria and Microorganisms
No medium known for culture of M. leprae, requires a host cell obligatorily

Developed an *in-vitro* system for study of permissive growth:

Monocyte-derived macrophages from peripheral blood

- Slow grower – division time 13 days

- M. leprae source – Human lepromas containing unknown number of live and dead bacteria

- Devised a selective approach for incorporation of Methyl $^3$H-thymidine into DNA by M. leprae without interference of host macrophages

Mycobacterial multiplication in cultivated macrophages derived from peripheral blood monocytes of Leprosy patients

<table>
<thead>
<tr>
<th>Patient no</th>
<th>Clinical status</th>
<th>CPM $^3$H-thymidine incorporated per $5 \times 10^5$ Phagocytic cells</th>
<th>Macrophages + Lymphocytes + M. leprae</th>
<th>Macrophages + M. leprae</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>LL</td>
<td>36,458</td>
<td>45,628</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>LL</td>
<td>53,929</td>
<td>59,596</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>LL</td>
<td>52,354</td>
<td>83,476</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>TT</td>
<td>6,332</td>
<td>54,969</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>TT</td>
<td>32</td>
<td>78,447</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>TT</td>
<td>381</td>
<td>26,260</td>
<td></td>
</tr>
</tbody>
</table>

Nature of defect is the specific inability to mount CMI response of the TH1 type to M. leprae


An Immuno-therapeutic vaccine
Developed For Multi bacillary Leprosy
based on Heterologous Approach

(14 papers in Golden Jubilee issue of Leprosy In India Oct. 1978)

Strategy adopted – Search for an immunologically cross-reactive mycobacteria eliciting immune response from not only TT but also LL patients; in-vitro followed by in-vivo investigations
A non-pathogenic fast growing Mycobacterium coded as ‘M.w’, sharing antigens with M. leprae and M. tuberculosis discovered.

Key antigens heat stable; usable as autoclaved vaccine; Simplification of toxicology studies.

Preclinical toxicology ➔ Phase I human safety ➔ Phase II, Phase III efficacy ➔ to field trials with approvals of Drugs Controller General of India and Ethics committees.
Approved by DCGI, India and US FDA
Mw vaccine
Combined Immunotherapy with Chemotherapy

BENEFITS
- Faster bacterial clearance
- Shortens recovery time
- Clears granulomas
- Reactions (number and severity) reduced
- Lepromin conversion to positivity
- Effective in “slow responders”
Patient code: MD

Initial

After 4 doses

There after 1 year
Patient code: CR

Initial

After 5 doses
Patient code: BWS

Initial vs. After 4 doses
Patient code: CHB

Initial | After 1 Year
Patient code: RAB

Initial

After 4 doses
Patient code: SNC

Initial After 2 doses (six months)
Protection test of M.w grown in Bio-reactor

(a) & (b) Guinea pigs challenged with M.tuberculosis H\textsubscript{37}R\textsubscript{v}
(c) & (d) Immunized with M.w.

Spleen
Lungs
Singh IG, Mukherjee R & Talwar GP. Vaccine, 9, 10 – 14, 1991
OUTCOME OF THERAPY
(CAT II Tuberculosis)

CURED

Mw + MDT  48/49*  97.96%

MDT alone  21/27**  77.77%

*  Defaulter for 6 doses, sputum negative after intensive phase
** 2 –2+  No effect of therapy
  4 –1+  No effect of therapy
## RELAPSE RATE (24 MTHS AFTER COMPLETION)

<table>
<thead>
<tr>
<th>CATEGORY</th>
<th>I</th>
<th>II</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDT + Mw</td>
<td>0/16 (0.0%)</td>
<td>2/46 (4.34%)</td>
</tr>
<tr>
<td>MDT alone</td>
<td>8/33 (24.24%)</td>
<td>11/20 (55%) *</td>
</tr>
</tbody>
</table>

*P=0.002

9TH NORTH AMERICAN IUATLD CONFERENCE-2005
CHRONIC TUBERCULOSIS

![Graph showing chronic tuberculosis progression](image)

- Mw: Red line
- Control: Yellow line
- Time points: 0, 2, 6, 12
Genomic analysis of M.w. done by a National consortium of Professors S. Hasnain, Akhlesh Tyagi and Anil Tyagi - laboratories
MIP ancestor of M. tuberculosis, M. leprae and M. avium intracellular complex (MAIC)

*PloS ONE* (2007), 10/e968, 1 – 8

M.w. named as Mycobacterium indicus pranii (MIP)

Landscape of genome evolution across the ‘generalist’ and ‘specialist’ mycobacterial lineages

PloS ONE (2007), 10/e968, 1 – 8
Ongoing therapeutic clinical trials on Mycobacterium indicus pranii (MIP)

- Phase III multicenter trials under DBT in tuberculosis
- Bladder Cancer (B Khamar, Ahemadabad; Pant, K G Medical College, Lucknow)
- Crohn’s disease (S. Hasnain and others)
- Psoriasis (Rath & Kar, 2003. Int. J. Dermat. 42, 756-57)
CREDITS

Dimensions of STIs and RTIs (WHO)

- 340 million cases of new sexually transmitted infections occurred in the year 1999 worldwide

New cases of various STDs each year (2006)

- 92 million of Chlamydia trachomatis
- 62 million of N. gonorrhoeae (Gonorrhea)
- 5.5 million of Human Papillomavirus (HPV)
- 5 million of Trichomoniasis
- 1 million of Genital Herpes
- 77,000 of Hepatitis B
- 300,000 – 500,000 of Candida albicans / year in the US only
- 300 million vaginosis / vaginitis

- Over 40 million people are living with human immunodeficiency virus (HIV) / AIDS worldwide and AIDS has assumed pandemic dimensions in Africa & South East Asia

http://www.who.int/reproductive-health/rtis/docs/sti_factsheet
## Two Microbicides

<table>
<thead>
<tr>
<th>PRANEEM Polyherbal</th>
<th>BASANT Polyherbal</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Tablet</td>
<td>Cream / gel</td>
</tr>
<tr>
<td>2. 15-30 min dispersion time</td>
<td>Ready to use</td>
</tr>
</tbody>
</table>

- Both have wide spectrum anti-microbial action
- Inhibit *N. gonnorrhoeae* (including Drugs resistant isolates); *E.coli, Staph. aureus*
- *Candida albicans* (including *Flucanozole resistant strain*) *C. tropicalis, C. krusei, C. glabrata*
- *Herpes simplex-2; Chlamydia trachomatis*
- *Anti-HPV-16*
- High virucidal action on *HIV-1*

Talwar et al. *Am Jour Reprod Immunology*, 43: 144-151; 2000
**Praneem:** Purified extracts of Neem leaves (Azadirachta indica); Saponins of Sapindus mukerosii; Mentha citrata oil

**BASANT:** Curcumin (Diferuloyl methane); purified extract of Amla (Emblica officinalis); Saponins of S. mukerossi; Aloe vera; Rose water

- All ingredients Quality controlled
- Tablets & Cream made under GMP conditions for clinical trials
- In these polyherbal formulations combination of ingredients extends the range
- Synergistic action, enhancing efficacy
Lack of action of Amla on various Candidas and Enhancement of Anti-Candida action of Saponins and Curcumin by Combination in total formulation BASANT

<table>
<thead>
<tr>
<th></th>
<th>Amla</th>
<th>Saponins</th>
<th>Curcumin</th>
<th>BASANT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Candida albicans</td>
<td>NZ</td>
<td>10</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>2. Candida glabrata</td>
<td>NZ</td>
<td>10</td>
<td>11</td>
<td>22</td>
</tr>
<tr>
<td>3. Candida krusei</td>
<td>NZ</td>
<td>10</td>
<td>NZ</td>
<td>18</td>
</tr>
<tr>
<td>4. Candida tropicalis</td>
<td>NZ</td>
<td>10</td>
<td>10</td>
<td>19</td>
</tr>
</tbody>
</table>

NZ = No zone of Inhibition
## Effect of Polyherbal formulation on prevention of Chlamydia vaginal infection

<table>
<thead>
<tr>
<th>Preparation</th>
<th># Positive on day 4/ # Inoculated</th>
<th># Positive on day 8 / # Inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyherbal Cream</td>
<td>00/12</td>
<td>00/12</td>
</tr>
<tr>
<td>PBS control</td>
<td>11/12</td>
<td>11/12</td>
</tr>
<tr>
<td>Polyherbal Pessary</td>
<td>02/12</td>
<td>02/12</td>
</tr>
<tr>
<td>PBS control</td>
<td>11/12</td>
<td>10/12</td>
</tr>
</tbody>
</table>

Investigations by K. Whalley, Johns Hopkins University
Effect on disease causation and Herpes Simplex virus-2 (HSV-2) multiplication

<table>
<thead>
<tr>
<th>Preparation</th>
<th># Shedding/ # Inoculated</th>
<th># with lesions/ # Inoculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyherbal Pessary</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>PBS control</td>
<td>8/8</td>
<td>8/8</td>
</tr>
<tr>
<td>Polyherbal Cream</td>
<td>0/6</td>
<td>0/6</td>
</tr>
<tr>
<td>PBS control</td>
<td>6/6</td>
<td>6/6</td>
</tr>
</tbody>
</table>

Investigations by K. Whalley, Johns Hopkins university
Phase II Efficacy Trials in women suffering from abnormal vaginal discharge due to RTIs

6 centers of the Indian Council of Medical Research at:-

- Postgraduate Institute Medical Education and Research, Chandigarh;
- AIIMS, New Delhi;
- Postgraduate Institute Kolkata;
- KEM Mumbai;
- KEM, Pune;

Subjects investigated = 128

Full or Partial Relief of symptoms in 124 women [96.8 %],

No Relief in 4 women [3.1%]

Extent of relief observed by Microbiology assays

- Trichomonas vaginalis
  - 8/8  100%
- Candida
  - 12/13  92.3%
- Bacterial vaginosis by Nugent score
  - 24/33  72.7%
Acceptability by both partners of Praneem Polyherbal vaginal Tablet for pre-intercourse use (NARI trials)

- 20 women & their partners
- Structured Questionnaire, focus group discussions
- 90% participants – 80% & higher acceptability score
- 95% liked the smell & reported that the product was easy to use

Phase II comparative safety and efficacy study of Praneem polyherbal tablet and Betadine in symptoms of chronic abnormal vaginal discharge in women employing WHO syndromic approach

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Patients treated (Number)</th>
<th>Cured after 2 weeks Rx one every night</th>
</tr>
</thead>
<tbody>
<tr>
<td>Praneem</td>
<td>50</td>
<td>46 / 50 (92%)</td>
</tr>
<tr>
<td>Betadine</td>
<td>49</td>
<td>40 / 49 (81.6%)</td>
</tr>
</tbody>
</table>

A PROSPECTIVE, COMPARATIVE, RANDOMIZED, MULTICENTRIC STUDY TO COMPARE THE EFFICACY AND SAFETY OF PRANEEM POLYHERBAL VAGINAL TABLET WITH BETADINE VAGINAL PESSARY IN SYMPTOMS OF ABNORMAL VAGINAL DISCHARGE

PHASE-III TRIAL in progress in 7 centers

Licensed to Panacea Biotech
### Inhibition of HIV NL4.3 replication by BASANT

<table>
<thead>
<tr>
<th>BASANT Dilution</th>
<th>In Human CD4$^+$ CEM-GFP cells</th>
<th>In Hela-CD4-LTR-βgal</th>
<th>Percent Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1000</td>
<td>98</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>1:10,000</td>
<td>81</td>
<td>64</td>
<td></td>
</tr>
<tr>
<td>1:20,000</td>
<td>51</td>
<td>53</td>
<td></td>
</tr>
<tr>
<td>1:40,000</td>
<td>22</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

Studies by Debashish Mitra at National Cell Science Center, Pune
INHIBITION OF HPV-16 TRANSDUCTION IN HeLa CELLS

Studies by Dr. John Schiller at NCI / NIH
Clearance of HPV DNA in Praneem-treated Women
(trials at Institute of Cytology and Preventive Oncology of ICMR)

Before Praneem Treatment

<table>
<thead>
<tr>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>+</td>
</tr>
</tbody>
</table>

~450bp

PCR L1 Consensus

After One (1) Course of Praneem Treatment

<table>
<thead>
<tr>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>+</td>
</tr>
</tbody>
</table>

~450bp

PCR L1 Consensus

After Two (2) Courses of Praneem Treatment

<table>
<thead>
<tr>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>+</td>
</tr>
</tbody>
</table>

450bp

PCR L1 Consensus
Women in Placebo-treated Arm showed Persistent HPV infection

**Placebo Group**

<table>
<thead>
<tr>
<th></th>
<th>Controls</th>
<th>Cases</th>
</tr>
</thead>
<tbody>
<tr>
<td>M</td>
<td>+</td>
<td>1, 2</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>3</td>
</tr>
</tbody>
</table>

Before Treatment

After Treatment

PCR L1 Consensus

450bp
Colposcopic Analysis of Regression of preneoplastic lesions in Women treated with Praneem

Before Treatment

After Treatment

Representative case showing abnormal transformation zone is characterized by acetowhite epithelium (flat warts indicated by arrows) on the posterior lip of the cervix with extensive ectopy.

Remission of acetowhite lesion (condylomatous changes) after Praneem treatment.
Cytological improvements in Pap Smears of Women treated with Praneem

**Before Treatment**

Pap Smear Showing intermediate and parabasal cells with rounded margins, thickening and separation of ectoplasm, and dark pyknotic eccentric nuclei, a hallmark feature of HPV infection. Pap Stain, original magnification 400X

**After Praneem Treatment**

Pap Smear Showing clearance of HPV infection and reversal of cellular changes in intermediate and parabasal cells characteristic of HPV infection. Pap Stain, original magnification 400X
SUMMARY

- Have developed Two Microbicides: Praneem Tablet; BASANT Cream
- Both have wide spectrum action against RTIs & STIs
- Both safe on 7 or 14 consecutive day use
- Praneem regresses AVDS in 92 - 96% women; Currently in Phase III trials; Licensed to Panacea Biotech
- Therapeutic Phase II / III trials in progress for elimination of HPV 16/18 in women with cervical dysplasia molecularly positive for HPV; Encouraging early results
## Credits

<table>
<thead>
<tr>
<th>Institution</th>
<th>Researchers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Talwar Research Foundation, New Delhi</td>
<td>GP Talwar, Sajad A Dar, Mahendra K Rai, Kavita Bansal</td>
</tr>
<tr>
<td>National Center for Cell Science, Pune</td>
<td>Debashish Mitra, Sujata V Kulkarni</td>
</tr>
<tr>
<td>CONRAD, Eastern Virginia Medical School, Virginia, USA</td>
<td>Gustavo Doncel</td>
</tr>
<tr>
<td>NCI / NIH, Bethesda, MD, USA</td>
<td>John Schiller, Christopher B Buck</td>
</tr>
<tr>
<td>Institute of Cytology &amp; Preventive Oncology (ICMR), Noida</td>
<td>Bhudev C. Das, Alok C. Bharti, Suresh Hedau, Shirish Shukla, Showket Hussain, Uma Kailash</td>
</tr>
<tr>
<td>National Institute for Research in Reproductive Health (ICMR), Mumbai</td>
<td>K V R Reddy</td>
</tr>
<tr>
<td>Johns Hopkins University, Baltimore, Maryland</td>
<td>K Whalley</td>
</tr>
</tbody>
</table>