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**Status of a Unique Vaccine against hCG for  
Contraception and Advanced Stage Cancers expressing  
ectopically hCG**

Talwar GP, Singh P, Gupta JC

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ENDOKRINOLOGIE & FERTILITÄT  
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# Status of a Unique Vaccine against hCG for Contraception and Advanced Stage Cancers expressing ectopically hCG

G. P. Talwar, P. Singh, J. C. Gupta

Dear Egon,

God bless you on your 95<sup>th</sup> Birthday! May you complete 100 years.

Being submitted in your honor is a brief article on my continuing work to make available a unique vaccine preventing pregnancy in women without blocking ovulation, her normal production of sex steroid hormones and retaining her regular menstrual cycles and bleeding profiles.

The vaccine is directed at the Human chorionic gonadotropin (hCG), which emerges following fertilization of the egg [1]. Healthy, non-pregnant women do not make it, the basis on which its detection in serum or urine serves as a reliable test for diagnosis of pregnancy. It plays a critical role in implantation of the embryo & thereby to the onset of pregnancy. The purpose of the vaccine is to generate bioeffective antibodies neutralizing hCG & thereby prevent the onset of pregnancy.

As you can imagine, the making of a workable vaccine, competent to make antibodies against a tolerant molecule to the woman's immune system (she literally bathes in hCG during pregnancy) was not simple. What was also demanded was high immunogenicity of the vaccine to make fairly high titres of antibodies to counteract the large amount of hCG made in early pregnancy. At each stage, it required testing in humans and before that could be done, appropriate toxicology studies & approval of Regulatory and Ethics Committees was needed each taking its time. Eventually the vaccine has to be amenable to industrial production to reach the public, hence a recombinant vaccine had to be developed. Given below is a brief write-up on the evolution of the vaccine against the human chorionic gonadotropin.

Yours, Pran

J Reproduktionsmed Endokrinol\_Online 2015; 12 (4): 268–73.

## ■ Linkage to a useful Carrier

hCG is composed of 2 subunits, alpha and beta (Fig.1). The alpha subunit is common to 3 other pituitary hormones: TSH, LH, FSH, hence  $\beta$  subunit of hCG was chosen to make the vaccine. hCG $\beta$  was linked to tetanus toxoid (TT), an immuno-prophylactic vaccine available in plenty at low cost, not only because it can mobilize T cell help to render B cells competent to make humoral response, but also because it can protect women from dying, following deliveries taking place in aseptic conditions. In the 1970's antenatal tetanus was a major killer of women in India.

hCG $\beta$  linked to TT indeed induced anti-hCG and anti-tetanus antibodies in all 4 women who opted for this investigation. They had completed their families and had undergone tubectomy [2, 3]. Figure 2 is representative of their immune response.

In order to test whether the circulating antibodies generated by the  $\beta$  subunit of hCG recognized the dimer, the biologically active hCG, a load test was performed. 5000 IU of authentic biological-

ly active hCG was given intravenously which led to decline in titer of anti-hCG antibodies indicating their binding with the administered hCG (Fig. 3). The titres of antibodies returned to previous levels

in course of time, which was again a desirable feature.

Thus by the carrier linkage approach, it was possible to render hCG immuno-

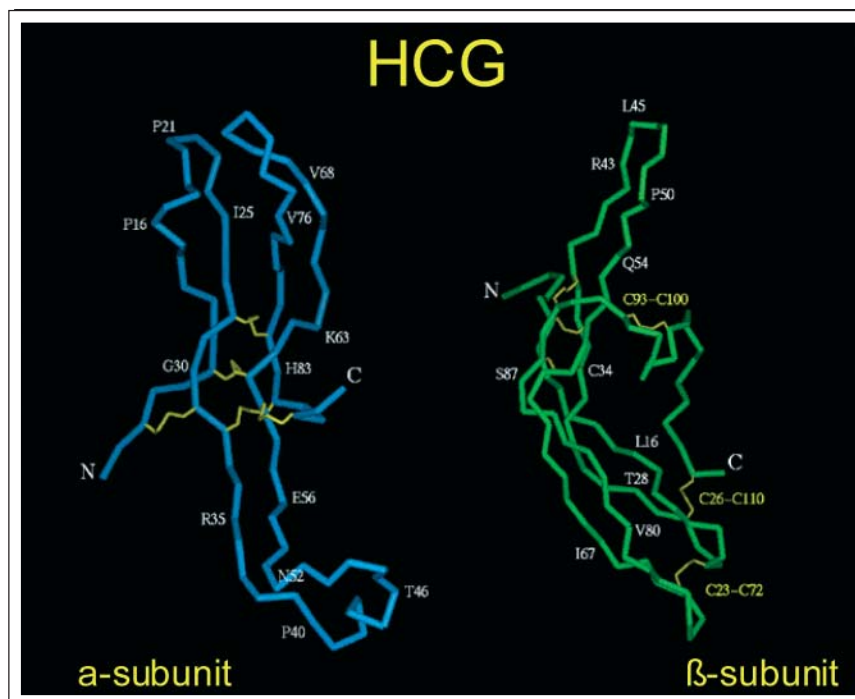
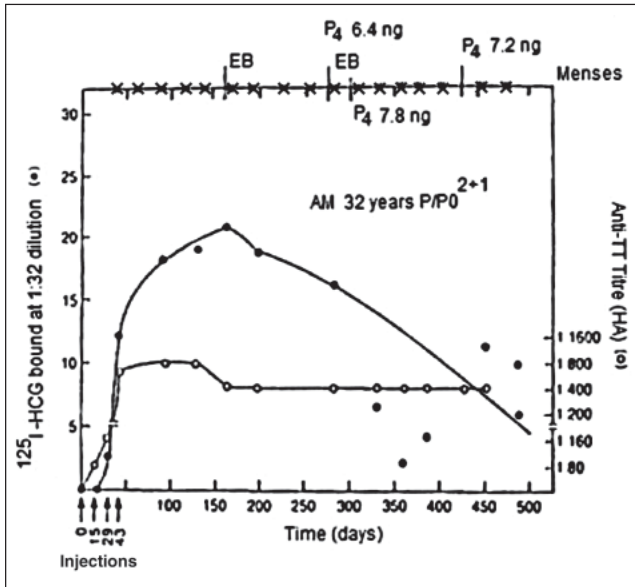


Figure 1. Human chorionic gonadotropin (hCG) is composed of 2 subunits.  $\alpha$  subunit is common to TSH, LH and FSH  $\beta$  subunit is hormone specific. © G. P. Talwar

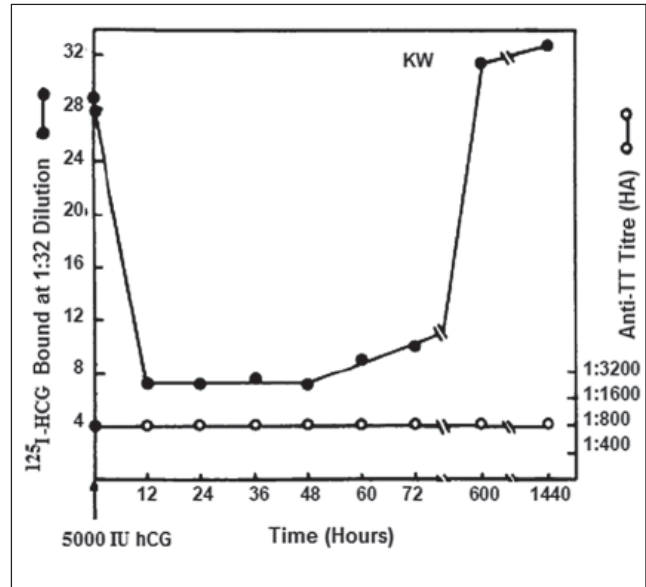
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**Figure 2.** Anti-hCG and anti-TT antibody responses in a subject (AM), with 2 live children and 1 elective termination of pregnancy, immunized with the  $\beta$ -hCG-TT vaccine. EB endometrial biopsy taken, P<sub>4</sub> progesterone in luteal phase to confirm ovulation. x are the menstrual cycles which remained regular. Adapted from [3]. © G. P. Talwar



**Figure 3.** Antibodies induced by the  $\beta$ -hCG $\beta$ -TT vaccine bound 5000 IU of hCG administered, returning back to previous titres in course of time. No change was noticed in anti-tetanus titres indicating 2 independent set of antibodies generated by the vaccine. Reprint from [2]. © G. P. Talwar

genic in women. However the titres of antibodies generated by hCG- $\beta$ -TT vaccine were fairly low. Thus the immunogenicity of the vaccine had to be improved.

**■ Hetero-Species Dimer**

We conceptualized the linkage of  $\beta$  subunit of hCG with alpha subunit of ovine LH [4]. The ability of  $\alpha$  and  $\beta$  subunits to

associate with each other is conserved across species in mammals. In this way, not only will a dimer be presented to the immune system to react against, but also a foreign alpha protein will be more strongly reacted against by the immune system. Indeed the Hetero-species dimer (HSD) linked to TT, or Diphtheria toxoid (DT) or Cholera toxin B subunit (CHB) generated higher titres [4] than hCG $\beta$ -TT (Tab. 1).

**■ Safety and Efficacy of the HSD-TT/DT Vaccine**

The next obvious question was whether this vaccine was safe for human usage, reversible and competent to prevent pregnancy in sexually active women.

After due Regulatory and Ethics committees approval, phase 1 followed by phase 2 clinical trials were conducted on the HSD-TT/DT vaccine in major institutions of India, such as the All India Institute of Medical Sciences, New Delhi & Postgraduate Institute of Medical Education & Research, Chandigarh.

The vaccine was devoid of toxicity and was reversible [5, 6]. The most important finding was that circulating antibodies against hCG indeed prevent women of proven fertility, who were sexually ac-

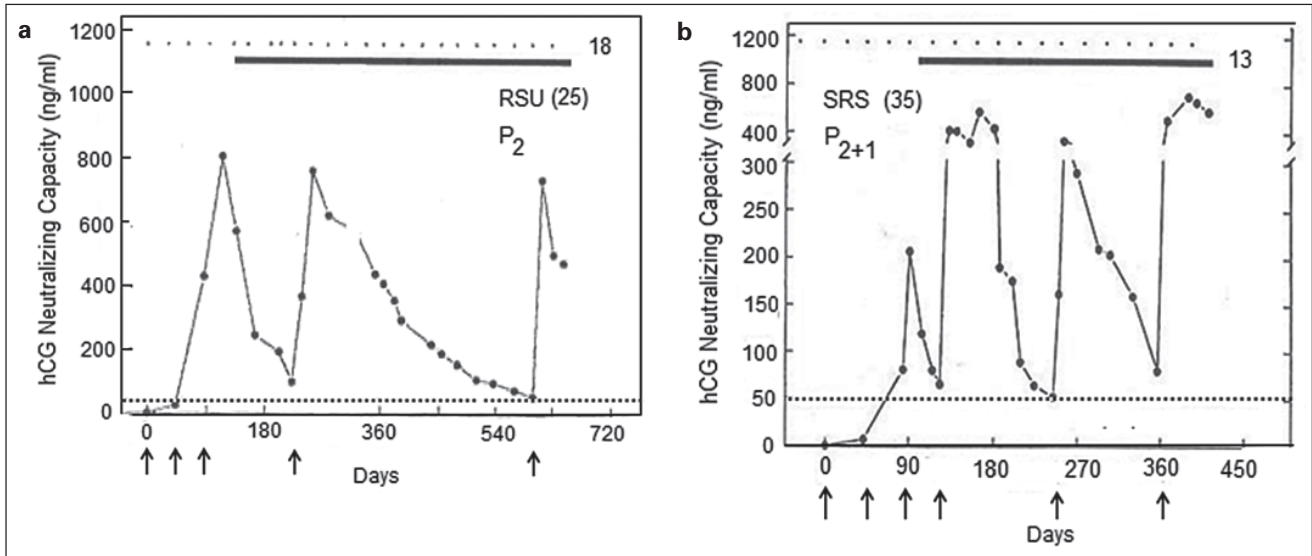
**Table 1.** Immunogenicity & Bionutralization capacity of Antibodies generated by HSD-TT/CHB (Hetero-species dimer linked to B subunit of Cholera toxin) as compared to hCG $\beta$ -CHB in rats (n = 6). Mod. from [3]. © G. P. Talwar

Immunogen	hCG binding capacity (pg) (Range)	hCG bio-neutralization potency (pg) (Range)
hCG $\beta$ -CHB	27.1 $\pm$ 1.7 (25-33)	17.1 $\pm$ 1.2 (13-21)
HSD-CHB	32.5 $\pm$ 1.4 (29-36)	26.1 $\pm$ 0.8 (23.5-28.0)

**Table 2.** Pregnancy in 4 women who conceived at low anti-hCG titres. All of them delivered normal children. © G. P. Talwar

Subject	Age (Yrs)	Parity	Antibody titres ng/ml hCG bionutralization	Antenatal history	Delivery	Gestation (weeks)	Birth weight (g)
NMT	28	3	20	No problem	Elective LSCS	38	2800
URM	28	2	5	One episode of swelling of parotids at 6 weeks gestation	SVD	35	2570
GTD	24	3 (2 live)	5	Recurrent vomiting and loose motions, PIH <sup>b</sup>	SVD	36	1840
ANT	35	2	10	No problem	Elective LSCS	38	2700

LSCS: Lower segment cesarian section; SVD: Spontaneous vaginal delivery



**Figure 4.** Representative illustration of the kinetics of antibody response and menstrual events in a woman (SRS, 35 years with 2 live children and 1 elective termination of pregnancy, P<sub>2+1</sub>) and in RSU (25 years with 2 live children). Arrows denote injections with the HSD-vaccine to generate and maintain antibody response above the threshold of 50 ng/ml. Rectangles near the top abscissa indicate the menstrual events, and the solid bar denotes the period (13 or 18 cycles) of protection from pregnancy in spite of unprotected intercourse. © G. P. Talwar.

tive, from becoming pregnant. Only one pregnancy took place in 1224 cycles in women having a titer of 50 ng/ml or more of anti-hCG antibodies. Eight women completed > 30 cycles without becoming pregnant, 9 between 24 and 29 cycles, 12 completed 18–23 cycles, 15, 12–17 cycles and 21 subjects had completed 6–11 cycles of continuous exposure to the risk of pregnancy without becoming pregnant. Women kept on ovulating normally and had regular menstrual cycles. Immunization had no adverse effect on ovulation. Luteal phase progesterone continued to indicate normal ovulation of the immunized women. Menstrual cycles were undisturbed and con-

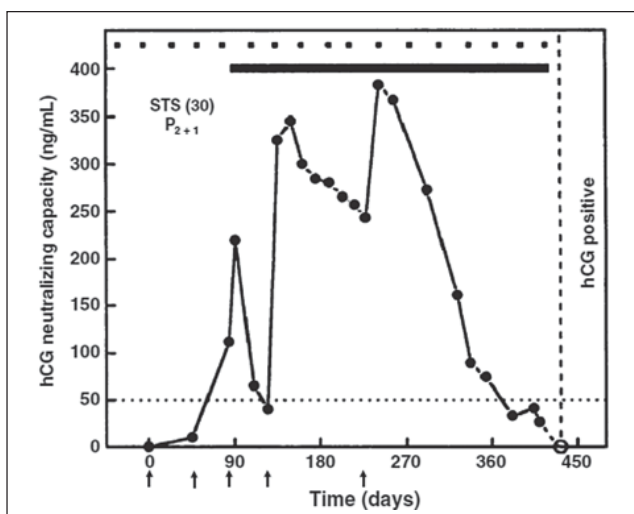
tinued to be as regular as before immunization (Fig. 4). No change was noticeable in bleeding profiles. These findings confirmed the achievements expected from choosing a target, which is not normally involved in reproductive functions of a non-pregnant healthy female.

### ■ Reversibility and Normalcy of Progeny born to previously Immunized Women

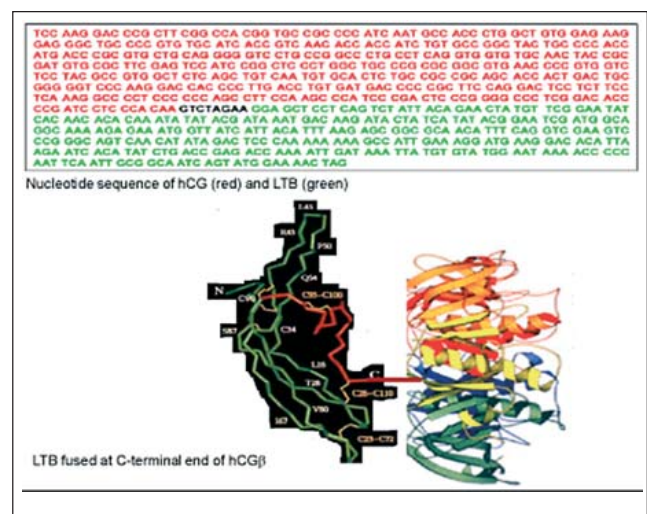
In case boosters were not given, the antibodies declined over time. They readily

conceived when titres went below 30 ng/ml of titres (Fig. 5).

A pertinent issue was whether women becoming pregnant at low titres would have normal pregnancy and whether the progeny born would not be adversely affected by prior immunization against hCG. A senior pediatrician surveyed carefully the record of 4 women, who had decided to have another child, after a gap of protection from pregnancy by the vaccine (Tab. 2). Two women delivered term babies by elective caesarian section and other two delivered full-term babies by spontaneous vaginal delivery. None of the babies suffered from birth asphyx-



**Figure 5.** Regain of fertility on decline of antibodies. STS 30-year with 2 children and 1 termination remained protected from becoming pregnant over 12 cycles. She conceived in the cycle when titres were below 20 ng/ml. Reprint from [5] with permission of National Academy of Sciences, USA. © 1994.



**Figure 6.** Conceptualized structure of hCGβ-LTB vaccine. The carrier B chain of heat labile enterotoxin of *E. coli* (LTβ) is fused at C-terminal glutamine of hCGβ. © G. P. Talwar.

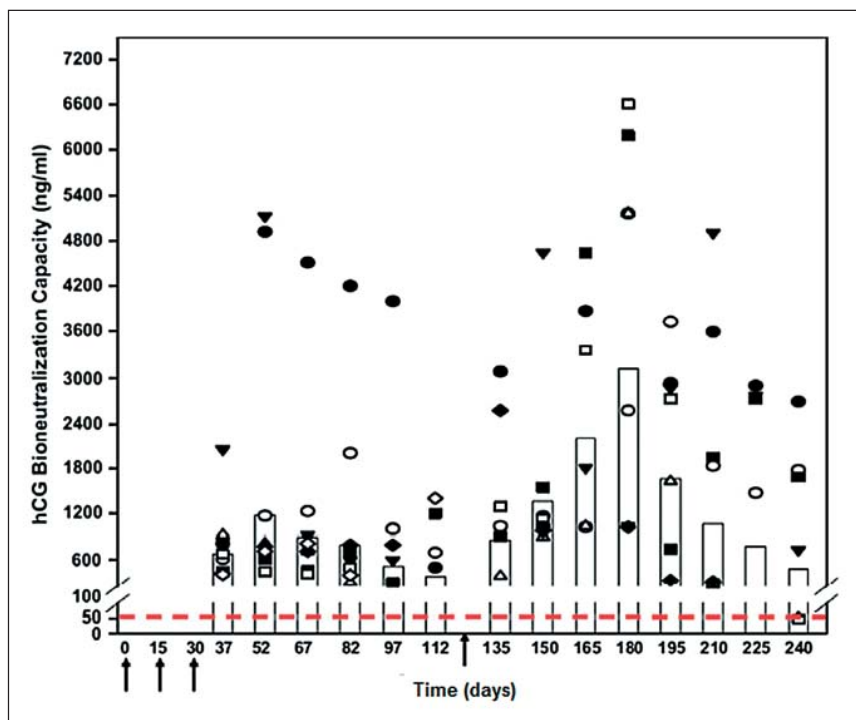
ia. There was no evidence of any congenital malformation in any of the babies. In conclusion, the anti-fertility effect of hCG vaccine is not only reversible at low titres of antibodies, but when desired the pregnancy progresses uneventfully to successful completion without any hazard to the mother [7].

### ■ Revival and Development of a Recombinant Vaccine

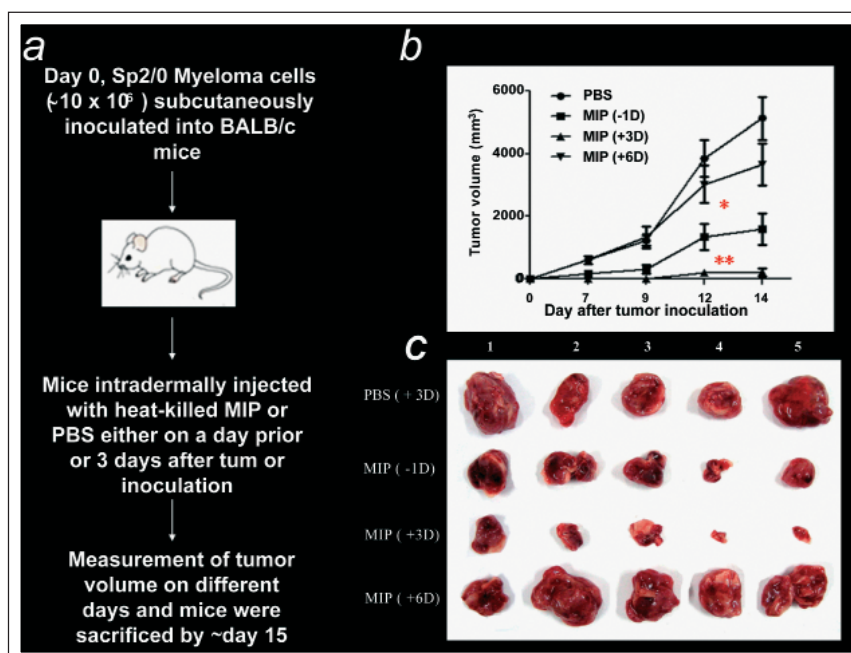
After a gap of 12 years, GPT was asked by an Indo-US Committee on Contraception Research to revive the vaccine. Meanwhile genetic engineering techniques had emerged. We decided to make a recombinant vaccine, which if successful, would be amendable to industrial production. We linked hCG- $\beta$  gene at C-terminal to B subunit of heat labile enterotoxoid of *E. coli* (Fig. 6). This carrier would have the additional advantage of imparting mucosal immunity. hCG $\beta$ -LTB is immunogenic and induces along with heat-killed *Mycobacterium indicus pranii* (MiP) as adjuvant, very high antibody titres in BALB/c mice (Fig. 7) [8]. It is also immunogenic in mice of 4 other genetic strains [9]. MiP is an atypical non-pathogenic mycobacteria developed by us as an immunotherapeutic vaccine against leprosy [10]. It is approved by the Drugs Controller General of India (DCGI) and by the US FDA. It is a potent invigorator of immune responses. It is being used as adjunct to multidrug regimen for treatment of category II (difficult to treat) tuberculosis patients. What is amazing is that it has both preventive & therapeutic action against SP2O Myeloma in mice (Fig. 8) as observed by Dipankar Nandi et al [11] at the Indian Institute of Science, Bangalore. This *Mycobacterium* has now been sequenced and being hitherto not listed in World Data Book, it has been named *Mycobacterium indicus pranii* [12]. Pran is my first name; NII is the National Institute of Immunology, where developmental work and trials on this mycobacterium were carried out.

### ■ Going back to Clinical trial on the Recombinant hCG $\beta$ -LTB Vaccine

Being a genetically engineered vaccine it was put up to the National Review Committee on Genetic Manipulation (RCGM). RCGM has approved the



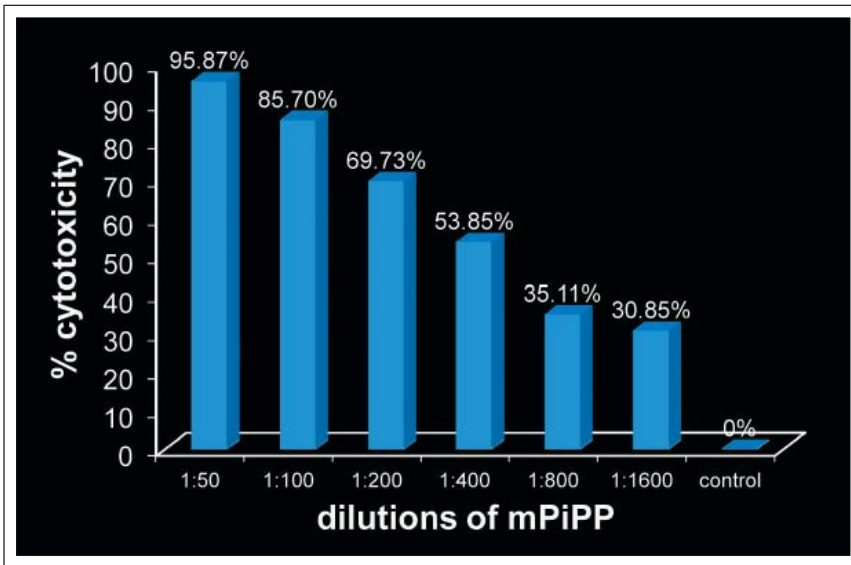
**Figure 7.** High bio-effective antibody response generated by hCG $\beta$ -LTB in Balb c mice given along with *Mycobacterium indicus pranii* (MiP) as adjuvant. Bars give the geometric mean of bionutralisation capacity determined by inhibition of 125 I-hCG binding to rat Leydig cell receptors. The symbols represent the titres in individual mice and bar the geometric means. 50 ng/ml is the protective threshold against pregnancy in women. Reprint from [8] with permission of Elsevier.



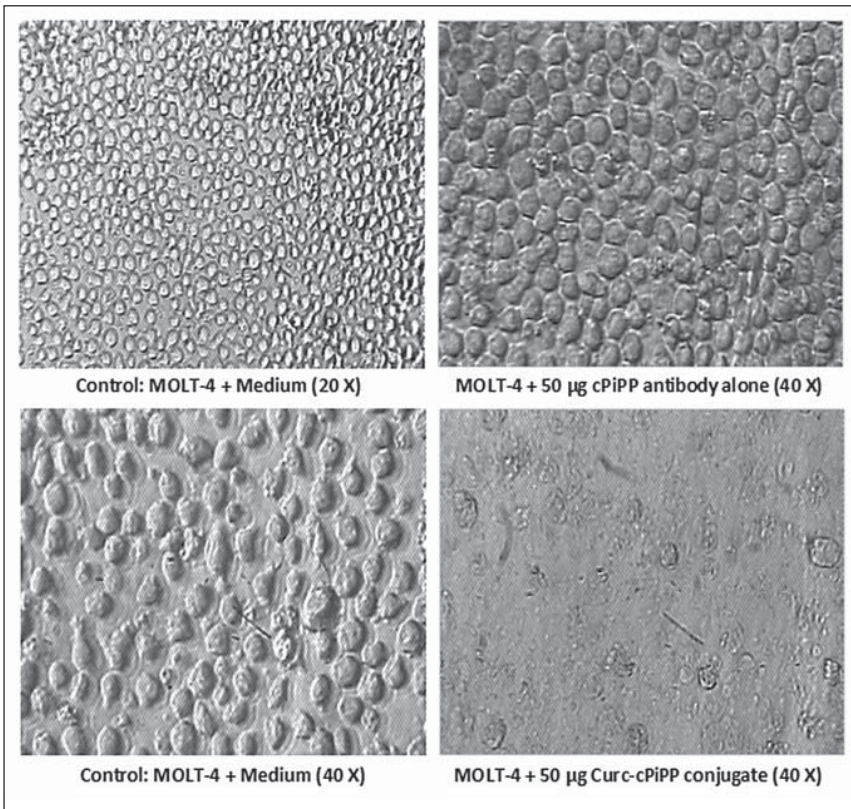
**Figure 8.** MiP treatment suppresses tumor growth and induces a Th1 cytokine response. (a): General outline of the in vivo experiment protocol. (b): Comparison of the anti-tumor effects of MiP administered at different time points. Cohorts of ten mice were inoculated s.c. with  $\sim 10^7$  Sp2/0 cells. Mice were injected i. d. with a single dose of MiP ( $\sim 5 \times 10^9$ ) either one day (-1D) before or 3 (+3D) or 6 (+6D) days after tumor inoculation. Mice injected i.d. with PBS on day 3 were included as controls. The growth of tumors (mean  $\pm$  SD mm $^3$ ) at indicated days post implantation. (c): Representative photographs of solid tumors from different treatment groups dissected on day 14. Reprint from [11] with permission of Wiley.

hCG $\beta$ -LTB recombinant vaccine after cross-checking the consistency of the Product made in 3 Batches of production. The Committee also approved the

fairly exhaustive Protocol for conduct of Toxicology in 2 species of rodents by a GLP company of international standard. Toxicology has been done. It is fully safe



**Figure 9.** Dose Dependent Cytotoxicity exercised by a monoclonal anti-hCG-antibody cPiPP on Lung Cancer cells A549. © G. P. Talwar



**Figure 10.** Microphotograph of MOLT-4 cells after incubation with cPiPP alone and cPiPP-curcumin conjugate. Cells incubated in culture medium are used as control. © G. P. Talwar

by all criteria and is also free of mutagenicity and causing skin sensitization. We were also able to conduct safety and immunogenicity studies on the recombinant hCG $\beta$ -LTB vaccine in a subhuman primate species, the marmosets at the National Institute of Research in Reproduction (NIRRH), Mumbai. These have also been completed and the vac-

cine found fully safe. Marmosets offered an additional opportunity to test whether the antibodies prevented pregnancy in marmosets. 8/9 immunized marmosets were protected from becoming pregnant on co-habitation with fertile males, whereas all non-immunized marmosets in the control group became pregnant.

Technology for producing the vaccine under GMP conditions has been transferred to a recognized biotech company. The vaccine prepared by the company has been cross-checked for physico-chemical traits and immunogenicity.

The Indian Council of Medical Research has constituted an Expert Committee to develop a protocol for safety & immunogenicity, the phase I trial of the hCG $\beta$ -LTB vaccine in women of proven fertility. The Committee has already met and finalized the protocol. It will now go to the Ethics Committees & Drugs Controller General of India for approval for starting the trial.

### ■ Potential utility of the Anti-hCG vaccine & Anti-hCG antibodies in Advanced stage Terminal Cancers

A number of reports have appeared in the literature on the expression of hCG or its subunits by a variety of cancers particularly at the advanced stage. Expression of alpha subunit of hCG was observed in lung carcinoma [13]. One third of transitional cell carcinomas of bladder ectopically produce trophoblastic hormones that are specifically correlated with stage and grade of the tumor [14]. hCG $\beta$  was made ectopically and reported as a poor prognostic marker in colorectal cancer [15]. More than 40% of pancreatic exocrine tumors produce hCG $\beta$  [16]. Determination of hCG $\beta$  in serum was considered as a potential marker in the prognostic evaluation of patients with squamous cell carcinoma of the oral cavity and oropharynx [17]. The survival of women suffering from cervical carcinoma in whom the tumor secreted hCG $\beta$  was poorer (14%) as compared to those negative for hCG $\beta$  (75%) [18]. Invariably at the stage that ectopic expression of hCG/subunits takes place, the cancer is advanced and refractory to the currently available drugs. Can active  $\pm$  passive immunization of the patients against hCG be helpful in prolonging their life?

A few studies done in our laboratory on cancer cells derived from patients dying of such cancers are encouraging. Anti-hCG antibodies were inhibitory to the growth of A549 cells *in vitro* in a dose-dependent manner (Fig. 9).

Many years back we reported the anti-cancerous effect of anti-hCG- $\beta$  antibodies on growth of Chago lung cancer expressing this subunit in nude mice [19]. The anti-cancerous effect of inactivation of hCG by bioeffective antibodies is understandable on the grounds that hCG promotes the multiplication of cells, overcoming apoptosis [20]. It promotes also angiogenesis and invasiveness [21].

### ■ Targeted delivery of Curcumin, a safe Anti-Cancerous Compound

Curcumin (Diferuloylmethane) has anti-inflammatory and anti-cancerous properties. It is highly safe, doses upto 8 mg per day show no ill effect of any sort. It is hydrophobic, but can be rendered hydrophilic by linking it to an antibody. PiPP, a monoclonal antibody against hCG linked to curcumin, kills 100% of MOLT-4 lymphoblastic leukemia cells in culture [22]. Figure 10 shows the cytolysis of MOLT-4 cells with PiPP-curcumin conjugate.

### ■ Summary & State of Art

Human chorionic gonadotropin (hCG) has proven to be a right target for immunological intervention. Pregnancy is prevented in sexually active women at and above 50 ng/ml of antibody titres without impairment of ovulation, regularity of menstrual cycles and normal bleeding profiles. Fertility is regained without delay on decline of antibodies below 30 ng/ml. Though observations are based on few numbers, progeny born to previously immunized women are normal in their developmental landmarks and cognitive abilities.

A recombinant vaccine (hCG $\beta$ -LTB) has been developed, which assures consistency of the linkage of the 'carrier' to

the  $\beta$  subunit of hCG in the product. The vaccine is highly immunogenic in mice. The vaccine has received the approval of the Regulatory Committee on Genetically Made Products. The technology has been transferred to a biotech company which will make available the vaccine prepared under GMP conditions. The recombinant vaccine has completed safety and toxicology studies in 2 species of rodents and in a subhuman primate Marmosets. It is poised to go back to clinical trials under the aegis of the Indian Council of Medical Research.

The vaccine is likely to find additional therapeutic use in advanced stage cancers expressing ectopically hCG, alone or along with monoclonal antibodies against hCG and those linked to curcumin.

### ■ Acknowledgements

Revival of the work on the hCG vaccine has benefited from research support of the Indo-US co-operation in contraception. Research grants from the Department of Biotechnology, Government of India and the Indian Council of Medical Research are gratefully acknowledged.

### ■ Conflict of Interest

The authors declare no conflict of interest.

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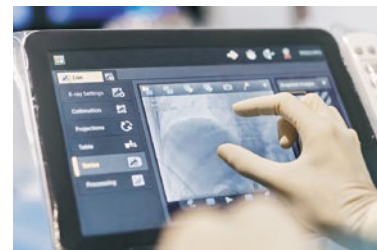
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