

**PRO2000 vaginal gel for the prevention of HIV infection: results of the
MDP301 Phase III randomised microbicide trial**

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Abstract

Background: There is an urgent need for effective HIV prevention strategies such as vaginal microbicides that can be used by women.

Methods: MDP301 assessed the safety and efficacy of 0.5% and 2% PRO2000 vaginal gels against HIV acquisition in a Phase III trial. Healthy HIV negative women recruited through six research centres in Africa were randomised to receive 0.5%, 2% or matching placebo gel for 52 weeks (up to 104 weeks in Uganda) to be applied within one hour before sex. HIV status was determined using rapid tests and/or ELISA at screening and weeks 12, 24, 40 and 52 (up to 104 in Uganda) and confirmed by a common algorithm in a central reference laboratory. The primary efficacy outcome was HIV acquisition by week 52, in a modified intention to treat (MITT) analysis, censored for pregnancy. The primary safety endpoint was adverse events of grade 3 or above. The 2% gel was discontinued in February 2008 on the recommendation of the Independent Data Monitoring Committee because there was only a small chance of demonstrating benefit.

Findings: Of 15818 women screened, 9385 were enrolled, 95% of whom contributed to the efficacy analysis. The mean reported gel use at the last sex act was 89%. In the primary efficacy analysis, the HIV incidence was 4.5/100 woman-years (wy) in the 0.5% PRO2000 group and 4.3/100wy in the placebo group. The HR for 0.5%:placebo was 1.05, 95% CI 0.82-1.34. For the 2%:placebo comparison, HIV incidence was 4.7 and 3.9/100wy respectively (HR=1.21, 95% CI 0.88-1.68). The rates of primary safety events were similar across all gel groups.

Interpretation: There was no evidence that either concentration of PRO2000 provided protection against HIV infection in this large Phase III trial. However both concentrations of the gel were safe.

Trial registration: Current controlled trials ISRCTN64716212.

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Introduction

With an estimated 1.9M new HIV infections in sub-Saharan Africa during 2008, innovative prevention technologies are urgently required.¹ Women in Africa are disproportionately affected by HIV and many are unable or unwilling to negotiate

condom use, have a desire to conceive or both. Vaginal microbicides are being investigated as a potential female-controlled HIV prevention method. After several disappointing trials,²⁻⁵ the Phase II/IIb trial, HPTN035, reported that the vaginal microbicide 0.5% PRO2000 showed a non-significant reduction in HIV incidence of 30% (HR=0.7; 95% CI 0.46-1.08; p=0.10).⁶

Here we report the results from the MDP301 trial (ISRCTN64716212), which evaluated the effectiveness and safety of 0.5% and 2% PRO2000 compared to the hydroxyethylcellulose (HEC) placebo gel,⁷ in the prevention of vaginally acquired HIV infection. MDP301 was conducted by the Microbicides Development Programme (MDP), a collaborative partnership of institutions in Africa and Europe.

PRO2000 is a synthetic naphthalene sulphonate polymer of ~5kD molecular weight, with anti-viral activity against HIV and other sexually transmitted infections as demonstrated in laboratory and animal studies including macaque challenge experiments in X4 and R5 SHIV models,⁸⁻¹³ and a favourable safety profile in Phase I/II clinical trials.¹⁴⁻¹⁷

Methods

Full details of the MDP301 design, sample size, research sites, study populations, study conduct including the randomisation and blinding, as well as the data underpinning the sample size assumptions, have been reported elsewhere.¹⁸ The design included a social science sub-study to assess the accuracy of the behavioural and adherence data described in detail in separate manuscripts.^{19 20}

Design and sample size

MDP301 was a Phase III randomised double-blind parallel group placebo-controlled trial, designed with 80% power to detect a 35% reduction in HIV incidence (90% for a 40% reduction), assuming an incidence in the placebo group of 4 per 100 woman-years (wy). Participants were randomised to 3 groups 2% PRO 2000, 0.5% PRO2000 and placebo in the ratio of 1:1:1. Following closure of the 2% arm, randomisation continued for the remaining two arms. The planned sample size of 9,673 women allowed for a 20% loss in woman-years by 52 weeks. The assumptions regarding incidence and loss to follow up were derived from data collected in preceding cohort studies. The social science sub-study had a planned sample size of 600 women (100 per research centre).

The protocol stated that the trial Independent Data Monitoring Committee (IDMC) could recommend early termination of the trial “if there was proof beyond reasonable

doubt that one of the trial interventions is clearly indicated or clearly contraindicated in terms of a net difference in sero-incidence or adverse events". The 2% PRO2000 gel was discontinued on 14th February 2008, following the review on the 8th February 2008 by the IDMC, using available data up to 15th January 2008. They advised there was little chance of this concentration showing benefit given the planned sample size and postulated effect size. However, the conditional power for demonstrating a significant benefit from the 0.5% PRO 2000 dose based on the original sample size assumptions, was sufficiently high to warrant the continuation of the trial.

The results for the 0.5% and placebo groups are reported here using all the available data, together with results for the 0.5%, 2% and placebo groups using data up to 14th February 2008.

Eligibility

Participants were enrolled at 13 clinics managed by six research centres in Africa: three in South Africa, one each in Tanzania, Uganda and Zambia. Eligible women were aged 18 or above (16 or above in Tanzania and Uganda); HIV negative at screening and willing to undergo HIV testing and receive the result; willing to undergo regular speculum examinations and urinary pregnancy tests; willing to use gel as instructed; likely to be sexually active; willing to receive health education about condoms; willing and able to give informed consent. Women were not eligible if they were unable or unwilling to provide a reliable method of contact; were likely to move out of the area within 12 months; were likely to have sex more than 14 times a week on a regular basis (due to the regulatory requirement that no more than 60 applicators be dispensed at each 4 weekly visit); used spermicides regularly; were pregnant or within six weeks postpartum; had a severe clinical or laboratory abnormality; required referral for assessment of a suspicious cervical lesion; had received treatment to the cervix or other gynaecological procedure within 30 days of enrolment; were allergic to latex; or were participating or had participated in another clinical trial likely to impact on the primary efficacy endpoint, within 30 days prior to enrolment.

Follow-up

Visits were scheduled every four weeks for 52 weeks (up to 104 weeks in Uganda to provide longer term safety data). Gel was dispensed in packs of 10 pre-filled single dose applicators up to a maximum of 60 at each visit, following a negative urinary pregnancy test. Women were instructed to apply gel within an hour before sexual intercourse. They were counselled to use condoms during all sex acts and received

unlimited free condom supplies at the research clinics. Gel was interrupted if a participant tested positive for pregnancy and could be resumed following a negative pregnancy test. At each 4-weekly visit, women were asked about gel and condom use at the most recent sex act and returned used and unused applicators were counted and recorded for adherence assessments.

HIV status was assessed at weeks 12, 24, 40, 52 (up to week 104 in Uganda) and if gel was interrupted or discontinued for pregnancy. A clinical interview to solicit genital symptoms and adverse events and a pelvic examination were also conducted at these visits, at week 4 and if a pregnancy was diagnosed. Solicited genital symptoms and signs were non-menstrual bleeding, genital sores or ulcers, genital discomfort (itching, burning or dryness), and external or internal epithelial disruption, genital erythema and genital oedema.

Routine haematology and biochemistry tests were performed on the first 500 participants enrolled in the Durban and Johannesburg centres and all 840 participants in Uganda at screening, and at weeks 12, 24, 52 (and 104 or at the final visit if this occurred earlier in Uganda). In addition, a plasma sample was collected from these participants at the final visit for PRO2000 analysis.

Laboratory confirmation of HIV status

Serum was collected at weeks -6, 0, 4, 12, 24, 40 and 52 (and week 72 and 104 in Uganda) and Buffy Coat (BC) at weeks 0, 24, 40 and 52 (and week 104 in Uganda). The HIV testing algorithm included parallel HIV rapid tests at the clinics, with discordant or positive tests after enrolment triggering ELISA testing at local laboratories and confirmation at a central laboratory located in South Africa. A second serum sample was collected at the subsequent visit after a single positive rapid test result. The central laboratory analysed samples from the visit at which a positive rapid test result was obtained and from all previous visits where samples were collected. This was to allow for the detection of acute seroconversion at or before enrolment. The algorithm was designed to confirm HIV infection based on two separate samples with two different methods of diagnosis. Serum samples were tested for HIV antibodies using the following assays: Abbott AxSYM HIV Ag/Ab Combo ELISA (4th generation ELISA), the Bio-Rad Genetic Systems HIV-1 ELISA (3rd generation ELISA), the Genetic Systems HIV 1 Western Blot. The Biomerieux Vironostika HIV-1 antigen ELISA was used as a confirmatory assay for p24 testing. BC samples were tested with the Roche qualitative DNA PCR Version 1.5 assay. In

some instances the Roche COBAS Amplicor HIV-1 Monitor was used for the detection of HIV RNA if the BC specimen was not satisfactory.

Randomisation and blinding

Randomisation lists were created using randomised permuted blocks of varying size, for each of the 13 clinics, by an independent statistician using a computerised random number generator, containing unique trial numbers matched to 9 sets of study product codes. Site pharmacists dispensed gel in identical applicators on the basis of the trial number using the assigned study product codes on the clinic randomisation list. No other site personnel had access to the list. At enrolment, women were assigned a unique trial number selected sequentially from the clinic trial register. Only statisticians responsible for preparing the IDMC reports and essential manufacturing and distribution staff had access to the list matching study product codes to gel.

Endpoints

Efficacy

The primary efficacy endpoint was HIV infection confirmed by the central reference laboratory in participants confirmed to be HIV negative at enrolment. Secondary efficacy endpoints were: acquisition of Herpes Simplex Virus-2 (HSV-2) infection by participants that were HSV-2 seronegative at enrolment, determined serologically and confirmed by the central laboratory at weeks 40 and 52; presence of *Neisseria gonorrhoea* (NG) or *Chlamydia trachomatis* (CT) determined by a positive nucleic acid amplification assay at 24 weeks.

Safety

The primary safety endpoint was a grade 3 (severe) or higher clinical or laboratory adverse event, regardless of relationship to study product. Secondary safety endpoints were solicited local (any grade of genital itching, burning, internal epithelial disruption, internal erythema or internal oedema, regardless of relationship to study product) and systemic toxicity (any increase in grade from baseline in a routine laboratory parameter). The rationale for systematically soliciting these data was an association with PRO2000 or other microbicides administered vaginally or systemically in preceding trials.

Statistical Analysis

All statistical tests were 2-sided and all analyses performed in Stata version 10.1.²¹

Efficacy

HIV efficacy analyses excluded women confirmed to be HIV positive at enrolment and those with no HIV follow-up data. In HIV seroconverters, the date of detection of HIV infection was determined by an endpoint committee. Woman-years of observation were censored at the date of seroconversion, estimated by the mid-point between the last negative and the date of detection, or at the last HIV-negative test (for those not becoming infected). The primary efficacy analysis was modified intention-to-treat (MITT): follow-up time was censored at 52 weeks (+ 6-week window) from enrolment, or when gel was discontinued due to pregnancy. For those that were HIV negative when gel was resumed after pregnancy, the additional time was added to the woman-years of follow-up. An intention to treat (ITT) analysis was also conducted based on all follow-up visits with no censoring for pregnancy. There were two further analyses conducted, censoring at 24 and 40 weeks (+ 4-week window) from enrolment.

There were two planned sub-group MITT analysis, including tests for interaction. Firstly, stratified by clinic and secondly using post randomisation data to categorise women according to the consistency of gel use with the expectation that the effect would be greater in consistent users. Gel use was pre-defined as 'consistent' if women reported using gel during the last sex act at 92% or more of visits (e.g. 12 of 13 visits); returned at least one used applicator to support their answer when appropriate and attended at least 7 of the expected 13 visits (unless they became pregnant or HIV positive during follow-up).

The primary efficacy endpoint was analysed as time-to-event and groups compared using hazard ratios (HR) relative to the placebo group, 95% confidence intervals (CI) and p-values, obtained using Cox proportional hazards regression stratified by clinic.

The secondary efficacy endpoints were analysed as binary outcomes using logistic regression: the proportion infected with HSV-2 at week 40 and week 52 in those HSV-2 negative at baseline; and cross sectional prevalence of CT and NG, in all those tested at week 24.

All analyses referring to the 2% PRO2000 arm were carried out with all three groups censored on 14th February 2008 when discontinuation of the 2% gel was implemented.

Safety

The safety analyses included all women with follow-up clinical data. Safety endpoints were analysed as time-to-first-event and groups compared as for the efficacy analysis.

Approvals

The protocol was approved by local and/or national ethics committees, in each participating country, as well as in the UK. Authorisation was obtained from the national regulatory authority in each participating country and from the US Food and Drug Administration.

Role of Funding Source

The main funders of MDP301 were the Department for International Development (DFID) and the UK Medical Research Council (MRC); they played no role in the study design, data collection, analysis or interpretation of data; they did not contribute to writing the paper. Endo Pharmaceuticals Solutions donated the study gels, provided regulatory support, and participated in the design and management of the study. The corresponding author had full access to the study data and had final responsibility for the decision to submit for publication.

Results

Between September 2005 and August 2008, 15818 women were screened for HIV with 9404 enrolments: 19 women enrolled twice and data for their second enrolment were excluded from all analyses, which included one seroconversion during the participant's second enrolment. Of 9385 women enrolled: 2734 were randomised to the 2% PRO2000 group; 3326 to 0.5% and 3325 to placebo (Figure 1). The evaluable number (% of maximum) of participants for the efficacy analyses were: 2591 (95%), 3156 (95%) and 3112 (94%) for the 2%, 0.5% and placebo groups respectively.

The groups were similar with respect to age, education, use of effective contraception, clinical and behavioural characteristics with the majority reporting only one partner in the preceding week, and just under half reporting condom use at the last sex act (Table 1).

The main results presented are for the 0.5% PRO2000 versus placebo comparison. Of 6651 women allocated to 0.5% or placebo, 81% attended the week 52 visit,

resulting in 85% of the maximum possible woman-years for 0.5% and 83% for placebo (data not shown).

Reported condom use at the last sex act increased over time, was similar across gel groups but varied between centres (Figure 2a). The mean percentage reported gel use at last sex act was 89% post enrolment. This changed little over time and did not differ between centres (Figure 2b) or between those using or not using a condom (Figure 2c). A drop in reported gel use was observed over time in those reporting sex acts without a condom in Durban and Johannesburg centres, but this was based on 78 and 198 sex acts respectively at week 52. During the trial, 181 (2%) women reported having anal sex in the last week at one or more of four visits; the majority (>90%) of these reports were from the South African sites where the HIV incidence was 5.4/100wy. Of the 181, 11 sero-converted, giving an incidence of 6.3/100wy.

Efficacy

In the primary efficacy analysis, the HIV incidence rates in the 0.5% and placebo groups were 4.5/100wy and 4.3/100wy, respectively (Table 2, left hand side). There was no significant difference in HIV incidence in either the primary efficacy analysis (HR 1.05; 95% CI 0.82-1.34; $p=0.71$) or the ITT analysis (HR 1.00; 95% CI 0.79-1.26; $p=0.99$). There was no significant difference in efficacy between centres ($p=0.19$ for interaction), nor between consistent (HR 0.97; 95% CI 0.87-1.35; $p=0.87$) and inconsistent (HR 1.17; 95% CI 0.42-1.72; $p=0.42$) gel users ($p=0.47$ for interaction). There was no significant difference in HIV incidence between the gel groups at weeks 24 or 40 (data not shown).

Up to February 2008, there was no significant difference in HIV incidence in the comparison of 2% to placebo (MITT analysis: HR 1.21; 95% CI 0.88-1.68; $p=0.24$) (ITT analysis: HR 1.11; 95% CI 0.82 -1.51; $p=0.50$) (Table 2, right hand side). At that time, the HRs for the 0.5% and placebo comparison were 0.99 (95% CI 0.70-1.39; $p=0.94$) and 0.90 (95% CI 0.65-1.24; $p=0.53$) for the MITT and ITT analyses, respectively.

Results from analyses of the secondary endpoints showed no evidence of differences between 0.5% and placebo gel groups (Table 3, left hand side). For HSV-2, 7% of both the 0.5% and placebo groups were HSV-2 seropositive at week 40 and 12% (0.5% group) and 13% (placebo group) at week 52. At week 24, 3% in each group tested positive for NG and 6% for CT. There were no differences between the three

gel groups for any of these secondary efficacy endpoints, based on data up to February 2008 (Table 3, right hand side).

Safety

Adverse events are summarised in Table 4. None of the deaths or other serious adverse events were considered to be related to study product. There were no significant differences in the rates of primary safety events between the 0.5%, 2% or placebo groups (Table 4).

1450 participants (16%) experienced at least one solicited local toxicity event post enrolment; the most commonly reported being genital itching. 595 (33%) of participants with routine laboratory data experienced at least one systemic toxicity event (usually raised aspartate aminotransferase or bilirubin). Rates for local and systemic toxicity were similar across all gel groups (data not shown). PRO2000 levels were below the lower limit of quantitation in all 1789 plasma specimens analysed.

Pregnancy incidence was 11.6/100wy (95% CI 10.9-12.4) ranging from 8.2 to 17.2 across the centres but similar across the three gel groups: 11.9/100wy in both the 0.5% and placebo groups; up to February 2008, the rates were 10.1, 11.3 and 10.9 /100wy for the 2%, 0.5% and placebo groups respectively.

Discussion

The MDP301 trial was conducted to the highest international standard in order to support a licence application, if indicated. As well as the clinical, laboratory and data management procedures for which international guidelines exist, social science was integrated in the trial protocol to an unprecedented degree,¹⁹ and a commitment to active community engagement and liaison made at all sites. Completion of this large, complex, multi-disciplinary, multi-country trial in resource limited settings is testimony to the success of the partnership. The result has shown conclusively that, despite high levels of reported adherence, PRO2000 was not effective in preventing vaginally acquired HIV infection or other sexually transmitted infections.

To ensure a Phase III HIV prevention trial is adequately powered, accurate incidence data in the target population are essential.⁴ In cohort studies conducted prior to MDP301, the weighted HIV incidence rates were estimated to be 6.2/100wy. The power calculations were based on a conservative estimate of 4/100wy as it was assumed HIV incidence in the target populations may have fallen over time and might be even lower due to more frequent visits with HIV counselling and testing.

This strategy was justified as the overall HIV incidence in the placebo group was 4.5/100wy. The power calculations allowed for a 20% loss of woman-years by 52 weeks, but in the event it was only 12%. Diligent promotion of effective methods of contraception, requiring extra clinical and laboratory effort, resulted in an additional loss for the primary MITT analysis of only 4% of woman-years due to pregnancy. The incidence of pregnancy was similar across the gel groups, justifying this modification to the ITT. In October 2007, following a review of blinded data, recruitment targets were revised to ensure that there was 75-80% power to detect efficacy at a lower level (30-35% reduction).

Self-reported adherence has been high in all microbicide trials to date, but scepticism remains regarding over-reporting in the absence of a reliable biomarker. In MDP301 self-reported adherence was corroborated by the used applicator returns, and participants were asked at every visit which provided more opportunity to report non-adherence, increasing the stringency of the definition of “consistent”. Information was also collected for 725 women randomly selected to take part in a social science sub-study. In addition to the clinic interviews and gel returns, they completed coital diaries and were interviewed in depth.¹⁹ Triangulation of adherence data from all these sources suggested that the adherence was high,²⁰ and the qualitative data implied that women and their partners genuinely enjoyed using the gel.²² There were no differences in reported adherence between the three gel groups, justifying the use of these data to define consistency for the pre-specified sub-group analysis.

There are a number of reasons why PRO2000 might not have provided protection in women despite promising results *in vitro* and in animal challenge models with 10 of 14 macaques protected. In earlier studies, active drug was recoverable from cervico-vaginal lavage (CVL) up to several hours after insertion, suggesting that it was released from the formulation, and not unduly diluted by vaginal secretions.^{23 24} However a more recent study in which CVLs were collected from 10 women who inserted 0.5% PRO2000 gel, significantly lower levels of PRO2000 were recovered following coitus without a condom (median (IQR) 14 (3, 27)) than in the absence of sex (28 (22, 110); $p=0.04$).²⁵ This could be due to redistribution of drug, binding to semen, loss due to leakage or difficulty assaying drug due to physical changes following interaction with the semen.

A number of HIV transmissions may have been due to unprotected anal intercourse, although reported anal sex on the case report form was very uncommon: 1% at

enrolment and 2% ever during the trial. The majority of reports were in the South African sites where the overall HIV incidence was high, and only marginally lower than the rate in women ever reporting anal sex during the trial. Although qualitative data from interviews and focus groups suggest that there may have been under-reporting of anal sex in the clinic, we consider it unlikely that anal sex explains the lack of efficacy observed in the trial.

The rationale for including two concentrations was that the higher concentration of PRO2000 may result in local inflammation, which in turn may inadvertently facilitate HIV transmission, offsetting any potential gain in biological potency against HIV. Although there was no evidence of this in the histology or colposcopy findings in the Phase I/II studies, it was plausible that symptoms and signs might emerge in the longer Phase III trial with cumulative exposure in a much larger population. The absence of an effect of PRO2000 on local toxicity was reassuring; the absence of an effect on systemic toxicity was expected and consistent with the failure to detect PRO2000 in plasma following prolonged use.

Seven different molecular candidates have been evaluated in 13 Phase IIb/III microbicide trials. One trial was designed to inform a decision algorithm,⁶ but only four of the remaining 12 had adequate statistical power to detect the effect of interest which ranged from 35-50% for a variety of reasons, including early termination³ and lower than expected incidence.⁴ One of the four, COL-1492,²⁶ observed a significant increase in HIV infections in the women using nonoxynol-9 (N-9) compared to placebo; two, including MDP301, observed no effect.² In July 2010, CAPRISA 004 reported a 39% reduction in HIV incidence in women using the anti-retroviral (ARV) agent tenofovir 1% formulated as a vaginal gel, compared to women using placebo gel.²⁷ Women were advised to administer gel before and after sex, and to use no more than two every 24 hours. Tenofovir acts specifically to block HIV replication at the intracellular level and is considerably more potent *in vitro* than any of the non-ARV candidates. CAPRISA provided the first proof of concept for ARV prophylaxis and for microbicides.

Five further trials evaluating the effectiveness of ARV prophylaxis are already in the field and will report over the next 4 years. All of these are assessing daily oral tenofovir based regimens; only one, the VOICE trial, will assess tenofovir 1% as a microbicide, administered daily. It will be important to confirm that coitally dependent dosing is effective, ideally in non-South African populations, as there are hypothetical advantages associated with the lower systemic drug levels in a coitally

dependent dosing regimen, such as less toxicity, and less risk of drug resistance emerging during break through infections.

MDP301 demonstrates that licensing trials can be conducted in resource limited settings, and the self-reported quantitative data collected in MDP301, supported by the return of the used applicators, as well as the quantitative and qualitative data collected in the social science sub-study, reinforce the acceptability of microbicides as potential prevention products. CAPRISA 004 demonstrates that microbicides can prevent HIV infection.

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Author contributions

All authors contributed to study design and conduct. SMC prepared the initial draft of the manuscript. AMC performed the statistical analysis and the initial draft of the tables. All authors contributed with substantive revisions to subsequent drafts. All authors read and approved the final manuscript.

Conflict of interest

AP was an employee of Endo Pharmaceuticals Solutions Inc. (formerly Indevus Pharmaceuticals Inc.), the owner of PRO2000, and held an equity interest in the company.

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