Plasma Nevirapine Levels and 24-week Efficacy of a Fixed-Dose Combination of Stavudine, Lamivudine and Nevirapine (GPO-VIR) among Thai HIV-Infected Patients

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Background: *A fixed-dose combination of stavudine, lamivudine, and nevirapine (GPO-VIR) is the most affordable antiretroviral therapy (ART) regimen in Thailand. The data of nevirapine (NVP) level and efficacy of this fixed-dose combination is limited.*
Material and Method: *Patients who were initiated GPO-VIR in 2004 were enrolled. NVP levels at 12 weeks were determined. Patients were followed for 24 weeks.*
Results: *Fifty-nine patients with a mean age of 36.4 years and 54% male were enrolled. Mean body weight was 54.7 kgs. Median baseline CD4 and HIV-RNA were 29 cells/mm^3 and 270,000 (5.4 log$_{10}$) copies/mL, respectively. Mean plasma NVP levels at 12 weeks was 6.4 mg/L. By linear regression, female gender (p = 0.042), and higher weight (p = 0.020) were associated with lower NVP levels. At 24 weeks, 78% achieved undetectable HIV-RNA and median CD4 was 156 cells/mm^3.*
Conclusion: *NVP levels and 24-week efficacy of GPO-VIR are favorable. According to the affordable cost, GPO-VIR should be an appropriate initial regimen for na ve HIV-infected patients in resource-limited settings.*

Keywords: *HIV, Nevirapine level, Nevirapine, GPO-VIR, Efficacy*

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HIV infection is a serious public health-threatening problem in many countries. Combined antiretroviral therapy (ART) can reduce the risk of HIV progressing to AIDS, morbidity, and mortality[1,2]. However, access to ART for HIV-infected patients in resource-limited setting is still a major obstacle[3,4]. Nevirapine (NVP) is a non-nucleoside reverse transcriptase inhibitor (NNRTI) that has shown effective antiretroviral efficacy even in advanced HIV disease[5-8]. NNRTI-based ART regimen has been widely used in the resource-limited countries since it is more accessible. The Thai Government Pharmaceutical Organization has produced a fixed-dose combination (GPO-VIR) of stavudine (d4T) 30 or 40 mg, lamivudine (3TC) 150 mg, and NVP 200 mg, which has been available in the market since 2002[9]. This combination formula makes simple dosing feasible by taking one tablet twice daily. However, the data of NVP level and efficacy of this fixed-dose combination is limited.

The primary objective of the present study was to determine NVP levels in HIV-infected patients receiving GPO-VIR. The secondary objective was to assess the efficacy at 24 weeks of ART, including the proportion of patients who achieved undetectable HIV RNA and the change of CD4 cell counts from baseline.

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Material and Method

The present study design was a prospective cohort study involving HIV-infected Thai patients in

the Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health, Nonthaburi, Thailand. Enrollment was from January to February 2004. Inclusion criteria were: (1) HIV-infected individuals ≥ 15 years of age, (2) CD4 cell count < 200 cells/mm^3 or had an AIDS defining illness (3) na ve to ART and (4) willing to participate and signed consent form. Exclusion criteria were: (1) pregnancy, (2) receiving a medication that has drug-drug interactions with NVP and (3) had abnormal liver function test, i.e. elevation of aminotransferase three times greater than normal upper limit. The present study was approved by the institutional ethics committees of the Bamrasnaradura Infectious Disease Institute and the Ministry of Public Health.

The dosage of stavudine was adjusted by body weight, i.e., GPO-VIR S30 (d4T 30 mg) and GPO-VIR S40 (d4T 40 mg) twice a day were given for patients with body weight ≤ 60 kg and > 60 kg, respectively. The demographic and general characteristics (e.g., gender, age, body weight, body mass index (BMI), and previous opportunistic infections) were recorded. The patients had follow-up visits at 2, 4, 8, 12, 18, and 24 weeks, at which time they were assessed clinically and evaluated for adverse events.

Blood samples were obtained to study CD4 cells count by flow cytometry and HIV-1 RNA by polymerase chain reaction using Roche Amplicor version 1.5 (Roche Diagnostics, Branchburg, NJ, USA) at baseline, 12 and 24 weeks after initiating ART; lower limit of detection, 50 copies/mL. After 12 weeks of ART, blood sample at 12 hours after taking GPO-VIR were obtained to analyze NVP levels at the HIV Netherlands-Australia-Thailand (HIV-NAT) Research Pharmacokinetic Laboratory located at Chulalongkorn Medical Research Center (Chula MRC) by high performance liquid chromatography (HPLC) assay.

Mean (± standard deviation, SD), median (interquartile range, IQR) and frequencies (%) were used to describe patients' characteristics. Chi-square test was used to compare categorical variables. Student's t-test was performed to assess differences between two means. When the variables were not normally distributed, the Mann-Whitney U test was used instead. Linear regression analysis was used to determine the predicting factors for NVP levels. A p value of < 0.05 was considered as statistically significant. All analyses were performed using SPSS version 11.5.

Results

Fifty-nine patients with a mean age of 36.4 years and 54% were male. Mean body weight was 54.7 kgs. Median (IQR) baseline CD4 cell count was 29 (8-91) cells/mm^3 and median (IQR) baseline HIV RNA was 270,000 (85,700-714,000) copies/mL or 5.4 (4.9-5.8) $\log_{10}$ copies/mL. Table 1 shows the baseline characteristics of 59 study patients.

Mean (± SD) plasma NVP levels at 12 weeks of ART was 6.4 ± 3.1 mg/L and 10% had NVP levels < 3.4 mg/L (ranged from 1.33 to 3.17 mg/L). Fig. 1 demonstrates the distribution of plasma NVP levels at 12 weeks of ART by different gender and body weight. The results of linear regression analysis are shown in

Table1. Baseline characteristics of 59 HIV-infected patients

Characteristics	Number (%)
Gender: Male	32 (54%)
Female	27 (46%)
Age, years, mean ± SD	36.4 ± 8.4
Body weight, Kgs, mean ± SD	54.7 ± 10.7
Body mass index, mean ± SD	20.6 ± 3.5
History of AIDS defining illness	11 (19%)
CD4 cell count, cells/mm^3, median (IQR)	29 (8-91)
Percent CD4 cells, %, median (IQR)	3 (1-6)
Plasma HIV RNA, copies/mL, median (IQR)	270000 (85700-714000)
Plasma HIV RNA, $\log_{10}$ copies/mL, median (IQR)	5.4 (4.9-5.8)
ALP, mg/dL, median (IQR)	85 (64-126)
AST, U/L, median (IQR)	34 (25-45)
ALT, U/L, median (IQR)	32 (21-48)
Total bilirubin, mg/dL, median (IQR)	0.5 (0.4-0.7)

ALP, alkaline phosphatase; AST, aspatate transaminase; ALT, alanine transaminase; IQR, interquartile range

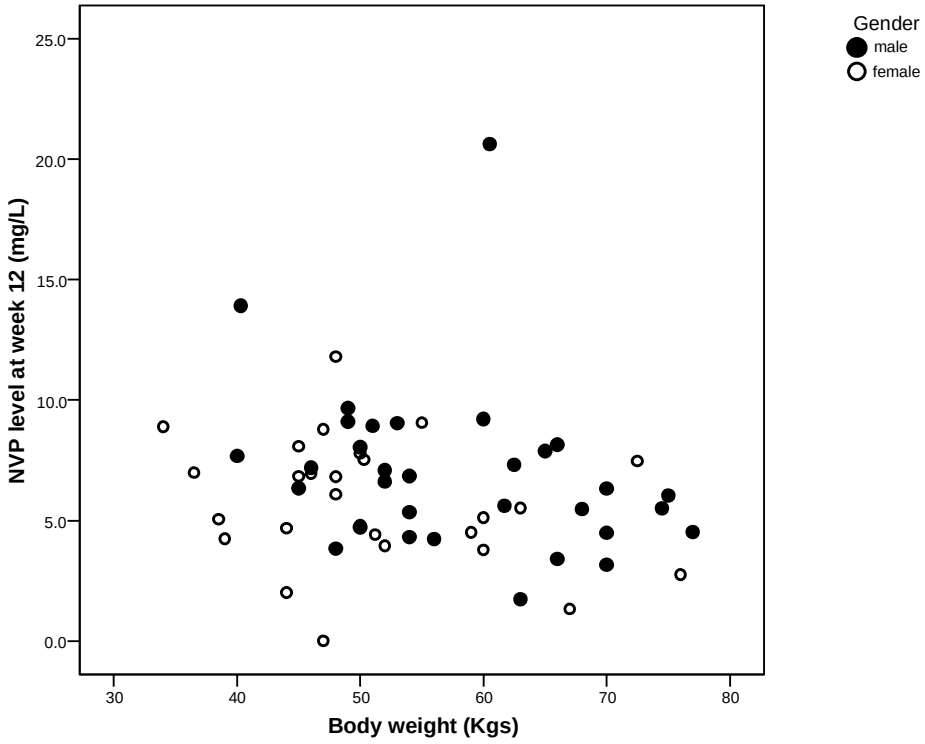

Fig. 1 Distribution of plasma NVP levels at 12 weeks of ART

Table 2. By linear regression, female gender ($p = 0.042$) and higher weight ($p = 0.020$) were significantly associated with lower NVP level.

During the follow-up period, one patient had discontinued NVP-based ART due to rashes and two patients were lost to follow-up before 24 weeks of ART. At 24 weeks, 78% and 88% of the study patients had achieved undetectable HIV RNA at < 50 and < 400 copies/mL, respectively. The intention-to-treat and on-treatment analysis of virological response at 24 weeks of ART are described in Table 3. From six patients who had NVP levels < 3.4 mg/L, five had HIV RNA < 400 copies/mL. Median (IQR) CD4 cell count at 24 weeks was 156 (104-273) cells/mm^3, which was significantly higher from the baseline ($p < 0.001$)(Fig. 2).

Regarding the adverse events, one patient had a rash and needed to change the ART regimen to efavirenz-based regimen. There was no difference in ALT levels at baseline and 12 weeks of ART ($p = 0.801$). No patient had clinical hepatitis.

Table 2. Factors associated with plasma NVP level at week 12 (linear regression analysis) (n = 59)

Factors	Beta	95%CI	p-value
Age	0.140	-0.046, 0.151	0.293
Female gender	-0.286	-3.498,-0.067	0.042
Body weight	-0.346	-0.485,-0.016	0.020
Baseline CD4 cell count	0.071	-0.008, 0.014	0.612

Beta, standardized coefficients; CI, confidence interval

Table 3. Virological response at 24 weeks of ART

	Patients with undetectable HIV RNA	
	< 50 copies/mL	< 400 copies/mL
Intention-to-treat analysis	78% (46/59)	88% (52/59)
On-treatment analysis	82% (46/56)	93% (52/56)

Discussion

In the present study, the authors have demon-

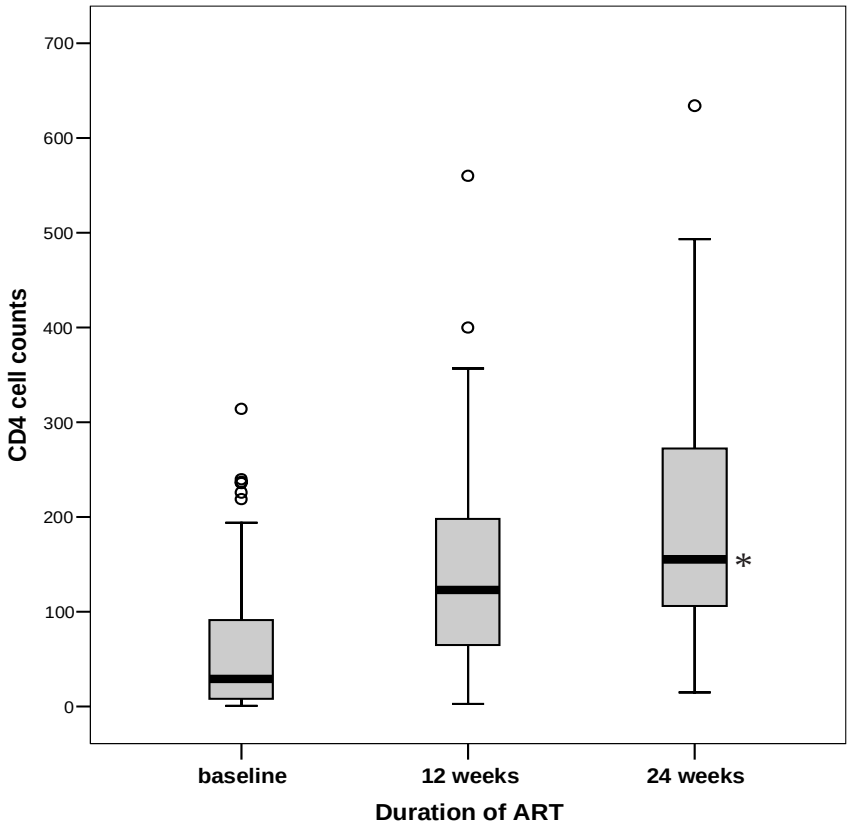

Fig. 2 Median (IQR) CD4 cell count response through 24 weeks of ART
* $p < 0.001$ from the baseline

strated that NVP levels in patients receiving a generic fixed-drug combination of d4T/3TC/NVP were in the favorable range. The majority (90%) of trough plasma NVP levels were in the recommended therapeutic NVP level (> 3.4 mg/L). Accordingly, about 80% of the patients had achieved undetectable HIV RNA, as well as higher CD4 cell counts. The results from the present study support the findings from previous studies in which NVP level was not performed[10,11]; this generic fixed-drug combination of d4T/3TC/NVP has a desirable efficacy.

Interestingly, the results from the present study also demonstrated that female gender and higher body weight were associated with lower NVP level. This finding warrants the necessity of close monitoring of treatment response in patients with high body weight or female gender. The previous studies demonstrated the effects of gender on the clearance of NVP[12,13]. While Zhou et al reported that body weight was not associated with NVP clearance[13], in contrast, the authors found that higher body weight was associated with lower NVP level. This discrepancy may be explained by the different range of the body weight of the study patients. The patients in the present study had a mean body weight of 54.7 kg whereas 73.9 kg in the previous study. Body weight may have effects on the NVP levels in patients with lower body weight. However, further large-scale study to confirm this and to determine the association of these two factors and treatment outcomes is needed. NVP are mainly metabolized by cytochrome P450 2B6 (CYP2B6). Allele 516 G > T (Gln172His) is associated with diminished activity of this isoenzyme, and may lead to differences in drug exposure[14]. The data regarding genetic polymorphism among the presented population is still limited. The present study enrolled Asian patients, which was dif-

ferent from the previous reports. Kappelhoff et al have demonstrated that Thais had less clearance of NVP compared with other ethnics[12]. Genetic variants in drug metabolism pathways have been shown to alter the safety and efficacy of other commonly prescribed medications. Furthermore, the previous studies demonstrated that there are differences in drug metabolism that may be related to genetic or environmental factors e.g. cytochrome P450C9 variant reduces the anticoagulant effect of warfarin[15]. Nonetheless, van Leth et al have demonstrated that race may not be associated with the pharmacokinetics of NVP[16]. To date, there is scanty data on differences in NVP metabolism based on genetic disposition.

Among patients who had trough plasma NVP levels < 3.4 mg/L, most of them achieved undetectable plasma HIV RNA at 24 weeks of ART. This is considered a desirable response. However, long-term virological and immunological outcomes are needed to monitor these patients.

Regarding tolerability, the prevalence of clinical hepatitis and ALT elevations in the present study is lower than that reported in the previous studies[17]. There may be two explanations for this discrepancy. First, the patients in the present study had very low CD4 cell counts. High CD4 cell count is a predictor of hepatotoxicity[17]. Second, this study included Asians, which was different from the previous studies[17,18].

NVP-based ART is a common regimen that is widely used for treatment of HIV-infected patients in resource-limited countries due to its affordability. Additionally, NVP is also recommended using two of the four World Health Organization-recommended generic combinations for the 3x5 program in resource-limited countries[19]. Until other options are more accessible, NVP-based ART is still a key regimen to scale up treatment of HIV infection in resource-limited countries. The present study provides the data of efficacy and safety, and supports the physician's use of a generic fixed-drug combination of d4T/3TC/NVP for the treatment of HIV disease in resource-limited settings.

The limitation of the present study is that the authors enrolled Asians with a low CD4 cell count. The results of NVP levels and adverse events from the present study may not be applicable for other populations. Long-term treatment outcomes are also needed to strengthen the efficacy data of this generic fixed-drug combination of d4T/3TC/NVP.

In conclusion, plasma NVP levels and 24-week efficacy of GPO-VIR in HIV-infected Thai patients are favorable. GPO-VIR may be an appropriate option for HIV-infected patients in resource-limited settings. Further study of long-term virological and immunological outcomes is needed.

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References

1. Hogg RS, Heath KV, Yip B, Craib KJ, O'Shaughnessy MV, Schechter MT, et al. Improved survival among HIV-infected individuals following initiation of antiretroviral therapy. JAMA 1998; 279: 450-4.
2. Palella FJ Jr, Delaney KM, Moorman AC, Loveless MO, Fuhrer J, Satten GA, et al. Declining morbidity and mortality among patients with advanced human immunodeficiency virus infection. HIV Outpatient Study Investigators. N Engl J Med 1998; 338: 853-60.
3. Dionisio D, Esperti F, Messeri D, Vivarelli A. Priority strategies for sustainable fight against HIV/AIDS in low-income countries. Curr HIV Res 2004; 2: 377-93.
4. Vermund SH, Powderly WG. Developing a human immunodeficiency virus/acquired immunodeficiency syndrome therapeutic research agenda for resource-limited countries: a consensus statement. Clin Infect Dis 2003; 37(Suppl 1): S4-12.
5. Floridia M, Bucciardini R, Ricciardulli D, Fragola V, Pirillo MF, Weimer LE, et al. A randomized, double-blind trial on the use of a triple combination including nevirapine, a nonnucleoside reverse transcriptase HIV inhibitor, in antiretroviral-naive patients with advanced disease. J Acquir Immune Defic Syndr Hum Retrovirol 1999; 20: 11-9.
6. Manosuthi W, Sungkanuparph S, Vibhagool A, Rattanasiri S, Thakkinstian A. Nevirapine- versus efavirenz-based highly active antiretroviral therapy regimens in antiretroviral-naive patients with advanced HIV infection. HIV Med 2004;5:105-9.
7. Sungkanuparph S, Manosuthi W, Kiertiburanakul S, Vibhagool A. Initiation of antiretroviral therapy in advanced AIDS with active tuberculosis: clinical experiences from Thailand. J Infect 2006; 52: 188-94.
8. van Leth F, Phanuphak P, Ruxrungtham K, Baraldi E, Miller S, Gazzard B, et al. Comparison of first-

line antiretroviral therapy with regimens including nevirapine, efavirenz, or both drugs, plus stavudine and lamivudine: a randomised open-label trial, the 2NN Study. Lancet 2004; 363: 1253-63.
9. Cohen J. Thailand's do-it-yourself therapy. Science 2003; 301: 1662.
10. Anekthananon T, Ratanasuwan W, Techasathit W, Sonjai A, Suwanagool S. Safety and efficacy of a simplified fixed-dose combination of stavudine, lamivudine and nevirapine (GPO-VIR) for the treatment of advanced HIV-infected patients: a 24-week study. J Med Assoc Thai 2004; 87: 760-7.
11. Tin EE, Bowonwatanuwong C, Desakorn V, Wilairatana P, Krudsood S, Pitisuttithum P. The efficacy and adverse effects of GPO-VIR (stavudine +lamivudine+nevirapine) in treatment-naive adult HIV patients. Southeast Asian J Trop Med Public Health 2005; 36: 362-9.
12. Kappelhoff BS, van Leth F, MacGregor TR, Lange J, Beijnen JH, Huitema AD. Nevirapine and efavirenz pharmacokinetics and covariate analysis in the 2NN study. Antivir Ther 2005; 10: 145-55.
13. Zhou XJ, Sheiner LB, D'Aquila RT, Hughes MD, Hirsch MS, Fischl MA, et al. Population pharmacokinetics of nevirapine, zidovudine, and didanosine in human immunodeficiency virus-infected patients. The National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators. Antimicrob Agents Chemother 1999; 43: 121-8.
14. Rotger M, Colombo S, Furrer H, Bleiber G, Buclin T, Lee BL, et al. Influence of CYP2B6 polymorphism on plasma and intracellular concentrations and toxicity of efavirenz and nevirapine in HIV-infected patients. Pharmacogenet Genomics 2005; 15: 1-5.
15. Aithal GP, Day CP, Kesteven PJ, Daly AK. Association of polymorphisms in the cytochrome P450 CYP2C9 with warfarin dose requirement and risk of bleeding complications. Lancet 1999; 353: 717-9.
16. van Leth F, Wit FW, Reiss P, Bleiber G, Buclin T, Lee BL, et al. Differential CD4 T-cell response in HIV-1-infected patients using protease inhibitor-based or nevirapine-based highly active antiretroviral therapy. HIV Med 2004; 5: 74-81.
17. Pollard RB, Robinson P, Dransfield K. Safety profile of nevirapine, a nonnucleoside reverse transcriptase inhibitor for the treatment of human immunodeficiency virus infection. Clin Ther 1998; 20: 1071-92.
18. Dieterich DT, Robinson PA, Love J, Stern JO. Drug-induced liver injury associated with the use of nonnucleoside reverse-transcriptase inhibitors. Clin Infect Dis 2004; 38(Suppl 2): S80-9.
19. World Health Organization and the Joint United Nations Programme on AIDS. *"3 by 5" progress report*. Geneva: WHO/UNAIDS; June 2005.

ระดับยาเนวิราปีนในพลาสมาและประสิทธิผลในการรักษาที่ 24 สัปดาห์ ของสูตรยาเม็ดผสมของ สตาวูดีน ลามิวูดีน และเนวิราปีน (จีพีโอ-เวียร์) ในผู้ป่วยไทยที่ติดเชื้อเอชไอวี

วีรวัฒน์ มโนสุทธิ, ศศิโสภิณ เกียรติบูรณกุล, อัจฉรา เชาวะวณิช, สมนึก สังฆานุภาพ

ที่มา: *สูตรยาเม็ดผสมของ สตาวูดีน ลามิวูดีน และเนวิราปีน (จีพีโอ-เวียร์) เป็นการรักษาด้วยยาต้านไวรัสเอชไอวีที่ใช้กันอย่างแพร่หลายที่สุดในประเทศไทย ข้อมูลการศึกษาเกี่ยวกับระดับยาเนวิราปีนในพลาสมาและประสิทธิผลในการรักษาของสูตรยานี้ยังมีอยู่จำกัด*

วัสดุและวิธีการ: *ได้ทำการศึกษาในผู้ป่วยไทยที่ได้รับการเริ่มยาด้วย จีพีโอ-เวียร์ ในปีพ.ศ.2547 ผู้ป่วยได้รับการเจาะเลือดตรวจหาระดับยาเนวิราปีนที่ 12 สัปดาห์และติดตามการรักษานาน 24สัปดาห์*

ผลการศึกษา: *มีจำนวนผู้ป่วย 59 ราย อายุเฉลี่ย 36.4 ปี และร้อยละ 54 เป็นเพศชาย น้ำหนักเฉลี่ยคือ 54.7 กก. ค่ากลางของปริมาณซีดีสี่และปริมาณไวรัสคือ 29 เซลล์/มม^{3}และ 270,000 ก็อปปี/มล.ตามลำดับ ระดับยาเนวิราปีนที่ 12 สัปดาห์คือ 6.4 มก/ล. โดยการวิเคราะห์วิธีลีเนียร์รีเกรสชัน พบว่าเพศหญิงและน้ำหนักที่มากขึ้นจะสัมพันธ์กับระดับยาเนวิราปีนที่ลดลงอย่างมีนัยสำคัญทางสถิติ ที่ 24 สัปดาห์ ร้อยละ 78 ของผู้ป่วยมีปริมาณไวรัสที่น้อยจนวัดไม่ได้ และค่ากลางซีดีสี่คือ 156 เซลล์/มม^3*

สรุป: *ระดับยาเนวิราปีนในพลาสมาและประสิทธิผลในการรักษาของ จีพีโอ-เวียร์ อยู่ในเกณฑ์ที่ดี ด้วยเหตุผลเรื่องราคายาที่ผู้ป่วยสามารถจ่ายได้ จีพีโอ-เวียร์ น่าจะเป็นยาต้านไวรัสเอชไอวีสูตรแรกที่เหมาะสมในผู้ป่วยที่ไม่เคยได้รับยาต้านไวรัสมาก่อน ในพื้นที่ที่มีทรัพยากรจำกัด*