Effective Program Against Mother-to-Child Transmission of HIV at Saint Camille Medical Centre in Burkina Faso

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The present research was aimed to prevent mother-to-child transmission of HIV; to use RT-PCR in order to detect, 6 months after birth, infected children; and to test the antiretroviral resistance of both children and mothers in order to offer them a suitable therapy. At the Saint Camille Medical Centre, 3,127 pregnant women (aged 15–44 years) accepted to be enrolled in the mother-to-child transmission prevention protocol that envisages: (i) Voluntary Counselling and Testing for all the pregnant women; (ii) Antiretroviral therapy for HIV positive pregnant women and for their newborns; (iii) either powdered milk feeding or short breast-feeding and RT-PCR test for their children; (iv) finally, pol gene sequencing and antiretroviral resistance identifications among HIV positive mothers and children. Among the patients, 227/3,127 HIV seropositive women were found: 221/227 HIV-1, 4/227 HIV-2, and 2/227 mixed HIV infections. The RT-PCR test allowed the detection of 3/213 (1.4%) HIV infected children: 0/109 (0%) from mothers under ARV therapy and 3/104 (2.8%) from mothers treated with Nevirapine. All children had recombinant HIV-1 strain (CRF06_CPX) with: minor PR mutations (M36I, K20I) and RT mutations (R211K). Among them, two twins had Non-Nucleoside Reverse Transcriptase Inhibitor mutation (Y18CY). Both mothers acquired a major PR mutation (V8IV), investigated 6 months after a single-dose of Nevirapine. Prevention by single-dose of Nevirapine reduced significantly mother-to-child transmission of HIV, but caused many mutations and resistance to antiretroviral drugs. Based on present study the antiretroviral therapy protocol, together with the artificial-feeding, might represent the ideal strategy to avoid transmission of HIV from mother-to-child. J. Med. Virol. 79:873–879, 2007.

KEY WORDS: HIV; mother-to-child transmission; nevirapine; antiretroviral therapy; drug-resistance; Burkina Faso

INTRODUCTION

Burkina Faso, a Western African country, is bordered by Mali (north and west), Niger (east) Ivory Coast, Ghana, Togo and Benin (south), and it is one of the Sub-Saharan countries with HIV/AIDS [Gregoire et al., 2000]. Since 1990, the Burkina Faso started a new strategy against HIV/AIDS through formation, information,

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awareness, assumption of responsibility, and compliance to treatment by the mothers.

In 1993, according to the WHO indications, the country was divided into 53 medical districts. Five national sentinel sites for HIV epidemiological monitoring were instituted in 1997 in the towns of Ouagadougou, Bobo-Dioulasso, Banfora, Ouahigouya, and Tenkodogo.

The “Comité National de Lutte contre le SIDA” was transformed into “Conseil National de Lutte contre le SIDA” under the presidency of Burkina Faso in 2001 and prevention programs of mother-to-child HIV transmission started.

After 15 years of efforts, epidemiological regression of HIV/AIDS was noted: 7.2% in 1997 [UNDP, 2001; CNLS IST, 2005]; 6.5% in 2001 [WHO 2004]; 4.2% in 2002 [ONUSIDA, 2004; CNLS IST, 2005]; 2.3% at the end of 2004 [OUNAIDS, 2005], particularly in the five sentinel sites (Table I) [Ministry for the Health of Burkina Faso, 2003, 2006].

These sentinel sites indicate that 5.1% of pregnant women were HIV seropositive in 2004. Considering that the number of annual births would account for 601,000 at Burkina Faso [Unicef-Statitiques, 2004], about 33,000 newborns per year were estimated to be exposed to the risks of mother-to-child transmission of HIV.

In Western countries, prevention programs eliminated mother-to-child transmission of HIV. By contrast, in sub-Saharan countries, several factors still keep to maintain this mode of transmission.

About 10% of these transmissions occur by transplacental passage during the antenatal period; 15% by exposure to maternal blood and vaginal secretions during labor; and 10% postpartum via breast-feeding. Thus, this HIV transmission occurs because of the high viral load in maternal blood, amniotic liquid, cervicovaginal secretions, and mother’s milk [Rouzioux et al., 1995; Meda et al., 1997; Shaheen et al., 1999]. In addition, chorioamnionitis (inflammation involving the chorion, its foetal vessels, umbilical cord, and amnios), viral co-infections (HBV, HCV, and HHV8) [Simpore et al., 2006a], parasitic diseases (toxoplasmosis and malaria) [Simpore et al., 2006b], and even obstetric factors, such as the premature membrane rupture, can increase significantly the risk of mother-to-child HIV infection [Landesman et al., 1996].

With these factors, the average rate of HIV transmission in Africa rises to 50% [Meda et al., 1997; Simpore et al., 2006c]. Thus, more than 600,000/per year babies are infected by HIV in the world [European Union Presidency Statement, 2001]. However, since 2002, a health-based prevention program has started in the Saint Camille Medical Centre, for the prevention of both HIV transmission to the child and unintended pregnancies in HIV infected women as primary objectives. The Saint Camille Medical Centre also organizes health education courses. A HIV test is also proposed to women and about 50% accept screening. The instruments of intervention for the mother-to-child HIV transmission are: prevention by caesarean section, antiretroviral

| TABLE I. HIV Seroprevalence Among Pregnant Women in Five Sentinel Towns in Burkina Faso From 1997 to 2004 |
|----------------|------|------|------|------|------|------|------|------|
| Bobo-Dioulasso | 7.6% | 6.2% | 5.7% | 6.2% | 5.7% | 6.2% | 4.3% | 2.3% |
| Ouagadougou | 6.7% | 7.7% | 7.7% | 6.3% | 6.3% | 6.3% | 4.9% | 2.4% |
| Ouahigouya | 6.5% | 6.5% | 6.5% | 5.9% | 5.9% | 5.9% | 4.7% | 2.4% |
| Gaoua | 4.0% | 6.0% | 5.4% | 5.9% | 5.9% | 5.9% | 4.3% | 2.3% |
| Total | 6.5% | 6.3% | 6.3% | 6.3% | 6.3% | 6.3% | 4.7% | 2.4% |


Simpore et al.
(ARV) drugs, breast-feeding suspension, and prevention of transmission of other infections by sexual route.

Prophylactic caesarean section could decrease the rate of HIV transmission [European Collaborative Study, 1994]. However, in poor nations, caesarean section poses many problems. It is rather expensive and there are several risks with this procedure: potential hemorrhage, wounding the child, exposure to infected maternal blood and, finally, anaesthetic and infectious complication risks for mothers [De Muylder, 1993].

For these reasons, we assumed that antiretroviral treatment of HIV positive pregnant women, and bottle-feeding of the infant, would be an alternative method to reduce mother-to-child transmission of HIV [Connor et al., 1994].

Therefore, the present study focuses on three aims:

1. Eradication of mother-to-child transmission of HIV at the Saint Camille Medical Centre using ARV therapy.
2. Use of qualitative RT-PCR technique to detect, 6 months after birth, babies infected by HIV.
3. Seek in infected children possible HIV mutations and antiretroviral resistance in order to start a suitable ARV therapy.

PATIENTS, MATERIALS, AND METHODS

Site of Research

The Burkina Faso Health Ministry, in agreement with both WHO and UNICEF, worked out a mother-to-child HIV transmission protocol and the Saint Camille Medical Centre (an officially agreed private medical structure) was asked to be the pilot center for this project. This center possesses a maternity unit with 100 beds, a neonatal pathology service, a maternal, and infant care service that currently follows more than 3,000 pregnant women per year and, finally, a laboratory for routine and molecular testing.

From July 5, 2004 to February 24, 2006, 3,127 pregnant women with less 6 months of pregnancy (15–44 years old, average age 26.6 \( \pm \) 4.6), agreed to have an HIV test and to follow the mother-to-child HIV transmission protocol in case of seropositivity for HIV.

Blood Samples

After informed consent, 10 ml of blood were collected from each pregnant woman and poured in two EDTA-containing tubes. The first tube was used for an HIV test and CD4\(^+\) count. The second tube was centrifuged at 3,000 rpm for 10 min for virus load. With the agreement of the HIV positive parents, 5 ml of blood was taken from their children at 6 months age. The plasma was kept at \(-80^\circ\)C until qualitative HIV RT-PCR genotype and antiretroviral resistance tests were undertaken.

HIV Test

Serological screening for HIV was carried out by using sequentially the two rapid Determine\(^\text{R}\) and Genie-II\(^\text{R}\) tests, employed to detect both HIV-1 and HIV-2, as described previously by Koblavi-Deme et al. [2001]. A third test was used in all cases in which the two rapid tests gave differing results. In such cases the samples were tested with enzyme immuno assay (EIA), using the Abbott IMX System (Abbott Laboratories, N. Chicago, IL), in order to confirm/exclude the HIV infection.

CD4\(^+\) T Cell Count and Virus Load

CD4\(^+\) T Cell count was carried out by the FACS Count (Becton Dickinson, San Jose, CA) and the virus load was

<table>
<thead>
<tr>
<th>Class ages</th>
<th>Age (years)</th>
<th>Total number of pregnant women</th>
<th>Number of HIV(^-) pregnant women</th>
<th>Number of HIV(^+) pregnant women</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>15–19</td>
<td>322</td>
<td>316</td>
<td>6 (1.9%)</td>
</tr>
<tr>
<td>2</td>
<td>20–24</td>
<td>1,110</td>
<td>1,051</td>
<td>59 (5.3%)</td>
</tr>
<tr>
<td>3</td>
<td>25–29</td>
<td>936</td>
<td>852</td>
<td>84 (9%)</td>
</tr>
<tr>
<td>4</td>
<td>30–34</td>
<td>491</td>
<td>434</td>
<td>57 (11.6%)</td>
</tr>
<tr>
<td>5</td>
<td>&gt;35</td>
<td>268</td>
<td>247</td>
<td>21 (7.8%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>3,127</td>
<td>2,900</td>
<td>227 (7.3%)</td>
</tr>
</tbody>
</table>

\[X_2: 1 \rightarrow 2 P = 0.009; X_2: 1 \rightarrow 3 P < 0.001; X_2: 2 \rightarrow 4 P < 0.001; X_2: 2 \rightarrow 5 P < 0.001.\]

\[X_2: 2 \rightarrow 3 P = 0.001; X_2: 2 \rightarrow 4 P < 0.001; X_2: 2 \rightarrow 5 P = 0.113 (NS); X_2: 3 \rightarrow 4 P = 0.113 NS.\]

\[X_2: 3 \rightarrow 5 P = 0.560 (NS); X_2: 4 \rightarrow 5 P = 0.102 (NS).\]
determined using the LCX system (Abbott Laboratories, North Chicago, IL).

**RNA Extraction, Qualitative RT-PCR, and Sequencing Test**

RNA was extracted from 1 ml of plasma using the QiaAmp Viral RNA (Qiagen GmbH, Hilden, Germany). RNA was recovered in 50 ml of sterile nuclease-free water and stored at −80 °C for later analyses. cDNA was synthesized from 10 ml of extracted RNA by RT-PCR kit (Viroseq 2, Abbott). Samples were amplified under the following conditions: 42 °C 60 min, 94 °C 5 min, and 50 cycles at: 93 °C for 30 sec, 60 °C for 30 sec, and 72 °C for 15 min for final extension. Electrophoresis was performed in 3% agarose gel in 1× TBE BUFFER (40 mM Sorting-Borate, 1 mM EDTA, pH 8.0) for 1 hr at a constant 120 V voltage. The fragments were visualized after staining with Ethidium bromide and photographed under UV light.

All procedures were carried out according to the manufacturer protocol. Sequencing reactions were run in the capillary automated DNA sequencer (ABI model 3100 Applera). Sequences were analyzed by the software program for HIV analysis, and the obtained reports were submitted into the Stanford web site for Drug Resistance Algorithm (http://hivdb2.stanford.edu/asi/deployed/hiv_central.pl?program=hivdb, Beta Test). The reference mutation list, used to evaluate resistance, was that reported in Stanford HIV Drug Resistance Database [http://hivdb.Stanford.edu].

**ARV Prophylaxis**

From July 5, 2004 to February 24, 2006, 223 HIV-1 seropositive pregnant women, diagnosed during this study, were clinically and biologically followed by the Saint Camille Medical Centre of Ouagadougou. The pregnant women, who presented indications for ARV therapy (CD4⁻⁺ <200/mL or Stage WHO III and CD4⁻⁺ <350/mL or Stage WHO IV) received, after the HIV positive tests, the AZT/3TC/NVP or, if anemic, the D4t/3TC/NVP tri-therapies. In HIV-2 infected mother, or unfavorable Nevirapine reactions, this drug was replaced by a protease inhibitor CRIXIVAN (Indinarate Sulfate). Pregnant women without indications for ARV therapy received a single Nevirapine dose (200 mg) during the labor. In the event of HIV-1/HIV-2 co-infection, pregnant women received 300 mg AZT every 12 hr, starting from the 36th week, and a 600 mg AZT single dose during labor. All the newborns, whose mothers were HIV seropositive, received a 2 mg/Kg of oral suspension Nevirapine single dose within the first 72 hr of life or (if infected by HIV-2) AZT syrup 4 mg/kg every 12 hr for 1 week. According to the national guidelines, all these women, weekly received, during their pregnancy, a 300 mg chloroquine single dose for malaria prevention. Women who showed less than 200 CD4⁻⁺/mL, also received 960 mg 3×/week Co-trimoxazole, starting from the 4th month of pregnancy.

**Breast-Feeding and Powered Milk Feeding**

For both ethic and cultural reasons, mothers were free to choose between breast-feeding or powered milk feeding.

**Ethical Committee**

The Ethics Committee of Saint Camille Medical Centre approved this study and each mother authorized orally the collection of blood.

**Statistical Analysis**

Demographic and clinical profiles were analyzed by the SPSS-12 for Windows and EpiInfo-6 standard softwares. Statistical significance was set at \( P < 0.05 \).

**RESULTS**

In the mother-to-child HIV transmission program, 3,127 pregnant women underwent voluntarily HIV testing. They represent 50% of those who were offered counseling and testing. All pregnant women who underwent the test came for results and post-test counseling. HIV seropositive pregnant women (227/3,127 (7.3%))

<p>| TABLE IV. Parameters of the Newborns Whose Mothers Were Following the Two Kind of Therapy |
|----------------------------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Babies born</th>
<th>Premature</th>
<th>Weight (kg)</th>
<th>Tinted amniotic liquid</th>
<th>Died babies 6 months of birth</th>
<th>Babies candidates with RT-PCR test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mono therapy mothers</td>
<td>108</td>
<td>0</td>
<td>2.819 ± 0.559</td>
<td>19/108 17.6% ( ^{k} )</td>
<td>4 (3.7%)</td>
<td>104</td>
</tr>
<tr>
<td>Tri-therapy mothers</td>
<td>115</td>
<td>4 (3.5%)</td>
<td>2.786 ± 0.658</td>
<td>14/115 12.2% ( ^{s} )</td>
<td>6 (5.2%)</td>
<td>109</td>
</tr>
<tr>
<td>Total</td>
<td>223</td>
<td>4 (1.8%)</td>
<td>2.808 ± 0.594</td>
<td>33/223 14.2%</td>
<td>10 (4.5%)</td>
<td>213</td>
</tr>
</tbody>
</table>

Student’s \( t \)-test \( * \rightarrow #; P = 0.688 \) (NS); \( \chi^2 \rightarrow \$; P = 0.255 \) (NS).

<p>| TABLE V. RT-PCR Results for Children Whose Mothers Were Following the Two Kind of Treatment |
|----------------------------------------|------------------|------------------|------------------|</p>
<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>RT-PCR negative</th>
<th>RT-PCR positive</th>
<th>Yates’ ( \chi^2 ) test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tri-therapy</td>
<td>109</td>
<td>109</td>
<td>0 (0.0%)</td>
<td>( P = 0.228 ) (NS)</td>
</tr>
<tr>
<td>Mono-therapy</td>
<td>104</td>
<td>101</td>
<td>3 (2.8%)</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>213</td>
<td>210</td>
<td>3 (1.4%)</td>
<td></td>
</tr>
</tbody>
</table>

were found; 221/227 (97.4%) were infected with HIV-1; 4/227 (1.8%) were infected with HIV-2, and 2/227 (0.9%) had mixed HIV-1/HIV-2 infections (Table II).

The average age between HIV seropositive women (27.88 ± 4.2) and negative (25.41 ± 5.3), gave a significant difference: \( P < 0.0001 \).

Table III shows a percentage progression of HIV infection with age: age group 15–19 (1.9%) up to 30–34 years (11.6%).

The 108 HIV seropositive pregnant women, who at the time of their inclusion in the mother-to-child HIV transmission program were treated with Nevirapine, had 513 ± 197 CD4+ /mL and 180,022 ± 81,252/ml copies /mL. The 115 women candidates for ARV therapy had 140 ± 51 CD4+/mL and 415,033 ± 212,490/ml viral load.

The four HIV-2 seropositive pregnant women did not follow the protocol of the mother-to-child HIV transmission prevention adopted by Saint Camille Medical Centre. Women who remained in our center, at the pregnancy end, delivered: 223 children including 4 premature who died within the first day of birth; others 6 babies died in 3 months because of bacterial diarrhea.

The average birth weight of children, whose mothers received either the mono-prophylaxis or ARV therapy, were 2.8 ± 0.5 and 2.7 ± 0.6 kg \( P = 0.688 \) (Table IV), respectively. The RT-PCR test detected 3/213 (1.4%) children infected with HIV vertically: 0/109 (0%) from mothers under ARV therapy and 3/104 (2.8%) from mothers under treatment with Nevirapine (Table V).

Some amniotic fluids, 33/213 (15.5%) were tinted with blood. Among the three HIV infected children, 2/3 born with blood tinted amniotic liquid (Table VI).

Concerning breast-feeding, 183/213 women (91.7%) of those under ARV therapy and 79.8% of those under mono therapy, \( P = 0.013 \) had chosen the bottle-feeding and 30/213 (14%) a short breast-feeding protocol for 4 months.

Among the 3/213 (1.4%) HIV positive children, two were twins and were fed with powdered milk while the other child was nourished by breast-feeding (Table VI).

Six months after a single dose of Nevirapine, the sequencing of HIV in the two mothers and their three children showed that all five were infected by the HIV-1. They had the HIV recombinant form CRF06_CPX 5/5.

DISCUSSION

Among the 3,127 pregnant women with less than 6 months of pregnancy, who accepted the mother-to-child HIV transmission program in the Saint Camille Medical Centre, 7.3% seroprevalence of HIV (Table II) was detected. This prevalence is equal (7.1%, \( P = 0.886 \)) to that found in 1997 in the three Burkina Faso sentinel sites and statistically higher than the average prevalence of the five sentinel sites (5.1%, \( P < 0.0001 \)) since 1997–2004 (Table I). The prevalence found in this study is still higher than that identified in 2004 at national level (4.2%). This can be explained by two reasons: (1) the Saint Camille Medical Centre is an official agreed social center taking care of poor people, and in this kind of population, the HIV incidence is very high; (2) the Saint Camille Medical Centre is a national pilot center for the mother-to-child HIV transmission program and some pregnant women, aware of their serologic status, come to this center in order to obtain good therapeutic services. The HIV prevalence increases up with the group age to reach a maximum in the 30–34 year range and decreases afterwards: 15–19 years (1.9%), 20–24 years (5.3%), 25–29 years (9%), 30–34 years (11.6%), and >35 years (7.8%) (Table III). Concerning the prevalence, of an age group to the other, significant differences were also found \( P < 0.01 \).

Before the ARV therapy and various prophylactic interventions, the HIV mother-to-child transmission was much higher (10.4%) at the Saint Camille Medical Centre [Simpore et al., 2006b].

Six months after birth, qualitative RT-PCR tests provided the following prevalence: 3/104 (2.8%) cases of HIV transmission among children whose mothers received Nevirapine (Table V). This HIV transmission prevalence is higher than that found recently (2005) in Colombia (1.8%) [Garcia et al., 2005] and almost similar to that identified in Johannesburg (South Africa) (2%) [McIntyre 2005] and in Sao Paulo (Brazil) (2.4%) [Matida et al., 2005]. In contrast, it is lower than that found in 2005 in Rio de Janeiro (6.8%) [Fernandez et al., 2005], in Cotonou (Benin) (7%) [Adeothy-Koumakpai et al., 2004], in Khayelitsha (South Africa) (8.8%) [Coetzee et al., 2005], in Ukraine (10%) [Malyuta et al., 2006], and at the Saint Camille Medical Centre (Burkina Faso) (10.4%), where the first protocol of the mother-to-child HIV transmission was adopted [Pignatti et al., 2006; Simpore et al., 2006c].

In the present study, we found no mother-to-child HIV transmission in mothers who received the ARV therapy, 0/109 HIV vertical transmission. In addition to the ARV
therapy, Co-trimoxazole (960 mg 3×/week) and chloroquine (300 mg) once weekly allowed also to obtain satisfactory results in prevention of infectious diseases and malaria, respectively.

Three children were infected with HIV: 3.3% (1/30) nursed by breast-feeding and 1.1% (2/183) nursed with powdered milk (Table VI). HIV seropositive women of Sub-Sahara countries have to face a double dilemma in feeding children. Powdered milk, in the absence of drinkable water and refrigerator, may cause a great risk to babies of death because of bacterial diarrhoea. On the other hand, breast fed babies could acquire HIV from the infected mother’s milk [Simpore et al., 2006c]. In addition, prevention of mother-to-child HIV transmission with Nevirapine determines antiretroviral resistance in breast fed children. Nevirapine inhibits HIV transcriptase reverse enzyme, induces mutations and ARV resistance into pol gene. Indeed, 6 months after Nevirapine two mothers and their three children acquired monotherapy-related resistances (Table VII). The mother of the two twins, 6 months after she took Nevirapine treatment, showed NNRTI V106I mutation. Several investigators confirm that Nevirapine can cause valine aminoacid substitution in the transcriptase enzyme in 106 (V106I) position [Bacherel et al., 2001; Ferris et al., 2005; Frederiksen et al., 2004; Hazen et al., 2005]. A further different RT NNRTI Y18C mutation was caused by Nevirapine among twins. We also found several other RT mutations: R211K (5/5), V35T (3/5), V21I (3/5), and K12E (3/5). Major and minor PR mutations were also found: V8IV (1/5), M36I (5/5), K20I (5/5), L63LP (4/5), I13V (5/5), K14R (5/5), H69K (5/5), and L89M (5/5) (Table VII). All five individuals showed a recombinant sub-type CRF06_cpx, the most widespread form in Burkina Faso (50%) [Ouedraogo-Traore et al., 2003; Nadembega et al., 2006].

Because of these mutations, WHO recommends that the Nevirapine monotherapy be discontinued in the prevention of mother-to-child HIV transmission in favor of the following combined therapy.

For HIV positive women: AZT starting from the 28th week of pregnancy, Nevirapine/AZT/3TC for the phase of labor and during the first postpartum week. The main goal of this AZT/3TC combination is “to protect” from Nevirapine resistance: for children monodose Nevirapine syrup plus 1 week of AZT syrup.

The mother-to-child HIV transmission protocol, based on mono prophylaxis and other kind of preventions, allowed us to reduce significantly the rate of HIV transmission (3/104). In addition, the ARV therapy and other different type of prevention against infection (vide supra), permitted prevention of HIV: 0 infection/109 children. Unfortunately, this protocol is financially expensive and employs several medical specialities. An anti-HIV/AIDS therapeutic vaccine should be the best strategy to prevent maternal to infant transmission of HIV.

ACKNOWLEDGMENTS

The authors are grateful to all the Ouagadougou SCMC laboratory technicians for their skilful

<table>
<thead>
<tr>
<th>Replacing Form Subtype</th>
<th>Major PR Changes</th>
<th>Minor PR Changes</th>
<th>Other PR Changes</th>
<th>Others RT Changes</th>
<th>NNRTI Others RT Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>M1, 30 years</td>
<td>390</td>
<td>CRF06_CPX</td>
<td>V8IV</td>
<td>M36I K20I L63LP I13V K14R E35D R41K H69K L89M K20I</td>
<td></td>
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<tr>
<td>E1, 6 months</td>
<td>450</td>
<td>CRF06_CPX</td>
<td>M36I K20I L63LP A71V I13V K14R E35D R41K H69K L89M Y18C R211K V35T V21I K12E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E1, 6 months</td>
<td>438</td>
<td>CRF06_CPX</td>
<td>M36I K20I L63LP I13V K14R E35D R41K H69K L89M Y18C R211K V35T V21I K12E</td>
<td></td>
<td></td>
</tr>
<tr>
<td>M2, 32 years</td>
<td>350</td>
<td>CRF06_CPX</td>
<td>M36I K20I L63LP I13V K14R E35D R41K H69K L89M K70R I151V R211K</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2, 6 months</td>
<td>420</td>
<td>CRF06_CPX</td>
<td>M36I K20I I13V K14R E35D R41K H69K L89M K70R R211K</td>
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</tr>
</tbody>
</table>
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