# รายงานวิจัยฉบับสมบูรณ์

## โครงการประเมินและติดตามเชื้อเอชไอวีดื้อยาปฐมภูมิในผู้ติดเชื้อเอชไอวี

(Evaluation and Monitoring of Primary Drug Resistance in Patients with HIV-1 Infection)

โดย รองศาสตราจารย์ นายแพทย์ สมนึก สังฆานุภาพ

๓๐ กันยายน ๒๕๕๔

สัญญาเลขที่ RMU5180018

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สนับสนุนโดยสำนักงานกองทุนสนับสนุนการวิจัย
และสำนักงานคณะกรรมการอุดมศึกษา

# บทคัดย่อ

**รหัสโครงการ:** RMU5180018

**ชื่อโครงการ:** โครงการประเมินและติดตามเชื้อเอชไอวีดื้อยาปฐมภูมิในผู้ติดเชื้อเอชไอวี

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**สถาบัน:** คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

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**ระยะเวลาโครงการ:** 15 พฤษภาคม 2551 ถึง 14 พฤษภาคม 2554

หลังจากที่มีการเร่งใช้การรักษาด้วยยาต้านไวรัสในผู้ป่วยติดเชื้อเอชไอวี มาเป็นเวลากว่าสิบปี ข้อมูลเกี่ยวกับการดื้อยาปฐมภูมิในผู้ติดเชื้อเอชไอวีก็ยังมีอยู่จำกัด การศึกษานี้มีจุดมุ่งหมายเพื่อประเมินความชุกของการดื้อยาไวรัสแบบปฐมภูมิในประเทศไทยและปัจจัยที่เกี่ยวข้อง การศึกษาเชิงสังเกตแบบไปข้างหน้าได้ทำขึ้นในผู้ติดเชื้อเอชไอวีไทยที่ไม่เคยได้รับยาต้านไวรัสมาก่อน ในช่วงปี พ.ศ. 2550 ถึง พ.ศ. 2553 โดยทำการศึกษาชนิด (subtype) และการกลายพันธุ์ (mutation) ของเอชไอวีด้วยการถอดรหัสพันธุกรรมบริเวณ pol gene ของไวรัสเอชไอวี และวิเคราะห์หาตำแหน่งการกลายพันธุ์ที่แนะนำให้ใช้ในการเฝ้าระวังการดื้อยาต้านไวรัสปฐมภูมิโดยองค์การอนามัยโลก (surveillance drug resistance mutations หรือ SDRMs) การดื้อยาปฐมภูมินิยามโดยการพบ SDRM $\geq$ 1 ตำแหน่ง จากจำนวนผู้ป่วย 466 รายที่มีอายุเฉลี่ย 38.8 ปี ร้อยละ 58.6 เป็นเพศชาย ความเสี่ยงของการติดเชื้อเอชไอวีส่วนใหญ่เป็นแบบรักต่างเพศ (heterosexual) คิดเป็นร้อยละ 77.7 ร้อยละ 16.7 เป็นรักร่วมเพศ (homosexual) และร้อยละ 5.6 ใช้ยาเสพติดชนิดฉีดเข้าเส้น ค่ากลางของระดับ CD4 และปริมาณไวรัส (HIV-1 RNA) คือ 176 เซลล์/ลบ.มม. และ 68,600 ก็อบปี/มล. ตามลำดับ subtype ของเอชไอวีส่วนใหญ่เป็นชนิด CRF01_AE (ร้อยละ 86.9) ที่เหลือเป็นชนิด B (ร้อยละ 8.6) และ recombinant อื่นๆ (ร้อยละ 4.5) ความชุกของการดื้อยาปฐมภูมิคือร้อยละ 4.9 ผู้ติดเชื้อที่มีการดื้อยาปฐมภูมิส่วนใหญ่ (ร้อยละ 73.9) มีการดื้อต่อยาต้านไวรัสเพียงกลุ่ม (class) เดียว ความชุกของการดื้อยาแต่ละกลุ่มพบว่าเท่ากับร้อยละ 1.9, 2.8, และ 1.7 สำหรับยากลุ่ม NRTI, NNRTI, และ PI ตามลำดับ จากการวิเคราะห์แบบ logistic regression พบว่าไม่มีปัจจัยทางคลินิกที่สัมพันธ์กับการดื้อยาปฐมภูมิอย่างมีนัยสำคัญทางสถิติ แต่มีแนวโน้มที่การดื้อยาปฐมภูมิจะพบสูงกว่าในเพศหญิง [odd ratio 2.18; 95% confidence interval, 0.896-5.304; $p$=0.086] โดยสรุปแล้ว การดื้อยาปฐมภูมิอุบัติขึ้นอย่างชัดเจนในประเทศไทยหลังจากที่มีการเร่งใช้การรักษาด้วยยาต้านไวรัสในผู้ป่วยติดเชื้อเอชไอวี มาเป็นเวลากว่าสิบปี ถึงแม้ว่าการตรวจหาการดื้อยาต้านไวรัสทางห้องปฏิบัติการก่อนการเริ่มรักษาด้วยยาต้านไวรัสในผู้ป่วยติดเชื้อเอชไอวีจะไม่เป็นที่แนะนำในประเทศไทย เนื่องจากข้อจำกัดเรื่องทรัพยากร ผลการศึกษานี้ได้แสดงให้เห็นถึงความเสี่ยงในผู้ป่วยติดเชื้อเอชไอวีจำนวนหนึ่งที่อาจประสบปัญหาการรักษาล้มเหลวแต่แรกเริ่ม ควรมีการหาแนวทางในการป้องกันการแพร่เชื้อดื้อยาและการเฝ้าระวังอย่างต่อเนื่อง

**คำหลัก:** เอชไอวี การดื้อยา การกลายพันธุ์ จีโนทัยป์ ประเทศไทย

## Abstract

**Project Code:** RMU5180018

**Project Title:** Evaluation and Monitoring of Primary Drug Resistance in Patients with HIV-1 Infection

**Investigator:** Associate Professor Somnuek Sungkanuparph

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**Project Period:** 15 May 2008 to 14 May 2011

After 10-year rapid scaling up of antiretroviral therapy (ART) in HIV-1-infected patients, the data of primary HIV-1 drug resistance (HIVDR) in Thailand is still limited. This study aims to determine the prevalence and associated factors of primary HIVDR in Thailand. A prospective observational study was conducted among antiretroviral-naïve HIV-1-infected Thai patients from 2007 to 2010. HIV-1 subtypes and mutations were assayed by sequencing a region of HIV-1 pol gene. Surveillance drug resistance mutations (SDRMs) recommended by WHO for surveillance of transmitted HIVDR in 2009 were used in all analyses. Primary HIVDR was defined as the presence of $\geq$ 1 SDRM(s). Of 466 patients with a mean age of 38.8 years, 58.6% of patients were males. Risks of HIV-1 infection included heterosexual (77.7%), homosexual (16.7%), and intravenous drug use (IDU, 5.6%). Median (IQR) CD4 cell count and HIV-1 RNA were 176 (42-317) cells/$mm^3$ and 68,600 (19,515-220,330) copies/mL, respectively. HIV-1 subtypes were CRF01_AE (86.9%), B (8.6), and other recombinants (4.5%). The prevalence of primary HIVDR was 4.9%; most of these (73.9%) had SDRM(s) to only one class of antiretrovirals. The prevalence of patients with NRTI-, NNRTI-, and PI-SDRMs was 1.9%, 2.8%, and 1.7%, respectively. From logistic regression analysis, there was no factor significantly associated with primary HIVDR. There was a trend toward higher prevalence in females [odd ratio 2.18; 95% confidence interval, 0.896-5.304; $p$=0.086]. In conclusion, there is a significant emergence of primary HIVDR in Thailand after a decade of rapid scaling-up of ART in Thailand. Although HIV-1 genotyping prior to ART initiation is not routinely recommended in Thailand, our results raise concerns about the risk of early treatment failure. Interventions to prevent the transmission of HIVDR and continuation of surveillance for primary HIVDR in Thailand are indicated.



**Keywords:** HIV-1; drug resistance; mutations; genotype; Thailand

# กิตติกรรมประกาศ

การวิจัยครั้งนี้ ได้รับทุนสนับสนุนจากสำนักงานกองทุนสนับสนุนการวิจัย (สกว) และสำนักงานคณะกรรมการอุดมศึกษา (สกอ) โดยความร่วมมือของแพทย์และพยาบาลในหน่วยโรคติดเชื้อและระบาดวิทยา ภาควิชาอายุรศาสตร์ และนักวิทยาศาสตร์ในหน่วยไวรัสวิทยาและจุลชีววิทยาโมเลกุล ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล ที่เล็งเห็นความสำคัญของการศึกษาปัญหาการดื้อยาปฐมภูมิในผู้ติดเชื้อเอชไอวีในประเทศไทย เพื่อนำไปเป็นข้อมูล ในการวางแผนแนวทางการดูแลรักษาผู้ติดเชื้อเอชไอวีและผู้ป่วยเอดส์ในประเทศไทยและนำไปสู่การวางแผนเฝ้าระวังการดื้อยาต้านไวรัสปฐมภูมิในระดับชาติ คณะผู้วิจัยขอขอบคุณสำนักงานกองทุนสนับสนุนการวิจัย และสำนักงานคณะกรรมการอุดมศึกษา ที่จัดสรรงบประมาณเพื่อสนับสนุนโครงการวิจัยครั้งนี้ คณะผู้วิจัยขอขอบคุณคณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดลที่สนับสนุนในทุกๆด้าน เพื่อให้คณะผู้วิจัยได้ทำการวิจัยครั้งนี้จนสำเร็จลุล่วงและบรรลุตามวัตถุประสงค์

คณะผู้วิจัยหวังเป็นอย่างยิ่งว่า การวิจัยครั้งนี้ จะเป็นประโยชน์สำหรับแพทย์ พยาบาลและบุคลากรทางสาธารณสุข ในการนำข้อมูลนี้ไปวางแผนการดูแลผู้ติดเชื้อเอชไอวีและผู้ป่วยเอดส์ในระดับท้องถิ่น และเป็นข้อมูลสำหรับหน่วยงานต่างๆที่เกี่ยวข้อง ที่จะได้นำไปเป็นแนวทางในการวางแผนการฝ้าระวังการดื้อยาต้านไวรัสปฐมภูมิในระดับประเทศ และพัฒนาแนวทางการดูแลรักษาผู้ติดเชื้อเอชไอวีและผู้ป่วยเอดส์ในประเทศไทย หากมีข้อผิดพลาดประการใดในการทำวิจัยครั้งนี้ คณะผู้วิจัยขอน้อมรับ เพื่อนำมาพัฒนาต่อไป

รองศาสตราจารย์ นายแพทย์ สมนึก สังฆานุภาพ

๓๐ กันยายน ๒๕๕๔

**หน้าสรุปโครงการ (Executive Summary)**

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**ชื่อโครงการ**

(ภาษาไทย) โครงการประเมินและติดตามเชื้อเอชไอวีดื้อยาปฐมภูมิในผู้ติดเชื้อเอชไอวี

(ภาษาอังกฤษ) Evaluation and Monitoring of Primary Drug Resistance in Patients with HIV-1 Infection

**นักวิจัย:** รองศาสตราจารย์ นายแพทย์ สมนึก สังฆานุภาพ

**สถาบัน:** คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี มหาวิทยาลัยมหิดล

**ระยะเวลาโครงการ:** 15 พฤษภาคม 2551 ถึง 14 พฤษภาคม 2554

**งบประมาณทั้งโครงการ** 1,200,000 บาท (หนึ่งล้านสองแสนบาทถ้วน)

## 1. ความสำคัญ และที่มาของปัญหาที่ทำวิจัย

การรักษาผู้ติดเชื้อเอชไอวีและผู้ป่วยเอดส์ด้วยยาต้านไวรัสในปัจจุบัน สามารถลดปริมาณไวรัสได้อย่างมีประสิทธิภาพ ทำให้อัตราการตายและอัตราการเจ็บป่วยจากโรคติดเชื้อเอชไอวีลดลงอย่างชัดเจน ผู้ป่วยมีคุณภาพชีวิตที่ดีขึ้นและสามารถกลับไปทำงานและดูแลครอบครัวได้ อย่างไรก็ตาม ในกลุ่มประเทศอุตสาหกรรมที่มีการใช้ยาต้านไวรัสมาหลายปีแล้ว มีการตรวจพบเชื้อเอชไอวีดื้อยาต้านไวรัสในผู้ติดเชื้อที่ยังไม่เคยได้รับยามาก่อน (การดื้อยาปฐมภูมิ) ในประเทศสหรัฐอเมริกาและยุโรปมีรายงานความชุกของการเกิดเชื้อดื้อยาปฐมภูมิที่ร้อยละ 4-25 การเกิดเชื้อดื้อยาปฐมภูมินี้จะทำให้การรักษาด้วยยาต้านไวรัสสูตรแรกไม่ได้ผล กระทรวงสาธารณสุขของประเทศสหรัฐอเมริกาและยุโรปจึงได้กำหนดให้มีการตรวจหาการดื้อยาปฐมภูมิในผู้ติดเชื้อที่เพิ่งได้รับเชื้อมาใหม่ทุกราย

การทราบลักษณะการดื้อยาของผู้ติดเชื้อเอชไอวีจะทำให้สามารถเลือกใช้สูตรยาต้านไวรัสที่ได้ผลสำหรับผู้ป่วยแต่ละรายได้ ข้อมูลที่ได้จะมีประโยชน์ในการประเมินการใช้ยาต้านไวรัสสูตรมาตรฐานที่เป็นสูตรแรก และเพื่อเป็นเกณฑ์ในการตัดสินใจว่าควรจะตรวจหาเชื้อดื้อยาก่อนให้ยาต้านเอดส์ในประชากรหรือไม่ นอกจากนี้ ยังสามารถใช้เป็นข้อมูลในการพิจารณายาสำหรับการป้องกันการติดเชื้อจากการปฏิบัติงาน (post-exposure prophylaxis) และการป้องกันการติดเชื้อจากแม่สู่ลูก (vertical transmission prophylaxis) และใช้เป็นข้อมูลในการประเมินและสร้างวิธีป้องกันการแพร่เชื้อเอชไอวีในสังคมได้ องค์การอนามัยโลกได้กำหนดมาตรการในการลดการเกิดเชื้อดื้อยาต้านไวรัสไว้หลายข้อ เช่น ให้ผู้ติดเชื้อสามารถเข้าถึงยาต้านไวรัสเอดส์ได้อย่างทั่วถึง ให้รับประทาน

ยาอย่างสม่ำเสมอ การป้องกันการถ่ายทอดเชื้อไวรัส (ที่อาจดื้อยา) รวมถึงการสุ่มตรวจทางห้องปฏิบัติการในการตรวจหาเชื้อดื้อยาในกลุ่มประชากรที่ติดเชื้อใหม่ เป็นต้น

การตรวจหาเชื้อดื้อยาต้านไวรัสเอดส์ที่ใช้มี 2 วิธีคือ Genotypic HIV Drug Resistance Testing ซึ่งดูที่ความเปลี่ยนแปลงของรหัสพันธุกรรมในส่วนของ Reverse transcriptase (RT) และ Protease (Pr) เมื่อเทียบกับรหัสพันธุกรรมของไวรัสที่ไวยา โดยรายงานผลเป็นตำแหน่งของกรดอะมิโนที่เปลี่ยนไปเมื่อเทียบกับชนิดของยาที่ได้รับ และ Phenotypic HIV Drug Resistance Testing ซึ่งดูความสามารถในการเจริญเพิ่มจำนวนของเชื้อเอชไอวีในหลอดทดลอง ที่ใส่ยาต้านไวรัสที่ความเข้มข้นต่างๆ กัน โดยรายงานผลเป็นความเข้มข้นของยาที่จะยับยั้งการเจริญเติบโตของไวรัสได้ร้อยละ 50 ถ้าต้องใช้ยาที่มีความเข้มข้นเพิ่มขึ้น แสดงว่าไวรัสเกิดดื้อต่อยาแล้ว ซึ่งการทดสอบโดยใช้วิธี Phenotypic HIV Drug Resistance Testing นี้มีวิธีการทำยุ่งยาก ใช้เวลามาก และเสียค่าใช้จ่ายสูงกว่าวิธี Genotypic HIV Drug Resistance Testing องค์การอนามัยโลกจึงแนะนำให้ใช้วิธี Genotypic ในการสำรวจหาเชื้อดื้อยาต้านไวรัสเอดส์ สิ่งส่งตรวจที่ใช้ในการตรวจหาเชื้อดื้อยาต้านไวรัสเอดส์ คือ พลาสมา หรือ ซีรั่ม

อย่างไรก็ตาม การตรวจดูการดื้อยาของเอชไอวีนี้มีราคาแพง จึงไม่สามารถส่งตรวจในผู้ติดเชื้อรายใหม่ได้ทุกรายคนไทย การใช้ยาต้านไวรัสเป็นที่แพร่หลายในประเทศไทยมาประมาณ 5-8 ปีแล้ว ซึ่งเป็นส่วนสำคัญที่ทำให้ผู้ติดเชื้อในประเทศไทยมีอายุยืนยาวขึ้นและมีคุณภาพชีวิตที่ดีขึ้น ทั้งนี้ สำหรับในประเทศไทยยังไม่เคยมีการศึกษาถึงการดื้อยาปฐมภูมิในผู้ติดเชื้อเอชไอวี ทำให้ยังไม่สามารถประเมินความสำคัญและผลกระทบของปัญหาการดื้อยาปฐมภูมิของเชื้อเอชไอวีในผู้ติดเชื้อเอชไอวีในประเทศไทย

## 2. วัตถุประสงค์

<u>วัตถุประสงค์หลัก</u>

1) เพื่อประเมินความชุกของการเกิดเชื้อเอชไอวีดื้อยาปฐมภูมิในผู้ติดเชื้อเอชไอวี ซึ่งเป็นดัชนีที่แสดงถึงการถ่ายทอดเชื้อดื้อยาในประเทศไทยที่มีการใช้ยาต้านไวรัสเพิ่มมากขึ้นอย่างรวดเร็ว

<u>วัตถุประสงค์รอง</u>

1) เพื่อศึกษาปัจจัยเสี่ยงของการมีเชื้อเอชไอวีดื้อยาปฐมภูมิในผู้ติดเชื้อเอชไอวี

2) เพื่อศึกษารูปแบบการดื้อยาปฐมภูมิของเชื้อเอชไอวี

3) เพื่อศึกษาความชุกของการดื้อยาปฐมภูมิในผู้ที่เพิ่งติดเชื้อเอชไอวีมาไม่นาน

4) เพื่อเปรียบเทียบการดื้อยาปฐมภูมิระหว่าง subtype B และ non-B

5) เพื่อศึกษารูปแบบการดื้อยาปฐมภูมิของเชื้อเอชไอวีที่มีผลต่อความไวของยาในกลุ่ม NNRTI ซึ่งได้แก่ efavirenz, nevirapine, etravirine และ rilpivirine ซึ่งเป็นยาใหม่

**3. ระเบียบวิธีวิจัย**

<u>รูปแบบการวิจัย</u>

การศึกษานี้เป็นการศึกษาแบบไปข้างหน้า (prospective cohort) โดยเก็บข้อมูลจากตัวอย่างที่เข้าเกณฑ์ของผู้ติดเชื้อเอชไอวีที่มารับการรักษาที่โรงพยาบาลรามาธิบดี โดยคัดเลือกผู้ติดเชื้อที่เข้าเกณฑ์จำนวน 400 ราย และคาดว่าน่าจะมีผู้เข้าร่วมโครงการปีละ 120-150 ราย ผู้เข้าร่วมโครงการได้รับการติดตามการเกิดเชื้อดื้อยาเป็นเวลา 1-3 ปี โดยจะได้รับการตรวจปริมาณไวรัสในเลือดและการดื้อยาเป็นข้อมูลพื้นฐาน ซึ่งผู้ป่วยจะได้รับการตรวจดังกล่าวเป็นส่วนหนึ่งของการดูแลรักษาที่ผู้ป่วยต้องมาพบแพทย์ตามปกติ

การตรวจการดื้อยา (HIV-1 genotypic resistance assay) จะทำการตรวจที่ห้องปฏิบัติการของหน่วยไวรัสวิทยาและจุลชีววิทยาโมเลกุล ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี โดยถอดรหัสพันธุกรรมของ RT และ Pr gene Sequence ทั้งหมดที่ได้ ข้อมูลทางคลินิกของผู้ติดเชื้อแต่ละรายจะได้รับการรวบรวมเพื่อวิเคราะห์ประเมินผลต่อไป

<u>ประชากรที่ศึกษา</u>

ขนาดประชากรที่จะศึกษาโดยประมาณ 400 ราย ประชากรที่ศึกษาคือผู้ติดเชื้อเอชไอวีที่ไม่เคยได้รับยาต้านไวรัสมาก่อน

<u>เกณฑ์การคัดเลือกผู้ป่วยเข้าร่วมโครงการ</u>

1. ผู้ป่วยยินยอมเข้าร่วมโครงการ และได้ลงชื่อในใบแสดงความยินยอมเพื่อเข้าร่วมในโครงการแล้ว
2. มีหลักฐานการติดเชื้อเอชไอวี
3. อายุมากกว่า 15 ปี
4. ไม่เคยได้รับการรักษาด้วยยาต้านไวรัสมาก่อน.
5. สามารถติดตามการรักษาได้ทุก 3 เดือน

<u>เกณฑ์การคัดเลือกผู้ป่วยออกจากการเข้าโครงการ</u>

1. ผู้ป่วยที่ได้รับการรักษาด้วยยาต้านไวรัสมาก่อน
2. ผู้ป่วยที่เคยได้รับยาป้องการแพร่เชื้อสู่ลูกในระหว่างตั้งครรภ์

<u>ขั้นตอนการปฏิบัติตัวของผู้ป่วยที่เข้าร่วมโครงการ</u>

ผู้ป่วยที่เข้าได้ตามเกณฑ์การคัดเลือกจะได้รับการตรวจมีปริมาณไวรัส และตรวจดูการดื้อยาโดยวิธี genotypic assay ผู้ป่วยต้องมาพบแพทย์โดยสม่ำเสมอเพื่อประเมินอาการทางคลินิกและตรวจปริมาณ CD4 ทุก 3 เดือนจนสิ้นสุดการวิจัย ผู้ป่วยทุกคนจะได้รับการดูแลรักษาตามปกติ

ตลอดโครงการวิจัย ข้อมูลส่วนใหญ่ของการวิจัยนี้จะถูกเก็บรวบรวมอย่างสม่ำเสมอทุกครั้งที่ผู้ป่วยมาพบแพทย์เพื่อติดตามการรักษา

<u>จุดสิ้นสุดการวิจัย</u>

จุดสิ้นสุดการวิจัยหลักคือการลดลงของปริมาณ CD4 จนน้อยกว่าหรือเท่ากับ 250 เซล/มคล. หรือมีอาการเกิดโรคติดเชื้อฉวยโอกาส (อาการแสดงของการติดเชื้อฉวยโอกาสตามการให้นิยามของอาการแสดงของโรคเอดส์โดย CDC 1993) ซึ่งเป็นข้อบ่งชี้ของการเริ่มยาต้านไวรัส จุดสิ้นสุดการวิจัยที่สำคัญอื่น ๆ ได้แก่ การอยู่รอด ระยะเวลาของการเกิดโรคติดเชื้อฉวยโอกาส และการเปลี่ยนแปลงของปริมาณ CD4

<u>การวิเคราะห์ข้อมูล</u>

ความชุกของการเกิดเชื้อเอชไอวีดื้อยาปฐมภูมิในผู้ที่เพิ่งติดเชื้อได้ไม่นานจะวิเคราะห์โดยใช้ความถี่ และร้อยละ 95 ของความเชื่อมั่น (95% confidence interval) ปัจจัยเสี่ยงของเชื้อเอชไอวีดื้อยาปฐมภูมิในผู้ที่เพิ่งติดเชื้อได้ไม่นานจะคำนวณโดยการเปรียบเทียบ odd ratio และ ร้อยละ 95 ของความเชื่อมั่น รูปแบบของการดื้อยาปฐมภูมิของเชื้อเอชไอวีจะวิเคราะห์โดยใช้ความถี่ และร้อยละ 95 ของความเชื่อมั่นของการกลายพันธุ์ที่สัมพันธ์กับการดื้อยา (drug resistance mutation)

<u>ตารางการวิจัย</u>

| นัดการตรวจในส่วนของโครงการ* | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| | วันตรวจคัดกรอง วันตรวจคัดกรองและวันที่เข้าโครงการวิจัย | เดือนที่ 12 | เดือนที่ 24 | เดือนที่ 36 |
| ลงชื่อในหนังสือยินยอมเข้าโครงการ | X | | | |
| เกณฑ์คัดเลือกผู้ป่วย (Inclusion/Exclusion criteria) | X | | | |
| ซักประวัติผู้ป่วย | X | X | X | X |
| ตรวจร่างกาย | X | X | X | X |
| ตรวจวัดระดับ HIV RNA | X | | | |
| ตรวจการดื้อยาของเชื้อไวรัส | X | | | |

*ประเมินทางคลินิกและตรวจปริมาณ CD4 ทุก 3 เดือน ตามการดูแลรักษาตามปกติ

**4. แผนการดำเนินงานวิจัย**

| แผนงาน | ระยะเวลาดำเนินงาน (เดือน) | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 6 | 12 | 18 | 24 | 30 | 36 | 38 |
| 1. เตรียมโครงร่างวิจัย | ■ | | | | | | | | | |
| 2. เสนอโครงร่างวิจัยต่อคณะกรรมการจริยธรรม | ■ | | | | | | | | | |
| 3. ขอทุนวิจัยจากสกว. | | ■ | | | | | | | | |
| 4. ดำเนินการวิจัย | | | ■ | ■ | ■ | ■ | ■ | ■ | ■ | |
| 5. วิเคราะห์ข้อมูลเบื้องต้น | | | | | ■ | | | | | |
| 6. วิเคราะห์ข้อมูลทั้งหมด | | | | | | | | | ■ | |
| 7. เตรียมต้นฉบับและส่งตีพิมพ์ข้อมูลทั้งหมด | | | | | | | | | | ■ |

**5. ชื่อเรื่องและชื่อวารสารที่คาดว่าจะตีพิมพ์**

HIV-1 Drug Resistance Mutations among Antiretroviral-naïve HIV-1-infected Patients in Thailand

วางแผนที่จะตีพิมพ์ในวารสาร Journal of International AIDS Society

**6. รายชื่อและสังกัดของผู้ร่วมวิจัย**

(1) รองศาสตราจารย์นายแพทย์สมนึก สังฆานุภาพ หัวหน้าโครงการวิจัย
หน่วยโรคติดเชื้อ ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี

(2) ผู้ช่วยศาสตราจารย์แพทย์หญิงศศิโสภิณ เกียรติบูรณกุล
หน่วยโรคติดเชื้อ ภาควิชาอายุรศาสตร์ คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี

(3) ศาสตราจารย์ ดร.วสันต์ จันทราทิตย์
หน่วยไวรัสวิทยาและชีววิทยาโมเลกุล ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี

(4) ดร.ชลภัทร สุขเกษม
หน่วยไวรัสวิทยาและชีววิทยาโมเลกุล ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์โรงพยาบาลรามาธิบดี

(5) ดร.เอกวัฒน์ ผสมทรัพย์

หน่วยไวรัสวิทยาและชีววิทยาโมเลกุล ภาควิชาพยาธิวิทยา คณะแพทยศาสตร์ โรงพยาบาลรามาธิบดี

**7. งบประมาณโครงการ**

| หมวดงบประมาณ | ปีที่ 1 | ปีที่ 2 | ปีที่ 3 | รวม |
|---|---|---|---|---|
| 1. หมวดค่าตอบแทน | 180,000 | 180,000 | 180,000 | 540,000 |
| 2. หมวดค่าจ้าง | - | - | - | - |
| 3. หมวดค่าวัสดุการตรวจเลือดของผู้ป่วย | | | | |
| - HIV-1 RNA | 36,000 | 36,000 | 36,000 | 108,000 |
| - HIV-1 Genotype | 180,000 | 180,000 | 180,000 | 540,000 |
| 4. หมวดค่าใช้สอย | | | | |
| - ค่าจัดพิมพ์รายงาน | 500 | 500 | 500 | 1500 |
| - ค่าสืบค้นข้อมูล | 400 | 400 | 400 | 1200 |
| - ค่าวิเคราะห์ทางสถิติ | 3000 | 3000 | 3000 | 9000 |
| - ค่าถ่ายเอกสาร | 100 | 100 | 100 | 300 |
| รวมงบประมาณโครงการ | 400,000 | 400,000 | 400,000 | 1,200,000 |

## 8. ผลการศึกษา

ผลการศึกษาเรื่อง “Emergence of HIV-1 Drug Resistance Mutations among Antiretroviral-naïve HIV-1-infected Patients in Thailand After a Decade of Scaling-up Antiretroviral Therapy”

(เอกสารหน้าถัดไป)

**Emergence of HIV-1 Drug Resistance Mutations among Antiretroviral-naïve HIV-1-infected Patients in Thailand After a Decade of Scaling-up Antiretroviral Therapy**


**Abstract**

**Background:** After 10-year rapid scaling up of antiretroviral therapy (ART) in HIV-1-infected patients, the data of primary HIV-1 drug resistance (HIVDR) in Thailand is still limited. This study aims to determine the prevalence and associated factors of primary HIVDR in Thailand.

**Methods:** A prospective observational study was conducted among antiretroviral-naïve HIV-1-infected Thai patients from 2007 to 2010. HIV-1 subtypes and mutations were assayed by sequencing a region of HIV-1 pol gene. Surveillance drug resistance mutations (SDRMs) recommended by WHO for surveillance of transmitted HIVDR in 2009 were used in all analyses. Primary HIVDR was defined as the presence of $\geq$1 SDRM(s).

**Results:** Of 466 patients with a mean age of 38.8 years, 58.6% of patients were males. Risks of HIV-1 infection included heterosexual (77.7%), homosexual (16.7%), and intravenous drug use (IDU, 5.6%). Median (IQR) CD4 cell count and HIV-1 RNA were 176 (42-317) cells/$mm^3$ and 68,600 (19,515-220,330) copies/mL, respectively. HIV-1 subtypes were CRF01_AE (86.9%), B (8.6), and other recombinants (4.5%). The prevalence of primary HIVDR was 4.9%; most of these (73.9%) had SDRM(s) to only one class of antiretrovirals. The prevalence of patients with NRTI-, NNRTI-, and PI-SDRMs was 1.9%, 2.8%, and 1.7%, respectively. From logistic regression analysis, there was no factor significantly associated with primary HIVDR. There was a trend toward higher prevalence in females [odd ratio 2.18; 95% confidence interval, 0.896-5.304; $p$=0.086].

**Conclusion:** There is a significant emergence of primary HIVDR in Thailand after a decade of rapid scaling-up of ART in Thailand. Although HIV-1 genotyping prior to ART initiation is not routinely recommended in Thailand, our results raise concerns about the risk of early treatment failure. Interventions to prevent the transmission of HIVDR and continuation of surveillance for primary HIVDR in Thailand are indicated.

## Introduction

In Thailand, the disease burden from HIV/AIDS resulting from the epidemic in the 1990s remains high [1]. Although the change in the incidence rate of HIV infection in Thailand, from 2001 to 2009, is over 25% decreasing [2], the accumulated number of HIV-infected persons is still high. Currently, an estimated number of 530,000 people are living with HIV Thailand [2]. Combination antiretroviral therapy (ART) has significantly reduced mortality and morbidity since its introduction in Thailand [3-5]. Since 2001, the government committed to providing ART free of charge to people living with HIV under the National Access to Antiretroviral treatment Program for People Living with HIV/AIDS (NAPHA) [6]. The subsequent production and use of generic drugs led to more than an eight-fold expansion in treatment provision between 2001 and 2003 [7]. Since 2006, NAPHA has been transformed to National AIDS Program (NAP) under the management of National Health Security Office with the rapid growing of the program. According to UNAIDS 2010 report, the number of people receiving ART in December 2009 is 216,118 persons and life years among adults gained due to ART between 1996 and 2009 is 389,000 [2]

Despite these successes, HIV-1 drug resistance (HIVDR) is a major reason for treatment failure during rapid scaling up of ART in Thailand [8,9]. Approximately 5% to 10% of patients receiving ART have experienced treatment failure and HIVDR [10]. Recently, a study in Thailand had demonstrated the transmission of HIVDR in antiretroviral-naïve HIV-1-infected patients in Thailand [11]. This threatens the effectiveness of rapidly scaled up first-line ART in the country. Primary HIVDR means increase resistance of HIV-1 to antiretroviral drugs seen in individuals who have never received ART and presumably have been infected with drug-resistant virus [12]. The prevalence of primary HIVDR has been well reported in the United States and Europe, ranged from 6.2% to 21% [13-16]. A study in Asia has recently reported the prevalence of primary HIVDR at 13.8% [17]. In resource-limited settings where ART is being scaled-up, the World Health Organization (WHO) recommends the surveillance of primary HIVDR [18]. To date, after a decade of ART scaling up, there is limited published information regarding primary HIVDR in Thailand. This study was aimed to determine the prevalence of HIVDR and associated factors among antiretroviral-naïve patients in Thailand.

## Methods

A cross-sectional study was conducted among antiretroviral-naïve HIV-1-infected patients who newly visited an infectious disease clinic in a university hospital between January 2007 and December 2010. Patients with a history of any exposure to antiretroviral drugs including mono- or dual-therapy, or prevention of mother-to-child-transmission were excluded. Ethics approvals were obtained from local institutional review boards. Informed consent was obtained prior to genotypic resistance testing.

All plasma samples, HIV-1 pol nucleotide sequencing of reverse transcriptase and protease region was carried out using TRUGENE HIV-1 Genotypic Assay in conjunction with the Open Gene automated DNA sequencing system (Visible Genetics, Toronto, Canada). Testing involved simultaneous clip sequencing of protease and codons 35-244 of the RT from the amplified cDNA in both the 3´ and 5´ directions. Sequences were aligned and compared with a lymphoadenopathy-associated virus type 1 (HIV-B-LAV1) consensus sequence using Visible Genetics Gene Librarian software [19,20]. Surveillance drug resistance mutations (SDRMs) recommended by WHO for surveillance of transmitted HIVDR in 2009 [21] were used in all analyses. HIVDR in a patient was defined as the presence of at least one SDRM.

Mean (± standard deviation, SD), median (interquartile range, IQR) and frequencies (%) were used to describe patients' characteristics. Categorical variables between the two groups were compared using Chi square or Fisher's exact test as appropriate. Continuous variables between the two groups were compared using Student's t test and Mann-Whitney U test as appropriate. Logistic regression analysis was used to determine factors associated with HIVDR. A *p*-value at $<0.05$ was considered as statistically significant. All analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, Illinois, U.S.A).

## Results

A total of 466 patients were included in this analysis. The mean (SD) age was 38.8 (11.4) years. Two hundred and sixty-three (58.6%) patients were males. Risks of HIV-1 infection were heterosexual (77.7%), homosexual (16.7%), and intravenous drug use (IDU, 5.6%). Forty-six (9.9%) and 32 (6.9%) patients had co-infection of hepatitis B virus and hepatitis C virus, respectively. Median (IQR) CD4 cell count and HIV-1 RNA were 176 (42-317) cells/$mm^3$ and 68,600 (19,515-220,330) copies/mL, respectively. Of 466 patients, 405

(86.9%) were infected with HIV-1 subtype CRF01_AE. Subtype B was found in 40 (8.6) patients. Other subtypes (4.5%) were CRF07_BC, CRF03_AB, CRF02_AG, CRF12_BF, D, and K.

The prevalence of primary HIVDR was 4.9%. The prevalence of patients with nucleoside reverse transcriptase inhibitor (NRTI)-, non nucleoside reverse transcriptase inhibitor (NNRTI)-, and protease inhibitor (PI)-SDRMs was 1.9%, 2.8%, and 1.7%, respectively. Seventeen (3.8%) patients had SDRM(s) to only one class of antiretroviral drugs. Five (1.1%) patients had both NRTI- and NNRTI-SDRMs. Only one patient had SDRMs to 3 classes of antiretroviral drugs. Table 1 shows SDRMs observed in 23 patients with HIVDR. The comparison of characteristics between patients with and without HIVDR is summarized in Table 2. From logistic regression analysis, there was no factor significantly associated with HIVDR. There was a trend toward higher prevalence in females [OR=2.18; 95%CI, 0.896-5.304; *p*=0.086].

**Discussion**

Primary HIVDR represents a challenge for the treatment of HIV-1 infection because it can reduce the efficacy of first line antiretroviral therapy and may impact clinical outcomes. Emergence of primary HIVDR in resource-limited settings is a concerning consequence of global scaleing-up of ART. It will be seen first in the region where ART has been widely available for years [18]. After a decade of rapid scaling-up of ART in Thailand, primary HIV is inevitably anticipated.

The results from the present study have demonstrated that there is an emergence of primary HIV in Thailand. The prevalence is as high as 4.9% and approaching WHO's first threshold (5%) of transmitted HIV. Blower's model had previously predicted that transmitted HIVDR will reach 5% after approximately 10 years of ART scaling-up [18,22]. Although the term 'transmitted HIVDR' is generally applied only to HIVDR detected in recently infected individuals, the prevalence of primary HIVDR among patients with chronic HIV-1 infection may be even underestimated. Thus, the results from the present study provide data about the likely efficacy of first-line ART in Thailand. For instance, about 5% of patients initiating first-line ART regimen, which is NNRTI-based regimens, in Thailand may have early treatment failure. Although most of the patients (3.8%) with primary HIVDR had SDRMs to only one class of antiretroviral drugs, the treatment response can be markedly reduced. NNRTI-based regimens generally have low genetic barrier for development of

resistance and early treatment failure is likely if the regimen does not consist of 3 fully active drugs [23,24].

Recently, various multicenter cohort studies have demonstrated that primary HIVDR is associated with poor treatment outcomes and/or clinical complication [25-27]. They all support the use of genotypic resistance test prior to initiation of ART. Since 1998, the International AIDS Society--USA Panel had suggested considering resistance testing for antiretroviral-naïve patients in areas with a prevalence of resistance of $\geq$5% [28]. However, a cost-effectiveness study of genotypic resistance testing for antiretroviral-naïve patients with chronic HIV-1 infection has reported that it is cost-effective if the prevalence of primary HIVDR is >1%.

Thailand is an area with predominance of HIV-1 subtype CRF01_AE. Although the prevalence of HIVDR in patients with subtype CRF01_AE is twice of that in patients with subtype B (5.2% vs. 2.5%), there was no statistically significant difference. There were no significant differences in demographic or clinical factors between those with/without primary HIVDR. There was only a trend toward higher prevalence in females from multivariate analysis. Therefore, there is no risk group to consider genotypic testing for primary HIV in Thailand. As ART continues to be scaled-up rapidly, it is likely that the prevalence of primary HIV continues to increase. It's a national priority to intervene with the intervention to prevent further transmission of HIVDR. To minimize primary HIVDR in Thailand, strengthening of health care system, supporting adherence to therapy, and ensuring a continuous supply of antiretroviral drugs are crucial. At some point, the National AIDS Program in Thailand has to carefully consider the advantages and disadvantages of genotypic testing for primary HIVDR and decide when and how to implement. The future plans have to include the strategies to make genotypic testing more accessible with the newer technologies, such as point mutation assays or short sequencing of some specific regions of RT gene.

There are some limitations in the present study. Although the patients in the present study were those who newly presented to the infectious disease clinic, some patients presented late. They were tested for HIV-1 genotypes at the stage of chronic infection. Some resistance mutations may have reverted to wild type. Thus, the prevalence of primary HIVDR could be underestimated. However, transmitted HIVDR among antiretroviral-naïve patients has been reported to be persistent, ranged from 4 years to longer than the lifetime of the patient [30].The prolonged persistence of transmitted HIVDR strongly supports the use of genotypic resistance test in newly presented patients.

In summary, primary HIVDR is emerging in Thailand after a decade of rapid scaling-up of ART. Although HIV-1 genotyping prior to ART initiation is not routinely recommended in Thailand, our results raise concerns about the risk of early treatment failure. Interventions to prevent the transmission of HIVDR and continuation of surveillance for primary HIVDR in Thailand are indicated.

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Table 1. Distribution of SDRMs in 23 patients with primary HIVDR*

| SDRMs | Number of patients | Prevalence (%) |
|---|---|---|
| NRTI-SDRMs | 9 | 1.9 |
| M41L | 3 | 0.6 |
| K65R | 1 | 0.2 |
| D67N | 1 | 0.2 |
| T69D | 1 | 0.2 |
| V75M | 1 | 0.2 |
| M184V | 3 | 0.6 |
| M184I | 1 | 0.2 |
| L210W | 1 | 0.2 |
| T215Y | 1 | 0.2 |
| T215S | 1 | 0.2 |
| K219Q | 1 | 0.2 |
| K219R | 1 | 0.2 |
| NNRTI-SDRMs | 13 | 2.8 |
| K101E | 1 | 0.2 |
| K103N | 3 | 0.6 |
| K103S | 1 | 0.2 |
| V106A | 1 | 0.2 |
| V106M | 1 | 0.2 |
| Y181C | 4 | 0.9 |
| Y181I | 1 | 0.2 |
| Y188L | 1 | 0.2 |
| G190S | 1 | 0.2 |
| PI-SDRMs | 8 | 1.7 |
| M46I | 1 | 0.2 |
| M46L | 1 | 0.2 |
| I47V | 1 | 0.2 |
| G48M | 1 | 0.2 |
| I54L | 1 | 0.2 |
| I54T | 1 | 0.2 |
| I84A | 1 | 0.2 |
| L90M | 6 | 1.3 |

Table 2. Comparison of characteristics between patients with and without primary HIVDR

| Characteristics | Primary HIVDR | | *P* value |
|---|---|---|---|
| | Yes *(n=23)* | No *(n=443)* | |
| Age, years, mean ± SD | 37.3 (7.9) | 38.8 (11.5) | 0.517 |
| Male gender, number (%) | 9 (60.9) | 264 (59.6) | 0.080 |
| Risk of HIV-1 infection, number (%) | | | 0.489 |
| Heterosexual | 19 (82.6) | 343 (77.4) | |
| Homosexual | 2 (8.7) | 76 (17.2) | |
| IVDU | 2 (8.7) | 24 (5.4) | |
| HBV co-infection, number (%) | 2 (8.7) | 44 (9.9) | 0.579 |
| HCV co-infection, number (%) | 2 (8.7) | 30 (6.8) | 0.326 |
| CD4, cells/$mm^3$, median (IQR) | 197 (35-307) | 173 (43-318) | 0.784 |
| HIV-1 RNA, log copies/mL, median (IQR) | 29,600 (3,580-214,000) | 70,150 (20,490-220,740) | 0.271 |
| HIV-1 subtypes, number (%) | | | 0.551 |
| CRF01_AE | 21 (91.4) | 384 (86.7) | |
| B | 1 (4.3) | 39 (8.8) | |
| Others* | 1 (4.3) | 20 (4.5) | |

IVDU=intravenous drug use

*including CRF07_BC, CRF03_AB, CRF02_AG, CRF12_BF, D, and K

8.2 ผลการศึกษาเรื่อง “Surveillance of Transmitted HIV Drug Resistance in Antiretroviral-naïve Patients Aged <25 Years in Bangkok, Thailand”
(เอกสารหน้าถัดไป)

**Surveillance of Transmitted HIV Drug Resistance in Antiretroviral-naïve Patients Aged <25 Years in Bangkok, Thailand**


**Abstract**

Emergence of transmitted HIV resistance (TDR) is a concern after global scale-up of antiretroviral therapy (ART). WHO had developed threshold survey method for surveillance of TDR in resource-limited countries. ART in Thailand has been scaling up for 10 years. To evaluate the current TDR in Thailand, a cross-sectional study was conducted among antiretroviral-naïve HIV-infected patients aged <25 years who newly visited an infectious disease clinic in a university hospital. HIV genotypic resistance test was performed. WHO 2009 surveillance drug resistance mutations (SDRMs) were used to define TDR. Of 49 patients, the prevalence of TDR was 4.1%. Of 2 patients with TDR, one had K103N and the other had Y181I mutation. TDR is emerging in Thailand after a decade of rapid scale-up of ART. Interventions to prevent the transmission of drug resistant virus in population level are essentially needed in Thailand. Surveillance for TDR in Thailand has to be regularly performed.

## Introduction

Emergence of transmitted HIV resistance (TDR) is a concern after global scale-up of antiretroviral therapy (ART). TDR is associated with poor treatment outcomes and/or clinical complication [1-5]. TDR is anticipated in the areas where ART has been widely available for years. The prevalence of TDR has been reported in the United States and Europe, ranged from 6.2% to 21% [6-9]. In resource-limited settings, TDF has been reported from sub-Saharan Africa after scale-up of ART and showed the prevalence from 3.5% to 11.6%, depended on the countries [10]. The higher prevalence of TDR in Uganda than in other African countries is probably related to the earlier start of ART scale-up in Uganda. In the countries scaling up ART, the World Health Organization (WHO) recommends the surveillance of TDR [11]. The WHO HIV drug resistance threshold survey method had been developed for surveillance of TDR in resource-limited countries. To minimize costs, WHO suggests that each survey requires < or =47 specimens from individuals consecutively diagnosed with HIV to categorize resistance to each relevant drug class as <5%, 5-15% or >15% [11].

In Thailand, ART has reduced mortality and morbidity since its introduction in the country [12-14]. Scaling up of ART has started in since 2001 and the National AIDS Program (NAP) continues to expand. According to UNAIDS 2010 report, the number of people receiving ART in December 2009 is 216,118 persons and life years among adults gained due to ART between 1996 and 2009 is 389,000 [15]. The first threshold survey in Thailand had been done in Bangkok, involving blood donors and counseling and testing centre (VCT) clients during 2005-2006 [16]. The findings showed no mutations associated with TDR. Recently, a multinational study in Asia including Thailand has demonstrated the prevalence of primary HIV drug resistance among antiretroviral naïve patients at 13.8% [17]. Although this study did not use surveillance drug resistance mutations (SDRMs) recommended by WHO for surveillance of TDR [18], it bring to a concern of TDR in Thailand. To evaluate the current situation of TDR in Thailand, especially in the patient care center, this study was conducted using WHO threshold survey for resource-limited settings.

## Methods

A cross-sectional study was conducted among antiretroviral-naïve HIV-infected patients who newly visited an infectious disease clinic in a university hospital between

January 2007 and December 2010. According to WHO threshold survey methods to minimize inclusion of ARV-experienced individuals and individuals infected before ART was available [11], the patients eligibility criteria included 1) laboratory confirmation of HIV infection, 2) age <25 years at HIV diagnosis, and 3) if female, no previous pregnancy. Eligible patients were consecutively enrolled from an infectious clinic during the study period. Ethics approvals were obtained from local institutional review boards. Informed consent was obtained prior to genotypic resistance testing.

All plasma samples, HIV pol nucleotide sequencing of reverse transcriptase and protease region was carried out using TRUGENE HIV Genotypic Assay in conjunction with the Open Gene automated DNA sequencing system (Visible Genetics, Toronto, Canada). Testing involved simultaneous clip sequencing of protease and codons 35-244 of the reverse transcriptase from the amplified cDNA in both the 3´ and 5´ directions. Sequences were aligned and compared with a lymphoadenopathy-associated virus type 1 (HIV-B-LAV1) consensus sequence using Visible Genetics Gene Librarian software [19,20]. SDRMs recommended by WHO for surveillance of TDR in 2009 [18] were used in all analyses. TDR in a patient was defined as the presence of at least one SDRM.

**Results**

A total of 49 patients were included in this analysis. The mean (SD) age was 21.7 (3.8) years. Thirty (61.2%) patients were males. Risks of HIV infection were heterosexual (65.3%), homosexual (30.6%), and intravenous drug use (IDU, 4.1%). Median (range) CD4 cell count and HIV RNA were 214 (10-782) cells/$mm^3$ and 68,150 (1,023->1,000,000) copies/mL, respectively. Of 49 patients, 39 (79.6%) were infected with HIV subtype CRF01_AE. Other subtypes were B (12.2%), CRF07_BC (4.1%), CRF12_BF (2.0%), and K (2.0%).

The prevalence of TDR was 4.1%. Of these two patients with TDR, both had only one non nucleoside reverse transcriptase inhibitor (NNRTI)-SDRM; one had K103N and the other had Y181I mutation. Nucleoside reverse transcriptase inhibitor (NRTI)- and protease inhibitor (PI)-SDRMs were not observed in this study. Both patients were males; one was heterosexual and the other was homosexual. They aged 23.4 and 23.9 years old. CD4 cell counts and HIV RNA of these two patients were 22 and 408 cells/$mm^3$, and 1,700 and 176,950 copes/mL, respectively.

## Discussion

Surveillance of TDR can support implementation of prevention measures on a population level. TDR represents a challenge for the treatment of HIV infection because it can reduce the efficacy of first-line ART and impact clinical outcomes [1-5]. After a decade of rapid scale-up of ART in Thailand, TDR is inevitably anticipated. The results from the present study have demonstrated that there is an emergence of TDR in Thailand. Although the prevalence is less than WHO lower threshold (5%), it raises a concern of HIV care in Thailand. Although patients with TDR had SDRMs to only NNRTI, the treatment response can be markedly impacted. ART regimens in resource-limited settings are usually selected at the national level following a public health approach. In Thailand, the national guidelines recommend using NNRTI-based regimens as the first-line ART [21]. NNRTI-based regimens generally have low genetic barrier for development of resistance and early treatment failure is likely if the regimen does not consist of three fully active drugs [22]. Although we cannot demonstrate how our patients acquired drug resistance mutations in this study, it is likely that sexual transmission from their treatment-experienced partners may be the case. Our previous study has shown that Y181C/I and K103N are common drug resistance mutations after failing the first-line ART in Thailand [23]. To minimize TDR in Thailand, strengthening of HIV care system, supporting patient's adherence to therapy, and prevention of HIV transmission in both HIV-infected and –uninfected individuals are crucial. Regular surveillance of TDR in Thailand will inform evidence-based decision making regarding national AIDS program.

There are some limitations in the present study. Although we followed the mandatory criteria of participant eligibility for WHO threshold survey, it appears that many patients with age <25 years old does not accurately predict recent HIV infection. Many patients in the present study had low CD4 cell counts and may indeed have chronic HIV infection. Patients in resource-limited setting tend to present to the health care system late. In addition, a recent study also demonstrated that HIV drug resistance in antiretroviral-naïve patients aged >25 years is dramatically high [24]. Although mandatory criteria of 'age < 25 years' suggested by WHO is practical for resource-limited settings to follow, it may not be accurate. Nevertheless, TDR among antiretroviral-naïve patients has been reported to be persistent, ranged from 4 years to longer than the lifetime of the patient [25]. There is a value of study TDR even in chronic HIV infection, with a caution of possible underestimation.

**Conclusion**

TDR is emerging in Thailand after a decade of rapid scale-up of ART. Interventions to prevent the development of HIV drug resistance among treated patients and to prevent transmission of drug resistant virus are essentially needed in Thailand. To inform the national policy for HIV care, surveillance for TDR in Thailand has to be regularly performed.

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## 9. Output ที่ได้จากโครงการ

9.1 Sungkanuparph S, Sukasem C, Kiertiburanakul S, Piyavong B, Chantratita W. Emergence and risk factors of HIV-1 drug resistance in antiretroviral-naïve patients in Thailand. The 47th Annual Meeting of Infectious Disease Society of America (IDSA), Philadelphia, USA, 2009. P 265. [Poster presentation]

9.2 Sungkanuparph S, Sukasem C, Kiertiburanakul S, Chantratita W. Surveillance of transmitted HIV-1 drug resistance in Thailand: A four-year study. The 48th Annual Meeting of Infectious Disease Society of America (IDSA), Vancouver, Canada, 2010. P 1107. [Poster presentation]

9.3 Sungkanuparph S, Sukasem C, Kiertiburanakul S, Pasomsub E, Chantratita W. Emergence of HIV-1 Drug Resistance Mutations among Antiretroviral-naïve HIV-1-infected Patients in Thailand After a Decade of Scaling-up Antiretroviral Therapy. Submitted to *Journal of International AIDS Society* - status under reviewed

9.4 Sungkanuparph S, Pasomsub E, Chantratita W. Surveillance of Transmitted HIV Drug Resistance in Antiretroviral-naïve Patients Aged <25 Years in Bangkok, Thailand. Submitted to *AIDS Research and Treatment* - status under reviewed.

9.5 Sungkanuparph S, Pasomsub E, Kiertiburanakul S, Chantratita W. Etravirine Resistance-Associated Mutations in Antiretroviral-Naïve Patients Infected with HIV-1 Subtype CRF01_AE versus Subtype B. Submitted to *Conference of Retroviruses and Opportunistic Infection* - status under reviewed.

9.6 Sungkanuparph S, Kiertiburanakul S, Pasomsub E, Chantratita W. Resistance-Associated Mutations to Efavirenz, Etravirine, Nevirapine, and Rilpivirine among Antiretroviral-Naïve HIV-1-Infected Patients in Thailand. Submitted to *Conference of Retroviruses and Opportunistic Infection* - status under reviewed.

9.7 Sungkanuparph S, Kiertiburanakul S, Sukasem C, Chantratita W. Discrepancies between WHO 2009 and IAS-USA 2009 lists for determining the rate of transmitted HIV-1 drug resistance: A prospective study. *Journal of AIDS 2011* - status accepted and proof available.

**10. ภาคผนวก (Reprint จากวารสารทางการแพทย์ และ Abstract จากงานประชุมวิชาการ)**

10.1 Sungkanuparph S, Sukasem C, Kiertiburanakul S, Piyavong B, Chantratita W. Emergence and risk factors of HIV-1 drug resistance in antiretroviral-naïve patients in Thailand. The 47th Annual Meeting of Infectious Disease Society of America (IDSA), Philadelphia, USA, 2009. P 265.

# Emergence and Risk Factors of HIV-1 Drug Resistance in Antiretroviral-naïve Patients in Thailand

Somnuek Sungkanuparph[1*], Chonlaphat Sukasem[2], Sasisopin Kiertiburanakul[1], Bucha Piyavong[1], Wasun Chantratita[2]

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Poster 265

## BACKGROUND

HIV-1 infection is still a major health problem in Thailand. To date, up to 600,000 Thais are living with HIV. Antiretroviral therapy (ART) has decreased the rate of morbidity and mortality and increased the quality of life in HIV-1 infected persons, both in resource-rich and resource-limited settings. ART has been rapidly scaled up in HIV-1 infected Thai patients for more than 7 years. The data of prevalence and risk factors of primary drug resistance in Thailand is limited. This study aimed to determine the prevalence and risk factors of primary HIV-1 drug resistance in Thailand.

## METHODS

A prospective cohort study was conducted among antiretroviral-naïve Thai patients who visit Ramathibodi Hospital, a 1200-bed university hospital between 1st January 2007 and 30th December 2008. HIV-1 subtypes and HIV-1 mutations were assayed by sequencing a region of HIV-1 pol gene. We focused at the surveillance drug resistance mutations (SDRMs) recommended for surveillance of transmitted HIV-1 drug resistance by WHO in 2009 (Table 1). The prevalence and risk factors of primary drug resistance were determined using multivariate analysis.

**Table 1.** Surveillance drug resistance mutations recommended for surveillance of transmitted HIV-1 drug resistance by WHO 2009

HIV-1 Protease and Reverse Transcriptase Mutations For Drug Resistance Surveillance (2009)

| NRTI | | NNRTI | | PI | |
|---|---|---|---|---|---|
| Pos | Mut | Pos | Mut | Pos | Mut |
| M41 | L | L100 | I | L23 | I |
| K65 | R | K101 | E, P | L24 | I |
| D67 | N, G, E | K103 | N, S | D30 | N |
| T69 | D, Ins | V106 | M, A | V32 | I |
| K70 | R, E | **V179** | **F** | M46 | I, L |
| L74 | V, I | Y181 | C, I, V | I47 | V, A |
| V75 | M, T, A, S | Y188 | L, H, C | G48 | V, M |
| F77 | L | G190 | A, S, E | I50 | V, L |
| Y115 | F | P225 | H | F53 | L, Y |
| F116 | Y | M230 | L | I54 | V, L, M, A, T, S |
| Q151 | M | | | G73 | S, T, C, A |
| M184 | V, I | | | **L76** | **V** |
| L210 | W | | | V82 | A, T, F, S, C, M, L |
| T215 | Y, F, I, S, C, D, V, E | | | **N83** | **D** |
| K219 | Q, E, N, R | | | I84 | V, A, C |
| | | | | **I85** | **V** |
| | | | | N88 | D, S |
| | | | | L90 | M |

New mutations are in **bold**

## RESULTS

A total of 266 patients with a mean ±SD age of 38.1 ±10.6 years and 54.9% male were studied. Risks of HIV-1 infection included heterosexual (83.5%), homosexual (10.9%), and intravenous drug use (IVDU, 5.6%). Of all, 85% had HIV subtype A/E; 9.4% and 6.0% had HBV and HCV co-infection, respectively. Median (range) CD4 cell count and HIV-1 RNA were 218 (2-1877) cells/mm$^3$ and 4.89 (2.46-7.86) log copies/mL, respectively.

The prevalence of patients with ≥1 SDRM in all patients, patients who were heterosexual, homosexual, and IVDU were 5.3%, 4.5%, 3.4%, and 20.0%, respectively (Figure 1). Regarding drug class resistance, 3.4%, 2.3%, and 0.8% of patients had mutations contributing to NRTI-, NNRT-, and PI-resistance, respectively (Figure 2); only 1.2% had drug resistance in two classes.

**Figure 1.** Prevalence of patients with ≥1 SDRM in all and subgroup.

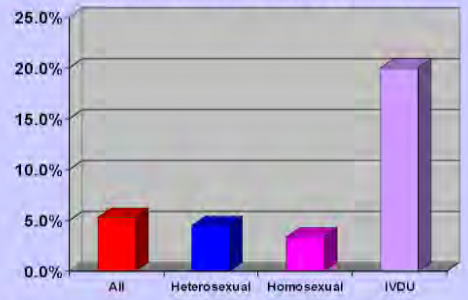


**Figure 2.** Prevalence of patients with drug class resistance.

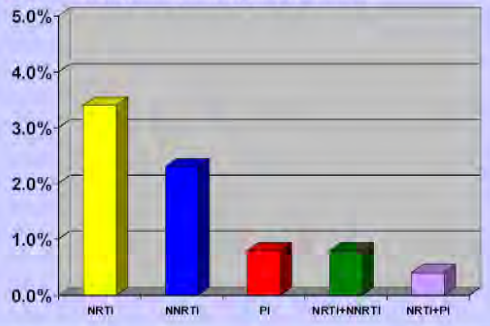


**Table 2.** Clinical characteristics of patients with and without SRDMs

| Characteristics | Total (n=266) | Presence of SRDMs | | P value |
|---|---|---|---|---|
| | | Yes (n=14) | No (n=252) | |
| Age, years, mean ± SD | 38.1 ± 10.6 | 33.9 ± 8.5 | 38.4 ± 10.7 | 0.122 |
| Male gender, number (%) | 146 (54.9) | 6 (42.9) | 140 (55.6) | 0.413 |
| Risk of HIV-1 infection, number (%) | | | | 0.001 |
| Homosexual | 29 (10.9) | 1 (7.1) | 28 (11.1) | |
| Heterosexual | 222 (83.5) | 10 (71.5) | 212 (84.1) | |
| IVDU | 15 (5.6) | 3 (21.4) | 12 (4.8) | |
| HBV co-infection, number (%) | 25 (9.4) | 1 (7.1) | 24 (9.5) | 0.947 |
| HCV co-infection, number (%) | 16 (6.0) | 2 (14.3) | 14 (5.6) | 0.469 |
| CD4, cells/mm$^3$, median (range) | 218 (2-1877) | 109 (13-464) | 220 (2-1877) | 0.399 |
| HIV-1 RNA, log copies/mL, median (range) | 4.89 (2.46-7.86) | 4.39 (3.26-4.94) | 4.91 (2.46-7.86) | 0.032 |

Table 2 shows the clinical characteristics between patients with and without SDRMs. From univariate and multivariate analysis, only IVDU [Odd Ratio (OR) 8.09; 95% confidence interval (CI), 1.76-37.08; $p$=0.007] and log HIV-1 RNA [OR 0.50; 95%CI 0.27-0.95; $p$=0.035] were significant factors positively and negatively associated with the occurrence of SDRMs. Other factors including age, gender, CD4 cell count, HBV or HCV co-infection, and HIV-1 subtypes were not significantly associated with the occurrence of SDRMs.

## CONCLUSION

There is a significant emergence of primary HIV-1 drug resistance in Thailand particularly in IVDU subgroup. Although resistance testing prior to ART initiation is not routinely recommended in resource-limited setting, it may be considered in this subgroup. Continuation of the surveillance for primary HIV-1 drug resistance in Thailand is indicated.

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10.2 Sungkanuparph S, Sukasem C, Kiertiburanakul S, Chantratita W. Surveillance of transmitted HIV-1 drug resistance in Thailand: A four-year study. The 48th Annual Meeting of Infectious Disease Society of America (IDSA), Vancouver, Canada, 2010. P 1107.

# Surveillance of Transmitted HIV-1 Drug Resistance in Thailand: A Four-Year Study

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IDSA 48TH ANNUAL MEETING VANCOUVER OCTOBER 21-24, 2010

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## BACKGROUND

HIV-1 infection is still a major health problem in Thailand. To date, up to 600,000 Thais are living with HIV. Antiretroviral therapy (ART) has decreased the rate of morbidity and mortality and has improved the quality of life in HIV-1 infected persons, both in resource-rich and resource-limited settings. After 8-year rapid scaling up of antiretroviral therapy (ART) among HIV-1 infected patients, the data of transmitted HIV-1 drug resistance in Thailand is still limited. The aim of this study was to determine the prevalence and trend of transmitted HIV-1 drug resistance in Thailand.

## METHODS

A prospective cohort study was conducted in antiretroviral-naïve Thai patients who visit Ramathibodi Hospital, a 1200-bed university hospital between January 2007 and May 2010. HIV-1 subtypes and mutations were assayed by sequencing a region of HIV-1 pol gene. We focused at the surveillance drug resistance mutations (SDRMs) recommended for surveillance of transmitted HIV-1 drug resistance by WHO in 2009 (Table 1). The prevalence and risk factors of primary drug resistance using multivariate analysis were determined.

Table 1. Surveillance drug resistance mutations recommended for surveillance of transmitted HIV-1 drug resistance by WHO 2009

HIV-1 Protease and Reverse Transcriptase Mutations For Drug Resistance Surveillance (2009)

| NRTI | |
|---|---|
| Pos | Mut |
| M41 | L |
| K65 | R |
| D67 | N, G, E |
| T69 | D, Ins |
| K70 | R, **E** |
| L74 | V, **I** |
| V75 | M, T, A, S |
| F77 | L |
| Y115 | F |
| F116 | Y |
| Q151 | M |
| M184 | V, I |
| L210 | W |
| T215 | Y, F, I, S, C, D, V, E |
| K219 | Q, E, N, R |

| NNRTI | |
|---|---|
| Pos | Mut |
| L100 | I |
| K101 | E, **P** |
| K103 | N, S |
| V106 | M, A |
| **V179** | **F** |
| Y181 | C, I, **V** |
| Y188 | L, H, C |
| G190 | A, S, E |
| P225 | H |
| M230 | L |

| PI | |
|---|---|
| Pos | Mut |
| **L23** | **I** |
| L24 | I |
| D30 | N |
| V32 | I |
| M46 | I, **L** |
| I47 | V, A |
| G48 | V, **M** |
| I50 | V, L |
| F53 | L, **Y** |
| I54 | V, L, M, A, T, S |
| G73 | S, T, C, A |
| **L76** | **V** |
| V82 | A, T, F, S, C, M, **L** |
| **N83** | **D** |
| I84 | V, A, C |
| **I85** | **V** |
| N88 | D, S |
| L90 | M |

New mutations are in **bold**

## RESULTS

A total of 374 patients with a mean (SD) age of 34.4 (11.6) years were studied. Of all, 214 (57.2%) patients were male. Risks of HIV-1 infection included heterosexual (78.6%), homosexual (16.3%), and intravenous drug use (IDU, 5.0%). Of all, 315 (84.2%) patients had HIV-1 subtype CRF01_AE; 7.3% and 5.3% had HBV and HCV co-infection, respectively. The median (IQR) CD4 cell count and HIV-1 RNA were 184 (45-338) cells/mm3 and 70,078 (19,021-214,500) copies/mL, respectively.

There were 39, 179, 129 and 27 patients in the year 2007, 2008, 2009 and 2010, respectively. The prevalence of patients with ≥1 SDRM(s) was 4.3%; from the year 2007 to 2010, the prevalence was 7.7%, 5.6%, 1.6%, and 3.7%, respectively (Figure 1). When categorized patients into 3 groups according to the risk of HIV-1 infection, the prevalence of patients with ≥1 SDRM(s) was 4.1%, 3.2%, and 10.5% in heterosexual, homosexual, and IDU groups, respectively (Figure 2). The prevalence in each group according to risk of HIV-1 infection from 2007 to 2010 is shown in Figure 2.

Figure 1. Prevalence of patients with ≥1 SDRM from 2007 to 2010.

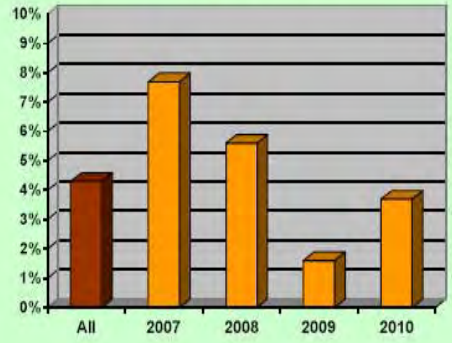
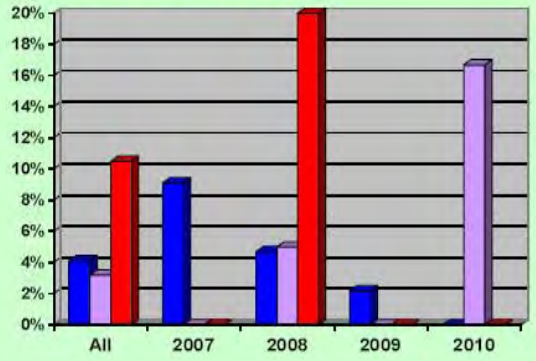



Figure 2. Prevalence of patients ≥1 SDRM from 2007 to 2010 in each group according to risk of HIV-1 infection: heterosexual, homosexual, and IVDU.




The prevalence of patients with NRTI-SDRMs, NNRTI-SDRMs, and PI-SDRMs was 2.1%, 3.5%, and 0.5%, respectively. Of 13 patients with NNRTI-SDRMs, 6 had only NNRTI-SDRMs; the others also had NRTI-SDRMs (6) or PI-SDRMs (1).

From logistic regression analysis, there was no significant factor to predict the occurrence of SDRMs. There was a trend toward higher risk for having SDRMs in patients with IDU [OR=2.87; 95%CI, 0.60-13.63].

## CONCLUSION

During rapid scaling up of ART in Thailand, transmitted HIV-1 drug resistance has been established from 2007 to 2010. Although there is no significant predicting factor, IDU appears to have a trend toward higher risk for transmitted HIV-1 drug resistance and further large-scaled study in this group is suggested. Continuation of surveillance for transmitted HIV-1 drug resistance in Thailand is indicated.

10.3 Sungkanuparph S, Sukasem C, Kiertiburanakul S, Pasomsub E, Chantratita W. Emergence of HIV-1 Drug Resistance Mutations among Antiretroviral-naïve HIV-1-infected Patients in Thailand After a Decade of Scaling-up Antiretroviral Therapy. Submitted to *Journal of International AIDS Society* - status under reviewed

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Subject: 8712811586062494 Emergence of HIV-1 Drug Resistance Mutations among Antiretroviral-naive HIV-1-infected Patients in Thailand After a Decade of Scaling-up Antiretroviral Therapy

Date: Fri, September 16, 2011 6:00 pm

To: "Prof Somnuek Sungkanuparph" <somnuek.sun@mahidol.ac.th>

Cc: "Wasun Chantratita" <rawctt@mahidol.ac.th>

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Authors : Somnuek Sungkanuparph, Chonlaphat Sukasem, Sasisopin Kiertiburanakul, Ekawat Pasomsub and Wasun Chantratita
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Emergence of HIV Drug Resistance in Thailand

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**Abstract**

**Background:** After 10-year rapid scaling up of antiretroviral therapy (ART) in HIV-1-infected patients, the data of primary HIV-1 drug resistance (HIVDR) in Thailand is still limited. This study aims to determine the prevalence and associated factors of primary HIVDR in Thailand.

**Methods:** A prospective observational study was conducted among antiretroviral-naïve HIV-1-infected Thai patients from 2007 to 2010. HIV-1 subtypes and mutations were assayed by sequencing a region of HIV-1 pol gene. Surveillance drug resistance mutations (SDRMs) recommended by WHO for surveillance of transmitted HIVDR in 2009 were used in all analyses. Primary HIVDR was defined as the presence of ≥1 SDRM(s).

**Results:** Of 466 patients with a mean age of 38.8 years, 58.6% of patients were males. Risks of HIV-1 infection included heterosexual (77.7%), homosexual (16.7%), and intravenous drug use (IDU, 5.6%). Median (IQR) CD4 cell count and HIV-1 RNA were 176 (42-317) cells/$mm^3$ and 68,600 (19,515-220,330) copies/mL, respectively. HIV-1 subtypes were CRF01_AE (86.9%), B (8.6), and other recombinants (4.5%). The prevalence of primary HIVDR was 4.9%; most of these (73.9%) had SDRM(s) to only one class of antiretrovirals. The prevalence of patients with NRTI-, NNRTI-, and PI-SDRMs was 1.9%, 2.8%, and 1.7%, respectively. From logistic regression analysis, there was no factor significantly associated with primary HIVDR. There was a trend toward higher prevalence in females [odd ratio 2.18; 95% confidence interval, 0.896-5.304; $p$=0.086].

**Conclusion:** There is a significant emergence of primary HIVDR in Thailand after a decade of rapid scaling-up of ART in Thailand. Although HIV-1 genotyping prior to ART initiation is not routinely recommended in Thailand, our results raise concerns

about the risk of early treatment failure. Interventions to prevent the transmission of HIVDR and continuation of surveillance for primary HIVDR in Thailand are indicated.

## Background

In Thailand, the disease burden from HIV/AIDS resulting from the epidemic in the 1990s remains high [1]. Although the change in the incidence rate of HIV infection in Thailand, from 2001 to 2009, is over 25% decreasing [2], the accumulated number of HIV-infected persons is still high. Currently, an estimated number of 530,000 people are living with HIV Thailand [2]. Combination antiretroviral therapy (ART) has significantly reduced mortality and morbidity since its introduction in Thailand [3-5]. Since 2001, the government committed to providing ART free of charge to people living with HIV under the National Access to Antiretroviral treatment Program for People Living with HIV/AIDS (NAPHA) [6]. The subsequent production and use of generic drugs led to more than an eight-fold expansion in treatment provision between 2001 and 2003 [7]. Since 2006, NAPHA has been transformed to National AIDS Program (NAP) under the management of National Health Security Office with the rapid growing of the program. According to UNAIDS 2010 report, the number of people receiving ART in December 2009 is 216,118 persons and life years among adults gained due to ART between 1996 and 2009 is 389,000 [2]

Despite these successes, HIV-1 drug resistance (HIVDR) is a major reason for treatment failure during rapid scaling up of ART in Thailand [8,9]. Approximately 5% to 10% of patients receiving ART have experienced treatment failure and HIVDR [10]. Recently, a study in Thailand had demonstrated the transmission of HIVDR in antiretroviral-naïve HIV-1-infected patients in Thailand [11]. This threatens the effectiveness of rapidly scaled up first-line ART in the country. Primary HIVDR means

increase resistance of HIV-1 to antiretroviral drugs seen in individuals who have never received ART and presumably have been infected with drug-resistant virus [12]. The prevalence of primary HIVDR has been well reported in the United States and Europe, ranged from 6.2% to 21% [13-16]. A study in Asia has recently reported the prevalence of primary HIVDR at 13.8% [17]. In resource-limited settings where ART is being scaled-up, the World Health Organization (WHO) recommends the surveillance of primary HIVDR [18]. To date, after a decade of ART scaling up, there is limited published information regarding primary HIVDR in Thailand. This study was aimed to determine the prevalence of HIVDR and associated factors among antiretroviral-naïve patients in Thailand.

**Methods**

A cross-sectional study was conducted among antiretroviral-naïve HIV-1-infected patients who newly visited an infectious disease clinic in a university hospital between January 2007 and December 2010. Patients with a history of any exposure to antiretroviral drugs including mono- or dual-therapy, or prevention of mother-to-child-transmission were excluded. Ethics approvals were obtained from local institutional review boards. Informed consent was obtained prior to genotypic resistance testing.

All plasma samples, HIV-1 pol nucleotide sequencing of reverse transcriptase and protease region was carried out using TRUGENE HIV-1 Genotypic Assay in conjunction with the Open Gene automated DNA sequencing system (Visible Genetics, Toronto, Canada). Testing involved simultaneous clip sequencing of protease and codons 35-244 of the RT from the amplified cDNA in both the 3´ and 5´ directions. Sequences were aligned and compared with a lymphoadenopathy-associated virus type 1 (HIV-B-LAV1) consensus sequence using Visible Genetics Gene Librarian software [19,20]. Surveillance

drug resistance mutations (SDRMs) recommended by WHO for surveillance of transmitted HIVDR in 2009 [21] were used in all analyses. HIVDR in a patient was defined as the presence of at least one SDRM.

Mean (± standard deviation, SD), median (interquartile range, IQR) and frequencies (%) were used to describe patients' characteristics. Categorical variables between the two groups were compared using Chi square or Fisher's exact test as appropriate. Continuous variables between the two groups were compared using Student's t test and Mann-Whitney U test as appropriate. Logistic regression analysis was used to determine factors associated with HIVDR. A *p*-value at <0.05 was considered as statistically significant. All analyses were performed using SPSS version 16.0 (SPSS Inc., Chicago, Illinois, U.S.A).

**Results**

A total of 466 patients were included in this analysis. The mean (SD) age was 38.8 (11.4) years. Two hundred and sixty-three (58.6%) patients were males. Risks of HIV-1 infection were heterosexual (77.7%), homosexual (16.7%), and intravenous drug use (IDU, 5.6%). Forty-six (9.9%) and 32 (6.9%) patients had co-infection of hepatitis B virus and hepatitis C virus, respectively. Median (IQR) CD4 cell count and HIV-1 RNA were 176 (42-317) cells/$mm^3$ and 68,600 (19,515-220,330) copies/mL, respectively. Of 466 patients, 405 (86.9%) were infected with HIV-1 subtype CRF01_AE. Subtype B was found in 40 (8.6) patients. Other subtypes (4.5%) were CRF07_BC, CRF03_AB, CRF02_AG, CRF12_BF, D, and K.

The prevalence of primary HIVDR was 4.9%. The prevalence of patients with nucleoside reverse transcriptase inhibitor (NRTI)-, non nucleoside reverse transcriptase inhibitor (NNRTI)-, and protease inhibitor (PI)-SDRMs was 1.9%, 2.8%, and 1.7%,

respectively. Seventeen (3.8%) patients had SDRM(s) to only one class of antiretroviral drugs. Five (1.1%) patients had both NRTI- and NNRTI-SDRMs. Only one patient had SDRMs to 3 classes of antiretroviral drugs. Table 1 shows SDRMs observed in 23 patients with HIVDR. The comparison of characteristics between patients with and without HIVDR is summarized in Table 2. From logistic regression analysis, there was no factor significantly associated with HIVDR. There was a trend toward higher prevalence in females [OR=2.18; 95%CI, 0.896-5.304; *p*=0.086].

**Discussion**

Primary HIVDR represents a challenge for the treatment of HIV-1 infection because it can reduce the efficacy of first line antiretroviral therapy and may impact clinical outcomes. Emergence of primary HIVDR in resource-limited settings is a concerning consequence of global scaleing-up of ART. It will be seen first in the region where ART has been widely available for years [18]. After a decade of rapid scaling-up of ART in Thailand, primary HIV is inevitably anticipated.

The results from the present study have demonstrated that there is an emergence of primary HIV in Thailand. The prevalence is as high as 4.9% and approaching WHO's first threshold (5%) of transmitted HIV. Blower's model had previously predicted that transmitted HIVDR will reach 5% after approximately 10 years of ART scaling-up [18,22]. Although the term 'transmitted HIVDR' is generally applied only to HIVDR detected in recently infected individuals, the prevalence of primary HIVDR among patients with chronic HIV-1 infection may be even underestimated. Thus, the results from the present study provide data about the likely efficacy of first-line ART in Thailand. For instance, about 5% of patients initiating first-line ART regimen, which is NNRTI-based regimens, in Thailand may have early treatment failure. Although most of the patients

(3.8%) with primary HIVDR had SDRMs to only one class of antiretroviral drugs, the treatment response can be markedly reduced. NNRTI-based regimens generally have low genetic barrier for development of resistance and early treatment failure is likely if the regimen does not consist of 3 fully active drugs [23,24].

Recently, various multicenter cohort studies have demonstrated that primary HIVDR is associated with poor treatment outcomes and/or clinical complication [25-27]. They all support the use of genotypic resistance test prior to initiation of ART. Since 1998, the International AIDS Society--USA Panel had suggested considering resistance testing for antiretroviral-naïve patients in areas with a prevalence of resistance of ≥5% [28]. However, a cost-effectiveness study of genotypic resistance testing for antiretroviral-naïve patients with chronic HIV-1 infection has reported that it is cost-effective if the prevalence of primary HIVDR is >1%.

Thailand is an area with predominance of HIV-1 subtype CRF01_AE. Although the prevalence of HIVDR in patients with subtype CRF01_AE is twice of that in patients with subtype B (5.2% vs. 2.5%), there was no statistically significant difference. There were no significant differences in demographic or clinical factors between those with/without primary HIVDR. There was only a trend toward higher prevalence in females from multivariate analysis. Therefore, there is no risk group to consider genotypic testing for primary HIV in Thailand. As ART continues to be scaled-up rapidly, it is likely that the prevalence of primary HIV continues to increase. It's a national priority to intervene with the intervention to prevent further transmission of HIVDR. To minimize primary HIVDR in Thailand, strengthening of health care system, supporting adherence to therapy, and ensuring a continuous supply of antiretroviral drugs are crucial. At some point, the National AIDS Program in Thailand has to carefully consider the advantages and disadvantages of genotypic testing for primary HIVDR and

decide when and how to implement. The future plans have to include the strategies to make genotypic testing more accessible with the newer technologies, such as point mutation assays or short sequencing of some specific regions of RT gene.

There are some limitations in the present study. Although the patients in the present study were those who newly presented to the infectious disease clinic, some patients presented late. They were tested for HIV-1 genotypes at the stage of chronic infection. Some resistance mutations may have reverted to wild type. Thus, the prevalence of primary HIVDR could be underestimated. However, transmitted HIVDR among antiretroviral-naïve patients has been reported to be persistent, ranged from 4 years to longer than the lifetime of the patient [30].The prolonged persistence of transmitted HIVDR strongly supports the use of genotypic resistance test in newly presented patients.

In summary, primary HIVDR is emerging in Thailand after a decade of rapid scaling-up of ART. Although HIV-1 genotyping prior to ART initiation is not routinely recommended in Thailand, our results raise concerns about the risk of early treatment failure. Interventions to prevent the transmission of HIVDR and continuation of surveillance for primary HIVDR in Thailand are indicated.

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**Authors' contributions**

SS and SK participated in the design of the study, enrolled patients, collected data on patient history, and drafted the manuscript. CS, EP, and WC carried out the viral load assays, genotypic drug-resistance test, and subtype analysis. All authors read and approved the final manuscript.

**Competing interests**

The authors declare that they have no competing interests.

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Table 1. Distribution of SDRMs in 23 patients with primary HIVDR*

| SDRMs | Number of patients | Prevalence (%) |
|---|---|---|
| NRTI-SDRMs | 9 | 1.9 |
| M41L | 3 | 0.6 |
| K65R | 1 | 0.2 |
| D67N | 1 | 0.2 |
| T69D | 1 | 0.2 |
| V75M | 1 | 0.2 |
| M184V | 3 | 0.6 |
| M184I | 1 | 0.2 |
| L210W | 1 | 0.2 |
| T215Y | 1 | 0.2 |
| T215S | 1 | 0.2 |
| K219Q | 1 | 0.2 |
| K219R | 1 | 0.2 |
| NNRTI-SDRMs | 13 | 2.8 |
| K101E | 1 | 0.2 |
| K103N | 3 | 0.6 |
| K103S | 1 | 0.2 |
| V106A | 1 | 0.2 |
| V106M | 1 | 0.2 |
| Y181C | 4 | 0.9 |
| Y181I | 1 | 0.2 |
| Y188L | 1 | 0.2 |
| G190S | 1 | 0.2 |
| PI-SDRMs | 8 | 1.7 |
| M46I | 1 | 0.2 |
| M46L | 1 | 0.2 |
| I47V | 1 | 0.2 |
| G48M | 1 | 0.2 |
| I54L | 1 | 0.2 |
| I54T | 1 | 0.2 |
| I84A | 1 | 0.2 |
| L90M | 6 | 1.3 |

*some patients had >1 SDRM

Table 2. Comparison of characteristics between patients with and without primary HIVDR

| Characteristics | Primary HIVDR | | *P* value |
|---|---|---|---|
| | Yes *(n=23)* | No *(n=443)* | |
| Age, years, mean ± SD | 37.3 (7.9) | 38.8 (11.5) | 0.517 |
| Male gender, number (%) | 9 (60.9) | 264 (59.6) | 0.080 |
| Risk of HIV-1 infection, number (%) | | | 0.489 |
| Heterosexual | 19 (82.6) | 343 (77.4) | |
| Homosexual | 2 (8.7) | 76 (17.2) | |
| IVDU | 2 (8.7) | 24 (5.4) | |
| HBV co-infection, number (%) | 2 (8.7) | 44 (9.9) | 0.579 |
| HCV co-infection, number (%) | 2 (8.7) | 30 (6.8) | 0.326 |
| CD4, cells/$mm^3$, median (IQR) | 197 (35-307) | 173 (43-318) | 0.784 |
| HIV-1 RNA, log copies/mL, median (IQR) | 29,600 (3,580-214,000) | 70,150 (20,490-220,740) | 0.271 |
| HIV-1 subtypes, number (%) | | | 0.551 |
| CRF01_AE | 21 (91.4) | 384 (86.7) | |
| B | 1 (4.3) | 39 (8.8) | |
| Others* | 1 (4.3) | 20 (4.5) | |

IVDU=intravenous drug use

*including CRF07_BC, CRF03_AB, CRF02_AG, CRF12_BF, D, and K

10.4 Sungkanuparph S, Pasomsub E, Chantratita W. Surveillance of Transmitted HIV Drug Resistance in Antiretroviral-naïve Patients Aged <25 Years in Bangkok, Thailand. Submitted to *AIDS Research and Treatment* - status under reviewed.

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Surveillance of Transmitted HIV Drug Resistance

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**Abstract**

Emergence of transmitted HIV resistance (TDR) is a concern after global scale-up of antiretroviral therapy (ART). WHO had developed threshold survey method for surveillance of TDR in resource-limited countries. ART in Thailand has been scaling up for 10 years. To evaluate the current TDR in Thailand, a cross-sectional study was conducted among antiretroviral-naïve HIV-infected patients aged <25 years who newly visited an infectious disease clinic in a university hospital. HIV genotypic resistance test was performed. WHO 2009 surveillance drug resistance mutations (SDRMs) were used to define TDR. Of 49 patients, the prevalence of TDR was 4.1%. Of 2 patients with TDR, one had K103N and the other had Y181I mutation. TDR is emerging in Thailand after a decade of rapid scale-up of ART. Interventions to prevent the transmission of drug resistant virus in population level are essentially needed in Thailand. Surveillance for TDR in Thailand has to be regularly performed.

## 1. Introduction

Emergence of transmitted HIV resistance (TDR) is a concern after global scale-up of antiretroviral therapy (ART). TDR is associated with poor treatment outcomes and/or clinical complication [1-5]. TDR is anticipated in the areas where ART has been widely available for years. The prevalence of TDR has been reported in the United States and Europe, ranged from 6.2% to 21% [6-9]. In resource-limited settings, TDF has been reported from sub-Saharan Africa after scale-up of ART and showed the prevalence from 3.5% to 11.6%, depended on the countries [10]. The higher prevalence of TDR in Uganda than in other African countries is probably related to the earlier start of ART scale-up in Uganda. In the countries scaling up ART, the World Health Organization (WHO) recommends the surveillance of TDR [11]. The WHO HIV drug resistance threshold survey method had been developed for surveillance of TDR in resource-limited countries. To minimize costs, WHO suggests that each survey requires < or =47 specimens from individuals consecutively diagnosed with HIV to categorize resistance to each relevant drug class as <5%, 5-15% or >15% [11].

In Thailand, ART has reduced mortality and morbidity since its introduction in the country [12-14]. Scaling up of ART has started in since 2001 and the National AIDS Program (NAP) continues to expand. According to UNAIDS 2010 report, the number of people receiving ART in December 2009 is 216,118 persons and life years among adults gained due to ART between 1996 and 2009 is 389,000 [15]. The first threshold survey in Thailand had been done in Bangkok, involving blood donors and counseling and testing centre (VCT) clients during 2005-2006 [16]. The findings showed no mutations associated with TDR. Recently, a multinational study in Asia including Thailand has

demonstrated the prevalence of primary HIV drug resistance among antiretroviral naïve patients at 13.8% [17]. Although this study did not use surveillance drug resistance mutations (SDRMs) recommended by WHO for surveillance of TDR [18], it bring to a concern of TDR in Thailand. To evaluate the current situation of TDR in Thailand, especially in the patient care center, this study was conducted using WHO threshold survey for resource-limited settings.

## 2. Methods

A cross-sectional study was conducted among antiretroviral-naïve HIV-infected patients who newly visited an infectious disease clinic in a university hospital between January 2007 and December 2010. According to WHO threshold survey methods to minimize inclusion of ARV-experienced individuals and individuals infected before ART was available [11], the patients eligibility criteria included 1) laboratory confirmation of HIV infection, 2) age <25 years at HIV diagnosis, and 3) if female, no previous pregnancy. Eligible patients were consecutively enrolled from an infectious clinic during the study period. Ethics approvals were obtained from local institutional review boards. Informed consent was obtained prior to genotypic resistance testing.

All plasma samples, HIV pol nucleotide sequencing of reverse transcriptase and protease region was carried out using TRUGENE HIV Genotypic Assay in conjunction with the Open Gene automated DNA sequencing system (Visible Genetics, Toronto, Canada). Testing involved simultaneous clip sequencing of protease and codons 35-244 of the reverse transcriptase from the amplified cDNA in both the 3´ and 5´ directions. Sequences were aligned and compared with a lymphoadenopathy-associated virus type 1 (HIV-B-LAV1) consensus sequence using Visible Genetics Gene Librarian software

[19,20]. SDRMs recommended by WHO for surveillance of TDR in 2009 [18] were used in all analyses. TDR in a patient was defined as the presence of at least one SDRM.

## 3. Results

A total of 49 patients were included in this analysis. The mean (SD) age was 21.7 (3.8) years. Thirty (61.2%) patients were males. Risks of HIV infection were heterosexual (65.3%), homosexual (30.6%), and intravenous drug use (IDU, 4.1%). Median (range) CD4 cell count and HIV RNA were 214 (10-782) cells/$mm^3$ and 68,150 (1,023->1,000,000) copies/mL, respectively. Of 49 patients, 39 (79.6%) were infected with HIV subtype CRF01_AE. Other subtypes were B (12.2%), CRF07_BC (4.1%), CRF12_BF (2.0%), and K (2.0%).

The prevalence of TDR was 4.1%. Of these two patients with TDR, both had only one non nucleoside reverse transcriptase inhibitor (NNRTI)-SDRM; one had K103N and the other had Y181I mutation. Nucleoside reverse transcriptase inhibitor (NRTI)- and protease inhibitor (PI)-SDRMs were not observed in this study. Both patients were males; one was heterosexual and the other was homosexual. They aged 23.4 and 23.9 years old. CD4 cell counts and HIV RNA of these two patients were 22 and 408 cells/$mm^3$, and 1,700 and 176,950 copes/mL, respectively.

## 4. Discussion

Surveillance of TDR can support implementation of prevention measures on a population level. TDR represents a challenge for the treatment of HIV infection because it can reduce the efficacy of first-line ART and impact clinical outcomes [1-5]. After a decade of rapid scale-up of ART in Thailand, TDR is inevitably anticipated. The results from the present study have demonstrated that there is an emergence of TDR in Thailand.

Although the prevalence is less than WHO lower threshold (5%), it raises a concern of HIV care in Thailand. Although patients with TDR had SDRMs to only NNRTI, the treatment response can be markedly impacted. ART regimens in resource-limited settings are usually selected at the national level following a public health approach. In Thailand, the national guidelines recommend using NNRTI-based regimens as the first-line ART [21]. NNRTI-based regimens generally have low genetic barrier for development of resistance and early treatment failure is likely if the regimen does not consist of three fully active drugs [22]. Although we cannot demonstrate how our patients acquired drug resistance mutations in this study, it is likely that sexual transmission from their treatment-experienced partners may be the case. Our previous study has shown that Y181C/I and K103N are common drug resistance mutations after failing the first-line ART in Thailand [23]. To minimize TDR in Thailand, strengthening of HIV care system, supporting patient's adherence to therapy, and prevention of HIV transmission in both HIV-infected and –uninfected individuals are crucial. Regular surveillance of TDR in Thailand will inform evidence-based decision making regarding national AIDS program.

There are some limitations in the present study. Although we followed the mandatory criteria of participant eligibility for WHO threshold survey, it appears that many patients with age <25 years old does not accurately predict recent HIV infection. Many patients in the present study had low CD4 cell counts and may indeed have chronic HIV infection. Patients in resource-limited setting tend to present to the health care system late. In addition, a recent study also demonstrated that HIV drug resistance in antiretroviral-naïve patients aged >25 years is dramatically high [24]. Although mandatory criteria of 'age < 25 years' suggested by WHO is practical for resource-limited settings to follow, it may not be accurate. Nevertheless, TDR among antiretroviral-naïve patients has been reported to be persistent, ranged from 4 years to

longer than the lifetime of the patient [25]. There is a value of study TDR even in chronic HIV infection, with a caution of possible underestimation.

## 5. Conclusion

TDR is emerging in Thailand after a decade of rapid scale-up of ART. Interventions to prevent the development of HIV drug resistance among treated patients and to prevent transmission of drug resistant virus are essentially needed in Thailand. To inform the national policy for HIV care, surveillance for TDR in Thailand has to be regularly performed.


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*Conflict of interests:* All authors declare that they have no conflict of interests.


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10.5 Sungkanuparph S, Pasomsub E, Kiertiburanakul S, Chantratita W. Etravirine Resistance-Associated Mutations in Antiretroviral-Naïve Patients Infected with HIV-1 Subtype CRF01_AE versus Subtype B. Submitted to *Conference of Retroviruses and Opportunistic Infection* - status under reviewed.


Your abstract entitled "**Etravirine Resistance-Associated Mutations in Antiretroviral-Naïve Patients Infected with HIV-1 Subtype CRF01_AE versus Subtype B**" has been submitted. Please keep this confirmation until you receive your disposition via email on or November 28, 2011. *Again this year*: edits to the abstract (other than designating a different category) may be made online after submission up until the deadline of 5 pm ET, October 5, 2011 (please note that you will not receive an updated confirmation email when making additional edits).

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| | |
|---|---|
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diagnostics)

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## Abstract

**Title** Etravirine Resistance-Associated Mutations in Antiretroviral-Naïve Patients Infected with HIV-1 Subtype CRF01_AE versus Subtype B

**Body**

**Background:** Susceptibility to etravirine (ETR) is determined by the number and positions of NNRTI resistance-associated mutations (RAMs). Non-B HIV-1 subtypes may have natural polymorphisms described as ETR RAMs. This study aims to determine the prevalence of ETR RAMs in antiretroviral-naive patients and to compare ETR RAMs and ETR susceptibility score between subtypes CRF01_AE and B.

**Methods:** A prospective observational study was conducted among antiretroviral-naive HIV-1-infected patients in Thailand where subtype CRF01_AE predominates. HIV-1 subtypes and mutations were assayed by sequencing a region of HIV-1 pol gene. In order to evaluate polymorphism between 2 subtypes, patients who had transmitted HIV-1 drug resistance defined by WHO 2009 surveillance RAMs were excluded. Seventeen ETR RAMs with weighted scoring system (3.0, Y181I/V; 2.5, L100I, K101P, Y181C, and M230L; 1.5, V106I, V179F, E138A, and G190S; and 1.0, V90I, A98G, K101E/H, V179D/T, and G190A) for ETR susceptibility were used in this analysis.

**Results:** Of 417 patients with a mean age of 38.9 years, 59.5% of patients were males. Risks of HIV-1 infection included heterosexual (77.5%), homosexual (17%), and intravenous drug use (IDU, 5.5%). Median (IQR) CD4 cell count and HIV-1 RNA were 169 (43-316) cells/mm$^3$ and 70,780 (21,900-221,000) copies/mL, respectively. The prevalence of ETR RAMs in 378 patients infected with subtype CRF01_AE and 39 with subtype B were 9% and 15.4%, respectively ($p$=0.246). The distributions of ETR RAMs between 2 subtypes are shown in Figure 1. All patients who harbored ETR RAMs had only one mutation. There were no differences for the frequencies of each ETR RAM between 2 subtypes ($p$>0.05). All patients who harbored ETR RAMs had score ranged from 1 to 1.5. ETR susceptibility scores between 2 subtypes are shown in Figure 2; the distributions of score between 2 subtypes were not significantly different ($p$=0.257).

**Conclusion:** Although different patterns of ETR RAMs are observed between HIV-1 subtype CRF01_AE and B, the prevalence of ETR RAMs and ETR susceptibility score are not different between antiretroviral-naive patients infected with subtypes CRF01_AE and B. The ETR susceptibility scores in both subtypes had no significant impact on ETR susceptibility.

Figure 1 ETR-RAMs between HIV-1 subtypes CRF01_AE and B

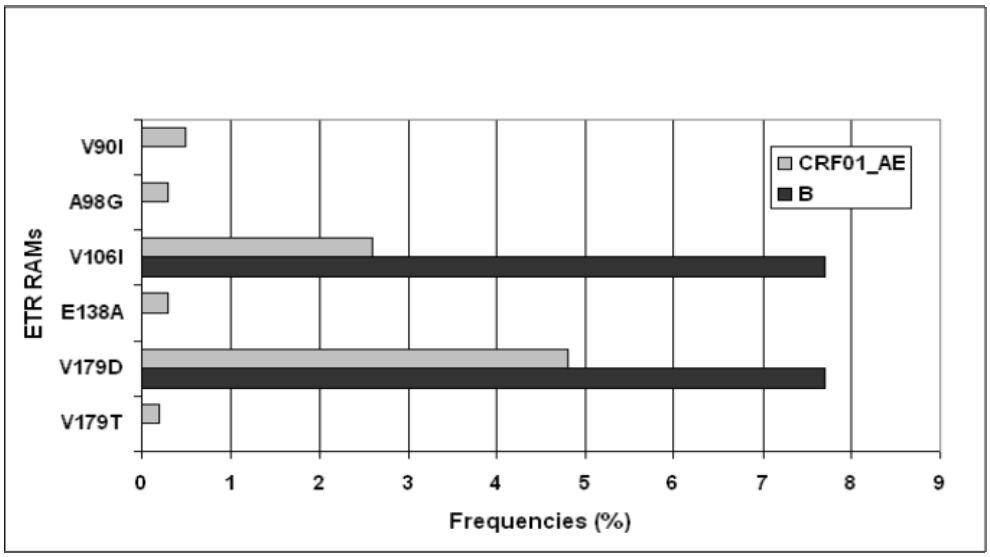
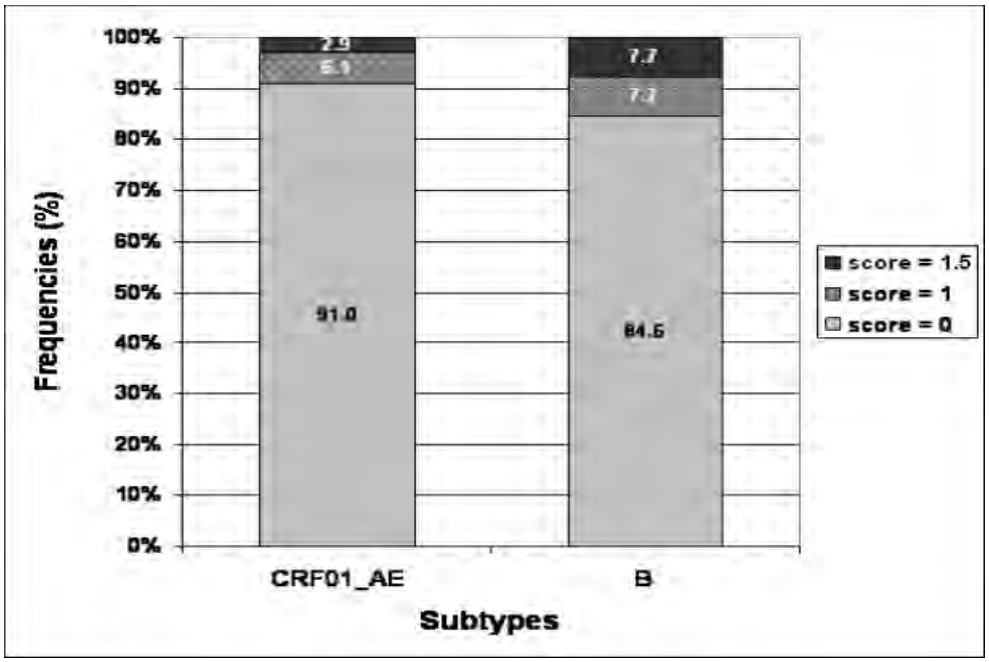



Figure 2 ETR susceptibility score between HIV-1 subtypes CRF01_AE and B

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10.6 Sungkanuparph S, Kiertiburanakul S, Pasomsub E, Chantratita W. Resistance-Associated Mutations to Efavirenz, Etravirine, Nevirapine, and Rilpivirine among Antiretroviral-Naïve HIV-1-Infected Patients in Thailand. Submitted to *Conference of Retroviruses and Opportunistic Infection* - status under reviewed.


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**Young Investigator** No

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## Abstract

**Title** Resistance-Associated Mutations to Efavirenz, Etravirine, Nevirapine, and Rilpivirine among Antiretroviral-Na ve HIV-1-Infected Patients in Thailand

**Body**

**Background:** NNRTI-based regimens are still mainstay of antiretroviral therapy (ART) in resource-limited settings. After 10-year rapid scale up of ART among HIV-1-infected patients in Thailand, primary HIV-1 drug resistance (HIVDR) has emerged. Susceptibility to efavirenz (EFV), etravirine (ETR), nevirapine (NVP), and rilpivirine (RPV) are determined by different number and positions of NNRTI resistance-associated mutations (RAMs). This study aims to determine the prevalence RAMs and resistance to each NNRTI among antiretroviral-naive HIV-1-infected patients in Thailand.

**Methods:** A prospective observational study was conducted among antiretroviral-naive HIV-1-infected Thai patients from 2007 to 2010. HIV-1 subtypes and mutations were assayed by sequencing a region of HIV-1 pol gene. Resistance to EFV and NVP were interpreted using the IAS-USA 2010 RAM list. DUET weighted scoring system was used for ETR susceptibility and the score of $\geq$ 2.5 was considered ETR resistance. RAMs recently described by Napolitano *et al* (ICAAC 2011) was used for RPV resistance.

**Results:** Of 466 patients with mean age of 38.8 years, 58.6% were males. Risks of HIV-1 infection included heterosexual (77.7%), homosexual (16.7%), and intravenous drug use (IDU, 5.6%). Median CD4 count and HIV-1 RNA were 176 cells/mm$^3$ and 68,600 copies/mL, respectively. HIV-1 subtypes were CRF01_AE (86.9%), B (8.6), and other recombinants (4.5%). The prevalence of patients with EFV-, ETR-, NVP-, and RPV-resistance were 3.2%, 1.3%, 3.2%, and 1.5%, respectively. All patients who had ETR resistance and all patients with RPV resistance, except one with E138G, had EFV and NVP resistance. The most common NNRTI-RAMs observed were V179D (5.2%), V106I (4.1%), Y181C (0.9%), K103N (0.6%), and V108I (0.6%). Y181C was significantly associated with resistance to all NNRTIs ($p$<0.001). K103N ($p$<0.001) and V108I ($p$=0.001) were significantly associated with only resistance to EFV and NVP. Although V179D and V106I were relatively common, most of the patients who harbored these two ETR-RAMs had score <2.5.

**Conclusion: A**fter a decade of ART scale-up in Thailand, there is significant primary resistance to NNRTIs. Although ETR has been limited accessible and RPV is not available in Thailand, there is also primary resistance to both ETR and RPV mainly due to Y181C mutations. Interventions to prevent the transmission of HIVDR and continuation of surveillance for primary HIVDR in Thailand are crucial for the National AIDS Program.

10.7 Sungkanuparph S, Kiertiburanakul S, Sukasem C, Chantratita W. Discrepancies between WHO 2009 and IAS-USA 2009 lists for determining the rate of



# Discrepancies Between WHO 2009 and IAS-USA 2009 Lists for Determining the Rate of Transmitted HIV-1 Drug Resistance: A Prospective Study

***To the Editors:***

HIV-1 infection in developing countries accounts for a major proportion of the global HIV-1 epidemic. After rapidly scaled-up of combination antiretroviral therapy (ART), the mortality and morbidity in the resource-limited settings have significantly reduced.[1–5] Unfortunately, some patients have experienced treatment failure, and this number is gradually accumulated in developing countries. HIV-1 drug resistance (HIVDR) is a major reason for treatment failure, and transmitted HIVDR threatens the effectiveness of first-line ART among HIV-1–infected patients in resource-limited settings.[6–8]

The prevalence of transmitted HIVDR varies from 6.2% to 21% in the United States and Europe.[6,7,9] In Brazil and Argentina, the rates of transmitted HIVDR are ranging from 3.3% to 22.2%.[10–13] The reported prevalence of HIVDR among naive HIV-1–infected persons in sub-Saharan Africa ranged from 4.3% to 14.8%.[14–17] In Asia, few studies show the prevalence of transmitted HIVDR ranged from 2.3% to 13.8%.[18,19] The wide range of reported prevalence of transmitted HIVDR depends on the characteristics of study population, HIV-1 subtype, use of ART in the region, and the lists of HIV-1 drug resistance mutations (DRMs) used in each individual study.

The International AIDS Society–USA (IAS-USA) list of DRMs[20] has been published and annually updated by the IAS-USA Drug Resistance Mutations Group, an independent volunteer panel of experts. This list includes mutations that may contribute to a reduced virologic response to a drug. The IAS-USA 2009 updated DRM list has 93 mutations including 20 nucleoside reverse transcriptase inhibitor (NRTI) resistance mutations at 16 reverse transcriptase (RT) positions, 24 nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance mutations at 14 RT positions, and 67 protease inhibitor (PI) resistance mutations at 36 protease positions. Most of the studies of transmitted HIVDR in the literatures, particularly before 2007, had used the IAS-USA list.[6,7,9–14,18,19]

To accurately compare transmitted drug resistance rates across geographic regions and times, the World Health Organization (WHO) has recommended the updated list of surveillance drug resistance mutations in March 2009.[21] The updated surveillance drug resistance mutation list has 93 mutations including 34 NRTI resistance mutations at 15 RT positions, 19 NNRTI resistance mutations at 10 RT positions, and 40 protease inhibitor (PI) resistance mutations at 18 protease positions. Both IAS-USA and WHO had updated the lists of DRMs in 2009.[20,21] They are different in terms of number, position, and mutations of RT and protease gene. To date, there is no direct comparison between IAS-USA and WHO lists in the study of transmitted HIVDR. This study aims to determine the discrepancies between these 2 lists for surveillance of transmitted HIVDR in the clinical setting.

A prospective study was conducted among antiretroviral-naive Thai patients who visit a 1200-bed university hospital between January 2007 and May 2010. All plasma samples, HIV-1 pol nucleotide sequencing of RT and protease region was carried out using TRUGENE HIV-1 Genotypic Assay in conjunction with the Open Gene automated DNA sequencing system (Visible Genetics, Toronto, Canada) to sequence the reverse RT and protease regions of the HIV-1 cDNA. Testing involved simultaneous clip sequencing of protease and codons 35–244 of the RT from the amplified cDNA in both the 3′ and 5′ directions. Sequences were aligned and compared with a lymphoadenopathy-associated virus type 1 (HIV-B-LAV1) consensus sequence using Visible Genetics Gene Librarian software. HIV-1 subtypes were determined by the sequences of HIV-1 pol gene. The study was approved by the institutional review board.

A total of 374 patients were studied. The mean (standard deviation) age was 34.4 (11.6) years, and 57.2% of patients were males. Risks of HIV-1 infection included heterosexual (78.3%), homosexual (16.6%), and intravenous drug use (5.0%). Of all, 84.2% of patients had HIV-1 subtype CRF01_AE. The median (IQR) CD4 cell count and HIV-1 RNA were 184 (45–338) cells per cubic millimeter and 70,078 (19,021–214,500) copies per milliliter, respectively. The prevalence of patients with ≥1 DRM(s) by IAS-USA and by WHO lists was 15.2% and 4.3%, respectively. The prevalence of patients with NRTI-DRMs, NNRTI-DRMs, and PI-DRMs by IAS-USA list was 2.1%, 15.0%, and 0.5%, respectively. The corresponding prevalence by WHO list was 2.1%, 3.5%, and 0.5%, respectively.

The discrepancies of the prevalence between the 2 lists arose from the different lists of NNRTI-DRMs, especially those DRMs listed by IAS-USA but not listed by WHO (Fig. 1). F1 These included V90I (prevalence, 0.5%), A98G (1.1%), V106I (4.3%), V108I (0.8%), E138A (0.3%), and V179D/T (6.2%), all of which were associated with decreased susceptibility of etravirine (Fig. 1). In contrast, NNRTI-DRMs listed by WHO but not listed by IAS-USA were observed only in 1 patient (K103S, 0.3%). Using IAS-USA list in logistic regression analysis, CD4 cell count <200 cells per cubic millimeter was the only factor associated with transmitted HIVDR (OR = 2.03; 95% CI: 1.11 to 3.70; $P = 0.021$). There was no

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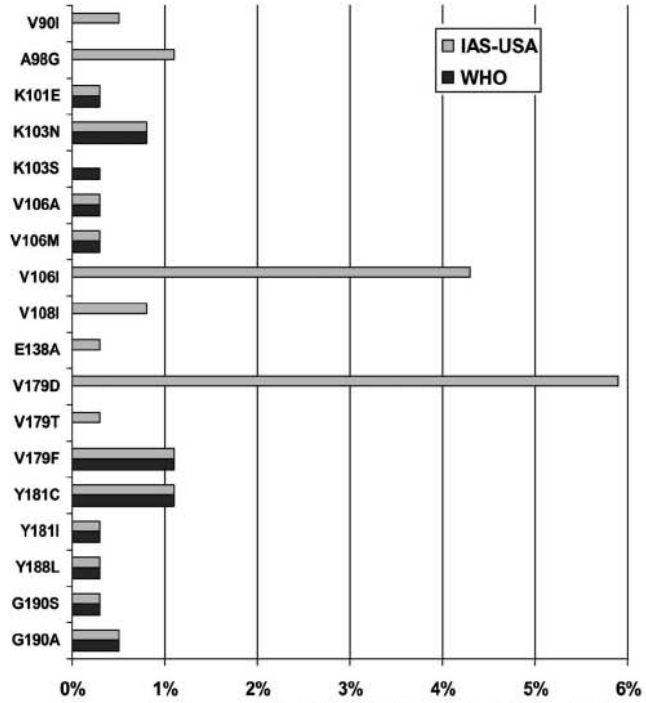


**FIGURE 1.** Prevalence of patients with NNRTI-DRM(s) by WHO 2009 and IAS-USA 2009 lists.

factor associated with transmitted HIVDR when WHO list was used in the logistic regression analysis.

The results from the present study have demonstrated the significant discrepancy of the overall prevalence of transmitted HIVDR when determined by 2 different DRM lists (15.2% vs. 4.3%). This difference was mainly driven by the high prevalence of NNRTI-DRMs by IAS-USA list. The 2 most common discrepant NNRTI-DRMs observed were V106I (4.3%) and V179D (5.9%), which are etravirine resistance–associated mutations. These mutations are more likely to be polymorphic mutations. Some DRMs occur commonly in the absence of drug-selective pressure. These polymorphic DRMs should not be used for surveillance of transmitted HIVDR because they could lead to overestimated prevalence of transmitted HIVDR. Of note, most of the patients in the present study had been infected with HIV-1 subtype CRF01_AE. A recent study has reported that non-B HIV-1 subtypes have natural polymorphisms described as etravirine resistance–associated mutations.[22] However, there were only 19 from 726 samples that were HIV-1 subtype CRF01_AE in this report. Whether V106I and V179D in the present study are real polymorphisms and related to CRF01_AE subtypes has to be further studied.

In summary, there are discrepancies of the prevalence rates and associated factor of transmitted HIVDR between using WHO 2009 and IAS-USA 2009 lists. These discrepancies may lead to the different recommendation of interventions, such as routine resistance test before ART initiation. Using IAS-USA, 2009 list is associated with higher prevalence of HIVDR, which is driven by the high rates of etravirine-associated resistance mutation and suspected polymorphisms. Further study to evaluate the potential of these polymorphisms in CRF01_AE subtypes is needed.

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