Development of Low-Cost Genotyping Assay for Routine Clinical Detection of HIV-1 Integrase Inhibitor Resistance

(MSS 284R)

Executive summary

- Background

The promising efficacy and excellent tolerability of integrase inhibitors (INIs) has soon led to its introduction into first line regimens. However, resistance to the first-generation integrase inhibitors, Raltegravir and Elvitegravir can arise rapidly as a result of single mutations or combination of mutations in the integrase gene and cross resistance with new generation drug, Dolutegravir, has recently been described. Furthermore, an increasing frequency of transmitted drug resistance to INI-naive patients have been observed. Surveillance of the INIs resistance is therefore crucial for optimizing the treatment regimens and routine monitoring the efficacy of integrase inhibitor-based antiretroviral therapy (ART).

- Aim and Objectives

1. To develop a simple and low-cost Kompetitive Allele-specific PCR (KASP) genotyping test for routine clinical detection of integrase inhibitor resistance.
2. To determine the current prevalence of major mutations associated with integrase inhibitor resistance among ART-naive, ART-experienced/INI-naive and INI-experienced patients.

- Project design

The diagnostic performance of KASP genotyping test was evaluated against integrase sequencing assay using archived plasma samples collected from a total of 160 HIV-1 infected individuals. The KASP genotyping test was then used to determine the prevalence of major mutations to integrase inhibitor resistance among 157 patients requesting for genotyping resistance test in the period between January and December 2016, including 120 ART-naive, 30 ART-experienced/INI-naive and 7 INI –experienced subjects.

- Target population

PLHIV/AIDS who had been followed up in ITC under Special Preventive Programme for at least three years.

- Main achievements

Five in-house Kompetitive Allele Specific PCR (KASP) genotyping assays were developed to detect the major drug resistance mutations (DRMS) at position T66, G140, Y143, Q148 and N155 of integrase gene in HIV genome respectively. An operational protocol of the KASP

genotyping assay for routine clinical detection of integrase inhibitor resistance was developed.

For evaluation of the diagnostic performance of KASP genotyping assays, a total of 160 plasma samples collected in 2010-2015 were analysed. A total of 142/160 (88.75%) samples successfully amplified by all the five KASP assays and were classified into wildtype and mutant groups. The genotyping result was found to be fully concordant with sanger sequencing.

In addition, a total of 157 plasma samples collected in 2016 were subjected to KASP genotyping. A total of 156/157 samples were classified as wildtype and one was typed as Q148R. The KASP result was fully concordant with Sanger sequencing. The prevalence of DRMs associated with INI resistance was found to be 0.6% (1/157) in 2016 cohort. The rate in INI-experienced groups was 14.29% (1/7), which was significantly higher than the INI-naïve group ($p < 0.01$).

- Conclusions

KASP genotyping tests for detection of DRM associated with integrase inhibitor resistance were developed. Considering its simplicity and comparable performance with Sanger sequencing, it is a good alternative laboratory assay for routine monitoring the efficacy of integrase inhibitor-based antiretroviral therapy.

In addition, based on the result obtained from 2016 cohort, the prevalence of DRMs associated with INI-resistance remained steadily low (0.6%).

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執行摘要

- 背景

由於整合酶抑製劑的功效和耐受性，它已成為治療愛滋病的一線藥物。 然而，愛滋病病毒的整合酶基因突變可迅速產生對整合酶抑製劑的抗藥性。 因此，我們需要常規監測病毒對整合酶抑製劑的抗藥性。

- 目標

1. 開發一種簡單和低成本的 Kompetitive Allele-specific PCR (KASP) 基因分型法，用於臨床常規檢測愛滋病病毒對整合酶抑製劑的抗藥性 。
2. 調查在已接受和未接受抗逆轉錄病毒療法的患者中，整合酶抑製劑的抗藥性相關突變的流行情況。

- 項目設計

用基因測序方法及 KASP 基因分型法， 分析來自 160 患者 的血漿樣品。透過比對兩者結果，去評估 KASP 的診斷性能。 然後， 使用 KASP 基因分型測試來 調查 2016 年間，157 名要求進行基因分型耐藥性測試的患者中，整合酶抑製劑 的抗藥性相關突變的流行情況 。

- 對象

在特殊預防計劃下，在 ITC 接受了至少三年的治療的 愛 滋病病毒感染者/愛 滋病患者

- 主要成就

開發了五種KASP基因分型法，分別檢測 愛 滋病病毒基因組中整合酶基因 T66，G140，Y143，Q148 和 N155 位點的主要 抗 藥突變。並制定了 KASP 基因分型法的操作方案，以用於常規臨床檢測整合酶抑製劑的 抗 藥性 。

為了評估 KASP 基因分型法的診斷性能，分析了 2010-2015 中收集的總共 160 個血漿樣品。通過所有五種 KASP 測定法，成功分析了總共 142/160（88.75％）樣品，並將其分類為野生型和突變體組。 基因分型結果發現與基因測序完全一致。

此外， 2016 年收集的 157 份血漿樣本進行了 KASP 基因分型 。 總共 156/157 個樣品被分類為野生型，一個樣品被確認有 抗 藥突變 Q148R。 KASP 結果與基因測序完全一致。在 2016 年，整合酶抑製劑 的抗藥性相關突變的流行 率為 0.6％（1/157）。 在已接受 療法的患者 比率為 14.29％（1/7），顯著高於未接受 療法的患者 組（$p < 0.01$）。

- 結論

我們已成功 開發用於檢測 整合酶基因 的 主要 抗 藥突變的 KASP 基因分型法。考慮到其簡單性和與基因測序相當的性能，它可用作 臨床常規檢測 病毒是否對 整合酶抑製劑產生抗藥性 。

此外，從 2016 樣本中得到的結果顯示，整合酶抑製劑 的抗藥性相關突變的流行 率為 0.6%。