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Short communication

# Reduced sensitivity to saquinavir: an update on genotyping from phase I/II trials

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**Abstract**

A genotypic analysis of the HIV-1 proteinase was performed on clinical specimen obtained from patients after different periods of Saquinavir (SQV) treatment. Proteinase genes of integrated proviral DNA from PBMC were isolated by PCR, cloned and individual sequences were obtained. Genotypic resistance was defined by the Gly48 → Val and Leu90 → Met exchanges. Frequencies and kinetics of resistance development will be reported for phase I/II trials V13330. V13329, O13328 and ACTG229 in patients on monotherapy or combination therapy with RT inhibitors. Data from V13330 have been analysed in more detail for correspondence of genotypic and phenotypic resistance and any correlation between resistance and changes in plasma viral RNA load. Furthermore, we will discuss the data from our extensive proteinase gene sequence collection with respect to mutational changes which would be indicative of resistance to other inhibitors of HIV-1 proteinase.

*Keywords:* HIV-1 protinease; Saquinavir; Inhibitors

## 1. Introduction

Selective and potent inhibitors of the HIV proteinase form a new class of antiviral agents with potential therapeutic benefit in the treatment of HIV infections. Several proteinase inhibitors have been decribed and their antiviral activity has been demonstrated in cell culture; some of them have already reached phase II/III clinical trials. Saquinavir (Ro 31-8959), a proteinase inhibitor with potent antiviral activity in vitro and proven antiviral activity in vivo, has now entered phase III testing. Previous studies have shown that prolonged in vitro culture in the presence of inhibitor selects for viral variants with significantly decreased drug sensitivity. Two key mutations in the proteinase gene have been identified consistently

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during in vitro which diminish drug sensitivity, i.e. a Gly48 → Val and a Leu90 → Met exchange. Each mutation by itself leads to a moderate decrease in susceptibility whereas the highly resistant phenotype requires both mutations.

## 2. Material and methods

Extensive genotypic analysis was carried out on clinical material obtained from patients after various times of saquinavir treatment, either as monotherapy or in combination with RT inhibitors. Total DNA was isolated from PBMC, the proteinase gene of the integrated provirus was isolated by nested PCR, cloned into pBSK and sequence determinations were done on individual clones using an Applied Biosystems Model 373A DNA Sequencer. The sequencing reaction employed Taq DNA polymerase and and fluorescent dyes labelled dideoxy-nucleotides. Samples from V13330 (SQV 600mg tid: SQV 600mg/ZDV 200mg tid), V13329 (SQV 600mg tid), O13328 (SQV 600mg tid) and ACTG229 (SQV 600mg/ZDV 200mg/ddC 0.75mg tid) have been analysed on a ~10 sequences per patient/per timepoint basis.

## 3. Results and discussion

After ca. 1 year of treatment approximately 50% of patients on saquinavir monotherapy showed genotypic resistance (18/33 $\approx$ 55%; combined data from V13330, V13329, O13328). The corresponding data for SQV/ZDV combination therapy (V13330) were 50% (7/14) and 22% (4/18) for the triple therapy arm of ACTG229. At intermediate timepoints of 4–6 months, the corresponding numbers were 30% (3/10, V13329) and 12% (3/25, ACTG229). The predominant resistance mutation was the Leu90 → Met exchange. Out of the 78 patients which were analysed, virus from 33 patients showed this mutation. The Gly48 → Val exchange was determined in 6 patients whereas viral proteinases carrying both mutations were observed in viruses from 2 patients. Out of > 600 sequences available after ca. 1 year treatment with saquinavir alone or in combination, 19% carry a Met90 and 2% a Val48. We also have sequence data from 4 patients treated for nearly 3 years with saquinavir theray. Three of these have Leu90 → Met mutant virus whereas the position 48 is still uniformly wild-type (Gly).

Genotype data from V13330 were compared with phenotypic susceptibility data performed on plasma virus which had been isolated at matching time points. Genotypic analysis confirmed phenotypic assays in 16 out of 23 samples where direct comparisons could be made. Phenotypic resistance was defined as a 10-fold decrease in drug sensitivity compared to baseline virus and genotypic resistance as presence of mutations at position 48 and/or 90. In five instances, a resistant phenotype was not matched by a corresponding genotype which probably reflects a higher turnover of virus in the plasma compartment compared to PBMC virus. However, in two other cases, we found genotypic resistance mutations with no corresponding phenotype.

No correlation was found between RNA levels in plasma or numbers of CD4 cells at onset of saquinavir treatment and the occurence of genotypic resistance at later timepoints. Sustained decreases in viral RNA load of greater than 1 log by approximately 1 year were observed only in the combination therapy arm and corresponded closely to the absence of saquinavir resistance. The incidence of ZDV phenotypic resistance was significantly lower in the combination treatment arm compared to the ZDV monotherapy arm (18% vs. 75%).

A loss of sensitivity after prolonged exposure of virus to the drug has been observed not only for saquinavir but also for other proteinase inhibitors. Although the set of critical mutations leading to resistance may differ for different inhibitors, there may be some positions in the proteinase polypeptide where mutations can affect sensitivity to several drugs, indicating potentially broad cross-resistance. Mutations at position 10 (Leu → Arg/Phe), position 63 (Leu → Pro), position 82 (Val → Ala/Thr/Ile) and 84 (Ile → Val) have been reported to cause cross-resistance against structurally dissimilar inhibitors including saquinavir. We have analysed a large sequence

data base for possible exchanges at these positions during saquinavir therapy of up to 1 year. We have found no mutations at position 10 and no trend towards Ala/Ile at position 82 and Val at position 84. Pro63 is already a major genotype in the baseline sequences and shows only a weak increase during treatment. On the other hand, Val48 and Met90 are completely absent from baseline sequences. Thus, based on genotypic data, there is no pre-existing resistance to saquinavir, and treatment does not appear to be associated with the emergence of a cross-resistance problem.