Abstract 100

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Using Classification Trees to Explore Relationships Between Viral Genotype and Response to Lopinavir/Ritonavir-Based Regimens

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BACKGROUND

Lopinavir/ritonavir (LPV/r, Kaletra) has demonstrated antiviral activity for up to three years in a variety of HIV-infected patient populations.[1-4] In a group of 112 patients with virus selected during therapy with protease inhibitors other than LPV/r, mutations at 11 positions in protease (10, 20, 24, 46, 53, 54, 63, 71, 82, 84, and 90) were found to be associated with reduced phenotypic susceptibility to lopinavir.[5] The total number of mutations in protease appeared to predict virologic response better than any individual mutation.[6] In an observational cohort of 792 patients, mutations at 10 positions in protease (10, 20, 24, 33, 36, 47, 48, 54, 82, and 84) were found to be associated with lowered rates of virologic response.[7] Classification trees may provide interesting insights in the prediction of virologic response to a LPV/r-containing regimen based on baseline genotype and other patient characteristics.

METHODS

Patients

- A group of 792 patients with baseline genotype and follow-up viral load data in the Kaletra ATU ("Authorisation Temporaire d'Utilisation," Provisional Authorization of Use) program conducted in France ("observational cohort").
- A group of 233 PI-experienced patients with baseline genotype and who were treated with LPV/r for a minimum of 8 weeks in Phase II/III clinical trials ("clinical trials group").

Virologic Response

- For the observational cohort, response was defined was any viral load (VL) <400 copies/mL or a VL decline from baseline of at least 1.0 $\log_{10}$ copies/mL.
- For the clinical trials group, in which all patients were NNRTI-naive and received either efavirenz or nevirapine, a stricter measure of response was used: VL <400 copies/mL at Week 24 or last observation carried forward (LOCF). A similar definition of response was also applied to Week 48.

Analysis

- Binary recursive partitioning (classification tree analysis) was implemented using CART® software (Salford Systems, Inc., San Diego, CA). The classification tree is created by recursively "splitting" the data into subgroups that are internally as homogeneous as possible and externally as separated as possible. Splits occur at "nodes"; nodes with no splits are "terminal nodes." The Gini splitting criterion was used, and the final or optimal tree was determined by "pruning" (successive removal of terminal nodes, balancing tree complexity vs. misclassification rate) the maximal tree.
- Logistic regression with (forward) stepwise selection was also used to analyze the association of response with baseline and concurrent treatment variables, using a p-value of 0.05 to enter and stay in the model.

Covariates

- Individual protease mutations previously associated with reduced lopinavir susceptibility or reduced response to lopinavir, as described above.
- Mutation scores: "LPV mutation score," consisting of the number of protease mutations among positions 10, 20, 24, 46, 53, 54, 63, 71, 82, 84, and 90; "ATU mutation score," consisting of the number of mutations among positions 10, 20, 24, 33, 36, 47, 48, 54, 82, and 84; and "DAP mutation score," consisting of the number of PI resistance mutations at positions identified by the Data Analysis Plan of the Resistance Collaborative Group (10, 20, 24, 30, 32, 33, 36, 46, 47, 48, 50, 54, 71, 73, 77, 82, 84, 88, and 90).[8]
- Baseline HIV RNA level.
- NNRTI resistance: mutations at positions 103 and 181 in reverse transcriptase, NNRTI mutation score (number of mutations among positions 100, 101, 103, 106, 181, 188, 190, 225, and 236 in RT).
- Concurrent and historical treatment: number of prior PIs used, prior NNRTI use (yes or no), number of concurrent PIs, number of concurrent NRTIs, number of concurrent NNRTIs, active NNRTI use (concurrent NNRTI use and no NNRTI mutations), and new NNRTI use (concurrent NNRTI use and no prior NNRTI use).
- Adherence (clinical trials group only): Pill count of returned medication over the first 24 weeks of treatment was used to determine adherence (percentage of pills not returned vs. the number expected to be consumed). Relative adherence rates, rather than the absolute pill count-based rates, were used since pill counts typically overestimate true adherence rates.

RESULTS

Observational Cohort

- In the observational cohort, mean baseline VL was 4.8 $\log_{10}$ copies/mL, mean CD4 count was 178 cells/mm^3, mean number of prior PIs used was 3.1, and 78% of patients were NNRTI-experienced.

- Results from a stepwise logistic regression analysis indicated that lower ATU mutation score, lower number of prior PIs, and use of a new NNRTI were strongly associated with a better chance of achieving a VL <400 copies/mL or a VL decline from baseline of 1.0 $\log_{10}$ copies/mL (Table 1).

- The significance of the V82 mutation is consistent with previous reports associating concurrent mutations at positions 10, 54, and 82 with decreased virologic response to LPV/r, although response was not precluded in patients with those mutations.[9]

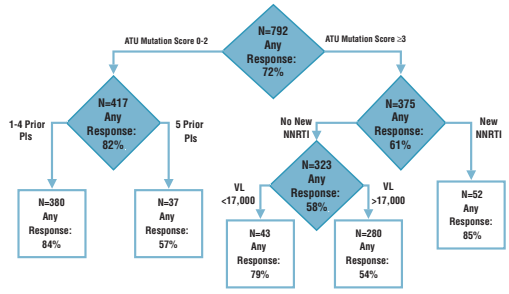
Table 1. Logistic Regression Analysis of Response in the Observational Cohort*

Covariate	Adjusted Odds Ratio (95% confidence interval)	p-value
ATU mutation score (per unit increase)	1.24 (1.10, 1.41)	<0.001
Number of prior PIs (per PI)	1.21 (1.04, 1.41)	0.012
New NNRTI use	0.43 (0.24, 0.80)	0.007
Baseline VL (per 1.0 $\log_{10}$ copies/mL increase)	1.28 (1.02, 1.62)	0.034
V82 mutation	1.54 (1.02, 2.34)	0.042

* Odds ratios above 1 indicate greater risk of failure with increasing values of the covariate.

- The final classification tree for the observational cohort contained covariates similar to those in the final stepwise logistic regression model, including ATU mutation score, prior PI use, use of a new NNRTI, and baseline VL (Figure 1).

Figure 1. Classification Tree for Any Response* (Observational Cohort)



* Any Response = VL decline of at least 1.0 $\log_{10}$ copies/mL or VL <400 copies/mL at any time.

Clinical Trials Group

- In the clinical trials group, mean baseline VL was 4.2 $\log_{10}$ copies/mL, mean CD4 count was 321 cells/mm^3, mean number of prior PIs used was 1.4, and all patients were NNRTI-naive.

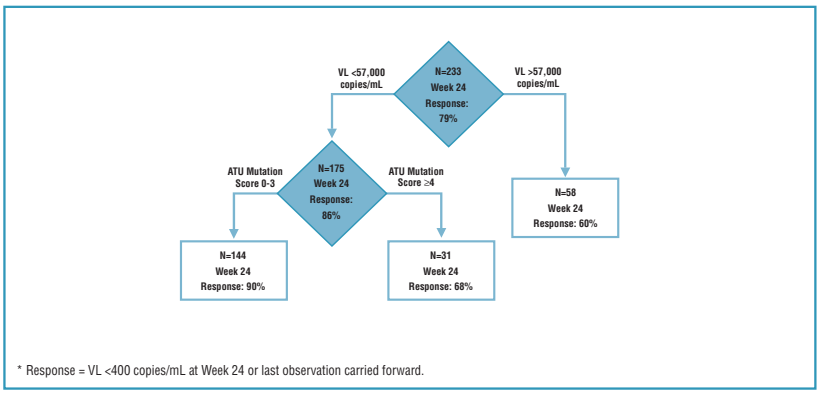
- Results from a stepwise logistic regression analysis indicated that an ATU mutation score of 3 or less and a lower baseline viral load were highly associated with a better chance of achieving HIV RNA <400 copies/mL at Week 24/LOCF (Table 2). After adjustment for these covariates, no individual mutation or other covariate was significantly associated with Week 24 response.

Table 2. Logistic Regression Analysis of Week 24/LOCF Response in the Clinical Trials Group

Covariate	Adjusted Odds Ratio (95% confidence interval)	p-value
ATU mutation score of 4 or more (vs. score of 0-3)	3.37 (1.59, 7.14)	0.001
Baseline VL (per 1.0 $\log_{10}$ copies/mL increase)	2.08 (1.33, 3.24)	0.002

- Results from the classification tree analysis identified a similar set of covariates as the logistic regression analysis (Figure 2).

Figure 2. Classification Tree for Week 24 Response* (Clinical Trials Group)

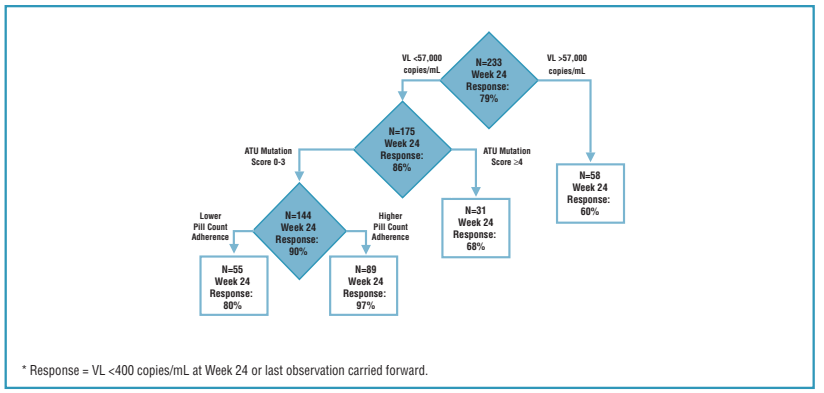


* Response = VL <400 copies/mL at Week 24 or last observation carried forward.

- The preceding analyses included only data available at baseline, and while adherence data are not available at baseline, adherence to therapy is likely to be an important factor in determining response.

- Adding adherence to the list of possible predictors results in the classification tree shown in Figure 3. Among patients with lower VL and fewer protease mutations, patients with higher pill count-based adherence (i.e., those with adherence in the highest 62% of all patients) had somewhat better virologic response.

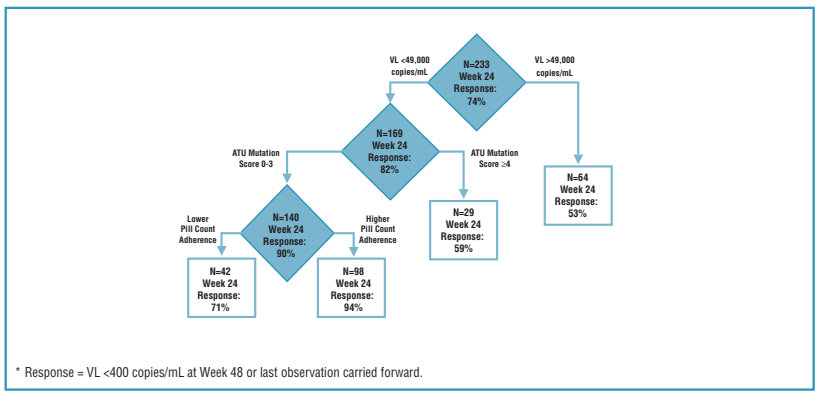
Figure 3. Classification Tree for Week 24 Response* Including Adherence (Clinical Trials Group)



* Response = VL <400 copies/mL at Week 24 or last observation carried forward.

- A classification tree was also created for Week 48 response (Week 48 or LOCF VL <400 copies/mL). The resulting tree has the same nodes as the tree for Week 24/LOCF shown in Figure 3, although the VL cutoff was slightly lower for predicting Week 48 response (Figure 4). Among patients with lower VL and fewer protease mutations, patients with higher pill count-based adherence (i.e., those with adherence in the highest 70% of all patients) had somewhat better virologic response.

Figure 4. Classification Tree for Week 48 Response* Including Adherence (Clinical Trials Group)



* Response = VL <400 copies/mL at Week 48 or last observation carried forward.

DISCUSSION

Covariates Associated with Lowered Response

In both groups of patients, the number of PI resistance mutations (ATU mutation score) was a more important predictor of response than any individual mutation. This result supports previous reports suggesting that an accumulation of several PI resistance mutations, rather than the appearance of mutations at one or two specific positions in protease, is necessary to significantly impact response to LPV/r therapy.[5,9]

In the observational cohort, use of a new NNRTI was important for patients with more PI mutations at baseline. One interpretation is that in NNRTI-naive patients with a larger number of PI resistance mutations, introduction of an NNRTI may compensate for a reduction in the antiviral activity of LPV/r: patients with an ATU mutation score of 0-2 had a response rate (82%) similar to that in patients with an ATU mutation score of 3 or more and new NNRTI use (85%).

A small subset of patients in the observational cohort had previously used 5 prior PIs but had a mutation score of 0-2, suggesting chronic poor adherence, although adherence data are not available to confirm this possibility.

In this analysis of PI-experienced patients, baseline viral load was observed to be a significant predictor of response. By contrast, in two previous studies of antiretroviral-naive patients, baseline viral load was not significantly associated with virologic response to LPV/r.[10,11] These differences may result from erosion of the genetic barrier and lowered susceptibility to LPV in PI-experienced patients such that replication of mutant virus is not inhibited as completely as wild type virus. In the absence of complete inhibition of replication, the magnitude of the initial viral load would be expected to impact the likelihood of response.

Classification Trees vs. Logistic Regression

In most cases, the classification tree analysis identified covariates similar to those identified with logistic regression analysis. However, the graphical presentation of the classification tree analysis may have the following advantages over the results of the logistic regression.

- Interactions may be easier to identify. For example, while use of a new NNRTI in the observational cohort was a significant covariate in both analyses, the classification tree suggests that it may be more important in patients with more PI mutations, as discussed above.
- It may be more intuitive to trace through the classification tree with a set of patient data rather than to predict response based on results from a logistic regression analysis.

Robustness of Classification Trees

While classification trees are gaining in popularity and can provide interesting insights, a number of caveats apply.

- The large number of parameters that can be adjusted results in an almost infinitely modifiable process of tree creation. For a given data set, changes in these settings may result in a number of different trees, complicating the interpretation of the data.
- In the absence of an external validation data set, it can be difficult to assess the applicability of a particular tree to future data, especially for smaller data sets, although cross-validation and bootstrap methods may provide some insight.

CONCLUSIONS

- Classification trees may provide insights into the relationship between viral genotype and response to LPV/r-based therapy that are difficult to discern using more traditional statistical methods such as logistic regression.
- The interpretability of classification trees may simplify the communication of such relationships.
- Mutation score was more strongly associated with response than any individual PI mutation in both patient populations. Baseline VL (both populations) and concurrent and prior treatment data (observational cohort) were also important predictors of response.
- Better adherence to LPV/r therapy was also associated with response in a subset of patients in the clinical trials group. Effects of adherence were more easily identified in patients with more susceptible virus.
- Virologic response appears more likely when LPV/r is used earlier in the treatment sequence.

REFERENCES

1. Thompson M, et al. 8th ECCATH, Athens, Greece, October 2001. Abstract P225.
2. Hicks C, et al. 8th ECCATH, Athens, Greece, October 2001. Abstract P220.
3. Walmsley S, et al. *N Engl J Med* 2002;346:2039-46.
4. Danner S, et al. 41st ICAAC, Chicago, IL, December 2001. Abstract I-1925.
5. Kempf D, et al. *J Virology* 2001;75:7462-7469.
6. Kempf D, et al. Analysis of the virologic response with respect to baseline viral phenotype and genotype in protease inhibitor-experienced HIV-1-infected patients receiving lopinavir/ritonavir therapy. *Antiviral Ther* 2002 (in press).
7. Isaacson J, et al. 9th CROI, Seattle, WA, February 2002. Abstract 559-T.
8. HIV Resistance Collaborative Group. Data analysis plan for resistance studies. 1999.
9. Kempf D, et al. *Antiviral Ther* 2001;6(Suppl 1):47.
10. White AC, et al. 1st IAS Conference on HIV Pathogenesis and Treatment, Buenos Aires, Argentina, July 2001. Abstract 217.
11. King M, et al. 9th CROI, Seattle, WA, February 2002, Abstract 470-M.