Author's response to reviews

Title: DR_SEQAN: a PC/Windows-based software to evaluate drug resistance using human immunodeficiency virus type 1 genotypes

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Author's response to reviews: see over
Dear Editor,

Thank you very much for your e-mail of October 18th, regarding our manuscript entitled “DR_SEQAN: a PC/Windows-based software to evaluate drug resistance using human immunodeficiency virus type 1 genotypes”. We are pleased to submit a revised version of the manuscript with the corresponding figure, tables and supplementary file.

In response to the comments of the reviewers, we have introduced modifications in both the manuscript and the software:

**Reviewer no. 1**

**Major compulsory revisions:**

1) Tables 2 and 3 of the manuscript showing the accuracy of DR_SEQAN in comparison with other publicly available algorithms have been included in the ‘Help’ of the program.

2) The current version of DR_SEQAN and its date of release are provided in the corresponding windows of the program.

3) In the revised version of the software we have introduced the title “Drug susceptibility data collected from the literature” for the report on the right side of the screen. The title of the report shown on the left side is “Resistance prediction”.

4) Programs whose predictions are based on objective quantitative modeling (SVM method, Beerenswinkel et al. 2003; or linear regression model, Wang et al. 2004) use training data sets. Therefore, drug resistance mutations under-represented in the corresponding data sets may not be detected with those algorithms (i.e. mutations tested using HIV clones obtained by site-directed mutagenesis). However, rule-based algorithms allow the introduction of specific rules considering mutations that may appear less frequently (this is now referred to in the revised manuscript at p. 3, last paragraph).

5) As correctly pointed out by the reviewer, there are situations where the results of the phenotypic assays (i.e. PhenoSense) are not consistent with the clinical response to antiretroviral drugs. For example, thymidine analogue resistance mutations (i.e. D67N, K70R, T215Y, etc.) are good predictors of failure to AZT and d4T therapy in the clinical setting, although there is no cross-reactivity between both drugs in phenotypic assays. The limitations of the use of phenotypic data for testing the accuracy of the prediction algorithms is now discussed in the revised version of the manuscript at p. 8, lines 1-5.

**Minor compulsory revisions:**

1) We have added the corresponding text to the symbols shown at the top of the screen.

2) We have replaced “Virologics” by “Monogram Biosciences”, where appropriate (i.e. at p. 7, 2nd paragraph).

**Discretionary revisions:**

1) The text “For optimal performance, display settings should be set at 1020 x 768” has been included at the bottom of the program’s main window.

2) We agree with the reviewer that for protease inhibitors, the accuracy of DR_SEQAN increases with a higher number of drug resistance mutations. However, we feel that a careful analysis of this question requires a larger data set, and will be studied in future.

**Reviewer no. 2**

**General**

In the revised manuscript we have included a small comment indicating that the output of DR_SEQAN shows the positions where sequence information is missing (this is a feature that the reviewer found
interesting in the program and we overlooked in our description) (see revised manuscript at p. 7, last two lines of the first paragraph).

In addition, in the revised version of the software, the user can check the complete alignment generated by BLAST. Reference strain in this alignment is HXB2, an HIV-1 subtype B virus.

**Major compulsory revisions:**

1) Certainly we agree with the reviewer that the interpretation software is not the appropriate place to do sequence editing. However, sequence ambiguities are not unusual in the clinical setting and we feel that we should try to provide users with a comprehensive tool. Therefore, we decided to keep the “heterogeneities” modules in the program, although modified according to the reviewers’ opinions:

- The window used for the analysis of heterogeneities has been modified in the revised version of the program to indicate the actual codon found in the sequence, together with the amino acid substitutions that could be relevant for resistance.

- Then, at a given position (i.e. residue 82 of the protease), the user can select either the wild-type sequence or the mutation that he/she thinks that is appropriate (wt, V82A, V82T, V82S, V82F, or V82L). If no selection is made, then the program assumes that the residue at this position should be an “X” (i.e. V82X). For assistance, a genetic code is provided in the ‘Help’ section of the program.

- Since it is prudent to assume the worst case when making a drug resistance prediction, we consider the worst scenario when an undefined position (i.e. V82X) is found in the sequence (in this case, the algorithm assumes V82A for indinavir, lopinavir and lopinavir resistance prediction, and V82T for tipranavir resistance prediction).

- The big bug found in the program regarding interpretation of sequences containing one ambiguous position has been corrected.

- The sentence “if ambiguities in the nucleotide…is displayed” has been deleted.

2) Within this specific point, the reviewer cautioned about two serious drawbacks:

2.1) First, he refers to concept of boosting protease inhibitors with low-dose ritonavir and the consequences for resistance interpretation. In our opinion, this is a difficult issue because the available literature is relatively scarce. Furthermore, boosting effects affect the levels of protease inhibitors, but do not result in different patterns of mutations. Only Retrogram makes slightly different predictions for boosted and non-boosted PR inhibitor treatments. Furthermore, the rules of the ANRS algorithm for saquinavir, nelfinavir and amprenavir are really designed for their corresponding ritonavir-boosted formulations. In addition, with the exception of lopinavir (provided as Kaletra) or atazanavir, the vast majority of the IC50 values obtained with phenotypic assays and reported in the literature have been obtained in the absence of low doses of ritonavir. Therefore, we believe that boosting effects is a clinically important issue but we feel that making different predictions for boosted and non-boosted regimens could be difficult and perhaps misleading until more extensive studies are done to pinpoint critical differences.

The problem of the cut-off values in the case of lopinavir and other protease inhibitors derives from limitations in the phenotypic assays. Although it is true that initially the FDA set up a 10-fold cut-off for reduced susceptibility to lopinavir, a recent paper published by Monogram Biosciences’ scientists (Parkin et al. Antimicrob Agents Chemother 2004; 48: 437-443) showed that the clinically relevant cut-off value for the PhenoSense assay was 1.6-fold for lopinavir resistance, which is consistent with the cut-off value we used in Tables 2 and 3 (a note mentioning this paper has been introduced under footnote ‘a’ to Table 2). Other cut-offs given by Parkin et al. for other inhibitors were similar to those used in our analysis.

2.2) We agree with the views of the reviewer on the substantial overestimation of resistance for protease inhibitors. We have modified the rules of DR_SEQAN’s algorithm for saquinavir, ritonavir, indinavir and nelfinavir to avoid some combinations of secondary mutations that reportedly had no significant effect on resistance. Interestingly, these modifications of the algorithm have improved the accuracy of DR_SEQAN in comparison with the other algorithms when measuring its ability to predict drug-susceptible isolates (see Table 2 in the revised manuscript).

3) We believe that treatment of antagonistic mutations is better in DR_SEQAN than in the other algorithms, mainly because of the larger number of antagonistic mutations used by DR_SEQAN’s algorithm in comparison to the other methods. To the best of our knowledge, antagonistic mutations have
not been included in any of the ANRS or RegaInst rules. It is true that the Stanford system makes a more sophisticated use of those mutations in predictions obtained using version 4.1.4, but only those affecting AZT (i.e. K65R, L74V, L100I, Y181C, M184I/V) and tenofovir (i.e. M184I/V) resistance are considered, without any mention of the antagonistic mutations for amprenavir or delavirdine, or the effects of foscarnet resistance mutations on AZT susceptibility. We have clarified this part of the discussion in the revised manuscript (p. 7, lines first paragraph).

**Minor compulsory revisions:**
Where appropriate, corrections indicated under points (1) to (5) have been introduced in the revised manuscript.

**Discretionary revisions:**

1) Our intention has been to include all mutations whose role in drug resistance is significant and has been documented. We agree with the reviewer that I47A and I50L should have been included. In the revised version of the program we have included those two mutations, while I47A and I50L have been incorporated into the lopinavir and atazanavir interpretation algorithms, respectively. We have decided not to include V82M since its role in protease inhibitor resistance has not been formally demonstrated yet. The only information on this specific mutation that we found was a recent abstract showing that in HIV-1 subtype G, an equivalent mutation (I82M) develops under drug pressure (Camacho et al. Antivir Ther 2005; 10: S151), and an older abstract referring to I82M and I82F as potential drug resistance mutations in HIV-2 (Descamps et al. Antivir Ther 2002; 7: S114).

Regarding other mutations such as R8Q or Q145M, we feel that they may not be relevant due to their low prevalence in the HIV-infected population, but in vitro evidence appears to be solid. We agree with the reviewer in his remark on the significance of K65R for lamivudine resistance. Therefore we have included this mutation in the corresponding algorithm (Supplementary file 1, p. 1).

2) As indicated in our response to major point 2.1, cut-offs for the PhenoSense assay have been determined by Parkin et al., based on the correlation of phenotypic data and clinical response. These cut-off values were used for testing the accuracy of the prediction methods since the phenotypic data in the reference data set were obtained with the PhenoSense assay (formerly provided by Virologic). When referring to the values given in the “Drug susceptibility data collected from the literature” on the right side of the program’s output, the cut-offs are different because there are different phenotypic assays involved and realistic cut-off values are difficult to assign. Arbitrary cut-off values (referred to in the revised manuscript, at p. 8, last paragraph) have been assigned assuming that we are dealing with different types of drug susceptibility determinations.

   The average-fold increase of the IC50 reported is usually a median value, although in cases where discrepancies were found we indicate the most trustable value.

3) We agree with the reviewer that it is perhaps odd not to include the red category for tenofovir and protease inhibitor resistance. For tenofovir, we think that available evidence is not sufficient and clear enough to define a “high-level” category. For protease inhibitors, our feeling is that the distinction between orange and red is not clear enough to define different sets of rules. In addition orange has been defined as “significant resistance”, and this should be enough to avoid the use of the corresponding inhibitor. The reviewer is right when noting the discrepancy in the nomenclature between Table 1 and the text and supplementary file, regarding the rules, and we have corrected this in the revised version of the manuscript (see Table 1).

4) We are working on the printing and storage functions of the program but this is something that we plan for the future.

In addition to the reviewer’s comments, we have updated the internal database of the program with more phenotypic data, collected from 20 more publications published in 2005, and the number of entries has gone up to 6,231 (see revised manuscript p. 5, last paragraph). We have also included an additional rule for partial resistance (yellow) to emtricitabine in the presence of K65R, based on data reported in a recent paper by White et al. AIDS 2005; 19: 1751-1760.

Finally, we are very grateful to both reviewers for their helpful comments. At this point we believe that we have responded to all their compulsory points, and almost all of their discretionary revisions, and this has
resulted in improved versions of the software and the manuscript. However, please let us know if additional modifications are necessary.

Thank you very much for your attention.

Sincerely,

Luis Menéndez-Arias
César Garriga