HIVDB Genotypic Resistance Test (GRT) Interpretation System

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Description and derivation

The purpose of the Stanford HIV Drug Resistance Database (HIVDB) genotypic resistance interpretation system is to provide educational material to physicians who order genotypic resistance tests (GRT) for nucleoside reverse transcriptase (RT) inhibitors (NRTIs), nonnucleoside RT inhibitors (NNRTIs), protease inhibitors (PIs), and integrase strand transfer inhibitors (INSTIs). One of the purposes of this educational material is to help physicians make treatment decisions for patients undergoing genotypic resistance testing. The HIVDB GRT interpretation system is updated as new studies on HIV drug resistance are published and as treatment guidelines evolve.

The HIVDB GRT interpretation system is a rules-based system that estimates NRTI, NNRTI, PI, and/or INSTI susceptibility using antiretroviral (ARV) penalty score for drug-resistance mutations (DRMs) in an HIV-1 protease, RT, or integrase sequence. ARV penalty scores (which are alternatively referred to as DRM penalty scores) have been created for both individual DRMs and combinations of DRMs. The interpretation system reports five different possible levels of drug resistance (alternatively described as reduced susceptibility) to each ARV: Susceptible, Potential low-level resistance, Low-level resistance, Intermediate resistance, and High-level resistance. The relationship between the DRM penalty scores and the five levels of reduced susceptibility are described in “DRM penalty scores” section of the Knowledgebase appendix.

The HIVDB GRT interpretation system classification "Susceptible" is assigned when a virus displays no evidence reduced susceptibility when compared with a wild-type virus. "Potential low-level resistance" is assigned when a virus has DRMs consistent with previous ARV exposure or contains DRMs associated with resistance only when they occur with other DRMs. "Low-level resistance" is assigned when a virus has DRMs associated with a reduction in vitro ARV susceptibility or a suboptimal virological response to ARV treatment. "Intermediate resistance" is assigned when, although there is a high likelihood that an ARV’s activity would be reduced in the presence of a virus’s DRMs, the ARV would likely still retain significant antiviral activity against the virus. "High-level resistance" is assigned when a virus has DRMs predicted to confer a level of resistance similar to that observed in viruses with the highest levels of reduced in vitro susceptibility or in viruses that have little or no virological response to ARV treatment.

The DRM penalty scores are created with two purposes in mind. First, they are meant to be educational in that the level of the score is an indicator of the magnitude of a DRM’s effect on the clinical activity of an ARV. More importantly, however, the scores are titrated so that when multiple mutations, the sum of the DRM penalty scores for an ARV will result in an estimate of reduced susceptibility for that ARV that would be consistent with the published literature and expert opinion. This is an important consideration in the design of penalties that only trigger when a combination of DRMs is present. It is for this reason that we have sections on our web site listing the summed DRM penalty scores for every pattern of DRMs that exists in HIVDB (“DRM pattern scores” section of the Knowledgebase appendix).

In addition to the DRM penalty scores and the estimated levels of reduced ARV susceptibilities, the mutation comments represent an essential component of the HIVDB GRT interpretation system (“DRM comments” section of the Knowledgebase appendix). Some users of the system have reported that they find the comments as informative if not more informative than DRM penalty scores and reduced ARV susceptibilities. The DRM comments should be considered an inseparable part of the HIVDB GRT interpretation system.

Finally, for each drug class there is a web page entitled “Notes” containing a frequently updated summary of the literature on NRTI, NNRTI, PI, and INSTI DRMs. The Note pages are expanded versions of the mutation comments. They also differ from the mutation comments in that they contain references to the peer-reviewed literature. Overall, there are more than 400 references cited pertinent to the effects of specific DRMs on ARV susceptibility.

The DRM penalty scores and comments are created and updated on the basis of the following six main types of data/considerations:

(i) The prevalence of a mutation in untreated (ARV-naïve) patients. As each of the NRTIs, NNRTIs, PIs, and INSTIs are highly active in ARV-naïve patients, there is a general consensus in the field that highly polymorphic mutations (e.g., those that occur in the absence of selective pressure) are unlikely to contribute greatly to reduced ARV susceptibility;

(ii) The prevalence of a mutation in treated patients. The vast majority of DRMs are nonpolymorphic mutations that occur significantly more often under ARV selection pressure. Therefore, in the absence of in vitro phenotypic data or clinical data, this type of prevalence data can indicate that a mutation may be associated with reduced susceptibility. These data are obtained from the published literature and are supplemented with data in HIVDB.

(iii) In vitro phenotypic data. Correlations between viral genotype and phenotype are the most straightforward form of data about HIV-1 drug resistance. Unfortunately, there are insufficient publicly available high-quality phenotypic data associated with many patterns of DRMs. Moreover, although many DRMs have a minimal effect on ARV susceptibility when they occur alone, they can be markers for the presence of other DRMs that are likely to emerge with continued selective drug pressure. These data are obtained from the published literature and are supplemented with data in HIVDB.

(iv) Correlations between genotype and virological suppression are relevant because sustained virological suppression is the main goal of ARV therapy. There have been several highly informative clinical trials that have demonstrated associations between specific pre-therapy DRMs and the risk of VF. However, many retrospective studies have had too few patients relative to the large number of covariates associated with response to therapy. Moreover, most retrospective studies have been confounded by the fact that the results of GRT were used to guide the choice of therapy. These data are obtained from the published literature.

(v) The FDA package insert often provides information on the most relevant DRMs identified during in vitro passage experiments with an ARV and in the ARV’s earliest clinical trials.

(vi) Expert opinion. Expert opinion is obtained from the published literature, the IAS-USA HIV Drug Resistance Mutation List updates (Gunthard et al, Clin Infect Dis 2018; PMID 30052811) a recent publication by Paredes et al entitled “Collaborative update of a rule-based expert system for HIV-1 genotypic resistance test interpretation.” (PLoS One 2017; PMID: 28753637), informal discussions, and feedback from many expert users.

In September 2019, we created an Advisory Committee to review and discuss updates to the HIVDB scoring system. The current members of the Committee are Jonathan Schapiro MD (Tel Hashomer Hospital, Tel Aviv, Israel), Daniel Kuritzkes MD (Harvard Medical School, Boston, USA), and Michele Moorehouse, MD (University of Witwatersrand, Johannesburg, South Africa). (https://hivdb.stanford.edu/pages/Committee_HIVdb.html)

In addition to the DRM penalty scores, comments, and Notes pages, the HIVDB knowledgebase includes the following additional material: (i) A classification system for each of the mutations (defined as differences from the subtype B consensus reference sequence ("DRM classification" section in the Knowledgebase appendix); (ii) A section with detailed notes about DRMs that includes literature citations; and (iii) tab-delimited files containing data that are used to assist with the quality control of sequences submitted to the HIVDB GRT interpretation system including lists of unusual mutations, signature APOBEC-associated mutations, and DRMs that can be caused by APOBEC each of which are available in the program's Release Notes.

The HIVDB GRT interpretation system has two main limitations. First, it lacks the heuristic power to provide specific ARV treatment recommendations because it does not integrate the additional clinical data needed by clinicians to choose therapy. These data include a patient's past treatment history, previous GRT results, plasma HIV-1 RNA levels, and information on the likelihood of adherence. Indeed, most of the studies demonstrating the predictive value of HIV-1 GRT rule-based systems have been performed using multivariate models that have at a minimum included patients' past treatment histories and plasma HIV-1 RNA levels. Second, the interpretation system is subjective in that It relies on expert opinion to prioritize the relative importance of the various forms of data underlying HIV drug resistance knowledge.

Program maintenance and data evaluations

The following circumstances lead to HIVDB GRT interpretation system updates: (i) the FDA approval of new NRTIs, NNRTIs, PIs, or INSTIs; (ii) the publication of new information about DRMs; (iii) feedback from users of the system; and (iv) informally scheduled comprehensive reviews of all of the system's DRM mutation penalty scores and mutation comments.

When DRM penalty scores are changed, changes are also usually made to one or more mutation comments and to one or more sections of the Notes (e.g., the addition of a new reference). The program is also run on every unique pattern of DRMs and each changed interpretation is reviewed to ensure that the change is considered superior to, or at least equivalent to, the pre-existing interpretation. For each drug class, there is also an interactive mutation penalty editor (e.g., https://hivdb.stanford.edu/dr-summary/rules-editor/INSTI/) makes it possible to evaluate the effect of new drug resistance rules (i.e., scores for individual mutations or combinations of mutations) on the complete set of distinct mutation patterns for a drug class.

Each of the changed scores and changed interpretations are posted on the Program Updates section of the system's Release Notes. Each of the web service users are notified of changes to the interpretation system a minimum of one week in advance.

Appendix I. Knowledgebase

(1) **DRM penalty scores.** For each ARV class, there is a table that lists each DRM and its associated scores for different ARVs, followed by a list of DRM combinations and their associated scores. Mutation penalty scores are multiples of 5 and ranged from -15 (increased ARV activity) to 60 (loss of ARV activity). ARV activity is estimated by adding the penalties for each DRM in a sequence and converting the total score to one of five interpretations: (i) "Susceptible", total score <10; (ii) "Potential low-level resistance", total score between 10 and 14; (iii) "Low-level resistance", total score between 15 and 29; (iv) "Intermediate resistance", total score between 30 and 59; and (v) "High-level resistance", total score ≥60. The complete set of DRM scores are available in tab-delimited files (Release Notes) and in the following web pages:

https://hivdb.stanford.edu/dr-summary/mut-scores/NRTI/
https://hivdb.stanford.edu/dr-summary/mut-scores/NNRTI/
https://hivdb.stanford.edu/dr-summary/mut-scores/PI/
https://hivdb.stanford.edu/dr-summary/mut-scores/INSTI/

(2) **DRM comments.** For each ARV class, there is a comment for each DRM that has a penalty score and for several mutations without penalty scores. The complete set of DRM comments are available in tab-delimited files (Release Notes) and in the following web pages:

https://hivdb.stanford.edu/dr-summary/comments/NRTI/
https://hivdb.stanford.edu/dr-summary/comments/NNRTI/
https://hivdb.stanford.edu/dr-summary/comments/PI/
https://hivdb.stanford.edu/dr-summary/comments/INSTI/

(3) **DRM Classifications.** PR mutations are classified as "Major", "Accessory"; or "Other. RT mutations are classified as "NRTI", 'NNRTI", or "Other"; IN Mutations were classified as "Major", "Accessory", or "Other". The classifications can be found in the tab-delimited DRM Comments files (Release Notes) and web pages.

(4) **DRM notes with literature references. F**or each ARV class, there is a web page a summary of the DRMs for that class along with literature references:

https://hivdb.stanford.edu/dr-summary/resistance-notes/NRTI/
https://hivdb.stanford.edu/dr-summary/resistance-notes/NNRTI/
https://hivdb.stanford.edu/dr-summary/resistance-notes/PI/
https://hivdb.stanford.edu/dr-summary/resistance-notes/INSTI/

(5) **DRM pattern scores.** A list of the calculated summed DRM penalty scores for every distinct DRM pattern present in HIVDB sequences. These are only available as web pages.

https://hivdb.stanford.edu/dr-summary/pattern-scores/NRTI/
https://hivdb.stanford.edu/dr-summary/pattern-scores/NNRTI/
https://hivdb.stanford.edu/dr-summary/pattern-scores/PI/
https://hivdb.stanford.edu/dr-summary/pattern-scores/INSTI/

(6) **Unusual mutations.** List of unusual mutations in PR, RT, and IN defined as mutations with a prevalence <0.01%. These are available as tab-delimited files (Release Notes).

(7) **Signature APOBEC-associated mutations.** Mutations in PR, RT, and IN strongly suggestive of G-to-A hypermutation. These are available as tab-delimited files (Release Notes).

(8) **DRMs that can arise from APOBEC G-to-A hypermutation.** These are available as tab-delimited files (Release Notes).

Appendix II. Documentation

(1) **The Release Notes.** The Release Notes provides high-level documentation for the HIVDB program (https://hivdb.stanford.edu/page/release-notes/)

(i) It describes the program's rationale, user interfaces, and output.

(ii) It contains tab-delimited files with DRM penalty scores, DRM comments and classifications, and lists of unusual mutations, signature APOBEC-associated mutations, and DRMs that can arise from APOBEC G-to-A hypermutation.

(iii) It contains the version history with detailed descriptions of each update.

(2) **Additional Supporting information.** The pages with the Drug Resistance Notes and Mutation Pattern Scores are parts of the Knowledgebase (described in the Appendix 1) not included in the Release Notes.

(3) **Software documentation.**

(i) The software is available on GitHub and is open-source (GNU General Public License v3.0)

(ii) Documentation for the HIVDB program's exposed API is in the process of being completed.

(iii) An API reference containing an explanation of every class and function is in the process of being completed.

Appendix III. Design Controls

(1) **J-unit tests.** J-unit tests are available >80% of classes and methods.

(2) **Continuous integration.** The program undergoes continuous integration to ensure that every commit is tested.

(3) **Regression testing.** The program undergoes several regression tests to ensure that code changes yield expected results.

(4) **Manual review of the effects of DRM penalty score updates.** When DRM penalty scores are changed, the program is run on every unique pattern of DRMs and the results are reviewed manually.

(5) **Code tracking.** Git.

(6) **Bug tracking.** GitHub.