

1. CORRELATES OF RESISTANCE TO LOPINAVIR

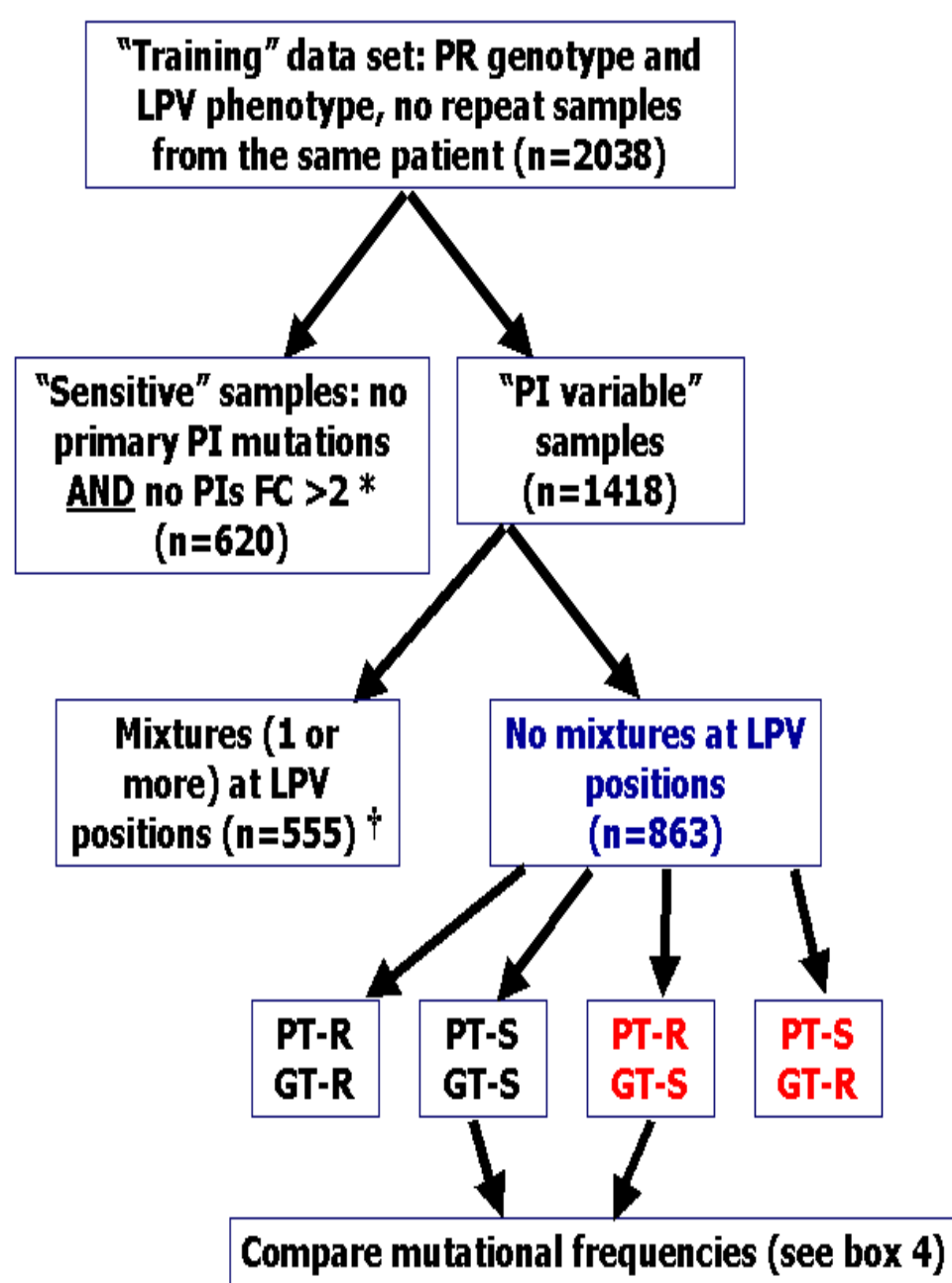
• Mutations associated with LPV phenotypic resistance from ABT clinical trial samples: L10F, I, R, or V, K20M or R, L24I, M46I or L, F53L, I54L, T, or V, L63P, A71I, L, T, or V, V82A, F, or T, I84V, L90M[†]

• Clinical response rates decrease when LPV FC > 10 or number of LPV mutations ("LPV mutation score") > 5[‡]

• **Do these results apply to the general population?**

• For this analysis, samples were defined as "resistant" by phenotype (PT-R) if the FC > 10 or by genotype (GT-R) if LPV mutation score > 5, even though some patients had a virologic response at higher levels of resistance[‡]. Thus, PT-R or GT-R refers to a virus with decreased (but not no) likelihood of response to LPV.

2. TRAINING DATA ANALYSIS



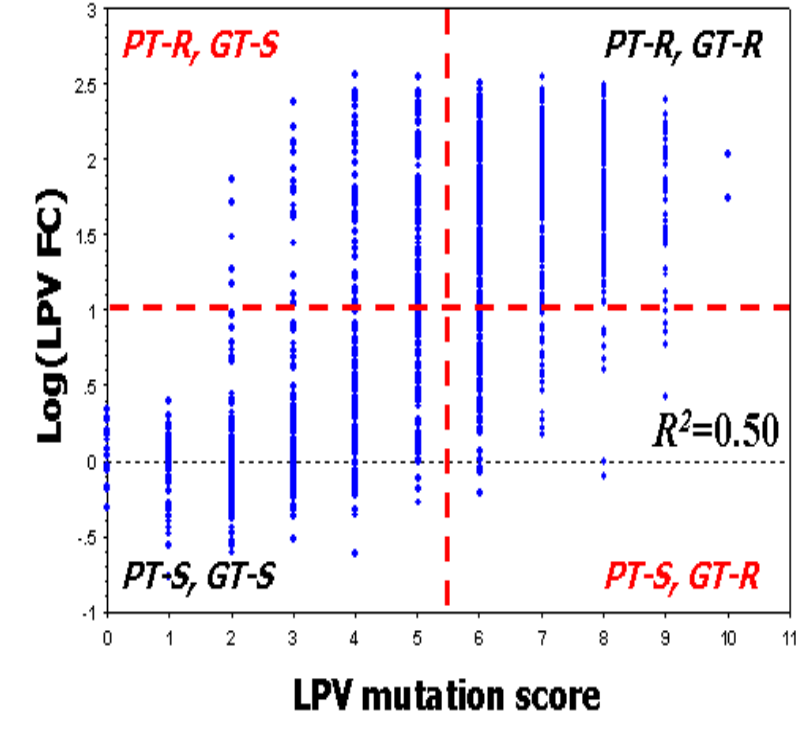
	N	PT-R, GT-R	PT-S, GT-S	PT-R, GT-S	PT-S, GT-R
All data	2038	637 (31%)	1109 (54%)	182 (9%)	110 (5%)
PI Variable*	1418	637 (45%)	489 (34%)	182 (13%)	110 (8%)
Unmixed [‡]	863	403 (47%)	282 (33%)	147 (17%)	31 (4%)

* Samples with no doubt about their LPV susceptibility are excluded i.e. no primary PI mutations (30, 32, 46, 48, 50, 54, 82, 84, 90) AND no PI with reduced susceptibility (FC > 2)

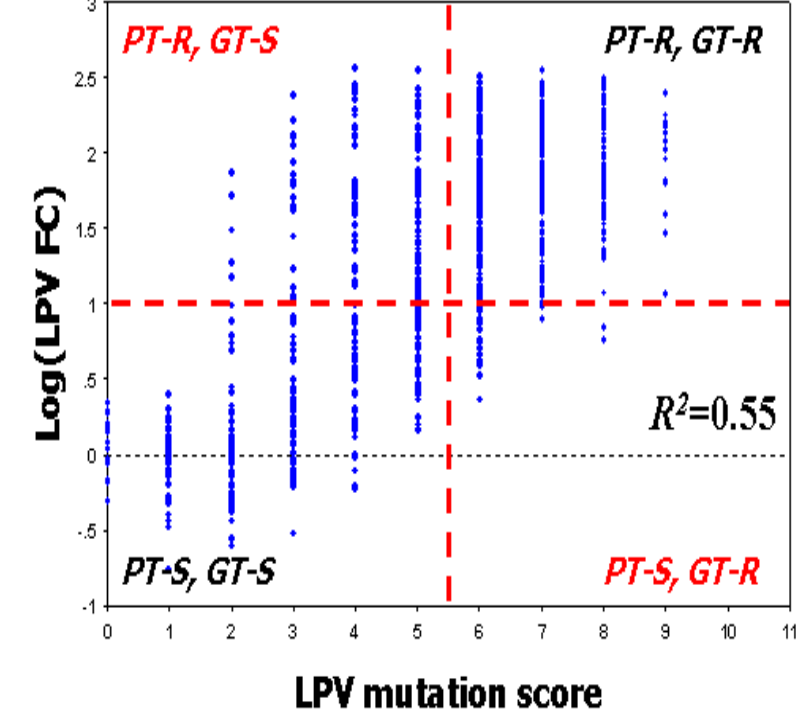
[‡] The number of samples in the PT-S, GT-R category is greatly reduced when samples with mixtures are excluded. Thus, mixtures are a large source of this type of discordance.

3. LPV SUSCEPTIBILITY VS. MUTATION SCORE

A. All samples (mixtures = mutant) n=1418



B. Mixtures excluded n=863



4. MUTATIONS ASSOCIATED WITH LPV FC > 10 IN PT-R, GT-S SAMPLES

A. Known[†] LPV mutations

Mutation	FC<10	FC>10	%R:%S	P value
I54T	0	5	>>>	0.0045
I54Y	7	47	13	<0.0001
V82A	14	73	10	<0.0001
E200M	4	10	5	0.0045
L108I	15	25	3	0.0001
M46L*	16	19	2	0.009
M46I*	49	57	2	<0.0001
L10I	105	97	2	<0.0001

B. New mutations at known positions

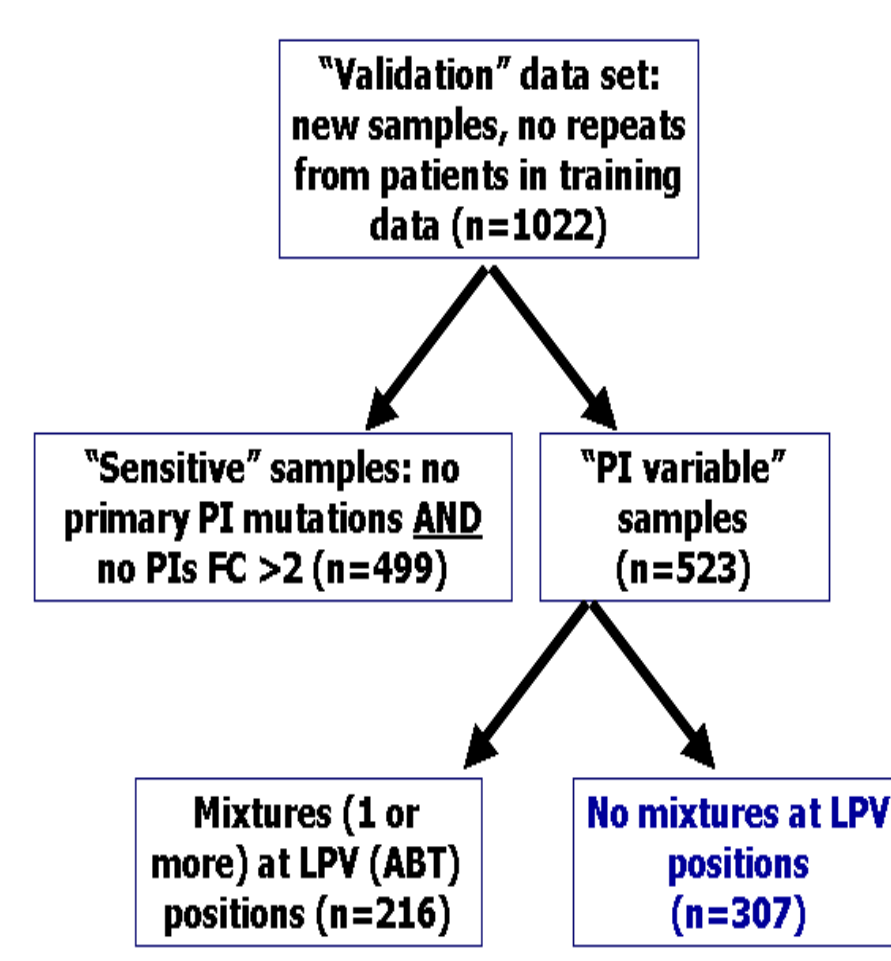
Mutation	FC<10	FC>10	%R:%S	P value
I54A	0	22	>>>	<0.0001
I54S	0	14	>>>	<0.0001
V82S	0	7	>>>	0.0005
E54M*	6	21	7	<0.0001
L63T	4	11	5	0.002
E20I	14	24	3	0.0001

C. "New" mutations

Mutation	FC<10	FC>10	%R:%S	P value
L89I	0	5	>>>	0.0045
I50V*	1	25	48	<0.0001
G48V	7	50	14	<0.0001
E34Q	2	12	12	0.0001
L76V	1	6	12	0.0076
E43T	4	22	11	<0.0001
I47V**	5	22	8	<0.0001
L33F	14	42	6	<0.0001
G14E [‡]	8	23	6	<0.0001
V32I*	9	18	4	0.0004
L89A	7	13	4	0.004
T74S	14	25	3	0.0001
Q58E	18	26	3	0.0003

* Mutations also important for APV resistance[‡]
[†] Mutations selected by ABT-378 *in vitro*, in combination with I84V[‡]

5. TESTING OF NEW LPV GENOTYPE INTERPRETATION ALGORITHM

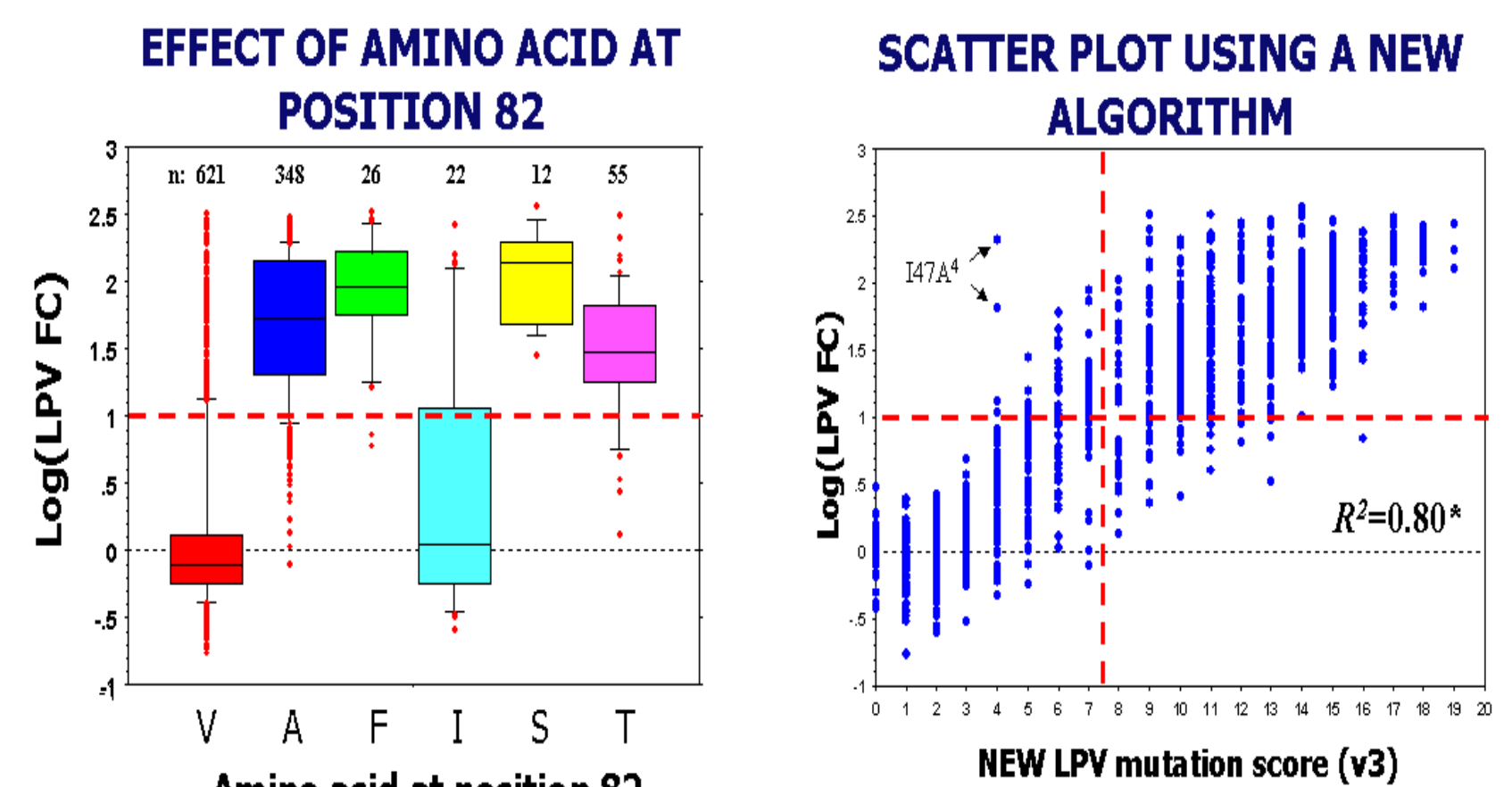
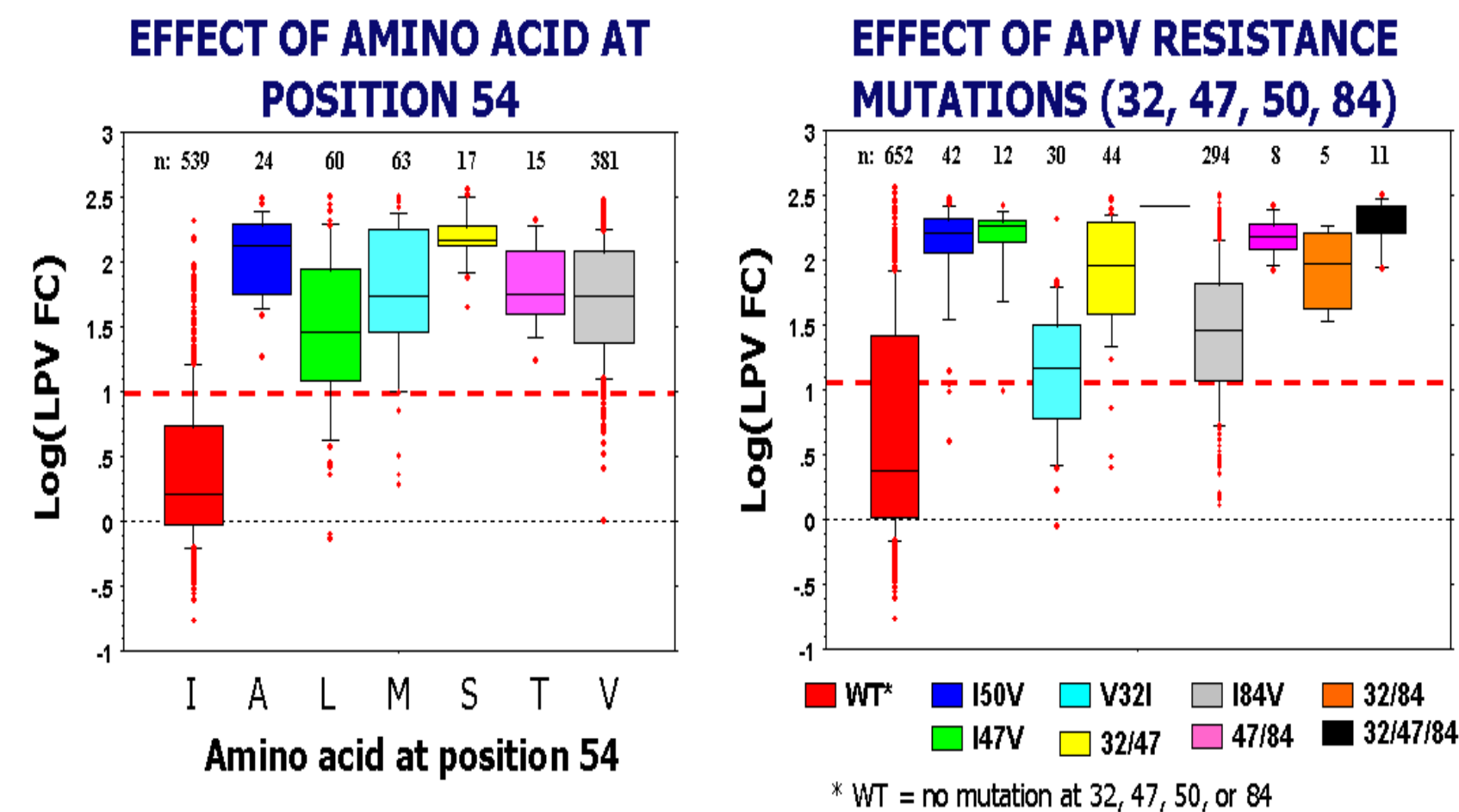


TRAINING DATA (n=863)				
rules	features	%PT-R, GT-S	%PT-S, GT-R	overall concordance
ABT	Abbott rules ¹	16.5%	3.7%	80.3%
GSv1*	add I50V, I54A/M/S, V82S	10.8%	4.2%	85.1%
GSv2	add mutations from list C	2.3%	7.4%	90.3%

VALIDATION DATA (n=307)				
rules	features	%PT-R, GT-S	%PT-S, GT-R	overall concordance
ABT	Abbott rules ¹	13.4%	2.6%	84.0%
GSv1*	add I50V, I54A/M/S, V82S	10.7%	2.6%	86.6%
GSv2	add mutations from list C	2.3%	8.1%	89.6%

*GeneSeq version 1 (implemented 6/2001)

6. ANALYSIS OF COMBINED (TRAINING + VALIDATION) DATA



• The combined data set has 2195 samples and excludes samples with mixtures at any position considered in the new algorithm (n=1100 after excluding "sensitive" samples)
• Different amino acids at the same position have variable effects on LPV susceptibility, suggesting that they should be considered differently by the interpretation algorithm
• Mutations associated with resistance to APV contribute to LPV resistance
• Longer list of mutations included in the algorithm reduced the variability in LPV susceptibility within categories defined by mutations score

7. CONCLUSIONS

1. Genotypic algorithms based on early clinical trial experience may be inadequate to account for all types of resistant variants eventually encountered.
2. Many mutations that contribute to LPV resistance are also involved in APV resistance, an observation which has implications for PI and boosted PI sequencing strategies.
3. The original ABT algorithm did not account for all pathways to LPV resistance, likely due to the treatment experience histories of the patients (n=112) studied in the clinical trials (i.e. lack of APV-experienced patients).
4. Simple counting and weighting algorithms may not be sufficient to fully explain phenotypic susceptibility. Other approaches such as regression tree analysis, as well as consideration of determinants in gag[‡], may be required for further improvement.

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REFERENCES

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