

Coexistence and coevolution of viral populations with distinct genotypes in patients failing treatment with protease inhibitors

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INTRODUCTION

The acquisition of viral resistance to protease inhibitors results from the accumulation of resistance mutations, but little data are available concerning the evolutionary pathway(s) followed during this process.
In particular, it is unclear to what extent mutants with genotypes different from the prevailing dominant resistance genotype can coexist for extended periods of time, and subsequently acquire additional mutations that permit their emergence as the dominant quasi-species.

METHODS

Seven patients were identified who experienced virologic treatment failure while continuously receiving treatment with protease inhibitors, and for whom standard genotyping ultimately demonstrated the appearance of resistant strains containing the L90M resistance mutation.

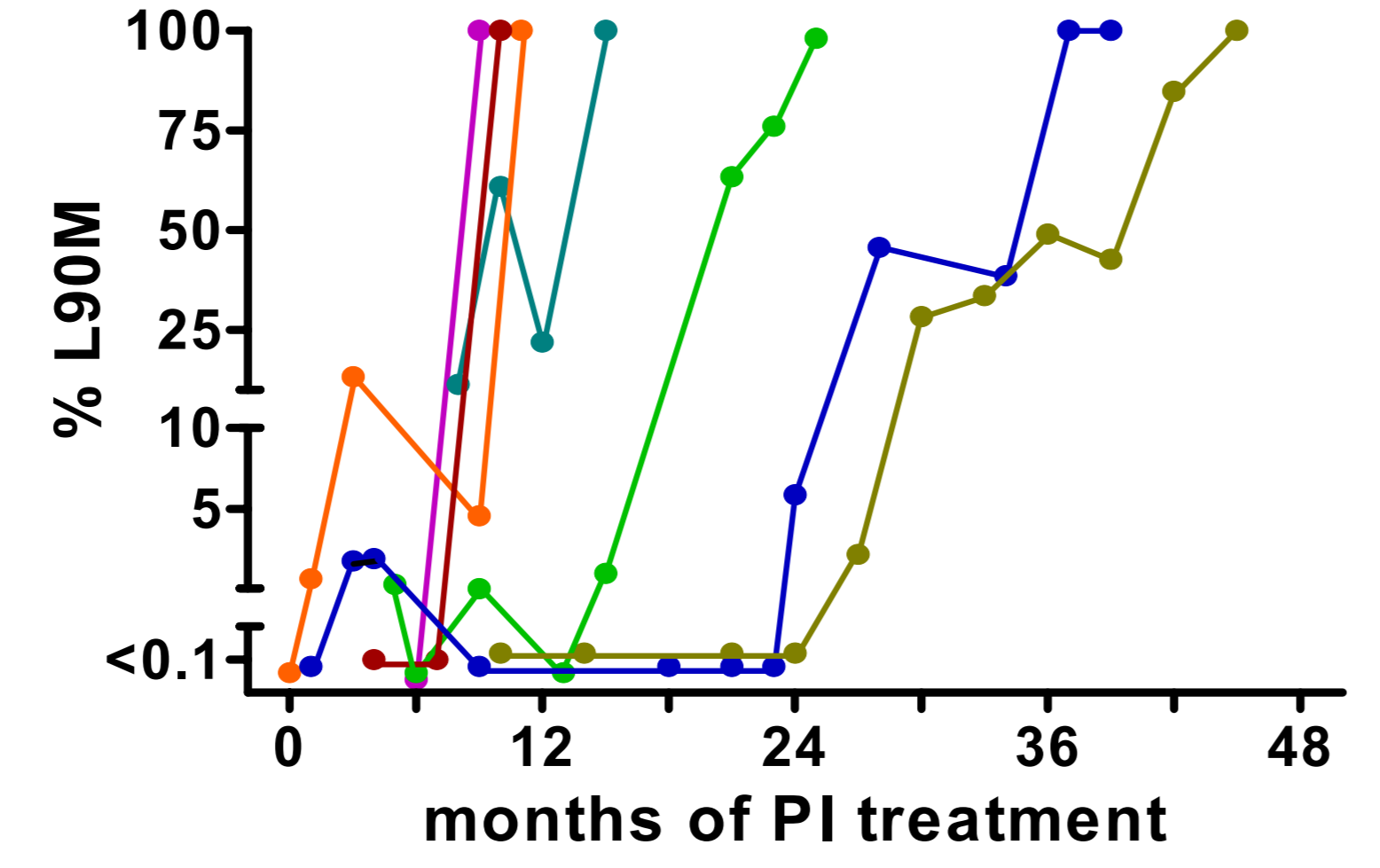
To evaluate the kinetics of appearance of the L90M mutation, viral mRNA was extracted from all archived serum samples obtained following the initiation of therapy with protease inhibitors, and the protease gene was amplified by RT-PCR. The proportion of sequences containing the L90M mutation was then determined using the previously described technique in which total viral sequences and those containing the L90M mutation are quantified by real-time PCR using primers that amplify either all viral sequences or amplify selectively sequences containing the L90M mutation. This approach can detect minority populations expressing L90M that represent as few as 0.1% of total virus.

At various times during the evolution of resistance in two patients, viral protease sequences present in plasma were amplified, using techniques that minimize recombination events (long extension times, avoidance of the plateau phase), and cloned. 50-100 clones were screened using the real-time PCR approach described above. Clones representative of the populations with and without the L90M mutation were sequenced.

The genotypes identified at various times after starting treatment with protease inhibitors are shown as dendrograms and in tabular form. The abundance of the viral populations is calculated on the basis of the percentage of clones with and without the L90M mutation. These values are generally concordant with the results obtained by evaluation of viral populations in plasma by real-time PCR.

RESULTS

For most patients, minority viral populations expressing the L90M mutation could be detected for many months before this mutation was present in the majority of plasma viruses (median 14 months, range 3-35 months). In 3 of the 7 patients studied, minority populations expressing the L90M mutation had been detectable for at least 1 year.



The evolution of resistance profiles was evaluated for two patients by sequencing clones that did and did not contain the L90M mutation. Several common features were observed in these two patients :

- 1) Soon after the beginning of treatment failure, clones emerged that gave rise to all subsequent variants. This "bottleneck" occurred at a time when relatively few resistance mutations were present.

• Patient 1 – addition of I54V + A71V + V82A
Patient 2 – addition of M46I + V82T

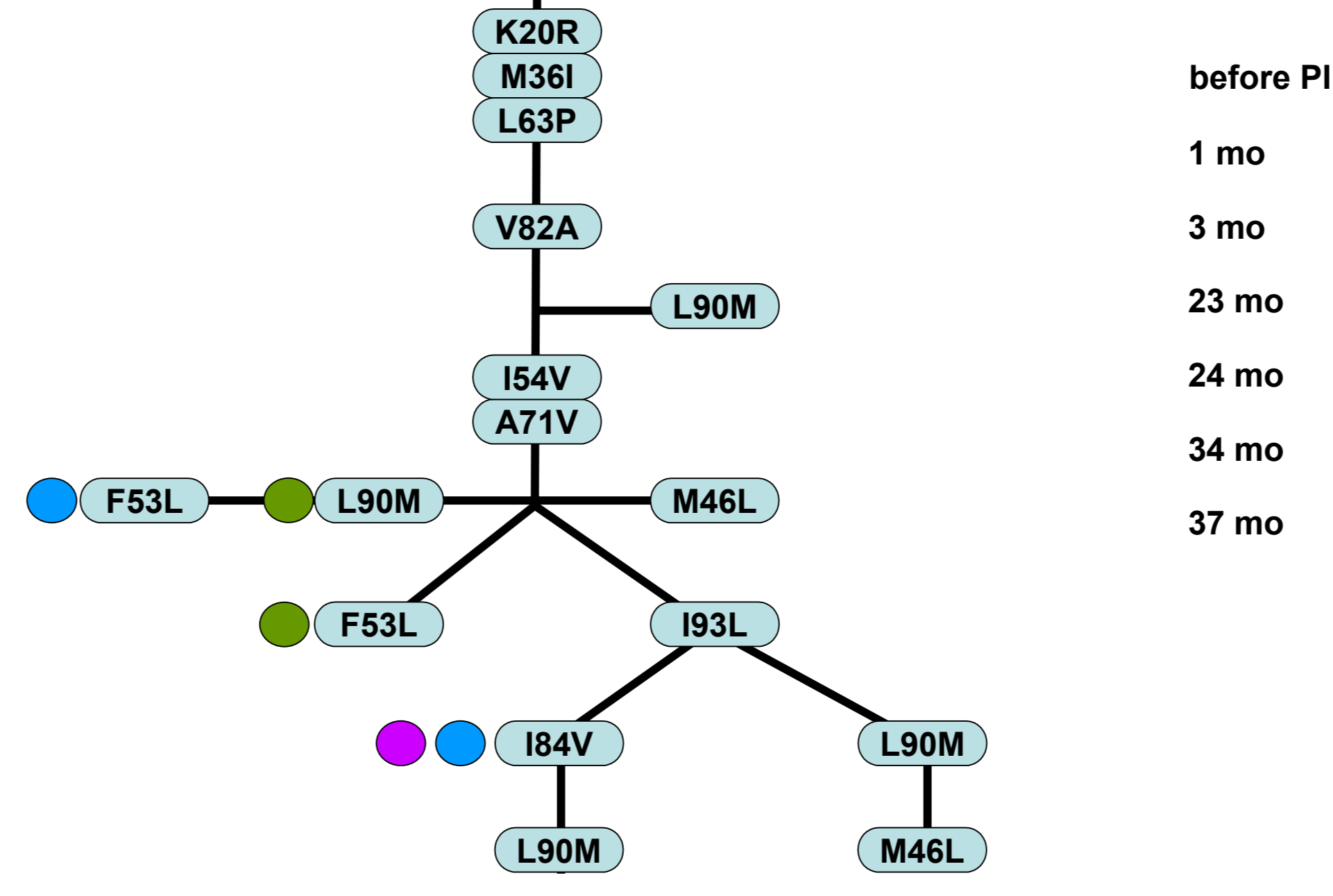
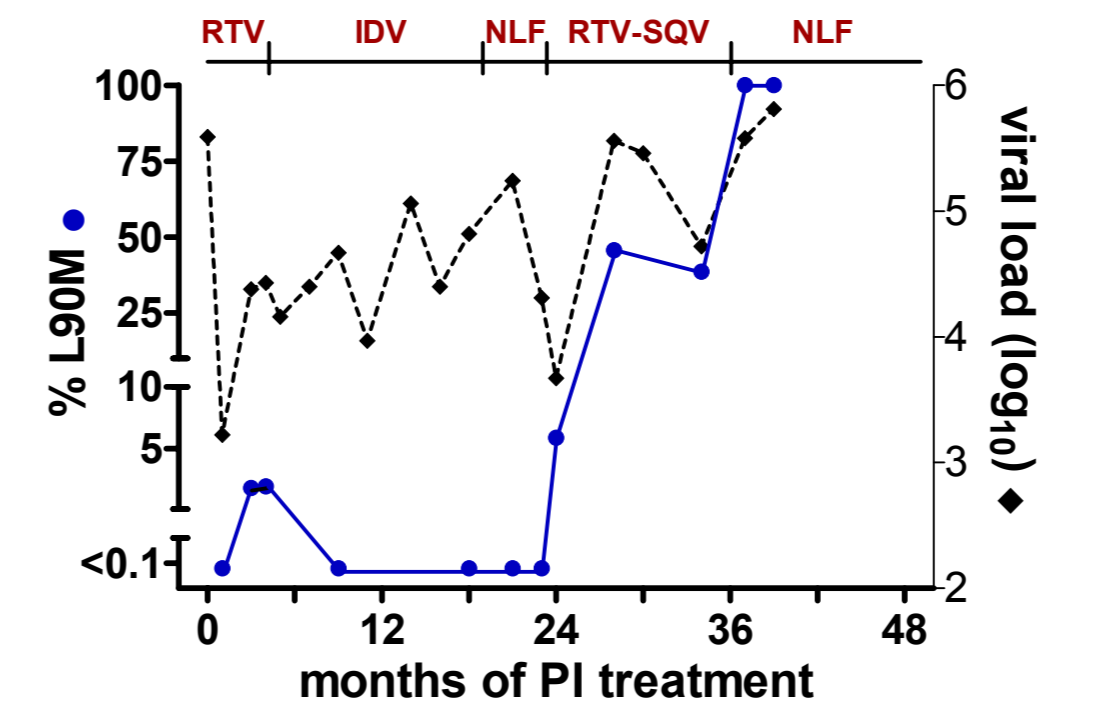
- 2) On this resistance backbone, multiple additional resistance mutations were added in varying combinations. Some mutations were only detected in minority populations (e.g., F53L and M46L in Patient 1 ; V82I in Patient 2).
- 3) In both patients, two different relatively abundant genotypes were found to coexist for several months.

Patient 1 K20R - M36I - I54V - L63P - A71V - V82A - I84V - I93L
vs
K20R - M36I - I54V - L63P - A71V - V82A - L90M - I93L

Patient 2 L24I - M46I - I54V - L63P - A71V - V77I - V82T - ± I93L
vs
M46I - I54V - L63P - A71V - V77I - V82T - L90M - I93L

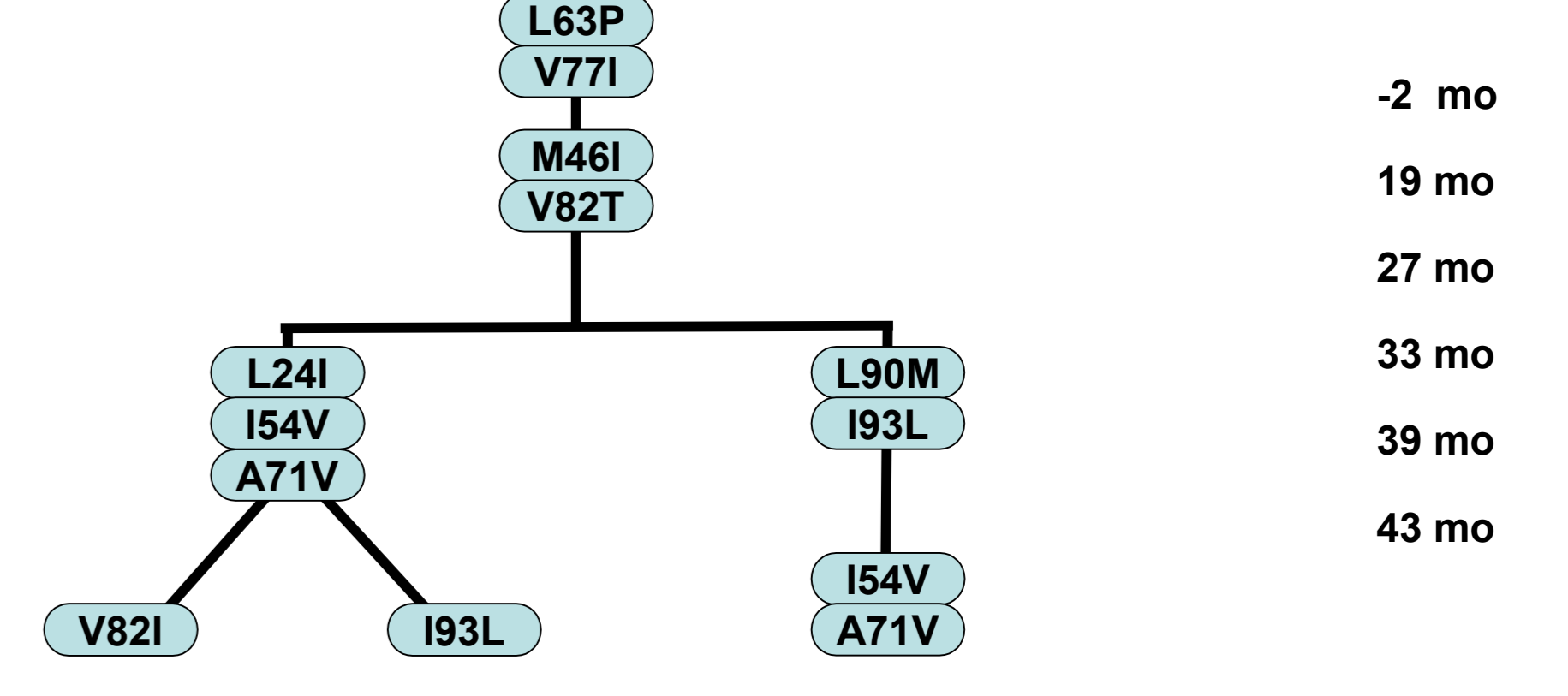
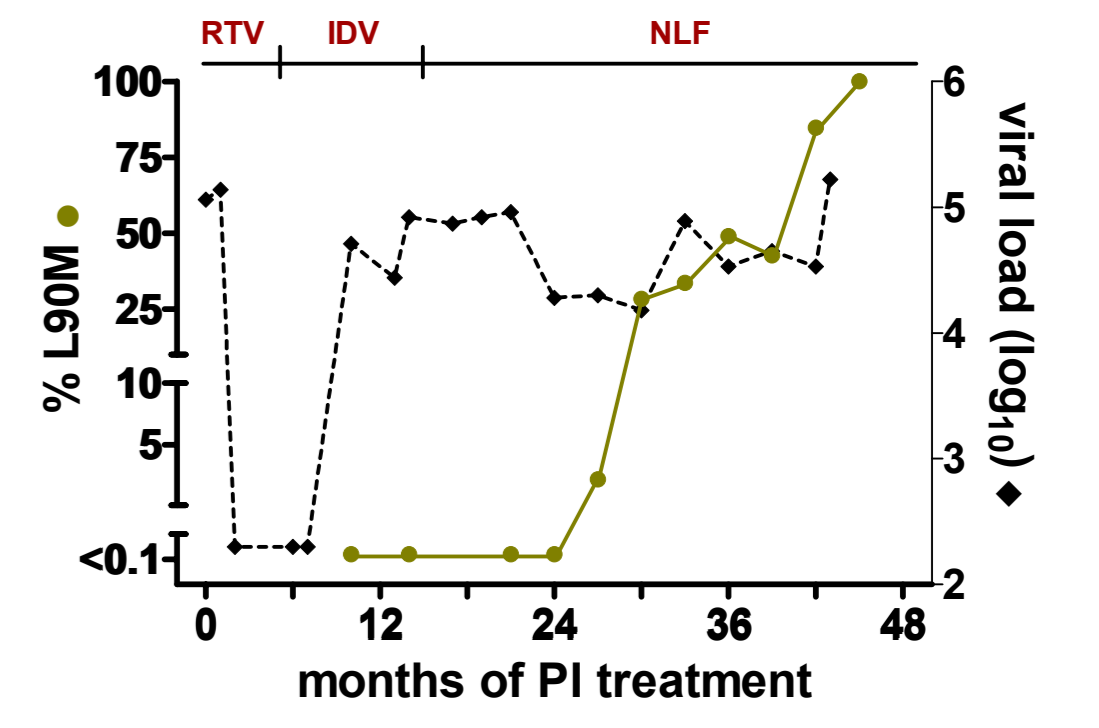
- 4) Ultimately, in the absence of changes in treatment, a single genotype became dominant.

Patient 1



| MO | GENOTYPES PRESENT | ABUNDANCE |
|------|---|------------|
| M 0 | K20R M36I L63P | 100% |
| M 3 | K20R M36I L63P V82A L90M | 98% 2% |
| M 23 | K20R M36I I54V L63P A71V V82A I93L | 25% 75% |
| M 24 | K20R M36I I54V L63P A71V V82A I93L | 89% |
| M 34 | K20R M36I I54V L63P A71V V82A I84V I93L | 73% |
| M 37 | K20R M36I I54V L63P A71V V82A I84V I93L | 11% 89% |

Patient 2



| MO | GENOTYPES PRESENT | ABUNDANCE |
|------|---|------------|
| M 19 | M46I L63P V77I V82T | 100% |
| M 27 | L24I M46I I54V L63P A71V V77I V82T M46I L63P V77I V82T L90M I93L | 99% 1% |
| M 33 | L24I M46I I54V L63P A71V V77I V82T L24I M46I I54V L63P A71V V77I V82T L90M I93L | 64% 36% |
| M 39 | L24I M46I I54V L63P A71V V77I V82T I93L M46I I54V L63P A71V V77I V82T L90M I93L | 55% 45% |
| M 43 | M46I I54V L63P A71V V77I V82T L90M I93L | 100% |

CONCLUSIONS

Quantitative evaluation of the emergence of the L90M mutation in patients failing therapy with PIs revealed two distinct patterns:

- Early and rapid emergence of the mutation in the majority viral population.
- Initial presence in minority population(s). These populations could either disappear, or, following subsequent evolution, become the dominant viral species.

Overall, these studies indicate that after an initial bottleneck, which can occur relatively early in the evolution of resistance, resistance genotypes become more diverse. Some resistance mutations are restricted to minority subpopulations that never become abundant. In other cases, minority populations with genotypes different from the prevailing majority genotype can coexist and coevolve for several months, before emerging as the dominant quasi-species.