DOCTORAL THESIS 2013

Evolution of the Human Immunodeficiency Virus type I Protease and Integrase: Effects on Viral Replication Capacity and Robustness.

Elena Capel Malo

IrsiCaixa
Institut de Recerca de la Sida

UAB
Universitat Autònoma de Barcelona

Discussion

Comparison of the sequence conservation of the Protease, Integrase and Gag genes

One of the hallmarks of HIV infection is the rapid development of a genetically complex population (quasispecies) from an initially limited number of infectious particles. Genetic diversity remains one of the major obstacles to eradication of HIV. The viral quasispecies can respond rapidly to selective pressures, such as that imposed by the immune system and antiretroviral therapy, and frustrates vaccine design efforts (Domingo *et al.*, 1997; Korber *et al.*, 2001; Más *et al.*, 2010). Thus, knowing how HIV genome diversifies remains an important issue. In this work, we compared the genetic diversification of three important genes of the virus, *gag* that encodes the viral structural proteins, and two *pol* genes, the protease that is essential for the maturation and release of new infectious particles and the integrase that is responsible for the persistence of the infection in the host by integrating the viral DNA into the host chromosome. A sequence conservation decrease was observed in the three studied genes, as shown by an increase in both mean nucleotide and amino acid p-distances from the early to late sequences. Greater genetic distance relative to the subtype B ancestral sequences were found in the protease and gag genes (mean ± SE nucleotide p-distance increase of 0.017 ± 0.003 and 0.016 ± 0.003, respectively, and mean ± SE amino acid p-distance increase of 0.016 ± 0.003 and 0.016 ± 0.002, respectively), whereas the integrase gene had a lower genetic distance (mean ± SE nucleotide p-distance increase of 0.009 ± 0.002, and mean ± SE amino acid p-distance increase of 0.006 ± 0.002), indicating that the integrase gene is more conserved. Moreover, the mean increase of nucleotide sequence diversification of proteases was also significantly higher than the one of gag sequences ($p = 0.0164$), but the mean increase of amino acid sequence diversification of proteases was not significantly higher than the one of gag sequences ($p = 1.000$). These trends confirm previous description that individual HIV-1 genes differ in their variability. Analysis of the conservation of the three genes' residues confirms that integrase protein is again more conserved than gag and protease: 91% of the integrase residues are conserved ≥97% , whereas the percentage of protease and gag conserved residues ≥97% is lower, 72% and 84%, respectively.

Estimation of synonymous and nonsynonymous substitution rates is important in understanding the dynamics of molecular sequence evolution (Kimura, 1983). As

synonymous (silent) mutations are largely invisible to natural selection, nonsynonymous (amino acid replacing) mutations may be under strong selective pressure. Comparison of the rates of fixation of those two types of mutations provides a powerful tool for understanding the mechanisms of DNA sequence evolution. For example, models of variable nonsynonymous/synonymous rate ratios among sites may provide important insights into functional constraints at different amino acid sites and may be used to detect sites under positive selection (Nielsen & Yang, 1998). In this study, we have examined the synonymous/nonsynonymous substitution rate of the protease, gag and integrase regions of two groups of HIV-1 patients infected 15 years apart. We find that the accumulation rates of synonymous substitutions are greater in patients who were lately infected for all the three studied genes. This result suggests that HIV-1 is under purifying selection, in other words, HIV-1 may be getting rid of the arising deleterious mutations.

The HIV-1 RNA genome is particularly rich in A-nucleotides while the C-content is low. HIV-1 is one of the most variable viruses known, yet it is able to maintain this highly biased nucleotide composition. In a recent study, Van der Kuyl *et al* (van der Kuyl & Berkhout, 2012) have compared HIV-1 genomes from the beginning of the epidemic with later isolates and shown that the nucleotide composition has been extremely stable over the past 30 years. Here, we have also analysed the nucleotide composition of three genes - gag, protease and integrase – from HIV-1 infected patients that were naïve to PIs or INSTIs. Our patients were all subtype B and were infected at two time points separated by 15 years. Our results show that the nucleotide content in HIV-1 subtype B sequences has remained constant over time for the gag gene. However, late proteases display a significant higher G-content, similarly, the late integrases are richer in C-nucleotide and the protease and integrase genes from late isolates have both a significant lower amount of U-nucleotide. Since these differences are significant, these results suggest that the precise nucleotide composition of the HIV-1 genome may change over time. Further studies should be performed in order to confirm these results. The study of the nucleotide composition of the HIV-1 genome is also important because the base composition of the HIV-1 genome has been linked to differences in pathogenicity. A recent study shows that a base composition that deviates most from that of the human host correlates with increased virulence (Li *et al.*, 2012; Vabret *et al.*, 2012). In that context, the slightly different base composition of HIV-1 compared to HIV-2 may also correlate with the increased pathogenicity of the former. The A-richness of

the HIV-1 genome may have been caused by a distinct mutation pattern of the viral RT polymerase, but also have been other pressures may be responsible for selecting an A-rich RNA genome. Further research is needed to identify possible RNA functions imposed by the A-abundance. No evidence has been reported that factors of the innate immune system shape the nucleotide composition of the viral genome, either by direct mutational activity or indirectly through viral escape (van der Kuyl & Berkhout, 2012).

Evolution of the human immunodeficiency virus type 1 protease: Effects on viral replication capacity and protease robustness

Previous work has provided evidence that the mean ex vivo relative RC of historical (1986–1989) HIV-1 isolates is significantly greater than that of more recent (2002–2003) isolates (Ariën *et al.*, 2005). Deleterious mutations introduced into the viral population via the continual introduction of new selective pressures and genetic bottlenecks may have reduced HIV-1 RC or virulence. Viral RC reduction could suggest that HIV-1 is adapting to the human host (Ariën *et al.*, 2007). Alternatively, other authors have suggested that instead of possible adaptation to the host, HIV-1 is shifting towards the possession of increasingly robust population characteristics at the expense of RC (Rolland *et al.*, 2007). Others have found that recent isolates have higher ex vivo RC than earlier isolates, suggesting that HIV-1 virulence may be increasing (Gali *et al.*, 2007). However, these studies have not focused on the impact of HIV-1 diversification on HIV-1 RC and mutational robustness or specifically on the impact of HIV-1 diversification on the fitness of individual viral proteins. In this thesis we tested whether HIV-1 diversification over time had affected ex vivo HIV-1 RC and protease robustness.

We found no significant ex vivo RC differences between viruses carrying naïve proteases from early or recent sample isolates, even though recent proteases have significantly diverged compared to a subtype B ancestral or consensus sequence. This finding is in good agreement with in vivo studies that have not found HIV-1 attenuation over time (Dorrucci *et al.*, 2005; 2007; Herbeck *et al.*, 2008; Müller *et al.*, 2006; Sinicco *et al.*, 1997; Troude *et al.*, 2009; van Manen *et al.*, 2011; Vanhems *et al.*, 1999). No changes in RC or even in disease progression were detected in these in vivo studies. Recently, a meta-analysis of trends in HIV-1 plasma viral load and CD4+

T-cell count, two prognostic markers of HIV disease progression, suggested that HIV-1 has become more virulent over the > 30-year history of the global HIV-1 epidemic (Herbeck *et al.*, 2012). Mathematical modelling has demonstrated that an increase in the parasite dispersal rate leads to selection for increased growth and to higher virulence (Wild *et al.*, 2009). As previously described, we found that viruses carrying protease resistance mutations had significantly lower ex vivo RC than naïve viruses (Martinez-Picado *et al.*, 1999; Nijhuis *et al.*, 1999). We also investigated whether the mutational robustness of naïve viral proteases was being affected by the progressive accumulation of new amino acid substitutions over time. Our results demonstrated that diversification of HIV-1 naïve proteases has not affected their robustness. Although robustness seems to be the opposite of evolvability, it has been shown recently that neutral diversity in a robust population, such as that observed in the recent proteases, can accelerate adaptation (Draghi *et al.*, 2010). Several studies have documented the relevance of neutral variation in allowing a population to access adaptive phenotypes (van Nimwegen, 2006). Our results also showed that PI resistant proteases are significantly less robust than naïve proteases. This finding, together with the RC cost of resistance mutations, explains why some resistance mutations revert to wild type residues in the absence of the specific drug (Martinez-Picado & Martínez, 2008).

Remarkably, to our knowledge this is the first study to investigate the impact of accumulation of amino acid substitutions over time in an individual viral protein (protease) on HIV-1 RC and mutational robustness. Our results provide convincing evidence that over time, HIV-1 protease diversification has not affected HIV-1 RC or protease mutational robustness and suggest that proteases carrying PI resistance substitutions are less robust than naïve proteases.

These results have some limitations. First, although our hypotheses were supported statistically, our study was restricted to a relatively epidemiologically homogeneous cohort of HIV-1 infected patients who sought care at our clinic. Second, analysis of other viral proteins or viral genomic regions may broaden our study conclusions. Third, RC differences can be substantial enough to have important evolutionary consequences, but nevertheless be too small to detect them experimentally. Further work should include an analysis of other viral genes to evaluate whether HIV-1 diversification over time is preferentially affecting other viral genomic regions. CTL escape mutations are gradually being imprinted in HIV-1 sequences as the epidemic

progresses. CTL escape mutations may differentially impact viral RC depending on the viral coding-region in which they emerge. For instance, CTL escape mutations in HIV-1 gag p24 are associated with significant RC costs, whereas most escape mutations in the env gene are neutral (Troyer *et al.*, 2009). A better understanding of how ongoing HIV-1 diversification affects viral RC and robustness has clinical implications for the design of effective therapeutic and vaccine strategies against circulating viruses.

Evolution of the human immunodeficiency virus type 1 integrase: Effects on viral replication capacity and integrase robustness

The previous results presented in this thesis show that the diversification of HIV-1 protease is not affecting viral RC. We decided to go a step further and study the impact of the HIV-1 diversification of another important gene, the viral encoded integrase, using the same cohort of naïve patients from our clinical unit. Our results suggest that HIV-1 integrase may have evolved to become less fit over time. Nevertheless, our results have some limitations, besides the geographical restriction to a relatively epidemiologically homogeneous cohort of HIV-1 infected patients who sought care at our clinic. First, our experimental design utilized recombinant viruses that focused on a single gene product, and as such, potential epistatic interactions with other viral proteins that could affect RC have not been taken into account (Buzón *et al.*, 2010b). Indeed, false-positive (and false-negative) results could arise due to the choice of NL4-3 as a backbone vector for this work, and studies using other viral strains and whole-virus isolates may be necessary to determine the full impact of the polymorphisms identified here on viral fitness. Second, the relative reductions in viral RC observed for patient-derived sequences were modest. For example, although the mean viral RC from late isolates is significantly lower than early isolates ($p = 0.0286$), when the viral RC of the less fit recombinant virus of the recent isolates is not included in the analysis; the former difference is no longer significant. Nevertheless, we have employed a well-acknowledged method of a tat-driven GFP reporter cell line to evaluate the viral RC (Brockman *et al.*, 2006; 2007; 2012; Miura *et al.*, 2008a; 2009). On the contrary to the previous study, where the viral replication capacity of a small number of proteases was evaluated (22), here we have measured a larger group of samples (94), making the results more statistically robust. Moreover, a recent study (Nomura *et al.*, 2012) indicates that gag-protease

associated HIV-1 replication capacity has decreased over the epidemic in Japan, using the same methodology, thus supporting our results. However, another recent study (Gali *et al.*, 2007) carried out in Amsterdam suggests that HIV-1 has evolved to become more fit over time. Therefore, larger studies with isolates from multiple geographical regions and that explore other viral genes will be required to confirm whether this is a local or global phenomenon.

Clinical markers of HIV infection such as plasma viral load and CD4+ T-cell count have been related to disease progression (Blaak *et al.*, 1998; Miura *et al.*, 2009; 2010; Quinones-Mateu *et al.*, 2000; Troyer *et al.*, 2005). However, no relationship was found between these parameters and the viral RC of the different recombinant viruses from our patients. Moreover, no relationship was found between the viral RC of the early and late recombinant viruses with their sequence conservation, although recent integrases have significantly diverged compared to a subtype B ancestral or consensus sequence.

In the present thesis, some integrase polymorphisms (S17N, I72V, S119P, and D256E) were found to be linked with viral RC reduction. In particular, mutations at HIV-1 integrase codon 119 are known to affect integration site selection (Harper *et al.*, 2003). A recent high-resolution structure of the prototype foamy virus (PFV) integrase bound to viral and target DNA demonstrated that residue A188 (which is homologous to HIV-1 integrase codon 119) formed a van der Waals interaction with a key cytosine in the minor groove of the target DNA sequence (Maertens *et al.*, 2010). The reduced viral RC that we observed with the S119P mutation is therefore consistent with a defect in integrase function at the step of target site recognition and strand transfer. Specifically, our data suggest that the substitution of a non-polar amino acid at this position (proline) may be more costly to integrase function than the more conservative changes that have been analysed previously, such as threonine, glycine, or alanine (Harper *et al.*, 2003).

CTL escape mutations have been shown to have a replicative cost. Therefore sites and pathways of Human Leukocyte-Antigen (HLA)-associated polymorphisms in HIV-1 have been broadly studied and identified through the analysis of population-level data (Brumme *et al.*, 2009). A recent study (Brockman *et al.*, 2012), suggests that the HLA-mediated impairment of HIV-1 integrase does not appear to be a general phenomenon during viral adaptation to host immune responses. Nevertheless,

Brockman and co-workers have identified uncommon immune-driven polymorphisms that are associated with reduced viral RC. Attenuating mutations are restricted by HLA class I alleles that are not conventionally regarded as being protective, including the S119R mutation, which is situated within a novel C*05 epitope. This study highlights the potential utility of population-based functional studies for immunogen discovery. Therefore, analysis of the relationship between the integrase polymorphisms found in this thesis and the HLA imprints woud be highly recommendable in order to decipher new escape pathways.

HIV-1 protease robustness determined by in vitro evolution

Robustness is defined as a reduced sensitivity to perturbations affecting phenotypic expression (Elena *et al.*, 2006), in other words, genetic robustness happens when mutations are inherited by the new viral genomes. There are many examples of proteins highly robust to mutations. They include several enzymes that can tolerate many amino acid changes (Guo *et al.*, 2004; Martinez *et al.*, 1996). Protein robustness seems to be a selectable trait because neutral mutations can be a key to future evolutionary innovations (Wagner, 2005). Mutational robustness allows a population to explore a range for genotypes that are neutral in one environment but potentially beneficial in another. A seminal evolution experiment demonstrated the evolutionary advantages of neutral mutations by showing that human or bacterial enzymes can acquire new functions without loosing their original functions (Aharoni *et al.*, 2004). Recent studies further suggest that the relationship between robustness and evolvability might be particulary important for viral pathogenesis (Lauring *et al.*, 2012).

The simplest measure of mutational robustness is to quantify the mutational fitness effect of individual mutations (Lauring *et al.*, 2013). The mutational fitness effect has been determined in a number of viruses by introducing random point mutations into the viral genome and measuring their effects on replicative efficiency (Sanjuán *et al.*, 2004a). By using this approach, we have previously demonstrated that most mutations have deleterious effects on the HIV-1 protease (Parera *et al.*, 2006). In this thesis, we have performed a directed evolution experiment with the HIV-1 protease in order to further study the enzyme robustness. We have constructed two random *in vitro* mutant libraries, one starting with the wild-type HIV-1 protease as a

template and another one starting with a mutated protease, 17a, previously generated in our laboratory. The 17a protease was chosen because displayed a good catalitic effiency in vitro and carried 4 mutations rarely found in nature. Furthermore, when the 17a protease was introduced in an HIV-1 infectious clone, viral growth was indistinguishable from wild type virus. Results show that up to 54% of random single amino acid substitutions were lethal in the proteases derived from the wild type protease and up to 47% in the proteases derived from the 17a clone. In vesicular stomatitis virus (VSV), 40% of random single-nucleotide mutants were lethal (Sanjuán *et al.*, 2004a). Similar results have been found in tobacco etch virus and the phages ΦX174 and Qβ (Sanjuan, 2010). Overall, these results show that our data on HIV-1 protease are in agreement to that found in other RNA viruses. This apparently high vulnerability to single mutations found in the HIV-1 protease and other RNA viruses contrasts with the high HIV-1 protease genetic variability found within infected individuals (Ceccherini-Silberstein *et al.*, 2004; Wu *et al.*, 2003). Especially intriguing is the extremely rapid evolvability displayed by this protein after protease inhibitor treatment (Condra *et al.*, 1995). HIV-1 protease can rapidly acquire mutations that lead to drug resistance but that barely affect its catalytic efficiency (Martinez-Picado *et al.*, 1999; Nijhuis *et al.*, 1999). Moreover, more than 20 (20%) different residues have been associated with drug resistance (Johnson *et al.*, 2009; Shafer *et al.*, 2001). Although the high mutation rates and large population size of HIV-1 are obviously favouring the rapid evolvability of the HIV-1 protease (Domingo *et al.*, 1997), protein evolution also depends on a reduced lethality of mutations (Aharoni *et al.*, 2004; Bloom *et al.*, 2006; Wagner, 2005).

The result that the artificial 17a protease is as vulnerable as the wild type protease to the addition of single random amino acid mutations is also intriguing. If mutational robustness is a heritable trait, that is, is adaptive, it will be expected that wild type protease would be more robust than an in vitro generated protease. Nevertheless, our results have the limitation that only one mutant, 17a, was analyzed. It cannot be discarded that the 17a mutant has a neutral sequence space neutral neighbourhood similar to wild type protease. Since wild type and 17a mutant have a comparable fitness, it cannot either be discarded, as previously suggested, that robustness and fitness are inversely correlated (Lauring *et al.*, 2013). To clarify this issue, random mutant libraries should be constructed on mutants displaying significant differences in fitness.

Comparing the fitness of wild type and 17a mutants carrying the same single amino acid mutation (e.g. the I70V mutation), we found that they have antagonist effects. Thus, enhancing the possible antagonistic epistatic relationship between two different mutations. In a number of recent studies carried out with RNA viruses, including HIV-1, a tendency toward antagonistic epistasis has been observed (Bonhoeffer, 2004; Burch & Chao, 2004; Sanjuán *et al.*, 2004b). Furthermore, analysis of epistatic interactions among pairs of deleterious mutations in the HIV-1 protease showed high frequencies of lethality and negative epistasis, thus indicating that the HIV-1 protease is highly sensitive to the effects of deleterious mutations (Parera *et al.*, 2009). The precise nature of the robustness mechanisms is far from well defined at the moment and, therefore, more experimental work is needed to better define the molecular mechanisms underlying robustness at the protein level.

Conclusions

- Sequence diversification of the HIV-1 protease, gag and integrase genes increases over time.

- The observed sequence diversification over time in the three HIV-1 genes (gag, PR and IN) has generated in the protease gene an increase in its G-base content, and in the integrase gene an increase in its C-base content, and a decrease in their U-base content in both enzymes.

- The HIV-1 protease diversification over time has neither influenced ex vivo viral RC or protein robustness.

- HIV-1 proteases carrying PI resistance substitutions are less robust than naïve proteases.

- The integrase genetic diversification over time has influenced in some cases the ex vivo viral replication capacity.

- Integrase polymorphisms S17N, I72V, S119P, and D256E are linked to viral RC reduction and their additive effect can contribute to impair the integrase function.

- A wild type natural HIV-1 protease is as vulnerable to the addition of single random amino acid mutations as an artificial in vitro-generated HIV-1 protease.

References

Adachi, A., Gendelman, H. E., Koenig, S., Folks, T., Willey, R., Rabson, A. & Martin, M. A. (1986). Production of acquired immunodeficiency syndrome-associated retrovirus in human and nonhuman cells transfected with an infectious molecular clone. *Journal of Virology* **59**, 284–291.

Aharoni, A., Gaidukov, L., Khersonsky, O., Gould, S. M., Roodveldt, C. & Tawfik, D. S. (2004). The 'evolvability' of promiscuous protein functions. *Nat Genet*.

Apetrei, C., Kaur, A., Lerche, N. W., Metzger, M., Pandrea, I., Hardcastle, J., Falkenstein, S., Bohm, R., Koehler, J. & other authors. **(2005).** Molecular Epidemiology of Simian Immunodeficiency Virus SIVsm in U.S. Primate Centers Unravels the Origin of SIVmac and SIVstm. *Journal of Virology* **79**, 8991–9005.

Apetrei, C., Lerche, N. W., Pandrea, I., Gormus, B., Silvestri, G., Kaur, A., Robertson, D. L., Hardcastle, J., Lackner, A. A. & Marx, P. A. (2006). Kuru experiments triggered the emergence of pathogenic SIVmac. *AIDS* **20**, 317–321.

Ariën, K. K., Troyer, R. M., Gali, Y., Colebunders, R. L., Arts, E. J. & Vanham, G. (2005). Replicative fitness of historical and recent HIV-1 isolates suggests HIV-1 attenuation over time. *AIDS* **19**, 1555–1564.

Ariën, K. K., Vanham, G. & Arts, E. J. (2007). Is HIV-1 evolving to a less virulent form in humans? *Nat Rev Micro* **5**, 141–151.

Arts, E. J. & Hazuda, D. J. (2012). HIV-1 Antiretroviral Drug Therapy. *Cold Spring Harb Perspect Med* **2**, a007161.

Autran, B. (1997). Positive Effects of Combined Antiretroviral Therapy on CD4+ T Cell Homeostasis and Function in Advanced HIV Disease. *Science* **277**, 112–116.

Avidan, O. & Hizi, A. (2008). Expression and characterization of the integrase of bovine immunodeficiency virus. *Virology* **371**, 309–321.

BA, B. (2012). Antiretroviral Drug Resistance in Brazilian Children Infected by Human Immunodeficiency Virus Type 1. *J Antivir Antiretrovir* **04**.

Baldanti, F., Paolucci, S., Gulminetti, R., Brandolini, M., Barbarini, G. & Maserati, R. (2010). Early emergence of raltegravir resistance mutations in patients receiving HAART salvage regimens. *J Med Virol* **82**, 116–122.

Barré-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vézinet-Brun, F. & other authors. **(1983).** Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **220**, 868–871.

Beckman, R. A., Mildvan, A. S. & Loeb, L. A. (1985). On the fidelity of DNA replication: manganese mutagenesis in vitro. *Biochemistry* **24**, 5810–5817.

Bedard, J. (2007). In vitro cross-resistance studies of five different classes of integrase inhibitors in recombinant HIV-1. *Antivir Ther (Lond)* **12**, S3.

Berkhout, B. & van Hemert, F. J. (1994). The unusual nucleotide content of the HIV RNA genome results in a biased amino acid composition of HIV proteins. *Nucleic Acids Res* **22**, 1705–1711.

Berthoux, L., Sebastian, S., Muesing, M. A. & Luban, J. (2007). The role of lysine 186 in HIV-1 integrase multimerization. *Virology* **364**, 227–236.

Betancor, G., Puertas, M. C., Nevot, M., Garriga, C., Martínez, M. A., Martinez-Picado, J. & Menéndez-Arias, L. (2010). Mechanisms involved in the selection of HIV-1 reverse transcriptase thumb subdomain polymorphisms associated with nucleoside analogue therapy failure. *Antimicrobial Agents and Chemotherapy* **54**, 4799–4811.

Billich, S., Knoop, M. T., Hansen, J., Strop, P., Sedlacek, J., Mertz, R. & Moelling, K. (1988). Synthetic peptides as substrates and inhibitors of human immune deficiency virus-1 protease. *J Biol Chem* **263**, 17905–17908.

Blaak, H., Brouwer, M., Ran, L. J., de Wolf, F. & Schuitemaker, H. (1998). In vitro replication kinetics of human immunodeficiency virus type 1 (HIV-1) variants in relation to virus load in long-term survivors of HIV-1 infection. *J Infect Dis* **177**, 600–610.

Bloom, J. D., Labthavikul, S. T., Otey, C. R. & Arnold, F. H. (2006). Protein stability promotes evolvability. *Proc Natl Acad Sci USA* **103**, 5869–5874. National Acad Sciences.

Bonhoeffer, S. (2004). Evidence for Positive Epistasis in HIV-1. *Science* **306**, 1547–1550.

Bouyac-Bertoia, M., Dvorin, J. D., Fouchier, R. A., Jenkins, Y., Meyer, B. E., Wu, L. I., Emerman, M. & Malim, M. H. (2001). HIV-1 infection requires a functional integrase NLS. *Mol Cell* **7**, 1025–1035.

Bremermann, H. J. & Pickering, J. (1983). A game-theoretical model of parasite virulence. *J Theor Biol* **100**, 411–426.

Brockman, M. A., Chopera, D. R., Olvera, A., Brumme, C. J., Sela, J., Markle, T. J., Martin, E., Carlson, J. M., Le, A. Q. & other authors. **(2012).** Uncommon Pathways of Immune Escape Attenuate HIV-1 Integrase Replication Capacity. *Journal of Virology* **86**, 6913–6923.

Brockman, M. A., Schneidewind, A., Lahaie, M., Schmidt, A., Miura, T., DeSouza, I., Ryvkin, F., Derdeyn, C. A., Allen, S. & other authors. **(2007).** Escape and Compensation from Early HLA-B57-Mediated Cytotoxic T-Lymphocyte Pressure on Human Immunodeficiency Virus Type 1 Gag Alter Capsid Interactions with Cyclophilin A. *Journal of Virology* **81**, 12608–12618.

Brockman, M. A., Tanzi, G. O., Walker, B. D. & Allen, T. M. (2006). Use of a novel GFP reporter cell line to examine replication capacity of CXCR4- and CCR5-tropic HIV-1 by flow cytometry. *Journal of Virological Methods* **131**, 134–142.

Bronson, E. C. & Anderson, J. N. (1994). Nucleotide composition as a driving force in the evolution of retroviruses. *J Mol Evol* **38**, 506–532.

Brown, P. O. (1990). Integration of retroviral DNA. *Curr Top Microbiol Immunol* **157**, 19–48. Springer.

Brumme, Z. L., John, M., Carlson, J. M., Brumme, C. J., Chan, D., Brockman, M. A., Swenson, L. C., Tao, I., Szeto, S. & other authors. **(2009).** HLA-Associated Immune Escape Pathways in HIV-1 Subtype B Gag, Pol and Nef Proteins. *PLoS ONE* **4**, e6687 (D. F. Nixon, Ed.).

Bukovsky, A. & Göttlinger, H. (1996). Lack of integrase can markedly affect human immunodeficiency virus type 1 particle production in the presence of an active viral protease. *Journal of Virology* **70**, 6820–6825.

Bull, J. J. (1994). Virulence. *Evolution* **48**, 1423–1437.

Burch, C. L. & Chao, L. (2004). Epistasis and its relationship to canalization in the RNA virus phi 6. *Genetics* **167**, 559–567.

Burke, C. J., Sanyal, G., Bruner, M. W., Ryan, J. A., LaFemina, R. L., Robbins, H. L., Zeft, A. S., Middaugh, C. R. & Cordingley, M. G. (1992). Structural implications of spectroscopic characterization of a putative zinc finger peptide from HIV-1 integrase.

Busschots, K., Voet, A., De Maeyer, M., Rain, J.-C., Emiliani, S., Benarous, R., Desender, L., Debyser, Z. & Christ, F. (2007). Identification of the LEDGF/p75 binding site in HIV-1 integrase. *Journal of Molecular Biology* **365**, 1480–1492.

Buzón, M. J., Massanella, M., Llibre, J. M., Esteve, A., Dahl, V., Puertas, M. C., Gatell, J. M., Domingo, P., Paredes, R. & other authors. (2010a). HIV-1 replication and immune dynamics are affected by raltegravir intensification of HAART-suppressed subjects. *Nat Med* **16**, 460–465. Nature Publishing Group.

Buzón, M. J., Dalmau, J., Puertas, M. C., Puig, J., Clotet, B. & Martinez-Picado, J. (2010b). The HIV-1 integrase genotype strongly predicts raltegravir susceptibility but not viral fitness of primary virus isolates. *AIDS* **24**, 17–25.

Cabana, M., Fernández, G., Parera, M., Clotet, B. & Martínez, M. A. (2002). Catalytic efficiency and phenotype of HIV-1 proteases encoding single critical resistance substitutions. *Virology* **300**, 71–78.

Cai, M., Zheng, R., Caffrey, M., Craigie, R., Clore, G. M. & Gronenborn, A. M. (1997). Solution structure of the N-terminal zinc binding domain of HIV-1 integrase. *Nat Struct Biol* **4**, 567–577.

Canducci, F., Marinozzi, M. C., Sampaolo, M., Boeri, E., Spagnuolo, V., Gianotti, N., Castagna, A., Paolucci, S., Baldanti, F. & other authors. (2010). Genotypic/phenotypic patterns of HIV-1 integrase resistance to raltegravir. *Journal of Antimicrobial Chemotherapy* **65**, 425–433.

Canducci, F., Sampaolo, M., Marinozzi, M. C., Boeri, E., Spagnuolo, V., Galli, A., Castagna, A., Lazzarin, A., Clementi, M. & Gianotti, N. (2009). Dynamic patterns of human immunodeficiency virus type 1 integrase gene evolution in patients failing raltegravir-based salvage therapies. *AIDS* **23**, 455–460.

Capel, E., Martrus, G., Parera, M., Clotet, B. & Martinez, M. A. (2012). Evolution of the human immunodeficiency virus type 1 protease: effects on viral replication capacity and protease robustness. *Journal of General Virology* **93**, 2625–2634.

Carr, A. (2003). Toxicity of antiretroviral therapy and implications for drug development. *Nat Rev Drug Discov* **2**, 624–634.

Casado, C., Colombo, S., Rauch, A., Martínez, R., Gunthard, H. F., Garcia, S., Rodríguez, C., Del Romero, J., Telenti, A. & Lopez-Galindez, C. (2010). Host and viral genetic correlates of clinical definitions of HIV-1 disease progression. *PLoS ONE* **5**, e11079.

Ceccherini-Silberstein, F., Erba, F., Gago, F., Bertoli, A., Forbici, F., Bellocchi, M. C., Gori, C., d'Arrigo, R., Marcon, L. & other authors. (2004). Identification of the minimal conserved structure of HIV-1 protease in the presence and absence of drug pressure. *AIDS* **18**, 11–19.

Ceccherini-Silberstein, F., Malet, I., d'Arrigo, R., Antinori, A., Marcelin, A.-G. & Perno, C.-F. (2009). Characterization and structural analysis of HIV-1 integrase conservation. *AIDS Rev* **11**, 17–29.

Ceccherini-Silberstein, F., Malet, I., Fabeni, L., Dimonte, S., Svicher, V., d'Arrigo, R., Artese, A., Costa, G., Bono, S. & other authors. (2010). Specific HIV-1 integrase polymorphisms change their prevalence in untreated versus antiretroviral-treated HIV-1-infected patients, all naive to integrase inhibitors. *Journal of Antimicrobial Chemotherapy* **65**, 2305–2318.

Centers for Disease Control (CDC). (1981). Pneumocystis pneumonia--Los Angeles. *MMWR Morb Mortal Wkly Rep* **30**, 250–252.

Chakrabarti, L., Guyader, M., Alizon, M., Daniel, M. D., Desrosiers, R. C., Tiollais, P. & Sonigo, P. (1987). Sequence of simian immunodeficiency virus from macaque and its relationship to other human and simian retroviruses. *Nature* **328**, 543–547.

Cherepanov, P., Sun, Z.-Y. J., Rahman, S., Maertens, G., Wagner, G. & Engelman, A. (2005). Solution structure of the HIV-1 integrase-binding domain in LEDGF/p75. *Nat Struct Mol Biol* **12**, 526–532. Nature Publishing Group.

Clavel, F., Guétard, D., Brun-Vezinet, F., Chamaret, S., Rey, M. A., Santos-Ferreira, M. O., Laurent, A. G., Dauguet, C., Katlama, C. & Rouzioux, C. (1986). Isolation of a new human retrovirus from West African patients with AIDS. *Science* **233**, 343–346.

Clumck, N., Van de Perre, P., Caral, M., Rouvroy, D. & Nzaramba, D. (1985). Heterosexual promiscuity among African patients with AIDS. *N Engl J Med* **313**, 182.

Coakley, E., Petropoulos, C. J. & Whitcomb, J. M. (2005). Assessing chemokine co-receptor usage in HIV. *Current Opinion in Infectious Diseases* **18**, 9–15.

Coffin, J. M. (1995). HIV population dynamics in vivo: implications for genetic variation, pathogenesis, and therapy. *Science* **267**, 483–489.

Coffin, J., Haase, A., Levy, J. A., Montagnier, L., Oroszlan, S., Teich, N., Temin, H., Toyoshima, K., Varmus, H. & Vogt, P. (1986). Human immunodeficiency viruses. *Science* **232**, 697.

Coffin, J., Hughes, S. H. & Varmus, H. E. (1997). *Retroviruses*. New York: Cold Spring Harbor Laboratory Press.

Collier, A. C., Coombs, R. W., Schoenfeld, D. A., Bassett, R. L., Timpone, J., Baruch, A., Jones, M., Facey, K., Whitacre, C. & McAuliffe, V. J. (1996). Treatment of human immunodeficiency virus infection with saquinavir, zidovudine, and zalcitabine. *N Engl J Med* **334**, 1011–1018. Mass Medical Soc.

Condra, J. H., Schleif, W. A., Blahy, O. M., Gabryelski, L. J., Graham, D. J., Quintero, J. C., Rhodes, A., Robbins, H. L., Roth, E. & Shivaprakash, M. (1995). In vivo emergence of HIV-1 variants resistant to multiple protease inhibitors. *Nature* **374**, 569–571.

Cooper, D. A., Steigbigel, R. T., Gatell, J. M., Rockstroh, J. K., Katlama, C., Yeni, P., Lazzarin, A., Clotet, B., Kumar, P. N. & other authors. (2008). Subgroup and resistance analyses of raltegravir for resistant HIV-1 infection. *N Engl J Med* **359**, 355–365.

Curran, J. W., Morgan, W. M., Hardy, A. M., Jaffe, H. W., Darrow, W. W. & Dowdle, W. R. (1985). The epidemiology of AIDS: current status and future prospects. *Science* **229**, 1352–1357.

D'Aquila, R. T., Hughes, M. D., Johnson, V. A., Fischl, M. A., Sommadossi, J. P., Liou, S. H., Timpone, J., Myers, M., Basgoz, N. & other authors. (1996). Nevirapine, zidovudine, and didanosine compared with zidovudine and didanosine in patients with HIV-1 infection. A randomized, double-blind, placebo-controlled trial. National Institute of Allergy and Infectious Diseases AIDS Clinical Trials Group Protocol 241 Investigators. *Ann Intern Med* **124**, 1019–1030.

da Silva, D., Van Wesenbeeck, L., Breilh, D., Reigadas, S., Anies, G., Van Baelen, K., Morlat, P., Neau, D., Dupon, M. & other authors. (2010). HIV-1 resistance patterns to integrase inhibitors in antiretroviral-experienced patients with

virological failure on raltegravir-containing regimens. *Journal of Antimicrobial Chemotherapy* **65**, 1262–1269.

Dalmau, J., Puertas, M. C., Azuara, M., Mariño, A., Frahm, N., Mothe, B., Izquierdo-Useros, N., Buzón, M. J., Paredes, R. & other authors. **(2009).** Contribution of immunological and virological factors to extremely severe primary HIV type 1 infection. *Clin Infect Dis* **48**, 229–238.

de Oliveira, T., Engelbrecht, S., Janse van Rensburg, E., Gordon, M., Bishop, K., Megede, zur, J., Barnett, S. W. & Cassol, S. (2003). Variability at human immunodeficiency virus type 1 subtype C protease cleavage sites: an indication of viral fitness? *Journal of Virology* **77**, 9422–9430.

Dean, M., Carrington, M., Winkler, C., Huttley, G. A., Smith, M. W., Allikmets, R., Goedert, J. J., Buchbinder, S. P., Vittinghoff, E. & other authors. **(1996).** Genetic restriction of HIV-1 infection and progression to AIDS by a deletion allele of the CKR5 structural gene. Hemophilia Growth and Development Study, Multicenter AIDS Cohort Study, Multicenter Hemophilia Cohort Study, San Francisco City Cohort, ALIVE Study. *Science* **273**, 1856–1862.

Deeks, S. G. (2012). Towards an HIV cure: a global scientific strategy. *Nature Reviews Immunology* 1–8. Nature Publishing Group.

Descamps, D., Lambert-Niclot, S., Marcelin, A. G., Peytavin, G., Roquebert, B., Katlama, C., Yeni, P., Felices, M., Calvez, V. & Brun-Vezinet, F. (2009). Mutations associated with virological response to darunavir/ritonavir in HIV-1-infected protease inhibitor-experienced patients. *Journal of Antimicrobial Chemotherapy* **63**, 585–592.

Deval, J., White, K. L., Miller, M. D. & Parkin, N. T. (2004). Mechanistic Basis for Reduced Viral and Enzymatic Fitness of HIV-1 Reverse Transcriptase Containing Both K65R and M184V Mutations. *Journal of Biological …*.

Domingo, E., Escarmis, C., Menéndez-Arias, L. & Holland, J. J. (1999). Viral Quasispecies and Fitness Variations. In *Origin and Evolution of Viruses*, pp. 141–161. Edited by E. Domingo, R. Webster & J. J. Holland. Academic Press.

Domingo, E., Escarmis, C., Sevilla, N., Moya, A., Elena, S. F., Quer, J., Novella, I. S. & Holland, J. J. (1996). Basic concepts in RNA virus evolution.

Domingo, E. & Holland, J. J. (1997). RNA virus mutations and fitness for survival. *Annu Rev Microbiol* **51**, 151–178.

Domingo, E., Menéndez-Arias, L., Quinones-Mateu, M. E., Holguín, A., Gutiérrez-Rivas, M., Martinez, M. A., Quer, J., Novella, I. S. & Holland, J. J. (1997). Viral quasispecies and the problem of vaccine-escape and drug-resistant mutants. *Prog Drug Res* **48**, 99–128.

Domingo, E., Sabo, D., Taniguchi, T. & Weissmann, C. (1978). Nucleotide sequence heterogeneity of an RNA phage population. *Cell* **13**, 735–744.

Dorrucci, M., Phillips, A. N., Longo, B., Rezza, G.Italian Seroconversion Study. **(2005).** Changes over time in post-seroconversion CD4 cell counts in the Italian HIV-Seroconversion Study: 1985-2002. *AIDS* **19**, 331–335.

Dorrucci, M., Rezza, G., Porter, K., Phillips, A.Concerted Action on Seroconversion to AIDS and Death in Europe Collaboration. **(2007).** Temporal trends in postseroconversion CD4 cell count and HIV load: the Concerted Action on Seroconversion to AIDS and Death in Europe Collaboration, 1985-2002. *J Infect Dis* **195**, 525–534.

Draghi, J. A., Parsons, T. L., Wagner, G. P. & Plotkin, J. B. (2010). Mutational robustness can facilitate adaptation. *Nature* **463**, 353–355.

Duffy, S., Shackelton, L. A. & Holmes, E. C. (2008). Rates of evolutionary change in viruses: patterns and determinants. *Nat Rev Genet* **9**, 267–276.

Dyda, F., Hickman, A. B., Jenkins, T. M., Engelman, A., Craigie, R. & Davies, D. R. (1994). Crystal structure of the catalytic domain of HIV-1 integrase: similarity to other polynucleotidyl transferases. *Science* **266**, 1981–1986.

Eigen, M. & Schuster, P. (1977). The hypercycle. A principle of natural self-organization. Part A: Emergence of the hypercycle. *Naturwissenschaften* **64**, 541–565.

Eigen, M. (1993). Viral quasispecies. *Sci Am* **269**, 32–32. CDS COMMUNICATIONS DATA SERVICES.

Eijkelenboom, A. P., Lutzke, R. A., Boelens, R., Plasterk, R. H., Kaptein, R. & Hård, K. (1995). The DNA-binding domain of HIV-1 integrase has an SH3-like fold. *Nat Struct Biol* **2**, 807–810.

Elena, S. F., Carrasco, P., Daròs, J.-A. & Sanjuán, R. (2006). Mechanisms of genetic robustness in RNA viruses. *EMBO Rep* **7**, 168–173.

Engelman, A. & Craigie, R. (1992). Identification of conserved amino acid residues critical for human immunodeficiency virus type 1 integrase function in vitro. *Journal of Virology* **66**, 6361–6369.

Engelman, A., Englund, G., Orenstein, J. M., Martin, M. A. & Craigie, R. (1995). Multiple effects of mutations in human immunodeficiency virus type 1 integrase on viral replication. *Journal of Virology* **69**, 2729–2736.

Engelman, A., Hickman, A. B. & Craigie, R. (1994). The core and carboxyl-terminal domains of the integrase protein of human immunodeficiency virus type 1 each contribute to nonspecific DNA binding. *Journal of Virology* **68**, 5911–5917.

Espeseth, A. S., Felock, P., Wolfe, A., Witmer, M., Grobler, J., Anthony, N., Egbertson, M., Melamed, J. Y., Young, S. & other authors. **(2000).** HIV-1 integrase inhibitors that compete with the target DNA substrate define a unique strand transfer conformation for integrase. *Proc Natl Acad Sci USA* **97**, 11244–11249.

Fauci, A. S., Pantaleo, G., Stanley, S. & Weissman, D. (1996). Immunopathogenic mechanisms of HIV infection. In *Ann Intern Med*, pp. 654–663. Presented at the Annals of internal medicine.

Felsenstein, J. (1985). Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791.

Fernandez, G., Clotet, B. & Martinez, M. A. (2007). Fitness Landscape of Human Immunodeficiency Virus Type 1 Protease Quasispecies. *Journal of Virology* **81**, 2485–2496.

Fikkert, V., Hombrouck, A., Van Remoortel, B., De Maeyer, M., Pannecouque, C., De Clercq, E., Debyser, Z. & Witvrouw, M. (2004). Multiple mutations in human immunodeficiency virus-1 integrase confer resistance to the clinical trial drug S-1360. *AIDS* **18**, 2019–2028.

Francis, D. P., Curran, J. W. & Essex, M. (1983). Epidemic acquired immune deficiency syndrome: epidemiologic evidence for a transmissible agent. *J Natl Cancer Inst* **71**, 1–4.

Frankel, A. D. & Young, J. A. (1998). HIV-1: fifteen proteins and an RNA. *Annu Rev*

Biochem **67**, 1–25. Annual Reviews 4139 El Camino Way, PO Box 10139, Palo Alto, CA 94303-0139, USA.

Fransen, S., Gupta, S., Danovich, R., Hazuda, D. & Miller, M. (2008). Loss of raltegravir susceptibility in treated patients is conferred by multiple non-overlapping genetic pathways. *XVII International HIV Drug Resistance Workshop, Sitges, Spain*.

Friedman-Kien, A. E. (1981). Disseminated Kaposi's sarcoma syndrome in young homosexual men. *J Am Acad Dermatol* **5**, 468–471.

Gali, Y., Berkhout, B., Vanham, G., Bakker, M., Back, N. K. T. & Ariën, K. K. (2007). Survey of the temporal changes in HIV-1 replicative fitness in the Amsterdam Cohort. *Virology* **364**, 140–146.

Gallo, R. C. (2002). HISTORICAL ESSAY: The Early Years of HIV/AIDS. *Science* **298**, 1728–1730.

Gallo, R. C., Salahuddin, S. Z., Popovic, M., Shearer, G. M., Kaplan, M., Haynes, B. F., Palker, T. J., Redfield, R., Oleske, J. & Safai, B. (1984). Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS. *Science* **224**, 500–503.

Gelderblom, H. R. (1991). Assembly and morphology of HIV: potential effect of structure on viral function. *AIDS* **5**, 617–637.

Gervaix, A., West, D., Leoni, L. M., Richman, D. D., Wong-Staal, F. & Corbeil, J. (1997). A new reporter cell line to monitor HIV infection and drug susceptibility in vitro. *Proc Natl Acad Sci USA* **94**, 4653–4658.

Gilbert, P. B., McKeague, I. W., Eisen, G., Mullins, C., Gueye-NDiaye, A., Mboup, S. & Kanki, P. J. (2003). Comparison of HIV-1 and HIV-2 infectivity from a prospective cohort study in Senegal. *Stat Med* **22**, 573–593.

Goethals, O., Clayton, R., Van Ginderen, M., Vereycken, I., Wagemans, E., Geluykens, P., Dockx, K., Strijbos, R., Smits, V. & other authors. (2008). Resistance mutations in human immunodeficiency virus type 1 integrase selected with elvitegravir confer reduced susceptibility to a wide range of integrase inhibitors. *Journal of Virology* **82**, 10366–10374.

Gottlieb, M. S., Schroff, R., Schanker, H. M., Weisman, J. D., Fan, P. T., Wolf, R. A. & Saxon, A. (1981). Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N Engl J Med* **305**, 1425–1431.

Grant, P. & Zolopa, A. (2008). Integrase inhibitors: a clinical review of raltegravir and elvitegravir. *Journal of HIV therapy* **13**, 36.

Guo, H. H., Choe, J. & Loeb, L. A. (2004). Protein tolerance to random amino acid change. *Proc Natl Acad Sci USA* **101**, 9205–9210.

Guyader, M., Emerman, M., Sonigo, P., Clavel, F., Montagnier, L. & Alizon, M. (1987). Genome organization and transactivation of the human immunodeficiency virus type 2. *Nature* **326**, 662–669.

Hahn, B. H., Shaw, G. M., De Cock, K. M. & Sharp, P. M. (2000). AIDS as a zoonosis: scientific and public health implications. *Science* **287**, 607–614.

Harper, A. L., Sudol, M. & Katzman, M. (2003). An amino acid in the central catalytic domain of three retroviral integrases that affects target site selection in nonviral DNA. *Journal of Virology* **77**, 3838–3845.

Hazuda, D. J., Felock, P., Witmer, M., Wolfe, A., Stillmock, K., Grobler, J. A.,

Espeseth, A., Gabryelski, L., Schleif, W. & other authors. **(2000).** Inhibitors of strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science* **287**, 646–650.

Hazuda, D. J., Anthony, N. J., Gomez, R. P., Jolly, S. M., Wai, J. S., Zhuang, L., Fisher, T. E., Embrey, M., Guare, J. P. & other authors. **(2004a).** A naphthyridine carboxamide provides evidence for discordant resistance between mechanistically identical inhibitors of HIV-1 integrase. *Proc Natl Acad Sci USA* **101**, 11233–11238.

Hazuda, D. J., Young, S. D., Guare, J. P., Anthony, N. J., Gomez, R. P., Wai, J. S., Vacca, J. P., Handt, L., Motzel, S. L. & other authors. **(2004b).** Integrase inhibitors and cellular immunity suppress retroviral replication in rhesus macaques. *Science* **305**, 528–532.

Hemelaar, J., Gouws, E., Ghys, P. D., Osmanov, S.WHO-UNAIDS Network for HIV Isolation and Characterisation. **(2011).** Global trends in molecular epidemiology of HIV-1 during 2000-2007. *AIDS* **25**, 679–689.

Herbeck, J. T., Gottlieb, G. S., Li, X., Hu, Z., Detels, R., Phair, J., Rinaldo, C., Jacobson, L. P., Margolick, J. B. & Mullins, J. I. (2008). Lack of evidence for changing virulence of HIV-1 in North America. *PLoS ONE* **3**, e1525.

Herbeck, J. T., Müller, V., Maust, B. S., Ledergerber, B., Torti, C., Di Giambenedetto, S., Gras, L., Gunthard, H. F., Jacobson, L. P. & other authors. **(2012).** Is the virulence of HIV changing? A meta-analysis of trends in prognostic markers of HIV disease progression and transmission. *AIDS* **26**, 193–205.

Hirsch, V. M., Olmsted, R. A., Murphey-Corb, M., Purcell, R. H. & Johnson, P. R. (1989). An African primate lentivirus (SIVsmclosely related to HIV-2. *Nature* **339**, 389–392. Nature Publishing Group.

Ho, D. D., Neumann, A. U., Perelson, A. S., Chen, W., Leonard, J. M. & Markowitz, M. (1995). Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. *Nature* **373**, 123–126.

Holland, J. J., La Torre, De, J. C. & Steinhauer, D. A. (1992). RNA virus populations as quasispecies. *Curr Top Microbiol Immunol* **176**, 1–20.

Holland, J., Spindler, K., Horodyski, F., Grabau, E., Nichol, S. & VandePol, S. (1982). Rapid evolution of RNA genomes. *Science* **215**, 1577–1585.

Hombrouck, A., De Rijck, J. & Hendrix, J. (2007). Virus evolution reveals an exclusive role for LEDGF/p75 in chromosomal tethering of HIV. *PLoS …*.

Hu, Z. & Kuritzkes, D. R. (2010). Effect of raltegravir resistance mutations in HIV-1 integrase on viral fitness. *J Acquir Immune Defic Syndr* **55**, 148–155.

Huet, T., Cheynier, R., Meyerhans, A., Roelants, G. & Wain-Hobson, S. (1990). Genetic organization of a chimpanzee lentivirus related to HIV-1. *Nature* **345**, 356–359.

Hütter, G., Nowak, D., Mossner, M., Ganepola, S., Müssig, A., Allers, K., Schneider, T., Hofmann, J., Kücherer, C. & other authors. **(2009).** Long-term control of HIV by CCR5 Delta32/Delta32 stem-cell transplantation. *N Engl J Med* **360**, 692–698.

Ibáñez, A., Clotet, B. & Martinez, M. A. (2000). Human immunodeficiency virus type 1 population bottleneck during indinavir therapy causes a genetic drift in the env quasispecies. *J Gen Virol* **81**, 85–95.

Ishima, R. (2001). Folded Monomer of HIV-1 Protease. *Journal of Biological Chemistry* **276**, 49110–49116.

Ishima, R., Freedberg, D. I., Wang, Y. X., Louis, J. M. & Torchia, D. A. (1999). Flap opening and dimer-interface flexibility in the free and inhibitor-bound HIV protease, and their implications for function. *Structure* **7**, 1047–1055.

Jacks, T., Power, M. D., Masiarz, F. R., Luciw, P. A., Barr, P. J. & Varmus, H. E. (1988). Characterization of ribosomal frameshifting in HIV-1 gag-pol expression. *Nature* **331**, 280–283.

Jaskolski, M., Alexandratos, J. N., Bujacz, G. & Wlodawer, A. (2009). Piecing together the structure of retroviral integrase, an important target in AIDS therapy. *FEBS Journal* **276**, 2926–2946.

Jenkins, T. M., Engelman, A., Ghirlando, R. & Craigie, R. (1996). A soluble active mutant of HIV-1 integrase: involvement of both the core and carboxyl-terminal domains in multimerization. *J Biol Chem* **271**, 7712–7718.

Johnson, V. A., Brun-Vezinet, F., Clotet, B., Gunthard, H. F., Kuritzkes, D. R., Pillay, D., Schapiro, J. M. & Richman, D. D. (2009). Update of the drug resistance mutations in HIV-1: December 2009. *Top HIV Med* **17**, 138–145.

Johnson, V. A., Calvez, V., Gunthard, H. F., Paredes, R., Pillay, D., Shafer, R., Wensing, A. M. & Richman, D. D. (2011). 2011 update of the drug resistance mutations in HIV-1. *Top Antivir Med* **19**, 156–164.

Jung, A., Maier, R., Vartanian, J.-P., Bocharov, G., Jung, V., Fischer, U., Meese, E., Wain-Hobson, S. & Meyerhans, A. (2002). Recombination: Multiply infected spleen cells in HIV patients. *Nature* **418**, 144.

Kaplan, A. H., Manchester, M. & Swanstrom, R. (1994). The activity of the protease of human immunodeficiency virus type 1 is initiated at the membrane of infected cells before the release of viral proteins and is required for release to occur with maximum efficiency. *Journal of Virology* **68**, 6782–6786.

Kimura, M. (1983). The Neutral Theory of Molecular Evolution - Motoo Kimura - Google Books. *Cambridge University Press*.

Kisic, M., Matamoros, T., Nevot, M., Mendieta, J., Martinez-Picado, J., Martínez, M. A. & Menéndez-Arias, L. (2011). Thymidine analogue excision and discrimination modulated by mutational complexes including single amino acid deletions of Asp-67 or Thr-69 in HIV-1 reverse transcriptase. *Journal of Biological Chemistry* **286**, 20615–20624.

Knight, S. C., Macatonia, S. E. & Patterson, S. (1990). HIV I infection of dendritic cells. *Int Rev Immunol* **6**, 163–175.

Kohl, N. E., Emini, E. A., Schleif, W. A., Davis, L. J., Heimbach, J. C., Dixon, R. A., Scolnick, E. M. & Sigal, I. S. (1988). Active human immunodeficiency virus protease is required for viral infectivity. *Proc Natl Acad Sci USA* **85**, 4686–4690.

Komanduri, K. V., Viswanathan, M. N., Wieder, E. D., Schmidt, D. K., Bredt, B. M., Jacobson, M. A. & McCune, J. M. (1998). Restoration of cytomegalovirus-specific CD4+ T-lymphocyte responses after ganciclovir and highly active antiretroviral therapy in individuals infected with HIV-1. *Nat Med* **4**, 953–956. Nature Publishing Group.

Korber, B. (2000). Timing the Ancestor of the HIV-1 Pandemic Strains. *Science* **288**, 1789–1796.

Korber, B., Gaschen, B., Yusim, K., Thakallapally, R., Kesmir, C. & Detours, V. (2001). Evolutionary and immunological implications of contemporary HIV-1 variation. *Br Med Bull* **58**, 19–42.

Kulkosky, J., Katz, R. A., Merkel, G. & Skalka, A. M. (1995). Activities and substrate specificity of the evolutionarily conserved central domain of retroviral integrase. *Virology* **206**, 448–456.

Kwong, P. D., Wyatt, R., Robinson, J., Sweet, R. W., Sodroski, J. & Hendrickson, W. A. (1998). Structure of an HIV gp120 envelope glycoprotein in complex with the CD4 receptor and a neutralizing human antibody. *Nature* **393**, 648–659.

Larder, B. A., Darby, G. & Richman, D. D. (1989). HIV with reduced sensitivity to zidovudine (AZT) isolated during prolonged therapy. *Science* **243**, 1731.

Lataillade, M., Chiarella, J. & Kozal, M. J. (2007). Natural polymorphism of the HIV-1 integrase gene and mutations associated with integrase inhibitor resistance. *Antivir Ther (Lond)* **12**, 563–570.

Lauring, A. S., Acevedo, A., Cooper, S. B. & Andino, R. (2012). Codon Usage Determines the Mutational Robustness, Evolutionary Capacity, and Virulence of an RNA Virus. *Cell Host and Microbe* **12**, 623–632. Elsevier Inc.

Lauring, A. S., Frydman, J. & Andino, R. (2013). The role of mutational robustness in RNA virus evolution. *Nat Rev Micro* 1–10. Nature Publishing Group.

Lederman, M. M., Connick, E., Landay, A., Kuritzkes, D. R., Spritzler, J., St Clair, M., Kotzin, B. L., Fox, L., Chiozzi, M. H. & other authors. **(1998).** Immunologic responses associated with 12 weeks of combination antiretroviral therapy consisting of zidovudine, lamivudine, and ritonavir: results of AIDS Clinical Trials Group Protocol 315. *J Infect Dis* **178**, 70–79.

Lee, S. P., Xiao, J., Knutson, J. R., Lewis, M. S. & Han, M. K. (1997). Zn 2+Promotes the Self-Association of Human Immunodeficiency Virus Type-1 Integrase in Vitro. *Biochemistry* **36**, 173–180. American Chemical Society.

Levy, J. A., Hoffman, A. D., Kramer, S. M., Landis, J. A., Shimabukuro, J. M. & Oshiro, L. S. (1984). Isolation of lymphocytopathic retroviruses from San Francisco patients with AIDS. *Science* **225**, 840–842.

Li, M., Kao, E., Gao, X., Sandig, H., Limmer, K., Pavon-Eternod, M., Jones, T. E., Landry, S., Pan, T. & other authors. **(2012).** Codon-usage-based inhibition of HIV protein synthesis by human schlafen 11. *Nature* **490**, 125–128. Nature Publishing Group.

Liu, R., Paxton, W. A., Choe, S., Ceradini, D., Martin, S. R., Horuk, R., MacDonald, M. E., Stuhlmann, H., Koup, R. A. & Landau, N. R. (1996). Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection. *Cell* **86**, 367–377.

Lodi, P. J., Ernst, J. A., Kuszewski, J., Hickman, A. B., Engelman, A., Craigie, R., Clore, G. M. & Gronenborn, A. M. (1995). Solution structure of the DNA binding domain of HIV-1 integrase. *Biochemistry* **34**, 9826–9833.

Loeb, D. D., Swanstrom, R., Everitt, L., Manchester, M., Stamper, S. E. & Hutchison, C. A., III. **(1989).** Complete mutagenesis of the HIV-1 protease. *Nature* **340**, 397–400.

Loveday, C. & Hill, A. (1995). Prediction of progression to AIDS with serum HIV-1 RNA and CD4 count. *Lancet* **345**, 790–791.

Low, A., Prada, N., Topper, M., Vaida, F., Castor, D., Mohri, H., Hazuda, D., Muesing, M. & Markowitz, M. (2009). Natural polymorphisms of human immunodeficiency virus type 1 integrase and inherent susceptibilities to a panel of integrase inhibitors. *Antimicrobial Agents and Chemotherapy* **53**, 4275–4282.

Lucas, S. B., Hounnou, A., Peacock, C., Beaumel, A., Djomand, G., N'Gbichi, J. M., Yeboue, K., Hondé, M., Diomande, M. & Giordano, C. (1993). The mortality and pathology of HIV infection in a west African city. *AIDS* **7**, 1569–1579.

Lutzke, R. A., Vink, C. & Plasterk, R. H. (1994). Characterization of the minimal DNA-binding domain of the HIV integrase protein. *Nucleic Acids Res* **22**, 4125–4131.

Lutzke, R. A. P. & Plasterk, R. H. A. (1998). Structure-Based Mutational Analysis of the C-Terminal DNA-Binding Domain of Human Immunodeficiency Virus Type 1 Integrase: Critical Residues for Protein Oligomerization and DNA Binding.

Maertens, G. (2003). LEDGF/p75 Is Essential for Nuclear and Chromosomal Targeting of HIV-1 Integrase in Human Cells. *Journal of Biological Chemistry* **278**, 33528–33539.

Maertens, G. N., Hare, S. & Cherepanov, P. (2010). The mechanism of retroviral integration from X-ray structures of its key intermediates. *Nature* **468**, 326–329. Nature Publishing Group.

Malet, I., Delelis, O., Valantin, M.-A., Montes, B., Soulie, C., Wirden, M., Tchertanov, L., Peytavin, G., Reynes, J. & other authors. **(2008).** Mutations associated with failure of raltegravir treatment affect integrase sensitivity to the inhibitor in vitro. *Antimicrobial Agents and Chemotherapy* **52**, 1351–1358.

Mammano, F., Trouplin, V., Zennou, V. & Clavel, F. (2000). Retracing the evolutionary pathways of human immunodeficiency virus type 1 resistance to protease inhibitors: virus fitness in the absence and in the presence of drug. *Journal of Virology* **74**, 8524–8531.

Mandal, D., Feng, Z. & Stoltzfus, C. M. (2008). Gag-Processing Defect of Human Immunodeficiency Virus Type 1 Integrase E246 and G247 Mutants Is Caused by Activation of an Overlapping 5′ Splice Site. *Journal of Virology*.

Mansky, L. M. & Temin, H. M. (1995). Lower in vivo mutation rate of human immunodeficiency virus type 1 than that predicted from the fidelity of purified reverse transcriptase. *Journal of Virology* **69**, 5087–5094.

Martinez, M. A., Cabana, M., Parera, M., Gutierrez, A., Este, J. A. & Clotet, B. (2000). A bacteriophage lambda-based genetic screen for characterization of the activity and phenotype of the human immunodeficiency virus type 1 protease. *Antimicrobial Agents and Chemotherapy* **44**, 1132–1139.

Martinez, M. A., Pezo, V., Marlière, P. & Wain-Hobson, S. (1996). Exploring the functional robustness of an enzyme by in vitro evolution. *The EMBO Journal* **15**, 1203–1210.

Martinez-Picado, J., Savara, A. V., Sutton, L. & D'Aquila, R. T. (1999). Replicative fitness of protease inhibitor-resistant mutants of human immunodeficiency virus type 1. *Journal of Virology* **73**, 3744–3752.

Martinez-Picado, J. & Martínez, M. A. (2008). HIV-1 reverse transcriptase inhibitor resistance mutations and fitness: a view from the clinic and ex vivo. *Virus Research* **134**, 104–123.

Martínez, M. A. & Clotet, B. (2003). Genetic screen for monitoring hepatitis C virus NS3 serine protease activity. *Antimicrobial Agents and Chemotherapy* **47**, 1760–1765.

Marx, P. A., Alcabes, P. G. & Drucker, E. (2001). Serial human passage of simian immunodeficiency virus by unsterile injections and the emergence of epidemic human immunodeficiency virus in Africa. *Philos Trans R Soc Lond, B, Biol Sci* **356**,

911–920.

Maschera, B., Furfine, E. & Blair, E. D. (1995). Analysis of resistance to human immunodeficiency virus type 1 protease inhibitors by using matched bacterial expression and proviral infection vectors. *Journal of Virology* **69**, 5431–5436.

Masur, H., Michelis, M. A., Greene, J. B., Onorato, I., Stouwe, R. A., Holzman, R. S., Wormser, G., Brettman, L., Lange, M. & other authors. **(1981).** An outbreak of community-acquired Pneumocystis carinii pneumonia: initial manifestation of cellular immune dysfunction. *N Engl J Med* **305**, 1431–1438.

Más, A., Lopez-Galindez, C., Cacho, I., Gómez, J. & Martínez, M. A. (2010). Unfinished Stories on Viral Quasispecies and Darwinian Views of Evolution. *Journal of Molecular Biology* **397**, 865–877. Elsevier Ltd.

McColl, D. J., Fransen, S., Gupta, S., Parkin, N. & Margot, N. (2007). Resistance and cross-resistance to first generation integrase inhibitors: insights from a phase II study of elvitegravir (GS-9137). *Antiviral …*.

McColl, D. J. & Chen, X. (2010). Strand transfer inhibitors of HIV-1 integrase: bringing IN a new era of antiretroviral therapy. *Antiviral Research* **85**, 101–118.

MF, G., S, K., S, G. & EW, B. (1983). On the Enzymatic Basis for Mutagenesis by Manganese. *J Biol Chem* **258**, 3469–3475.

Miller, M. D., Danovich, R., Ke, Y., Witmer, M. V. & Zhao, J. (2008). Longitudinal analysis of resistance to the HIV-1 integrase inhibitor raltegravir: results from P005, a phase 2 study in treatment experienced patients. *Antivir Ther*.

Miller, M., Schneider, J., Sathyanarayana, B. K., Toth, M. V., Marshall, G. R., Clawson, L., Selk, L., Kent, S. B. & Wlodawer, A. (1989). Structure of complex of synthetic HIV-1 protease with a substrate-based inhibitor at 2.3 A resolution. *Science* **246**, 1149–1152.

Miura, T., Brockman, M. A., Brumme, Z. L., Brumme, C. J., Pereyra, F., Trocha, A., Block, B. L., Schneidewind, A., Allen, T. M. & other authors. **(2008a).** HLA-Associated Alterations in Replication Capacity of Chimeric NL4-3 Viruses Carrying gag-protease from Elite Controllers of Human Immunodeficiency Virus Type 1. *Journal of Virology* **83**, 140–149.

Miura, T., Brockman, M. A., Schneidewind, A., Lobritz, M., Pereyra, F., Rathod, A., Block, B. L., Brumme, Z. L., Brumme, C. J. & other authors. **(2009).** HLA-B57/B*5801 Human Immunodeficiency Virus Type 1 Elite Controllers Select for Rare Gag Variants Associated with Reduced Viral Replication Capacity and Strong Cytotoxic T-Lymphotye Recognition. *Journal of Virology* **83**, 2743–2755.

Miura, T., Brockman, M. A., Brumme, C. J., Brumme, Z. L., Carlson, J. M., Pereyra, F., Trocha, A., Addo, M. M., Block, B. L. & other authors. **(2008b).** Genetic Characterization of Human Immunodeficiency Virus Type 1 in Elite Controllers: Lack of Gross Genetic Defects or Common Amino Acid Changes. *Journal of Virology* **82**, 8422–8430.

Miura, T., Brumme, Z. L., Brockman, M. A., Rosato, P., Sela, J., Brumme, C. J., Pereyra, F., Kaufmann, D. E., Trocha, A. & other authors. **(2010).** Impaired Replication Capacity of Acute/Early Viruses in Persons Who Become HIV Controllers. *Journal of Virology* **84**, 7581–7591.

Montagnier, L. (1985). Lymphadenopathy-associated virus: from molecular biology to pathogenicity. **103**, 689–693.

Montagnier, L. (2002). HISTORICAL ESSAY: A History of HIV Discovery. *Science* **298**,

1727–1728.

Muse, S. V. & Gaut, B. S. (1994). A likelihood approach for comparing synonymous and nonsynonymous nucleotide substitution rates, with application to the chloroplast genome. *Molecular Biology and Evolution* **11**, 715–724.

Müller, V., Ledergerber, B., Perrin, L., Klimkait, T., Furrer, H., Telenti, A., Bernasconi, E., Vernazza, P., Gunthard, H. F. & other authors. **(2006).** Stable virulence levels in the HIV epidemic of Switzerland over two decades. *AIDS* **20**, 889–894.

Myers, M. W. (1990). New antiretroviral agents in the clinic. *Rev Infect Dis* **12**, 944–950.

Navia, M. A., Fitzgerald, P. M., McKeever, B. M., Leu, C. T., Heimbach, J. C., Herber, W. K., Sigal, I. S., Darke, P. L. & Springer, J. P. (1989). Three-dimensional structure of aspartyl protease from human immunodeficiency virus HIV-1. *Nature* **337**, 615–620.

Nei, M. & Kumar, S. (2000). Molecular evolution and phylogenetics. *Oxford University Press*. New York.

Nevot, M., Martrus, G., Clotet, B. & Martínez, M. A. (2011). RNA Interference as a Tool for Exploring HIV-1 Robustness. *Journal of Molecular Biology* **413**, 84–96. Elsevier Ltd.

Nguyen, B.-Y. T., Isaacs, R. D., Teppler, H., Leavitt, R. Y., Sklar, P., Iwamoto, M., Wenning, L. A., Miller, M. D., Chen, J. & other authors. **(2011).** Raltegravir: the first HIV-1 integrase strand transfer inhibitor in the HIV armamentarium. *Annals of the New York Academy of Sciences* **1222**, 83–89.

Nguyen, D. H. & Hildreth, J. E. (2000). Evidence for budding of human immunodeficiency virus type 1 selectively from glycolipid-enriched membrane lipid rafts. *Journal of Virology* **74**, 3264–3272.

Nielsen, R. & Yang, Z. (1998). Likelihood models for detecting positively selected amino acid sites and applications to the HIV-1 envelope gene. *Genetics* **148**, 929–936.

Nijhuis, M., Schuurman, R., de Jong, D., Erickson, J., Gustchina, E., Albert, J., Schipper, P., Gulnik, S. & Boucher, C. A. (1999). Increased fitness of drug resistant HIV-1 protease as a result of acquisition of compensatory mutations during suboptimal therapy. *AIDS* **13**, 2349–2359.

Nomura, S., Hosoya, N., Brumme, Z. L., Brockman, M. A., Kikuchi, T., Koga, M., Nakamura, H., Koibuchi, T., Fujii, T. & other authors. **(2012).** Significant reductions in gag-protease mediated HIV-1 replication capacity over the course of the epidemic in Japan. *Journal of Virology* 1–66.

Pannecouque, C., Daelemans, D. & De Clercq, E. (2008). Tetrazolium-based colorimetric assay for the detection of HIV replication inhibitors: revisited 20 years later. *Nat Protoc* **3**, 427–434.

Pantaleo, G., Graziosi, C. & Fauci, A. S. (1993). New concepts in the immunopathogenesis of human immunodeficiency virus infection. *N Engl J Med* **328**, 327–335.

Parera, M., Clotet, B. & Martinez, M. A. (2004). Genetic Screen for Monitoring Severe Acute Respiratory Syndrome Coronavirus 3C-Like Protease. *Journal of Virology* **78**, 14057–14061.

Parera, M., Fernandez, G., Clotet, B. & Martinez, M. A. (2006). HIV-1 Protease

Catalytic Efficiency Effects Caused by Random Single Amino Acid Substitutions. *Molecular Biology and Evolution* **24**, 382–387.

Parera, M., Perez-Alvarez, N., Clotet, B. & Martínez, M. A. (2009). Epistasis among Deleterious Mutations in the HIV-1 Protease. *Journal of Molecular Biology* **392**, 243–250. Elsevier Ltd.

Phillips, P. C., Otto, S. P., Whitlock, M. C. & Wolf, J. B. (2000). *Beyond the Average: The Evolutionary Importance of Gene Interactions and Variability of Epistatic Effects*. Epistasis and the evolutionary process.

Piot, P., Quinn, T. C., Taelman, H., Feinsod, F. M., Minlangu, K. B., Wobin, O., Mbendi, N., Mazebo, P., Ndangi, K. & Stevens, W. (1984). Acquired immunodeficiency syndrome in a heterosexual population in Zaire. *Lancet* **2**, 65–69.

Polard, P. & Chandler, M. (1995). Bacterial transposases and retroviral integrases. *Mol Microbiol* **15**, 13–23.

Pond, S. L. K. & Frost, S. D. W. (2005). Not so different after all: a comparison of methods for detecting amino acid sites under selection.

Pond, S. L. K., Frost, S. D. W. & Muse, S. V. (2005). HyPhy: hypothesis testing using phylogenies. *Bioinformatics* **21**, 676–679.

Popovic, M., Sarngadharan, M. G., Read, E. & Gallo, R. C. (1984). Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science*.

Powderly, W. G. (2010). Integrase inhibitors in the treatment of HIV-1 infection. *Journal of Antimicrobial Chemotherapy*.

Quillent, C., Borman, A. M., Paulous, S., Dauguet, C. & Clavel, F. (1996). Extensive regions of pol are required for efficient human immunodeficiency virus polyprotein processing and particle maturation. *Virology* **219**, 29–36.

Quinones-Mateu, M. E., Ball, S. C., Marozsan, A. J., Torre, V. S., Albright, J. L., Vanham, G., van der Groen, G., Colebunders, R. L. & Arts, E. J. (2000). A Dual Infection/Competition Assay Shows a Correlation between Ex Vivo Human Immunodeficiency Virus Type 1 Fitness and Disease Progression. *Journal of Virology* **74**, 9222–9233.

Quiñones-Mateu, M. E., Moore Dudley, D. M., Jegede, O., Weber, J. & J Arts, E. (2008). Viral Drug Resistance and Fitness. In *Advances in Pharmacology*, Advances in Pharmacology, pp. 257–296. Elsevier.

Rahman, S., Lu, R., Vandegraaff, N., Cherepanov, P. & Engelman, A. (2007). Structure-based mutagenesis of the integrase-LEDGF/p75 interface uncouples a strict correlation between in vitro protein binding and HIV-1 fitness. *Virology* **357**, 79–90.

Rao, J. K., Erickson, J. W. & Wlodawer, A. (1991). Structural and evolutionary relationships between retroviral and eucaryotic aspartic proteinases. *Biochemistry* **30**, 4663–4671.

Ratner, L., Gallo, R. C. & Wong-Staal, F. (1985). HTLV-III, LAV, ARV are variants of same AIDS virus. *Nature* **313**, 636–637.

Reed, L. J. & Muench, H. (1938). A simple Method of Estimating Fifty per Cent Endpoints. *American Journal of Epidemiology*.

Reeves, J. D. & Doms, R. W. (2002). Human immunodeficiency virus type 2. *J Gen Virol* **83**, 1253–1265. Soc General Microbiol.

Rice, P., Craigie, R. & Davies, D. R. (1996). Retroviral integrases and their cousins. *Curr Opin Struct Biol* **6**, 76–83.

Robert W Shafer, K. D. M. A. W. S. H. E. (2001). A Guide to HIV-1 Reverse Transcriptase and Protease Sequencing for Drug Resistance Studies. *HIV sequence compendium* **2001**, 1–51.

Robertson, D. L., Anderson, J. P., Bradac, J. A. & Carr, J. K. (2000). HIV-1 nomenclature proposal. *Science*.

Rolland, M., Brander, C., Nickle, D. C., Herbeck, J. T., Gottlieb, G. S., Campbell, M. S., Maust, B. S. & Mullins, J. I. (2007). HIV-1 over time: fitness loss or robustness gain? *Nat Rev Micro* **5**, 1–2.

Samson, M., Libert, F., Doranz, B. J., Rucker, J., Liesnard, C., Farber, C. M., Saragosti, S., Lapoumeroulie, C., Cognaux, J. & other authors. **(1996).** Resistance to HIV-1 infection in caucasian individuals bearing mutant alleles of the CCR-5 chemokine receptor gene. *Nature* **382**, 722–725.

Sanjuan, R. (2010). Mutational fitness effects in RNA and single-stranded DNA viruses: common patterns revealed by site-directed mutagenesis studies. *Philosophical Transactions of the Royal Society B: Biological Sciences* **365**, 1975–1982.

Sanjuán, R., Moya, A. & Elena, S. F. (2004a). The distribution of fitness effects caused by single-nucleotide substitutions in an RNA virus. *Proc Natl Acad Sci USA* **101**, 8396–8401.

Sanjuán, R., Moya, A. & Elena, S. F. (2004b). The contribution of epistasis to the architecture of fitness in an RNA virus. *Proc Natl Acad Sci USA* **101**, 15376–15379.

Schneidewind, A., Brockman, M. A., Yang, R., Adam, R. I., Li, B., Le Gall, S., Rinaldo, C. R., Craggs, S. L., Allgaier, R. L. & other authors. **(2007).** Escape from the Dominant HLA-B27-Restricted Cytotoxic T-Lymphocyte Response in Gag Is Associated with a Dramatic Reduction in Human Immunodeficiency Virus Type 1 Replication. *Journal of Virology* **81**, 12382–12393.

Schröder, A. R. W., Shinn, P., Chen, H., Berry, C., Ecker, J. R. & Bushman, F. (2002). HIV-1 integration in the human genome favors active genes and local hotspots. *Cell* **110**, 521–529.

Seki, T. & Kobayashi, M. (2010). S/GSK1349572 is a potent next generation HIV integrase inhibitor and demonstrates a superior resistance profile substantiated with 60 integrase mutant molecular clones. *17th Conference on Retroviral and Opportunistic Infections, San Francisco, CA, USA*.

Shafer, R. W., Dupnik, K., Winters, M. A. & Eshleman, S. H. (2001). A Guide to HIV-1 Reverse Transcriptase and Protease Sequencing for Drug Resistance Studies. *HIV sequence compendium* **2001**, 1–51.

Shafer, R. W. & Schapiro, J. M. (2008). HIV-1 drug resistance mutations: an updated framework for the second decade of HAART. *AIDS Rev* **10**, 67–84.

Sharp, P. M., Robertson, D. L. & Gao, F. (1994). Origins and diversity of human immunodeficiency viruses. *AIDS* **8**, S27–S42.

Shimura, K., Kodama, E., Sakagami, Y., Matsuzaki, Y., Watanabe, W., Yamataka, K., Watanabe, Y., Ohata, Y., Doi, S. & other authors. **(2008).** Broad Antiretroviral Activity and Resistance Profile of the Novel Human Immunodeficiency Virus Integrase Inhibitor Elvitegravir (JTK-303/GS-9137). *Journal of Virology* **82**, 764–

774.

Sices, H. J. & Kristie, T. M. (1998). A genetic screen for the isolation and characterization of site-specific proteases. *Proc Natl Acad Sci USA* **95**, 2828–2833.

Sigal, A., Kim, J. T., Balazs, A. B., Dekel, E., Mayo, A., Milo, R. & Baltimore, D. (2011). Cell-to-cell spread of HIV permits ongoing replication despite antiretroviral therapy. *Nature* **477**, 95–98. Nature Publishing Group.

Sinicco, A., Fora, R., Raiteri, R., Sciandra, M., Bechis, G., Calvo, M. M. & Gioannini, P. (1997). Is the clinical course of HIV-1 changing? Cohort study. *BMJ* **314**, 1232–1237.

Smyth, R. P., Davenport, M. P. & Mak, J. (2012). The origin of genetic diversity in HIV-1. *Virus Research* **169**, 415–429. Elsevier B.V.

Sourgen, F., Maroun, R. G., Frère, V., Bouziane, M., Auclair, C., Troalen, F. & Fermandjian, S. (1996). A synthetic peptide from the human immunodeficiency virus type-1 integrase exhibits coiled-coil properties and interferes with the in vitro integration activity of the enzyme. Correlated biochemical and spectroscopic results. *Eur J Biochem* **240**, 765–773.

Staszewski, S., Miller, V., Rehmet, S., Stark, T., De Cree, J., De Brabander, M., Peeters, M., Andries, K., Moeremans, M. & other authors. **(1996).** Virological and immunological analysis of a triple combination pilot study with loviride, lamivudine and zidovudine in HIV-1-infected patients. *AIDS* **10**, F1–7.

Stevenson, M. (2003). HIV-1 pathogenesis. *Nat Med* **9**, 853–860.

Sundquist, W. I. & Kräusslich, H.-G. (2012). HIV-1 Assembly, Budding, and Maturation. *Cold Spring Harb Perspect Med* **2**, a006924.

Suzuki, Y. & Gojobori, T. (1999). A method for detecting positive selection at single amino acid sites. *Molecular Biology and Evolution* **16**, 1315–1328.

Tamura, K., Peterson, D., Peterson, N., Stecher, G., Nei, M. & Kumar, S. (2011). MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution* **28**, 2731–2739.

Taverna, D. M. & Goldstein, R. A. (2002). Why are proteins so robust to site mutations? *Journal of Molecular Biology* **315**, 479–484.

Tebit, D. M. & Arts, E. J. (2010). Tracking a century of global expansion and evolution of HIV to drive understanding and to combat disease. *The Lancet Infectious Diseases* **11**, 45–56. Elsevier Ltd.

Tindall, B. & Cooper, D. A. (1991). Primary HIV infection: host responses and intervention strategies. *AIDS* **5**, 1–14.

Tözsér, J., Bagossi, P., Boross, P., Louis, J. M., Majerova, E., Oroszlan, S. & Copeland, T. D. (1999). Effect of serine and tyrosine phosphorylation on retroviral proteinase substrates. *Eur J Biochem* **265**, 423–429.

Troude, P., Chaix, M.-L., Tran, L., Deveau, C., Seng, R., Delfraissy, J.-F., Rouzioux, C., Goujard, C., Meyer, L.ANRS Primo cohort. **(2009).** No evidence of a change in HIV-1 virulence since 1996 in France. *AIDS* **23**, 1261–1267.

Troyer, R. M., Collins, K. R., Abraha, A., Fraundorf, E., Moore, D. M., Krizan, R. W., Toossi, Z., Colebunders, R. L., Jensen, M. A. & other authors. **(2005).** Changes in Human Immunodeficiency Virus Type 1 Fitness and Genetic Diversity during Disease Progression. *Journal of Virology* **79**, 9006–9018.

Troyer, R. M., McNevin, J., Liu, Y., Zhang, S. C., Krizan, R. W., Abraha, A., Tebit, D. M., Zhao, H., Avila, S. & other authors. **(2009).** Variable fitness impact of HIV-1 escape mutations to cytotoxic T lymphocyte (CTL) response. *PLoS Pathog* **5**, e1000365.

Turner, B. G. & Summers, M. F. (1999). Structural biology of HIV. *Journal of Molecular Biology* **285**, 1–32. Elsevier.

Vabret, N., Bailly-Bechet, M., Najburg, V., Müller-Trutwin, M., Verrier, B. & Tangy, F. (2012). The biased nucleotide composition of HIV-1 triggers type I interferon response and correlates with subtype D increased pathogenicity. *PLoS ONE* **7**, e33502.

Van Baelen, K., Rondelez, E., Van Eygen, V., Ariën, K., Clynhens, M., Van den Zegel, P., Winters, B. & Stuyver, L. J. (2009). A combined genotypic and phenotypic human immunodeficiency virus type 1 recombinant virus assay for the reverse transcriptase and integrase genes. *Journal of Virological Methods* **161**, 231–239.

Van Baelen, K., Van Eygen, V., Rondelez, E. & Stuyver, L. J. (2008). Clade-specific HIV-1 integrase polymorphisms do not reduce raltegravir and elvitegravir phenotypic susceptibility. *AIDS* **22**, 1877–1880.

van der Kuyl, A. C. & Berkhout, B. (2012). The biased nucleotide composition of the HIV genome: a constant factor in a highly variable virus. *Retrovirology* **9**, 92.

van Hemert, F. J. & Berkhout, B. (1995). The tendency of lentiviral open reading frames to become A-rich: constraints imposed by viral genome organization and cellular tRNA availability. *J Mol Evol* **41**, 132–140.

van Manen, D., Gras, L., Boeser-Nunnink, B. D., van Sighem, A. I., Maurer, I., Mangas Ruiz, M. M., Harskamp, A. M., Steingrover, R., Prins, J. M. & other authors. **(2011).** Rising HIV-1 viral load set point at a population level coincides with a fading impact of host genetic factors on HIV-1 control. *AIDS* **25**, 2217–2226.

van Nimwegen, E. (2006). Epidemiology. Influenza escapes immunity along neutral networks. *Science* **314**, 1884–1886.

Vanhems, P., Lambert, J., Guerra, M., Hirschel, B. & Allard, R. (1999). Association between the rate of CD4+ T cell decrease and the year of human immunodeficiency virus (HIV) type 1 seroconversion among persons enrolled in the Swiss HIV cohort study. *J Infect Dis* **180**, 1803–1808.

Varmus, H. (1988). Retroviruses. *Science* **240**, 1427–1435.

Vartanian, J. P., Henry, M. & Wain-Hobson, S. (1996). Hypermutagenic PCR involving all four transitions and a sizeable proportion of transversions. *Nucleic Acids Res* **24**, 2627–2631.

Vink, C., Oude Groeneger, A. M. & Plasterk, R. H. (1993). Identification of the catalytic and DNA-binding region of the human immunodeficiency virus type I integrase protein. *Nucleic Acids Res* **21**, 1419–1425.

Wagner, A. (2005). Robustness, evolvability, and neutrality. *FEBS Letters* **579**, 1772–1778.

Wai, J., Fisher, T., Embrey, M., Egbertson, M. & Vacca, J. (2007). Next generation of inhibitors of HIV-1 integrase strand transfer inhibitor: structural diversity and resistance profiles. *14th Conference on Retroviral and Opportunistic Infections, Los Angeles, CA, USA*.

Wain-Hobson, S., Vartanian, J. P., Henry, M., Chenciner, N., Cheynier, R., Delassus,

S., Martins, L. P., Sala, M., Nugeyre, M. T. & Guétard, D. (1991). LAV revisited: origins of the early HIV-1 isolates from Institut Pasteur. *Science* **252**, 961–965.

Wang, J. Y. (2001). Structure of a two-domain fragment of HIV-1 integrase: implications for domain organization in the intact protein. *The EMBO Journal* **20**, 7333–7343. Nature Publishing Group.

Wei, X., Ghosh, S. K., Taylor, M. E., Johnson, V. A., Emini, E. A., Deutsch, P., Lifson, J. D., Bonhoeffer, S., Nowak, M. A. & Hahn, B. H. (1995). Viral dynamics in human immunodeficiency virus type 1 infection. *Nature* **373**, 117–122.

Weiss, R. A. (2003). HIV and AIDS in relation to other pandemics. Among the viruses plaguing humans, HIV is a recent acquisition. Its outstanding success as an infection poses immense scientific challenges to human health and raises the question "What comes nest?". *EMBO Rep* **4 Spec No**, S10–4.

White, K. L., Margot, N. A., Wrin, T., Petropoulos, C. J., Miller, M. D. & Naeger, L. K. (2002). Molecular mechanisms of resistance to human immunodeficiency virus type 1 with reverse transcriptase mutations K65R and K65R+M184V and their effects on enzyme function and viral replication capacity. *Antimicrobial Agents and Chemotherapy* **46**, 3437–3446.

Whittle, H., Morris, J., Todd, J., Corrah, T., Sabally, S., Bangali, J., Ngom, P. T., Rolfe, M. & Wilkins, A. (1994). HIV-2-infected patients survive longer than HIV-1-infected patients. *AIDS* **8**, 1617–1620.

Wild, G., Gardner, A. & West, S. A. (2009). Adaptation and the evolution of parasite virulence in a connected world. *Nature* **459**, 983–986.

Wills, J. W. & Craven, R. C. (1991). Form, function, and use of retroviral Gag proteins. *AIDS* **5**, 639.

Wlodawer, A., Miller, M., Jaskólski, M., Sathyanarayana, B. K., Baldwin, E., Weber, I. T., Selk, L. M., Clawson, L., Schneider, J. & Kent, S. B. (1989). Conserved folding in retroviral proteases: crystal structure of a synthetic HIV-1 protease. *Science* **245**, 616–621.

Wright, K. (1986). AIDS therapy. First tentative signs of therapeutic promise. *Nature* **323**, 283.

Wu, T. D., Schiffer, C. A., Gonzales, M. J., Taylor, J., Kantor, R., Chou, S., Israelski, D., Zolopa, A. R., Fessel, W. J. & Shafer, R. W. (2003). Mutation patterns and structural correlates in human immunodeficiency virus type 1 protease following different protease inhibitor treatments. *Journal of Virology* **77**, 4836–4847.

Wu, X., Liu, H., Xiao, H., Conway, J. A., Hehl, E., Kalpana, G. V., Prasad, V. & Kappes, J. C. (1999). Human Immunodeficiency Virus Type 1 Integrase Protein Promotes Reverse Transcription through Specific Interactions with the Nucleoprotein Reverse Transcription Complex. *Journal of Virology* **73**, 2126–2135.

Young, F. E. (1988). The role of the FDA in the effort against AIDS. *Public Health Rep* **103**, 242–245.

Zheng, R., Jenkins, T. M. & Craigie, R. (1996). Zinc folds the N-terminal domain of HIV-1 integrase, promotes multimerization, and enhances catalytic activity. *Proc Natl Acad Sci USA* **93**, 13659–13664.

Zhu, K., Dobard, C. & Chow, S. A. (2004). Requirement for integrase during reverse transcription of human immunodeficiency virus type 1 and the effect of cysteine mutations of integrase on its interactions with reverse transcriptase. *Journal of Virology* **78**, 5045–5055.

Global report: UNAIDS report on the global AIDS epidemic 2012. **(2012).** Global report: UNAIDS report on the global AIDS epidemic 2012. *wwwunaidsorg* 1–212.

Abbreviations used

AIDS	Acquired Immunodeficiency Syndrome
AZT	Azidothymidine
CA	Capsid
CD4	CD4 positive T lymphocyte
CTL	Cytotoxic T Lymphocyte
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
FACS	Fluorescence-Activated Cell Sorting
FBS	Fetal Bovine Serum
GFP	Green Fluorescent Protein
HAART	Highly Active Antiretroviral Therapy
HIV-1	Human Immunodeficiency Virus type 1
HLA	Human Leucocyte Antigen
HTLV-III	Human T-Lymphotropic Virus type 3
IN	Integrase
INIs	Integrase Inhibitors
INSTIs	Integrase Strand Transfer Inhibitors
IPTG	Isopropyl-beta-D-1-thiogalasctopyranoside
LB	Luria-Bertani
MA	Matrix
NC	Nucleocapsid
OD600	Optical density at 600 nanometer
PBS	Phosphate Buffer Saline
PIs	Protease Inhibitors
PR	Protease
pVL	Plasma Viral Load
RAL	Raltegravir
RNA	Ribonucleic acid
rpm	Revolution per minute
RPMI	Roswell Park Memorial Institute medium
RT	Reverse Transcription or Reverse Transcriptase
RTIs	Reverse Transcritptase Inhibitors
RT-PCR	Reverse transcription polymerase chain reaction
SU	Surface
TM	Transmembrane
VL	Viral Load

VRC	Viral Replication Capacity
wt	wild type
λ bacteriophage	Lambda bacteriophage

Annex I

Culture media and Solutions

Media

LB (Luria-Bertani): 10 g Tryptone
5 g Yeast extract
10 g NaCl
Fill until 1 litre with ultrapure water. (Autoclaving)

SOC: 25 ml LB
500 µl Glucose 20%
250 µl $MgCl_2$ 1M
62,5 µl KCl 1M

Bacto-Agar: LB broth
1,2% Agarose
(Autoclaving)

Top-Agar: LB broth
0,7% Agarose
(Autoclaving)

RPMI 1640® Gibco, Invitrogen
With glucose, non essential amino acids, sodium pyruvate, phenol red
Without L-glutamine, HEPES.

Solutions

SM: 5,8 g NaCl
2 g MgSO4·7H2O
50 ml Tris-Cl 1M (pH 7,5)
5 ml gelatine solution 2%
Fill until 1 litre with ultrapure water. (Autoclaving)

PBS® Gibco, Invitrogen 1,06 mM Potassium Phosphate monobasic (KH2PO4)
155.17 mM Sodium Chloride (NaCl)
2,97 mM Sodium Phosphate dibasic (Na2HPO4-7H2O)

DNA Purification: **Elution buffer** 10 mM Tris-Cl (pH 8,5)

Annex II

Primers

Primer	Sequence (5'-3')	HXB2 position
T3	AATTAACCCTCACTAAAGGG	-
T7	TCGAGGTCGACGGTATC	-
SP6	ATTTAGGTGACACTATAG	-
T3proL[a]	AATTAACCCTCACTAAAGGGAACAAAAGCTGGAGCTCCACCG CGGTGGCGGCCGCTCTAGAACTAGTGGATCCCCCGGGCTGCA GGAATTCT**TCCTTTAACTTCCCTCAG**	2241 - 2258
T7XHO[b]	TAATACGACTCACTATAGGGCGAATTGGGTACCGGGCCCCCCC TCGAGTCAA**AGGCCATCCATTCCTGGC**	2588 - 2605
5'prot1	AGGCTAATTTTTTAGGGAAGATCTGGCCTTCC	2078 - 2109
3'prot1	GCAAATACTGGAGTATTGTATGGATTTTCAGG	2703 - 2734
5'prot2	TCAGAGCAGACCAGAGCCAACAGCCCCA	2136 - 2163
3'prot2	AATGCTTTTATTTTTTCTTCTGTCAATGGCC	2620 - 2650
5'prot2L	TCAGAGCAGACCAGAGCCAACAGCCCCACCAGAAGAGAGCTT CAGGTCTGGGGTAGAGACAACAACTCCCCCTCAGAAGCAGGA GCCGATAGACAAGGAACTGTATCCTTTAACTTCCCTCAG	2136 - 2258
3'prot2R	TAATGCTTTTATTTTTTCTTCTGTCAATGGCCATTGTTTAACTTT TGGGCCATCCATTCCTGGCTTTAATTTTACTGGTACAGTCTCAA TAGGGCTAATGGG	2550 - 2650
5'gag1	AAATCTCTAGCAGTGGCGCCCGAACAG	623 - 649
3'gag1	TAACCCTGCGGGATGTGGTATTCC	2826 - 2849
5'gag2	GACTCGGCTTGCTGAAGCGCGCACGGCAAGAGGCGAGGGGC GGCGACTGGTGAGTACGCCAAAAATTTTGACTAGCGGAGGCT AGAAGGAGAGAGATGGG	695 - 794
3'gag2	GGCCCAATTTTTGAAATTTTTCCTTCCTTTTCCATTTCTGTACAA ATTTCTACTAATGCTTTTATTTTTTCTTCTGTCAATGGCCATTGT TTAACTTTTG	2605 - 2704
s5'gag2	GGGCGGCGACTGGTGAGT	732 - 749
S3'gag2	CTTTATTGTGACGAGGGGTCG	2274 - 2294
5protRv	AGGGGTCGTTGCCAAAGAGTG	2261 - 2281
1bwt	AGGTGGATTATTTGTCATCCATCCTATTTGTTCCTGAAGG	1515 - 1554
5I1	GGAGGAAATGAACAAGTAGAT	4176 - 4196
3I1	GGGATGTGTACTTCTGAAC	5195 - 5213
5I2	TTTTTAGATGGAATAGATAAGG	4230 - 4251
3I2	TAATCCTCATCCTGTCTAC	5077 - 5095
5IRv_int	TATGCTGTTTCCTGCCCTGT	4506 - 4525
3IFw_int	CAGGGACAGCAGAAATCCAC	4910 - 4929

[a]: EcoRI enzyme restriction site is underlined and HXB2 sequence is indicated in bold characters.

[b]: XhoI enzyme restriction site is underlined and HXB2 sequence is indicated in bold characters.

NB: Fw means forward and Rv means reverse.

Primer	Sequence (5'-3')	pNL4-3 position
AgeIFw	CTGGCAGAAAACAGGGAG	2834 – 2871
BstEIIRv[a]	TAC**C**ATTTCTTTTGCTACTAC	3698 – 3718
BstEIIFw[a]	GTAGTAGCAAAAGAAAT**G**GT**A**A**C**CGTAGTAATACAAGATAA TAG	4349 – 4372
EcoRIRv	GGATAAACAGCAGTTGTTGC	5128 – 5147

[a]: BstEII enzyme restriction site (G'GTNACC) is underlined and characters differing from pNL4-3 template are indicated in bold characters.

NB: Fw means forward and Rv means reverse.

Annex III

Cell types

Procariotic cells

Host strains	Genotype
XL1-Blue MRF´ [a]	Δ(mcrA)183 Δ(mcrCB-hsdSMR-mrr)173 *endA1 supE44 thi-1 recA1 gyrA96 relA1 lac* [F´ *proAB lacIqZΔM15* Tn10 (Tetr)]
JM109	e14–(McrA–) recA1 endA1 gyrA96 thi-1 hsdR17 (rK–mK+) supE44 relA1 Δ(lac-proAB) [F´ traD36 proAB lacIqZΔM15].
SOLR[a]	e14–(McrA–) Δ(mcrCB-hsdSMR-mrr)171 sbcC recB recJ uvrC umuC::Tn5 (Kanr) lac gyrA96 relA1 thi-1 endA1 λR [F´ proAB lacIqZΔM15] Su–

[a]: Host strains supplied with the Uni-ZAP XR vector kit (Stratagene).

Eukaryotic cells

MT4:

Human T cells isolated from a patient with adult T-cell leukemia. Cultured in RPMI (Gibco, Madrid, Spain) supplemented with 10% heat inactivated fetal calf serum (FCS), Life Technologies, Madrid, Spain.

CEM-GFP:

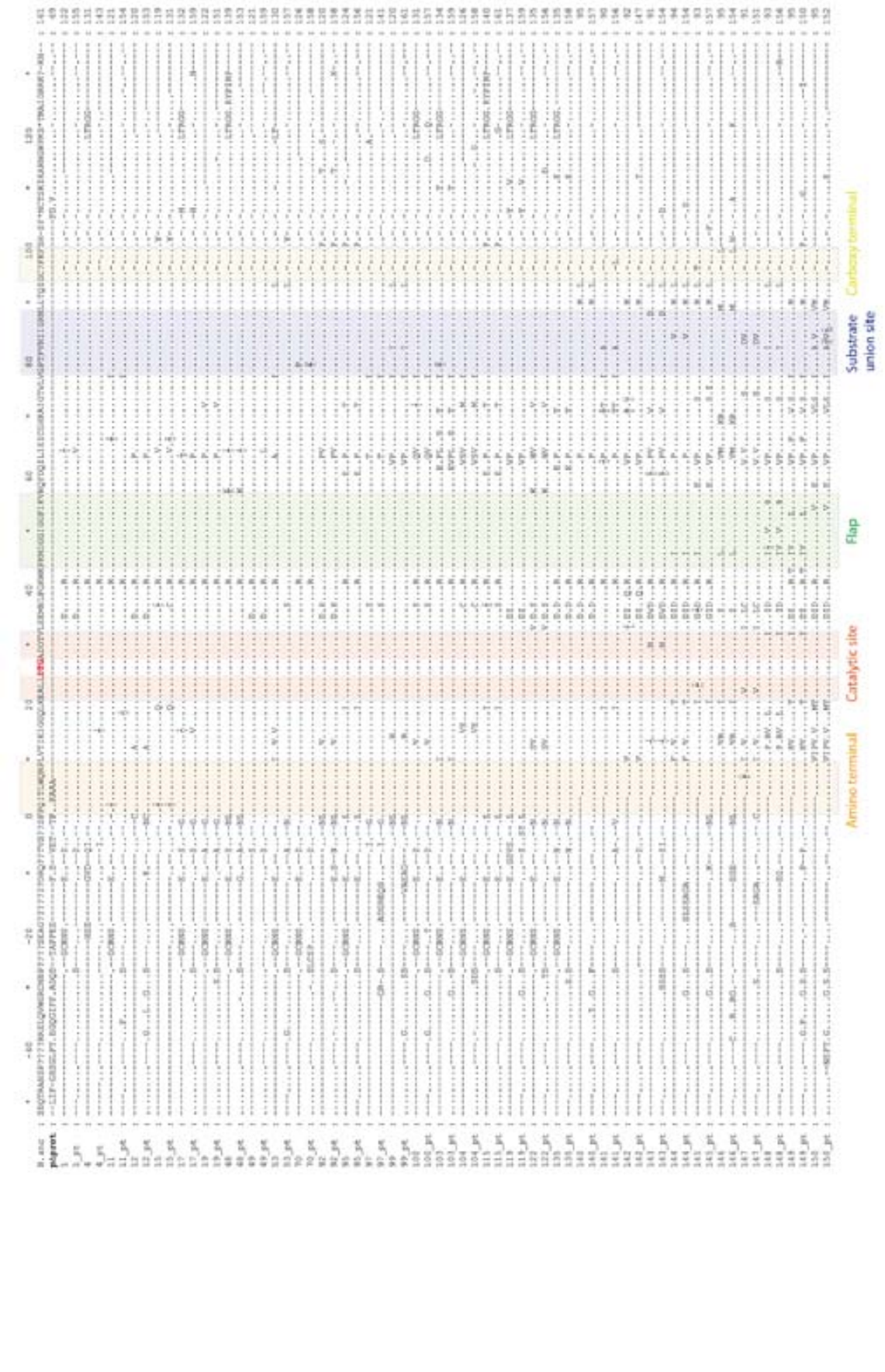
Derived from Human T-lymphoblastoid cell line CEM, expressing GFP under HIV-1 LTR promoter. Obtained from the AIDS Research and Reference Reagent Program, National Institutes of Health, Bethesda, MD. Cultured in RPMI (Gibco, Madrid, Spain) supplemented with 10% heat inactivated fetal calf serum (FCS), Life Technologies, Madrid, Spain.

Annex IV

Amino acid alignments of the plasma and recombinant HIV-1 protease and integrase sequences from study patients.

Amino acid sequence alignment of the HIV-1 protease sequences from plasma derived patients and from viral recombinant stocks at day 7 post-transfection (pt). Amino acid changes are indicated relative to the HIV-1 subtype B ancestral sequence obtained from Los Alamos HIV Sequence Database. The protease-deleted HXB2 clone, pΔprot, is also indicated. The catalytic triad of the protease formed by Asp 25, Thr 26, and Gly 27 is indicated in red bold cases. Dots indicate amino acid sequence identity and dash lines indicate sequences gaps. Colour boxes indicate the protease conserved regions: orange, amino terminal; red, catalytic site; green, flap; blue, substrate union site; and yellow, carboxyl terminal.

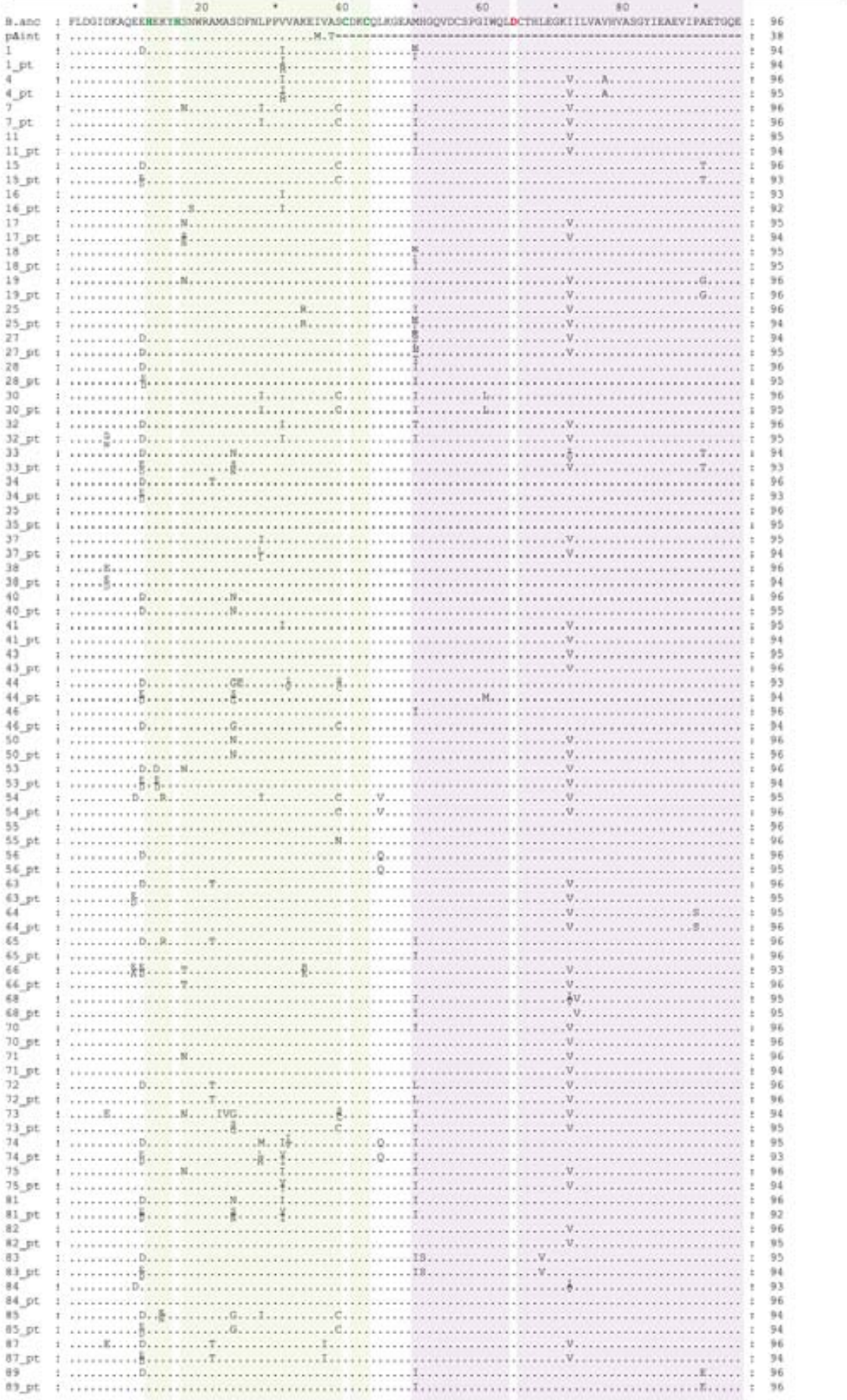
Annex IV: Amino acid alignments of the plasma and recombinant HIV-1 protease and integrase sequences from study patients



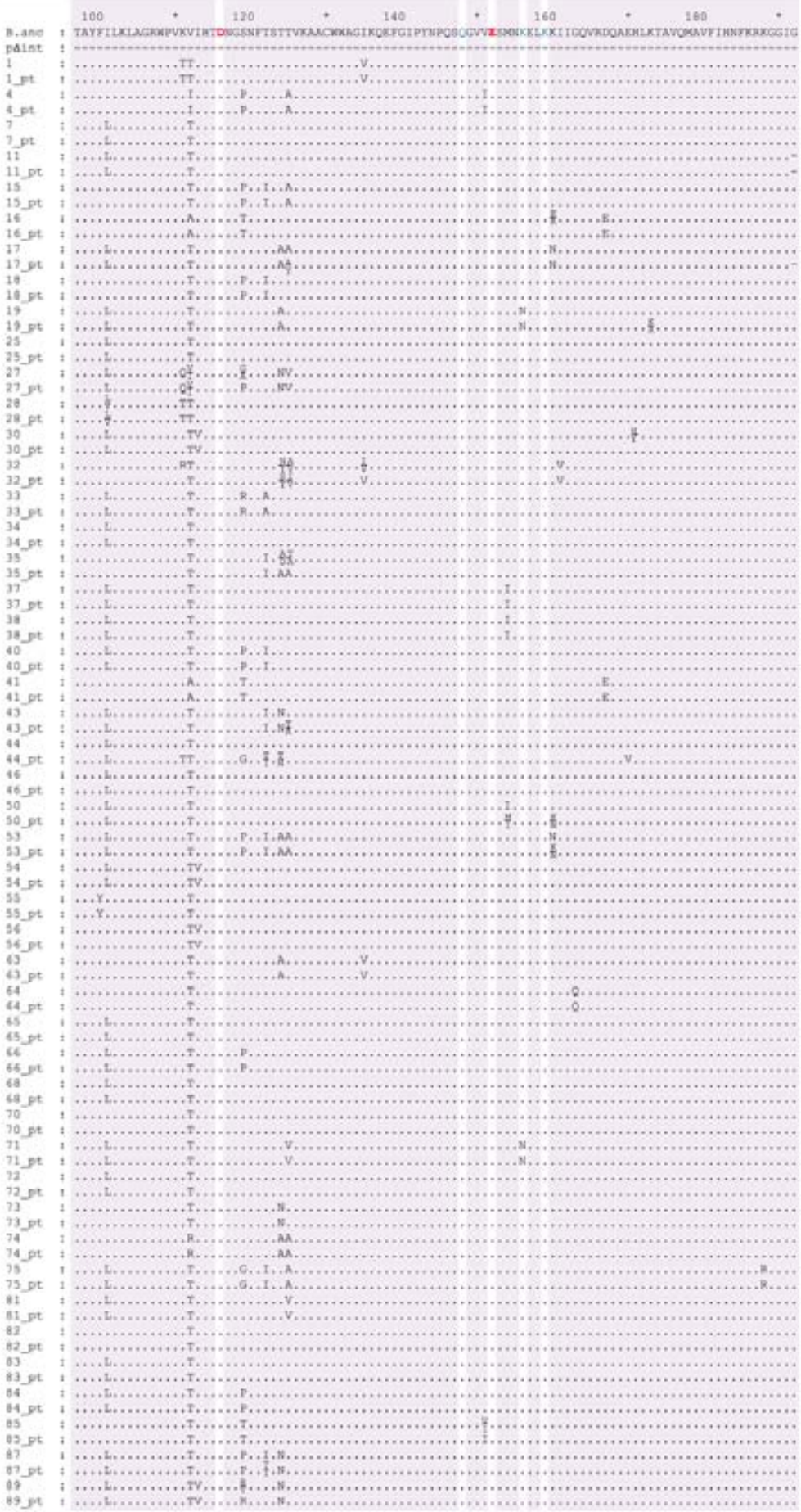
Amino acid sequence alignment of the HIV-1 integrase sequences from plasma derived patients and viral recombinant stocks at day 7 after transfection (pt). Amino acid changes are indicated relative to the HIV-1 subtype B ancestral sequence obtained from Los Alamos HIV Sequence Database. The protease-deleted pNL4-3 clone, pΔint, is also indicated. The HHCC domain formed by Hys 12 and 16, Cys 40 and 43 is indicated with green bold cases, the catalytic domain of the integrase formed by Asp 64 and 116, and Glu 152 is indicated with red bold cases, and the residues Gln 148, and Lys 156 and 159, that are also implicated in the integrase catalytic activity, are indicated in blue bold cases. Colour boxes indicate the integrase conserved regions: green, amino terminal or HHCC region; purple, catalytic domain; red, carboxyl terminal or DNA binding domain. Dots indicate amino acid sequence identity and dash lines indicate sequences gaps. Ambiguous positions are indicated with the two possible amino acid residues put on the top of each other.

Integrase sequences corresponding to the early infected patients, 1 to 89, in 1993-1994 appear in first order, followed by the integrase sequences corresponding to the late infected patients, 90 to 139, in 2006-2007.

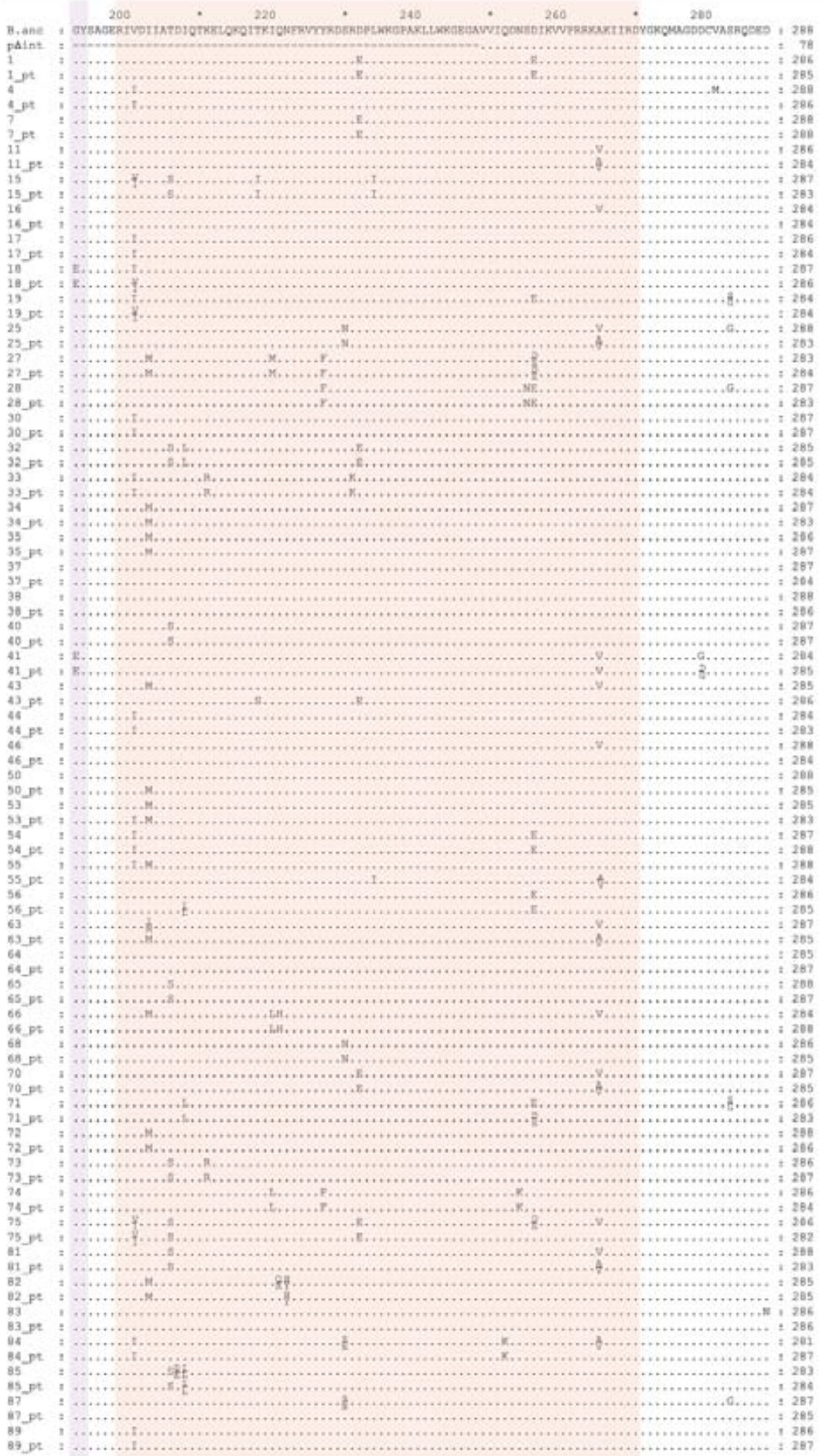
Annex IV: Amino acid alignments of the plasma and recombinant HIV-1 protease and integrase sequences from study patients



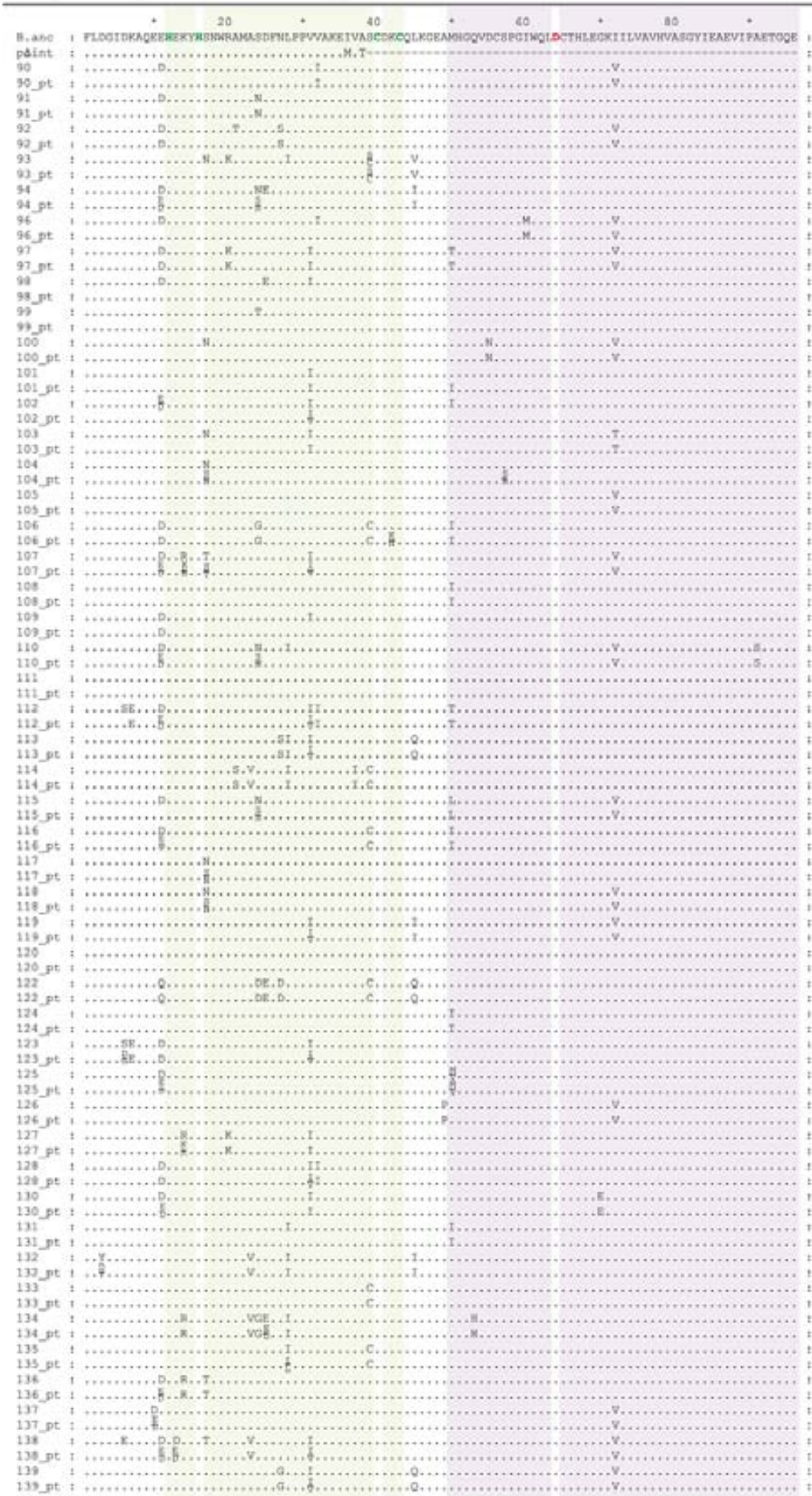
Annex IV: Amino acid alignments of the plasma and recombinant HIV-1 protease and integrase sequences from study patients



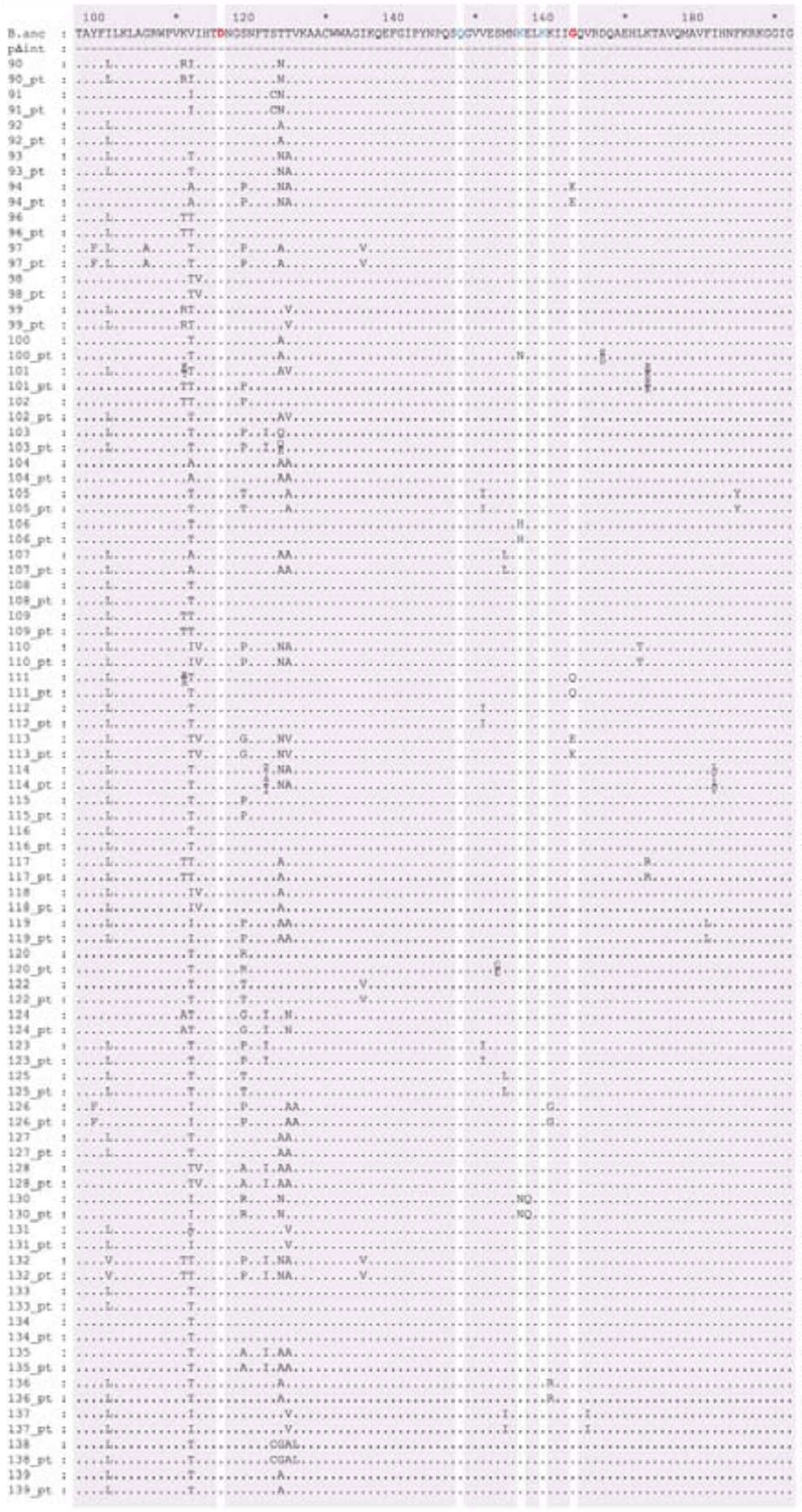
Annex IV: Amino acid alignments of the plasma and recombinant HIV-1 protease and integrase sequences from study patients



Annex IV: Amino acid alignments of the plasma and recombinant HIV-1 protease and integrase sequences from study patients



Annex IV: Amino acid alignments of the plasma and recombinant HIV-1 protease and integrase sequences from study patients



Annex IV: Amino acid alignments of the plasma and recombinant HIV-1 protease and integrase sequences from study patients

Acknowledgements

¡Por fin llegó mi parte favorita! Quiero aprovechar las siguientes líneas para agradecer a todas las personas que habéis contribuido de una manera u otra a la realización de este trabajo.

En primer lugar quería darle las gracias a Miguel Ángel Martínez, mi director de tesis, por darme la oportunidad de formar parte de sus proyectos y llevar a cabo uno de mis sueños: investigar. A parte de guiarme y acompañarme en este largo camino, he de decir que me sorprendió muy gratamente su calidez y trato al abrirnos las puertas de su casa y compartir sus artes culinarias con nosotras, las "molonas". Muchas gracias por todo lo que me has enseñado y sobretodo por tu sinceridad.

Antes de pasar a mis compañeras de grupo, quisiera agradecer también a Antonio Mas, mi tutor de prácticas en mi época de estudiante de Biología, por ser el primero en darme la oportunidad de conocer cómo funciona un laboratorio por dentro y meterme el gusanillo en el cuerpo para querer dedicarme a la investigación. Sabes que disfruté mucho de esa época, cuando tú estabas montando tu nuevo grupo de investigación en el Centro Regional de Investigaciones Biomédicas de Albacete. También contribuyeron a ello Itxaso, compañera de laboratorio, y aunque vieja conocida, amiga ganada en la poyata pero también, cómo no, en el día a día albaceteño. Así como todos los amigos que allí encontré y entre los que quiero hacer una especial mención a mi querida Clara, compañera de juergas, viajes (que espero a partir de ahora podamos reemprender, sino siempre puedo llamar al abogadooo!) y a la que quiero mucho! Y a Chiqui, que también estuvo ahí y del cual no deja de sorprenderme su extraordinaria habilidad de conciliar trabajo y vida recreativa… maldito;) A ver si aprendo a montármelo tan bien.

Ara sí, arribo a les "molones", les meves companyes de grup. Pensava que aquesta part seria més fàcil, perquè també és la que més il·lusió em feia. Però em costa trobar les paraules i em fa por no reflectir exactament tot el que sento. Noies, hem compartit tantes hores a la poyata, sota la flama, colze contra colze, han sorgit frecs, però sobretot una gran amistat. Com trobaré a faltar aquells matins comptant "calves" o vespres plaquejant screenings, mentres l'altra feia una mini, preparava seqüències o es barallava amb l'ordinador perquè se li quedava penjat o no trobava el que volia, tot amanit amb les nostres converses tant variades sobre "amoríos" o qualsevol tema quotidià, on cadascuna posava la seva part i feia que fos especial. Començo amb la Ester, la meva estimada sensak, que em va conquistar des de el

primer moment, ja quan vaig vindre a fer l'entrevista amb el Miguel Àngel. Sense tu no podria entendre Barcelona. Gràcies per compartir amb mi les teves ganes de viure i encomanar-me la teva alegria cada matí, vespre o nit! I pels riures... ja saps que sóc un públic agraït, però igualment he de reconèixer el teu "salero". I com no, merci per les classes de informàtica, disseny gràfic i sobretot donar-me a conèixer les OhDioses, equipaç de futbol, el poble de Sant Andreu... Gràcies també per escoltar-me sempre i donar-me tants bons consells quan tant els necessito. T'estimo molt i tot el que pugui dir es queda curt. Mariona, no et posis gelosa, que a tu també t'estimo molt! Ha estat un regal conèixer-te i compartir tants bons moments al laboratori amb el nostre estimat fag però també afora, a La Garriga, amb els teus nens o de concert amb La Troba Kung-Fú. La veu de l'experiència i la més organitzada! Que ens posa a totes rectes quan cal, però sempre atenta i carinyosa, gràcies també per estar ahí en tot moment, ja fos bó o dolent. Encara ens queda molt per compartir! N'estic segura, d'una manera o d'un altra. Sandra, encara me'n recordo també de quan em vas rebre al laboratori, fent-me de guia. Gràcies per ensenyar-me i ajudar-me amb tot, pels consells i per les xuxes que tant ens alegren a la Glòria i a mi... i pels viatges al cotxe portant-me a Sants, pels riures i pel teu caliu. Maria, gràcies a tu també pel teu carinyo i proximitat. És un gust raonar amb tu, tant sigui de PCRs o cultius, com de temes més personals. M'agrada molt quan rius amb les "burrades" que trec de tant en tant. I com no, no em puc oblidar de les curses per les muntanyes de Can Ruti! Encara que fes una calor tremenda o plogués, al vespre, migdia o ben d'hora al matí, i sempre com no amb la companyia de la Glo! Glòria, companya de congressos, penes i glòries (mai millor dit). Increïble la teva energia i ganes d'ajudar en tot moment. Què hauria fet sense tu els últims mesos, aquells vespres a la biblio de P3 analitzant i re-analitzant dades. Molts ànims en aquesta recta final que afrontes tu també ara mateix! Cristina, vas arribar a fer pràctiques i ens vas captivar. Tela, tela! Merci pel teu carinyo i suport. Et desitjo el millor i molts ànims per continuar lluitant per trobar el teu foradet al món de la recerca. També vull agrair a tots els estudiants en pràctiques que han passat pel laboratori i que amb els seus dubtes i ganes d'aprendre ens ajuden a acabar d'entendre el que fem. En especial volia agrair al Gerard Prats, que va estar al meu càrrec i em va ajudar molt quan començava els estudis de la integrasa.

Vull continuar amb companyes que ja han marxat de l'Irsicaixa, però que són amigues molt estimades, amb les que puc comptar i continuen sent properes. He de començar amb la meva estimada Curriu! Marta, que des de el primer dia m'ha

acompanyat i hem viscut moments entranyables. Et vas convertir de seguida en amiga de confidències i em vas obrir les portes de casa teva. I gràcies a això he conegut quasi tot Catalunya seguin-te amunt i avall, des de les terres de Osona al Priorat. Vam inclús viure juntes i després vam ser veïnes a Badalona. Quina enveja no poder expressar-me tant bé com tu per dir-te tot lo que t'estimo i lo molt que m'has aportat durant tot aquest temps. I part d'aquests bons moments els hem compartit amb la Isa. Ma chère Isa-Belle, encara que estiguis a Paris una part de tu encara està amb mi. Sempre em quedarà Isil o la màgica nit de Sant Joan a Roda de Barà! Quants riures m'has tret amb el teu bon humor. Com trobo a faltar els sopars-reunions amb vosaltres dues, amb embotits del país i un bon vi, però sobretot amb les xerrades interminables. I els viatges o excursions, a Formentera, Paris... on va aparèixer la An-Tània. Com et trobo a faltar a tu també Tània. Ara que ens havíem acostumat a dinar de tant en tant juntes. Gràcies per escoltar-me, per animar-me, pels riures, pels plors i per estar ahí. Ballarina, viatgera i somniadora incansable. Et desitjo el millor, però jo vull estar ahi i veure-ho, eh?! I no em puc deixar a la patinadora d'Irsi per excel·lència, la Sílvia, que quan pot troba un moment per juntar-nos i gaudir d'un vespre entre amigues. M'encanta el teu humor i la força per encarar les adversitats. Us estimo molt noietes! I per últim, vull agrair al meu estimat Emmanuel tots els bons moments que he passat amb ell. Tant al laboratori com a fora, on m'has tornat la il·lusió per continuar lluitant contra les injustícies i a ser més crítica amb el món que ens envolta. Vas deixar un vuit quan vas marxar, però per sort, gràcies a les noves tecnologies et continuo sentint ben a prop. Gràcies per donar-me ànims aquests últims mesos en que no veia el moment d'acabar el manuscrit. I espero algun dia no molt llunyà poder brindar amb tu a la nostra salut!

També vull agrair la estima de tota la resta de companys del laboratori amb els que tantes hores he compartit a dintre i a fora en els múltiples sopars de nadal, celebracions varies, partits de vollei-platja... Començant pels meus companys de la Biblio de P3, els "jaecitos". Als històrics, amb qui vaig començar, la Mer, la Moncu, el Jordi Senserrich (no hi ha jokes!) als que tant he trobat a faltar; i als actuals. Començant amb el meu popet, la Ester Ballana, no va durar gaire l'experiència nadadora, però sí que perdurarà la teva estima; la Alba, que té un cor i una gràcia que enamora; el Marc, treballador i corredor incansable i que té unes eixides que em fan molt riure; la Encarna, salerosa i molt curranta, mai m'oblidaré d'aquell festival a Burriana amb la Tània i tu (i crec que tu tampoc;); el Gerard, ai no, Roger! Jaja, quin gran fitxatge el Roger. Amb el teu humor i carinyo donava gust matinar per arribar a

treballar. Per cert, un secret, tinc whatsapp! I Eva, gran fichaje también, ha sido un gusto conocerte y compartir contigo aunque sea unos meses de trabajo y buen humor. I l'Edu, amb qui obro i tanco cicle. Ara hem compartit una mica més que quan vaig començar i espero que et vagi molt bé. Ah, i no em puc deixar a la Mari Pau Mena, que va passar fugaçment per Irsi però ens va omplir de tendror. También quiero agradecer a José Esté su espíritu crítico y trato amable durante todo este tiempo.

I abans de la biblio de P3, vaig compartir lloc de treball amb las "grecas" i gent de VIC. Judith, la inesgotable i acollidora, que sempre està ahi quant necessites el que sigui. Em quedo amb els riures i sopars a Sabadell. Mari Carmen y Jorge que siempre están atentos también y me han hecho sentir como en casa. Gracias por cuidarme tan bien y sobretodo por las risas. Quién se mete ahora contigo en P3 Jorge? Y quién me pincha a mi? Espero continuar compartiendo tertulias y buenas comidas con vosotros. De momento os digo que en mi nueva casa vuelvo a tener cocina de gas donde seguir cocinando paellas y donde os espero. La Eli, la auténtica e inconfundible, que también me ha dado su cariño a su manera. Y con la que espero poder seguir descubriendo japoneses. La Ruth, que té un cor enorme i et fa veure el sol amb el seu somriure. La Maria José, rebautizada María en Boston, que aunque pare un poco más fría, siempre ha estado ahí para echar una mano o unas risas. Al Gerard, Yeral, s'ha acabat el bròquil? Ni de lluny! A tu també t'he trobat molt a faltar, i no només pel teu catxondeo sinó sobretot pel teu carinyo. Quant fem la propera birra? La Itziar, siempre dispuesta a echar un cable y que me enseñó a cuidar al Ruper y saber entenderme con él cuando de repente no daba señal. La Nuria, investigadora tenaz que seguro tendrá una carrera exitosa pero que también sabe disfrutar de la vida y con la que es un gusto ir de cañas. Siempre recordaré aquella tarde en que celebramos improvisadamente con Itziar mi primer contrato! Julia, otra investigadora a la que admiro, que tanto me ha ayudado y animado en esos momentos críticos en los que pierdes la esperanza y piensas que nada tiene sentido. La MT, MªTeresa, frikipedia, volleibolera... què més? Què guai quant venies a la biblio de P3 als vespres a despedir-te de la Glòria i de mi. Molt ànims per tu també en aquesta recta final! I com no la Maria Pino, que va portar aire fresc al laboratori amb la seva arribada, sempre amb el somriure a la cara. Espero que et vagi molt bé la teva tesi. També vull agrair al Javier Martínez-Picado el seu suport i bon humor tot aquest temps. Així com al Julià Blanco, que sempre està atent i disposat a donar un cop de mà quan el necessites. A la Martona, la Mas-Bella, una

altra curranta, a la que poques vegades he vist perdre el somriure. Merci pel teu suport en la lluita per aconseguir unes condicions de treball dignes, però sobretot pels moments a fora del lab, encara hem d'anar a ballar salsa! I merci per la teva ajuda i consells inavaluables al moment de començar la escriptura d'aquesta tesi. Al Francesc, aquest company tant ben plantat i llest, que a sobre té un gran sentit de l'humor i comparteix l'afició de jugar a futbol al mateix lloc que las Ohdiosas. A la Ceci Cabrera, que te sorprendre a horas y momentos insospechados en el laboratorio y que me hace reír mucho con su humor ácido. Al Christian que, a banda d'alegrar-nos els dinars amb el seus acudits tant dolents, però amb els que petem de riure, també es fa estimar. I les seves inseparables Rocío i Maria Casadellà i amb les que sempre era un gust compartir el cafè de després de dinar. També vull agrair al Roger Paredes el seu caliu i bon humor. I al Marc Noguera pel seu humor histriònic. I com no, gràcies a la Tere, que es va incorporar fa poquet, però s'ha fet estimar moltíssim. A la Esther Giménez, salerosa ella también y aventurera incansable. Siempre recordaré aquel Raid en la Barceloneta haciendo equipo contigo y Ester Aparicio. ¿Quién me manda a mi madrugar un domingo? Valió la pena con creces! A pesar de las agujetas...

També vull agrair el companyerisme de l'equip del Christian Brander, which has such a great sense of humor, a la Anuska, la Bea, el Álex Olvera, la Vanessa, la Marta Ruiz, la Susana, el Pep i la Sandra. I would also like to thank Ai Kawama, who worked with us for a year and made much warmer to work in the P3 lab. Thanks for approaching us your japanese culture and for being so kind and lovely.

I també vull fer una menció especial a la Margarita Bofill, amb qui sempre m'he sentit molt a gust i a la que li desitjo el millor en aquesta nova etapa i sobretot molta salut.

Com no, vull agrair també a les responsables de l'èxit dels serveis cientifico-tècnics que amb la seva cura i ben fer ens proveeixen les mostres que després ens permeten fer estudis com el que he presentat en aquesta tesi. Però sobretot vull destacar el seu costat humà i lo bé que m'he sentit amb vosaltres. Amb la Teresa, compartint xerrades a l'hora de dinar o un té al vespre i sempre et sorprèn amb alguna curiositat que mai haguessis sospitat; la Rafi, tan cariñosa, siempre recordaré tu ofrecimiento de hacerme calditos para que yo me centrara en escribir la tesis; Samandhy, chamita, también cariñosa y atenta y con la que siempre es un gusto charlar o salir

de marcha; la Eulàlia, la millor coordinadora del Màster i amb la que sempre és un gust xerrar; Cristina Ramírez, otro solete del laboratorio; Lucía, más reservada pero igual de entrañable. Y gracias también a Lydia por los momentos compartidos. No me puedo dejar a mi querida Susana, que nos hace más liviana la organización del laboratorio, pero sobretodo por sus abrazos matutinos y cachondeo que te alegran la mañana.

I gràcies a l'equip de comunicació que tant bona feina fan. Primer a la Rosina, que em va permetre participar en la elaboració de la web divulgativa i em va apropar el món de la comunicació científica. I a la Matilde, que em va descobrir el racó de la terreta a Barcelona i amb qui m'hagués agradat compartir més temps.

També vull agrair als companys que van passar de manera més fugaç per Irsi, però que van deixar la seva imprenta i amb els que em vaig sentir molt a gust: el Javier Ibarrondo, la Jenny, el Paco, la Elisenda. I al Raúl Hernández, cuyo paso por Irsi no fue fugaz, pero con el que coincidí solo al inicio de mi tesis, y tan bien me acogió cuando todavía no conocía a gente en Barcelona. Gracias amigo! I al Mattia, que a banda d'alegrar-nos amb les seves estupendes lasanyes i tiramisús, va ser un gran company que em fa molt de gust trobar-me després en xerrades reivindicatives.

Gracias también Cris y Penélope por ser tan organizadas y tan majas! Y gracias Lourdes por tu comprensión y esfuerzo en estos momentos tan delicados que vivimos ahora mismo.

Gràcies també al Ventura per mostrar-se atent quan l'he necessitat, tot i la seva complicada agenda. Però sobretot gràcies per haver dut la recerca de la SIDA a tant bon punt.

Gràcies també als membres de la Unitat clínica que tant bé cuiden als pacients, però amb qui també he pogut compartir molts bons moments i sempre estan disposats a ajudar-te, en especial al Jordi, la Cristina Miranda i el José Santos.

Pero fuera del laboratorio hay vida, y mucha! Y quiero agradecer a todos los amigos y amigas que me habéis hecho disfrutar y querer tanto a Barcelona. Empezando por mi queridísima Aura, compañera y amiga de piso;) Convivimos más de 3 años que dieron mucho de sí, pero sobretodo una gran amistad... y que no lo pierda! América,

con la que disfruté tanto no solo de los vermuts en la Boquería o la Barceloneta, sino también de su preciosa amistad. Como no, mi querida Ana, que llegó al poco que yo a Barcelona para hacer su tesis, que fue un gran apoyo y con la que tanto me divertí. Y gracias a la que conocí a los que ahora son grandes amigos, Régis y Nicolas, a los que quiero mucho. Y como no, a Pancho, que pese a todos los vaivenes también es un gran amigo al que quiero mucho. Y no me puedo dejar a mis compañeros de clase de alemán y afines, con los que tan bien me lo he pasado estos últimos tiempos: Xavi, Eva, Magaly, Silvia, Vir, José, Marcel, Elena-Arantxa, Raquel, Katty... però sobretodo a mi Cris, a la que quiero mucho! Ah, y a mis amigos de la facultad de Biología que siguen estando ahí presentes. En especial a Fernando, con él que tan bien me lo he pasado en los congresos de Virología y al que quiero mucho.

I no m'he oblidat, vull agrair a les meves companyes "Ohdiosas" tot el bon rotllo i estima al llarg de quasi 4 anys cada dimarts al camp de futbol. Mai hagués pensat que jugaria al futbol, però en jugar un partit amb vosaltres em vau conquistar. Us estimo molt i us portaré sempre al cor: Ester, Èlia, Marieta, Nùria, Anna Peuets, Txemiqui, Gabi, Maria "A18", Elba, Alba, Carla, Gemita, Mar, Curriu...

Y no puedo dejar de agradecer a mis amigas gatunas, algunas todavía en la terreta y otras dispersas por el mundo, pero que siempre logramos reunirnos y a las que quiero mucho: Alba, Elia, Elena, Rocío, San, Oli y Rosa. Es un gusto poder seguir compartiendo con vosotras quedadas, cenas, viajes y ahora también nacimientos y bodas!

Y ya por último quiero agradecer a mi familia su apoyo incondicional. Empezando por las abuelas, que tanto nos han cuidado y tanto cariño nos han dado. A mi abuela Marina y a la tía Rosa, Rosigües, os quiero mucho y siempre será y sabrá a poco el tiempo que pueda pasar ahora con vosotras. Sí, sempre tenim raó, com ens dieu... Y a mi abuela Nieves, que ya nos dejó, pero que siempre fue muy cariñosa y de alguna manera sigue estando presente. Y salto a una generación más joven. Gracias a mis tíos, Marina y Jesús, por estar también siempre ahí presentes y quererme tanto. Y a mi tía Lola, que aunque hablemos menos de lo que me gustaría, también tengo muy presente y quiero mucho. Y a mis primos, Marina, Carmen y Xuso, que nos hemos criado casi como hermanos y a los que tanto quiero. Y también quiero agradecer a mi cuñado Devis, todo su cariño y comprensión durante estos años. Magari torniamo

presto a fare una cena insieme, come cuando venevi a Barcelona, per parlare de tante chose…

Y me dejo lo mejor para el final. Siempre me han dicho que soy una pelota, pero todo lo que pueda decir de mis padres y de mi hermana se queda corto. A vosotros os dedico este trabajo. Gracias por vuestra paciencia, amor, comprensión y sobretodo por estar ahí a las malas y a las maduras. Gracias por haberme enseñado a ser perseverante, aunque a veces me diluya, eh mamá? Pero aquí estoy papá quemando este último cartucho, de esta tanda, que muchos me quedan por quemar todavía. Mi queridísima sister, eres todo un ejemplo a seguir para mi y te quiero mucho, como la trucha al trucho! Y a mi sobrina Alba, que nos tiene a todos enamorados y seguro que dará mucho que hablar el día de mañana.