Hot News

Multi-Step Inhibition against HIV Lifecycle-Underlying the "Magic" of Protease Inhibitors

HIV protease inhibitors exhibit an exceptional antiviral efficacy and high genetic barrier for the development of resistance among anti-HIV drugs. This profile makes protease inhibitors key molecules for the treatment of HIV infection for both initial antiretroviral regimens and rescue therapies. The molecular mechanisms that might explain these unique features have been recently published in a fascinating study (Rabi, et al. J Clin Invest. 2013;123:3848-60.) evaluating the contribution of protease inhibitors to block each relevant step of the HIV lifecycle including entry, reverse transcription, and post-reverse transcription events.

The mechanism of action of protease inhibitors is well known at the structural and biochemical level. These molecules are substrate analogues for the HIV protease enzyme which cleaves viral polyproteins ($Pr55^{Gag}$ and $Pr166^{Gag\text{-}Pol}$) into multiple mature virion proteins to finally produce mature HIV particles capable of infecting new cells. However, it was unknown at which point of the viral cycle the inhibition of virus maturation became manifest. The inhibition of the proteolytic cleavages necessary to produce mature virions could affect early post-entry steps including un-coating and reverse transcription. In addition, studies performed in mutant viruses without completing the proteolytic cleavages have suggested that immature particles are defective in entry. In addition, the interactions occurring between the cytoplasmic tail (CT) of gp41 and the un-cleaved polyprotein $Pr55^{Gag}$ appear to inhibit the fusion of immature particles to the cell surface.

Rabin, et al. have conducted a detailed analysis of those steps within the HIV lifecycle affected by the inhibition of HIV protease to provide a mechanistic explanation for the exceptional pharmacodynamics and efficacy of protease inhibitors. Among the main findings, they proved that immature particles can be released efficiently from cells treated with protease inhibitors. However, the immature particles are not able to efficiently complete the entry, reverse transcription, and post-entry transcription steps. They have experimentally isolated and measured the dose-response curves of protease inhibitors for each of these stages. Then, by combining the curves obtained for each step, they were able to reconstruct the overall dose-response curves for protease inhibitors. These experiments showed that through the independent inhibition of multiple distinct steps in the lifecycle, protease inhibitors generate highly cooperative dose-response curves that make them uniquely effective. Interestingly, almost half of the inhibitory potential of protease inhibitors is observed at the entry step, while inhibition at the reverse transcription and post-reverse transcription steps account for smaller and variable fractions of the total inhibition.

This research also demonstrated that the presence of drug resistance mutations at the HIV protease alters both entry and post-entry dose-response curves. Moreover, mutations at the envelope gene, specifically at the CT domain of gp41, can confer resistance to protease inhibitors even in the context of wild-type *gag* and *pol* genes. These findings can also provide an explanation for those cases of patients failing a protease inhibitor-based therapy without the selection of resistance mutations at the HIV protease. Likewise, these observations highlight the potential relevance of HIV-1 envelope for a more accurate assessment of resistance to protease inhibitors. Since the most common commercial genotypic and phenotypic assays used for the analysis of resistance to HIV protease inhibitors do not consider the *env* gene, the overall estimation of resistance to protease inhibitors might be underestimated. However, at this time, it is still unknown how common this phenomenon is and therefore its relevance to accurately quantify protease inhibitor resistance.

Eva Poveda
Division of Clinical Virology
INIBIC-Complejo Hospitalario Universitario de A Coruña
A Coruña, Spain

New Promises for Hepatitis C Cure in HIV Coinfection

One of the most eagerly awaited news of 2013 was the approval in December of another two direct-acting antivirals for hepatitis C, Janssen's HCV protease inhibitor simeprevir (Olysio®) and Gilead's HCV polymerase inhibitor sofosbuvir (Sovaldi®). Sofosbuvir plus ribavirin is the first interferon-free combination approved for patients infected with HCV genotypes 2 or 3 and those with HCV genotype 1 who cannot tolerate the side effects of interferon.

The advent of direct-acting antivirals has brought a revolution in hepatitis C treatment, with a wealth of promising new data. Further advances are expected over the next couple of years as more drugs become available, including the first NS5A inhibitors daclatasvir and ledipasvir. Major expectations

are for a sofosbuvir/ledipasvir co-formulation from Gilead and all-oral combinations from AbbVie (ABT-450, ABT-267, ABT-333), Bristol-Myers-Squibb (daclatasvir, asunaprevir, BMS-791325), and Merck (MK-5172, MK-8742).

Very soon it will be possible to cure most chronic hepatitis C patients – usually with interferon-free therapy that lasts 8-12 weeks – including most difficult-to-treat groups, such as prior interferon null responders, people with liver cirrhosis, liver transplant recipients, and specially HIV/HCV coinfected patients.

The high cost of the new antivirals for hepatitis C, however, may limit a rapid and wide access to these medications. In many places, there is a prioritization of therapy for subjects with a more urgent need to be treated, such as those with advanced liver fibrosis. Hopefully, the paradox of interferon (that works less and is poorly tolerated in cirrhotics) will finally be put to rest thanks to these oral regimens.

Pablo Labarga
Department of Infectious Diseases
Hospital Carlos III
Madrid, Spain

Bone Marrow Transplant to Fight Cancer and HIV Infection

More than 30 years after the first AIDS cases were described, there is still no cure for HIV infection. Treatment is based on antiretrovirals that maintain viral replication suppressed, but need to be taken indefinitely since they are not able to eradicate the infection. In 2009 a ray of light gave hope to the HIV field, with the first case of HIV cure being reported (Hütter, et al. N Engl J Med. 2009;360:692-8). Timothy Ray Brown, better known as "the Berlin patient", was an HIV-infected individual diagnosed with acute myeloid lymphoma who underwent a myeloablative bone marrow transplant from a donor harbouring the Δ32 mutation at the CCR5 gene in homozygosis. This rare mutation confers resistance to HIV infection by the disruption of CCR5, the major coreceptor used by HIV to enter host cells. More than six years after the transplant, there are no signs of the virus in the blood or in other tissues, despite extensive sampling and lack of antiretroviral therapy (ART). Several factors may have contributed to HIV cure in this person, which is still matter of discussion, including the myeloablative conditioning performed, graft vs. host disease, or full engraftment of CCR5-negative cells.

More recently, another hematopoietic stem cell (HSC) transplantation approach raised the interest of the international scientific community. In July 2012, a report from the XIX International AIDS Conference described similar cases in Boston. Two HIV-infected individuals were diagnosed with Hodgkin's lymphoma and underwent bone marrow transplantation from wild-type CCR5 donors (Henrich, et al. IAC 2012. THAA0101). The milder conditioning followed as preparation for the transplant made it possible to maintain ART during the whole procedure and beyond, which likely helped to protect the infused donor cells. Up to four years after transplantation there were no signs of HIV infection in peripheral blood, although the patients were kept on ART during the whole period. These exciting results drove the investigators to consider a controlled treatment interruption in order to assess a possible clearance of the virus.

One year after, preliminary data was presented about the controlled treatment interruption of these patients (Henrich, et al. IAC 2013. WELBA05). Briefly, eight and 15 weeks after treatment interruption, no HIV was found in peripheral blood, using rigorous and very sensitive techniques for viral detection. Two possible new cases of HIV cure were in sight, but investigators recommended avoiding hasty conclusions since latent HIV reservoirs could still be present. Indeed, this was the case. At the Sixth International Workshop on HIV Persistence during Therapy, held in December 2013, investigators reported that HIV had re-emerged in the two Boston patients, four and eight months after ART cessation (Henrich, et al. Int Works HIV Persist 2013. Abstract 94). The hope for a cure of two more HIV-infected patients had vanished.

Although obviously disappointing, these results are very informative about how profoundly the HIV reservoir is established after infection, regardless of ART, and how insufficient our current detection techniques are. Latent reservoirs, undetectable using the most sensitive techniques, were enough to re-launch infection when antiretroviral pressure was off. A detailed analysis of both cases is eagerly awaited to shed light on important questions: Where did the virus rebound from? Why was one of the patients able to hold HIV resurgence significantly longer than the other? What level of reservoir reduction could be needed for a long-lasting effect?

In the meantime, some characteristics of these cases are worth highlighting. Two major differences are present between the successful "Berlin patient" and the failed "Boston patients". First, a thorough myeloablative conditioning for bone marrow transplant in the Berlin patient most likely fully depleted the infected immune system and HSC in the patient. In contrast, a much gentler conditioning was performed for the Boston patients, which was likely to leave residual infected host cells. A more aggressive conditioning might reduce the HIV reservoir to a greater extent, increasing the chances for complete

viral clearance. Secondly, and perhaps more importantly, the donor of the Berlin patient was homozygous for the Δ32 mutation at the CCR5 gene, which was not the case for either of the Boston patients. As a result, the immune system developed from CCR5-negative HSC after transplantation was resistant to HIV infection. This aspect most likely played an essential role in the success of the Berlin patient.

The disappointing outcome of the Boston patients stresses the importance of HIV-resistant phenotypes, which may be essential for any effective anti-HIV strategy involving HSC transplantation. Several groups are pursuing modulation of host cell susceptibility to HIV infection by the disruption of CCR5 using gene therapy, either in lymphocytes or HSC. The use of HSC has obvious advantages over lymphocytes, given that HSC will produce all cells of the immune system, including T-cells, the main target of HIV, but also other potential targets such as macrophages. Early results using the CCR5 disruption strategy showed protection in animal models, and clinical trials are underway to evaluate this protective effect in humans. Therefore, despite the initial disappointment from the Boston patients experience, important knowledge has come from it, and more is about to arrive, which may bring us one step closer to a definitive HIV cure.

Eduardo Seclén
Molecular Microbiology and Immunology Department
Keck School of Medicine
University of Southern California
Los Angeles, USA

New IDSA Guidelines for Vaccination of Immunocompromised Patients

The Infectious Diseases Society of America (IDSA) has recently updated the guidelines for vaccination of immunocompromised patients (Rubin, et al. Clin Infect Dis. 2014;58:309-18), with specific recommendations for HIV-infected individuals. Besides stressing the importance of completion and updating infant vaccination schedules, these guidelines highlight the need for administration of certain inactivated vaccines to adults. Moreover, principles that should guide the administration of attenuated vaccines in immunocompromised patients are given.

Briefly, HIV-infected patients should be vaccinated following the CDC vaccination schedules (CDC. MMWR Surveill Summ. 2013;62(Suppl 1):1-19). Besides ensuring prior vaccination for diphtheria-tetanus-pertussis, hepatitis A and B, and polio schedules, HIV-infected patients should receive a yearly dose of inactivated influenza vaccine. Pneumococcal vaccine should be administered every five years, while patients aged 11-26 years should receive a quadrivalent human papillomavirus vaccine.

The safety and efficacy of live attenuated vaccines is satisfactory in HIV-infected patients with CD4 counts > 200 cells/μl (> 15% in children). Hence, non-immune patients with stable HIV infection should receive the appropriate vaccination doses against mumps, rubella, measles, and varicella. However, the use of the quadrivalent mumps-measles-rubella-varicella vaccine is still discouraged in the HIV population regardless of immune status. Other live attenuated vaccines, mainly for international travel purposes, such as yellow fever, cholera, or typhoid fever, can be safely administered in patients with CD4 counts > 200 cells/μl.

The IDSA guidelines remind us of the need for correct vaccination of household contacts as well as the use of appropriate boosting, since HIV-infected persons tend to experience quicker antibody waning (Kerneis et al. Clin Infect Dis. 2014. E-pub ahead of print).

José Vicente Fernández-Montero
Department of Infectious Diseases
Hospital Carlos III
Madrid, Spain

HIV Drug Resistance Testing Still Important

The proportion of HIV-infected individuals on antiretroviral therapy exhibiting undetectable viremia has increased up to 90% in most HIV clinics, mainly as a result of the introduction of the newest antiretrovirals, namely integrase strand transfer inhibitors (INSTI). Conversely, the proportion of HIV-positive patients experiencing virological failure has drastically declined, making the request for drug resistance testing less frequent (Cescon A, et al. J Acquir Immune Defic Syndr. 2014;65:107-14).

Despite the potency, tolerability, and durability of first-generation INSTIs (i.e. raltegravir and elvitegravir), resistance mutations arise in a substantial proportion of HIV-infected individuals experiencing virological failure on these drugs. Interestingly, INSTI mutants are detected in up to 60% of treatment-experienced patients but only in 8% of drug-naive individuals.

A recent report examined all clinically requested tests for integrase genotypic resistance performed at a national reference laboratory in the USA, covering four years from 2009 through 2012 (Hurt C, et al. Clin Infect Dis. 2014;58:423-31). Overall, 15.6% of patients harbored ≥ 1 raltegravir or elvitegravir resistance mutation. Changes Q148 and N155 were equally represented (42% for each pathway), whereas Y143 was recognized in only 18%. The Q148 pathway was accompanied by other integrase mutations in 98% of cases (Q148 + G140 in 56%), whereas N155 appeared alone in 44%. Interestingly, the predicted high-level resistance to dolutegravir using Stanford scores (> 60) was found in 12% of

patients exhibiting raltegravir or elvitegravir resistant viruses. Although the authors concluded that dolutegravir is likely to remain active in most patients that fail first-generation INSTIs, a major concern derives from these data. Significant dolutegravir resistance (> 30 using Stanford rules) was noticed in 53% of patients harboring ≥ 2 INSTI resistance mutations. Accordingly, rescue interventions based on dolutegravir could fail in around half of these patients. Therefore, resistance testing should remain crucial for designing the best antiretroviral regimen as rescue intervention.

As technology improves, the value of introducing more sensitive methods able to detect HIV minority variants has given rise to huge debate. A recent study (Boltz, et al. J Infect Dis. 2014 Jan 16 [Epub ahead of print]) examined the prevalence and impact of nevirapine-resistant minority variants in African women with and without prior exposure to single-dose nevirapine for prevention of mother-to-child HIV transmission. Interestingly, the rate of virological failure did not differ according to the presence or absence of baseline nevirapine resistance changes detected by allele-specific polymerase chain reaction in women never exposed to nevirapine that began first-line nevirapine-based antiretroviral therapy. In contrast, virological failure was more frequent in women that began similar regimens after being previously exposed to single-dose nevirapine (OCTANE/A5208 trials 2 and 1, respectively). However, the rate of virological failure did not differ when the proportion of nevirapine resistance mutants was < 1%. Thus, the clinical impact of HIV minority variants seems to become manifest only for changes present > 1%. Thus, using population sequencing (Sanger) technologies that exhibit a sensitivity for minority variants around 15-20%, there is a narrow window between 1% and 15-20% that may account for potentially meaningful false-positive results. However, this is only true when prior drug exposure has occurred, leading to a so-called residual quasispecies "memory" (Briones C, et al. AIDS Rev. 2008;10:93-109). Thus, antiretroviral exposure is critical for assessing the significance of low-frequency HIV-resistant variants, being only potentially meaningful when present above 1% in the virus population.

Carmen de Mendoza
Department of Internal Medicine
Hospital Universitario Puerta de Hierro
Puerta de Hierro Research Institute
Madrid, Spain