Hot News

Welcome to "Hot News", a section of AIDS Reviews written by the editors and invited experts which focuses on recently reported information believed to be of both impact and higher interest to the readership.

The RESIST Trials – Superiority of Tipranavir over other PIs

The next antiretroviral agent likely to be approved is a protease inhibitor (PI) called tipranavir (TPV), which has been specifically developed to address the urgent need for new active agents for people with limited treatment options due to multidrug resistance. TPV is active against many HIV variants that have developed resistance to currently available PIs. In November, the interim results at week 24 of two identical phase 3 clinical trials of TPV, called RESIST, were released. RESIST-1, conducted in the USA, Canada and Australia was presented at the 44th ICAAC, which took place in Washington, while RESIST-2, conducted in Europe, Argentina, Brasil and Mexico, was presented at the 7th Drug Therapy Congress, which took place in Glasgow.

All participants in RESIST-1 received an optimised standard-of-care regimen selected by their physicians and were randomized to include either TPV/ritonavir or an alternative boosted PI. Although the study offered expert analysis of baseline genotype data to help physicians construct the best possible regimen, only approximately 30% of the treating clinicians took advantage of this assistance. Lopinavir/ritonavir was selected as a PI component of the standard-of-care regimens in 61% of the patients, saquinavir/ritonavir was used in 21% of regimens, and amprenavir/ritonavir was used in 14%.

To be eligible for the study, patients had to have prior exposure to antiretrovirals from all three classes; at baseline, they had to have taken a median of four prior PIs, two prior NNRTIs, and six prior NRTIs. Participants also had to have a viral load of > 1000 copies/ml for at least one month while receiving their current regimen. There was no restriction on CD4+ cell count.

At 24 weeks, 42% of patients receiving a TPV-containing regimen achieved a treatment response, defined as a ≥ 1 log reduction in HIV-RNA level, compared with 22% of those who did not add TPV to their "best available" regimens ($p < 0.0001$). Viral load was suppressed below 400 copies/ml in 35% of those receiving TPV vs. 16% of patients in the comparator arm ($p < 0.001$), and below 50 copies/ml in 25 and 10% of these groups, respectively ($p < 0.001$). The median decrease in viral load was –0.88 log copies/ml in the TPV arm compared with –0.26 log copies/ml in the control group ($p < 0.001$).

Changes in CD4+ cell counts at week 24 were modest, with a median increase of 36 cells/mm^3 among

Table 1. RESIST trials

	RESIST-1 (US, Canada, Australia)		RESIST-2 (Latin America, Europe)	
	TPV/r	CPI/r	TPV/r	CPI/r
No.	311	309	435	428
Hepatitis B/C	8%	11%	12%	18%
Median baseline CD4	123	123	175	196
Median baseline HIV-RNA (log)	4.8	4.8	4.8	4.8
Enfuvirtide use	36%		11.5%	
Discontinuation at w24 due to adverse events	9%	5%	7%	5%
Virological response (> 1 log HIV-RNA drop) at w24 (ITT)	42%	22%	41%	15%
Median HIV-RNA log drop (ITT)	–0.88	–0.28	–0.72	–0.22
Undetectable HIV-RNA (ITT)				
< 400 copies/ml	35%	16%	33%	13%
< 50 copies/ml	25%	10%	22%	9%
Median CD4 change from baseline (ITT)	+36	+6	+31	+1

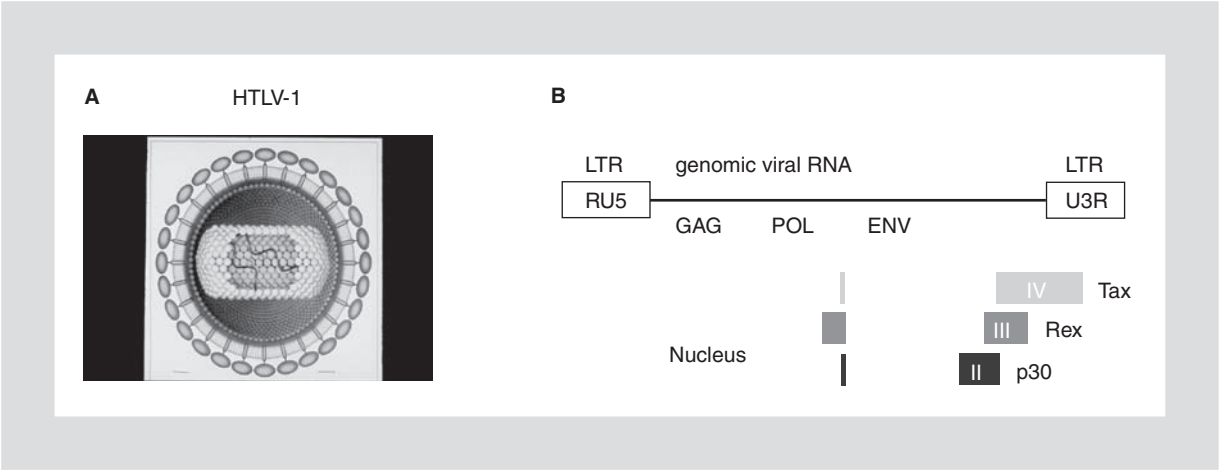

Figure 1. *HTLV-1 morphology (A) and genomic organization and depiction of the spliced mRNA encoding the tax, rex, and p30^{II} proteins (B).*

those receiving TPV vs. an increase of 6 cells/mm^3 in the comparator arm ($p < 0.001$).

Enfuvirtide (T-20) was used in 36% of the regimens and played an important role in contributing to treatment success in the trial, in both the TPV and the comparator arms. Among patients who received both T-20 and TPV, 47% achieved plasma HIV-RNA < 400 copies/ml compared with 35% in the overall TPV group.

Grade 3 or 4 adverse events were more common in the TPV arm, mainly due to increased rates of nausea, and there were more discontinuations due to adverse events than in the comparator arm. Grade 3 or 4 elevations of ALT levels were three-times more frequent among TPV recipients ($p < 0.001$), although elevations of transaminases were asymptomatic and did not result in study discontinuations. Elevations in cholesterol ($p < 0.001$) and triglycerides ($p < 0.01$) levels were also significantly more common in those receiving TPV. The use of 200 mg bid of ritonavir along with TPV most likely explains the higher rate of transaminase elevations and dyslipidemia in patients receiving TPV when compared with other boosted PIs (which were given with half the ritonavir dose).

The results of the RESIST-2 trial were quite similar. All together, the RESIST trials prove for the first time, in the largest studies conducted so far in heavily antiretroviral-experienced patients, that TPV is the most potent PI available. Its approval is eagerly awaited by the growing population of HIV-infected individuals who have failed prior antiretroviral regimens.

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Human T-Cell Leukemia/Lymphoma Virus Type 1: Playing Hide-and-Seek

Humans have evolved specialized immune functions, such as cytolytic T-cells, cytokines, chemokines, and antibodies, to fight intracellular and extracellular pathogens. On the other hand, infectious agents able to persist in the host have evolved sophisticated mechanisms to escape host immune surveillance.

The human T-cell leukemia/lymphoma virus type 1 (Fig. 1A) is transmitted sexually or through breastfeeding and causes adult T-cell leukemia/lymphoma (ATLL), or a progressive demyelinating disease designated tropical spastic paraparesis/HTLV-1-associated myelopathy (TSP/HAM).

The HTLV-1 genome (Fig. 1B) is composed of two copies of a single-stranded RNA virus whose genome is copied into a double-stranded DNA form that integrates into the host cell genome (provirus). Molecular epidemiological studies indicate a direct correlation between the proviral DNA level and disease occurrence, and it is thought that the proviral amplification occurs mainly through cell division.

HTLV-1 infects memory (antigen-experienced) CD4+ T-cells that are programmed to quickly enter the cell cycle and undergo cell division upon antigen reencounter. Thus, at any moment the provirus could be expressed and the infected cells recognized and eliminated by the host immune system.

HTLV-1 genome expression begins by transcription from the viral promoter within the long terminal repeat (LTR) (Fig. 1B). The genomic RNA encodes the structural/enzymatic proteins *gag* and *pol*, whereas a singly spliced messenger RNA (mRNA) encodes the *env* protein. A unique doubly spliced mRNA encodes two positive regulators of viral ex-

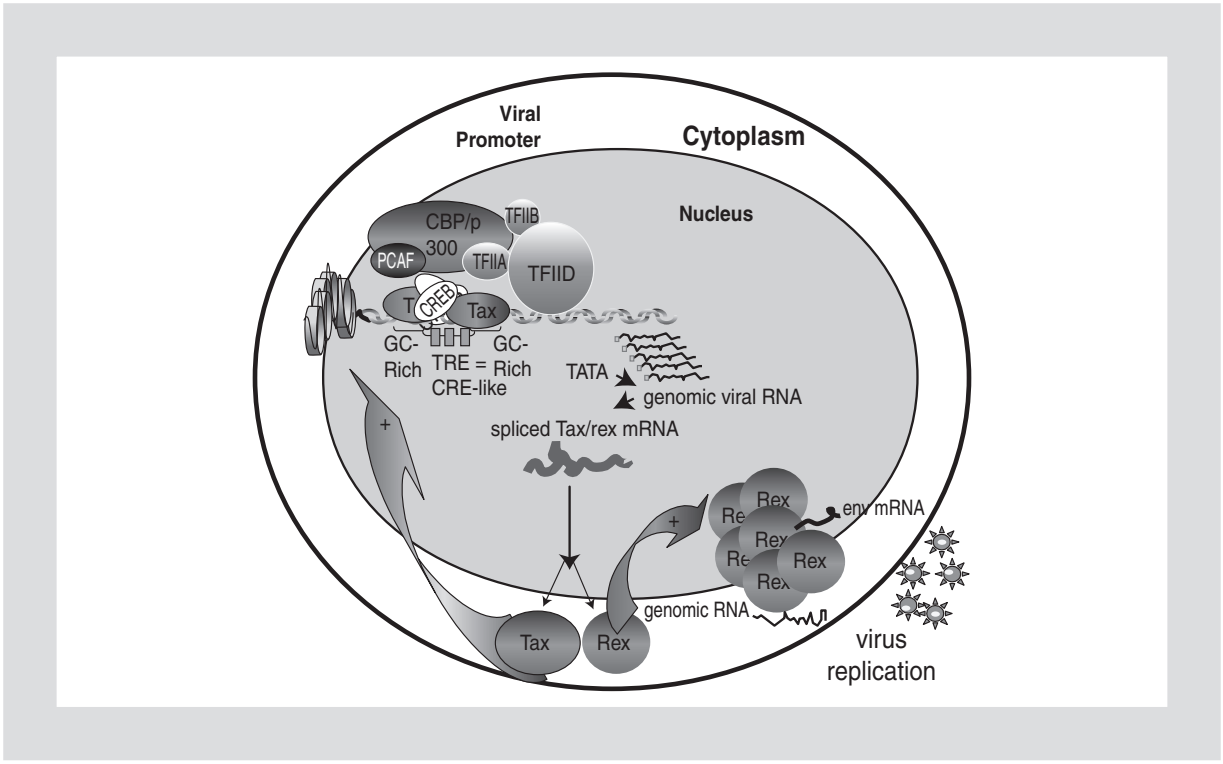

Figure 2. *Top left: a simplified transcription complex on the viral promoter. Bottom: tax and rex production and the effect of rex on the transport of singly spliced and unspliced viral RNAs.*

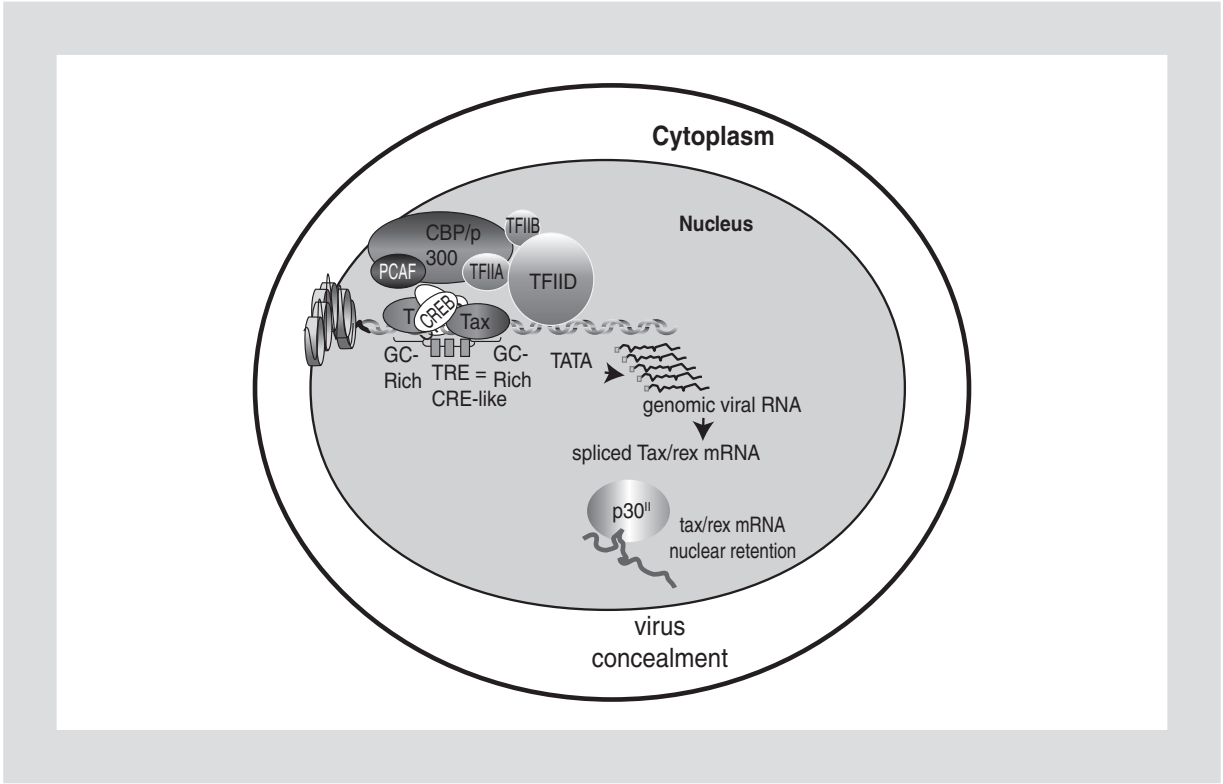

Figure 3. *The p30II protein is depicted binding to the tax/rex mRNA (it is unknown whether the binding is direct or indirect) and retaining it in the nucleus.*

pression, *tax* and *rex* (Fig. 1B). Increasing levels of *tax* during viral expression leads to the recruitment of highly effective transcription complexes to the viral promoter (Fig. 2), and fully fledged viral expression occurs. The genomic RNA that encodes the protease, reverse transcriptase, and integrase, as well as the *env* protein, are necessary for the production of infectious viral particles. The *rex* function is essential to transport the singly spliced *env* and the unspliced genomic mRNAs to the cytoplasm (Fig. 2).

We have previously found that HTLV-1 encodes a protein, $p30^{II}$, generated from a doubly spliced mRNA from the HTLV-1 ORF II (Fig. 1B) (Ciminale, et al. J Virol 1992;66:1737-45; Koralnik, et al. Proc Natl Acad Sci USA 1992;89:8813-7). More recently, we have uncovered its function and demonstrated that $p30^{II}$ is a negative regulator of viral expression (Nicot, et al. Nat Med 2004;10:197-201). The negative effect of $p30^{II}$ on viral replication is not due to interference with *tax*-mediated transcription from the viral promoter, as overexpression of *tax* or *rex* cDNA alone is unable to counteract the negative effect of $p30^{II}$ on proviral expression. However, when *tax* and *rex* are expressed from the unique doubly spliced mRNA derived from the proviral clone, $p30^{II}$ exerts a negative post-transcriptional effect. Quantitative RT-PCR analysis of viral messenger mRNA species demonstrated that the cytoplasmic level of the *tax*/*rex* mRNA is decreased by $p30^{II}$. Importantly, we found that $p30^{II}$ is a nuclear-resident protein unable to shuttle in and out of the nucleus. In addition, we demonstrated that $p30^{II}$ binds to the doubly spliced *tax*/*rex* mRNA. Thus, by retaining the *tax*/*rex* mRNA in the nucleus, $p30^{II}$ negatively regulates viral expression (Fig. 3). As expected, overexpression of $p30^{II}$ in HTLV-1-infected T-cell lines also decreases viral replication by decreasing the level of *tax*. Interestingly, a protein of 28 kD encoded by HTLV-2, a virus genetically related to HTLV-1, also exerts a negative regulatory effect on viral replication by a similar posttranscriptional mechanism (Younis, et al. J Virol 2004;78:11077-83).

Immune T-cells continuously patrol lymphoid and nonlymphoid tissues in search of "foreign" signals. A reasonable hypothesis appears to be that $p30^{II}$ may help HTLV-1 to hide better when cells divide. This hypothesis could explain the paradox that most T-cells carrying the provirus do not produce detectable viral transcripts. How $p30^{II}$ expression is regulated, and whether inhibition of $p30^{II}$ function may reveal hidden infected cells to immune surveillance, are unknown. Thus, further investigation on the basic molecular mechanisms of $p30^{II}$ activity might teach us how to reduce or even eradicate virus-infected cells and prevent disease.

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Recombination Theories Refuted by Positive Epistasis

As an integral part of the HIV life cycle, recombination has significantly shaped HIV diversity. It has, however, been a longstanding scientific question whether recombination exists because it is itself beneficial or as the result of some extrinsic process. In a recent issue of Science, Bonhoeffer, et al. investigated whether negative epistasis could lead to the emergence of recombination for HIV (Science 2004;304:1547-50). Epistasis refers to the fitness effects of the interaction of different genes within the genome. Negative epistasis describes a situation where the combination of two detrimental mutations results in a greater loss of fitness than expected from the single mutations (synergy) and where beneficial mutations may act antagonistically. The opposite situation is termed positive epistasis; this would imply no beneficial role for recombination.

An analysis of 9466 HIV protease and partial RT sequences with associated fitness values, obtained by a single-round replication assay, indicated that there is a predominant signal of positive epistasis. These findings contrast with the theory that recombination is an adaptation to purge deleterious mutations in the genome. A related study on the non-recombining vesicular stomatitis virus (VSV), by Sanjuan and colleagues (PNAS 2004;101:15376-9), showed that the patterns of epistasis are also not compatible with such theories.

What explanations for the emergence of recombination are we left with then? For HIV, Bonhoeffer, et al. refer to drift-based explanations or template switching as a means to overcome single-strand breaks. The latter would imply that recombination is the consequence of a mechanistic process rather than a trait selected because of its possible fitness benefits.

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