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# Hot News

## Exosomes as New Players in HIV Pathogenesis - New Data from the IAS 2017

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Exosomes are nanovesicles that can be released into the extracellular medium by different cell types and are considered an important system of intercellular communication. In some instances, on secretion, exosomes break down and release their content into the extracellular space. Alternatively, intact exosomes can interact with other cells and discharge their content directly into the target cell cytoplasm. Exosomes are rich in endosome-associated proteins (i.e., the tetraspanin family) but also carry different molecules in their lumen including proteins, RNAs (i.e., microRNAs), and pathogen-derived cargo. Indeed, the different biological functions of exosomes might depend on their cargo components. Several recent publications have identified exosomes as new players in the pathogenesis of HIV infection. This is an emerging field of growing interest that deserved a satellite symposium at the 9th IAS Conference held in Paris in July 2017, sponsored by the International Society for Extracellular Vesicles (EVs).

Although the role of exosomes in HIV infection is not yet clarified, accumulating data suggest that exosomes may act at different levels of HIV pathogenesis by modulating immune responses, infectivity, or even by activating the latent viral reservoir. However, there are still methodological limitations that need to be resolved to advance our understanding of the role of exosomes in HIV infection. One particular limitation is to define an accurate and reproducible procedure for their isolation. A new strategy to separate exosomes from HIV-1-infected cells was proposed at this symposium. Martin-Jaular and colleagues (Institut Curie, France) used velocity centrifugation gradients and antibody labeling of specific endosome-associated protein markers (i.e., CD45+ and AChE+) to isolate pure exosomes from complex preparations of EVs. Separately, Arakelyan and colleagues (NIH, USA) used flow cytometry coupled with magnetic nanoparticles to characterize the antigenic composition of EVs and demonstrate that EVs carrying HIV envelope proteins facilitated HIV infection. This is an interesting observation that could lead to the discovery of new antiviral drugs targeting EVs that contain HIV-Env.

Exosomes might also have an effect on the viral reservoir. Kashanchi et al. (George Mason University, USA) presented interesting results demonstrating how exosomes from HIV-uninfected cells could reactivate latent HIV-1 in infected cells. They proposed a mechanism in which exosomes might increase RNA polymerase II loading onto the HIV-1 promoter in the infected cells, thereby facilitating transcription and leading to an increase of cellular activation.

These presentations represent just a small piece of the growing body of research focused on the role of exosomes in HIV infection. We stand at the beginning of a new source of knowledge of HIV pathogenesis that may provide novel strategies to control HIV infectivity, regulate HIV-reactive immune responses, and act against the HIV reservoir. The impact of exosomes on HIV infection at all these levels should be considered in ongoing and future approaches to achieve HIV remission and cure.

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## Gene Therapy with CRISPR/Cas9 Coming to Age for HIV Cure

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The huge success of current antiretroviral therapy is mediated by a triple effect: (i) Halting progression to AIDS in infected persons; (ii) reducing the risk of transmission to contacts (treatment as prevention); and (iii) minimizing the risk of HIV acquisition treating uninfected persons at risk (pre-exposure prophylaxis). However, UNAIDS has estimated that only 70% of infected people globally are diagnosed, only 53% are treated, and overall 44% have undetectable viral load, which is the necessary request for ensuring any anti-

retroviral benefit. Thus, with 37 million people currently living with HIV worldwide and more than 2 million new infections per year, the prospects for global HIV eradication are far on the horizon.

Over the past couple of years, rapid development has been seen for technologies enabling modification of gene expression, either by direct inhibition by RNA interference (RNAi) or by genomic modification at DNA level. In particular, genome-editing endonucleases have significantly improved our ability to make precise changes in the DNA of eukaryotic cells. Notably, first-generation genome-editing technologies (i.e., ZFNs and TALENs) have been replaced by clustered regularly interspaced short palindromic repeats (CRISPR/Cas9), which work with a short guide RNA (gRNA) to hybridize to a target DNA site and recruit the Cas9 endonuclease.

Once integrated into the host genome, HIV gene expression is regulated by the LTR promoter. Hypothetically, gene editing of the HIV promoter might have the potential to deactivate viral transcription by the introduction of mutations or fragment excision. HIV gene therapy progressed very slowly until recent breakthroughs in gene-editing methods using CRISPR/Cas9 (Liao et al. Nat Commun 2015;6:6413).

Using a shorter version of the Cas9 endonuclease ensembled into an adenoviral vector, critical segments of thAQ!e viral DNA genome spanning between the LTR and gag regions were successfully removed in HIV transgenic mice. Excision was confirmed in all examined tissues as well as in circulating lymphocytes and resulted in a drastic reduction of HIV-RNA (Kaminski et al. Gene Ther 2016;23:690-5). Moreover, using latently infected CD4+ T lymphocytes from HIV-infected persons, lentiviral-delivered CRISPR/Cas9 precisely removed the entire HIV genome spanning between the 50 and 30 LTRs of integrated HIV proviral DNA (Kaminski et al., Sci Rep 2016;6:22555), providing a proof of concept of the high potential of genome-editing technologies.

Before moving to the clinic, the CRISPR/Cas9 technology must solve several major issues in the HIV scenario. First, generation of resistance is a major concern. Mutations may occur surrounding the targeted site and result in the selection of strains that are no longer recognized nor cleaved by CRISPR (Badia et al. Curr Opin Virol 2017;24:46-54). The efficacy of the anti-HIV CRISPR/Cas9 strategy is highly dependent on the gRNA sequence, yet some mutant viral strains show poor or no cleavage at all. Higher CRISPR/Cas9 pressure could delay but not eliminate viral replication when using a combination of distinct gRNAs targeting distinct HIV proviral genes. In this case, although the reading frame may remain unaltered, an accumulation of insertions and/or deletions may occur in the target sequence, rendering new viral strains insensitive to CRISPR/Cas9 cleavage. Finally, double-strand breaks resulting from CRISPR/Cas9 activity and subsequent cellular non-homologous end joining machinery may introduce mutations in sequences that are no longer recognized by the gRNA, and therefore not susceptible to Cas9 cleavage.

A second consideration is a need for developing safe and effective mechanisms of delivery. Adenoviral vectors have long been studied in gene therapy and represent an ideal viral vector for transduction at different tissues. However, the packaging size of adenoviral vectors is a limiting factor, especially for CRIPSR/Cas9.

Third, HIV has a genome of about 10 kb while a gRNA generally only targets 20 bp of the DNA molecule, which means that there are thousands available targeting sites for the provirus in latently infected cells. To date, there is no platform established solely for gRNA candidate evaluation in HIV provirus eradication.

A final consideration is an access to all tissues and cells potentially harboring the HIV provirus, including reservoirs as the central nervous system. In this regard, efforts are being focused in the development of Cas9/gRNA nanoparticle formulations.

To overcome these problems, a group in Florida recently developed human transgenic cells that may be used for gene-editing studies and as platform for high-throughput screen of HIV provirus disrupters (Huang et al. Sci Rep 2017;7:5955). Of note, Cas9 protein instead of a Cas9 plasmid was used. Compared to a plasmid introduction, Cas9 protein agents could be easily quantitatively applied and standardized, mimicking better real clinic scenarios.

In summary, RNAi-based technologies have widely dominated gene therapy research during the past decade, with overall slow progress. However, the advent of new gene-editing technologies, and especially the CRISPR/Cas9 system, has revolutionized the field. In the HIV context, CRISPR/Cas9 applications might go further than those of RNAi, for example, enabling excision of segments of integrated proviral DNA from latently infected cells and allowing complete provirus elimination, or it may be used to reverse HIV latency. Although important challenges still need to be overcome, a promising pathway to HIV cure seems to have been found.

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## Ready for HIV Dual Therapy? - New Data from IAS 2017

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The introduction of combination antiretroviral therapy (ART) in the 1990s has fundamentally transformed the landscape of HIV clinical practice, greatly improved disease morbidity and mortality, and reduced transmission rates across all demographic groups. Central to this success is the idea that in order to achieve best outcomes and prevent the emergence of drug resistance, at least three antiretroviral agents be used in HIV treatment. This therapeutic strategy is a core tenet of HIV medicine, backed by incontrovertible scientific evidence and made easy to deploy by the high adherence levels with once-daily coformulations, which have generally been well tolerated.

However, there has been increasing support in favor of a paradigm shift towards dual therapy in recent years, particularly in the maintenance phase of treatment. This concept advocates that once virologic suppression has been achieved with three or more antiretroviral drugs during the treatment initiation phase, switching to a two-drug regimen for maintenance therapy should be possible. Notably, the results of the LAMIDOL *(Joly et al.,* Abstract 458) and phase III SWORD 1&2 *(Llibre et al.,* Abstract 44LB) trials presented at the 2017 Conference on Retroviruses and Opportunistic Infections (CROI 2017) earlier this year seemed to lend support this hypothesis.

More new data was recently presented at the 9th IAS Conference on HIV Science (IAS 2017) that adds to the growing body of evidence in support of a two-drug regimen approach in maintenance therapy. The LATTE-2 study *(Eron et al.,* Abstract 5628) was of major interest because of the exciting new therapeutic options that long-acting injectable antiretroviral agents may offer in the near future. But more than that, the findings of comparable response between a traditional three-drug oral regimen and a novel injectable two-drug regimen at 96 weeks were quite noteworthy. In this phase II multicenter open-label study of 286 HIV-infected ART-naïve patients, once daily oral cabotegravir/abacavir/lamivudine achieved virologic suppression in 84% of study subjects. In comparison, 87% in the injectable cabotegravir/rilpivirine once four-weekly group and 94% in injectable cabotegravir/rilpivirine once eight-weekly group remained suppressed at 96 weeks. Crucially, no drug resistance mutations were seen in study participants who remained adherent to their treatment regimen.

While the two-drug regimen strategy has been entertained in maintenance therapy, there has been little willingness to consider this approach in the initiation of ART in treatment-naïve HIV-infected patients because of the justifiable concerns that exist around the emergence of drug resistance. Despite this, new data presented at the IAS 2017 meeting suggests that the idea is not without merit. In a proof of concept single-arm pilot study (ACTG A5353) which looked at 120 treatment naïve HIV-infected subjects with high study entry viral load (VL ≥ 1,000 and < 500,000 copies/mL), it was shown that once daily dolutegravir/lamivudine had virologic efficacy of 90% at 24 weeks, with 96% of the as-treated study population achieving VL < 50 copies/mL *(Taiwo et al.,* Abstract MOAB0107LB). The regimen was well tolerated, with no reported drug resistance mutations while study participants remained on treatment.

There may be many real-world advantages to the two-drug regimen approach, among them lower costs (crucial in resource-poor settings where affordability may be a limiting factor), fewer adverse effects or drug toxicities, improved tolerability, and possibly better adherence to treatment regimen. These are all increasingly important considerations in clinical decision-making, given that improved mortality means patients are now expected to remain on ART for longer than has been previously seen. But how the two-drug regimen approach will stack up against tradition and firmly established norms is far from clear, and it is almost certain that the uneasiness that understandably surrounds this idea is likely to persist. Until the case for the two-drug regimen approach is made more convincingly in ongoing and future trials, the “three or more” rule will reign in HIV medicine, not merely as orthodoxy, but in fact as the cornerstone of good practice.

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