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Antiviral treatment strategies based on gene silencing and genome editing

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The ability of some viruses to establish latently infected chronic reservoirs that escape to immune control becomes a major roadblock that impedes the cure of these infections. Therefore, new alternatives are needed to pursuit the eradication of viral persistent infections. Gene silencing technologies are in constant evolution and provide an outstanding sequence specificity that allows targeting any coding sequence of interest. Here we provide an overview of the development of gene silencing technologies ranging from initially RNA interference to the recently developed CRISPR/Cas9 and their potential as new antiviral strategies focusing on the eradication of HIV.

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Introduction

Over the last two decades, technologies enabling modification of gene expression, either by direct inhibition of gene expression by RNA interference (RNAi) or by genomic modification at DNA level (*i.e.*, zinc finger nucleases (ZFNs), transcription activator like-effector nucleases (TALENs) and RNA-guided gene editing with CRISPR/Cas9) have contributed to an enormous progress in molecular biology (Figure 1), and both strategies are currently under study for the treatment of a broad range of infectious and non-infectious diseases. Ongoing clinical trials using RNAi and genome editing tools as potential anti-human immunodeficiency virus (HIV) therapy aim at generating cells resistant to infection, in an attempt to mimic the effect observed in the often called *Berlin patient*, an HIV+ individual with apparent eradication of the virus after receiving a stem cell transplant from a CCR5 Δ32 donor, and the unique reported case of an HIV-1 functional cure [1••]. Here, we summarize the most important milestones accomplished and the putative applications of gene silencing and genome editing technologies as an alternative therapy for HIV eradication.

Challenges of RNA interference for therapeutic applications

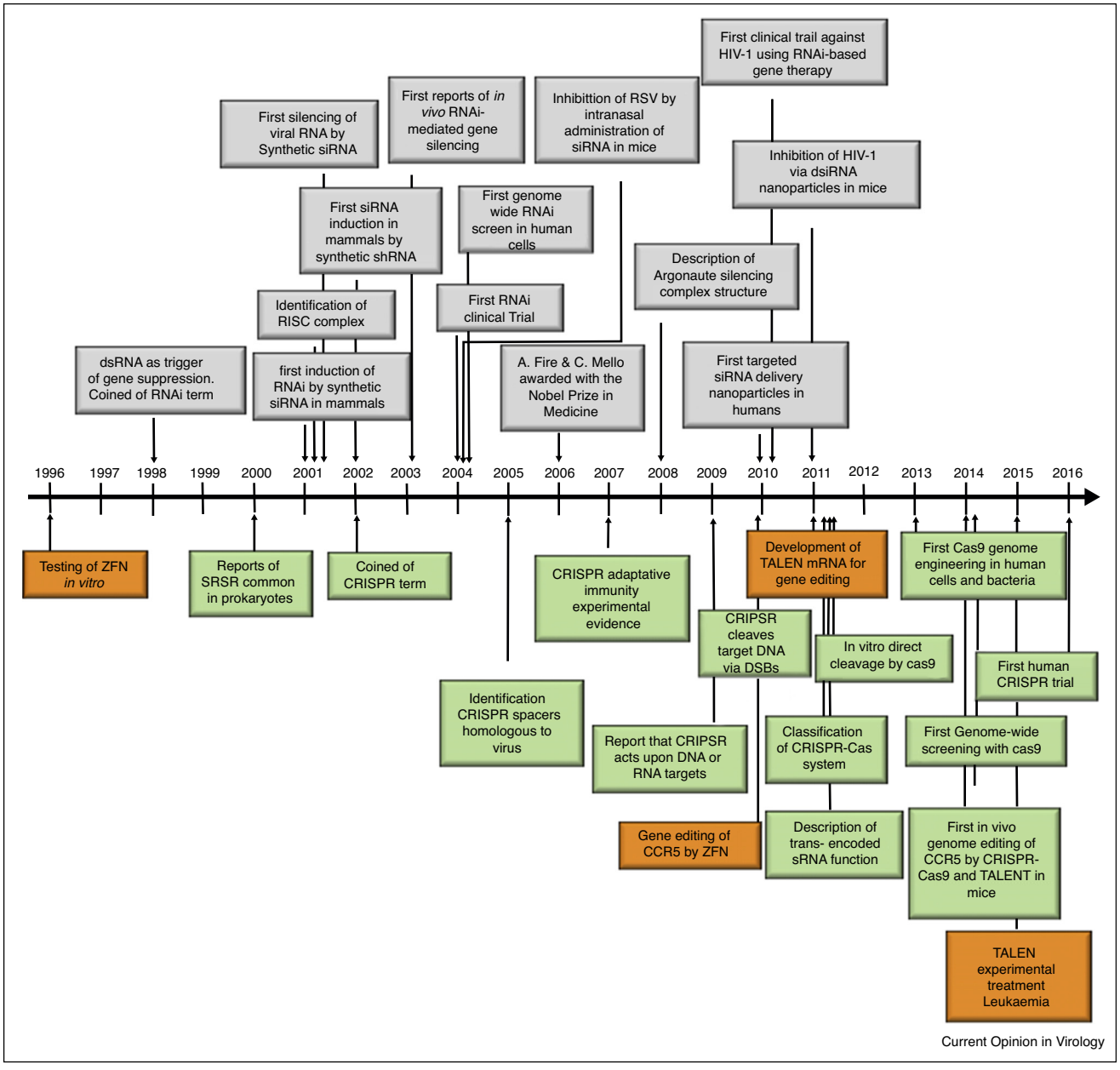
One of the most attractive applications of the RNAi approach is the feasibility of its delivery into whole organisms or tissues/organs to target disease related proteins considered as 'non-druggable' [2,3]. Therapeutic applications of RNAi have been challenged by several limitations specially related to the existence of nonspecific off-target effects, including the induction of innate immune responses; RNA stability; and the efficiency of delivery [4]. Some of these limitations can be overcome with an optimal design and/or the introduction of chemical modifications: modification of the 2′ position of the ribose moiety (2′-*O*-methyl, LNA, 2′-deoxy) avoids Toll like receptor (TLR) recognition and subsequent innate immune system stimulation in response to RNAi, whereas modification of the RNAi seed region limits off-target effects and degradation by nucleases [5,6]. Nowadays, most of the efforts are centered into achievement of a safe, efficient and targeted delivery of RNAi to cells and tissues using low toxicity non-viral vectors such as cationic lipids and polymers, cholesterol, liposomes, antibodies and nanoparticle formulations [7,8].

RNA interference as an antiviral therapy: the case of HIV

The availability of genome wide siRNAs libraries enable large-scale, high-throughput RNAi screens which have identified key factors involved in the life cycle of HIV and other viruses, such as influenza virus and human hepatitis C virus (HCV), among others [9]. These findings may help uncover new potential therapeutic targets and expand our knowledge of the interplay between cellular factors and viral infection. RNAi also represents a promising technique to promote sequence-specific degradation and silencing of viral RNA [10], as (i) it avoids off-target effects of host genes; (ii) siRNA presents lower toxicity; (iii) it may be suitable for long-treatment therapy; and (iv) may be combined with other antiviral therapies.

Several approaches have used RNAi-based technology to target viral or host genes at all stages of HIV replication, such as viral entry, reverse transcription, integration and transcription [11•,12,13]. Nevertheless, these promising

Figure 1



Timeline of milestones for RNAi and genome editing technologies.
Above, milestones in the RNAi field, colored in grey. Below, milestones in the CRISPR-Cas9 (green), and ZFN and TALENS (orange) technologies.

results of RNAi-based technology obtained in HIV *in vitro* assays have not been efficiently translated to the clinical setting, mostly due to problems associated with the use of RNAi for long-term viral infections. A complication is the emergence of RNAi-resistant strains [14]. This mutational escape can be avoided by targeting highly conserved regions of the HIV genome, or combining multiple viral target sites and/or host factors required for the HIV infection and replication [15]. For instance, multiple shRNAs delivered with a lentiviral vector have been used to target CCR5 and CXCR4 co-receptors, providing resistance to infection [16,17]. In addition, HIV replication can be restricted by targeting cellular cofactors involved in viral integration such as LEDGF/p75, Chaperonin or Importin 7 [18]; viral transcription such as P-TEFb, SPT5, and ZNRD1 [19,20]; or nuclear entry such as TNPO3 [21], although none of them seems a suitable candidate since modifying their normal gene expression may affect essential cellular functions.