

After more than 25 years since acquired immunodeficiency syndrome (AIDS) was first recognized, the human immunodeficiency virus (HIV) continues to present an ongoing challenge for academic, government, and pharmaceutical industry researchers to find effective treatments for this devastating infection.

To date in the US, about 30 drugs - as single agents or as combination products - for the treatment of HIV infection have been introduced by more than ten different companies. But collectively, these agents represent only six different classes working at just five different sites of action. And despite the enhanced potency of the more recently introduced products, resistance continues to be a major challenge for managing patients with HIV. Adding to this is the complexity of the multi-drug regimens that are now required, which often leads to significant drug interactions, untold side effects, and poor adherence or compliance. Thus, a continuous search for new classes of antiretroviral drugs ensues.

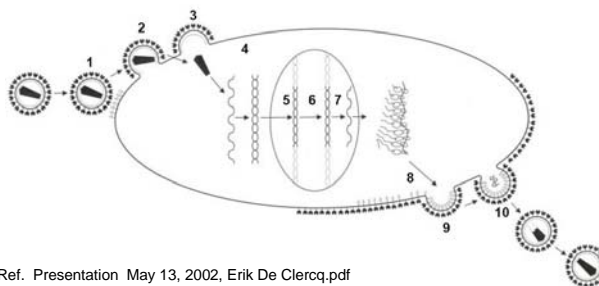
Responding to this challenge, numerous companies are investigating totally new therapeutic approaches such as integrase inhibitors and co-receptor antagonists, the first of which were recently approved. As expected, more than one company is in the race to pursue drug development programs for each of these advanced targets.

Meanwhile, there remains an unexploited target for antiretroviral drugs – the disruption of the use of human transfer RNA (tRNA) by the virus during HIV replication. The role of tRNA is essential for the replication and survival of HIV, but until recently several barriers had prevented exploration of tRNA as a drug target. However, Trana Discovery has developed technology that overcomes those barriers and opens the way to discover and help develop HIV drugs that work by means of this novel mechanism of action.

### The Viral Replication Cycle for HIV Involves a Number of Critical Steps

According to HIV expert Erik De Clercq, there are 10 major points of intervention in the HIV replicative cycle at which chemotherapeutic agents could be targeted. These ten steps are: (1) viral adsorption to the cell membrane; (2) fusion between the viral envelope and the cell membrane; (3) uncoating of the viral nucleocapsid; (4) reverse transcription of the viral RNA to proviral DNA; (5) integration of the proviral DNA into the cellular genome; (6) DNA replication; (7) transcription of the proviral DNA to RNA; (8) translation of the viral precursor mRNA to mature mRNA; (9) maturation of the viral precursor proteins by proteolysis, myristoylation, and glycosylation; and (10) budding, virion assembly, and release. Because each of these steps is critical for virus survival, interruption of any step should stop the replication process, resulting in an effective antiretroviral mechanism.

### HIV Replicative Cycle



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Currently available antiretroviral agents work at step 1 (viral adsorption - co-receptor antagonists), step 2 (the available fusion inhibitor), step 4 (reverse transcription – nucleoside and non-nucleoside reverse transcriptase inhibitors), step 5 (integration – integrase inhibitors), and step 9 (maturation – the protease inhibitors). Because of the importance of tRNA in HIV replication, an effective inhibitor of tRNA would appear to act uniquely at step 10 (assembly) as well as at step 4 (reverse transcription). This dual point of intervention of tRNA inhibitors potentially offers significant advantages over other agents in both potency and resistance.

## The Role of tRNA in HIV Replication

All organisms need tRNA for normal protein synthesis. Specifically, HIV uses human tRNA<sup>Lys3</sup><sub>SUU</sub> from the host cell to form complexes with the HIV genome RNA during replication. At step 4, reverse transcription, tRNA<sup>Lys3</sup><sub>SUU</sub> is used to prime or start the copying of the genome. Then, at step 10, the assembling HIV particles package tRNA<sup>Lys3</sup><sub>SUU</sub> molecules together with viral RNA prior to release from the host cell. Inhibition of tRNA during RNA replication or blocking its recruitment during assembly should stop the replicative cycle, and the virus would not be able to survive.

## Identifying tRNA Inhibitors

Scientists at Trana Discovery have invested years of research to develop the technology that forms the basis for the assays used to screen molecular libraries for tRNA inhibitors. The technology centers on the anticodon stem loop (ASL) of tRNA and the importance of nucleotide modifications within the ASL. The ability to synthesize exact copies or mimics of the ASL with the modifications, just as they occur in nature, is what overcomes previous barriers and enables further research and the application of Trana Discovery technology to methodically search for inhibitors of tRNA.

The composition and sequence of the nucleotides that make up the ASL, along with its natural chemical modifications, are species specific and therefore provide a unique code to design an assay probe for most any targeted pathogen. The nucleotide modifications are not only important for selectivity and specificity but have been shown to increase the binding affinity of this RNA:RNA interaction by several orders of magnitude. As such, a molecule capable of disrupting this interaction would be a potent tRNA inhibitor.

For the HIV assay specifically, a mimic of the ASL of tRNA<sup>Lys3</sup><sub>SUU</sub> is used as the probe and synthetic viral RNA serves as the substrate to which the ASL naturally binds. The assay process itself is a bimolecular interaction that involves the introduction of tRNA<sup>Lys</sup> mimic labeled with a fluorescent tag to a solution containing the viral RNA and the respective test compounds. If an inhibitor is present, the tRNA<sup>Lys</sup> will not be able to bind to the viral RNA, and no fluorescence will be detected. Trana's HIV assay can be performed manually or scaled to high-throughput screening formats.

Typically, Trana Discovery or its partner will screen a collection of compounds from a partner organization's molecular library. If an inhibitor is detected, further testing by Trana or the partner company would be necessary to determine the compound's bioactivity against HIV, as well as several determinants of "drugability." Structural activity and property optimization by the partner organization would follow in order to select the best candidate(s) for product development. The Trana assay would again be employed to validate the tRNA inhibitory mechanism of the lead compounds. The partner would then assume full responsibility for drug development and commercialization.

Through research of the inhibition of tRNA, Trana Discovery and its technology opens the way for the discovery and development of new anti-infectives for the treatment of HIV.