

Trana Discovery develops and licenses high-throughput drug discovery assays that utilize RNA oligomers which contain modified base residues. One such example is the use of the human tRNA^{Lys3} by HIV where the hairpin region of the anticodon stem loop (ASL) contains 3 modified bases. Our assay structurally mimics the viral complex that forms between this segment of the tRNA^{Lys3} and the HIV RNA. In viral function the tRNA in the ASL hairpin is unfolded and adopts a bimolecular duplex conformation. This radical modification of the tRNA conformation alters the structure of the target molecule and changes the spatial distribution of the R groups making the site of the RNA-RNA interaction accessible to intervention by therapeutic compounds.

What data validates tRNA as a HIV drug target?

Sequence analysis of HIV genome collected from human isolates reveals that the primer binding site, PBS, is the most conserved region of the viral genome. The 18 nucleotides of the PBS are universally conserved in all mutants. Deletion of the PBS blocks the ability of the mutant to replicate in culture; thus, one can infer that blocking the PBS with a therapeutic compound would also be lethal to HIV. Mutation of the site by transferring the priming function to another tRNA results in poor viral transcription within the infected cell and marked loss in the ability to form infectious particles. All of the PBS mutants quickly revert back to tRNA lysine by selective mutation. Validation that the disruption of the PBS complex disrupts viral function has been demonstrated both by use of antisense oligonucleotides and siRNA.

Can HIV prime with any other tRNA albeit at lower efficiency?

No, HIV cannot prime with any other tRNA. Analysis of the RNA sequences for HIV isolates in the public domain contain no examples of a PBS complementary to any tRNA other than tRNA^{Lys3}. The exclusive use of tRNA^{Lys3} as a primer extends to all lentiviruses. Extensive efforts to study mutated PBS found rapid reversion back to tRNA^{Lys3} in culture. Reversion back to Lys3 is observed, even when the entire sequence is mutated to one that is complementary to another tRNA.

Has anyone made a tRNA^{Lys3} knock out cell (wobbling should make them viable)?

Over 450 transfer RNA (tRNA) genes have been annotated in the human genome. For tRNA^{Lys}, 27 copies have been annotated, 12 for CTT anticodon and 15 for UUU. To our knowledge no attempt has been made to make a tRNA knockout in any organism. Related research has shown that while U is a wobble base, C is not. Knocking out of tRNA^{Lys3} would prevent the translation of the AAA lysine codon and likely be lethal to the cell.

Has there ever been a small molecule drug that binds a structural RNA motif?

Many effective antibiotics bind exclusively to RNA structural sites in the ribosome. Crystal structures of the drug and ribosome complex provide insight into mechanism of action of drug with RNA targets.

Do the prototype hits not intercolate into nucleic acids in general?

We have no specific data at this time in regards to these compounds. Please recall our objective is to demonstrate that this assay will identify compounds which inhibit this assay and that can serve as the basis for a drug discovery program. As with any drug discovery program, there will be classes of compounds that are more 'druggable' than others.

Do the hits bind to other tRNAs?

There are two basic reasons as to why we currently believe that our hits do not bind to other tRNAs.

First, based on our limited data set these compounds are not indiscriminate toxins; thus, they are most likely not indiscriminate binders to tRNA even though the compounds are active in our assay and inhibit HIV in a PBMC assay.

Second, HIV does not recognize normally configured tRNA^{Lys3}, thus, the site of potential interaction of these compounds with other tRNA is not readily accessible. The normal configuration of the ultra conserved ASL loop of tRNA^{Lys3} looks much like the AIDS red ribbon label pin. This configuration is different when the HIV viral RNA forms a complex with the tRNA^{Lys3}. The virus uncoils the "red ribbon" described above into a bimolecular duplex conformation that looks much like a short piece of a double helix.