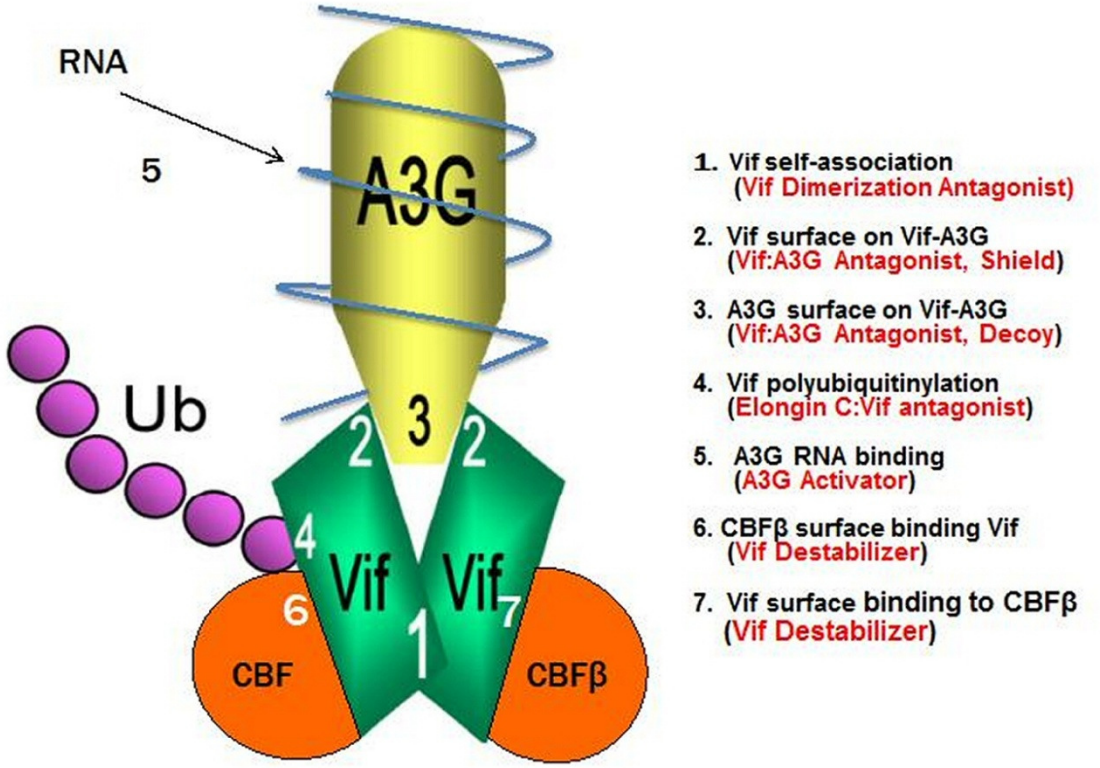
Assay Update



Vif dimerization antagonists (Target No.1)

OyaGen's lead therapeutic target for HIV is Vif Dimerization Antagonist (VDA). Dimerization of Vif has been shown by several labs to be essential for viral infectivity in nonpermissive cell types due to the requirement for Vif to bind to the host defense factor APOBEC3G (A3G) and induce ubiquitination and degradation of A3G. Vif dimerization also is required for Vif-dependent regulation of cell cycle progression required for viral infectivity. After validating the target as essential for viral infectivity using peptide mimetics and assembling a battery of primary and secondary assays to screen for this target, OyaGen is now engaged in the 2nd round of medicinal chemistry to optimize the molecules (hits) identified as having the greatest potency vs. HIV as well as a safety profile that is acceptable. OyaGen's optimization activity has been conducted using performance standards provided to OyaGen by Pharma industry leaders in the HIV marketplace who are potential strategic partners for further development and commercialization. Current activity has been focused on hits derived from high throughput screening (HTS) at OyaGen using its proprietary small molecule library and

NIH funded HTS conducted at the Broad Institute (MIT/Harvard) using OyaGen's proprietary assay systems under a Material Transfer Agreement (MTA). OyaGen is working on medicinal chemistry one a hit from a small screen and The Broad has recently completed a primary screen of 350,000 compounds of which 250 have been selected based on computational chemical analysis. Optimization work on molecules on this target is expected to continue to mid-year when a lead will be selected. It is anticipated that Vif dimerization antagonists will be a first in class antiviral and become part of HAART.

A3G Activators (Target No.5)

Upon viral infection, cytokine release induces A3G to bind to cellular RNAs resulting in the inactivation of its host defense capability. This raises the question of whether inhibitors of Vif alone will be sufficient to enable A3G antiviral activity. Assay development for compounds that activate A3G deaminase activity (A3G Activators) by antagonizing A3G binding to RNA was funded by the Bill and Melinda Gates foundation and have now yielded a hit that stimulated DNA deaminase activity and antiviral activity by reducing the ability of A3G to bind cellular RNA Yet A3G retained its ability to package with viral particles. This hit is in the second round of medicinal chemistry. NIH funding has enabled additional screening of OyaGen's proprietary library and those hits are being evaluated. The assay will be transferred for HTS to the Broad Institute under an MTA. It is anticipate that the A3G Activator will be a first in class HIV prophylactic as well as part of HAART.

Decoy and Shield (Targets No. 2 and 3)

Vif and A3G must bind to one another in order for Vif to degrade A3G. Peptide molecular mimics to the Vif and A3G contact points demonstrate their requirement for viral infectivity. OyaGen has validated an HTS assay for these two targets in a pilot screen where Vif binds to A3G but does not degrade it. OyaGen will soon begin hit identification. We anticipate that these targets will yield two different groups of molecule hits identified through this screen that will prevent A3G degradation. The compounds identified will be of interest as potential first in class antivirals and to become components of HAART along with VDA and AA

CBF-beta

CBF-beta is the non-DNA binding component of a dimeric protein transcription factor of the PEBP2/CBF transcription factor family which master-regulates a host of genes specific to hematopoiesis (e.g., RUNX1) and osteogenesis (e.g., RUNX2). CBF-beta allosterically enhances DNA binding by the DNA binding subunit when the dimeric complex binds to various enhancers and promoters, including murine leukemia virus, polyomavirus enhancer, T-cell receptor enhancers and GM-CSF promoters. CBF-beta shuttles between the cytoplasm to nucleus as part of how the cell regulates transcription activation. Recent proteome interaction analysis of HIV proteins and host cell proteins

has revealed that CBF-beta (And A3G/A3F) was predicted to interact with Vif. RNAi knockdown of CBF-beta reveal a marked reduction in the abundance of Vif which was shown to be due to Vif protein destabilization and degradation. Knockdown of CBF-beat was correlated with an increase in A3G abundance and antiviral activity. Further co-immunoprecipitation analyses revealed that CBF-beta does not bind to A3G/A3F. Amino acids within the central region of CBF-beta that were not involved in transcription activation were shown to bind within the N-terminus of Vif. The consequence of CBF-beat binding to Vif and enhancing the abundance of Vif was that Vif bound better to Elongin C and increased its efficiency of A3G degradation. OyaGen seeks to identify compounds that selectively inhibit CBF-beta binding to Vif as these are anticipated to reduce the amount of Vif available to bind to A3G and inhibit the ability of Vif to degrade A3G. OyaGen has developed cell-based primary, secondary and counter screening assays for the CBF-beta mechanism using similar methods as those used for the VDA, Shield and Decoy. We anticipate commencing screening within a few months and that inhibitors of CBF-beta binding to Vif will also be first in class drugs.

A3G Degradation Assay

The ability of Vif to induce the degradation of fluorescently tagged A3G has been engineered for maximal Vif potency and is used by OyaGen as a secondary HTS assay for VDA, Shield, Decoy and CBF-beta inhibitor hits.

Other Targets

A3G is a host defense factor in liver against hepatitis virus. We anticipate that A3G activators against HIV will also have utility as anti-hepatitis virus treatments.

OyaGen is also developing assays to HTS anticancer compounds based on their ability to inhibit activity of editing enzymes that are believed to trigger uncontrolled chromosomal mutations and translocations in B cell cancers and several solid tumors of the gastrointestinal and urogenital systems.

OyaGen is evaluating A3G activators as antiviral against several RNA viruses and diseases for which there are no current treatments.