The HBV Ribonuclease H as a new drug target

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Disclosure: I am an inventor on patent applications covering the data in this presentation.
Our Target: The HBV RNaseH

- HBV reverse transcription is catalyzed by coordinated action the viral DNA polymerase and RNaseH activities
- The RNaseH destroys the RNA in the RNA:DNA heteroduplex intermediate made during reverse transcription
- Blocking the RNaseH stalls minus-polarity DNA strand synthesis and inhibits plus-polarity DNA synthesis
- No drugs exist against the RNaseH

RNaseH drugs would block both cccDNA formation and infection of new cells
• We can purify 2 forms of enzymatically active RNaseH from *E. coli*
• A fluorescent molecular beacon RNaseH assay was developed where loss of fluorescence indicates RNaseH activity
• The molecular beacon assay can be used for RNaseH inhibition studies and kinetic assays

**Mechanistic evaluation of the HBV RNaseH is beginning**
HBV replication inhibition assay specific for RNaseH inhibitors

- Inhibiting RNaseH activity blocks synthesis of the (+) polarity DNA strand and truncates most (-) strand DNAs
- A strand-preferential quantitative PCR assay was developed that takes advantage of HBV’s unusual DNA structure
- HBV expression is measured in cells that inducibly express HBV
- The assay has been miniaturized to a 96-well format with Z’ values >0.6

Replication inhibition assays are the primary screening tool being used to improve RNaseH inhibitors
Compounds were screened in chemotypes containing inhibitors of the HIV RNaseH.
93 of ~410 compounds inhibited HBV replication at ≤ 20 µM.
17 inhibitors had EC_{50}s ≤ 1.0 µM.
Low-to-sub-micromolar EC_{50}s were found among the α-hydroxytropolones, N-hydroxyisoquinolinonediones, N-hydroxynapthyridinones, and N-hydroxypyridinediones.
Cellular toxicity is present and must be reduced during development.

The best compound has an EC_{50} of 50 nM and a TI of 720.
RNaseH inhibitors: Sensitivity to HBV’s genetic diversity and synergy with other drugs

- 13 recombinant genotype B, C or D RNaseHs were equivalently sensitive to compounds #1, 12, or 46
- 2 chemotypes of HBV RNaseH inhibitors were synergistic with Lamivudine, additive with Hap12, and synergistic with each other

**RNaseH inhibitors are likely to be active against a wide range of clinical isolates and to be suitable for combination with other drugs**
RNaseH inhibitors suppress HBV titers in infected FRG-KO chimeric mice

- FRG-KO mice carrying humanized livers were infected with HBV and serum viremia was measured weekly.
- The animals were treated daily with the maximum tolerated doses of #110 (αHT) or #208 (HPD) by IP injection for 2 weeks and followed for 3 more weeks.
- Viremia was significantly suppressed without affecting HBsAg or HBeAg levels.
- All mice survived, but adverse effects were evident with both #208 and #110.

RNaseH inhibitors can work in vivo

Experiment done in collaboration with Seventh Wave Laboratories and Yecuris
Ongoing drug discovery efforts

- Hit-to-lead optimization of the αHTs, HIDs, and HPDs
- Mid-throughput, hypothesis-driven phenotypic screening for novel chemotypes
- Improvements to the recombinant RNaseH to enable high-throughput RNaseH assays
- RNaseH:inhibitor binding studies to guide compound optimization and perhaps provide an alternate compound screening approach

Optimization of existing hits and screening for new chemotypes of RNaseH inhibitors is ongoing
Summary

• Active recombinant HBV RNaseH can be produced
• Generation of active RNaseH led to development of a suite of screening assays for RNaseH inhibitors
• The RNaseH can be pharmacologically inhibited and suppressing the RNaseH blocks HBV replication
• RNaseH inhibitors with low-nanomolar EC$_{50}$ values and TI values >700 have been identified
• The RNaseH has been validated as a viable drug target in vivo
• Hit-to-lead medicinal chemistry campaigns to generate clinical candidates are underway
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