Bemnifosbuvir is a potent HCV NS5B inhibitor with a favorable antiviral profile and high resistance barrier

SAT-402

Qi Huang, Dawei Cai, Steven Good, Nancy Agrawal, and Jean-Pierre Sommadossi
Atea Pharmaceuticals, Inc., Boston, MA, USA

INTRODUCTION

- Bemnifosbuvir (BEM) is an orally bioavailable, modified guanosine nucleotide prodrug under development for the treatment of people with COVID-19 (as monotherapy) or HCV infections (combined with ruzasvir)
- BEM, a direct-acting antiviral (DAA), is a hemisulfate salt of AT-511, a phosphoramidate protide that is converted after multi-step activation to the active 5'-triphosphate metabolite, AT-9010[1]
- AT-9010 selectively targets the RNA-dependent RNA polymerase (RdRp), a nonstructural protein (NS)5B essential for viral replication in flaviviruses, such as HCV
  - AT-9010 is a potent inhibitor of the HCV genotype (GT)1b RdRp, with a mean IC_{50} value of 0.15 μM[2]
- The potency of BEM (AT-511) to inhibit viral replication of HCV has been demonstrated in replicon-based cellular assays
  - In HCV laboratory strains and clinical isolates with genotypes 1–5, the EC_{50} of AT-511 ranged from 6.2–28.5 nM across the genotypes, which is ~10- to 20-fold more active than sofosbuvir (SOF)[2]
- Viral resistance is an important consideration for DAA use as it may impact the efficacy of treatments for HCV infection
- We performed *in vitro* resistance selection using HCV GT1a and GT1b replicon cells to identify NS5B Resistance-Associated Substitutions (RASs) that could potentially impact the antiviral activity of BEM. Following selection, the antiviral activity of BEM was profiled against a panel of potential BEM RASs selected *in vitro* by transient transfection assays

METHODS

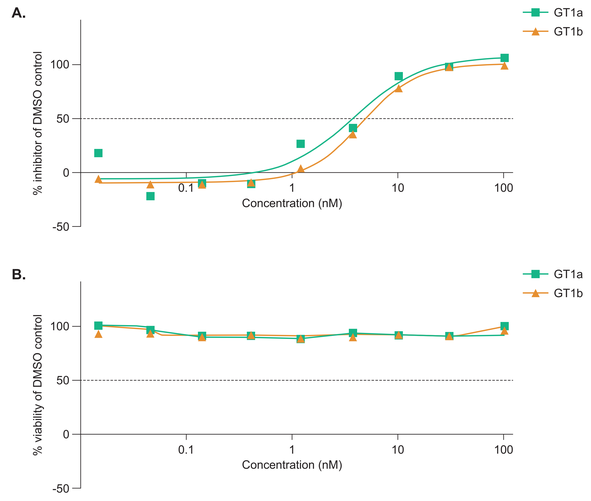
- *In vitro* resistance selection was conducted in the presence of G418 and gradually increasing concentrations of AT-511, and cells from each passage were collected for genotypic analysis
- Emerged NS5B substitutions (single or linked) were selected for phenotyping in transient replicon assays

RESULTS

AT-511 potency in HCV GT1a and GT1b replicons

- To determine the concentrations for resistance selection, EC_{50} values for AT-511 against HCV GT1a and GT1b replicon cells were first measured:
  - AT-511 had potent antiviral activity against HCV GT1a and GT1b replicon cells with EC_{50} values of 3.8 nM and 4.5 nM, respectively (**Figure 1A**)
  - No obvious toxicities were observed at the highest dose tested (100 nM), as indicated by CC_{50} values (**Figure 1B**)

Figure 1. Determination of AT-511 EC_{50} (A) and CC_{50} (B) in HCV GT1a and GT1b replicon cells



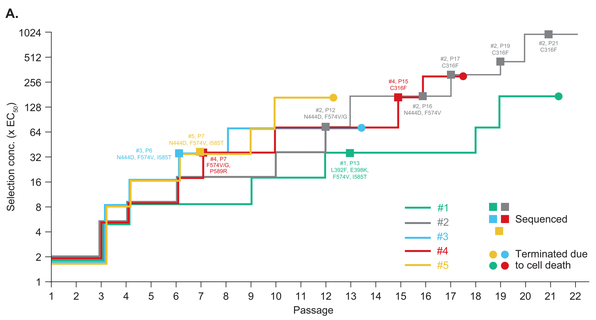
AT-511 *in vitro* resistance selection in HCV GT1a and GT1b replicon cells

- Since a previous attempt to select resistance failed with AT-9010 prodrugs in GT1a or GT1b replicons,[3,4] both colony formation and pooled passage experiments were performed for BEM (AT-511) resistance selection in HCV GT1a and GT1b replicons
  - In GT1a replicons, ten HCV GT1a replicon colonies survived (passage 6; 20 × EC_{50}) and multiple amino-acid substitutions or changes within the NS5B region emerged, including A117G, L314I, N444D, I520T, G543D, F574V, L577F and R591X
  - In pooled passage experiments, five HCV GT1a replicon lineage pools were sequenced (**Figure 2A**)
  - In GT1b replicons, no replicon colonies survived
  - In pooled passage experiments, six HCV GT1b replicon lineage pools were sequenced (**Figure 2B**)

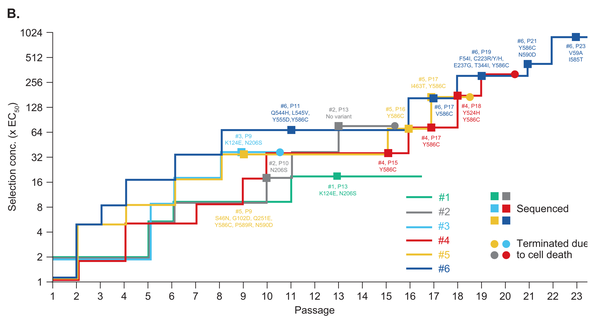
Identification of AT-511 key RASs by phenotyping

- To identify AT-511 RASs, NS5B variants that emerged from the HCV GT1a and GT1b resistance selection were introduced to HCV GT1a and GT1b replicon plasmids, respectively, as single, or linked mutation constructs and EC_{50} values were determined (**Figure 3A and 3B**)
  - The H77 GT1a replicons containing single or linked NS5B substitutions showed good replication levels, ranging from 13.7% (C316F) to 102% (C316N) replication fitness vs wild type
  - The single C223H mutation did not significantly affect AT-511 activity (1.5-fold EC_{50} shift), similar to published results[3,4]
  - Other single or linked mutants that emerged during GT1a selection did not show high resistance levels either (range of 1.2- to 1.7-fold shift in EC_{50})
  - SOF demonstrated a similar pattern of activity (albeit ~20 times less potent than BEM) against all tested GT1a NS5B variants (range of 0.5- to 1.3-fold EC_{50} shift), indicating no cross-resistance to BEM
  - The Con1 GT1b replicons containing single or linked NS5B substitutions showed varied replication levels, ranging from 1.1% (F54I + C223H + E237G) to 200% (C316N) replication fitness
  - Overall, the single NS5B substitutions that emerged in GT1b selection did not show a high resistance level (range of 0.9- to 1.7-fold EC_{50} shift); the key RAS C223H identified in GT1b selection did not significantly affect AT-511 activity (1.7-fold decrease), whereas when linked with some emergent variants, increased resistance (3.6- to 5.9-fold EC_{50} shift) was observed
  - SOF demonstrated a similar pattern of activity (albeit ~20 times less potent than BEM) against all tested GT1b NS5B variants (range of 0.6- to 1.7-fold shift in EC_{50}), indicating no cross-resistance to BEM RASs

Figure 2. *In vitro* resistance selection in HCV GT1a (A) and GT1b (B) replicons

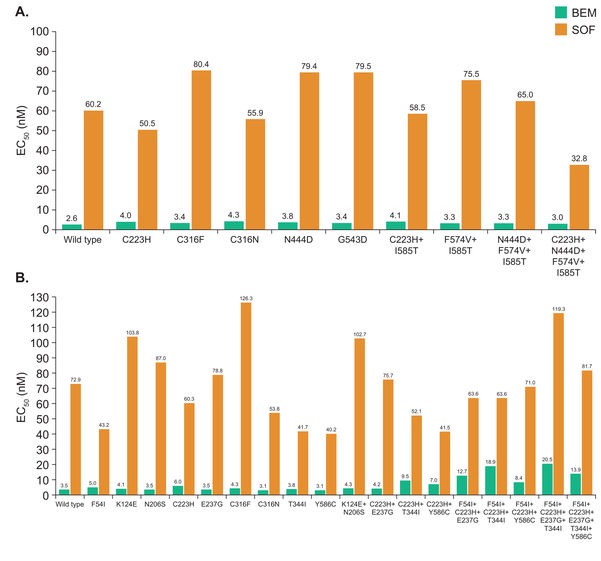


Selection started from 2 × EC_{50} and escalated up to 1000 × EC_{50} and HCV RNA from five pooled replicon cells was amplified and sequenced at the indicated passages. Multiple amino acid variants emerged within the NS5B region: L392F, E398K, F574V, and I585T (Lineage #1); N444D, F574V, and I585T (Lineages #3 and #5), and F574V/G, P589R, and C316F (Lineage #4). For Lineage #2, NS5B variants N444D, F574V/G, and C316F were identified at up to 22 passages and up to 209 days of selection, after reaching the highest concentration of 1000 × EC_{50} (3.8 μM).



Selection started from 1 or 2 × EC_{50} and escalated up to 1000 × EC_{50} and HCV RNA from six pooled replicons was amplified and sequenced at the indicated passages. Multiple NS5B substitutions (S46N, G102D, K124E, N206S, Q251E, I463T, Y524H, Y586C, P589R, and N590D) emerged in Lineages #1 to #5 at various time points and selection pressure. Lineage #6 survived to 23 passages and 167 days after selection under 1000 × EC_{50} (4.5 μM), and a series of potential AT-511 NS5B RASs – F54I, V59A, C223R/Y/H, E237G, T344I, Q544H, L545V, Y555D, I585T, Y586C, and N590D were identified.

Figure 3. Bemnifosbuvir and sofosbuvir activities (EC_{50}) against NS5B variants that emerged during AT-511 resistance selection in GT1a (A) and GT1b (B) replicons



CONCLUSIONS

- C223H was found to be the primary BEM RAS in GT1b and multiple substitutions at other NS5B regions were required to confer meaningful resistance, suggesting BEM provides a very high barrier to resistance
- In addition, BEM and SOF did not display cross-resistance in a panel of NS5B RASs tested *in vitro*
- BEM is currently being evaluated in combination with ruzasvir, a highly potent pan-genotypic NS5A inhibitor, in a Phase 2 clinical trial (NCT05904470)[5]
  - Based upon the data demonstrated to date, it is expected that the BEM/RZR combination should have a more compelling antiviral profile against major HCV NS5A and NS5B RASs than the current standard of care

References

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Disclosures

QH, SG, DC, NA and JS are employees of and may own stock in Atea Pharmaceuticals, Boston, MA, USA.

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