MZ-301 is a small molecule inhibitor of APOL1 pore function that attenuates albuminuria in a mouse model of APOL1-mediated kidney disease

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Introduction

APOL1 genetic variants (G1 and G2) increase risk for a spectrum of progressive kidney diseases in people of African ancestry. To date, no APOL1-targeted therapies are available that address the underlying genetic driver of disease. Recent human genetic findings suggest that pharmacologic inhibition of APOL1 pore function may prevent APOL1-mediated kidney disease (ASN 2022, FR-PO316). Here we describe the in vitro and in vivo activity of MZ-301, a small molecule APOL1 pore blocker that reduces APOL1-driven toxicity in multiple cell systems and attenuates albuminuria in a mouse model of APOL1-mediated kidney disease.

MZ-301 blocks APOL1 cell surface cation flux

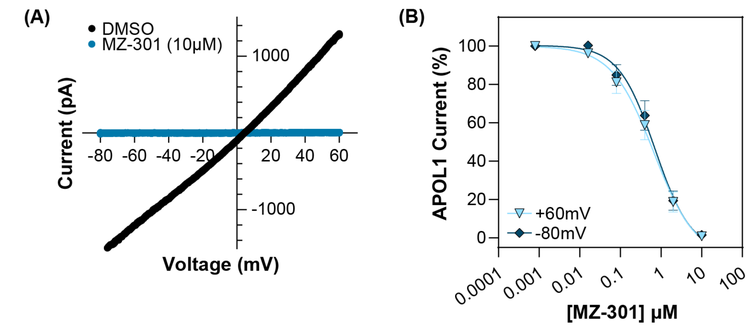

Figure 1. (A) Representative data of electrophysiological current in response to a voltage ramp in HEK293 cells expressing APOL1 G2 with and without MZ-301 treatment. (B) APOL1-mediated current measured in HEK293 cells expressing APOL1 G2 in the presence of various concentrations of MZ-301. Data represents the average of three independent experiments performed in triplicate or quintuplicate. Error bars represent the SEM.

MZ-301 rescues APOL1-mediated cell death

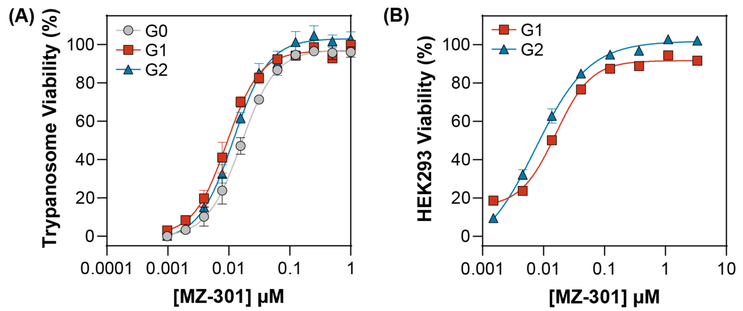

Figure 2. *Trypanosoma brucei* Lister 427 VSG221 and HEK293 cell viability determined using a CellTiter-Glo® assay. Error bars represent the SEM. (A) Trypanosomes were incubated with MZ-301 at different concentrations and a concentration of APOL1 (G0, G1, or G2) recombinant protein sufficient to cause ~90% cell death. Trypanosome viability (%) was measured at 20 hours. Data represents the average of two independent experiments performed in duplicate. (B) Cell viability was measured 18 hours after doxycycline induction of APOL1 (G1 or G2). Data represents the average of at least ten independent experiments performed in duplicate.

MZ-301 rescues APOL1 disease variant toxicity in human immortalized podocytes

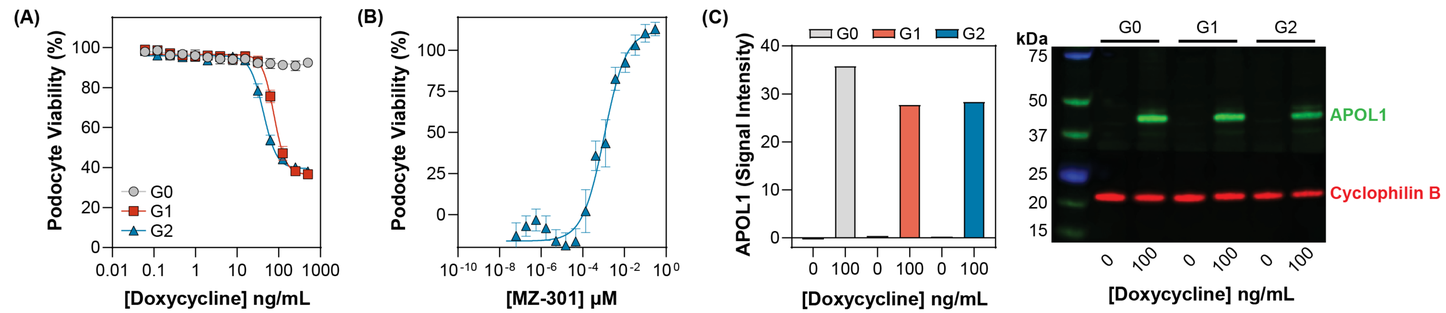

Figure 3. (A) APOL1 disease variants, G1 and G2, are toxic when overexpressed in human immortalized podocytes. APOL1 G0 overexpression does not affect podocyte cell viability. Differentiated podocytes were treated with a dose titration of doxycycline for 96 hours and cell viability was determined using a CellTiter-Glo® assay. Data represents the average of at least 3 independent experiments performed in triplicate. Error bars represent the SEM. (B) APOL1 G2 podocyte cell viability was determined using a CellTiter-Glo® assay. Cell viability was measured 96 hours after doxycycline induction. Data represents the average of two independent experiments performed in duplicate. Error bars represent the SEM. (C) Equivalent protein expression of APOL1 G0, G1, and G2 was observed in human immortalized podocytes, as measured by western blot. Undifferentiated cell lines were exposed to 100 ng/mL of doxycycline for 24 hours prior to lysate generation.

IFNγ induces albuminuria in APOL1 mice

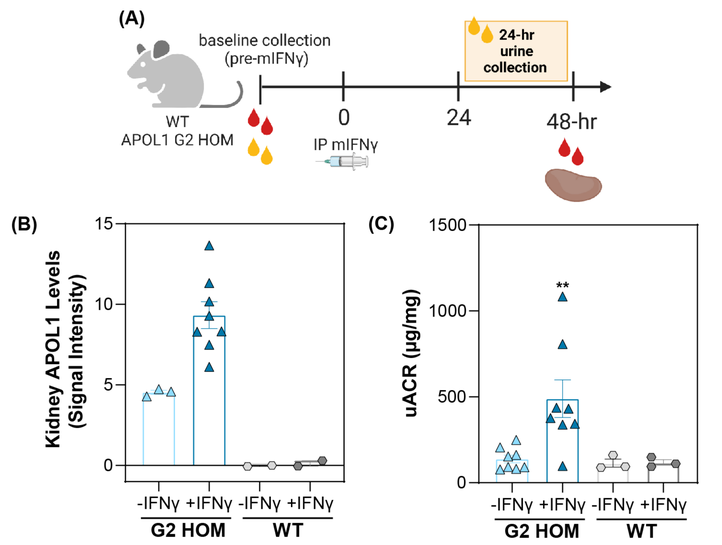

Figure 4. APOL1 transgenic (Tg) mice were generated using a bacterial artificial chromosome (BAC) containing APOL1 G2 and its upstream and downstream genomic regions. Mice were generated and provided by Taconic Biosciences Inc on behalf of Merck Sharp & Dohme LLC, Rahway, NJ. APOL1 Tg mice were bred to homozygosity. (A) Schematic highlighting the administration of interferon gamma (IFNγ) and urine/tissue collections. (B) Quantified western blot of kidney tissue homogenate from G2 HOM and WT mice dosed with IFNγ or vehicle (PBS). (C) G2 HOM and WT mice were injected with IFNγ or vehicle (PBS), urine was collected for 24 hours between days 1 and 2, and the urinary albumin-to-creatinine ratio (uACR) was measured. Error bars represent the SEM. An unpaired t-test was performed (**p=0.0073, G2 HOM -IFNγ vs. G2 HOM +IFNγ).

MZ-301 reduces uACR in an APOL1 renal model

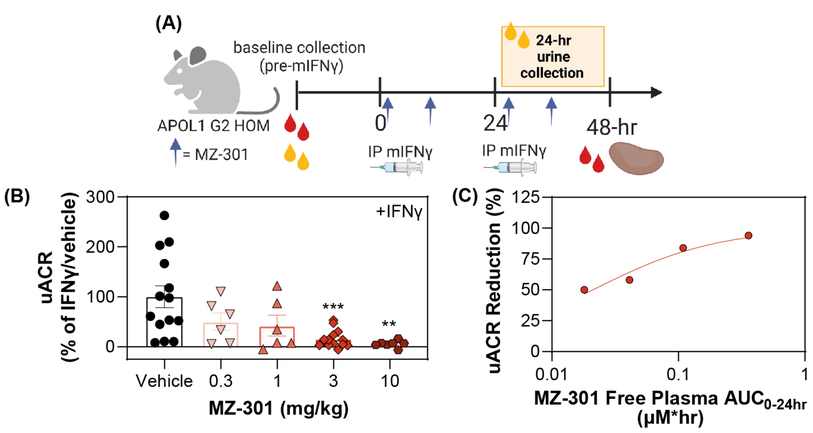

Figure 5. (A) Schematic highlighting the administration of MZ-301 (PO BID), IFNγ, and urine/tissue collections. (B) MZ-301 dose-dependently reduced IFNγ induced albuminuria in APOL1 G2 HOM mice. Error bars represent the SEM. A one-way ANOVA with Dunnett's multiple comparisons test was performed (***p=0.0004, Vehicle vs. 3 mg/kg; **p=0.0013, Vehicle vs. 10 mg/kg). (C) Graph depicting the relationship between free plasma concentration of MZ-301 and % uACR reduction in APOL1 G2 HOM mice. Data represents the mean value from each dose group.

Conclusions

- MZ-301 is a potent orally bioavailable small molecule inhibitor of APOL1 pore function that blocks APOL1 lytic activity and reduces APOL1-mediated cytotoxicity in kidney cells.
- MZ-301 ameliorates albuminuria in a mouse model of APOL1 kidney disease.
- These findings support the further development of MZ-301 for APOL1 nephropathies.