# Toxicity of three antisense oligonucleotide drugs and eighteen of their impurities in primary human hepatocytes

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## Abstract

**Background:** Antisense oligonucleotide (ASO) drugs are synthetic polymers of RNA, DNA, or modified nucleic acids that bind to RNA or protein targets, regulating protein expression or function. Though approximately 20 ASOs have been approved around the globe, quality control and safety assessment pose significant challenges to regulatory agencies, with hepatotoxicity associated with many ASOs.

**Purpose:** This study used pooled primary human hepatocytes (p-PHHs), a commonly used in vitro model for drug hepatotoxicity, to assess the potential for increased risk of hepatotoxicity with ASO impurities compared to the parent compounds.

**Methodology:** Three FDA-approved ASO drugs representing different levels of hepatotoxicity risk were selected in this study: mipomersen, inotersen, and nusinersen. Mipomersen was initially approved by FDA but later discontinued from market, and was denied twice by European Medicines Agency (EMA) due to hepatotoxicity. The label for inotersen includes a warning for hepatotoxicity. In contrast, nusinersen has not been associated with liver injuries. Eighteen of the commonly-found ASO impurities, such as n-1, for each drug were investigated in parallel. Commercial cryopreserved p-PHHs were maintained in sandwich culture and treated for 3 days with ASOs at concentrations normalized to human peak blood concentrations (Cmax). Cellular ATP, albumin secretion, and urea production were measured to reflect toxicity.

**Results:** At 100-fold Cmax, mipomersen and inotersen, but not nusinersen, caused significant inhibition of albumin; however, none of the parent compounds showed noticeable effects on ATP and urea production, consistent with decreased assay sensitivity. For most of the impurities tested, in addition to remarkable inhibition of albumin, sharp deceases in ATP and urea production were also induced by some impurities. Notably, half of the ASO impurities led to a decrease of albumin by >90%, with several triggering >90% ATP inhibition. Compared to the parent compounds, some impurities showed similar toxicity whereas others were more toxic.

**Conclusion:** The p-PHHs appeared to be a useful in vitro model in ranking the relative hepatoxicity risk of ASO impurities to parent ASOs, wherein some impurities were remarkably more toxic than the parent drug themselves. These data provide novel insights into hepatotoxicity risks of ASO impurities, highlighting the importance of quality control in ASO manufacturing.

## Introduction

- Hepatotoxicity and quality control of antisense oligonucleotide (ASO) drugs are two major challenges to regulatory agencies.
  - Mipomersen: initially approved by FDA in 2013 with box warnings for hepatotoxicity, then withdrawn from market in 2019; rejected twice by EMA due to hepatotoxicity and cardiovascular risks
  - Inotersen: FDA approved in 2018 with warnings for hepatotoxicity and recommendations for monitoring liver risks during treatment
  - Impurity concentrations can be as high as 10%
- It is unclear if toxicity is due to the primary drug alone, or if the impurities contribute
- It is unknown if different sequences or subclasses of impurities are associated with greater risks for adverse toxicities, such as hepatotoxicity
- Primary human hepatocytes are generally considered as the gold standard in vitro model for drug hepatotoxicity, but most FDA-approved ASO drugs have not been examined in this model.

## Materials and Methods

- Pooled primary human hepatocytes were obtained from XenoTech-BioIVT. The catalog number is 1810050. These cells were from 5 male and 5 female Caucasians within the age range of 9 to 63 years. The cells were cultured in 24-well plates according to vendor instructions and maintained in sandwich format.
- The three test drugs and 18 impurities were purchased from GenScript Biotech. All test materials were freshly prepared immediately before being applied to the cells.
- The Cmax of the three test drugs was obtained from the FDA-approved drug labels and NDA reviews retrieved from https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm
- The test concentrations were 100-fold Cmax for all drugs and impurities: mipomersen and mipomersen impurities, 56.6 μM; inotersen and inotersen impurities, 89.0 μM; nusinersen and nusinersen impurities, 11.1 μM
- Cells were treated for 3 days. The medium was collected and replaced with new drug-containing medium every 24 h after treatment.
- Albumin, urea and ATP were measured using commercial kits from ABCAM, BioChain, and Promega, respectively.

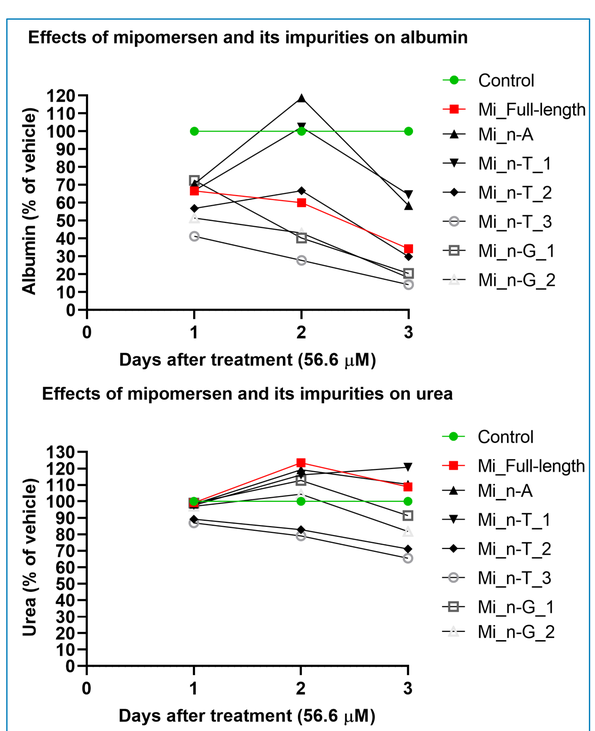


**Figure 1.** Effects of mipomersen and 6 impurities on albumin and urea in p-PHHs. Cells were treated with 56.6 μM mipomersen or impurities for 3 days. Medium was collected every 24 h for the measurement of albumin and urea. n=1.

## Results

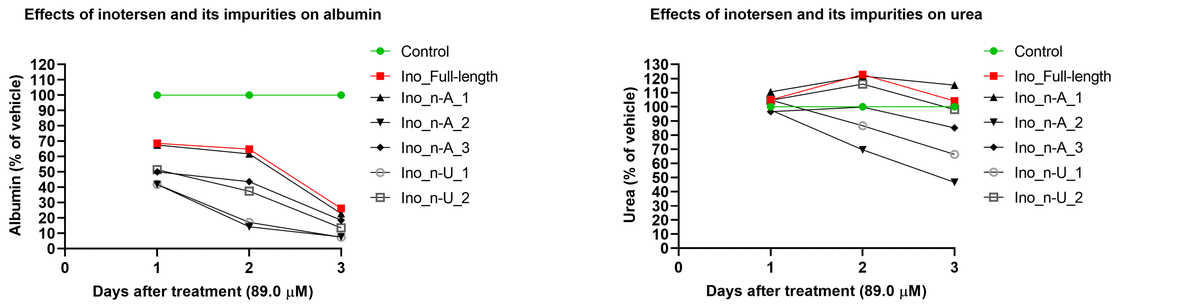


**Figure 2.** Effects of inotersen and 5 impurities on albumin and urea in p-PHHs. Cells were treated with 89.0 μM inotersen or impurities for 3 days. Medium was collected every 24 h for the measurement of albumin and urea. n=1.

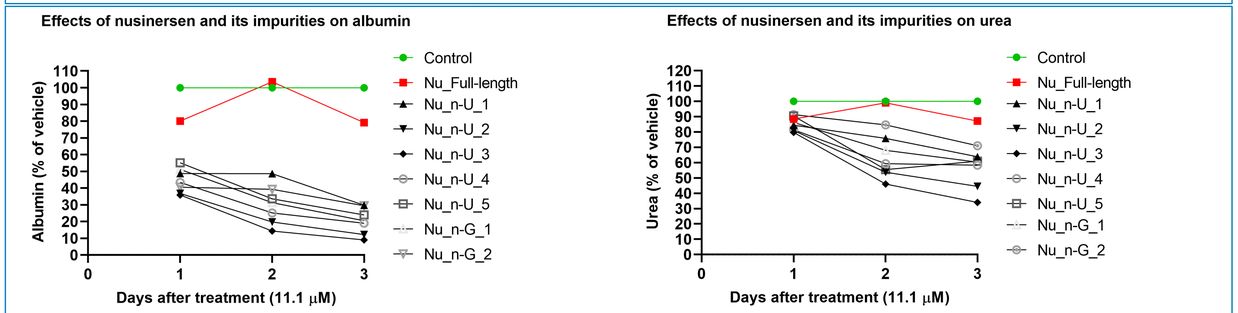


**Figure 3.** Effects of nusinersen and 7 impurities on albumin and urea in p-PHHs. Cells were treated with 11.1 μM nusinersen or impurities for 3 days. Medium was collected every 24 h for the measurement of albumin and urea. n=1.

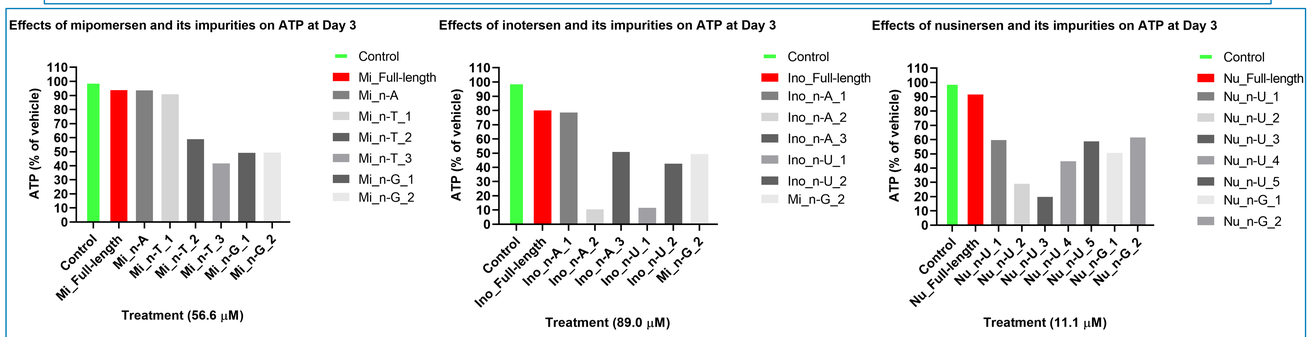


**Figure 4.** Effects of three antisense oligonucleotide drugs and 18 of their impurities on ATP in p-PHHs. The test concentrations were 100-fold Cmax and treatment duration was 3 days. n=1.

## Conclusions

This preliminary study suggests that pooled primary human hepatocytes appear to be a useful in vitro model for ranking the relative hepatoxicity risk of ASO impurities to parent ASOs, wherein some impurities were remarkably more toxic than the parent drug themselves.

## Disclaimer

This presentation reflects the views of the authors and does not necessarily reflect those of the U.S. Food and Drug Administration. Any mention of commercial products is for clarification only and is not intended as approval, endorsement, or recommendation.