American Heart Association’s
High Blood Pressure Research 2014 Scientific Sessions

ALN-AGT, an RNAi Therapeutic in Development for the Treatment of Hypertensive Disorders of Pregnancy

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Hypertensive disorders of pregnancy (HDP)

- Over half a million women in U.S. and EU suffer from HDP

Associated with increased risk of preeclampsia

- One of most common complications of pregnancy in U.S.
- One of the most common causes of maternal death in developed countries
- Occurs in over 200,000 pregnant women in the U.S. and EU
- Associated with increased risk of maternal mortality, perinatal fetal mortality, infant prematurity, neonatal intensive care, and infant morbidity
  - Presentation with severe preeclampsia before fetus is viable may necessitate termination of pregnancy

Khan et al., Lancet 2006
Angiotensinogen (AGT)

- AGT is constitutively expressed primarily in liver
- Initiation of cascade leads to Angiotensin II
  - Angiotensin I is cleaved from N-terminus by Renin (rate-limiting step)
  - Angiotensin Converting Enzyme (ACE) processes Ang I to Ang II
- Angiotensin II signaling targeted by existing small molecule therapeutics
  - ACE inhibitors
  - Angiotensin receptor blockers (ARBs)
  - Renin inhibitors

AGT is associated with Hypertensive Disorders of Pregnancy (HDP)

- Gain of function M235T AGT mutations more prevalent in preeclamptic women than in uneventful pregnancies\(^1\)
- Oxidation of AGT, similar to L10F mutation of the cleavage site, is associated with enhanced Ang II liberation and more prevalent in preeclamptic women\(^2\)

Angiotensin II signaling is dysregulated in HDP

- Hypersensitivity to Ang II has been identified in women who will become hypertensive during pregnancy\(^3\)
- Elevated levels of AT\(_1\)R, as well as heterodimerization of AT\(_1\)R with bradykinin receptor B2 has been identified in preeclamptic women and contribute to Ang II hypersensitivity\(^4\)
- AT\(_1\)-AA, (agnostic receptor auto-antibodies) which can be produced in an AGT-driven model of preeclampsia, also promotes AT\(_1\) signaling\(^5\)

GalNAc-siRNA Conjugates as RNAi Therapeutics

Asialoglycoprotein Receptor (ASGPR)
- Highly expressed in hepatocytes
- High rate of uptake
- Recycling time ~15 minutes
- Conserved across species

GalNAc-siRNA Conjugates
- siRNA conjugated to N-acetylgalactosamine (GalNAc) ligand
- Efficient delivery to hepatocytes following subcutaneous administration
- Includes ALN-TTRsc, ALN-AT3, ALN-CC5
- “Enhanced stabilization chemistry” (ESC) used with ALN-AT3 and ALN-CC5
  » Significantly improved potency and durability compared with ALN-TTRsc
ALN-AGT for Treatment of HDP
Rat Model of Preeclampsia (PE)

Robust hAGT knockdown in maternal liver in established rat model of preeclampsia

- ALN-AGT administration led to >90% knockdown of hAGT mRNA in maternal liver
- Dose regimen: 10 mg/kg, q3d x5 (gestation day 3 through day 15)
- ALN-AGT does not cross placental barrier
  - No significant hAGT knockdown in placenta
  - No significant fetal liver drug exposure
ALN-AGT Reduces Blood Pressure and Albuminuria
Rat Model of Preeclampsia

Elevated blood pressure and albuminuria are hallmarks of preeclampsia (PE)

- ALN-AGT significantly reduced mean arterial pressure (MAP) by ~ 20mmHG
  » Reduced blood pressure may reduce cardiovascular events associated with PE
- ALN-AGT treatment resulted in a >80% decrease in proteinuria
  » Reduced albuminuria indicative of improved renal function

1WHO Recommendations for Prevention and Treatment of Pre-Eclampsia and Eclampsia, 2011
ALN-AGT resulted in reduced sFLT1 and PLGF expression in maternal kidney; no significant change in placenta

- sFLT1 (soluble VEGF receptor) mRNA decreased by ~75% in maternal kidney and was not significantly changed in placenta
- PLGF (member of VEGF family) mRNA decreased by ~50% in maternal kidney and remained unchanged in placenta (the primary source of PLGF)
- sFLT1:PLGF ratio diagnostic for disease severity (higher = worse)
ALN-AGT Reduces AT\textsubscript{1} Agonistic Autoantibodies

Agonistic autoantibodies to Angiotensin II receptor type I (AT\textsubscript{1}-AA) identified in preeclampsia
- Pregnant rodents exposed to AT\textsubscript{1}-AA produce a preeclampsia-like syndrome\textsuperscript{1}
- Activation of AT\textsubscript{1} associated with vasoconstriction, aldosterone secretion, ROS generation, sFlt-1 expression, and other effects associated with the preeclamptic phenotype

ALN-AGT reduction of AT\textsubscript{1}-AA improves hypertension
- Activity levels of AT\textsubscript{1}-AA reduced by approximately 90% by ALN-AGT

\textsuperscript{1}Zhou, Nat Med (2008)
ALN-AGT Positive Impact on Placental Structure

Administration of ALN-AGT resulted in enhanced nutritional exchange

- Larger labyrinth (site of nutritional exchange between fetal and maternal blood) and placenta may underlie observed improvement in fetal growth
- No changes observed to mesometrial triangle (maternal portion of the placenta) or trophospongium (uniform layer of cells which are precursors of differentiated trophoblasts)
ALN-AGT Resulted in Improved Fetal Outcome

Preeclampsia associated with reduced placental size
- Poor placentation leads to reduced placental perfusion, and subsequent impaired fetal growth

ALN-AGT administration improved fetal development
- Increased uteroplacental unit weight
- Increased fetal weight
- Normalized fetal brain:liver ratio
Summary and Conclusions

ALN-AGT is an RNAi therapeutic targeting angiotensinogen (AGT) for treatment of hypertensive disorders of pregnancy, including preeclampsia

- ALN-AGT employs Alnylam’s GalNAc-siRNA conjugate technology for subcutaneous dose administration and a wide therapeutic index
- In rodent model of preeclampsia, ALN-AGT showed significant efficacy in reducing preeclamptic phenotype
  - >90% silencing of maternal hAGT with no detectable fetal exposure
  - ~20 mmHg improvement in mean arterial pressure
  - >80% reduction in proteinuria and improvement in other biomarkers
  - Significant improvements in placenta and fetal outcomes