A SINGLE SUBCUTANEOUS DOSE OF KU-AS-272* ELICITS COMPLETE LOSS OF SPERMATOCYTOGENIC SUPPORT IN ADULT MALE RATS

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Outline
- KU-AS-272 mechanism of action
- Single subcutaneous dose finding in adult male rats
  - Study design
  - Side effects
  - Testicular effects
  - Hormone effects
  - Epididymal clearance
- Ongoing studies
- Conclusions
- Acknowledgements

KU-AS-272 Mechanism of Action

A Non-hormonal Approach to Sterilization: Target the Testis

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Male Sterilization by Disruption of Sertoli cell-Spermatogenic cell Junctions

Normal Spermatogenesis

Post-treatment loss of spermatogenic cells

Maintenance of Spermatogenesis Requires Actin-rich Junctional Complexes

KU-AS-272 Mechanism of Action: Unbundling of Actin that Stabilizes apical ES and BTB

F-actin filaments which form a vital structural component of the aES are bundled by eEF1A-1.

KU-AS-272 binds to eEF1A-1 causing unbundling and instability of the apical and basil ES, causing release of spermatogenic cells.

Electron Microscopy of Junctional Targets of KU-AS-272 in Testis

Untreated Control

Rapid KU-AS-272 Elicited Loss of Actin

Single SQ Dose Finding Study Design

Efficacy and Safety Methodology

Controls

Single dose of vehicle (0.1 M Captisol) administered SQ

Treated

Single SQ dose of 6, 12, 25, or 50 mg/kg KU-AS-272

Body weight recorded weekly

For each dose, 6 rats from each control and treated group were euthanized for tissue harvest on days 5, 30, and 58 post-dose, respectively

Tissue harvest day: Body weight, tests and epididymis weight, tissues fixed in Bouin's for histology

No Side Effects at 6 and 25 mg/kg, Minor Transient Effects Observed at 25 and 50 mg/kg

- Vehicle control, 6mg/kg and 12 mg/kg KU-AS-272 appeared normal after dosing.
- 25 mg/kg: slight lethargy onset at 20 min ~12 hrs, total recovery by 24 hr.
- 50 mg/kg: slight lethargy and wobbly onset within 15-20 min appeared. By next morning, all rats showed good mobility, though slight tremor and imbalance was visible. On day 2 all 50 mg/kg treated rat behavior was comparable to control.
Body Weight Was Not Significantly Different Between Control and All Treatment Groups

Testicular Effects

Testis were smaller in all treated rats and cauda epididymis were devoid of sperm in most of the treated rats (all pictures at same magnification)
Significant Drop In Epididymal Weight Was Seen At All Doses of KU-AS-272 At All Time Points (P*/**=0.05)

**Remarkable Efficacy at All Doses By Day 30 and Continued at Day 58 Post Dose**

Spermatozogenic Index At Day 5, 30 And 58 Post-Dose Indicated Irreversible Spermatogenic Arrest

Based on Whitsell et al. (1984)

KU-AS-272 Has Very Rapid Effect After Single SQ Dose

* = n=5 for 25 mg/kg, one outlier had a partial response, but all animals at this dose had no spermatogenic cells at this dose at day 58.
Hormone Effects

Serum Testosterone Levels Measured at Day 58 show “Normal” Levels

Serum Inhibin B Levels Were Undetectable at All Doses Above 6 mg/kg at Day 58

Epididymal Clearance
KU-AS-272 Elicits Complete Loss of All Spermatogenic Cells In Adult Male Rats

Testicular sperm must transit and mature through the ducts of the epididymis and enter the ductus deferens before they are capable of being ejaculated.

In the normal male this take 10-14 days, depending on species.

Sperm Were Absent in the Caput Epididymis Ducts at All Doses Above 6 mg/kg at All Time Points

KU-AS-272 (12 mg/kg)

Time-dependent clearing of epididymal ducts is occurring, but slower than expected.
Transition of Epididymal Duct Contents

Ongoing and Future Directions

- Are the retained epididymal sperm “fertile” even the testis is no longer producing flow
- Other active analogues have been identified
- Target species:
  - Complete adult male dog study
  - Other target species

Conclusions

- Single SQ dose of KU-AS-272 at 12 mg/kg and higher achieved the desired total loss of all spermatogenic cells 58 days post single dose
- Testis histopathology suggests that these animals are sterile after a single SQ dose
- No adverse side effects at 6-12 mg/kg. Minor transient side-effects at 25-50 mg/kg
- There were no significant changes in body weight gain at any doses

Conclusions – contd.

- Day 58 data identified no significant changes in circulating testosterone at all doses.
- Serum Inhibin B levels measured at day 58 were undetectable at all doses above 6 mg/kg, suggesting that sterilizing doses were achieved.
- Clearance of epididymal sperm appears to be slower than normal following “shutdown” of testis
For Addition Details About KU-AS-272

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