**Introduction**

- Hemophilia A arises from mutations in the F8 gene, affecting ~1/5000 males.
- Treatment options include frequent FV factor and SC non-factor therapies; however, they have limitations such as:
  - breakthrough bleeds and joint disease due to suboptimal adherence
  - non-ideal factor kinetics
  - inhibitor generation
  - risk of thrombotic events and coagulation test interference with newer non-factor therapies
- Alternative modalities such as cell therapies with genetically modified, optimized protein

**Methods and Results**

**Figure 1. Cell Line Selection & Construct Optimization to Produce Engineered Cells Expressing hFVIII Using a Non-Viral Method**

- **Cell Line Features**
  - **Safety**
  - **Durability**
  - **Time and Cost Savings**
  - **Efficacy**

**Figure 2. Inner Compartment Matrix Optimization Drives Higher in vivo hFVIII Levels**

- Control or inner compartment modified spheres with hFVIII expressing cells were placed in the IP space of nude mice via laparotomy (n=5 mice/group). Mice were sacrificed for blood collection on day 7. hFVIII antigen level in plasma was measured by ELISA.

**Figure 3. Outer Layer Optimization To Avoid Pericapsular Fibrotic Overgrowth**

- Alginates polymers were prepared with varying levels of small molecule conjugation (0-45.5%). Spheres were prepared with 20 million cells/ml of matrix forming solution, and implanted into C5/BL6 mice (n=4 per group). Spheres were explanted at day 7 and assessed for pericapsular fibrotic overgrowth using bright-field microscopy.

**Figure 4. Final Product SIG-001: Cell Line Modified with a Non-Viral Vector to Express hFVIII, Encapsulated within Alginate Spheres**

- Blue color for illustration purposes only. Each sphere is ~1.5mm in diameter.

**Figure 5. SIG-001 Dose-Dependent Production of hFVIII in Hemophilia A Mice**

- SIG-001 was placed in the IP space of nude mice via laparotomy (n=5 mice per group). Control group included mice receiving sham surgery. Mice were observed daily and sacrificed for blood collection on day 6 and hFVIII activity in plasma was measured by a chromogenic assay.

**Figure 6. SIG-001 Corrects the Tail Bleeding Phenotype in vivo**

- SIG-001 was placed in the IP space of male FVIII Hemophilia A (HA) mice via laparotomy (SIG-001, n=8). Control groups included male wild-type mice (wild-type, n=7), and FVIII HA mice with spheres containing unmodified control (control, n=8). Mice were observed daily. The bleeding time assay was conducted on day 7.

**Conclusions**

- The Shielded Living Therapeutics™ platform can be used to develop a new category of medicines for chronic diseases including rare blood disorders such as Hemophilia A.
- The platform overcomes the significant challenge of cell therapy: pericapsular fibrotic overgrowth.
- Our most advanced program, SIG-001, is able to produce functionally active hFVIII in a dose-dependent manner, and remains stable in animals sacrificed at 6 months.
- SIG-001 has the potential:
  - To eliminate the need for regular factor or non-factor injections, lowering the patient burden and providing consistent factor levels without the peaks and troughs observed with factor and non-factor therapies.
  - For use in pediatric patients, and
  - Re-dosing and retrieval, if needed.
- SIG-001 has been granted orphan status by the FDA; first in human clinical trial is planned for 2020.