Correcting Rare Blood Disorders Using Coagulation Factors Produced *in vivo* by Shielded Living Therapeutics™ Products

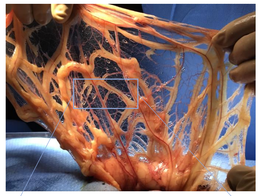
Guillaume Carmona, Lauren Barney, Jared Sewell, Kevin Lai, Ryan Newman, Christine Carroll, Michael Beauregard, Marley Kapsimalis, Janet Huang, Richard Heidebrecht, Deya Corzo, David Moller, Devyn Smith, David Peritt and Rogerio Vivaldi

Sigilon Therapeutics, Cambridge, MA, United States

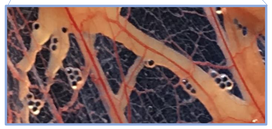
Introduction

- Hemophilia A arises from mutations in the F8 gene, affecting ~ 1/5000 males.
- Treatment options include frequent IV 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, human cells are being investigated.
- Allogeneic cells either need to be physically shielded and/or the host immunosuppressed to avoid a cytotoxic immune response by the host
- Various biomaterials, e.g. hydrogels, could serve as the physical barrier avoiding the need for immunosuppression altogether.
- However, the host can still activate a foreign body response, targeting the biomaterial, which significantly limits cell survival and durability of cell therapies due to pericapsular fibrotic overgrowth (PFO).
- We have successfully identified a library of proprietary small molecules, which when conjugated to alginate used to create encapsulating spheres, avoid the PFO (Bochenek, Nat Biomed Eng 2018)

Afibromer™ Biomaterials Avoid Pericapsular Fibrotic Overgrowth in Host Organisms



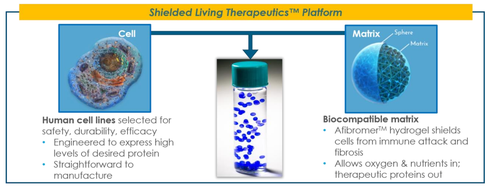
Empty spheres were placed in the omentum of non-human primates. Spheres were retrieved two weeks after placement.* They appeared translucent and no pericapsular fibrotic overgrowth was observed.



Close-up of the retrieved spheres

**Similar results were reported after 1 month and 4 months post-placement using spheres that contained allogeneic cells (Bochenek, Nat Biomed Eng 2018)*

The Shielded Living Therapeutics™ Platform



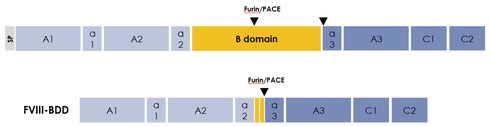
Methods and Results

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

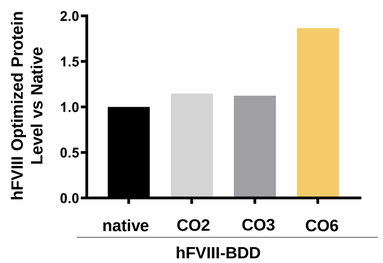
Cell Line Features

Safety	• Previous human exposure • Easily identifiable gene insertion sites • Human line • Non-tumorigenic
Durability	• Contact inhibited • Phagocytic • Hardy cell line
Time and Cost Saving	• Commercial scale production • Allogeneic
Efficacy	• High expression levels • Versatility of proteins expressed • High viability in spheres

Final hFVIII Construct in SIG-001

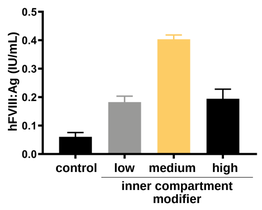


hFVIII Construct Optimization



hFVIII-BDD coding sequence was optimized in order to stabilize mRNA and increase protein secretion in the chosen cell line. Codon usage and GC content were changed in each construct to identify top performing sequence combinations. Driven by a strong promoter specially chosen for our cell line, we stably engineered cells to express hFVIII-BDD by a non-viral method. Secreted protein levels were then determined by ELISA to identify top performing constructs.

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.

Cells were encapsulated at 5, 20, 35 and 50 million cells/ml of matrix forming solution and implanted into nude mice. FVIII levels were assessed by ELISA at day 7. Density of 20 million cells/ml was chosen for further development.

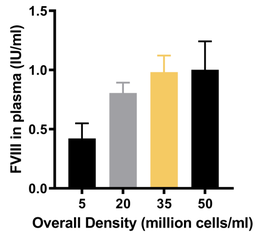
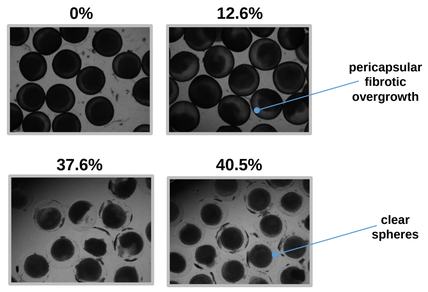
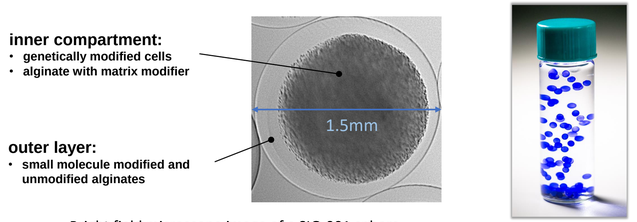


Figure 3. Outer Layer Optimization to Avoid Pericapsular Fibrotic Overgrowth



Alginate polymers were prepared with varying levels of small molecule conjugation (0-40.5%). Spheres were prepared with 20 million cells/ml of matrix forming solution, and implanted into C57/BL6 mice (n=4 per group). Spheres were explanted at day 7 and assessed for pericapsular fibrotic overgrowth using bright-field microscopy.

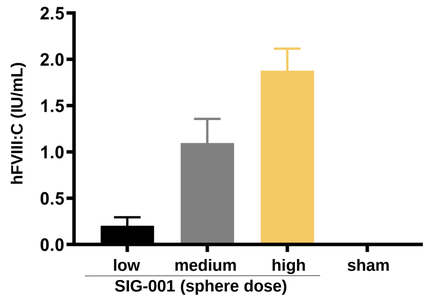
Figure 4. Final Product SIG-001: Cell Line Modified with a Non-Viral Vector to Express hFVIII, Encapsulated within Alginate Spheres



Bright field microscope image of a SIG-001 sphere

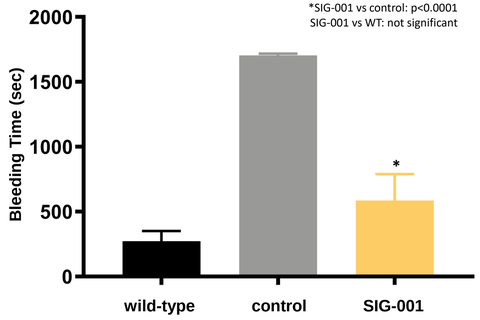
Blue color for illustration purposes only
Each sphere is ~1.5mm in diameter

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



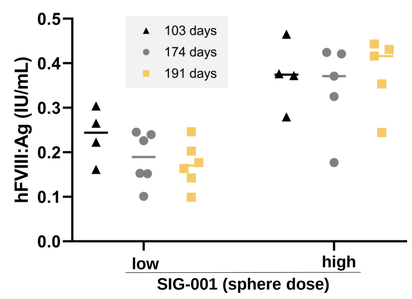
SIG-001 was placed in the IP space of mice via laparotomy (n=6 mice per group). Control group included mice receiving sham surgery . Mice were observed daily and sacrificed for blood collection on day 6 and hFVIII activity in plasma was measured by a chromogenic assay.

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 cells (control, n=8). Mice were observed daily, The bleeding time assay was conducted on day 7.

Figure 7. SIG-001 hFVIII Production in NSG Mice at 6 Months



Given the expected immune response to longer term human FVIII exposure in mice, NSG mice were used in this experiment. Low or high doses of SIG-001 were placed in the IP space of mice via laparotomy. hFVIII levels were measured at 103 days (terminal bleed, 4 mice per group), at 174 days (interim bleed, 5-6 mice per group) and at 191 days (terminal bleed, 5-6 mice per group). hFVIII levels were measured using ELISA.

Conclusions

- **The Shielded Living Therapeutics™ platform can be used to develop a new category of medicines for severe chronic diseases including rare blood disorders such as Hemophilia**
- **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**

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