Appendix D. PCORI Safety Study Results

Because our study involved using readily-available *LGG* via an off-label delivery mechanism (intravesical instillation), regulatory oversight was required by our Institutional Review Board. We submitted an Investigational New Drug (IND) application with the Food and Drug Administration (FDA) and for which we received approval. As part of that process, the FDA required completion of a "safety study" prior to the proposed prospective study.

The initial FDA-required safety study included 5 adults and 5 children. All study subjects were required to endorse no USQNB-IC items (have no urinary symptoms) at the time of instillation. Adult participants had a mean age of 35 years, were all male, and were, on average, 4.1 years post-SCI. Two participants had a cervical injury, two had a thoracic injury, and one had a lumbar injury, all of which were incomplete. Pediatric participants had a mean age of 8.4 years and were 80% male. All pediatric participants in the safety trial were born with SB (See Table 8).

Table 8. Safety study participant demographics

	Adults (n=5)	**Pediatrics (n=5)**
Age (years)	35.4 (12.7)	8.4 (2.3)
Male (n (%))	5 (100)	4 (80)
Level of Spinal Cord Injury		
Cervical (n (%))	2 (40)	-
Thoracic (n (%))	2 (40)	-
Lumbar (n (%))	1 (20)	-
Year Post Injury	4.1 (3.6)	-
Myelomeningocele Level		
L4	-	1 (20)
L5-S1		4 (80)

Data presented as: mean(SD) unless otherwise specified

LGG instillations were well-tolerated by all safety study participants, with no immediate adverse events. In the pediatric group, one child developed upper respiratory symptoms following instillation, and two children reported transient cloudy and malodorous urine that self-resolved during the week following instillation. No adults reported urinary symptoms in the week following probiotic instillation.

7 of the 10 participants had a decrease in urinary pH following probiotic instillation, (mean change (95% confidence interval): -0.45 (-0.99, 0.01) and one subject had no change in pH. No children had positive nitrites on their pre-instillation urinalysis whereas four of the five

children had positive nitrites following the instillation. Two adults had nitrites present on their pre-instillation urinalysis, and one had nitrites following instillation. One pediatric participant and three adults had pyuria present prior to instillation, and four pediatric participants and two adults had pyuria after the instillation (See Table 9). There were no changes in results from the pre-instillation urine culture to the post-instillation urine culture in any adult participants. Of the five pediatric participants, two had no changes between the pre- and post-instillation urine cultures, two had an increased colony count of the same bacteria in the post-instillation culture compared to the pre-instillation culture, and one participant had a negative pre-instillation urine culture, and a positive urine culture post-instillation (See Table 9).

Table 9. Pre- and Post-Instillation Urinalysis and Urine Culture Results Safety Trial

	Pre-Instillation						Post-Instillation					
	pH	Nitrites	Leukocyte Esterase	Urine WBC	Culture Result, Colony Count (CFU/mL)	Culture Result, Organism	pH	Nitrites	Leukocyte Esterase	Urine WBC	Culture Result, Colony Count (CFU/mL)	Culture Result, Organism
Children	7.0	Absent	1+	0-5	1,000-10,000	E. coli	6.0	Present	2+	20-40	>100,000	E. coli
	7.5	Absent	0	0-5	No Growth	-	6.0	Present	1+	10-20	>100,000	E. coli
	6.0	Absent	2+	10-20	10,000-50,000	E. coli	7.0	Present	1+	6-10	>100,000	E. coli
	6.5	Absent	0	0-5	>100,000	E. coli	6.0	Present	1+	10-20	>100,000	E. coli
	6.5	Absent	0	0-5	No Growth	-	7.0	Absent	0	0-5	No Growth	-
Adult	6.0	Absent	0	1-2	No Growth	-	5.5	Absent	0	0-5	No Growth	-
	6.0	Present	1+	10-20	>100,000	E. coli	6.0	Present	2+	10-20	>100,000	E. coli
	7.5	Absent	2+	0-5	>100,000	Pseudomonas	7.0	Absent	2+	0-5	>100,000	Pseudomonas
	7.0	Absent	2+	6-10	>100,000	E. coli	6.0	Absent	1+	6-10	>100,000	E. coli
	7.0	Absent	2+	6-10	>100,000	E. coli	6.0	Absent	Trace	0-5	>100,000	E. coli

Once the safety study was completed and the FDA reviewed the results and approved proceeding with the proposed trial, we proceeded with aims 2 and 3.