

1 **Supplemental Material**

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3 **Supplemental Table 1. Individual Viral Loads, Symptom Scores, and Respiration Rates in Individual bRSV-infected Calves**
4 **from Study 1.**

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| Parameter | Group 1 GS1 (2mg/kg BID) Individual Calves | | | | Group 2 Placebo Individual Calves | | | |
|---|---|------------|------------|------------|--|------------|------------|------------|
| | 1-1 | 1-2 | 1-3 | 1-4 | 2-1 | 2-2 | 2-3 | 2-4 |
| AUC of log ₁₀ Viral Load (log ₁₀ bRSV/GAPDH×day/mL) | 10.13 | 2.06 | 6.20 | 5.26 | 8.23 | 21.69 | 23.91 | 21.74 |
| AUC of Symptom Score (score×day) | 758.0 | 135.0 | 364.0 | 208.0 | 1399.5 | 1523.5 | 3648.5 | 1078.0 |
| AUC of Respiration Rate (breaths×day/min) | 318.0 | 279.0 | 325.0 | 250.0 | 434.5 | 480.0 | 720.0 | 400.0 |

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7 **Supplemental Table 2. Individual Viral Loads, Symptom Scores, and Respiration Rates in Individual bRSV-infected Calves**
 8 **from Study 2.**

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| Parameter | Group 1 GS1 at 4 mg/kg/day (Initiated 24 h.p.i. ^a) Individual Calves ^b | | | Group 2 GS1 at 4 mg/kg/day (Initiated 72 h.p.i.) Individual Calves | | | | | | Group 3 Placebo Individual Calves | | | |
|--|--|-------|-------|---|--------|-------|-------|-------|-------|---|-------|--------|--------|
| | 1-1 | 1-2 | 1-4 | 2-1 | 2-2 | 2-3 | 2-4 | 2-5 | 2-6 | 3-1 | 3-2 | 3-3 | 3-4 |
| AUC of log ₁₀ Viral Load (log ₁₀ bRSV/GAPDH ×day/mL) | 1.2 | 7.4 | 17.1 | 6.8 | 19.3 | 23.5 | 23.9 | 27.7 | 11.4 | 16.2 | 8.9 | 23.6 | 37.7 |
| AUC of Symptom Score (score×day) | 451.0 | 725.0 | 797.0 | 856.0 | 1087.0 | 368.0 | 508.5 | 458.0 | 361.0 | 835.0 | 648.0 | 2087.0 | 1033.0 |
| AUC of Respiration Rate (breaths×day/min), mean | 226 | 235 | 202 | 261 | 377 | 258 | 206 | 218 | 256 | 250 | 213 | 457 | 358 |

10 ^a hpi: hours post-inoculation

11 ^b Calf 1-3 was excluded from analysis due to secondary infection

12 **Supplemental Table 3. Study 1: Gross Pathology and Lung Histopathology**

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| Animal ID | Gross Pathology | Lung Histopathology |
|-----------------------------------|--|---|
| Group 1 GS1 4mg/kg/day BID | | |
| *1-1 | Mild bilateral areas of atelectasis (<5% lung); 0% consolidation | Minimal neutrophilic bronchopneumonia with pleocellular bronchiolitis |
| 1-2 | No gross lesions: 0% consolidation | Miminal scattered foci of neutrophilic bronchiolar exudate |
| 1-3 | No gross lesions: 0% consolidation; a focal fibrous adhesion on the thoracic wall. | Mild neutrophilic bronchial exudate; occasional lymphoid aggregates in bronchial and bronchiolar epithelium; no active bronchopneumonia or exudation in lung. |
| 1-4 | Mimimal focal unilateral multilobular discoloration, right middle lobe (RML), 0% consolidation | Mainstem bronchus has mild diffuse lymphoplasmacytic mucosal infiltrate; multilobular areas of atelectasis with peribronchiolar septal and focal pleural infiltrate of lymphocytes and plasma cells in RML; other areas unremarkable |
| Group 2 – Vehicle | | |
| 2-1 | 30% of total lung consolidated | Neutrophilic bronchointerstitial pneumonia; epithelial hyperplasia of bronchioles, obliterating bronchiolitis; type II epithelial hyperplasia with occasional multinucleated giant cells in alveoli; lymphofollicular hyperplasia in bronchial lymph node. bRSV positive on lung immunostain. |
| 2-2 | 25% of total lung consolidated | Confluent bronchointerstitial pneumonia with neutrophilic infiltrate in bronchioles and alveoli of consolidated areas; hyperplastic epithelium and focal necrosis of bronchial epithelium; expansion of alveolar septa and proliferation of type II epithelial cells. |
| 2-3 | 60% of total lung consolidated | Widespread bronchointerstitial pneumonia with neutrophilic exudation in which bronchioles have hyperplastic epithelium with focal necrosis and surrounding lymphoplasmocytic infiltrate; in bronchioles multifocal epithelial syncytia and syncytia in alveolar spaces; type II epithelial cell hyperplasia. bRSV positive on lung immunostain. |
| 2-4 | 20% of total lung consolidated | In consolidated areas patchy to lobular and confluent bronchointerstitial pneumonia with mild to moderate neutrophilic exudation into alveolar spaces; hyperplastic bronchiolar epithelium in grossly affected areas and type II alveolar epithelial hyperplasia. bRSV positive on lung immunostain. |

14 *This calf had an umbilical abscess visible at necropsy that may have caused systemic signs of inflammation.

15 **Supplemental Table 4. Study 2: Gross Pathology and Lung Histopathology**

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| Animal ID | Gross Pathology | Histopathology |
|---|---|---|
| Group 1- GS1 4 mg/kg Day 1 start | | |
| 1-1 | Minimal multifocal atelectasis (<5% lung) | Focal lobular atelectasis and bronchopneumonia with neutrophilic exudate into bronchioles; mild lymphoplasmacytic infiltrate in mainstem bronchus; <i>Mycoplasma bovis</i> isolated; bRSV negative on immunostain |
| 1-2 | Minimal multifocal multilobular atelectasis (<1% lung) | Mild bilateral neutrophilic histiocytic bronchopneumonia; <i>Mycoplasma bovis</i> isolated; bRSV negative on immunostain |
| 1-3* | Moderate bilateral consolidation (15% lung) | Acute suppurative bronchopneumonia, multifocal necrotic foci with <i>Mannheimia haemolytica</i> and <i>Mycoplasma bovis</i> isolated; bRSV negative on immunostain |
| 1-4 | Mild bilateral multifocal consolidation (8-10% lung) | Regional multilobular bronchopneumonia with mild neutrophilic and histiocytic exudate in to bronchioles and alveolar spaces. No bronchiolitis observed. <i>Mycoplasma bovis</i> isolated; bRSV negative on immunostain |
| Group 2 – GS1 4mg/kg Day 3 start | | |
| 2-1 | No gross lesions | No histologic lesions. <i>Mycoplasma bovis</i> isolated |
| 2-2 | Mild multifocal pulmonary consolidation with abscessation (5% lung) | Mild lymphoplasmacytic infiltrate in large bronchi; chronic lesions of fibrinoneutrophilic exudation with macrophages. <i>Mycoplasma bovis</i> and <i>Pasteurella multocida</i> isolated; bRSV negative on immunostain |
| 2-3 | Moderate to severe pulmonary consolidation (35% lung) | In areas of gross consolidation multilobular consolidation with exudation of neutrophils and macrophages within bronchioles andn alveolar spaces. Occasional peribronchiolar lymphoplasmacytic infiltrate with fibroplasia; no lesions in other areas; <i>Mycoplasma bovis</i> isolated bRSV positive on immunostain. |
| 2-4 | Minimal focal pulmonary atelectasis (1% lung) | Minimal scattered areas of atelectasis; in these areas minimal neutrophilic exudate in collapsed bronchioles; remainder of lung unremarkable. <i>Mycoplasma bovis</i> isolated; bRSV negative on immunostain |
| 2-5 | Mild bilateral multifocal lobular atelectasis (5% lung) | Disseminated neutrophilic histiocytic proliferative bronchiolitis with mild histiocytic neutrophilic bronchopneumonia and atelectasis. <i>Mycoplasma bovis</i> and <i>Mycoplasma bovirhinis</i> isolated; bRSV negative on immunostain |
| 2-6 | Minimal focal multilobular consolidation, right middle lobe, (<1% lung) | Regional focal multilobular atelectasis with minimal histiocytic neutrophilic bronchopneumonia. <i>Mycoplasma bovis</i> isolated; bRSV negative on immunostain |
| Group 3 – Vehicle day 1 start | | |
| 3-1 | Severe bilateral anteroventral pulmonary consolidation (35% lung) | Extensive neutrophilic histiocytic bronchopneumonia with mild necrotizing bronchiolitis, caseonecrotic foci; <i>Pasteurella mutocida</i> and <i>Mycoplasma bovis</i> isolated; bRSV negative on immunostain |

| | | |
|-----|--|--|
| 3-2 | Minimal focal multilobular consolidation (<2% lung) | Focal neutrophilic bronchopneumonia with bronchiolar epithelial hyperplasia; bRSV negative on immunostain |
| 3-3 | Severe bilateral anteroventral pulmonary consolidation (45-50% lung) | Affected lung lobes show extensive multilobular areas of neutrophilic bronchopneumonia with multifocal necrosuppurative bronchiolitis and focal necrosis; <i>Arcanobacterium pyogenes</i> ; <i>Mycoplasma bovis</i> isolated; bRSV negative on immunostain |
| 3-4 | Minimal bilateral lobular to linear atelectasis and focal consolidation (<5% lung) | Widespread neutrophilic histiocytic bronchointerstitial pneumonia with proliferative bronchiolitis and alveolitis with mild obliterating bronchiolitis and focal lobular neutrophilic bronchopneumonia; <i>Mycoplasma bovis</i> isolated; bRSV positive on immunostain |

17 * Excluded from analysis due to secondary infection
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19 **Supplemental Table 5. EC₅₀ values (μM) for RSV inhibitors against human and bovine RSV**

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| Compound | Mechanism of action | RSV A2 | bRSV | |
|-----------------|----------------------------|---------------|------------------|-----------------|
| | | (HEp2 cells) | (BAT2 cells) | (BT cells) |
| Ribavirin | Polymerase | 13.7 | 5.7 | 19.4 |
| RSV-604 | Nucleocapsid | 2.1 | > 100 | > 100 |
| YM-53403 | Polymerase | 0.2 | > 43.7 | > 100 |
| BI-D | Polymerase | 0.12 | 0.06 | 0.02 |
| TMC-353121 | Fusion | 0.001 | 0.030 | 0.014 |
| BMS-433771 | Fusion | 0.005 | 54.5 | 3.4 |

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22 N = 2