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Brief Communication

In-vivo and human evidence for potential efficacy of therapeutic polyclonal RSV neutralizing antibodies for palivizumab-resistant RSV infections

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ABSTRACT

Background: Monoclonal antibody (palivizumab), intravenous immune globulin (IGIV), or respiratory syncytial virus (RSV)-polyclonal-hyperimmune-globulin (RSV-IG as Respigam®, RI-001, RI-002) are used with ribavirin in RSV-infected immunocompromised patients, with debated efficacy. Palivizumab-resistance (PR) can arise during treatment of persistent infections in this population. RSV-IG may confer benefit in PR-RSV infection.
Methods: RSV-IG [RI-001] was provided for an immunocompromised infant with RSV-pneumonitis refractory to ribavirin and palivizumab. RSV-neutralizing antibody, respiratory RSV load (qPCR), and F-gene-sequence-detection of PR was determined. Prophylactic RSV-IG [RI-002] or palivizumab was administered in a cotton-rat model infected with wild-type and PR-RSV. Lung RSV load and neutralizing antibody were measured.
Results: As protective RI-001-neutralizing antibody titers waned in the infant, a subpopulation of PR-escape mutants were detected with a fatal RSV-burden in the lungs. In PR-RSV-infected cotton rats, prophylactic RI-002 reduced RSV-load in the lungs (2.45 vs 0.28 $\log_{10}$ PFU/g lung-tissue reduction, respectively, $p < 0.05$) and provided protective RSV-neutralizing antibody.
Conclusions: RSV-IG and ribavirin use in immunocompromised patients requires further study.

1. Introduction

Respiratory syncytial virus (RSV) causes significant morbidity/mortality in young infants and patients of all ages with compromised immune function. It is the most common cause of serious lower respiratory infection (LRTI) in infants and young children, and is the leading cause of hospitalization in those <1 year of age, with an estimated average annual rate of 2381 (per 100,000 children) of RSV-associated hospitalization in the US in this age group (Goldstein et al., 2019). Immune compromised patients, particularly lung or hematopoetic stem cell transplant (HSCT) recipients, are at higher risk of severe RSV disease progressing from upper to LRTI (25–55%) and death (7–40%) (Science et al., 2019; Renaud and Campbell, 2011).

Monthly intramuscular prophylaxis during an RSV season with monoclonal antibody, palivizumab, is recommended in high-risk infants with prematurity and/or underlying cardiopulmonary disease, and may be considered for children younger than 24 months who will be immune-compromised during the RSV season (Red Book Committee on Infectious Diseases, 2014). Treatment of already-established RSV disease in immune competent children is supportive, as lack of clinically significant outcome effect, high cost, cumbersome delivery methods and potential toxicity of the inhaled antiviral ribavirin precludes its use

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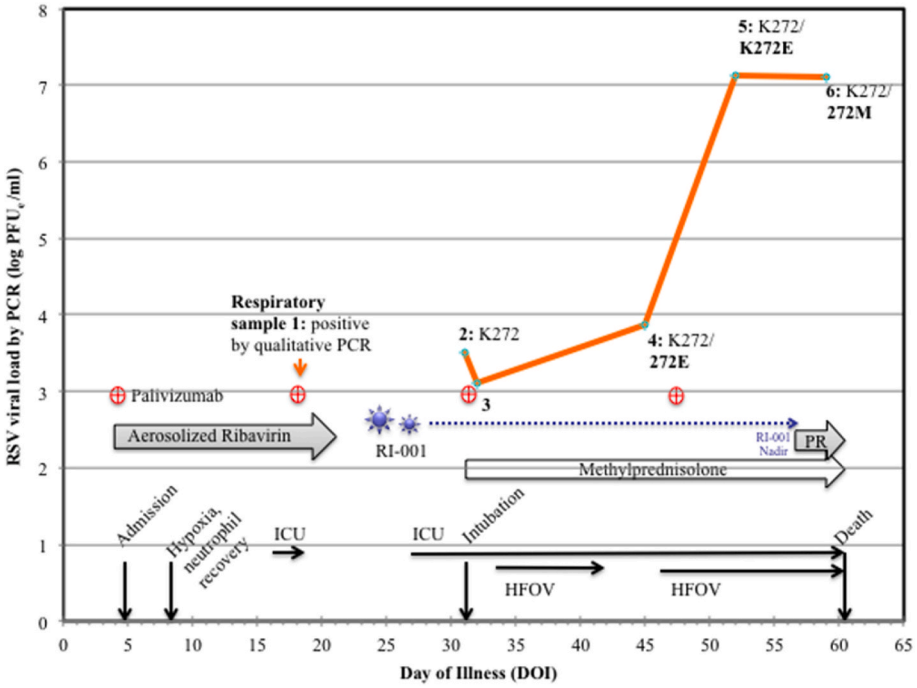


Fig. 1. Illustrated clinical course, therapeutic interventions, respiratory viral dynamics, and evolution of a subpopulation of palivizumab-resistant escape mutants detected in an RSV-infected immunocompromised infant with LRTI.
An immunosuppressed, lymphopenic 11 month-old infant with ALL and RSV-LRTI is illustrated. LRTI and RSV detection persisted despite three prophylactic doses (not shown) and four treatment doses (shown) of palivizumab, and inhaled ribavirin. The patient received two doses of RSV hyperimmune globulin (RSV-IG/RI-001) on DOI-24 and 26. Pre- and serial post-RI-001 serum antibody concentrations were measured with respect to ability to neutralize wild-type RSV in vitro (Table 1). Subpopulations of palivizumab-resistant (PR) escape mutants (K272E and K272M) emerge in the LRT (endotracheal samples 4–6) after selective pressures of repetitive doses of palivizumab, and as effective RSV-neutralizing titers wane. This palivizumab escape mutant evolution is concomitant with a rising RSV load (log PFU$_e$/ml) as determined by PCR and post-mortem specimens (Fig. 2). The patient died from respiratory failure with extremely high LRT viral loads and PR-mutations.

(Ventre and Randolph, 2007). Albeit with debated efficacy, rational potential treatment options in immunocompromised patients include inhaled ribavirin (Permpalung et al., 2020), with or without available monoclonal and/or polyclonal antibody preparations (Hirsch et al., 2013; Dignan et al., 2016).

RSV hyperimmune globulin (RSV-IG) preparations (Respigam®, RI-001, RI-002) are polyclonal intravenous hyperimmune globulins made from pooled plasma collected from healthy adults which is selected to contain high neutralizing anti-RSV titers. RI-002 (Asceniv™, ADMA Biologics, NJ, USA) is currently licensed and available by prescription (Asceniv package insert) for prophylactic use in primary immunodeficiency and other immune-compromised populations (Wasserman et al., 2017). Palivizumab, standard IGIV, and Asceniv™ remain the only available adjunctive antibody-based treatment options for seriously-ill immunocompromised patients with RSV infection.

Palivizumab resistance is a rare but appreciable concern, particularly in the prolonged infections that can arise in the severely immunocompromised patient. Palivizumab resistant RSV (PR-RSV) escape mutants have been found in 5–10% of children hospitalized with RSV breakthrough infections while receiving monthly palivizumab prophylaxis. These RSV escape mutations may or may not confer measurable impaired viral fitness, and to date, they appear to not play a major role in promoting breakthrough hospitalization (DeVincenzo et al., 2004; Papenburg et al., 2012; Zhu et al., 2011; Oliveira et al., 2015) of immunocompetent infants receiving monthly prophylaxis. However, treatment emergent virus may develop in the immunocompromised state in which higher viral loads and prolonged viral replication occurs (Grad et al., 2014). The therapeutic benefit of RSV-IG for PR-RSV infections is unknown. We report a case of an immunocompromised child with prolonged RSV infection in which a quasi-species of PR-RSV evolved after waning serum detection of RI-001 polyclonal antibodies and selective pressure of repeated doses of palivizumab, which ultimately preceded an increasing, fatal RSV burden in the lungs. In a separate cotton rat model, the effect of prophylactic RI-002 in those infected with PR-RSV was evaluated.

2. Materials and methods

2.1. RSV-IG compassionate use protocol

IRB-approved investigator-requested Emergency Investigational New Drug Application (EIND #32116) was obtained and RI-001 was made available for compassionate use given the immunocompromised infant's poor response to supportive care, inhaled ribavirin, and palivizumab for RSV infection.

Treatment was initiated with RI-001 1500 mg/kg on day of illness (DOI)-24 and with 750 mg/kg on DOI-26 (Fig. 1). Serum RSV neutralizing antibody titer samples were collected pre- and post-treatment doses. Nasal wash specimens or deep tracheal aspirates for detection and clinical reporting of RSV were collected throughout the patient's hospitalizations as clinically indicated and placed into viral transport media for further testing including direct fluorescent antibody detection (DFA) and quantitative real-time reverse transcription PCR [(RT)-PCR] (Fig. 1).

All samples (1–6) were then extracted on the EZ-1 instrument (Qiagen, Germantown, MD) and molecularly quantified utilizing a (RT)-PCR assay which amplifies an RSV N-gene sequence, as described elsewhere (Perkins et al., 2005; DeVincenzo et al., 2014). To assure assay precision, RSV quantitative standards (RSV-A-long-ATCC#VR-26) of known quantity were run in parallel with the subject's aspirate. Results were expressed as the means of duplicates in $\log_{10}$ plaque forming unit equivalents per milliliter (log PFU$_e$/mL).

Sample 1 did not amplify, so eluent from extracted samples 2–6 was sent to Laval University Research Center for F-gene amplification, traditional Sanger sequencing, and phylogenetic analyses (Papenburg et al., 2012). Reverse transcription was performed on 10 μL of RNA using 1 μL of 50 ng/μL random primers (Amersham, Piscataway, NJ) and the Superscript II RT Kit (Invitrogen, Carlsbad, CA) as per manufacturer's recommendations. Conventional PCR amplification was performed with QuantiFast Probe PCR + ROX Vial Kit (QIAGEN, Mississauga, Ontario, Canada). Both strands of each RSV-F amplicon were sequenced using an ABI3730xl DNA Analyzer (Applied Biosystems, Foster City, CA). Sample 3 did not amplify.

BioEdit 7.0.5 (http://www.mbio.ncsu.edu/bioedit/bioedit.html) was used to visually inspect chromatograms and prepare contigs for

sequence analysis. Newly-generated sequences were analyzed alongside a previously described dataset of genogroup A RSV-F sequences. Multiple-sequence alignment was performed with ClustalW in MEGA5 (www.megasoftware.net). A 1524-bp region (positions 79–1602 in the prototype A2 RSV-F gene) was translated into amino acid sequences, and the palivizumab binding site (residues 262–276) of sequences from samples 2, 4, 5, and 6 were assessed for variations. Using standard Sanger sequencing practices, the presence of a mixed population of viral sequences at specific sites were assessed by visually observing chromatograms. No attempt at relative quantification of the different quasi-species was nor should have been attempted.

3 cm-sized post-mortem specimens from each mid-lung were stored in formalin and cuts were stained with hematoxylin and eosin (H&E). Paraffin-fixed tissue from the same specimens were analyzed for RSV antigen via immunohistochemical (IHC) staining, though these images are not available.

Serum neutralizing antibody titers were performed as previously described (Walsh and Falsey, 2004; Falsey et al., 2017). The study was performed in compliance with institutional laws and guidelines and approved by the appropriate institutional review board (Approval #32116, and 09-00399-XP).

Prophylactic administration of RI-002 in the palivizumab-resistant (PR)-RSV/A/Tracy-infected cotton rat model

Inbred six-to eight-week-old female *S. hispidus* cotton rats were housed in large polycarbonate cages and fed a standard diet of rodent chow and water ad libitum, and RSV infection and evaluation of animals was performed as previously described (Gilbert et al., 2018). All studies were conducted under applicable laws and guidelines and after approval from Baylor College of Medicine Institutional Animal Care and Use Committee (Gilbert et al., 2018). RI-002 (1500 mg/kg) or palivizumab (15 mg/kg) were administered prophylactically at −24 h (Day −1) by intraperitoneal (IP) or intramuscular (IM) injection (left anterior tibialis), respectively, and compared for their relative activity to prevent RSV LRTI in cotton rats (n = 30, ~130 g bodyweight) infected intranasally (100 μL) (Day 0) with wild type (wt)-RSV/A/Tracy (strain P3.9/26/2013 grown in HEp-2 cells inoculated at 1.35×10^5 PFU) and palivizumab-resistant (PR)-RSV/A/Tracy (strain P8w.p.11/22/2013, inoculated at 2.04×10^5 PFU). Endpoints after euthanasia with CO_2 included the demonstration of reduced virus concentrations by semi-quantitative plaque assay in nasal wash (2 ml) and lung lavage (3 ml) fluids of the infected cotton rats on Day+4 compared to untreated cotton rats. Additionally, RI-002 RSV-IG (RSV-neutralizing antibody titers) in sera were determined by microneutralization assay on days 0 and +4 (Piedra et al., 1993). Control animals were inoculated with IP saline.

3. Results

3.1. Clinical case summary

An 11-month-old female diagnosed at 4 months of age with acute Pre-B lymphoblastic leukemia (ALL) was admitted to the hospital in January with RSV-LRTI. She achieved minimal residual disease after several courses of chemotherapy, but had remained lymphocytopenic. She was still receiving voriconazole antifungal suppression after completing three months of successful therapy for aspergillosis. Her pulmonary lesions had resolved as assessed by resolution of lesions on pulmonary computerized topography (CT) and undetectable serum galactomannan. She had received three monthly prophylactic palivizumab (15 mg/kg) doses, the most recent given ten days prior to RSV-illness onset.

Four days prior to hospital admission (DOI-0) she developed cough and rhinorrhea. In clinic (DOI-3), RSV was detected in nasopharyngeal washings by rapid antigen detection test (RAST), direct fluorescent antigen (DFA) and PCR. The day of hospital admission (DOI-4), she had tachypnea without hypoxia, neutropenia [absolute neutrophil count (ANC) 400 cells/mm,^{3} nl range 1500–8500 cells/mm^3] and lymphopenia [absolute lymphocyte count (ALC) 48 cells/mm,^{3} normal range 3000–9500 cells/mm^3]. Chest radiograph (CXR) showed a dense, band-like right paratracheal density as well as haziness in the left upper lobe consistent with atelectasis or pneumonia. Antimicrobials included aerosolized ribavirin [20 mg/ml (6 g diluted in 300 ml distilled water) over 12–18 h daily], palivizumab (30 mg/kg), cefepime, vancomycin, azithromycin and voriconazole.

Table 1
Serum RSV neutralizing antibody concentrations and fold increase between pre- and post- RSV hyperimmune globulin (RSV-IG/RI-001) infusion titers in an RSV-infected immunocompromised infant.

Day of Illness (DOI)	RSV neutralizing antibody concentration (Ln/0.05 ml)	Fold increase from day 1 pre-dose
24 (Pre-Dose 1 infusion)	6.7	Not applicable
24 (Post-Dose 1 infusion)	7.9	3
26 (Pre-Dose 2 infusion)	7.2	2
32	6.5	1
42	6.7	1
57	5.4	Not available

An immunocompromised patient with prolonged RSV infection received RSV hyperimmune globulin (RSV-IG/RI-001) for compassionate use. Serum antibody concentrations prior and subsequent to Doses 1 and 2 are measured (expressed as Ln/0.05 ml) with respect to ability to neutralize wild-type RSV in vitro. A three-fold increase in RSV-neutralizing antibody titers are appreciated immediately after the initial loading dose, and then wane significantly within 33 days.

DOI-8 she began to require 1 L (L) of nasal cannula supplemental oxygen. During this time, she had partial reconstitution of neutrophils (ANC 2800/mm,^{3} nl range 1500–8500 cells/mm^3), but remained lymphopenic. She was transferred to the Intensive Care Unit (ICU) (DOI-16) for increasing oxygen requirements with bilateral interstitial and patchy opacities on CXR. Nasal wash remained positive for RSV by PCR (DOI-17). She received a second dose of palivizumab (30 mg/kg). She was transferred out of the ICU on 0.5 L nasal cannula oxygen supplementation (DOI-18) and inhaled ribavirin was discontinued (DOI-20). She remained hypoxic, tachypneic, and her lymphopenia worsened (ALC 0/mm,^{3} normal range 3000–9500 cells/mm^3). Request for RI-001 was made for compassionate use given refractory RSV-LRTI in the setting of profound lymphopenia and treatment began on DOI-24 (Fig. 1). She received two doses, each given 48-h apart.

On DOI-26 she was transferred back to the ICU for respiratory distress and a new left lower lobe airspace opacity, which was felt to be due to natural progression of RSV disease. She was intubated (DOI-31). Chest computed topography (CT) showed persistent consolidation of perihilar and bilateral lower lobes concerning for co-infection, however, alternative causative organisms were not identified from endotracheal samples. She remained profoundly lymphopenic, started on methylprednisolone, and received another dose of palivizumab. She continued to require high-frequency oscillatory ventilation on DOI-33 to 41.

She was transitioned back to the conventional ventilator, however, on DOI-45 she had ongoing molecular detection of RSV in the LRT. Genetic sequencing detected a subpopulation of palivizumab-resistant mutants (F-gene analysis in Results, and Fig. 1). She then developed fevers, worsening hypoxemia and increasing bilateral consolidative infiltrates on chest radiograph. Repeat endotracheal testing for alternative infectious etiologies remained negative. A fourth dose of palivizumab and IGIV was given on DOI-47 and 49, respectively. Antimicrobial therapy was broadened and she transferred to a different facility in anticipation of need for extracorporeal membrane oxygenation (DOI-51), but was determined not to be a candidate. On DOI-52, LRT RSV load dramatically increased. A subpopulation of palivizumab escape mutants was still present.

On DOI-57, serum anti-RSV titers were measured at their nadir

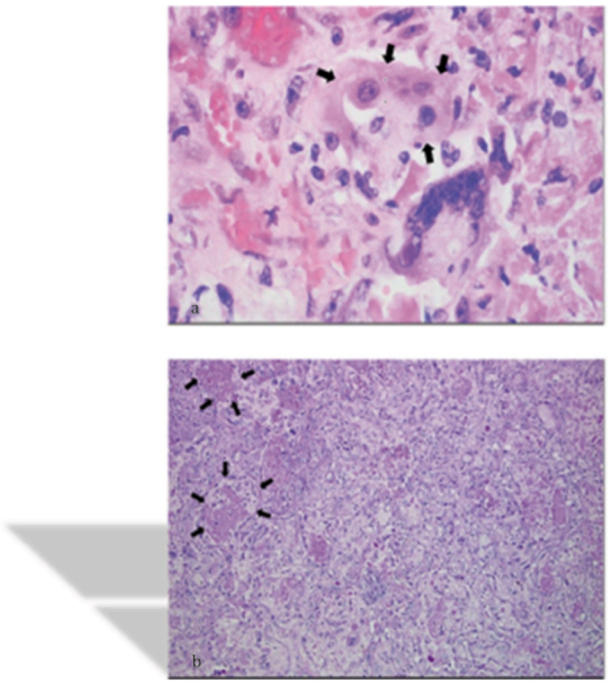


Fig. 2. Hematoxylin and Eosin (H&E) staining of post-mortem lung tissues in an RSV-infected immunocompromised infant.
Common post-mortem characteristics of RSV-infected lungs include robust infection, polypoid bronchiolar epithelial cells with syncytia formation, and predominantly polymorphonuclear (but some macrophage) inflammatory cell infiltrates (Pickles and DeVincenzo, 2015). Immunohistochemical staining (not shown) of our patient's lung post-mortem specimens confirmed an abundant presence of RSV. H&E staining is magnified at 60× (Panel a) and 10× (Panel b). Panel a (arrows) demonstrates syncytia formation (multinucleated giant cells). In Panel b (arrows), representative air spaces are filled with sloughed epithelial cell debris, some of which have formed syncytia. There are few inflammatory cells secondary to her profound lymphopenia, and potentially contributed by immunosuppression with methylprednisolone, which may attenuate recruitment of neutrophils.

(Table 1). She remained critically ill with hypoxia and hypotension, and was started on parenteral ribavirin. She required thoracotomy for left pneumothorax (DOI-58). Her LRT-RSV load remained elevated (Fig. 1). The next day she became bradycardic and persistently hypoxic. After a second cardiopulmonary decompensation she expired on DOI-60.

3.2. Molecular quantification and RSV-neutralizing antibody titers

Six respiratory samples were selected representing key time points with respect to alterations in immune phenomenon, monoclonal and polyclonal antibody administration, clinical disease and viral kinetics. Additionally, preference was given for samples with viral loads above the threshold for successful sequencing. RSV RNA was molecularly detected via qualitative PCR in the first respiratory sample prior to RSV-IG (Sample 1, in Fig. 1), however it did not amplify in quantitative RT-PCR. Thus, the first recorded respiratory viral load was collected several days after two doses of RSV-IG (Sample 2, Fig. 1). Viral load was sufficient for successful F-gene amplification, Sanger sequencing, and phylogenetic analysis from four extracted specimens (samples 2, 4, 5, and 6), as sample 3 did not amplify for sequencing.

Serum RSV neutralizing antibody titers were measured pre- and post-infusion Dose 1 given on DOI-24, pre-infusion Dose 2 given DOI-26, then on DOI-32, 42 and 57 (Table 1).

3.3. RSV-F gene analyses

Phylogenetic analysis of respiratory samples 2, 4, 5 and 6 (all from the LRT, except for Sample 2), confirmed that the infecting strain belonged to RSV genotype A with nucleotide and amino acid homologies with the RSV-A2 reference strain of 94.3–94.4% and 97.7–97.9%, respectively. F gene sequencing of sample 2 was wild-type for the entire palivizumab binding site. Sample 3 did not amplify. Samples 4 and 5 were predominantly wild-type (K272); however a guanine to adenine substitution at nucleotide 814 of AA 272 known to confer resistance to palivizumab (K272E) was observed as a minor variant. Finally, an adenine to thymine substitution at nucleotide 815 of AA 272, also known to confer resistance to palivizumab (K272 M), was observed in sample 6, in a mixed population with near equal proportions to wild type.

3.4. Descriptive post-mortem lung pathology

Post-mortem biopsy specimens from each mid-lung were grossly heavy and congested. H&E staining of all specimens revealed diffuse alveolar damage (DAD), syncytia formation (multinucleated giant cells), RSV inclusion bodies, and airways filled with sloughed epithelial cell debris. No additional infectious organisms, and only minimal inflammatory infiltrates (polymorphonucleur lymphocytes) were appreciated (Fig. 2). Immunohistochemical staining (not shown) was abundantly positive for RSV antigen.

3.5. Effect of intraperitoneal RSV hyperimmune globulin (RI-002) vs palivizumab on nasal wash and lung lavage viral load in wt- and PR-RSV/A/Tracy infected cotton rats

As expected (Siber et al., 1994), because of the low penetration of IgG antibodies into the upper respiratory tract, the antiviral effects of RI-002 (RSV-IG) and palivizumab administered by IP injection 24 h before RSV/A/Tracy challenge were minimal in the upper respiratory tract (Fig. 3a).

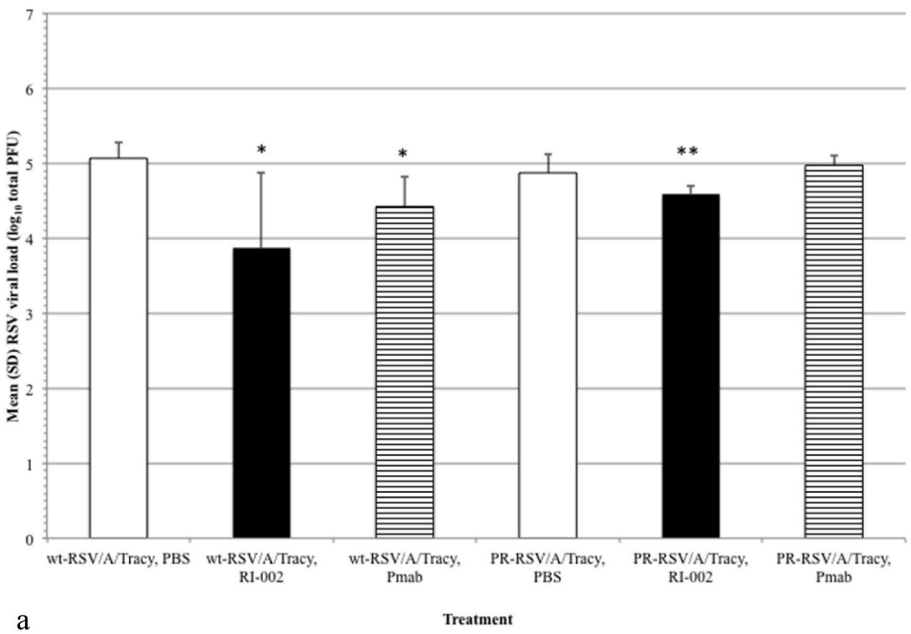
There was a significant antiviral effect of RI-002 and palivizumab in the LRT when administered 24 h before challenge with wt-RSV/A/Tracy (mean reductions of 2.8 and 3.0 $\log_{10}$ PFU/g of lung tissue, respectively) (~99% reduction) (Fig. 3b). However, RI-002 inhibited virus replication in PR-RSV/A/Tracy infected cotton rats, while palivizumab did not (reductions of 2.5 vs 0.3 $\log_{10}$ PFU/g of lung tissue, respectively ($p < 0.05$). The lower limit of detection by this method is 0.70 $\log_{10}$ total PFU for nasal fluids and approximately 1.4 $\log_{10}$ PFU/g lung for the lung fluids.

3.6. Effect of intraperitoneal injection of RI-002 on RSV/A/tracy serum neutralizing antibody titers

After administration of RI-002 at 1500 mg protein/kg, detectable serum RSV neutralizing antibody concentrations were achieved in both wt and PR-RSV/A/Tracy infected cotton rats on day 0 (8.9 and 9.0 Ln/0.05 ml) and day 4 (8.5 and 8.0 Ln/0.05 ml), respectively.

4. Discussion

Despite lack of definitive randomized trials, passive antibody therapies such as monoclonal palivizumab, intravenous immune globulin (IGIV), or RSV hyperimmune globulin (RSV-IG) have been used with ribavirin (RBV) in immunocompromised patients with severe RSV infection (Hirsch et al., 2013; Dignan et al., 2016). We illustrate a case of an infant with ALL in which sub-populations of PR-escape mutants emerged during persistent RSV infection after the selective pressure of repeated doses of palivizumab. Interestingly, the evolution coincided with an increase in RSV load, clinical decompensation, and ultimately death (Fig. 1, Samples 4 and 5). These viral escape mutants were first



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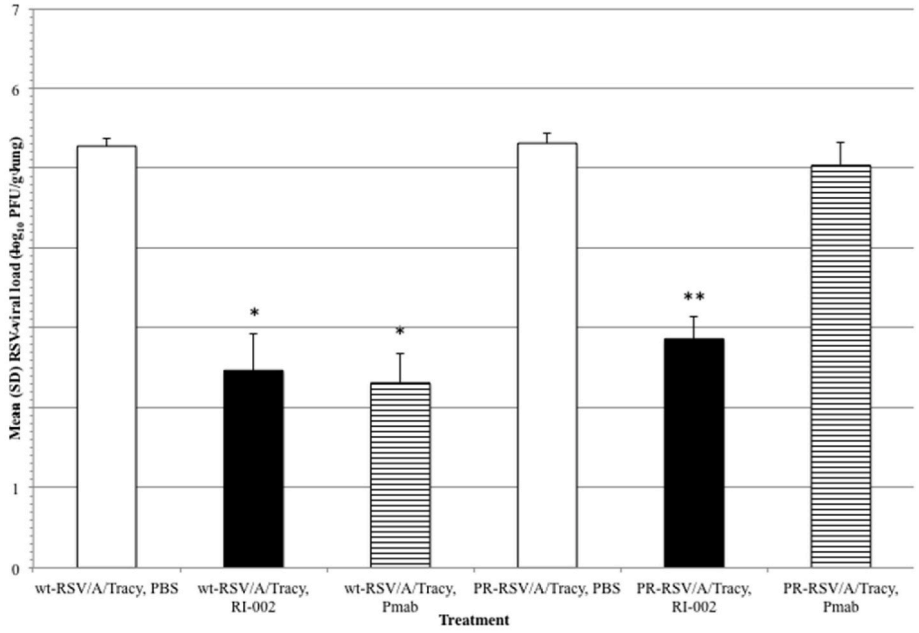

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Fig. 3. Effect of intraperitoneal RSV hyperimmune globulin (RSV-IG/RI-002) vs palivizumab on nasal wash and lung lavage viral load in wt- and PR-RSV/A/Tracy infected cotton rats

RI-002 (1500 mg/kg) or palivizumab (15 mg/kg) was administered prophylactically (day −1) in cotton rats infected (day 0) with either wild type (wt)- or palivizumab resistant (PR)-RSV/A/Tracy, and the antiviral effects (day 4) in nasal (2 ml) and lung lavage (3 ml) fluids were measured (mean $\log_{10}$ PFU). The lower limit of detection by this method is 0.70 $\log_{10}$ total PFU for nasal fluids and approximately 1.4 $\log_{10}$ PFU/g lung for the lung fluids.

Panel 3a demonstrates the minimal antiviral effects of RI-002 and palivizumab in the URT (RI-002–90% [~1 Log_{10}] reduction and palivizumab [~0.5 Log_{10}] reduction (*$p < 0.05$ vs wt-RSV, PBS)). RI-002 minimally inhibited virus in the PR-RSV infected cotton rat, while palivizumab had no activity (**$p < 0.05$ vs wt-RSV, PBS; PR-RSV, PBS; and PR-RSV, Pmab, respectively).

Panel 3b demonstrates the significant antiviral effects of RI-002 and palivizumab in the LRT of wt-RSV infected cotton rats (~3 Log_{10} reduction, *$p < 0.05$ vs wt-RSV, PBS), while only RI-002 demonstrates antiviral activity in PR-RSV infection (**$p < 0.05$ vs wt-RSV, PBS; PR-RSV, PBS; and PR-RSV, Pmab, respectively).

detected after protective serum titers of polyclonal antibody RI-001 waned (Table 1). In parallel, we demonstrated that RI-002 in a murine cotton-rat model can achieve adequate neutralizing antibody concentrations and effectively neutralizes PR virus in the LRT in vivo, highlighting the potential benefits of its use as a prophylactic and therapeutic drug during active and prolonged RSV infections seen in severely immunocompromised patients.

The RSV epitope for the neutralizing monoclonal antibody palivizumab includes amino acid (AA) residues 262–276 of the RSV-F (fusion) protein, which mediates fusion to respiratory epithelium and syncytia formation (Mason et al., 2018; Zhu et al., 2012). Natural polymorphisms in these residues are rare (<1%) (Zhu et al., 2012), with acquired palivizumab resistance reported in only 5–10% of RSV infected infants following 4–5 monthly prophylaxis doses during RSV season (DeVincenzo et al., 2004; Papenburg et al., 2012; Zhu et al., 2011). However, the development of mutations is facilitated by prolonged drug exposure during states of ongoing viral replication, particularly in the immunocompromised host. Evolving nucleotide substitution mutations at AA272 (K272E and M) conferring PR emerged after at least two weeks of selective pressure of palivizumab in our patient with a protracted immunocompromised state (Grad et al., 2014). Some evidence in infants receiving palivizumab prophylaxis suggests that resistant isolates have reduced viral fitness, and the association between break-through hospitalizations and palivizumab resistance has not been firmly established (Oliveira et al., 2015). While characteristics of these escape mutants are needed to further assess RSV fitness during treatment in the

Summary/key points

A fatal lung burden of palivizumab-resistant (PR) RSV evolved in an immunocompromised infant as protective RSV-Immune Globulin (RSV-IG) antibodies waned. A cotton-rat model demonstrates reduction of lung PR-RSV load after RSV-IG. RSV-IG adjunctive therapy in immunocompromised patients requires further study.

immunocompromised host, it is a noteworthy observation that their emergence in our patient coincided with an increase in lung viral load, which was temporally-associated with worsening respiratory function and death (Fig. 1).

While treatment-emergent PR may be a concern in the immunocompromised state, our results suggest RSV-IG could be a beneficial adjunct to antivirals in this clinical scenario. An immune globulin preparation containing high titers of RSV neutralizing antibodies is now available commercially for prophylactic use (Asceniv package insert). Small retrospective case-series including immunocompromised or HSCT patients (n = 13 and 15, respectively) treated with inhaled RBV and the now-discontinued RSV-hyperimmune globulin, Respigam® for compassionate use showed favorable mortality rates compared to RBV alone (DeVincenzo et al., 2000) and similar mortality rates to RBV and RI-001 (Falsey et al., 2017). The high-titer RSV polyclonal immune globulins (RI-001, RI-002, and RSV-IG) share similar antiviral properties and also have IGIV anti-inflammatory properties. Unlike standard IGIV, RSV-immune globulins are prepared from donors pre-selected to have high RSV neutralizing titers (Asceniv package insert), and thus provide higher concentrations of anti-RSV antibodies. Administration can therefore achieve up to a 76-fold increase in serum RSV-neutralizing titers (Falsey et al., 2017; Boukhvalova et al., 2016; Wasserman et al., 2016). Low serum RSV-neutralizing antibody is a risk factor for severe disease in adults (Walsh and Falsey, 2004; Luchsinger et al., 2012; Bagga, 2016). Higher serum antibody levels lead to greater transudation of protective IgG into the LRT as previously demonstrated in an immunosuppressed cotton rat model (Boukhvalova et al., 2016), and further showed a reduction in lung RSV load after therapeutic RI-002 administration at doses similar to the dose given to this study patient.

Reported risk factors for unfavorable outcome after RSV infection in our patient includes age less than two years, malignancy, and profound lymphopenia (El Saleeby et al., 2008). Inhaled ribavirin use was unfortunately limited to two weeks. Viral load in the respiratory tract was likely low prior to discontinuation, given RSV was detected only by exquisitely sensitive molecular methods, and could not, in duplicate, be subsequently molecularly quantified. Methylprednisolone may have prevented demargination of neutrophils into the lungs and suppressed lymphocytic T-helper cell (CD8) mediated cell damage (Siefker et al., 2020), while ultimately contributing to the fatal viral load rebound in the lungs which would occur in the absence of effective pharmacologic or immune viral control (El Saleeby et al., 2004). Our cotton rat model demonstrates that RI-001 is capable of generating adequate serum antibody titers and antiviral activity in the lungs, particularly during palivizumab-resistant RSV infections. Our patient demonstrated that after waning RI-001 neutralizing antibody detection, a fatal increase in respiratory viral load occurred, with evident subpopulations of PR-resistant RSV.

RSV-specific antibodies have been shown to reduce viral load within the LRT of infected children in a randomized, blinded trial, thus establishing the first human proof of concept that antibodies can treat an established viral infection. However, no specific reduction could be detected within the nasal compartment (Malley et al., 1998; De Vincenzo et al., 1996). This is thought to be a purely pharmacokinetic affect reflecting the poor penetration of systemically-delivered IgG into the nasal respiratory compartment (Malley et al., 1998, 2000). Similarly, systemic administration of RSV-IG to immune-compromised adults provides substantial RSV-neutralizing activity within serum, but relatively low RSV-neutralizing activity within the nasal compartment (Devincenzo, 2000). The relatively low RSV-neutralizing activity within the nasal compartment in conjunction with the relative lower viral fitness previously observed in-vitro in this F-gene resistance mutation, may explain genetic heterogeneity (mixed populations of viruses containing or not containing the palivizumab resistant mutation(s) at the K273 AA site), a viral-evolution phenomenon which we previously observed by deep-sequencing in another severely immunocompromised patient (Grad et al., 2014; El Saleeby et al., 2004). The elimination plasma half-life of IgG in immunocompromised patients with RSV ($T^{1/2}$ 10.8 days in allogeneic HSCT recipients) is significantly shorter than in healthy people because of vascular leak and other phenomena (Boeckh et al., 2001). This suggests that LRT viral replication in our patient was being driven by PR mutation-containing RSV quasi-species, especially once the polyclonal antibody suppressive effect waned ~ two weeks after RI-001 infusion (Table 1).

Efficacy of noteworthy therapeutic interventions for RSV are difficult to assess due in large part to the small populations of immunosuppressed patients unlucky enough to acquire RSV infection. Thus, there is a general dearth of randomized controlled trials. Clearly, the generalizability of our results remain speculative as expected in case reports supported by animal models. However, our carefully-studied single patient provides a wealth of longitudinal data supporting the relevance of high-titer RSV immune globulin in the treatment of RSV infection in severely immunocompromised patients. RSV-IG and ribavirin use in these patients requires further study.

5. Conclusion

A systematic study evaluating high-titer immune globulins in addition to ribavirin in the management of immunocompromised patients is warranted, particularly when palivizumab resistance is a concern.

CRediT authorship contribution statement

Kacy A. Ramirez: Writing – original draft, Writing – review & editing, Investigation. **James Mond:** Conceptualization, Writing – review & editing. **Jesse Papenburg:** Writing – original draft, Writing – review & editing, Formal analysis, Investigation. **Guy Boivin:** Writing – review & editing, Formal analysis, Investigation. **Brian E. Gilbert:** Writing – review & editing, Formal analysis, Investigation. **Ann R. Falsey:** Writing – review & editing, Investigation. **Bindiya Bagga:** Writing – review & editing, Investigation. **John P. DeVincenzo:** Conceptualization, Writing – original draft, Writing – review & editing, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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