RSV Antivirals: Problems and Progress

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Respiratory syncytial virus (RSV) is a significant cause of illness in children less than two years of age, resulting in 2-3% of infected children requiring hospitalization\(^1\). Globally, RSV was estimated in 2005 to have caused approximately 34 million cases of acute lower respiratory tract (LRT) illness and 66,000–199,000 deaths in children, with 99% of these deaths occurring in developing countries\(^2\). With the development of more sensitive diagnostic techniques, particularly polymerase chain reaction, the recognition of high disease burden and associated mortality in immunocompromised hosts and in older adults has increased\(^3\)-\(^5\). In spite of disease burden, specific antiviral treatment for RSV disease remains an unmet medical need. Here we summarize problems and progress in achieving successful therapeutic interventions.

In 1986 aerosolized ribavirin became the first and only approved antiviral for therapy of RSV infections in the United States, although it is no longer recommended\(^6\) and rarely used for its FDA-approved indication of treating RSV bronchiolitis and pneumonia in hospitalized infants. Instead aerosolized or increasingly oral ribavirin has been administered principally for treating RSV-infected immunocompromised patients, especially hematopoietic cell transplant (HCT) and lung transplant recipients\(^3\). Clinical practice varies widely and has been based on observational reports suggesting that timely ribavirin delivery can reduce the risks of progression to lower respiratory tract disease and mortality in such patients\(^3\).

Despite clear medical needs, subsequent efforts to develop more effective, better tolerated, less costly\(^7\) and more easily administered antivirals has been challenging for multiple reasons\(^8\)-\(^10\). Studies of an inhaled small inhibitory RNA targeting the RSV polymerase (L protein) (ALN-RSV01) in RSV-infected lung transplant recipients yielded promising results with respect to
reducing the risk of subsequent bronchiolitis obliterans syndrome\textsuperscript{11} and an oral RSV nucleoprotein (N) inhibitor (A-604) appeared to inhibit RSV replication and benefit a subgroup of RSV-infected HCT patients in whom adequate drug exposure was obtained\textsuperscript{12}, but both development programs were ultimately abandoned. Systemic administration of the anti-F (fusion) protein monoclonal antibodies (palivizumab, motavizumab) are effective for prophylaxis in premature infants but not for treatment of established RSV infection\textsuperscript{13,14}. Recently, inhaled delivery of the anti-F trivalent nanobody ALX-0171 showed dose-related antiviral effects in hospitalized RSV-infected infants but no consistent differences in clinical outcome measures (NCT02979431); a second trial in Japanese children was terminated early because of the lack of clinical efficacy (NCT03418571)\textsuperscript{15}.

Within the past few years orally administered antivirals that target the F protein (presatovir [GS-5806], JNJ-53718678, sisunatovir [RV521]), the L protein (lumicitabine [ALS-008176]), or the N protein (EDP-983) have shown antiviral and clinical efficacy, as well as acceptable tolerability, in adult volunteers experimentally infected with RSV\textsuperscript{16-20}. Several other F protein inhibitors (enzaplatovir [BTA-C585], BTA-9881, MDT-687) and the L protein inhibitor PC786 (NCT03382431) have undergone or are undergoing proof-of-concept studies in an RSV challenge model\textsuperscript{15}. However, despite the positive findings in experimentally infected adults, the development of lumicitabine has since been suspended due to toxicity concerns in infants\textsuperscript{21}. Further, a recent trial of inhaled PC786 in HCT patients was terminated due to practical issues leading to recruitment difficulties (NCT03715023).

Presatovir has received the most comprehensive evaluation to date in a series of phase 2 placebo-controlled, double-blind, randomized trials that utilized upper respiratory tract (URT) viral load reductions as the primary endpoint. A single 200mg dose of presatovir failed to reduce measures
of viral replication or improve clinical outcomes in RSV-infected hospitalized adults\textsuperscript{22}. Small trials with multiple dose regimens showed no clear antiviral effects or clinical benefits in HCT recipients with lower respiratory tract disease\textsuperscript{23} or in lung transplant recipients\textsuperscript{24}, and a larger trial in HCT recipients with URT illness also failed to meet its primary endpoint\textsuperscript{25}. Arguably, inclusion criteria in these trials allowed for randomization well into the course of disease at a time when benefit would be less likely. However, post hoc analysis of HCT patients with lymphopenia found that presatovir decreased the risk of developing LRT complications by day 28 (2/15 [13.3\%] vs 9/14 [64.3\%], \(p = 0.008\)). Encouragingly, another oral F protein inhibitor (zirosovir [AK5029]), not studied in the challenge model, was recently reported to have dose-related antiviral and clinical effects in hospitalized infants with RSV\textsuperscript{21,26}. Studies in adult outpatients with RSV infection are ongoing (NCT03699202), and the sponsor has indicated that phase 3 registrational studies are planned to start in 2020. Also, a phase 1 study of another orally administered F protein inhibitor JNJ-53718678 showed no safety signals and a clear trend for an early antiviral effect in RSV-infected infants\textsuperscript{27}. Randomized, controlled phase 2 studies in hospitalized infants and young children (NCT03656510), outpatient infants (NCT04068792), outpatient adults (NCT03379675), and adult HCT patients (NCT04056611) are in progress, and a phase 2 trial of sisunatovir (RV521) in hospitalized infants (NCT04225897) has just initiated recruitment\textsuperscript{15}.

While antivirals targeting the F protein have had the most extensive clinical testing to date, one liability of this inhibitor class is the relatively frequent emergence of F protein amino acid substitutions that confer reduced drug susceptibility. The current study\textsuperscript{28} involving adults with experimental RSV infections found 28 unique treatment-emergent F protein amino acid substitutions among 24 (17\%) presatovir recipients. One-half of the substitutions occurred at or
near amino acid positions previously selected by other F protein inhibitors, an observation highlighting the problem that some F protein substitutions result in loss of susceptibility to other inhibitors across the class\textsuperscript{29,30}. Susceptibility testing and replication fitness studies were done on recombinant viruses engineered on the RSV A2 backbone to express the F substitutions of interest. Of 17 RSV recombinants expressing a single substitution, in vitro testing in HEP-2 cells found that 13 had highly (38- to >410-fold) and 3 others had modestly (2.9- to 5-fold) reduced susceptibility to presatovir. Not surprisingly, all of these variants remained susceptible to ribavirin and palivizumab, but testing of variants for inhibition by other small molecule F protein inhibitors was not reported. Direct susceptibility testing of clinical isolates would be of great interest, especially as they might contain additional F protein or other substitutions that could affect susceptibility or replication fitness.

Key questions for any antiviral-selected amino acid substitutions in the target proteins are effects on replication fitness, associated virulence, and potential transmissibility and to what extent other permissive or enabling substitutions in the target or other RSV proteins might affect these properties. In vitro studies of five recombinant viruses with substitutions conferring highly reduced presatovir susceptibility found that replication kinetics in Hep-2 cells were similar to wild-type control virus; limited competitive co-infection assays indicated no fitness loss for one substitution (V360A) but reduced replication fitness for two others (L141F and T400I). However, replication fitness studies in such continuous cell lines and with recombinant viruses may not predict results in primary human respiratory epithelial cell systems or with clinical isolates. In the current study treatment-emergent F protein variants were associated with significant loss of antiviral efficacy but did not affect clinical outcomes, perhaps because of their
relatively late emergence (median of 6 days post-treatment [range, 1-10 days]) and the mild nature of these infections in RSV-experienced, immunocompetent adults. However, it would be helpful in the current study to know whether emergence of variants was associated with rebounds in viral titers in individual participants, as observed with oseltamivir in experimentally infected adults with influenza. Of course, animal model studies would be helpful in assessing the in vivo replication fitness and potential transmissibility of RSV clinical isolates with key F protein substitutions, as recently reported for baloxavir-resistant A(H3N2) viruses in the influenza ferret model. One engineered virus containing an F protein substitution (D401E) conferring reduced susceptibility was replication competent and virulent in a murine RSV model. Of note, this substitution is adjacent to the T400I/V/A-substituted variants associated with over 200-fold reductions in in vitro susceptibility found in the current study.

The frequency of treatment-emergent F protein substitutions leading to reduced presatovir susceptibility in the current trial is substantially higher than the two instances (4%) observed with oseltamivir treatment of influenza in adults experimentally infected with a seasonal A(H1N1) virus. The potential for treatment-related emergence of replication-competent RSV variants that also have highly reduced susceptibility (e.g., V360A in current study) is particularly concerning. While such variants might not cause clinical problems in immunocompetent patients, they could lead to protracted replication and illness in infants and young children and in immunocompromised hosts. The randomized trials of presatovir treatment in natural RSV infections used various dose regimens. Treatment-emergent F protein substitutions were detected in only 1/80 (1.3%) of hospitalized adults and 1/35 (2.9%) of lung transplant recipients but in
10/89 (11.2%) of HCT with URT illness and 6/29 (20.7%) of HCT patients with LRT illness\textsuperscript{23,25,33}. Of note, HCT patients with lymphopenia (ALC < 200) at randomisation were significantly more likely to develop presatovir-associated substitutions (7/15 [47%] vs 3/74 [4%], \(p < 0.0001\))\textsuperscript{24}. However, in HCT patients with LRT disease these substitutions were detected a median of 25 (range, 7–56) days after starting treatment in nasal samples\textsuperscript{23}. Thus, the contribution of resistance to the limited efficacy observed in these studies remains to be clarified.

In future an obvious treatment strategy will be the use of combinations of RSV antivirals with differing mechanisms of action to increase potency and reduce the risk of resistance emergence, especially in immunosuppressed patients. In the presatovir studies in HCT and lung transplant recipients, patients were stratified at randomization based on receipt of ribavirin treatment. It would be interesting to know if the combined use of these agents might have reduced the frequency of variant emergence.

Development of RSV inhibitors is infants and young children is particularly challenging, in part because of the potentially increased risk of antiviral resistance emergence. Presatovir has not been studied in this population, and details from the ziresovir and JNJ-53718678 studies are awaited with interest. In contrast, influenza prospective therapeutic studies of oseltamivir\textsuperscript{34} and, recently, baloxavir\textsuperscript{35} reported high frequencies of treatment-emergent influenza viruses with reduced susceptibility in children. In the case of baloxavir these were seen more common in young children with low influenza-specific antibody titers and were associated with both more prolonged viral replication and illness. Depending on transmission fitness, such variants could spread to close contacts as reported for baloxavir-resistant influenza A(H3N2) virus\textsuperscript{36}. In the worst case scenario, they might become established in the circulating virus population.
Deployment of RSV antivirals will require ongoing surveillance of drug susceptibility profiles, as is currently being done for influenza antivirals by US Centers for Disease Control and Prevention and other organizations involved in WHO’s Global Influenza Surveillance and Response System (GISIRS).

Another issue with presatovir and possibly other F protein inhibitors is the apparent disconnect between their potent nanomolar inhibitory activity in vitro and modest or negligible effects on virus replication in the URT of patients naturally infected with RSV. The primary mechanism of action of F protein inhibitors appears to be binding to prefusion F and interfering with F-mediated membrane fusion, an essential step in RSV entry. Delayed time to treatment, perhaps pharmacokinetic factors (reduced absorption in seriously ill patients, limited respiratory mucosal distribution, inactivation by pulmonary secretions) leading to inadequate exposure in the respiratory tract, or insufficient inhibition of cell-to-cell spread of virus may be contributory. The delayed emergence of variants in presatovir-treated HCT patients and the low frequency observed in hospitalized adults and lung transplant recipients raises concerns about adequate drug exposure. Post-hoc analysis of pharmacokinetic parameters found no correlation between plasma presatovir exposure and resistance development in the current report. Also presatovir does not appear to inhibit viral replication as rapidly or as potently as the polymerase inhibitors. Respiratory tract secretion concentrations in RSV-infected subjects have not been reported, although pre-clinical studies demonstrated increased lung distribution of presatovir relative to plasma in healthy rats and bovines. This raises the possibility that alternative presatovir dosing regimens might be more effective.
In summary, several studies with the F protein inhibitors presatovir in HCT patients with URT illness and ziresovir and JNJ-53718678 in hospitalized infants have yielded encouraging results. Importantly, a number of RSV antiviral agents are advancing in clinical study, although no RSV therapeutics are listed as having progressed to the phase 3 trials required for regulatory approval.

The current state of RSV antiviral development illustrates the need for better understanding of the role of ongoing viral replication across time in disease pathogenesis for different RSV patient populations, of pharmacokinetic-pharmacodynamic relationships with currently available agents, and of the effectiveness of RSV inhibitors with differing mechanisms of antiviral action.

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