Discovery of potent small molecule inhibitors of RSV Fusion protein

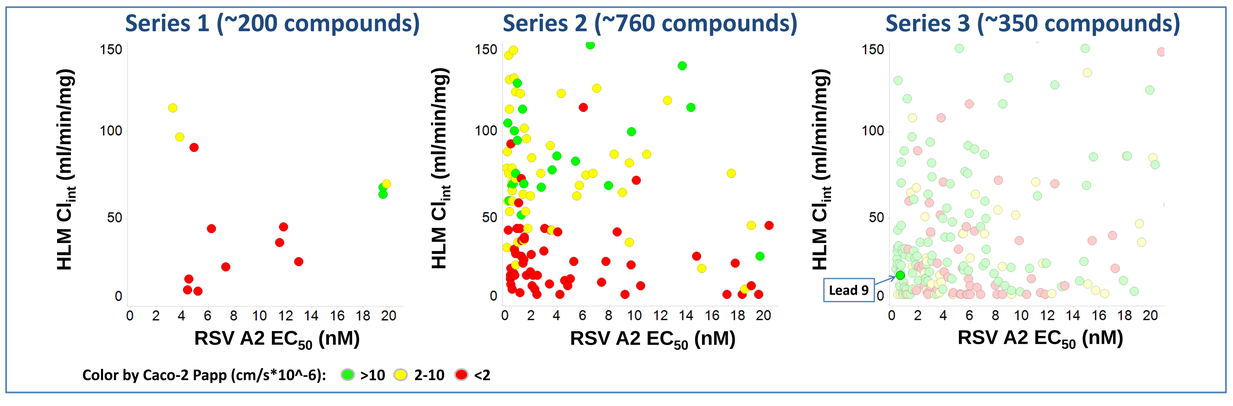
Karolina Ersmark[1], Christina Rydergård[1], Helen Kylefjord[1], Erwin Brenndörfer[1], Susana Ayesa Alvarez[1], Stina Lundgren[1], Fernando Sehgelmeble[1], Horst Wähling[1], Lourdes Salvador Oden[1], Mikael Pelcman[1], Karin Leijondahl[1], Dean Derbyshire[1], Jonna Stålring[1], Neera Borkakoti[1], Michael Battles[2], Jason McLellan[2], Sara Moses[1], Tom J. Goldschmidt[1], Johan Bylund[1], Richard Bethell[1], Paul Targett-Adams[1]. *[1]Medivir AB, Huddinge, Sweden 141 22. [2]Geisel School of Medicine, Dartmouth College, Hanover, NH 03755-3844*

Background

Respiratory syncytial virus (RSV) infections of infant, elderly, and immunocompromised patients represent substantial unmet medical need.[1,2] Tractable options for the development of anti-RSV therapies include inhibition of RSV-encoded fusion (F) protein.[3] We report the discovery of orally bioavailable RSV F inhibitors exhibiting highly potent and balanced activities against diverse RSV isolates, large cytotoxicity indices, and promising *in vivo* pharmacokinetics. The profile of a front-running candidate from this program ('**Lead 9**') is presented below.

Program development

Lead optimization was instigated on three novel 6,6-bicyclic cores (series 1-3) with the aim of selecting a candidate drug capable of sustaining therapeutic drug exposures against a broad range of RSV infections in humans. Inhibitors synthesized early in the lead optimization campaign achieved potencies <10 nM against a primary RSV A screening strain (RSV A2) but were often associated with lower activities against additional RSV strains and non-optimal ADME profiles. Subsequent optimizations resulted in several promising molecules from **series 3** with picomolar EC_{50} values against both RSV A and B subtypes, cytotoxicity indices >50,000, favorable ADMET properties, and encouraging *in vivo* PK profiles in rat and dog . **Lead 9** was identified as one of several special interest molecules from series 3 and was profiled extensively.



Methods

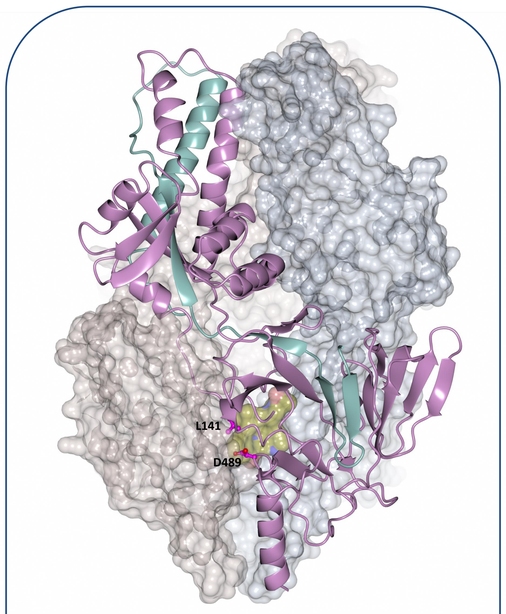
Established virology, molecular/structural biology, and drug metabolism/pharmacokinetic assays were used to screen and profile F inhibitors generated from the internal chemistry program.

Results

Mechanism of action for series 1-3 molecules

Time-of-addition studies and the generation of specific resistance-associated substitutions in the F protein using series 1-3 examples indicated the mechanism of action for these molecules was mediated by targeting the RSV F protein. Co-crystallization of series 1-3 examples with preF revealed compounds bound in a pocket of preF created at the interface of the 3 monomeric subunits:

- Medivir example compounds from all 3 series bind in the same pocket with the same stoichiometry: 1 inhibitor per preF trimer
- Binding pocket contains residues involved in conferring resistance to fusion inhibitors e.g. L141 and D489.
- The inhibitors are likely ***'triggering antagonists'***: they tether and stabilize 2 structurally labile regions of F (heptad repeat B and fusion peptide) to prevent release of the fusion peptide during the conformational change required to initiate the membrane fusion process.



Co-crystal structure of a series 1 inhibitor (yellow) bound to preF timer. L141 and D489 residues are highlighted in pink.

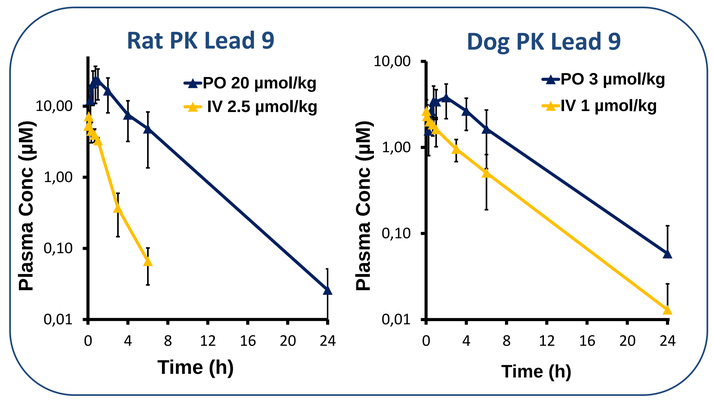
Broad-ranging anti-RSV activities of Lead 9 determined in Hep2C cells (5 day assay, XTT end-point)

Assay	EC_{50} (nM)	
	Series 1 example	Lead 9
RSV A/A2	1	0.6
RSV A/Long	180	0.5
RSV A/Memphis 37	140	0.5
RSV B/Washington	22	0.6
RSV A clinical isolates (n=8)	ND	0.4
RSV B clinical isolates (n=8)	ND	0.2
Hep2C CC_{50} (nM)	>50 000	>50 000

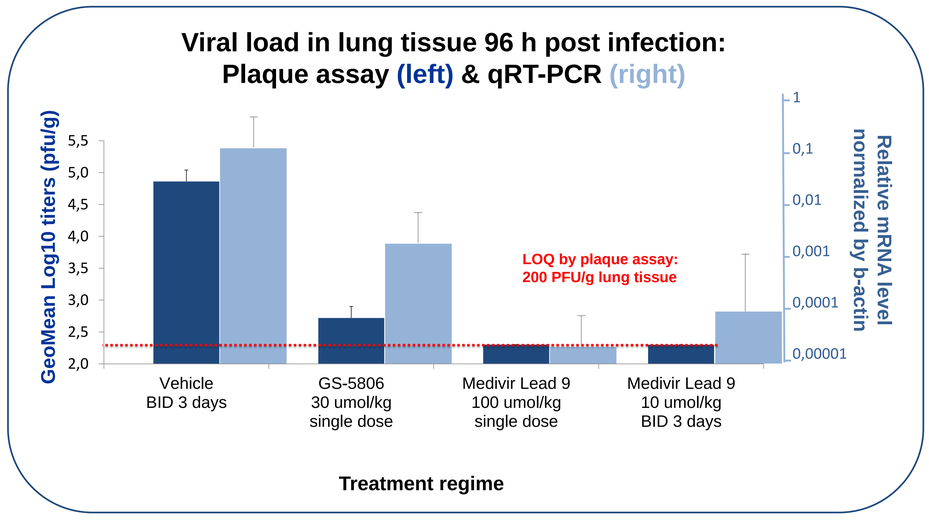
Favourable *in vitro* and *in vivo* DMPK properties of Lead 9

In vitro DMPK Lead 9	
Solubility pH 6.5 (µM)	110
Caco-2 P_{app} (10^{-6}cm/s)	13
Cl_{int} Hum Hep (µL/min/10^6 cells)	1.2
Plasma Protein Binding Hum (%)	75

In vivo DMPK Lead 9	Rat	Dog
Clearance Plasma (mL/min/kg)	4.5	1.5
Half-life Plasma iv (h)	1.2	3.0
Bioavailability (%)	100	92



Lead 9 demonstrated robust antiviral efficacy in the cotton rat model for human RSV infection



Male cotton rats (*Sigmodon hispidus*, n=8 per group) were treated by oral gavage with **Lead 9** 2 h before intranasal infection by 10^5 pfu human RSV/A/Long.

In vitro safety assessments for Lead 9 revealed benign safety profiles

Assay	Description	Result Lead 9
Cytotoxicity evaluations	Hep3B/HUH7/MT4 cell lines	CC_{50} >50µM
High content multi-parameter toxicity assessment (CellCiphr®)	HepG2 and rat primary hepatocytes	No significant effects on any parameter tested (top concentration 200 µM)
Secondary pharmacology target screen (87 targets)	*In vitro* binding to GPCR, ion channels, transporters, nuclear receptors, kinases and other non-kinase enzymes.	No hits (tested at 10 µM)

Conclusions

- **A lead optimization campaign directed upon three novel 6,6-bicyclic cores (series 1-3) resulted in the identification of Lead 9, which demonstrated:**
  - ✓ **Biology data consistent with inhibition of RSV Fusion protein.**
  - ✓ **Balanced picomolar EC_{50}s against a broad range of RSV A and B isolates.**
  - ✓ **Favourable human *in vitro* DMPK properties.**
  - ✓ **Excellent oral bioavailability in rat and dog.**
  - ✓ **A robust antiviral effect in the cotton rat model for human RSV infection.**
  - ✓ **A benign *in vitro* safety profile.**
- **The profile of Lead 9 supports progression to preclinical development with the aim of developing a safe and effective treatment against RSV infections in humans.**

References

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2. Falsey A.R. et al Respiratory syncytial virus infection in elderly and high-risk adults. *N. Engl. J. Med.* **2005**, 352, 1749-59.
3. DeVincenzo J.P. et al Oral GS-5806 activity in a respiratory syncytial virus challenge study. *N. Engl. J. Med.* **2014**, 371, 711-22.