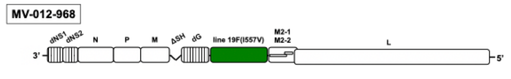
The codon deoptimized, intranasally delivered, live attenuated RSV vaccine MV-012-968 is well tolerated and increases RSV preF specific IgA levels in healthy adults

Oliver F. Medzihradsky[1], Carlos Fierro[2], Barbara L. Schlingmann-Molina[1,3], Xing Cheng[1], Aditya Garg[1], Jorge C.G. Blanco[4], Roderick S. Tang[1], Karen S. Slobod[1], and Martin L. Moore[1]
[1]Meissa Vaccines Inc., Redwood City, CA, [2]Johnson County Clin-Trials, Lenexa, KS, [3]Present Address: Merck Sharp & Dohme, West Point, PA, [4]Sigmovir Biosystems Inc., Rockville, MD

Introduction

RSV is a leading cause of infant lower respiratory tract infection and hospitalization as well as elderly pulmonary disease. There is no approved RSV vaccine. We describe here the first in human study of the live attenuated RSV vaccine candidate MV-012-968. A derivative of the RSV A2 OE4 strain [Stobart, C. C. et al. *Nat. Commun.* 7, 13916 (2016)], MV-012-968 was designed to attenuate RSV without substantial impairment of immunogenicity, via codon-deoptimization of NS1, NS2, and G genes. The virus was also attenuated by deletion of SH, which increased expression of the F gene. Mutation was introduced to ablate expression of secreted G protein. The wt RSV A2 F was substituted with Line19 F, which favored the meta-stable pre-fusion conformation and conferred thermostability.

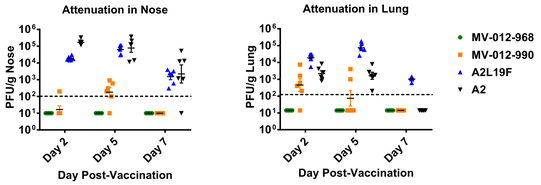


Symbol	Modification
dNS1 / dNS2	Codon-deoptimized NS1 and NS2 for human expression
ΔSH	Deletion of SH protein
dG	Codon-deoptimization of G for human expression
Line19 F	Chimeric expression of line19 F

Candidates were evaluated for attenuation, immunogenicity, and protective efficacy in the cotton rat model. Under a US IND, a Ph1, dose-ranging, open-label study was conducted in healthy adults with serum RSV nAb levels in the lowest quartile of screened population (NCT04227210). Tolerability, safety, nasal viral shedding, and immunogenicity were followed for 6 months after 1 intranasal (i.n.) dose of 10^5 (n=10) or 10^6 (n=10) PFU of MV-012-968.

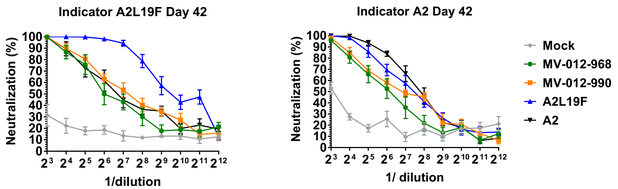
Results

MV-012-968 was highly attenuated in upper/lower respiratory tracts of cotton rats



Cotton rats (CR) were inoculated i.n. with $5x10^5$ PFU of MV-012-968 (n=24), MV-012-990 (n=24; DB1-Quad, another research candidate), wt A2L19F (n=24), wt A2 (n=24), mock agent (Mn. Essen. Media; n=12), or no agent (n=6) on Day 1. Viral titers in nose and lungs were determined by plaque assay (n=6 CR/group/day) on Days 2, 5, 7. No infectious MV-012-968 was detected in nose or lungs at any time point. On Days 2 and 5, CR inoculated with MV-012-968 had significantly lower viral loads in upper and lower respiratory tracts than CR inoculated with wt A2 or A2L19F (p≤0.0001, two-way ANOVA, Tukey multiple comparisons test).

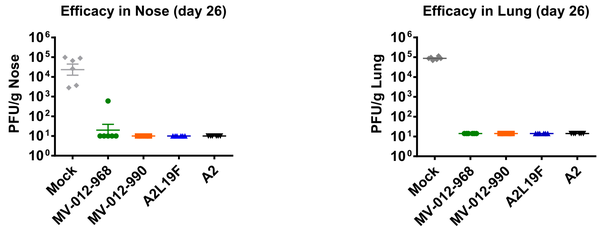
Despite heavy attenuation MV-012-968 elicited serum neutralizing antibodies in cotton rats comparable to levels induced by wild type RSV



Day 42	MV-012-968		MV-012-990		A2L19F		A2	
Indicator	EC50	CI	EC50	CI	EC50	CI	EC50	CI
A2L19F	105.8	77.5 to 146	191.2	151.1 to 242.8	966.7	785.7 to 1199	157.4	115.5 to 216.2
A2	74.36	57.27 to 96.76	130.4	104.4 to 162.9	185.9	152.2 to 227.8	227.6	196.2 to 264.4

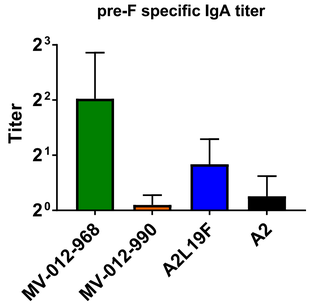
Serum samples were taken on Day 42 from CR vaccinated on Day 1 with $5x10^5$ PFU of MV-012-968 (n=18). Samples were pooled (n=6, 3 animals/pool) and serum nAb titers against reporter-expressing RSV A2 and A2L19F determined via microneutralization. Percent neutralization was calculated: [100-(# plaques at counted dilution/max # plaques*100)]. EC50 and confidence intervals (CI) were determined by non-linear curve fit. Despite high levels of attenuation MV-012-968 produced levels of serum RSV nAb comparable to wt RSV.

MV-012-968 protected cotton rats against wt RSV challenge 26 days after vaccination



CR were vaccinated ($5x10^5$ PFU of test article) on Day 1, then challenged ($1x10^6$ PFU of A2L19F) on Day 21. Titers of A2L19F in nose and lungs were measured by plaque assay on Day 26 (n=6 CR/group). CR vaccinated with MV-012-968 were completely protected in lung and significantly protected in nose against challenge. Only unvaccinated animals showed detectable virus replication 5 days post challenge in nose and lung tissue (p≤0.0001, one-way-ANOVA, Tukey multiple comparisons test).

MV-012-968 elicited pre-F specific IgA in nasal wash samples of cotton rats on day 47 post vaccination



Pre-F specific IgA titers were measured in nasal wash (NW) samples of CR on Day 47 post-vaccination (Day 5 post-challenge with RSV A2L19F). The cutoff to determine IgA titer was defined as absorbance measured at the lowest dilution (1:2) + 1x SD in the Mock (MEM) sample. The highest dilution in test samples that exceeded cutoff absorbance for each pool was chosen as IgA titer. Total protein in samples was determined by use of micro BCA kit. Log_2-transformed IgA titer for each sample was adjusted to a total protein of 100 μg/mL. MV-012-968 elicited the highest preF-specific igA response, though differences were not statistically significant relative to other groups. Shown are mean ± SEM (one-way-ANOVA with Tukey's multiple comparisons test).

MV-012-968 was well-tolerated and boosted nasal mucosal RSV preF-specific IgA in healthy 'sero low' adults

At a single US center, 20 participants 18-38 years of age with serum RSV nAb in the lowest quartile determined by a microneutralization assay were randomized to receive 10^5 (n=10) or 10^6 (n=10) PFU of open-label MV-012-968 on Day 1. Participants were followed to Day 180 (6 months) for safety. Nasal vaccine virus shedding, serum RSV IgG and nAb, and RSV specific nasal mucosal IgA were measured to Day 56.

Characteristic	10^5 PFU Inoculation Group (n=10)	10^6 PFU Inoculation Group (n=10)	Inoculation Groups Combined (n=20)
Age (mean, SD in years)	29.3 (5.3)	25.5 (6.0)	27.4 (6.0)
Sex (female, n, %)	6 (60)	5 (50)	11 (55)
Race (n, %)			
White	4 (40)	6 (60)	10 (50)
Black/African American	5 (50)	4 (40)	9 (45)
American Indian	1 (1%)	0	1 (5)
Ethnicity (n, %)			
Hispanic/Latino	1 (10)	0	1 (5)
Non-Hispanic Latino	9 (90)	10 (100)	19 (95)

Solicited AE	Subjects n (%)	
	10^5 PFU (n=10)	10^6 PFU (n=10)
Subjects with any solicited AE	2 (20)	3 (30)
Fever	0	0
Rhinorrhea	2 (20)	2 (20)
Pharyngitis	0	1 (10)
Cough	0	0
Hoarseness	0	0
Difficulty breathing	0	1 (10)
Muscle ache/pain	1 (10)	0
Headache	1 (10)	3 (30)

Solicited adverse events (AE), collected to Day 7, were reported by 2/10 and 3/10 of 10^5 and 10^6 PFU recipients respectively. All solicited AE were mild; none persisted beyond Day 7. Unsolicited AE, collected to Day 7, were all mild and reported by 2/10 and 0/10 of 10^5 and 10^6 PFU recipients respectively. There were no serious or severe AE. One medically attended AE was reported (influenza, Grade 2, > 30 days after MV-012-968 inoculation). No dosage-dependent pattern of AE or clinical laboratory abnormality was observed.

No infectious virus was recovered from any nasal swab through Day 56 as measured by plaque assay.

Serum (RSV nAb and preF specific binding) and nasal mucosal (preF specific binding) Ab responses were measured through post-inoculation Day 56. As anticipated in naturally seropositive adults, no substantive change in RSV-specific serum Ab titers was observed. An increase in RSV-specific nasal IgA (≥ 2-fold over baseline) was detected in 6/9 recipients at the 10^6 PFU dosage and 3/10 recipients at the 10^5 PFU dosage during the 14 days following study vaccination:

Dosage of MV-012-968 (n)	≥ 2-fold increase in RSV preF nasal mucosal IgA (n [%])
10^5 PFU (10)	3 (30%)
10^6 PFU (9)	6 (66.7%)

Summary

- LAV MV-012-968 has been rationally designed to attenuate RSV without compromising immunogenicity
- MV-012-968 has a highly attenuated replication phenotype and provided protection against wt RSV challenge in the cotton rat model
- MV-012-968 was immunogenic in cotton rats, eliciting serum nAb responses comparable to wt RSV and inducing mucosal IgA, which has been correlated with protection
- MV-012-968 was well tolerated in healthy 'sero low' adults, with no serious or severe adverse events, and with infrequent post-vaccination adverse events that were, when present, mild and short-lived
- No infectious vaccine virus was recovered through Day 56 after vaccination of adults with MV-012-968
- ≥2-fold increase over baseline in RSV preF specific nasal IgA was detected in the majority of adult vaccinees after receiving 10^6 PFU through Day 14 post vaccine dosing
- MV-012-968 has advanced to evaluation in seropositive children

Acknowledgments

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