Safety, tolerability and biological activity of repeated intranasal administration of Ampligen (Poly I:Poly C12U) as potential antiviral treatment in healthy subjects – preliminary results

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AIM ImmunoTech

Disclosures

- Johan L. van der Plas, Lisanne C.A. Smidt, Aliede E. in ’t Veld, Christina Yfanti, Ingrid M.C. Kamerling, Naomi B. Klarenbeek, Manon A.A. Jansen, Matthijs Moerland have nothing to declare
- David R. Strayer, Diane L. Young: employees of AIM ImmunoTech
- Study was sponsored by AIM ImmunoTech Inc., Ocala, Florida, United States

Introduction

- COVID-19 therapeutic arsenal limited
- Route of infection: upper respiratory tract
  - Potential target location for early local treatment or post-exposure prophylaxis
- Intranasal Ampligen® has potential as early treatment or prevention of COVID-19
  - Immunomodulatory effects on mucosal level
  - Antiviral activity against DNA and RNA viruses (including SARS-CoV-2 *in vitro*)
  - Also potential for other respiratory viruses

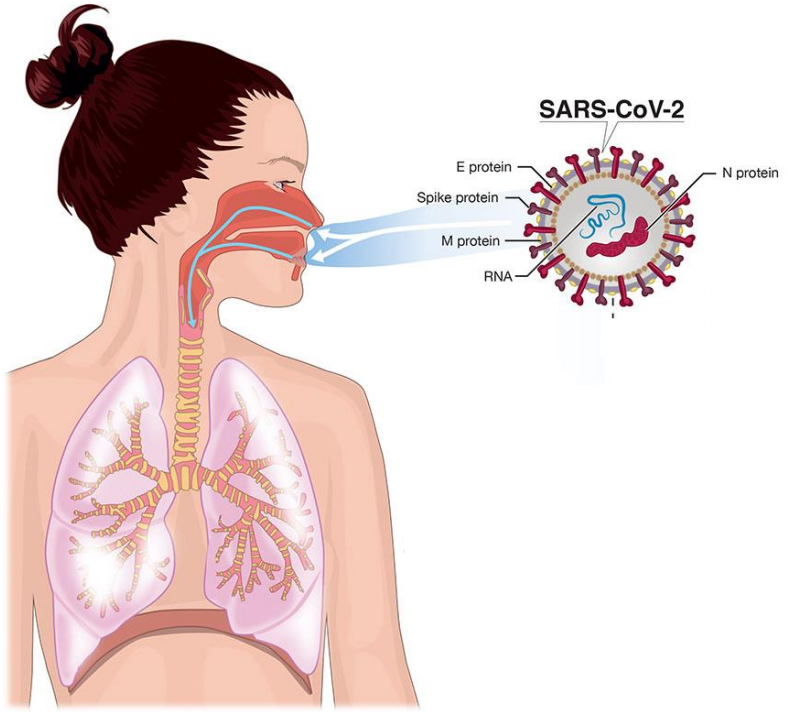
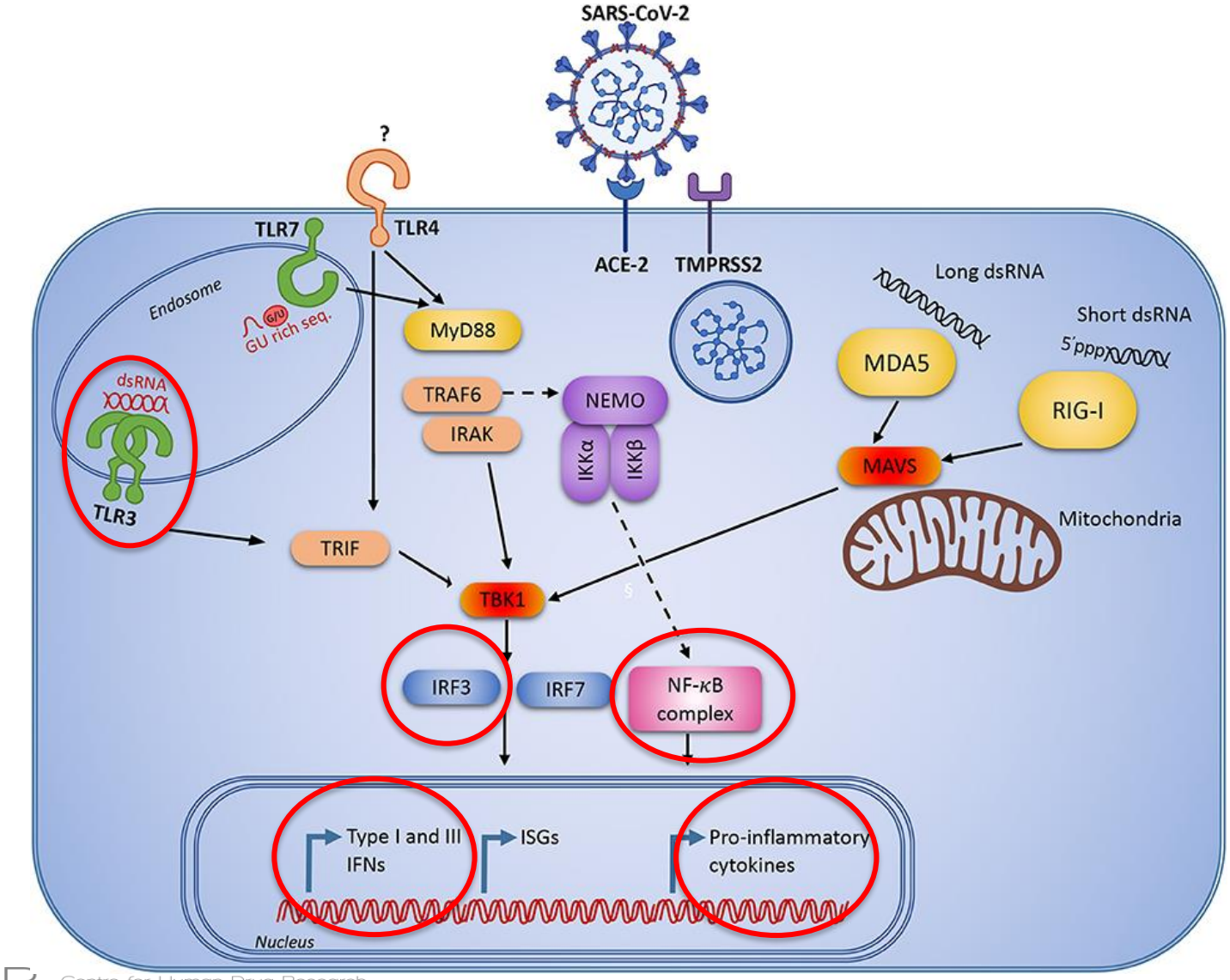


Image adjusted from: Funk CD, Laferrière C and Ardakani A (2020) A Snapshot of the Global Race for Vaccines Targeting SARS-CoV-2 and the COVID-19 Pandemic. Front. Pharmacol. 11:937. https://doi.org/10.3389/fphar.2020.00937

Innate immune signaling pathways



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Source image: Carter-Timofte ME, Jørgensen SE, Freytag MR, Thomsen MM, Brinck Andersen N-S, Al-Mousawi A, Hait AS and Mogensen TH (2020) Deciphering the Role of Host Genetics in Susceptibility to Severe COVID-19. *Front. Immunol.* 11:1606. doi: 10.3389/fimmu.2020.01606

Ampligen® (rintatolimod)

- Synthetic mismatched double-stranded RNA (Poly I: Poly C12U)
- Molecular mimic of viral infection → activation of innate immunity
- Selective TLR3 agonist → type 1 interferon production
  - No NFκB activation

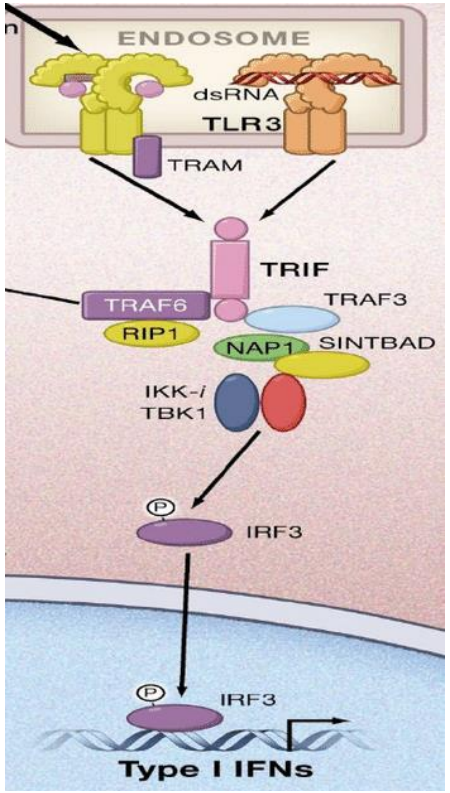


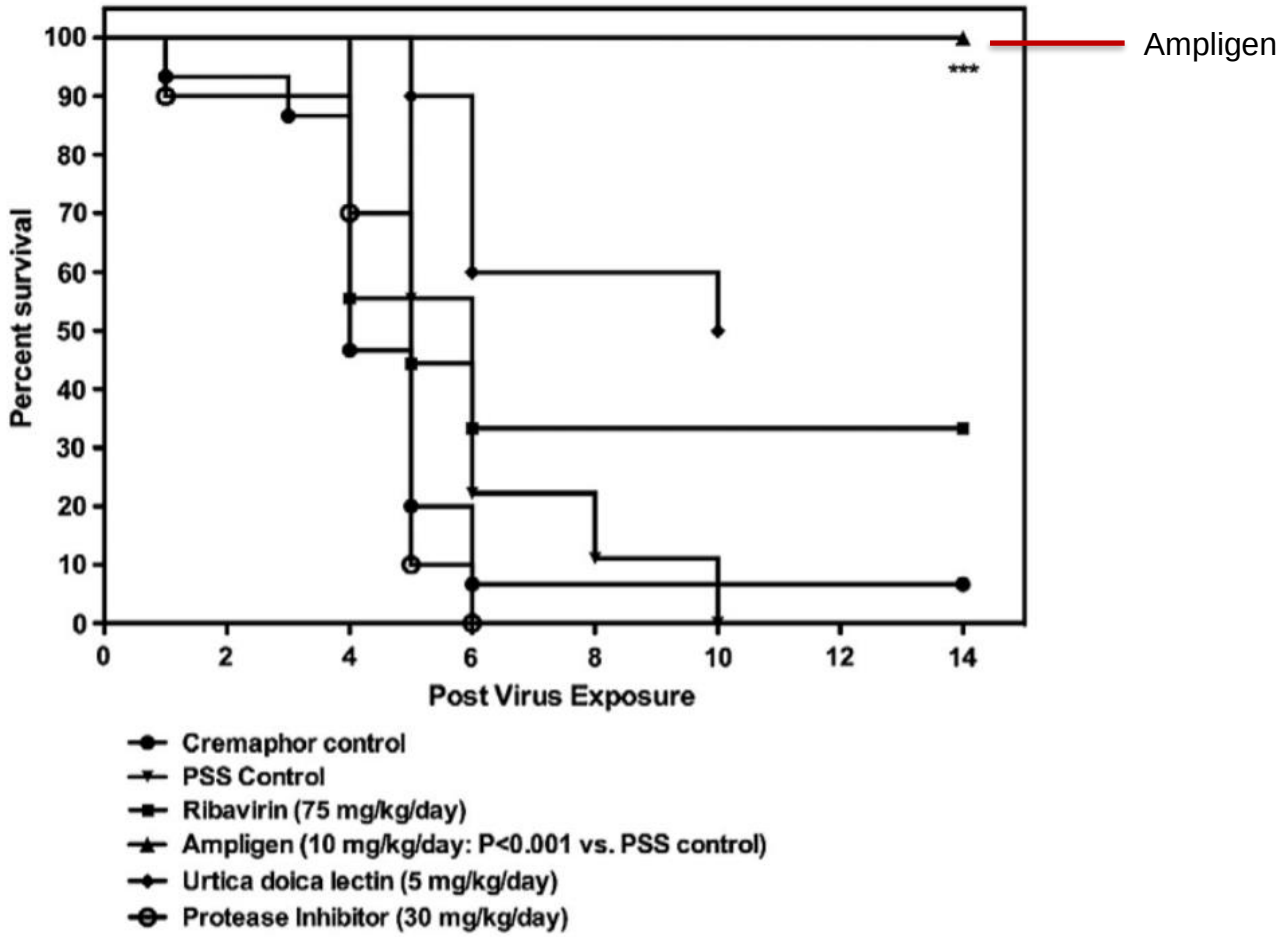
Image adjusted from: Abouelmaatti, Reham & Elfeil, Wael & Wang, Yu & Liu, Shanshan. (2013). Pattern recognition receptors mini review. Global Animal Science Journal.1(1):1118-1127, 2013

Ampligen® pre-clinical/clinical data

- Pre-clinical
  - Survival SARS-CoV-1 infected mice 100% 14 days post virus exposure[1]

1. Day CW, Baric R, Cai SX, et al. A new mouse-adapted strain of SARS-CoV as a lethal model for evaluating antiviral agents in vitro and in vivo. *Virology*. 2009;395(2):210-222. doi:10.1016/j.virol.2009.09.023

Ampligen® – anti-SARS-CoV-1 effect in mice



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Ampligen® pre-clinical/clinical data

- Pre-clinical
  - Survival SARS-CoV-1 infected mice 100% 14 days post virus exposure[1]
  - *In vitro* direct antiviral effect against SARS-CoV-2 in human tracheal/bronchial epithelial cells[2]

- Clinical
  - Systemic: clinical trials for several indications (malignancies, chronic fatigue syndrome, HIV), up to doses of 1200mg (iv) twice weekly
  - Intranasal: adjuvant to intranasal live attenuated influenza vaccine, 3 times with 4-week interval, dose up to 1250ug → well tolerated[3]

1. Day CW, Baric R, Cai SX, et al. A new mouse-adapted strain of SARS-CoV as a lethal model for evaluating antiviral agents in vitro and in vivo. *Virology*. 2009;395(2):210-222. doi:10.1016/j.virol.2009.09.023
2. AIM ImmunoTech Press release. *Decreases SARS-CoV-2 Infectious Viral Yields by 90% Using New In Vitro Model; Supports Further Testing of Ampligen as an Intranasal Prophylactic to Prevent COVID-19.*; Aug, 2020. Available at: https://aimimmuno.com/press-release/aim-immunotech-inc-decreases-sars-cov-2-infectious-viral-yields-by-90-using-new-in-vitro-model-supports-further-testing-of-ampligen-as-an-intranasal-prophylactic-to-prevent-covid-19/. Accessed November 16, 2021.
3. Overton ET, Goepfert PA, Cunningham P, Carter WA, Horvath J, Young D, et al. Intranasal seasonal influenza vaccine and a TLR-3 agonist, rintatolimod, induced cross-reactive IgA antibody formation against avian H5N1 and H7N9 influenza HA in humans. Vaccine. 2014;32(42):5490-5.

Study design

- Randomized, double-blind, placebo-controlled, dose-escalation study
- Healthy subjects (18-70 yr, male/female)
  - Exclusion criteria: recent/current respiratory tract infection, nasal abnormalities, smoking, active allergic or chronic rhinitis
- Ampligen intranasally every other day (q.o.d.) for 13 days (7 doses)
- 4 dose levels: 75µg, 200µg, 500µg, 1250µg in 0.5 ml
  - 10 subjects per cohort → 8 active : 2 placebo (normal saline)
  - Dose escalation after interim safety analyses

Endpoints: safety, local tolerability and mucosal immune response

Safety and tolerability of intranasal rintatolimod q.o.d. for 13 days

- Adverse events (AE)
- Solicited local AEs (upper respiratory symptoms)
- Nasal pain
- Vital signs
- Laboratory values
- Physical examination
- Integrity of nasal mucosa (anterior rhinoscopy)

Pharmacodynamic endpoint: nasal mucosal immune response over time

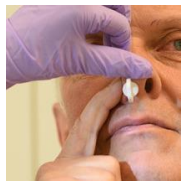
- Mucosal lining cytokines
  - Type I Interferons: IFN-α, IFN-β
  - NFκB-mediated cytokines: IFN-γ, IL-6, IL-8, TNFα
  - Chemokines: CXCL10, RANTES, MCP-1

- Mucosal immune cell characterization by flow cytometry
  - Granulocytes, T-cells, B-cells, dendritic cells, macrophages/monocytes

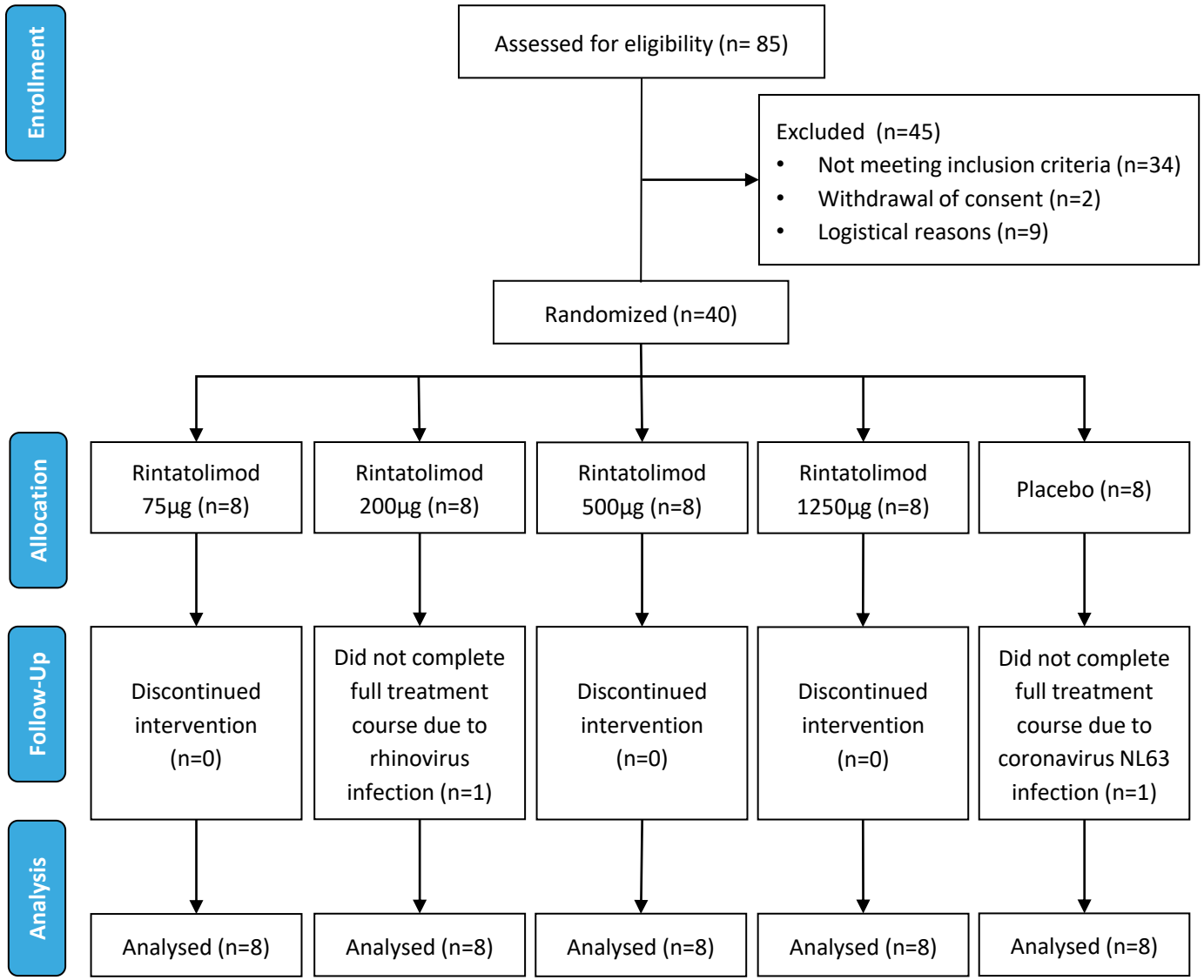
Simplified study schedule

Nasal swab PCR resp viruses

	Baseline (day -4 to -1)	Treatment period														Follow-up (day 28 ± 3)
		Day 1			Day 2	Day 3	Day 5	Day 7	Day 9	Day 11	Day 13			Day 14	Day 15	
		0h	3h	6h							0h	3h	6h			
Intranasal administration		x				x	x	x	x	x	x					
Mucosal lining fluid (cytokines) - nasosorption		x[1]	x	x	x	x		x		x	x	x	x	x	x	
Mucosal immune cells - nasal scrape		x[1]		x	x	x		x		x	x		x	x	x	
Safety and local tolerability monitoring throughout																



[1]Pre-dose


Enrollment
Assessed for eligibility (n= 85)
Excluded (n=45)
• Not meeting inclusion criteria (n=34)
• Withdrawal of consent (n=2)
• Logistical reasons (n=9)
Randomized (n=40)
Allocation
Rintatolimod 75µg (n=8)
Rintatolimod 200µg (n=8)
Rintatolimod 500µg (n=8)
Rintatolimod 1250µg (n=8)
Placebo (n=8)
Follow-Up
Discontinued intervention (n=0)
Did not complete full treatment course due to rhinovirus infection (n=1)
Discontinued intervention (n=0)
Discontinued intervention (n=0)
Did not complete full treatment course due to coronavirus NL63 infection (n=1)
Analysis
Analysed (n=8)
Analysed (n=8)
Analysed (n=8)
Analysed (n=8)
Analysed (n=8)

Baseline characteristics

- Total: 40 subjects, median age 24 (18-69), 40% male

	Rintatolimod				Placebo
	75µg	200µg	500µg	1250 µg	
N	8	8	8	8	8
Age - median (range)	22 (18 - 33)	24 (20 - 69)	29 (19 - 58)	25 (20 - 51)	23 (19 - 37)
Male – N (%)	3 (38%)	4 (50%)	2 (25%)	4 (50%)	3 (38%)

Safety and Tolerability

No safety or tolerability findings of clinical concern

- No severe or serious adverse events
- 38 post-dose AEs reported by 16 (50%) exposed subjects → all mild-to-moderate
  - 25 AEs possibly or probably related (44% of exposed subjects)

Adverse events			Rintatolimod dose administered				Total (all 4 dose levels)
			75ug	200 ug	500 ug	1250 ug	
			Number of AEs (in % of subjects)				
Relatedness		**Probable**	**-**	**-**	**6 (25%)**	**1 (13%)**	**7 (9%)**
	Severity	Mild	-	-	6 (25%)	1 (13%)	7 (9%)
	Severity	Moderate	-	-	-	-	-
	Severity	Severe	-	-	-	-	-
		Possible	**3 (38%)**	**5 (50%)**	**4 (25%)**	**6 (50%)**	**18 (41%)**
	Severity	Mild	3 (38%)	5 (50%)	4 (25%)	5 (50%)	17 (41%)
	Severity	Moderate	-	-	-	1 (13%)	1 (3%)
	Severity	Severe	-	-	-	-	-
		Total	**3 (38%)**	**5 (50%)**	**10 (38%)**	**7 (50%)**	**25 (44%)**

- Solicited AEs (upper respiratory symptoms) similar in placebo and treatment groups
- No abnormalities in anterior rhinoscopy
- No clinical concerns in vital signs or laboratory assessments

Pharmacodynamic outcomes: mucosal immune response

Analysis still pending, results expected soon

Conclusions

- Intranasal Ampligen® q.o.d. for 13 days (7 doses) well tolerated
- Analysis of mucosal immune response expected soon
- Pending pharmacodynamic analyses in this phase I trial to provide early mechanistic insight in pharmacodynamic effect
  - May aid in dose selection for future trials
- Safety and tolerability profile of doses up to 1250 µg indicates intranasal doses of Ampligen warrant examination in phase II efficacy studies

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