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Inhaled Sargramostim (rhu GM-CSF) Leads to Enhanced SARS-CoV-2 Virus-Specific Immune Response and Viral Clearance: Results of the Biomarker Cohort of a Randomized, Double-Blind, Placebo-Controlled Phase 2b Trial in Non-Hospitalized Patients with COVID-19

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Background

Progression from mild to severe coronavirus disease (COVID-19) correlates with humoral and cellular immune signatures that adjust to a multiplying SARS-CoV-2 viral load.[1,2] Enhancing immunity with host-directed therapy, such as sargramostim (yeast-derived, recombinant human granulocyte-macrophage colony-stimulating factor [rhu GM-CSF]), may prevent disease progression and reduce severity. The aim of this study was to evaluate the effect of sargramostim on the progression of mild/moderate COVID-19 to severe disease.

Methods

This prospective, randomized, double-blind, placebo-controlled study enrolled symptomatic vaccinated and unvaccinated non-hospitalized patients with mild/moderate COVID-19 at high risk for progression. Patients received daily inhaled sargramostim 250 mcg or placebo, via nebulizer, for 5 days. The proportion of patients with any emergency room visit, hospitalization, or death by day 28 was the overall endpoint. Biomarker measurements were recorded up to day 28 in a subset of patients. Humoral response serology profiling analysis (SARS-CoV-2 antigen-specific antibodies and antigen-specific antibody Fc receptor binding) was done by computational modeling. The trial is registered on clinicaltrials.gov (NCT04707664).

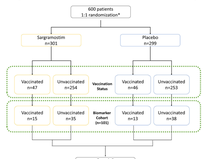
Primary Objective: to determine if inhaled sargramostim can prevent progression to more severe disease in symptomatic outpatients with mild or moderate COVID-19 at a higher risk for progression to severe disease (proportion of patients who experienced any emergency room visit or hospitalization or death by day 28).
Secondary Objectives: to explore the time to clinical progression of COVID-19; change from baseline in overall symptom score; and safety of inhaled sargramostim.
Exploratory Biomarker Cohort Analysis: to investigate the effect of inhaled sargramostim on biological COVID-19 response (SARS-CoV-2 viral load in nasopharyngeal swabs up to day 14; cytokine blood concentration; proportion of patients generating anti-SARS-CoV-2 antibodies up to day 28 and the humoral response).
There was no α adjustment for multiple endpoints multiplicity, including the biomarker analyses.

Clinical Results

From April 28, 2021, to January 31, 2022, 600 patients were randomized. 93 patients were stratified based on COVID-19 vaccination status or participation in a COVID-19 vaccine clinical trial (n= 47 in vaccinated-sargramostim arm; n=46 in vaccinated-placebo arm). Most received an mRNA COVID vaccine.

No difference was found in the overall study primary endpoint (n=21/301 sargramostim, n=16/299 placebo, p=0.4079) (Table 1). The number of patients with any treatment-emergent adverse event (TEAE) was similar in both arms. Three deaths occurred and were considered COVID-related. The number of treatment-related adverse events (TRAE) was low, and severity limited to grade 1 (mild) or grade 2 (moderate). There were no clinically meaningful changes from baseline between treatment arms in ferritin, D-dimer, or C-reactive protein levels (data not shown).

Figure 1. Patient enrollment, allocation, and analysis



*Stratified based on (yes/no) completion of a COVID-19 vaccination regimen or participation in a COVID-19 vaccine clinical trial

Table 1. Study Endpoints	Sargramostim (n=301)	Placebo (n=299)	P-value*
Efficacy			
Primary: patients who experienced any ER visit or hospitalization or death by day 28; n (%)	21 (7.0)	16 (5.4)	0.4079
Safety**	(n=297)	(n=290)	
Number of patients with any TEAE; n (%)	54 (18.2)	63 (21.7)	-
Number of patients with any fatal TEAE; n (%)	1 (0.3)	2 (0.7)	-
Number of patients with any TRAE; n (%)	7 (2.4)	9 (3.1)	-
Number of patients with TRAE >1% Dry mouth (grade 1) Paresthesia (grade 1)	3 (1.0) 2 (0.7)	1 (0.3) 3 (1.0)	-

*p-value from Chi-square test comparing the proportion of patients between treatment arms
**74 patients in the sargramostim arm and 9 patients in the placebo arm were randomized but did not receive treatment

Secondary Endpoint: Sargramostim reduced the overall symptom score, which was more pronounced in vaccinated patients

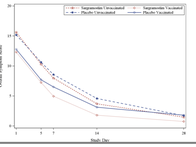
To evaluate the change in symptoms during the study, patients completed an electronic symptom score questionnaire at approximately the same time every day up to day 28. The questionnaire assessed 14 common symptoms of COVID-19 (*i.e.,* cough, headache, body aches, etc.) with patients rating each symptom over the prior 24 hours (0=none, 1=mild, 2=moderate, 3=severe). The overall symptom score was calculated as a sum of the individual scores from the 14 questions each day. Data analyzed at day 7, 14, and day 28.

The sargramostim arm had a greater reduction in overall symptom score than the placebo arm (Table 2). This effect was more pronounced in the vaccinated-sargramostim arm (Figure 2). All vaccinated patients had a lower overall symptom score at baseline than unvaccinated patients.

Table 2. Overall Symptom Score Results	Sargramostim		Placebo		
Overall Symptom Score, mean score (SD)	Result	Change From Baseline	Result	Change From Baseline	P-value*
Baseline	15.1 (6.76)		14.9 (6.93)		
Day 7	7.5 (6.09)	-7.7 (7.02)	8.2 (7.01)	-6.7 (7.33)	0.013
Day 14	3.4 (4.29)	-11.7 (6.62)	4.3 (5.18)	-10.6 (7.35)	0.023
Day 28	1.4 (2.73)	-13.8 (6.69)	1.7 (3.38)	-13.3 (7.19)	NS

*p-value from mixed model repeated measures analysis comparing the change from baseline between treatment arms
NS: not significant

Figure 2. Overall Symptom Score Over Time by Treatment Arm and Vaccination Status



Exploratory Endpoints: Biomarker Results

Sargramostim reduced the viral load by day 14

101 patients consented and provided samples for assessment of biomarkers and viral load (sargramostim n=50, placebo n=51). Treatment groups were well-balanced for mean age, sex, race, ethnicity, body mass index (BMI), and vaccination status. Patients in the biomarker cohort were comparable to the overall study population in terms of demographic and baseline disease characteristics. Most patients in the biomarker cohort had the delta SARS-CoV-2 variant.

Viral load clearance (change from baseline) was greater in the sargramostim arm at day 14 (p=0.0137). Viral load was undetectable in a larger proportion of patients on the sargramostim arm by day 14 (87.5% vs 65.2%).

Table 3. Viral Load Results	Sargramostim		Placebo		
Viral Load, Log10 RNA copies/mL, mean (SD)	Result	Change From Baseline	Result	Change From Baseline	P-value*
Baseline	5.1 (2)	-	4.4 (2.36)	-	-
Day 5	3.2 (1.89)	-1.8 (1.56)	2.7 (2.13)	-1.7 (1.61)	NS
Day 14	0.7 (0.50)	-4.4 (2.06)	1.2 (1.09)	-3.2 (2.16)	0.0137
Patients with Undetectable Viral Load, %	Result		Result		
Baseline	10		14		NS
Day 5	23.3		30.6		NS
Day 14	87.5		65.2		NS

*p-value from Wilcoxon two-sample tests comparing the change from baseline between treatment arms
NS: not significant

Sargramostim reduced the viral load more than placebo in vaccinated patients

Despite higher baseline viral load levels, a pronounced reduction in viral load from baseline was seen in the vaccinated-sargramostim arm.

Figure 3. Baseline Log10 RNA copies/mL

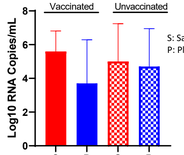
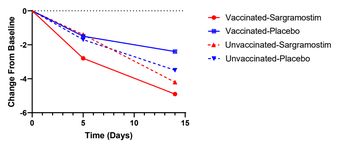


Figure 4. Change from Baseline in Log10 RNA copies/mL



Inflammatory cytokine levels did not increase with sargramostim

Inflammatory cytokine levels did not increase in the sargramostim arm.

Table 4. Cytokine Results	Sargramostim			Placebo		
Cytokine, pg/mL, mean (SD)	Baseline	Day 5	Day 14	Baseline	Day 5	Day 14
IFN-γ	110.3 (116.90)	115.3 (115.67)	104.8 (94.85)	90.3 (78.66)	76.9 (83.30)	73.4 (63.37)
IL-10	78.7 (74.64)	77.2 (79.50)	44.4 (48.22)	70.6 (65.27)	45.9 (48.04)	31.1 (34.20)
IL-6	64.9 (52.03)	66.7 (53.53)	52.8 (42.68)	71.3 (44.55)	67.3 (50.32)	60.1 (39.51)
TNF-α	68.3 (52.69)	74.1 (61.81)	60.7 (50.97)	68.8 (45.95)	68.3 (60.52)	49.5 (31.23)

IL-6: interleukin 6; IL-10: interleukin 6; IFN-γ: interferon γ; TNF-α: tumor necrosis factor α

The vaccinated-sargramostim arm had the greatest proportion of patients with reactive anti-SARS-CoV-2 antibodies

The proportion of patients with anti-SARS-CoV-2 IgG reactivity was more pronounced in the vaccinated-sargramostim arm despite baseline IgG non-reactivity.

Figure 5. Serum IgG Reactivity at Baseline

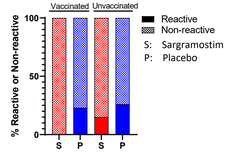
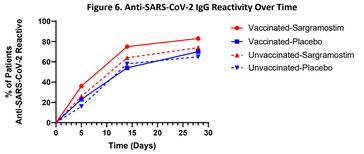


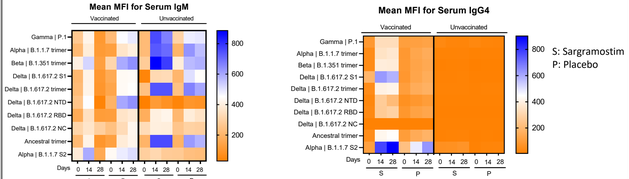
Figure 6. Anti-SARS-CoV-2 IgG Reactivity Over Time



Sargramostim modulates the humoral kinetics and magnitude of IgM and IgG4 titers associated with isotype-class switching in vaccinated patients

The humoral kinetics and magnitude of antibody response against SARS-CoV-2 antigens differed by group and vaccination status. After an initial IgM-titer peak at day 14, IgM titers at day 28 were lower in the vaccinated-sargramostim arm than the vaccinated-placebo arm. The unvaccinated-sargramostim arm maintained higher IgM titers than the unvaccinated-placebo arm. Overall IgG titers were higher for vaccinated patients across both treatment arms. Further, the vaccinated-sargramostim arm had higher IgG4 titers associated with IgG4-isotype-class switching than the vaccinated-placebo arm.

Figure 7. Mean Median Fluorescence Intensity (MFI) Values



Sargramostim enhances IgG4 expression and FcGR2B binding in vaccinated patients

Computational models of sargramostim and vaccination status highlight IgG4 expression and FcGR2B binding may be important in a synergistic immune response against COVID-specific antigens.

Area under the curve (AUC) is a measure of the association between two subsections of the cohort, vaccinated-sargramostim *versus* vaccinated-placebo. An AUC>0.6 indicates that either IgG4 concentration or FcGR2B binding was higher in the vaccinated-sargramostim arm whereas an AUC<0.4 indicates that either feature was higher in the vaccinated-placebo arm. AUCs between 0.4 and 0.6 are indicative of no difference.

Figure 8. AUC IgG4 Trimers

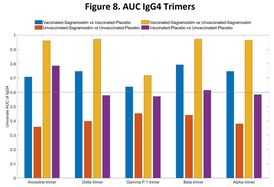
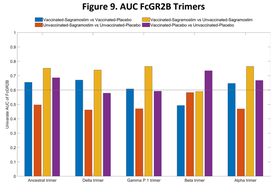


Figure 9. AUC FcGR2B Trimers

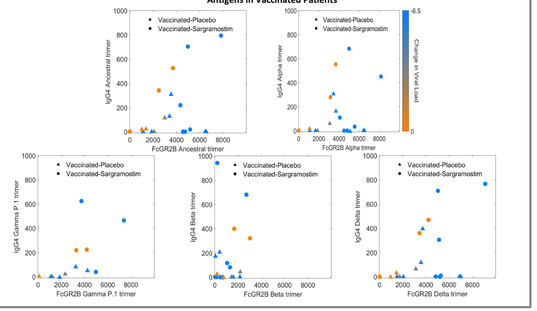


Sargramostim treatment of vaccinated patients was correlated with an increase in FcGR2B binding and greater viral load clearance

In vaccinated patients, the sargramostim arm had an increased correlation between FcGR2B binding and viral load clearance. Patients with low viral load clearance tended to be in the vaccinated-placebo arm with lower FcGR2B binding.

IgG4 did not correlate with viral load clearance.

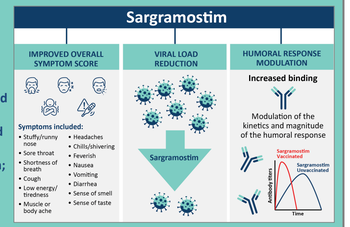
Figure 10. Comparison of Change in Viral Load, IgG4 Expression, and FcGR2B Binding Across SARS-CoV-2 Antigens in Vaccinated Patients



Conclusions

Treatment with inhaled sargramostim:

- Overall study
  1. Failed to meet the primary clinical endpoint evaluating if inhaled sargramostim could prevent progression to more severe disease in symptomatic outpatients with mild or moderate COVID-19 at a higher risk for progression to severe disease via measuring the number of patients who experienced any emergency room visit or hospitalization or death by day 28.
  2. Reduced the overall symptom score, which was more pronounced in vaccinated patients
  3. Resulted in similar treatment-emergent adverse events as placebo.
  4. Did not result in a hyperinflammatory response (*e.g.,* no meaningful changes from baseline between treatment arms in ferritin, D-dimer, or C-reactive protein levels).
- Biomarker cohort
  1. Enhanced SARS-CoV-2 viral load clearance.
  2. Did not increase inflammatory cytokine levels.
  3. Modulated the humoral kinetics and magnitude against SARS-CoV-2 antigens, more so in the vaccinated patients.
  4. Suggests enhanced IgG4 expression; FcGR2B binding in vaccinated patients that correlates with increased viral clearance.



The biomarker data indicate a synergy between sargramostim treatment and COVID-19 vaccination. These results suggest the potential of sargramostim as a virus-agnostic, host-directed immunomodulator.

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References: 1. Galipeau Y, Greig M, Liu G, Driedger M, Langlois MA. Humoral Responses and Serological Assays in SARS-CoV-2 Infections. *Front Immunol.* 2020;11:610688. Published 2020 Dec 18. doi:10.3389/fimmu.2020.610688; 2. Zhou X, Ye Q. Cellular Immune Response to COVID-19 and Potential Immune Modulators. *Front Immunol.* 2021;12:646333. Published 2021 Apr 30. doi:10.3389/fimmu.2021.646333