Cancer Cell, Volume *40*

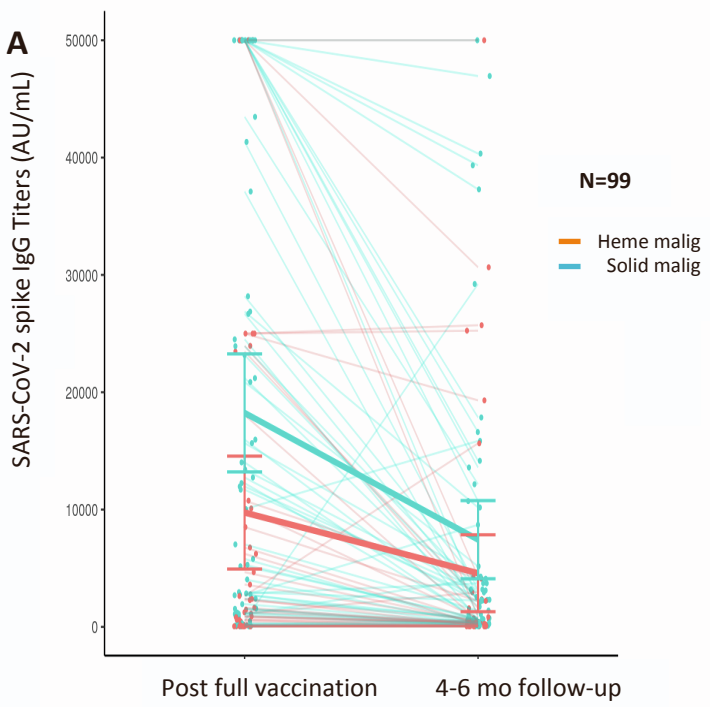
Supplemental information

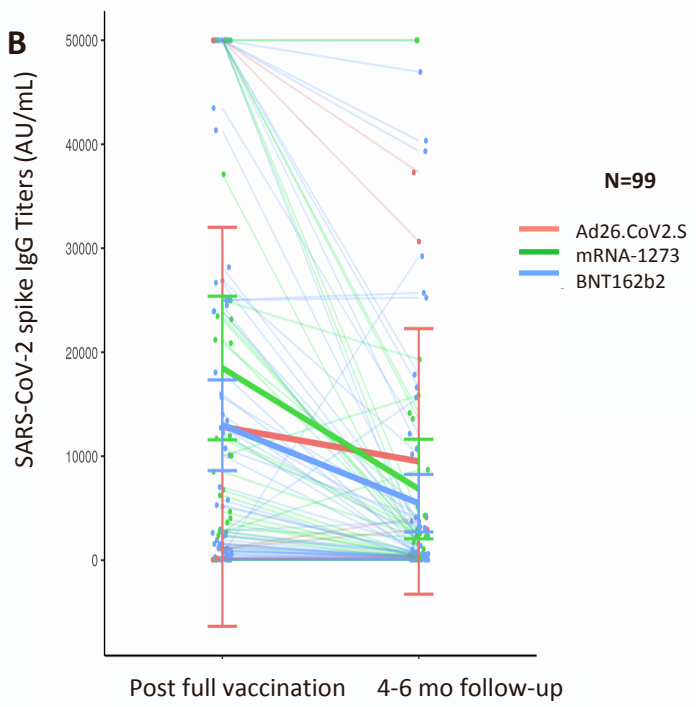
Efficacy of booster doses

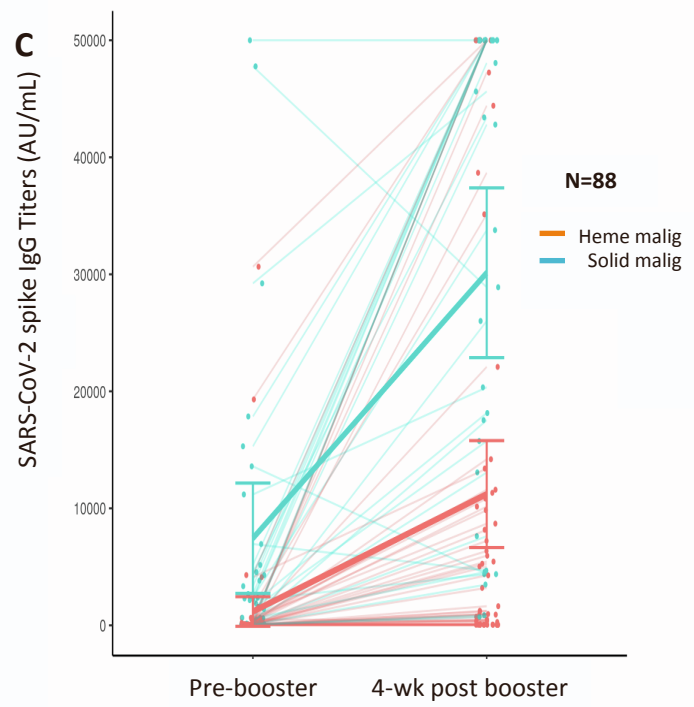
in augmenting waning immune responses

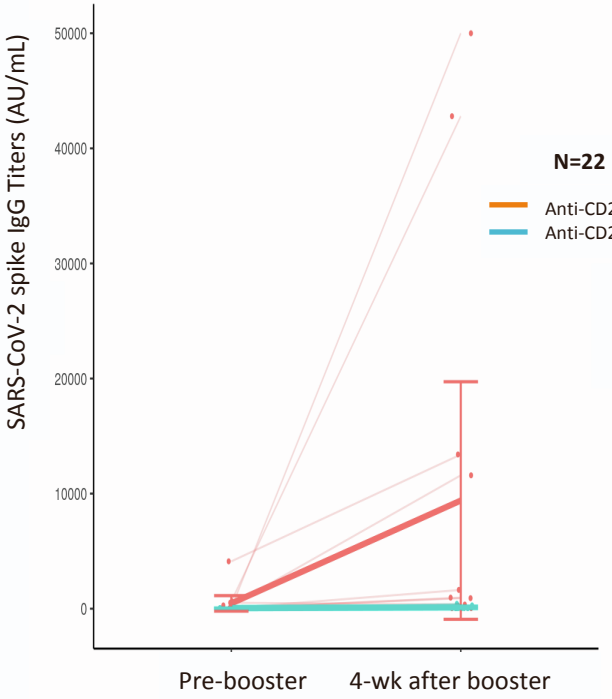
to COVID-19 vaccine in patients with cancer

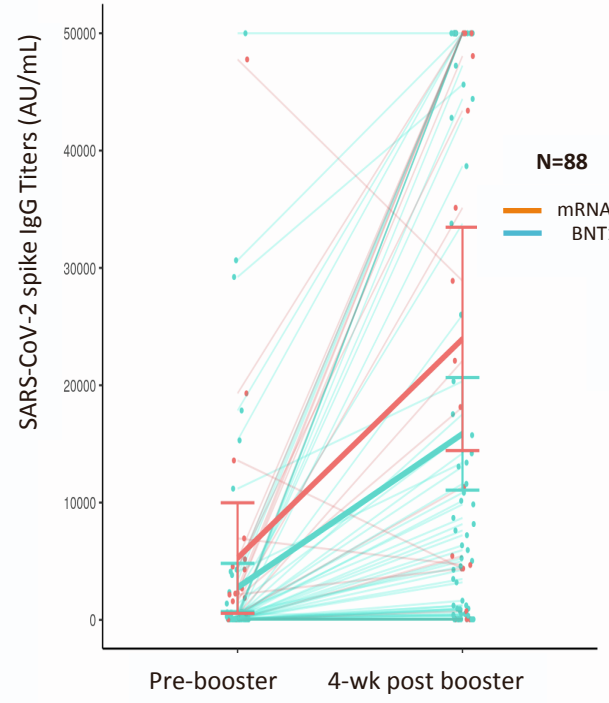
Lauren C. Shapiro, Astha Thakkar, Sean T. Campbell, Stefanie K. Forest, Kith Pradhan, Jesus D. Gonzalez-Lugo, Ryann Quinn, Tushar D. Bhagat, Gaurav S. Choudhary, Margaret McCort, R. Alejandro Sica, Mendel Goldfinger, Swati Goel, Jesus D. Anampa, David Levitz, Ariel Fromowitz, Akash Pradip Shah, Charlotte Sklow, Gregory Alfieri, Andrew Racine, Lucia Wolgast, Lee Greenberger, Amit Verma, and Balazs Halmos

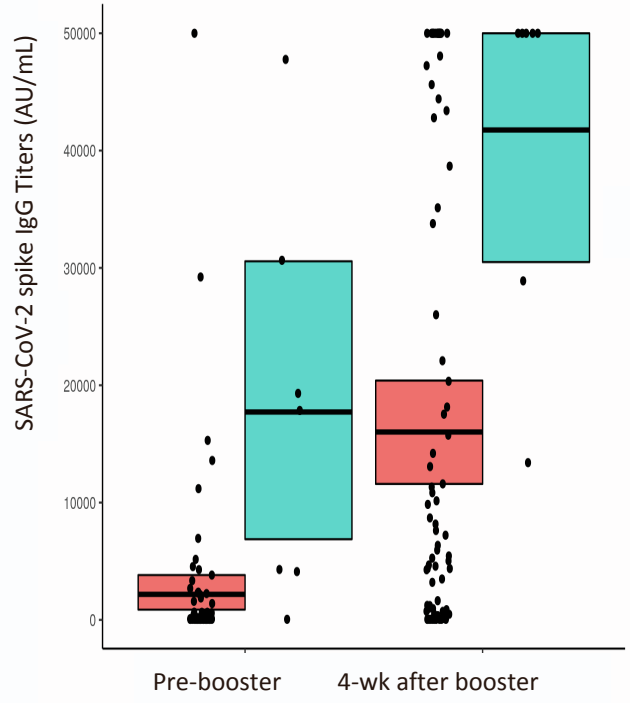

A
SARS-CoV-2 spike IgG Titers (AU/mL)
50000
40000
30000
20000
10000
0
N=99
Heme malig
Solid malig
Post full vaccination
4-6 mo follow-up

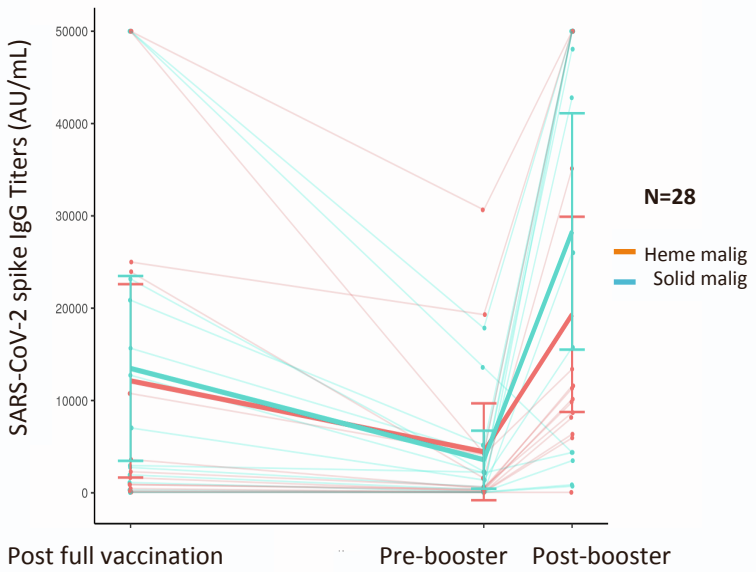

B
SARS-CoV-2 spike IgG Titers (AU/mL)
50000
40000
30000
20000
10000
0
N=99
Ad26.CoV2.S
mRNA-1273
BNT162b2
Post full vaccination
4-6 mo follow-up


C
SARS-CoV-2 spike IgG Titers (AU/mL)
50000
40000
30000
20000
10000
0
N=88
Heme malig
Solid malig
Pre-booster
4-wk post booster


D
SARS-CoV-2 spike IgG Titers (AU/mL)
50000
40000
30000
20000
10000
0
N=22
Anti-CD20 >6 mo
Anti-CD20 <6 mo
Pre-booster
4-wk after booster


E
SARS-CoV-2 spike IgG Titers (AU/mL)
50000
40000
30000
20000
10000
0
N=88
mRNA-1273
BNT162b2
Pre-booster
4-wk post booster


F
SARS-CoV-2 spike IgG Titers (AU/mL)
50000
40000
30000
20000
10000
0
N=87
No COVID
Prior COVID
Pre-booster
4-wk after booster


G
SARS-CoV-2 spike IgG Titers (AU/mL)
50000
40000
30000
20000
10000
0
N=28
Heme malig
Solid malig
Post full vaccination
Pre-booster
Post-booster

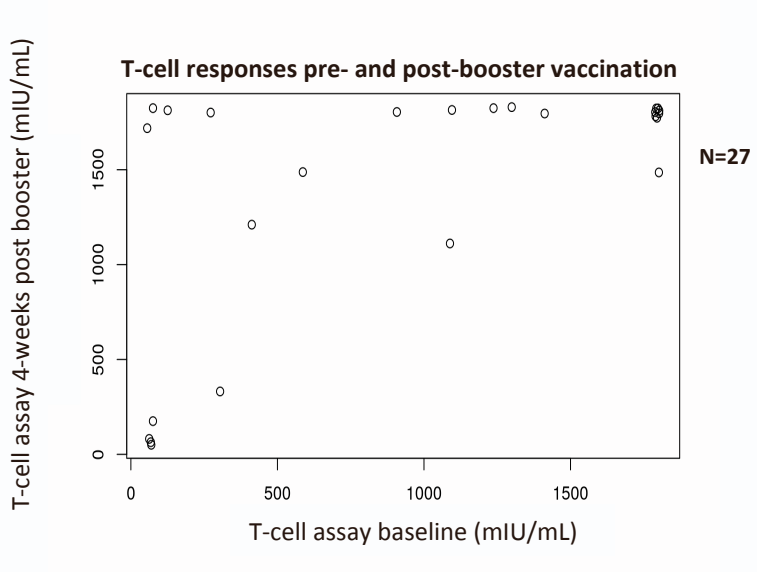

H
T-cell responses pre- and post-booster vaccination
T-cell assay 4-weeks post booster (mIU/mL)
1500
1000
500
0
N=27
0
500
1000
1500
T-cell assay baseline (mIU/mL)

Fig S1. Dynamics of immune responses against SARS-CoV-2 after standard and booster vaccination in patients with cancer.

A: Plot showing waning immunity over time in 99 patients with solid (mean after standard vaccination = 18239 AU/mL, 13210-23269; mean after 4-6 month follow-up = 7434 AU/mL, 4104-10763) and hematologic cancer (mean after standard vaccination = 9742 AU/mL, 4929-14554; mean after 4-6 month follow-up = 4573 AU/mL, 1295-7851). Mean values are denoted with solid thicker lines with error bars denoting 95% confidence interval (p=0.009151, Kruskal Wallis test).

B: Plot showing waning immunity over time in 99 patients with mean values for types of vaccines denoted with solid thicker lines with error bars denoting 95% confidence interval. Mean for BNT162b2 after standard vaccination = 12979 AU/mL, 95% CI 8617-17340; mean for BNT162b2 after 4-6 month follow-up = 5483 AU/mL, 95% CI 2713-8252. Mean for mRNA-1273 after standard vaccination = 18483 AU/mL, 95% CI 11577-25389; mean for mRNA-1273 after 4-6 month follow-up = 6844 AU/mL, 95% CI 2053-11365. Mean for Ad26.CoV2.S after standard vaccination = 12828 AU/mL, 95% CI -6356-32013; mean for Ad26.CoV2.S after 4-6 month follow-up = 9503, 95% CI -3269-22274 (p=0.03099, Kruskal Wallis test).

C: Plot showing increase in anti-SARS-CoV-2 spike (S) antibody titers at 4 weeks after the booster vaccine in 88 patients with solid (mean prior to booster vaccination = 7446 AU/mL, 95% CI 2732-12159; mean after booster vaccination = 30132 AU/mL, 95% CI 22877-37386) and hematologic cancer (mean prior to booster vaccination = 1183 AU/mL, 95% CI -81.83-2448; mean after booster vaccination = 11217 AU/mL, 95% CI 6649-15786). Mean values are denoted with solid thicker lines with error bars denoting 95% confidence interval (p=0.00263, Kruskal Wallis test).

D: Plot showing increase in anti-S antibody titers at 4 weeks after the booster vaccine. Mean values for hematologic malignancy patients who received anti-CD20 antibodies within 6 months (mean prior to booster vaccination = 51.84 AU/mL, 95% CI 47.59-56.1; mean after booster vaccination = 118.4, 95% CI 12.82-223.9) of booster vaccine or not (mean prior to booster vaccination = 462.6 AU/mL, 95% CI -211.8-1137; mean after booster vaccination = 9401 AU/mL, 95% CI -920.6–19723) are denoted with solid thicker lines with error bars denoting 95% confidence interval (p=0.04566, Kruskal Wallis test).

E: Plot showing increase in anti-S antibody titers at 4 weeks after the booster vaccine. Mean values for type of booster vaccine, BNT162b2 (mean prior to booster vaccination = 2762 AU/mL, 95% CI 707.4-4816; mean after booster vaccination = 15858 AU/mL, 95% CI 11060-20656) and mRNA-1273 (mean prior to booster vaccination = 5272 AU/mL, 95% CI 561.2-9983; mean after booster vaccination = 23948 AU/mL, 95% CI 14429-33468) are denoted with solid thicker lines with error bars denoting 95% confidence interval (p=0.6, Kruskal Wallis test).

F: Box plot showing increase in anti-S antibody titers at 4 weeks after the booster vaccine with mean values in patients that had prior SARS-CoV-2 infection (mean prior to booster vaccination = 17719 AU/mL, 95% CI 1923-33515; mean after booster vaccination = 41757 AU/mL, 95% CI 28095-55418) or not (mean prior to booster vaccination = 2177 AU/mL, 95% CI 654.7-3700; mean after booster vaccination = 16014 AU/mL, 11705-20323) denoted with solid thicker lines with error bars denoting 95% confidence interval (p=0.14, Kruskal Wallis test).

G: Plot showing trend in titers for patients (n=28) that had anti-S antibody titers at 3 time-points: 1) Post full vaccination 2) 4-6 month follow-up and 3) 4-weeks post-booster vaccine. Mean values are denoted with solid thicker lines with error bars denoting 95% confidence interval.

H: Plot showing T cell assay results at baseline and 4 weeks after booster vaccination in evaluable pre-booster seronegative cohort.

Abbreviations: 4-wk: 4 week, malig: malignancy, mo: months

Supplemental Table S1. Consort Diagram and Patient Characteristics Prior to SARS-CoV-2 Booster Vaccination.

Supplemental Methods

Anti-SARS-CoV-2 spike (S) IgG assay

The AdviseDx SARS-CoV-2 IgG II assay was used for the assessment of anti-S IgG antibody. AdviseDx is an automated, two-step chemiluminescent immunoassay performed on the Abbott i1000SR instrument. The assay is designed to detect IgG antibodies directed against the receptor binding domain (RBD) of the S1 subunit of the spike protein of SARS-CoV-2. The RBD is a portion of the S1 subunit of the viral spike protein and has a high affinity for the angiotensin converting enzyme 2 (ACE2) receptor on the cellular membrane (Pillay, 2020 ;Yang et al., 2020). The procedure, in brief, is as follows. Patient serum containing IgG antibodies directed against the RBD is bound to microparticles coated with SARS-CoV-2 antigen. The mixture is then washed of unbound IgG and anti-human IgG, acridinium-labeled, secondary antibody is added and incubated. Following another wash, sodium hydroxide is added and the acridinium undergoes an oxidative reaction, which releases light energy which is detected by the instrument and expressed as relative light units (RLU). There is a direct relationship between the amount of anti-spike IgG antibody and the RLU detected by the system optics. The RLU values are fit to a logistic curve which was used to calibrate the instrument and expresses results as a concentration in AU/mL (arbitrary units/milliliter). This assay recently has shown high sensitivity (100%) and positive percent agreement with other platforms including a surrogate neutralization assay (Bradley et al., 2021) and also demonstrated high specificity both in the post COVID-19 infection and post vaccination settings. The cutoff value for this assay is 50 AU/mL with <50 AU/ml values reported as negative and the maximum value is 50000 AU/mL.

SARS-CoV-2 Interferon Gamma Release Assay

The Euroimmun SARS-COV-2 Interferon Gamma Release Assay (Quan-T-Cell SARS-CoV-2) was used for the assessment of patient's T-cell response to SARS-CoV-2 antigens before and after the third vaccine dose, through analysis of the production of interferon gamma by patient T-cells after exposure to SARS-CoV-2 specific proteins. The assay does not differentiate between vaccine- or infection-induced T-cell responses. The SARS-CoV-2 IGRA assay is performed in two steps as per manufacturer instructions, and a brief protocol follows. First, patient samples from lithium heparin vacutainers are aliquoted into three separate tubes each. These tubes contain either nothing ('blank'), general T-cell activating proteins ('mitogen'), or components of the S1 domain of SARS-CoV-2 ('SARS-CoV-2 activated'). These samples were incubated at 37 degrees for 24 hours before being centrifuged and the plasma separated and frozen at -80 degrees for later analysis. Samples were then batched to be run as a full 96 well plate along with calibrators and controls. Paired samples from a single patient before and after a booster dose were run together on a single plate in order to lower possible analytical variability between plates. Plasma samples were unfrozen and added to an ELISA plate, which was prepared with monoclonal interferon-gamma binding antibodies, along with calibrators and controls. After incubation at room temperature the plate was washed and biotin-labeled anti-interferon gamma antibody was added to bind the patient interferon gamma bound to the plate. The plate was again incubated before being washed of excess antibody and a streptavidin-bound horseradish peroxidase (HRP) enzyme added, which binds strongly to the biotin-labeled antibodies present. This was again incubated and then washed of excess enzyme before a solution of H_2O_2 and TMB (3,3', 5,5;-tetramethylbenzidine, a peroxide-reactive chromogen) is added and allowed to react in the dark for 20 minutes. The reaction is then stopped through the addition of sulfuric acid and the results read at 450 nM with background subtraction at 650 nM. Results

for controls and samples were quantified by the calibration curve generated on the same plate, and results were interpreted as long as controls were within the pre-specified range. Blank results for each specimen set were subtracted from each tube in the set and the mIU/mL for both the mitogen and SARS-CoV-2 activated samples were determined with the calibration curve. Samples with mitogen results below 400 mIU/mL were considered 'invalid', as the overall T-cell activity for that set was too low, and excluded from analysis. All other sample sets were interpreted as per manufacturer's instructions based on the SARS-CoV-2 activated sample results: less than 100 mIU/mL were denoted as negative, and greater than or equal to 100 mIU/mL were denoted as positive.

Statistical Analysis

The primary endpoint of this study was to assess the rate of booster induced seroconversion amongst patients who remained seronegative at least 28 days following standard set of FDA authorized COVID-19 vaccinations. We hypothesized that booster dosing would convert at least 30% of the enrolled seronegative patients to seropositive as defined by our institutional CLIA certified SARS-CoV-2 spike IgG assay (as compared to 10% as our null hypothesis). In a pre-specified analysis, at least 26 evaluable seronegative patients were required to have sufficient power to be able to reach this assessment. A McNemar's test was used to determine the equality of marginal frequencies for paired nominal data with the aid of a homogeneity of stratum effects (HSE ref1) test to check if the effect was the same across all levels of a stratifying variable (Zhao et al., 2014). A Wilcox test was used to determine if titers of two paired observations have changed over time subsequently using a Kruskal Wallis test to determine if this difference is associated with another variable. An alpha < 0.05 was considered statistically significant. All analysis was performed in R (version 3.6.2). This study was approved by The Albert Einstein College of Medicine Institutional Board Review.

Supplemental References

Bradley, B.T., Bryan, A., Fink, S.L., Goecker, E.A., Roychoudhury, P., Huang, M.-L., Zhu, H., Chaudhary, A., Madarampalli, B., Lu, J.Y.C., et al. (2021). Anti-SARS-CoV-2 antibody levels are concordant across multiple platforms but are not fully predictive of sterilizing immunity. J. Clin. Microbiol. 59, e0098921.

Pillay, T.S. (2020). Gene of the month: the 2019-nCoV/SARS-CoV-2 novel coronavirus

spike protein. J. Clin. Pathol. 73, 366–369.

Yang, J., Petitjean, S.J.L., Koehler, M., Zhang, Q., Dumitru, A.C., Chen, W., Derclaye, S., Vincent, S.P., Soumillion, P., and Alsteens, D. (2020). Molecular interaction and inhibition of SARS-CoV-2 binding to the ACE2 receptor. Nat. Commun. 11, 4541.

Zhao, Y.D., Rahardja, D., Wang, D.H., Shen, H. (2014). Testing homogeneity of stratum effects in stratified paired binary data. J. Biopharm. Stat. 24, 600-607.