UNC SCHOOL OF MEDICINE

COVID-19 NC Collaboratory Projects

Final Narrative
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COVID-19 Convalescent Plasma (CCP) Program

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Project summary

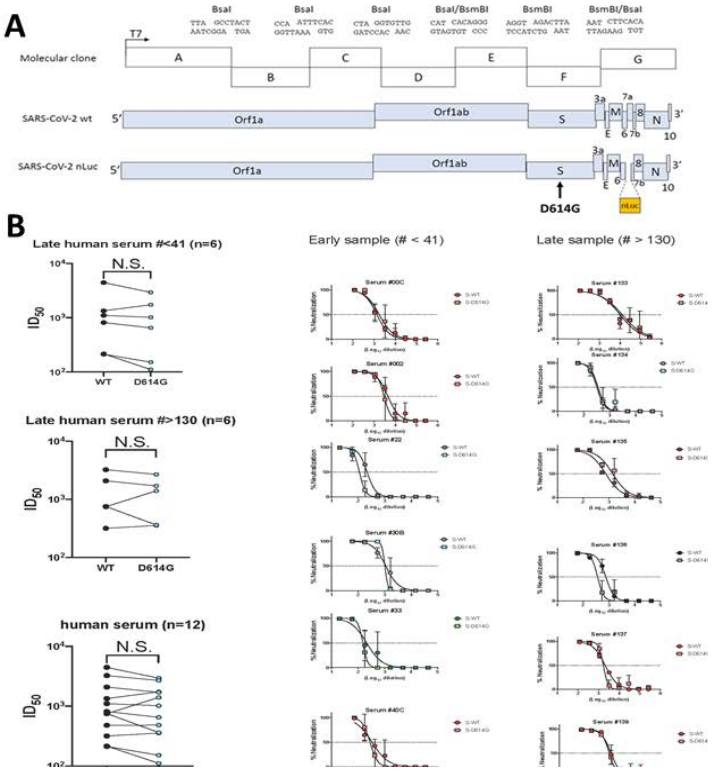
This project aimed to collect convalescent plasma from individuals who have recovered from COVID-19 (CCP) and use this CCP as treatment for patients hospitalized with severe COVID-19. The primary objective of the project was to specifically study the effectiveness of neutralizing antibodies (nAbs) present in COVID-19 convalescent plasma (CCP) as therapy for severe COVID-19 in a randomized control trial (FDA IND 22282; https://clinicaltrials.gov/ct2/show/NCT04524507) at UNC Medical Center in Chapel Hill (Bartelt/Margolis). Additionally, the project supported the development of novel assays of measuring highly specific anti-SARS-CoV-2 antibody binding titers (deSilva) and neutralizing antibody titers to authentic wild-type SARS-CoV-2 and emerging dominant mutant strains (Baric). The program treated >250 patients with severe COVID-19 with CCP including 55 patients in a randomized control trial, contributed to 5 peer-reviewed reports including 3 published manuscripts and several more in preparation, a federally funded U54 Serocenter of Excellence Award (>$3,000,000 in total) with another federal grant under review, and new collaborations with partners in biotech industry.

Methods/Results:

Assays development (Baric and DeSilva):

Neutralizing antibodies (nAb) in the Baric Laboratory: The Baric laboratory assessed the functional ability of convalescent plasma to neutralize SARS-CoV-2 using their novel authentic nLUC assay. All units of convalescent plasma collected in the program were assayed. In addition, as new SARS-CoV-2 spike variants emerged (specifically the D614G mutation), the Baric Laboratory used convalescent plasma from donors in this program to evaluate the impact of D614G mutation on neutralization (**Figure 1**). The laboratory reported that both viruses had very similar neutralization ID50 and ID80 values across populations of sera. This work was published in *Hou et al., Science, 2020*.

Figure 1. Neutralization Assays with D614G SARS-CoV2 Indicator Viruses. **A**: Organization of SARS-CoV2 reporter viruses, **B**: Both of the D614G reporter viruses were equally sensitive neutralization, supporting the continued use of convalescent plasma in our patient cohort. *Hou, et al. Science, 2020*



Total antibody and SARS-CoV-2 specific antigen binding (deSilva, Markmann): The de Silva laboratory has generated four SARS-CoV-2 proteins/antigens for use in ELISA format. These include the full-length spike protein, two spike protein domains, and the nucleocapsid protein. Two of these antigens have been validated in the ELISA format and found to be highly specific and sensitive assays for quantifying SARS-COV-2 binding antibodies. These two ELISA format assays detect specific

regions of the SARS-CoV-2 Spike protein that are critical for viral attachment to host ACE-2 Receptor: the receptor binding domain (RBD) (*Premkumar, Sci Immunology, 2020*) and the N-terminal domain (NTD). Both assays have also been engineered to quantify isotype specific responses to IgA, IgM and IgG. This project has characterized the durability and breadth of antibody responses to SARS-CoV-2 during convalescence among the first 101 CCP donors. First, RBD and to a lesser extent, NTD, show stronger correlations with either WT virus neutralization in the Baric laboratory or a pseudovirus assay (Monogram, LabCorp) than IgG to nucleocapsid (N) protein done by the Abbott assay (McLendon Laboratory) (**Fig. 2**). Additionally, both nAb and total Ig RBD titers are durable through at least 6 months after infection in this cohort (**Fig. 3**). These data have been submitted for publication: *Markmann et al., medRxiv, submitted for peer-review, Feb. 3, 2021*.

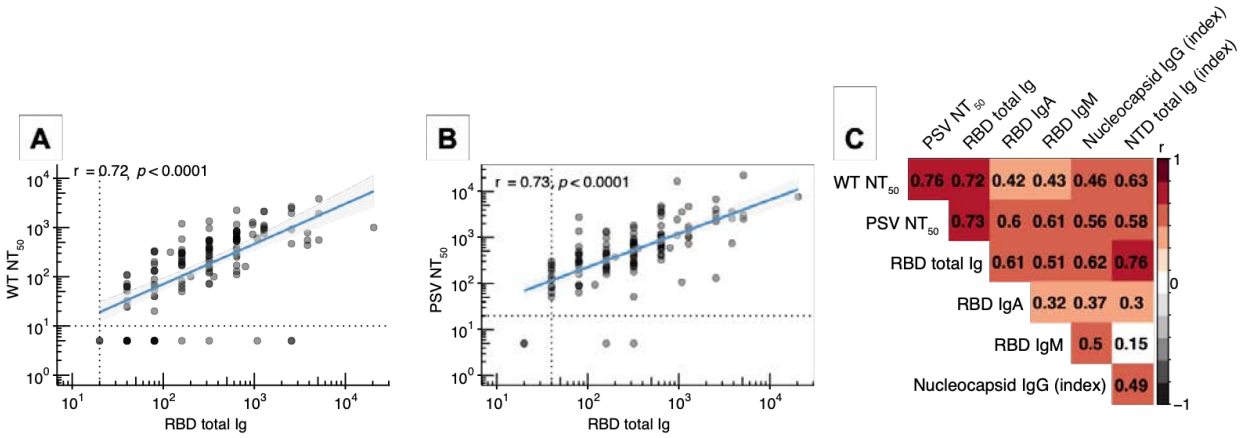


Figure 2: Correlations between neutralization assays and antibody binding to different SARS-CoV2 antigens. **A:** Correlation between nLuc SARS-CoV2 wild-type (WT) neutralization and total Ig to RBD. **B:** Correlation between pseudovirus neutralization and total Ig to RBD. **C:** Correlation heat map inclusive of all assays reported to date. *Markmann, et al. submitted, 2021*.

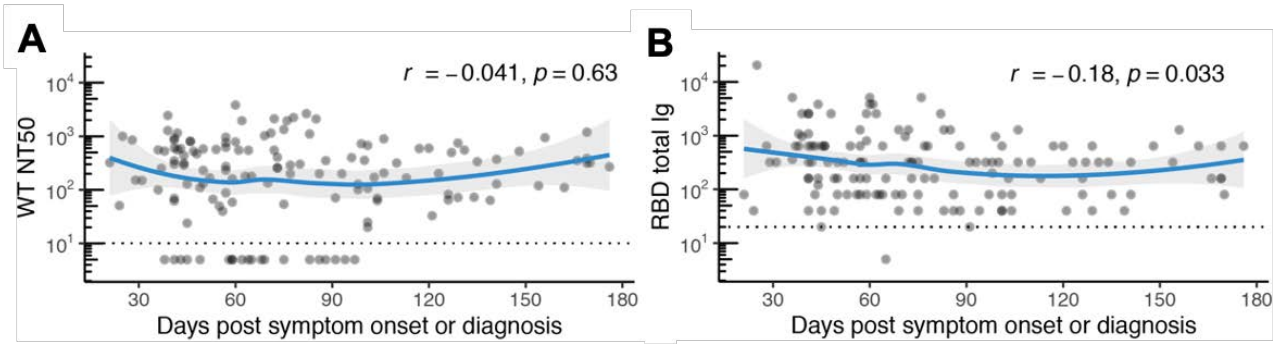


Figure 3*:* Durability of neutralizing antibody (A) and RBD total Ig (B) responses in convalescent plasma donors (N=101). *Markmann, et al., submitted, 2021*

Finally, through this funding we have developed partnerships with local biotechnology companies. First, viral pseudoneutralization assays were performed by Monogram, a subsidiary of LabCorp. Secondly, we have begun correlating the end-titer antibody binding assays to recombinant highly-specific SARS-CoV-2 antigens in the deSilva laboratory with a quantitative, high-throughput lateral flow (LFA) point-of-care (POC) assay developed by Biomedomics. Our preliminary data shows a strong correlation with the Biomedomics LFA and our RBD IgG results and good correlation with Baric NT50 results (**Fig. 4A, B**). Furthermore, 28/29 donor CCP plasma tested

that have a Biomedomics result of > 1000, or exceeding the linear curve of the assay, have a > 1:160 Baric NT50, suggesting possible use to qualify moderate to high titer CCP (**Fig. 4C**). Through these partnerships we hope to facilitate the transfer of assays developed in the Baric and de Silva laboratories into platforms adapted for clinical care

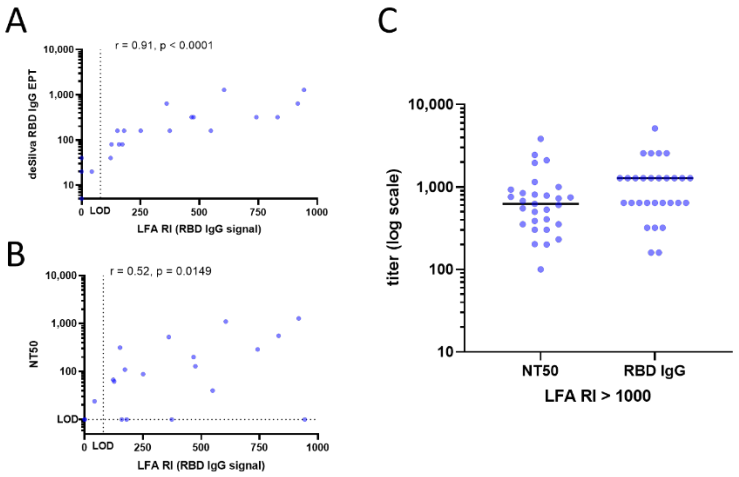


Figure 4. Preliminary results comparing Biomedomics RBD IgG lateral flow assay. (A) Correlation plot between in-house RBD IgG ELISA and Biomedomics assay. (B) Correlation plot between LFA and Baric NT50. (C) Baric NT50 and in-house RBD IgG titers for LFA results that were > 1000. r = Spearman correlation coefficient. RI, reflective index.

CCP donor collection program (Bartelt/Margolis; Root, Weiss, van Duin, Lachiecwicz, Napravnik): Funding supported a key plasma donation program and clinical trial personnel. Among >1,000 potential CCP donors contacted by the study staff, 504 total units (200-250 mL of plasma per unit) of CCP was collected from a total of 194 unique donors and 258 total donations at the UNC Blood Donation Center (BDC) (**Table 1**). There was a median of 9 donors and a median of 23 units collected per week throughout this time period.

Donor demographics and sustainability: A goal of this program was to collect CCP from a wide range of donor demographics and to sustain a stable CCP collection program to provide adequate supply for patients admitted with severe Covid-19. **Table 2** shows the demographic breakdown of donors. Importantly, donor blood type representation was within the range of the general population. CCP collections were sustained in sufficient quantity to meet inpatient therapy demands. The advantages of a local collection program were most apparent early in the project when national and regional blood bank CCP supply was limited. As such, we were able to leverage our CCP donation capabilities to reach other UNC affiliate hospitals primarily through patient transfer to UNC MC in June-July. We intended to transition to deploy UNC MC CCP to other UNC Health hospitals; however, by late July, the American Red Cross and other blood banks had adequate supplies to meet needs at other hospitals as admission rates fluctuated between August-December.

Table 1: UNC Blood Donation Center Convalescent Plasma Cumulative Inventory

Total Donors	Total Donations	Units Collected	Units infused	Units in storage
194	258	504	320	184

Correlates of neutralizing antibody responses: Basic demographics and clinical data collected on donors revealed that male donors and donors with a history of cardiometabolic disease were more likely to have higher neutralizing antibody titers (**Fig. 5**). There was also an interaction with age and male sex wherein nAb titer increased with age in male donors, an effect that was not seen among female donors. Most donors had mild-moderate disease (**Table 2**), yet a small effect of disease severity on nAb titer was seen, although only in males. Taken together, these data suggested that clinical and demographic factors in this cohort had more influence on nAb in males than females. The preliminary findings for these data were presented that the International AIDS Society (IAS) conference in July 2020 and the complete dataset was submitted for publication: *Markmann et al, medRxiv, Feb 3 2021.*

Finally, Dr. Bhatt is leading plasma proteomics (UNC Proteomics Core) and metabolomics (Metabolon) analyses to further explore the relationship between cardiometabolic diseases, sex, and innate immune-mediated effects on development of anti-SARS-CoV-2 antibody responses. Work is performed in the UNC Proteomics Core. The Proteomics Core applied an innovative

pipeline using Tandem Mass Tag 10-plex to label and then fractionate trypsin-digested samples using reverse phase HPLSC to generate high-quality peptide profiles inclusive of proteins in both high and low concentrations in the convalescent plasma samples. The core uses a state-of-the-art Thermo QExactive HF LC-MS/MS capable of large-scale protein profiling. This instrument is supported by the University Cancer Research Fund established by NC General Assembly. This method identified 577 total proteins, of which 452 had >1 peptide identified. The first set of pilot data from these analyses has identified 23 putative peptides that discriminate between high and low titer CCP (7 peptides that increased in high titer CCP and 16 decreased) (**Fig. 6-volcano plot**). Interestingly, several of these peptides map to markers of innate immune responses, including complement proteins, an evolutionarily ancient branch of immunity with potential to neutralize viruses independent or in conjunction with antibodies. Validation of these findings with additional samples and targeted assays for specific peptides are ongoing.

Table 2: Convalescent plasm donor characteristics at time of donation (n=101 unless otherwise specified)

Age		**Race (n=98)**	
18-39	39	White/Caucasian	75
40-64	53	Black/African American	7
65-79	9	Asian	5
80+	0	Pacific Islander	1
		Other	10
Sex			
M	52	**Ethnicity (n=98)**	
F	49	Hispanic	15
		Non-Hispanic	82
Parity (n=48)		Unknown	1
Parous	26		
Nulliparous	22	**ABO (n=99)**	
		A+	36
Comorbid conditions		A-	7
None	64	B+	7
One	18	B-	1
Two or more	16	AB+	6
Unknown	3	AB-	0
		O+	36
		O-	6

COVID-19 disease characteristics	**(n)**
RT-PCR diagnosed	79
Antibody diagnosed	22
Diagnostic test unknown	1
Symptomatic	93
Asymptomatic	8
Overall symptom grade (n = 71)	
1 (mild)	24
2 (moderate)	33
3 (severe)	11
4 (potentially life-threatening)	3
Supplemental oxygen required (n = 71)	6
	(days, range)
Median time from symptom onset or RT-PCR diagnosis to donation (n = 95)	57, 21-121
Median time of symptom duration (n=70)	16, 2-107

CCP infusion program (Bartelt/Margolis; Root, Weiss, van Duin, Lachiecwicz, Napravnik):
We treated 253 total patients with at least one unit of CCP and most (>90%) were treated with at least 2 units of CCP. There were 3 overlapping mechanisms by which the FDA allowed use of CCP: An expanded access program operated by the Mayo Clinic (April 25- August 29, 2020), the UNC-initiated *Coronavirus inactivating plasma (CoVIP) randomized control trial* (FDA IND 22282; August 22 – December 30, 2020), and an emergency use authorization (EUA) program (August 23, 2020 - present). The breakdown of patients receiving CCP through these various mechanisms is shown in **Table 3 (next page).** Our program also engaged hospitalists caring for patients with COVID-19 at multiple hospitals affiliated with UNC Health and Dr. Root led discussions regarding optimal use of CCP for hospitalized patients and was a liaison for hospitals wishing to participate in the EAP.

The Mayo EAP metrics and outcomes: CCP was used for treatment of patients hospitalized with severe COVID-19 or non-severe COVID-19 in the setting of risk factors for progression to severe disease. Of the 163 patients treated in this program, 90 were treated with CCP collected from the UNC donation collection program. Outcomes from these patients contributed to Mayo-led analyses of the safety and efficacy of CCP in a large observational database. UNC

investigators Drs. Bartelt and Root are also directly involved in another Mayo-led project comparing treated patients to untreated matched controls.

Early data in the Mayo program supported that early treatment (within 3 days of diagnosis) and treatment with higher titer CCP showed a signal for greater benefit than CCP infused later in disease or with lower titers of antibody (*Joyner, et al, NEJM, 2020*). Among patients treated in the EAP CCP program at UNC-MC, the median duration of symptoms onset to admission was 7.0 days (IQR 4.0) and from diagnosis to admission was 2.0 days (IQR 5.0). Consequently, for maximal benefit the CCP infusion program needed to efficiently infuse CCP as soon after admission as possible. Among all EAP CCP recipients, 47.5% of patients were treated within 3 days of diagnosis. We infused CCP at a median of 29.6 hours (range: 5.5-303.4 hours) from admission, which improved after the first one to two weeks (median 55.8 hours) of the program to remain stable each subsequent month (median time 27.6, 27.2, 34.2, and 26.8 hours, p<0.05). The total time from admission to infusion was significantly shorter at UNC-MC (n=163) than other UNC affiliate hospitals (n=153) (median 47.6 hours, range 5.1-207.0 hours, p<0.0001). The latter relied exclusively on regional blood banks for CCP rather than being locally collected and distributed from an in-hospital Blood Donation Center (BDC). The majority of the time delay included the steps from admission to enrollment (median 22.6 hrs,ange: 1.7-300.7 hrs) whereas the median time from enrollment to infusion was 5.3 hrs (range 1.1-50.5 hours). At UNC-MC, CCP from the UNC BDC bank was infused approximately two times faster than when CCP was ordered from a nearby vendor (median time 3.4 hrs (1.1-32.2) vs. 6.8 hrs (1.1-50.5); p<0.0001). The primary determinant of between-patient differences in time from admission to infusion were patient location (ICU = 26.1 hours vs floor status 35.3 hours (p<0.05), an effect that was at least partially driven by greater representation of participants unable to sign consent. Finally, the use of Spanish Interpreters did not delay time from admission to infusion in Spanish-only speaking recipients (Spanish-speaking 27.4 hrs (n=36) vs. not Spanish-speaking 28.2 hrs (n=64) (ns)).

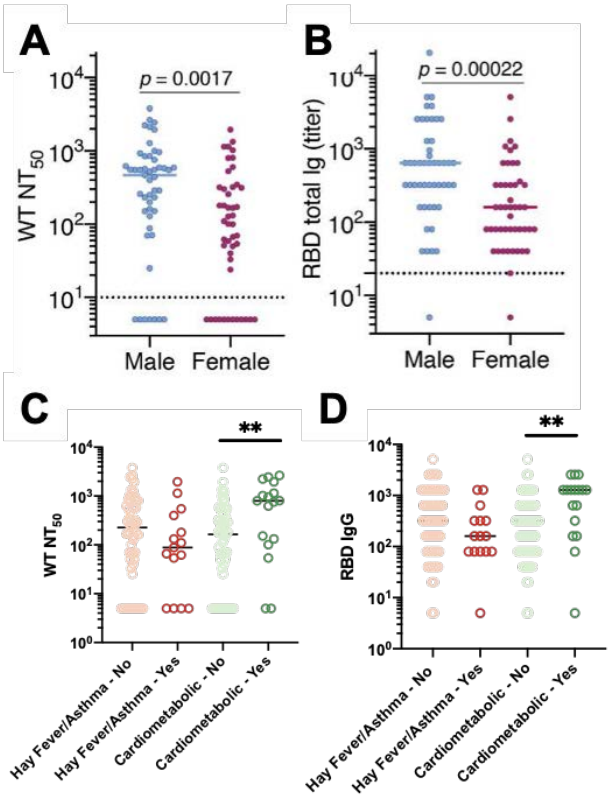


Figure 5: Clinical-demographic correlates of neutralizing antibody titers to SARS-CoV2. Male donors (A, B) and history of cardiometabolic disease (C, D) associate with higher titers of neutralizing antibody and anti-RBD binding antibodies Cardiometabolic disease = any 1 of hypertension, obesity, diabetes mellitus, and/or cardiovascular disease

Among patients treated with CCP collected at the UNC BDC, 80% were infused with CCP at or exceeding the FDA-recommended nAb titer of 1:160. Some experts have suggested that a titer of 1:640 would be more appropriate to qualify CCP as sufficient therapeutic, and 37% of UNC patients were treated with CCP with a titer of 1:640). Titer data was unavailable at the time of infusion, and titer data was not reported by ARC. These data reporting the reach and efficiency of the CCP infusion program implementation have been presented at the American Society of Tropical Medicine and Hygiene (ASTMH) conference and a full publication is in preparation. Outcomes analyses of the role of neutralizing antibody titer and other characteristics of CCP is

ongoing. On August 28th the FDA converted the EAP to an EUA program. We continue to provide CCP collected during this program in specific but more restricted situations under the EUA provisions.

The Coronavirus-inactivating plasma (CoVIP) randomized control trial of Standard (1:160-1:640) versus High-Titer (>1:640) CCP enrolled 56 participants (https://clinicaltrials.gov/ct2/show/NCT04524507).

From August 22 through December 2020, we reached our goal of 56 enrollees into a randomized clinical trial designed to provide CCP to all participants and discern the clinical efficacy of higher titer CCP compared with CCP in the standard titer range. The primary outcome of this study is recovery from severe Covid-19 defined as discharge to home or equivalent of discharge to home (ie. prolonged hospitalization only for the purposes of infection control). Secondary outcomes include in-hospital mortality, clinical status at days 14 and days 28, and immunological and virological outcomes through 6 months post-treatment. We continue to accrue these outcomes data and expect to have a primary analysis of the first 28 days for all participants by the end of February 2021. Importantly, we have confirmed that in comparison to the EAP we were able to operate this RCT with similar efficiency in regards to time from admission to infusion (median time, CCP EAP UNC units=29.6 hrs, CoVIP=31.4, ns). In addition, participants enrolled in CoVIP were treated with CCP as quickly as patients receiving the streamlined EUA authorized CCP at other hospitals (data not shown). Thus, this randomized clinical trial functioned as efficiently as compassionate-use care CCP delivery without compromising time to delivery of therapies to severely ill patients, guaranteed CCP of a minimum titer of 1:160 and provided higher level of evidence for the potential safety and efficacy of the therapy. Thus, in addition to forthcoming data regarding the clinical efficacy of high versus standard titer plasma, the data from the program contribute important findings to the capabilities of operating randomized control trials for rapid therapies in the hospital setting amidst a pandemic.

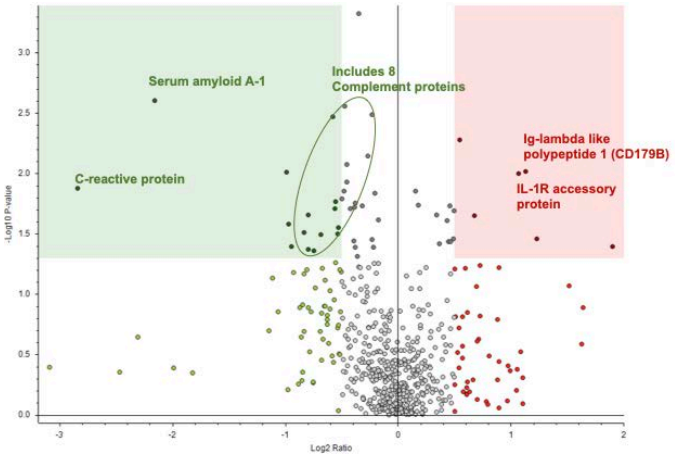


Figure 6 Valcano plot of 452 proteins identified in convalescent plasma donors. Proteins differentially increased in donors with low nAb-titer plasma are to the left of 0 (green) and proteins differentially increased in high nAb titer plasma (red) are on the right of 0 on the x-axis. Colored boxes indicate proteins differentially detected by at least >.5 log and at p-value <0.05.

Table 3: Patients admitted to UNC-MC treated with CCP by different mechanisms (4/25-12/30 2020) and source: ARC=American Red Cross. **UNC and ARC CCP units distributed at UNC-MC under the FDA EUA were labeled "Investigational Drug" (IND) given that the FDA-approved platform to measure antibody titers was not available.*

Mechanism (source)	Mayo EAP (ARC)	Mayo EAP (UNC)	CoVIP (UNC)	EUA (UNC or ARC)
Total: 253	75 (142 units)	88 (164 units)	55 (110 units)	35 (*IND) (66 units)

Discussion/Conclusions:

The SARS-CoV-2 pandemic has swept the globe and significantly impacted the population of North Carolina. An early deployable therapy biological plausibility, convalescent plasma, has shown promise for benefit in observational studies. Randomized control trials continue to show equipoise regarding whether CCP is beneficial to patients with severe COVID-19 and/or patients with more mild disease at risk of progression. Our efforts have contributed substantially to the understanding of the reptoire of antibodies present in CCP, their antiviral activity, and their

potential benefit when transferred to a patient with active SARS-CoV-2 infection. This NC Collaboratory funding stream allowed us to expand and accelerate our efforts in a very short time. The preliminary data generated from this project led to successful federal funding opportunities to continue this work.

Personnel Supported by the Project:
This project was made possible by the contributions from a large interdisciplinary team of 32 people supported by project funds. This included faculty, research associates, a project manager, clinical fellows, graduate assistants, and research technicians.

Budget Summary:
Personnel: Grand Total = $434,679.93
Transactions (including supplies, equipment, assays in primary labs and cores, and clinical care costs): $621,003.38

External funding and publications:
In addition to the progress outlined above, this program has succeeded in securing additional funding from external sources, 5 publications in print (listed below), 1 publication under review (*Markmann et al., mBio, February 2021*), 2 additional publications in preparation, and 2 published abstracts.

Funding Received:
NIH NCI U54CA260543 North Carolina Seronet Center for Excellence. $3,974,612. Projects 1 (Baric) and 2 (Bartelt/Margolis) build upon the human studies supported initially by the CCP program. This program allows for further investigations of antibody and B cell responses to SARS-CoV-2 infection (Core B, de Silva).

Funding pending:
NIH NIAID GRANT13244664 A RANDOMIZED, PHASE II STUDY COMPARING THE EFFICACY AND SAFETY OF STANDARD VERSUS HIGH-TITER ANTI-SARS-COV-2 NEUTRALIZING ANTIBODY PLASMA IN HOSPITALIZED PATIENTS WITH COVID-19 (Bartelt/Margolis Co-PI). This Clinical Trial competitive funding mechanism supports the extension of duration and participant enrollment (up to 150) in CoVIP. The grant is scheduled to be reviewed by study section in April 2021.

Publications (including published abstracts) to date
(personnel supported with the NC Collaboratory funds are indicated in bold type):

Yixuan J. Hou, Shiho Chiba, Peter Halfmann, Camille Ehre, Makoto Kuroda, Kenneth H Dinnon III, Sarah R. Leist, Alexandra Schäfer, Noriko Nakajima, Kenta Takahashi, Rhianna E. Lee, Teresa M. Mascenik, Rachel Graham, Caitlin E. Edwards, Longping V. Tse, Kenichi Okuda, **Alena J. Markmann**, **Luther Bartelt**, **Aravinda de Silva**, **David M. Margolis**, Richard C. Boucher, Scott H. Randell, Tadaki Suzuki, Lisa E. Gralinski, Yoshihiro Kawaoka and **Ralph S. Baric**. SARS-CoV-2 D614G Variant Exhibits Enhanced Replication *ex vivo* and Increased Transmission *in vivo*. *Science, 12 Nov 2020: eabe8499*

Root H, **Gurney B**, **Kuruc J**, **Markmann A**, Evans M, **van Duin D**, Herce M, Wohl D, **Margolis D**, Park Y, **Weiss S**, **Bartelt L**, Implementation of a local COVID-19 convalescent plasma program: Implications for implementation in resource limited settings. Abstract #3521. American Society of Tropical Medicine and Hygiene, 69th Annual Meeting, November 19, 2020

Schwartz S, Thompson P, Smith M, Lercher DM, **Bartelt L**, Park Y, **Weiss S**, **Markmann A**, Raut R, Lakshmanane P, **Kuruc J**, Willis Z, Convalescent Plasma Therapy in 4 Critically Ill Pediatric Patients with COVID-19: A Case Series, Critical Care Explorations. 2020; 2:e2037 (7 pages).

Markmann A, Martinez D, **Kuruc J**, Weiss S, **Park Y**, Premkumar L, Segovia-Chumbez B, Raut R, **van Duin D**, **Baric R**, **de Silva A**, **Margolis D**, **Bartelt L**, (2020) COVID-19 convalescent plasma donors demonstrate a wide range of antibody titers to highly specific anti-SARS-CoV-2 neutralization and receptor binding domain assays. Oral Presentation, International AIDS Society Virtual Covid-19 Conference; Abstract C-AIDS2020-11564, July 10.

Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH 3rd, Kato T, Lee RE, Yount BL, Mascenik TM, Chen G, Olivier KN, Ghio A, Tse LV, Leist SR, Gralinski LE, Schäfer A, Dang H, Gilmore R, Nakano S, Sun L, Fulcher ML, Livraghi-Butrico A, Nicely NI, Cameron M, Cameron C, Kelvin DJ, **de Silva A**, **Margolis DM**, **Markmann A**, **Bartelt L**, Zumwalt R, Martinez FJ, Salvatore SP, Borczuk A, Tata PR, Sontake V, Kimple A, Jaspers I, O'Neal WK, Randell SH, Boucher RC, **Baric RS.** SARS-CoV-2 Reverse Genetics Reveals a Variable Infection Gradient in the Respiratory Tract. Cell. 2020 Jul 23;182(2):429-446.e14.

Submitted for peer-review publication

Markmann AJ, Biallourou N, Bhowmik DR, Hou YJ, Lerner A, Martinez DR, **Premkumar L**, **Root H**, **van Duin D**, **Napravnik S**, Graham SD, Guerra Q, Raut R, Petropoulos CJ, Wrin T, Cornaby C, Schmitz J, **Kuruc J**, **Weiss S**, Park Y, **Baric R**, **de Silva AM**, **Margolis D**, **Bartelt LA**. Sex disparities and neutralizing antibody durability to SARS-CoV-2 infection in convalescent individuals, *medRxiv*, 3 February, 2021 (*submitted for peer review*)