Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Aydillo T, Gonzalez-Reiche AS, Aslam S, et al. Shedding of viable SARS-CoV-2 after immunosuppressive therapy for cancer. N Engl J Med. DOI: 10.1056/NEJMc2031670

Supplemental Appendix

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SUPPLEMENTAL METHODS

Study population

Memorial Sloan Kettering Cancer Center (MSKCC) is a 514-bed tertiary care cancer center that performed 458 HCT in 2019, including 282 autologous and 176 allogeneic transplants. Also, 85 Chimeric Antigen Receptor T-Cell Therapy (CAR-T) treatments were given during the year. HCT and cellular therapy recipients or HCT candidates who were diagnosed with COVID-19 during the peak of the New York City epidemic between March 10 and April 20, 2020 and had serially collected respiratory samples were included. Identification of case-patients and their medical background and clinical course during COVID-19 illness were abstracted from electronic medical records. The MSKCC Institutional Review Board granted a Health Insurance Portability and Accountability Act waiver of authorization to conduct this study.

Laboratory methods

SARS CoV-2 RNA was detected by a Real-time reverse transcription PCR using a modified Centers for Disease Control and Prevention (CDC) protocol. Nasopharyngeal (NP) swab samples were collected using flocked swabs (Copan Diagnostics, Murrieta, CA) and placed in viral transport media. Samples were stored at -20ºC. SARS-CoV-2 IgG were measured using the Abbott Architect test (Abbott Laboratories, Abbott Park, IL) that targets the nucleocapsid protein of SARS-CoV-2.

Viral culture of positive NP swabs was used to assess the presence of infective particles. Briefly, 50 µL supernatant was inoculated onto Vero E6 cell monolayers containing SARS-CoV-2 optimal virus growth media (DMEM w/ L-Glutamate, Sodium Pyruvate, 2% FBS, 100 U Penicillin/ml, and 100 mg Streptomycin/ml, 10 mM Non-Essential Amino Acids, 1 mM Sodium Pyruvate and 10 mM HEPES) . Cells were incubated at 37 °C, 5% CO2 for a week, and monitored daily for potential cytopathic effect (CPE). Cell culture supernatants were collected,

and positive isolation was confirmed by plaque assay and specific immunostaining against SARS-CoV2 by using a mouse anti-SARS NP antibody, 1C7 (an in-house mAb, provided by Dr. Thomas, Moran, Thomas.Moran@mssm.edu). Isolation experiments were performed under Biosafety Level 3 (BSL-3) conditions.

For whole genome sequencing, nucleic acid isolation was performed on SARS-CoV-2 viral isolates and NP swabs using QIAmp Viral RNA kit (Qiagen) according to the manufacturer's instructions. Whole-genome amplification of tiling amplicons and short read Illumina sequencing, genome assembly. Single nucleotide variant profiles relative to the SARS-CoV-2 Wuhan reference genome (RefSeq NC_045512.2) were identified by NextClade v0.4.7 (https://clades.nextstrain.org/), and lineages were assigned using PANGOLIN v.1.1.14, and lineage data from 2020-05-19.

Figure S1 Graph showing collection time of 57 analyzed samples from 20 patients relative to the time of laboratory confirmation of SARS CoV-2 by PCR. At diagnosis, 10/14 patients with samples had viral isolation in culture. Eight additional samples from five patients were positive as shown in the grey bars. Overall, 11 unique patients had at least one positive isolation.

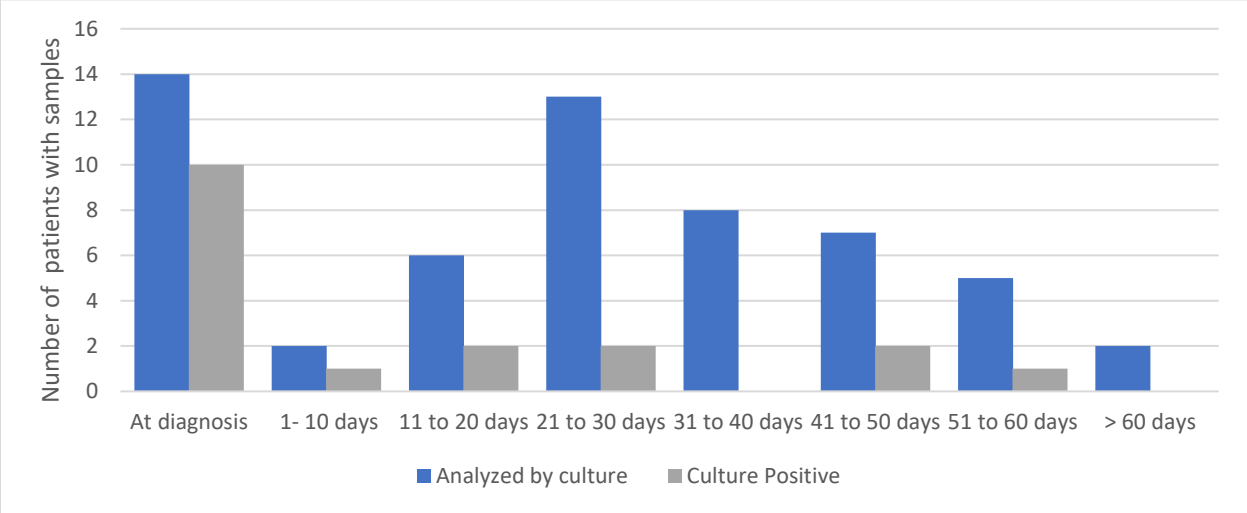

Table S1. Demographic, transplant, and clinical characteristics of study patients (n=20)

Median age	61 years (range 35-77)
Sex	Male, 11 (55%)
Underlying disease	
Acute Leukemia/MDS	4 (20%)
Chronic Leukemia	1 (5%)
Lymphoma	8 (40%)
Multiple Myeloma	7 (35%)
Hematopoietic Stem Cell Transplant (HCT)	16 (80%)
Allogeneic	6 (38%)
Autologous	10 (62%)
Time from HCT (n=16)	
< 100 days	4 (25%)
3 months- 1 year	4 (25%)
1-2 years	3(19%)
> 2 years	5 (31%)
Active Graft vs host disease (GVHD)	6
Non-HCT (n=4)	4(20%)
CAR-T therapy	2
Other	2
Active immunosuppressive or immunomodulatory drugs, or recent chemotherapy (30 days)	15 (75%)
Lenalidomide	4
Fludarabine, Cyclophosphamide	1

Tacrolimus, Mycophenolate, Steroids	2
Tacrolimus, Steroids, Methotrexate	1
Blinatumomab	1
Tacrolimus, Budesonide	1
Cyclosporine, Ruxolitinib	1
BTK-inhibitor	1
Mycophenolate, Cyclosporine, Prednisone	1
Duvelisib	1
G/R-CHOP	1
Time from COVID-19 symptom onset to diagnosis	median 3 days (range -3 to 11 days)
COVID-19 severity	
Asymptomatic	0
Mild	6 (30%)
Moderate	3 (15%)
Severe	11(55%)
Hospitalized	19 (95%)
Death within 30 days	4(20%)
Treatment	15(75%)
Hydroxychloroquine	13
Remdesivir	2
Azithromycin	5
Convalescent plasma	8
Tocilizumab	5
Corticosteroids	9
N-acetyl cysteine	1

Seropositivity (Anti SARS CoV-2 IgG)	
Positive/ Tested	7/15 (47%)
Duration of viral shedding in days (PCR)	symptom onset, laboratory detection
Median	51 days, 47 days
Range	12-82 days, 9 -78 days

CAR- T, Chimeric Antigen Receptor T-Cell Therapy; MDS, Myelodysplastic syndrome;

*Severe, High oxygen requirement with NRB or mechanical ventilation

Moderate, Oxygen requirement limited to nasal cannula < 6 liters

Mild, no oxygen requirement

BTK, Bruton's Tyrosine Kinase Inhibitors

G/R-CHOP, Obinutuzumab or Rituximab Plus Cyclophosphamide, Doxorubicin, Vincristine, and Prednisone

Acknowledgement

We thank Randy A. Albrecht for oversight of the conventional BSL3 biocontainment facility at the Icahn School of Medicine at Mount Sinai.