Response to Pegylated Interferon in a COVID-19 Positive Male with Metastatic Jejunal Neuroendocrine Tumor Treated with Everolimus

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Abstract

A 61 years old male had minimally symptomatic SARS-CoV-2 infection while taking everolimus. He remained RT-PCR positive for viral RNA for 52 days. Pegylated interferon for 4 weeks led to viral RNA clearance. The observations support consideration and further evaluation of combination therapy with everolimus plus interferon for COVID-19.

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The study was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained for the interferon therapy and case report.

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Key Clinical Message

A 61 years old male had minimally symptomatic SARS-CoV-2 infection while taking everolimus. He remained RT-PCR positive for viral RNA for 52 days. Pegylated interferon for 4 weeks led to viral RNA clearance. The observations support consideration and further evaluation of combination therapy with everolimus plus interferon for COVID-19.

Introduction

SARS-CoV-2 is a positive-sense, single stranded RNA in the*Coronaviridae* family of viruses[1]. While most cases of infection present with mild to moderate symptoms consisting of fever, fatigue, dry cough, headache, hypogeusia, anosmia, nausea and diarrhea[2]; roughly 15% of patients develop a severe disease phenotype requiring hospitalization, most commonly due to dyspnea and hypoxia[3,4]. Laboratory findings of severe infection include leukopenia, prolonged prothrombin time, and elevated serum concentrations of D-dimer,

lactate dehydrogenase (LDH), ferritin, and c-reactive protein (CRP)[5]. Chest computed tomography classically demonstrates bilateral ground glass opacities[1]. Severe pathology associated with SARS-CoV-2 infection involves a hyperactive immune response to the virus resulting in a sudden, acute increase in pro-inflammatory cytokines, termed "the cytokine storm"[6]. Key pro-inflammatory cytokines upregulated in this process include interleukin 6 (IL-6) and tumor necrosis factor-alpha (TNF-α)[6]. Elevated cytokine levels prompt an influx of various immune cells into the site of infection, leading to tissue destruction, acute respiratory distress syndrome, septic shock and multiorgan failure[7]. Several mRNA and adenovirus vaccines targeting the spike protein have been approved[8], but they appear to have reduced efficacy against the multiple higher infectivity and immune evading variants (e.g., spike protein mutations A222V, N501Y, or E484K/N501Y/K417N) that have arisen[9,10]. Thus, general SARS-CoV-2 therapies are needed. Early administration of monoclonal antibodies to the spike protein, RNA-dependent RNA polymerase inhibitor remdesivir and dexamethasone and late administration of enoxaparin may modify the natural history of the infection[11-15], but additional treatments are needed. We previously reported the activity of a ruxolitinib plus interferon combination[16]. In this case report, we examine another combination that may show utility for SARS-CoV-2 infections.

Well differentiated small intestinal neuroendocrine tumors arise from serotonin-producing enterochromaffin cells and show epigenetic alterations frequently leading to PI3K/mTOR activation[17]. Upon metastases to liver and other sites, serotonin escapes liver metabolism, and the carcinoid syndrome occurs with flushing, diarrhea, and abdominal pain[18]. Treatments include sandostatin analogues, telotristat ethyl,^{177}Lu-DOTATATE, everolimus, sunitinib, interferon-α, and surgical resection or ablation of metastases[19]. Metastatic small intestinal NET patients have a 70% 5-year survival[20].

PI3K/mTOR pathway inhibitors have been proposed as a therapy to target viral protein synthesis and the hyperinflammation associated with SARS-CoV-2[21]. mTOR is the serine/threonine protein kinase catalytic subunit of the mTORC1 complex with LST8, PRA540 and raptor. Targets of mTOR include phosphorylation inactivation of 4E-BP-1 and LARP-1[22,23]. The result is release of eIF-4E translation initiation factor and stimulation of cap-dependent, 5'-terminal oligopyrimidine tract viral RNA translation. SARS-CoV-2 proteins N and nsp1 assist in this seminal virus infectivity step[24,25]. Inhibitors of mTOR block viral RNA synthesis[23]. SARS-CoV-2 hyper-inflammation is associated with the NLRP3 inflammasome containing the tripartite NLRP3 sensor protein, ASC adapter protein, and caspase-1[26]. Infection produces a "priming" signal with viral RNA binding and signaling through Toll-like receptors or NOD-like receptors leading to NF-ϰB nuclear translocation, and NLRP3 and procaspase gene expression[27]. Then SARS-CoV-2 viroporins E, Orf3a and Orf8a cause cellular potassium efflux, NLRP3 oligomerization, ASC recruitment, and, finally, procaspase-1 recruitment and cleavage[27]. Orf8b aids the steps by binding the leucin-rich repeat domain of NLRP3 facilitating oligomerization. Gasdermin D is cleaved by caspase 1 and pyroptosis occurs along with inflammatory cytokine release. mTOR stimulates the NLRP3 inflammasome by increased mitochondrial reactive oxygen species formation and inhibiting autophagy[28]. As predicted, mTOR inhibitors inhibit NLRP3 inflammasome induced hyper-inflammation *in vitro* and*in vivo* [29,30].

Interferons signal through cell surface interferon receptors to JAK and STAT proteins and ultimately induce interferon-stimulated genes (ISGs) that halt viral translation via protein kinase receptor (PKR) and block viral NLRP3-induced inflammation via STAT1[31,32]. SARS-CoV-2 proteins block interferon activity via multiple pathways. SARS-CoV-2 induced inflammasome caspases degrade interferon and interferon signaling polypeptides[33]. Over a dozen SARS CoV-2 proteins block early interferon induction or activity[31]. Late in the natural history, infection is associated with excessive inflammatory cytokinemia including interferons with dysfunctional T and NK cell responses[34].

We present the case of a 61-years-old male found to be SARS-CoV-2 positive who was relatively asymptomatic while taking everolimus for co-existing metastatic neuroendocrine tumor but displayed a prolonged period of nasal swab PCR positivity. Administration of pegylated interferon was followed by prompt clearance of viral RNA by PCR. We hypothesize that the combination of everolimus with interferon may be useful in the acute COVID-19 setting to induce viral clearance with reduced risk of cytokine storm.

Methods

Informed consent to monitor, treat and report the subject was obtained from the patient and approved by the West Palm Beach VAMC Research & Development Committee. Nasopharyngeal swab specimens were obtained from the patient. Samples were shipped to Orlando for performance of the Gene Xpert Cepheid Innovation XPRSARS-COv2-10 RT PCR assay measuring viral N2 sequence abundance. The limit of detection was 0.01 plaque forming units/mL (CFU/mL) or 250 viral RNA copies/mL with first cycle number above background (Cq) of 39. Blood samples were collected using a serum separator tube and serum also shipped to Orlando for performance of the Abbott Architect anti-N SARS CoV-2 IgG assay with results calculated as chemiluminescence ratios of sample to control with negative ratios being <1.4.

Results/Case Presentation

A 61-year-old Caucasian male veteran developed diarrhea, flushing, pruritis, and abdominal pain in June, 2019. Computerized tomography scans showed a jejunal mass and liver lesions. Liver biopsy confirmed well differentiated neuroendocrine tumor. Blood counts and chemistries were normal, but serotonin was 1246ng/mL (normal 50-200ng/mL), chromogranin A 781ng/L (normal <39ng/L) and 24 hour urine 5-hydroxyindoleacetic acid was 91mg (normal <6mg). He was treated with lanreotide 120mg subcutaneously weekly and everolimus 10mg orally daily. He did well until June 27, 2020 when he noted mild lightheadedness, nausea, cough, headache, clear sputum production, hypogeusia, and anosmia and had a positive SARS-CoV-2 nasal swab RT-PCR test (Table 1). He remained at home, and his symptoms resolved within several days. However, his RT-PCR assay remained positive repeatedly for 52 days (Table 1). Because of hospital restrictions at the time on RT-PCR positive patients, he was unable to receive his monthly clinic lanreotide injections. His carcinoid symptoms recurred, and he required breakthrough octreitide acetate 200mcg subcutaneous injections every 8 hours at home. To facilitate viral RNA clearance, we elected to try pegylated interferon alpha-2a as treatment for both his neuroendocrine tumor and his COVID-19. After informed consent and approval by the West Palm Beach VA Medical Center Administration, Pharmacy and Research & Education Committee, the patient received four weekly subcutaneous injections of 90 mcg pegylated interferon-α-2a. His RT-PCR rapidly cleared within one week of treatment, and he was able to resume somatostatin analogue therapy at the oncology clinic. Initial anti-N SARS-CoV-2 IgG and IgM antibodies were absent from his blood (IgG 0.03 and IgG 0.02), but by January, 2021 he had measurable antibodies (IgG 2.07 and IgM 2.18). He remained asymptomatic and is undergoing additional treatments for his metastatic neuroendocrine tumor.

Discussion

SARS-CoV-2 is associated with a wide range of symptoms ranging from a mild clinical phenotype with fever and cough to severe respiratory and/or multi-organ failure. SARS-CoV-2 has considerable morbidity and mortality, particularly among older patients with co-morbidities[35]. A significant factor contributing to the morbidity and mortality of this infection is the pulmonary and systemic inflammatory response as noted above[36]. We were struck by the minimal clinical findings in this high-risk, male with a co-existing malignancy. Our patient was on chronic everolimus therapy for metastatic neuroendocrine tumor. As noted above, everolimus is an mTOR inhibitor that may target both viral replication and inflammation. We speculate whether our patient's minimal clinical symptoms throughout his infection could be linked to the anti-inflammatory effect of the drug. Everolimus may reduce the SARS-CoV-2 inflammatory state, improve the quality of life, and perhaps prolong survival from this devastating disease. This speculation is supported by several case studies. Among 18 hospitalized COVID-19 positive renal transplant patients, three were on chronic everolimus[37]. A 60 years old male and a 40 years old female were maintained on everolimus and did well; one 40 years old male had everolimus stopped and died from infection complications. Among 10 COVID-19 tuberous sclerosis/lymphangioleiomatosis patients, five were on sirolimus or everolimus and only one was briefly hospitalized and recovered; four of five patients not on mTOR inhibitors were hospitalized and one died[38]. A 45 years old T3 paraplegic pancreas-kidney transplant male with asthma and chronic renal insufficiency maintained on everolimus developed RT-PCR positive SARS-CoV-2 and had an eight days hospitalization with transient symptoms[39]. Among 111 SARS-CoV-2 positive liver transplant

patients, immunosuppression with mycophenolate was associated with severe disease (risk ratio = 5.3); immunosuppression with mTOR inhibitors—tacrolimus or everolimus yielded fewer severe cases (tacrolimus risk ratio = 0.54 and everolimus risk ratio = 0.77)[40].

An interesting facet of this case is the sustained positivity of the patient's SARS-CoV-2 test. He was repeatedly tested for viral RNA clearance by nasal swab RT-PCR secondary to his immunocompromised state, and because he required a negative test prior to treatment at the oncology clinic. Many SARS-CoV-2 infected individuals have persistently positive RT-PCR tests for weeks to months after clinical recovery[41]. Based on viral culture, the percent of these individuals who remain infectious approaches zero by 10 to 15 days after the onset of symptoms[41-43]. However, shedding of infectious SARS-CoV-2 has been demonstrated by viral culture or inferred by the presence of subgenomic RNA in a subset of individuals, including immunosuppressed hosts, for months following infection[44,45]. We lack Cq values for multiple tests on our patient, but the reported positivity implies a Cq less than 39. Likely our patient had low quantities of viral RNA or viral RNA fragments that were non-infectious.

The persistent positivity of his SARS-CoV-2 testing may be potentially secondary to the immunosuppressive effects of everolimus[46]. mTOR inhibitors inhibit dendritic cell maturation and function and T cell proliferation. In our case, possible hampered anti-viral defense was reversed with interferon supplementation with subsequent T cell activation to fight the SARS-CoV-2 infection. Our patient was able to clear the viral RNA after the administration of the first of a total of four treatments of pegylated interferon-α2a while continuing treatment with everolimus. Presence of RT-PCR positivity for one to two months after SARS-CoV-2 infection is not rare[47], and does not always represent infectious virions.

Interferons have been successfully used in the treatment of viral infections, such as hepatitis C, multiple sclerosis, hematologic malignancies, in particular the Philadelphia-negative myeloproliferative neoplasms and neuroendocrine tumors[48-50]. In SARS-CoV-2, interferon therapy in phase 2 and phase 3 randomized clinical trials have shown reduced the duration of virus infection, reduced inflammatory markers including IL6 and CRP and reduced mortality when administered early[51-58]. As a note of caution, type I interferons administered in later stages may cause progressive tissue damage leading to a deleterious hyperinflammation characterized by the excessive macrophage activation and hypercoagulation seen in patients with acute disease[36]. Interestingly, pharmacologic interferon treatment inhibits inflammation early by repressing the NLRP3 inflammasome via STAT1[32]. We hypothesized that administration of interferon in our patient who was minimally symptomatic would strengthen anti-viral defense and potentially lead to viral RNA clearance. Our results support the hypothesis.

Conclusion

The availability of vaccines will reduce the number of acute cases of COVID-19. However, acute cases will continue to exist, requiring therapeutic interventions to reduce toxicities and improve survival. Reducing the cytokine storm appears to be crucial in preventing end organ damage which is associated with high mortality[59]. Genetic and immunologic studies of hospitalized COVID-19 subjects showed mutations yielding increased TYK2 or decreased IFNAR2 expression or inactivating mutations in interferon pathway genes—IRF3, IRF7, IFNAR1/2, TBK1 or TLR3 or autoantibodies to interferons had more severe disease[60-62]. These subjects suffered increased inflammatory cytokines and absent anti-viral interferons. Targeted immune regulation to reverse this state may provide substantial benefit in SARS-CoV-2 infection. Our case suggests that everolimus plus pegylated interferon is a potential regimen for SARS-CoV-2 patients. This treatment combination may benefit select patients if used early in the disease. Future studies are needed to elucidate the potential therapeutic benefits and side effects of this regimen.

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Disclosure of Conflicts of Interest

None of the authors has a relevant conflict of interest.

Author Contributions

Author 1: Conceived of study, obtained permissions, accessed agents, provided patient care, wrote draft manuscript.

Author 2: Assisted in patient care and obtained SARS-CoV-2 nasal swabs and reviewed manuscript.

Author 3: Assisted in collecting patient data and reviewed manuscript.

Author 4: Aided in planning patient treatment and in review of manuscript.

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Table 1. RT-PCR Cq values *

Day
-17
1
15
25
38
52
69
97
180
191

RT-PCR Cq, reverse transcriptase polymerase chain reaction cycle at which fluorescence detected above baseline. The value