The BTK-inhibitor ibrutinib may protect against pulmonary injury in COVID-19 infected patients

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Abstract:

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Dear Editor:

The BTK-inhibitor ibrutinib is used to treat indolent B-cell malignancies and chronic graft versus host disease. The potential for ibrutinib to abrogate pulmonary inflammatory cytokines, lung injury and death was previously demonstrated in a highly relevant, lethal flu animal model. We therefore sought to clarify the impact of ibrutinib in COVID-19 patients. We care for 600-800 Waldenstrom’s Macroglobulinemia (WM) patients per year; approximately 300 of whom are on a BTK-inhibitor. We identified 6 patients receiving ibrutinib for Waldenstrom’s Macroglobulinemia who were diagnosed with COVID-19; these patients consented to the use of their data. Their clinical characteristics appear in Table 1. Their median age was 66 years, and five were on the recommended treatment dose of 420 mg/day; the sixth patient was on a reduced dose of 140 mg/day because of arthralgias. For all patients, the median time on ibrutinib was 52 months. Their median time with COVID-19 related symptoms prior to diagnostic testing was 5 days, and since diagnosis of COVID-19 was 22 days. All 6 patients experienced cough and fever as prodromal symptoms. The 5 patients on ibrutinib at 420 mg/day experienced no dyspnea and required no hospitalization. Their course was marked by steady improvement, and resolution or near resolution of COVID-19 related symptoms in all five of these patients during the follow-up period.

The patient on reduced dose ibrutinib (Patient 6; Table 1) experienced progressive dyspnea and hypoxia prompting hospitalization. Chest CT showed bilateral ground glass opacities and a pleural effusion on admission prompting a hold on ibrutinib during which his hypoxia acutely worsened necessitating supplemental oxygen use. Hydroxychloroquine (HCQ) and azithromycin were administered. Azithromycin was stopped after 3 days due to wide QRS complex tachyarrhythmia, HCQ was given for a total of 5 days. Hypoxia worsened and fever persisted during HCQ course. Ibrutinib was restarted at 140 mg/day and tocilizumab 400 mg was co-administered on hospital day 5 with improved oxygenation, and decreased C-reactive protein (CRP) levels (83 to 9 mg/L). Intravenous immunoglobulin was also given on hospital days 6-10. On day 10 of hospitalization, the patient experienced worsening hypoxia accompanied by increased CRP (28 mg/L) and required mechanical ventilation. Given the lack of hypoxia in the other COVID-19 infected WM patients on full dose ibrutinib, ibrutinib was increased to 420 mg/day on days 11 and day 12. A rapid improvement in oxygenation followed, and the patient was successfully extubated late on day 12 and maintained oxygen saturations of 94-96% on 3 liters/min supplemental oxygen by nasal cannula. The next day supplemental oxygen was decreased to 2 liters/min, with oxygen saturations of 96-98%, and CRP level of 10 mg/L. On day 14, oxygen saturation was 95% on room air, repeat CRP level was 6 mg/L, and he was discharged home off supplemental oxygen on 420 mg/day of ibrutinib. Seven days later he continues to do well, without fever, cough or dyspnea at rest. He remains on ibrutinib at 420 mg/day and tolerating therapy well.

Discussion

Pulmonary failure is the main cause of mortality related to COVID-19 infection. Up to 80% of patients hospitalized for COVID-19 infection require supplemental oxygenation, of whom 30-40% may require mechanical ventilation. SARS-CoV-2 binds via the ACE2-receptor that is highly expressed on Alveolar Type II (ATII) cells in the lung. ATII cells constitute 5-15% of the lung epithelium. While Alveolar Type I cells are highly adapted for gas exchange, Alveolar Type II cells have a specialized role in innate immune response. ATII cells express Toll receptors (TLRs) and can trigger inflammatory cytokines and chemo-attractants in response to pathogens that recruit and activate other immune cells including macrophages and neutrophils. Highly relevant to coronavirus infection, expression of pro-
inflammatory and chemo-attractant cytokines IL1-B, IL6, IP10/CXCL10, MCP-1 and TNF-a were identified in the ACE2-positive cells from autopsy tissue of SARS-CoV-1 infected patients, that appeared causally related to the acute lung injury and pathogenesis observed with SARS-CoV-1.

SARS-CoV-1 shares 86% homology with SARS-CoV-2. SARS-Cov-2 patients requiring intensive care also showed elevated plasma levels of inflammatory cytokines and chemo-attractants such as IL-2, IL-6, IL-7, IL-10, G-CSF, IP-10/CXCL-10, MCP-1/CCL2, MIP-1a/CCL3, and TNF-a. The importance of inflammatory cytokines to lung injury in SARS-CoV-2 infected patients has been suggested by reports of benefit with IL-6 and IL6-receptor blocking antibodies, and clinical trials to examine their use have been initiated (NCT04317092, NCT04306705, NCT04315298).

We and others previously showed that BTK, and its upstream activator HCK, were involved in TLR-mediated signaling. Both BTK and HCK are triggered by MYD88, a TLR-adaptor protein that signals for all Toll receptors except TLR3 in response to viral and bacterial pathogens, including coronaviruses. ATII cells express TLRs, as do alveolar macrophages that coordinate inflammatory responses with ATII cells. As components of TLR/MYD88 signaling, BTK and HCK can drive inflammatory cytokine production through ERK1/2.

In a transgenic mouse model, activated HCK over-expression promoted extensive pulmonary inflammation and an enhanced innate immune response, particularly in older mice. Elevated levels of TNF-a were identified in the bronchoalveolar lavage fluids of these mice following LPS challenge. The pulmonary pathology findings from these mice show great overlap with those described in the lungs of patients with COVID-19 infection which showed serous and fibrin exudation with alveolar infiltration consisting mostly of macrophages and monocytes.

Ibrutinib is a highly potent, covalent inhibitor of BTK (biochemical IC<sub>50</sub> 0.5 nM). Ibrutinib is also a potent reversible inhibitor of HCK (IC<sub>50</sub> 49 nM). The IC<sub>50</sub> levels for BTK and HCK are within the pharmacologically attainable dosimetry of orally administered ibrutinib. Serially collected blood samples from patients with chronic lymphocytic leukemia (CLL), Waldenstrom’s Macroglobulinemia (WM), and chronic graft versus host disease (cGVHD) on ibrutinib monotherapy showed marked reductions in pro-inflammatory and chemo-attractant cytokines that greatly overlapped with those reported elevated in the plasma of SARS-Cov-1 and SARS-COV-2 patients, and in ACE2+ cells from lung tissue of SARS-CoV-1 patients (Table 2). In the iLLUMINATE randomized study, CLL subjects treated with ibrutinib immediately prior to infusion with obinutuzumab also showed significantly decreased levels of inflammatory cytokines associated with infusion related reactions (a cytokine release syndrome). These findings are consistent with a shift from an M1 to M2 polarized macrophage response following ibrutinib, and are supported by pre-clinical and clinical studies showing dependence of macrophage lineage commitment on BTK function.

The potential for ibrutinib to abrogate lung injury and death was also demonstrated in an experimental model wherein mice challenged with a lethal intranasal inoculum of a mouse adapted strain of H1N1 influenza virus were protected against lung injury. Control mice developed respiratory failure, along with histological and CT findings consistent with lung injury in sharp contrast to the mice that received ibrutinib. Control mice also lost weight and died, whereas those treated with ibrutinib recovered their
weight after a brief loss and all survived.\(^1\) Notably, mice treated with ibrutinib also showed decreased inflammatory cell infiltration as well as pro-inflammatory cytokines in lung tissues that included pro-inflammatory and chemo-attractant cytokines such as IL-1β, IL-6, KC/CXCL1, TNFα, and MCP-1 observed in SARS-CoV-1 and SARS-CoV-2 patients.\(^1\) The findings provide rationale that an exaggerated cytokine release syndrome triggered by ATII cells and resident macrophages by SARS-CoV-2 may underlie pulmonary injury associated with COVID-19.

Ibrutinib and possibly other BTK-inhibitors may therefore provide protection against lung injury, and even improve pulmonary function in hypoxic patients with COVID-19 as we observed in this series of WM patients on ibrutinib. These findings should be considered as hypothesis generating and preliminary in nature. Patients on ibrutinib, and possibly other BTK-inhibitors may well benefit with continuation of their therapy despite the diagnosis of COVID-19. It will be important to further validate these findings in other patient populations on BTK-inhibitors, including CLL patients. Clinical trials examining the benefit of BTK-inhibitors are being initiated by us and others in COVID-19 patients in pulmonary distress, and the outcome of these prospective, randomized studies will be needed to confirm these preliminary observations.

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**Author contribution**

SPT conceptualized and designed the study and wrote the first draft. All authors provided editorial review. JJC, AS, IMG, KM, provided patient care and data. ST, JS, MLS, GY provided input for supportive basic science studies.

**Conflict of Interest**

SPT has received research funding and/or consulting fees from Pharmacyclics Inc., Janssen Pharmaceuticals, Beigene Pharmaceuticals, and LOXO Pharmaceuticals. JJC has received research funds and/or consulting fees from Pharmacyclics Inc., AbbVie, Beigene, Janssen Pharmacueticals, and Merck. AS have received research funding and consulting fees from Pharmacyclics, and Abbvive (PCYC parent company). JDS has received research support/and or consulting fees from Abbvie, AstraZeneca, Beigene Pharmaceuticals, TG Therapeutics, and BostonGene. IMG has received research funding and/or consulting fees from GSK, Sanofi, Janssen, Takeda, Celgene, Karyopharm, AbbVie, GNS, Cellectar, Medscape, Genentech, Adaptive, BMS, Aptitude, and Curio Science.
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Table 1. Clinical characteristics of 6 patients with Waldenstrom’s Macroglobulinemia on ibrutinib with COVID-19 infection. WM, Waldenstrom’s Macroglobulinemia; HC, hydrochloroquine; AZ, azithromycin; TOCI, tocilizumab.

| Demographics                  | Pt-1 | Pt-2 | Pt-3 | Pt-4 | Pt-5 | Pt-6 |
|-------------------------------|------|------|------|------|------|------|
| Age (years)                   | 65   | 61   | 72   | 67   | 71   | 58   |
| Gender M/F                    | M    | M    | F    | F    | M    | M    |
| Time since B-cell Diagnosis (months) | 39   | 54   | 95   | 202  | 52   | 107  |
| Received treatment prior to 1brutinib for WM | N    | N    | Y    | Y    | N    | Y    |
| Time on 1brutinib (months)    | 39   | 54   | 83   | 50   | 47   | 85   |
| Dose of 1brutinib (mg/day)    | 420  | 420  | 420  | 420  | 420  | 140-HELD-420 |

| COVID-19 Symptoms             |      |      |      |      |      |      |
| Time with symptoms prior to COVID-19 diagnostic testing (days) | 5    | 2    | 6    | 7    | 10   | 5    |
| Time since COVID-19 diagnostic testing (days)                   | 24   | 20   | 17   | 28   | 13   | 29   |
| Cough                        | Y    | Y    | Y    | Y    | Y    | Y    |
| Fever                        | Y    | Y    | Y    | Y    | Y    | Y    |
| Dyspnea                      | N    | N    | N    | N    | N    | Y    |
| Sore throat                   | Y    | N    | N    | N    | N    | Y    |
| Taste loss                    | N    | N    | Y    | N    | Y    | N    |
| Smell loss                    | N    | N    | Y    | N    | Y    | N    |
| Hospitalization              | N    | N    | N    | N    | N    | Y    |
| Required Intensive Care Unit | Y    | N    | N    | N    | N    | Y    |
| Required Supplemental O2      | N    | N    | N    | N    | N    | Y    |
| Required Mechanical Ventilation | N    | N    | N    | N    | N    | Y    |
| Other COVID-19 symptoms      | N    | Anorexia | Diarrhea | Headache | N    | N    |
| Other Meds for COVID-19       | HC, AZ | NA   | N    | NA   | N    | HC, AZ, TOCI |

| Disposition                  |      |      |      |      |      |      |
| COVID-19 Symptoms Resolved   | N    | Y    | Y    | Y    | Y    | N    |
| COVID-19 Symptoms Persist    | Y    | N    | Y    | Y    | N    | Y    |
| COVID-19 Symptoms Improved   | Y    | Y    | Y    | Y    | Y    | Y    |
Table 2. Summary of pro-inflammatory and chemo-attractant cytokine patterns in patients infected with SARS-CoV-1 and SARS-CoV-2 (*highlighted in red*), and following ibrutinib treatment in patients with CLL, WM, and cGVHD (*highlighted in green*).

| STUDY | HE⁹ | JIANG¹⁰ | HUANG¹² | NIEMANN²⁰ | GREIL²³ | VOS²¹ | MIKLOS²² |
|-------|-----|---------|---------|-----------|---------|--------|---------|
| PATIENT POPULATION | CoV-1 | CoV-1 | CoV-2 | CLL ON IBRUTINIB | CLL ON IBRUTINIB | WM ON IBRUTINIB | cGVHD ON IBRUTINIB |
| TISSUE | ACE2+ cells | Plasma | Plasma | Plasma | Plasma | Plasma | Plasma |

| | GMCSF | IL1B | IL2 | IL6 | IL7 | IL8 | IL10 | IP10/CXCL10 | MCP-1/CCL2 | MIP-1A/CCL3 | MIP1B/CCL4 | TNF-a |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ | ↑ |
| | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ | ↓ |

Note: ↑ indicates increased levels, ↓ indicates decreased levels, and Variable indicates variable levels.