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Rapid and sustained decline in CXCL-10 (IP-10) annotates clinical outcomes following TNFα-antagonist therapy in hospitalized patients with severe and critical COVID-19 respiratory failure

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Abstract

Background: A feedforward pathological signaling loop generated by TNFα and IFN-γ synergy in the inflamed lung, driving CXCL-10 (IP-10) and CXCL-9 chemokine-mediated activated T-cell and monocyte/macrophage tissue recruitment, may define the inflammatory biology of lethal COVID-19 respiratory failure. *Methods:* To assess TNFα-antagonist therapy, 18 hospitalized adults with hypoxic respiratory failure and COVID-19 pneumonia received single-dose infliximab-abda therapy 5 mg/kg intravenously between April and December 2020. The primary endpoint was time to increase in oxygen saturation to fraction of inspired oxygen ratio (SpO2/FiO2) by ≥50 compared to baseline and sustained for 48 h. Secondary endpoints included 28-day mortality, dynamic cytokine profiles, secondary infections, duration of supplemental oxygen support, and hospitalization. *Findings:* Patients were predominantly in critical respiratory failure (15/18, 83%), male (14/18, 78%), above 60 years (median 63 years, range 31–80), race-ethnic minorities (13/18, 72%), lymphopenic (13/18, 72%), steroid-treated (17/18, 94%), with a median ferritin of 1953 ng/ml. Sixteen patients (89%) met the primary endpoint within a median of 4 days; 14/18 (78%) were discharged in a median of 8 days and were alive at 28-day follow-up. Three deaths were attributed to secondary lung infection. Mean plasma IP-10 levels declined sharply from 9183 to 483 pg/ml at Day 3 and 146 pg/ml at Day 14/discharge. Significant Day 3 declines in IFN-γ, TNFα, IL-27, CRP, and ferritin occurred. IP-10 and CXCL-9 declines were strongly correlated among patients with lymphopenia reversal (Day 3, Pearson *r*: 0.98, *P*-value 0.0006). *Interpretation:* Infliximab-abda may rapidly abrogate pathological inflammatory signaling to facilitate clinical recovery in severe and critical COVID-19.

Introduction

Preclinical and clinical evidence indicates that TNFα may fundamentally orchestrate the hyperinflammatory cytokine signature and adverse disease course of COVID-19 [1,2]. Elevated circulating levels of TNFα and its known regulatory targets including interleukin-6 (IL-6) and ferritin have been correlated with indices of disease severity, including intensive care needs, multisystem organ failure, and death [3–5]. A marked quantitative reduction in lymphocytes and natural killer cells in severe COVID-19 illness is accompanied by markers of immune exhaustion, which are inversely associated with levels of IL-6, IL-10, and TNFα [6]. An aberrant hyperimmune response triggered by the synergy between TNFα and IFN-γ may underpin the markedly elevated T-cell chemokines CXCL-10 (IP-10) and CXCL-9, associated with immune exhaustion and adverse clinical outcomes [1,7,8]. TNFα has been previously implicated as a master regulator of immune exhaustion in other biological contexts [9]. In preclinical animal models of H1N1 influenza and lymphocytic choriomeningitis virus infection, anti-TNFα monotherapy resolved inflammation, restored immune surveillance, suppressed or cleared viral infection, and prolonged survival [9,10]. Based on the hypothesis that TNFα-antagonists may abrogate the adverse inflammatory cytokine profile of COVID-19 disease and reduce the need for advanced cardiorespiratory support, a pilot study of the infliximab biosimilar, infliximab-abda, was initiated in COVID-19 respiratory failure (NCT04425538). The goal of the study was

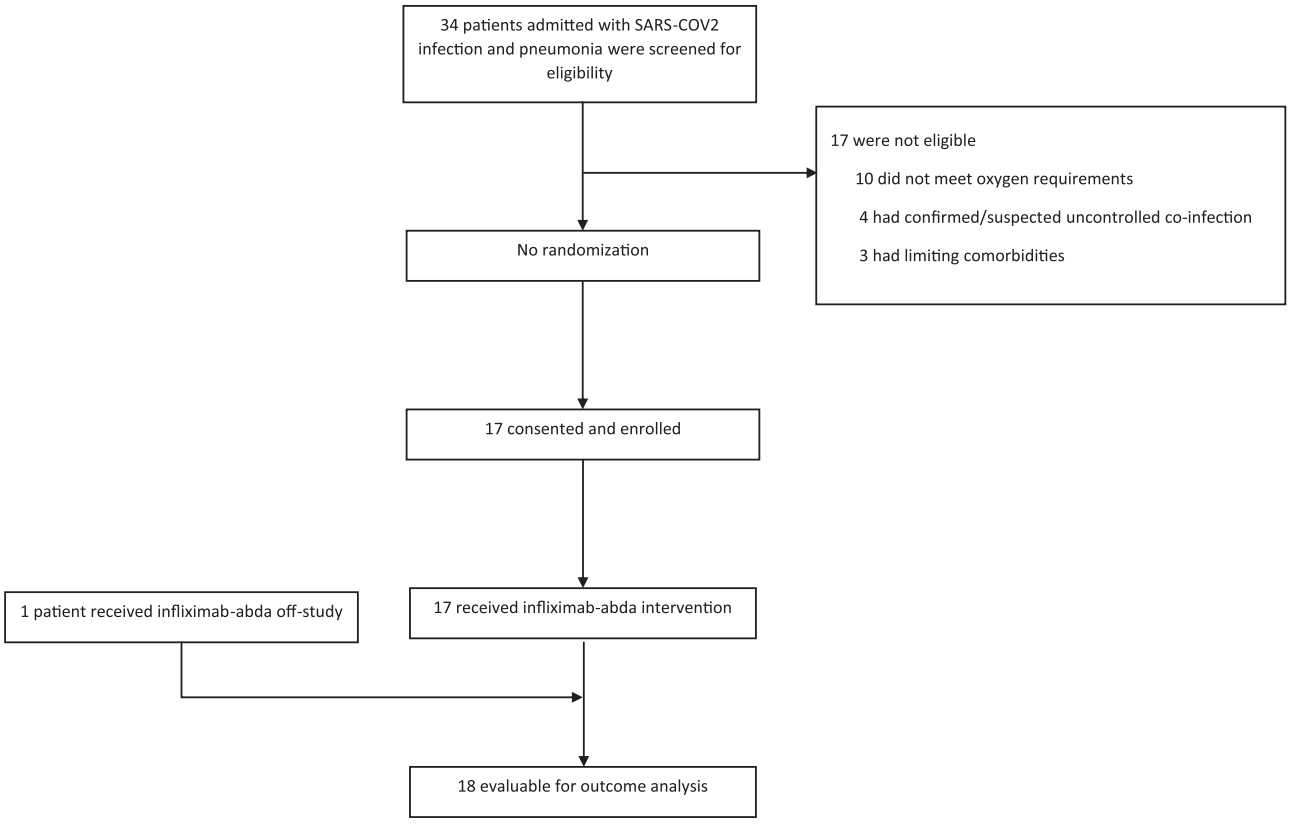

Fig. 1. Consort diagram. Hospitalized patients with SARS-COV2 infection and pneumonia were referred to the infliximab-abda study team for evaluation.

to generate early efficacy, toxicity, and cytokine correlative data to inform the next generation of studies.

Methods

Patients (Fig. 1): Eligible subjects were at least 18 years old, could provide informed consent, had pneumonia evidenced by chest X-ray or computerized tomography, and laboratory (reverse transcriptase-polymerase chain reaction) confirmed infection for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with at least one of the following: respiratory frequency ≥30/min, blood oxygen saturation ≤93% on room air, partial pressure of arterial oxygen to fraction of inspired oxygen ratio (PaO2/FiO2) < 300, worsening of lung involvement defined as an increase in number and/or extension of pulmonary areas of consolidation, need for increased FiO2 to maintain stable oxygen saturation, or worsening oxygen saturation of >3% with stable FiO2. These represent patients with both severe and critical COVID-19 illness as defined by the Food and Drug Administration (https://www.fda.gov/media/137926/download; Appendix A). Exclusion criteria included treatment with any TNFα inhibitor in the past 30 days, absolute neutrophil count less than 1000 mm^3, hemoglobin < 7.0 g/L, platelets < 50,000 per mm^3, or aspartate transaminase or alanine transaminase greater than five times the upper limit of normal, known active or latent hepatitis B, known or suspected active tuberculosis (TB) or a history of incompletely treated or latent TB, pregnancy, uncontrolled systemic bacterial or fungal infections (prior positive bacterial or fungal cultures on appropriate therapy with negative repeat cultures was permissible), myocardial infarction (within last month), moderate or severe heart failure (New York Heart Association class III or IV), acute stroke (within last month), uncontrolled malignancy, Stage 4 severe chronic kidney disease or requiring dialysis (i.e. estimated glomerular filtration rate (eGFR) < 30 ml/min/1.73 m^2) at baseline. Prior and concurrent remdesivir, dexamethasone, and convalescent plasma therapy were permitted. No limits to the level and duration of oxygenation support or hospitalization were specified. Infliximab-abda therapy was sanctioned as part of a COVID-19 care pathway for hospitalized patients by an institutional task force and by infectious disease and critical care medicine consultation for each patient. Patients who were judged to be improving on standard therapy or suspected to have active concomitant infection were not sanctioned for study consideration.

Therapy

Treatment with infliximab-abda 5 mg/kg IV was planned within 6 h of enrollment, and no more than 24 h following enrollment. Premedication with acetaminophen 650 mg once, 30 min prior to infusion, was recommended. Retreatment with infliximab-abda was permitted at the treating physician's discretion 7–21 days following primary therapy. One patient (TMC-Pre) who met the eligibility criteria and was treated on the care pathway prior to the formal activation of the pilot study was included in the analysis and reporting. The choice of TNFα-antagonist for the study was based on availability, cost, rapid onset of action when delivered parenterally, potency, and a relatively short half-life (7–10 days for infliximab-abda, https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/761054orig1s000lbl.pdf) [11,12].

Laboratory Studies

Included complete blood counts and leucocyte differential, complete metabolic panels which included electrolytes, creatinine, and liver function tests, inflammatory markers including serum ferritin and C-reactive protein (CRP) and a panel of cytokines performed at baseline prior to infliximab-abda (Day 1) in all enrolled patients and on Days 3 and 14 or discharge if earlier. Luminex xMAP technology was employed for multiplexed quantification of 48 human cytokines, chemokines, and growth factors. The multiplexing analysis was performed using the Luminex™ 200 system (Luminex, Austin, TX, USA) by Eve Technologies Corp. (Calgary, Alberta). Forty-eight markers were simultaneously measured in the samples using Eve Technologies' Human Cytokine 48-Plex Discovery Assay® (MilliporeSigma, Burlington, Massachusetts, USA) according to the manufacturer's protocol. The 48-plex consisted of sCD40L, EGF, Eotaxin, FGF-2, FLT-3 Ligand, Fractalkine, G-CSF, GM-CSF, GROα, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1RA, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12 (p40), IL-12(p70), IL-13, IL-15, IL-17A, IL-17E/IL-25, IL-17F, IL-18, IL-22, IL-27, IP-10, MCP-1, MCP-3, M-CSF, MDC, MIG/CXCL9, MIP-1α, MIP-1β, PDGF-AA, PDGF-AB/BB, RANTES, TGFα, TNF-α, TNF-β, and VEGF-A. Assay sensitivities of these markers range from 0.14 to 50.78 pg/ml.

Endpoints

The primary endpoint of the pilot study was time to improvement in oxygenation (increase in oxygen saturation to fraction of inspired oxygen ratio SpO2/FiO2 of 50 or greater compared to the baseline SpO2/FiO2) sustained for a minimum of 48 h. Secondary endpoints included 28-day mortality, assessment of dynamic changes in cytokine and inflammatory profile after therapy, qualitative and quantitative toxicity, incidence and duration of supplementation oxygen support including mechanical ventilation (MV) and extracorporeal membrane oxygenation (ECMO), duration of hospitalization, and frequency and spectrum of secondary infections. COVID-19-associated pulmonary aspergillosis (CAPA) diagnoses were annotated by consensus criteria [13]. Common Terminology Criteria for Adverse Events were used to annotate toxicity including infusion reactions, anaphylaxis, and unanticipated outcomes in the critical care setting potentially attributable to the study therapy.

Statistics

Mean ferritin, CRP, and cytokine levels on Day 1 and the mean dynamic change on Day 3 and Day 14 were compared using pairwise ratio *t*-tests (GraphPad Prism). Given the number of comparisons (50 at each timepoint), a 5% false-discovery rate (FDR) was applied using the Benjamini–Hochberg method, and those cytokines that met statistical significance at the adjusted thresholds are also reported. Given that post-steroid therapy IL-6 levels at different cut-off levels have been shown to correlate with adverse outcomes [14,15], the impact of infliximab-abda on IL-6 levels at Day 3 and Day 14 stratified by degree of baseline elevation in the largely steroid-pretreated population was separately analyzed. Pearson correlation coefficients were calculated for fold changes (log2 transformed ratio) in expression from baseline to Day 3 between each pair of cytokines. In all statistical comparisons, values for samples whose cytokine levels were below the assay detection limits were replaced by the sensitivity of the assay for each respective cytokine. Descriptive statistics annotate the remaining endpoints.

Study Approval

The study was approved by the institutional review board and written informed consent was received from participants or designees prior to inclusion.

Results

The demographics, comorbidities, clinical features, inflammatory markers, and outcomes of 18 patients with COVID-19 respiratory failure treated with infliximab-abda between April and December 2020 are represented in Table 1. The median age was 63 years (range 31–80), largely male (78%), and race-ethnic minority (Asian, South Asian, Black, Hispanic, or Latino, 72%), with at least one significant medical comorbidity (72%). Comorbidities included hypertension (67%), diabetes (28%), smoking history (22%), or chronic lung disease, including asthma (22%). Half were obese or morbidly obese; the median body mass index was 30.3 kg/m^2 (range 18.0–41.8). The median Charlson Comorbidity Index was 2.5 (range 0–9). Sixteen patients (89%) had received both remdesivir and steroid therapy (median 2 days, range 0–7 days) with infliximab-abda therapy supported by the infectious disease consultative service. Five patients (28%) received prior convalescent plasma (median 2 days, range 0–4 days). Fifteen patients (83%) were on high-flow nasal cannula (HFNC), MV, and/or ECMO and four (22%) were receiving vasopressor support. The median ferritin level was 1953 ng/ml and 13/18 (72%) of patients were lymphopenic. The median baseline SpO2/FiO2 was 202 (range 110–336). The median duration from symptom onset to infliximab-abda therapy was 10 days (range: 5–29 days), and the median duration from hospitalization to infliximab-abda therapy was 2 days (range: 0–7 days).

Following infliximab-abda therapy, 16 patients (89%) met the primary endpoint of sustained improvement in SpO2/FiO2 of ≥50 for at least 48 h and 15 patients (83%) had resolution of respiratory failure to room air or nasal cannula (NC) oxygen supplementation. The median time to the primary endpoint was 4 days (range: 1–12 days). The median support duration among patients requiring NC was 3 days (range: 1–4 days), for HFNC 2.5 days (range: 2–6 days), and MV 10 days (range: 5–45 days) (Fig. 2). One patient on ECMO was successfully de-cannulated after 16 days. One patient on MV, with prior steroid and tocilizumab-failure, was successfully extubated to full recovery of respiratory failure on room air. No patient received a second dose of infliximab-abda. Fifteen patients (83%) were alive at the end of the 28-day study period, with one death occurring on Day 34. Fourteen patients (78%) were discharged after a median of 8 days (range 1–52 days), 3 on oxygen supplementation via NC, and 11 on room air.

By Day 3, there was a significant decline from elevated baseline levels in a subset of pro-inflammatory and immunomodulatory cytokines, which have been implicated in the pathogenesis and/or adverse outcomes from severe COVID-19 illness, including IP-10, IL-10, IL-27, TNF-α, and IFN-γ [1,5,8,16–18] (Fig. 3, Supplemental Table 1). Among patients with elevated IL-6 levels at baseline, including one without steroid exposure, sharp declines at Day 3 were uniformly observed (Fig. 3, Supplemental Figs. 1 and 2). CRP and ferritin were significantly lower as well. Reduction of the CXCR3-ligands IP-10 and CXCL-9 [8,16], IL-27, CRP, and ferritin at Day 14 were sustained although only IP-10 stood up to adjustment for multiple comparisons among

Table 1. *Patient characteristics and clinical outcomes following infliximab-abda*

	TMC-OFF	TMC-001	TMC-002	TMC-003	TMC-004	TMC-005	TMC-006	TMC-007	TMC-008	TMC-009	TMC-010	TMC-011	TMC-012	TMC-013	TMC-014	TMC-015	TMC-016	TMC-017
Age (range, years)	>60	>60	21–40	>60	41–60	>60	41–60	>60	41–60	>60	>60	41–60	>60	>60	41–60	41–60	>60	41–60
Sex	Female	Male	Male	Male	Male	Male	Female	Male	Male	Male	Male	Female	Male	Male	Female	Male	Male	Male
Race or ethnic group minority (Yes/No)	Yes	Yes	No	Yes	Yes	No	Yes	No	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	Yes
Charlson Comorbidity Index	6	6	0	4	1	3	0	3	1	2	3	1	9	4	2	2	5	2
Baseline laboratory values																		
Lymphocyte vount (K/ul)	0.8	0.8	1.4	0.7	1.3	0.2	0.4	0.4	0.5	0.9	0.7	0.8	1	1.2	0.4	0.5	0.7	1
LDH (IU/l)	503	372	544	403	375	313	431	493	415	321	613	490	595	835	313	361	599	339
Ferritin (ng/ml)	4773	1407	2244	1598	1273	2013	352	2128	343	6279	2063	2008	2782	1898	221	2369	1122	626
D-dimer (ng/ml)	1969	710	264	420	183	353	343	267	491	<150	485	998	2199	531	176	194	843	<150
CRP (mg/l)	33.6	243.2	64.1	200.1	54.4	108.6	233.6	104.3	58.4	57.9	164.4	54.3	140.3	29.7	214.3	26.9	50.7	7.87
Vasopressor support	Yes	No	No	Yes	No	Yes	No	No	No	No	No	No	No	Yes	No	No	No	No
Baseline SpO2/FiO2	230	188	332	111	214	145	261	209	196	182	134	271	110	93	336	220	243	139
Oxygenation support*																		
Time of enrollment	MV	HFNC	NC	MV	NC	MV	HFNC	HFNC	MV	HFNC	MV	HFNC	ECMO	MV	NC	HFNC	HFNC	HFNC
Maximum support	MV	HFNC	NC	MV	NC	MV	HFNC	HFNC	MV	HFNC	MV	HFNC	ECMO	MV	NC	MV	MV	HFNC
Discharge or death	NC	RA	RA	RA	RA	RA†	RA	NC	RA	NC	MV	NC	RA†	MV	RA	RA†	MV	RA
Symptom onset to infliximab-abda (days)	29	15	12	13	5	27	5	9	10	10	7	8	10	12	11	8	20	12
Admission to Infliximab-abda (days)	6	3	5	1	3	7	2	2	2	2	0	1	2	3	1	2	1	5
Hospital duration following infliximab-abda (days)	21	8	1	14	5	47	7	12	12	7	22	4	52	10	4	37	34	9
Primary endpoint met (days)	Yes (12)	Yes (3)	Yes (1)	Yes (3)	Yes (3)	Yes (3)	Yes (6)	No	Yes (6)	Yes (3)	Yes (6)	Yes (5)	Yes (4)	Yes (2)	Yes (4)	No	Yes (12)	Yes (4)
Secondary infections	Yes	No	No	Yes	No	Yes	No	No	No	No	Yes	No	Yes	Yes	No	Yes	Yes	No
Survival to discharge	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes	No	Yes	Yes‡	No	Yes

RA, Room air; NC, Nasal Cannula; HFNC, High-Flow Nasal Cannula; MV, mechanical ventilator; ECMO, Extracorporeal membrane oxygenation.
*Within 24 h of trial enrollment.
†Via tracheostomy.
‡Ventilated at Day 28.

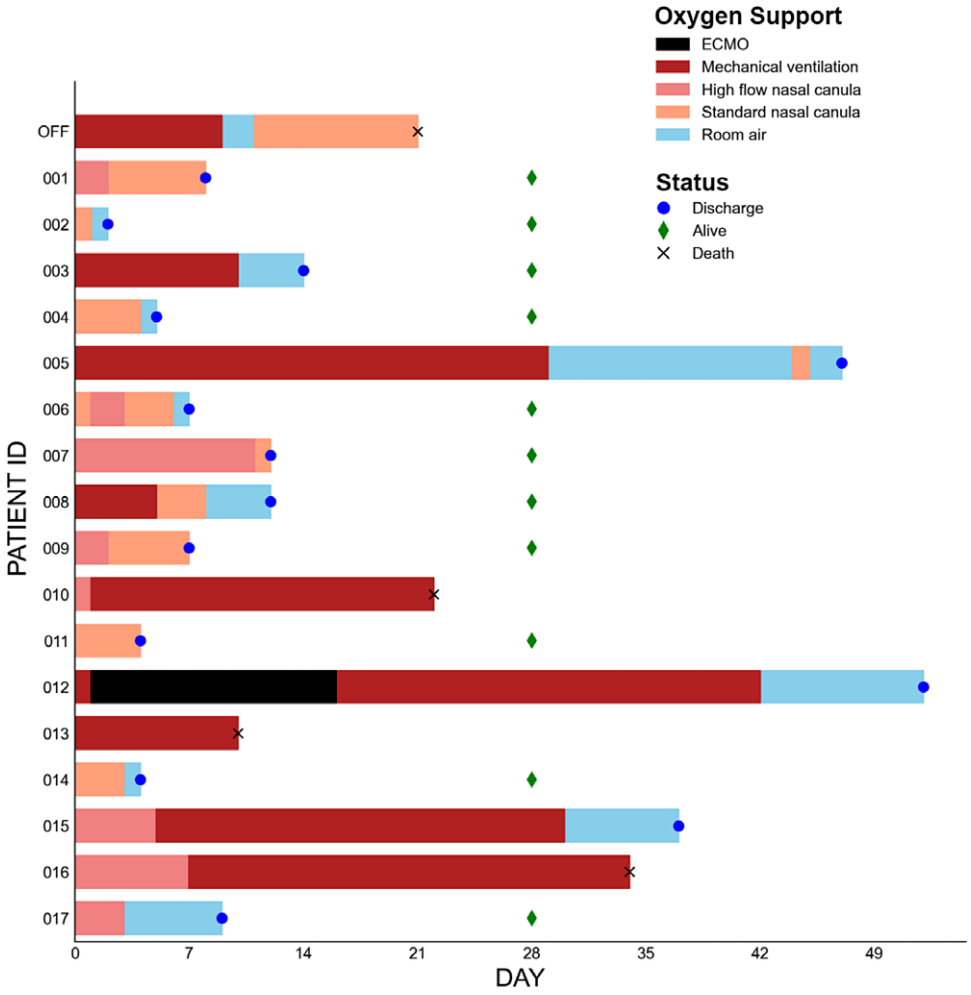

Fig. 2. Changes in oxygen support status following infliximab-abda treatment. Colored bars indicate the maximal level of oxygen support for each individual following treatment with infliximab-abda. The status of the patient at the last follow-up (discharged, alive, or dead) is indicated. ECMO, extracorporeal membrane oxygenation.

the 50 cytokines and markers assessed. The majority of cytokines assessed did not change significantly, either on Day 3 or Day 14 (Fig. 3, Supplemental Table 1).

Among 13 patients who were lymphopenic at baseline, six (46%) had complete resolution of lymphopenia by Day 3, and 11 recovered by Day 14. Absolute lymphocyte counts (normal 1.0–4.0 K/μl) increased from an average baseline of 0.8–1.0 K/μl by Day 3 ($P = 0.038$, n = 17) and 1.7 K/μl by Day 14/discharge ($P = 0.0004$, n = 13). Absolute monocyte counts (normal 0.2–0.8 K/μl) also increased from an average baseline of 0.5–0.8 K/μl by Day 3 ($P = 0.0045$, n = 17) and 1.1 K/μl by Day 14/discharge ($P = 0.002$, n = 13). Accordingly, the strongest correlation between dynamic changes in IP-10 among all tested cytokines between baseline and Day 3 (Supplemental Table 2) was with CXCL-9 (Pearson r: 0.653, *P*-value: 0.004, n = 17), in particular among lymphopenic patients (Pearson r: 0.843, *P*-value: 0.0003, n = 13) and those that recovered by Day 3 (Pearson r: 0.98, *P*-value: 0.0006, n = 6) and Day 14 (Pearson r: 0.85, *P*-value 0.008, n = 8 evaluable), whereas this correlation was not detectable among the small number of non-lymphopenic patients (Pearson r: −0.217, *P*-value: 0.782, n = 4). Among the non-lymphopenic patients, IP-10 decline correlated instead with IL12p40, a homodimeric inhibitory subunit of IL-12.

Infliximab-abda was well tolerated. Two patients experienced transient Grade 1 infusion-related reactions. Transaminase (aspartate aminotransferase and alanine aminotransferase) elevations occurred in 11 patients (16 events, 2 Grade 2, 1 Grade 3, 1 Grade 4), but the association with infliximab-abda could not be clearly inferred given the comorbidities and concurrent remdesivir use. The Grade 3 and Grade 4 events resolved fully. Two cases of transient asymptomatic bradycardia (Grade 1) were noted. There were no cases of deep venous thrombosis or pulmonary thromboembolism; all patients were managed per institutional guidelines for prophylaxis with low-molecular-weight heparin. Two cases of asymptomatic bradycardia (Grade 1) were noted, of uncertain association with infliximab-abda. Transient clinical worsening among two patients following rapid lymphocyte recovery in the absence of identifiable infection raised suspicions of an immune reconstitution inflammatory syndrome (IRIS). One patient with suspected IRIS received high-dose steroids with subsequent clinical improvement, but later succumbed to secondary infection.

All active infections were considered as controlled at the time of infliximab-abda administration. Seventeen secondary infections were encountered in eight patients (44%), all of whom were mechanically ventilated or on ECMO for critical respiratory failure. Four patients were diabetic, three had chronic lung disease, seven had hypertension, and all had prior steroid therapy for a median duration of 5 days. The majority of enrolled patients with

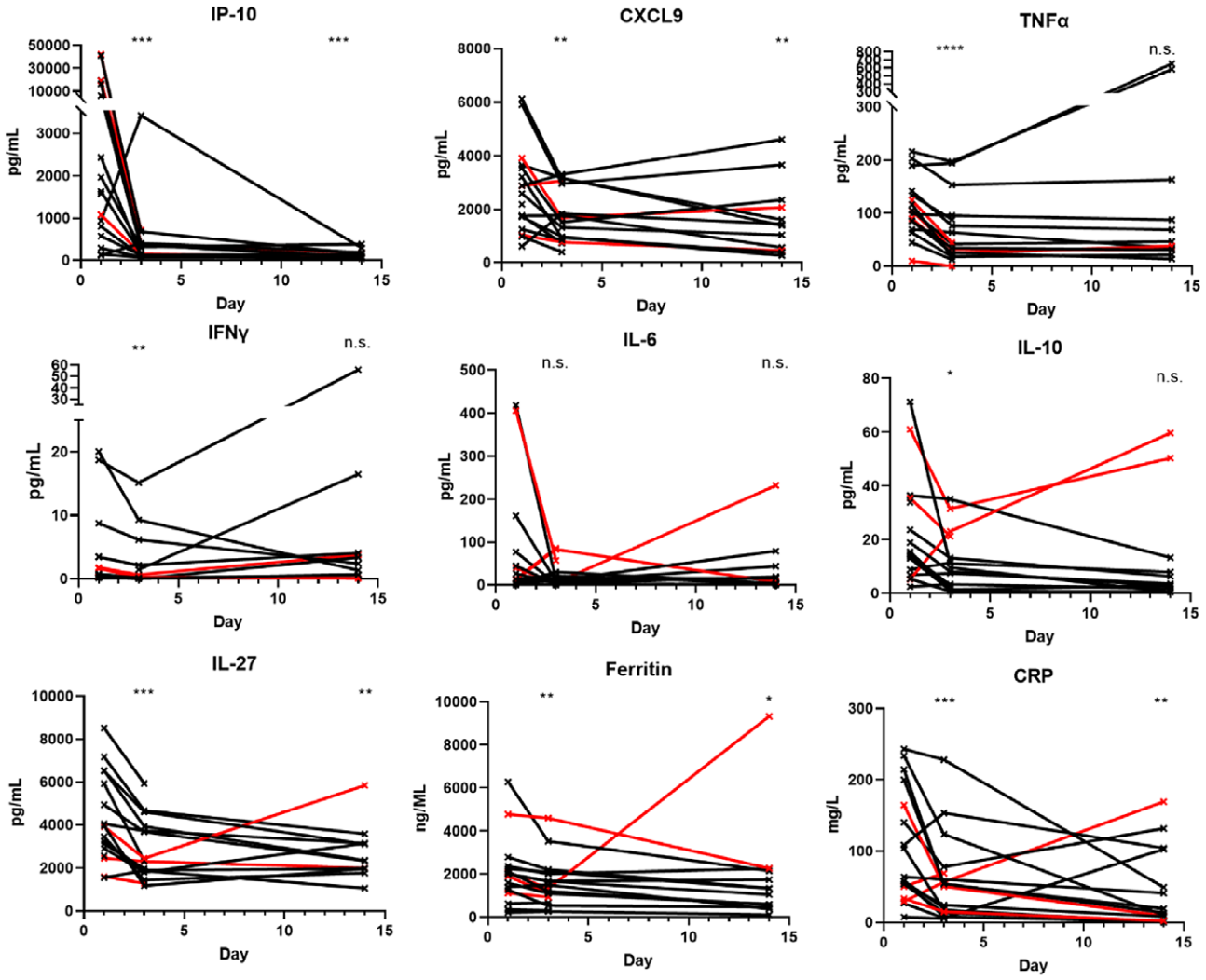

Fig. 3. Decline in key cytokines and inflammatory markers following infliximab-abda therapy. Values from individuals are connected with solid lines, with deceased individuals indicated in red. Statistics: n = 18, paired ratio *t*-test compared to baseline; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$, n.s., not significant.

diabetes (4/5), chronic lung disease (3/4), hypertension (7/12), and those on MV/ECMO support (8/9) were eventually diagnosed with secondary infections. Nine infections (53%) were bacterial, four (24%) were fungal, and four (24%) were viral in etiology (Supplemental Table 3). Median time to diagnosis was 9 days (range: 1–35 days). The most common diagnosis was ventilator-associated pneumonia (41%). Two instances of herpes simplex virus pneumonitis were observed, one of which was diagnosed at autopsy with concomitant necrotizing pyogenic infection in a patient with underlying diabetes, hypertension, and obesity who had received high-dose parenteral steroids. One possible and two probable instances of COVID-19-associated CAPA were encountered, of whom two died. Both deaths occurred in patients with preexisting chronic lung disease. Two patients (11%) were diagnosed with low-level cytomegalovirus viremia, not requiring treatment. One death was attributed to aspiration and upper gastrointestinal hemorrhage in a frail elderly patient, following recovery after prolonged MV. The deaths in the study did not correlate with the improvements with oxygenation as specified in the primary endpoint (Table 1), but with late-emerging events during hospitalization as described.

Discussion

Notwithstanding the uncontrolled nature of these observations, the clinical and translational outcomes following infliximab-abda administration are consistent with the hypothesis that TNFα is a major regulator of the adverse cytokine signature and pathobiology of severe and critical COVID-19 respiratory failure. One of the most striking outcomes was the rapid and sustained decline in the markedly elevated levels of IP-10, potentially generated by the aberrantly amplified synergy between IFN-γ and TNFα as previously proposed from preclinical modeling [7], and which has strongly correlated with adverse outcomes [8,19]. IP-10 has been mechanistically implicated in the pathogenesis of adult respiratory distress syndrome (ARDS) [20] and elevations of IP-10 in COVID-19 may predict the duration of MV [21]. Our pilot data predict that TNFα-antagonist therapy, specifically infliximab-abda, may rapidly control IP-10 and associated pathological inflammatory signaling. Rapid resolution of lymphopenia in our study may reflect the concordant drop in IP-10 and CXCL-9 which drive tissue redistribution of activated memory T-lymphocytes via CXCR3 receptors [7,22]. Upregulation of these CXCR3 ligands in the lung

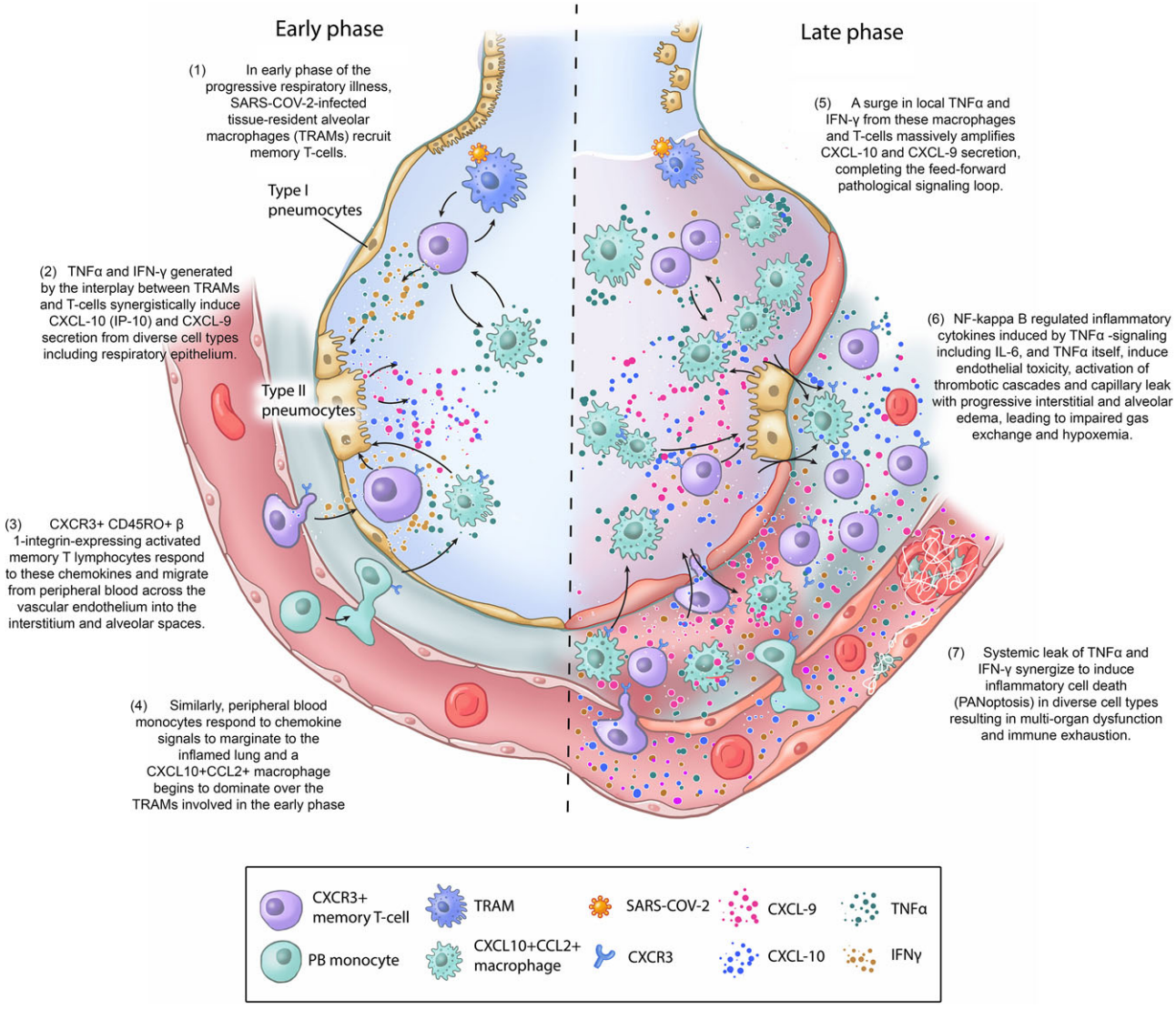

Fig. 4. Concept map of a feed-forward signaling loop implicating TNFα in the pathogenesis of COVID-19 respiratory failure.

microenvironment may similarly drive peripheral depletion of CXCR3+ monocytes with induction of macrophage differentiation, which progressively replaces resident alveolar macrophages [23,24]. Further release of IFN-γ and TNFα by extravasated T-cells [24] and monocytes/macrophages [25] respectively, sustain and amplify the feedforward pathological signaling loop, driving a major escalation in IP-10 and inflammatory cytokines. In this regard, single-cell transcriptomic profiling of bronchoalveolar lavage specimens has identified a striking abundance of CXCL10 + CCL-2+ inflammatory macrophages, a phenotype that is shared across tissues from inflammatory diseases that respond to TNF-antagonists, including rheumatoid arthritis synovium, inflamed Crohn's disease ileum, and ulcerative colitis specimens. Furthermore, this transcriptional phenotype was experimentally induced in human blood-derived macrophages by costimulation with IFN-γ and TNFα specifically, and demonstrated the activation of a combination of JAK-STAT and NF-kappa B signaling pathways which upregulate multiple inflammatory mediators [26]. As with peripheral blood lymphocyte counts, increasing monocyte counts following infliximab-abda therapy in our study may reflect the reversal of this process. The study observations and mechanistic considerations described here (Fig. 4), align with a proposed chemokine immune signature in severe COVID-19, dominated by IP-10/CXCL-9 [19]. Prospective validation of chemokine signatures may allow disease stratification by severity, predict survival outcomes and permit future individualization of therapeutic strategies.

The rapid decreases in TNFα and IL-6 from their elevated baseline values by Day 3 appeared to correlate well with the median time to improvement in oxygenation as defined by the primary endpoint, at Day 4. TNFα and IL-6 may exert endothelial toxicity, generate widespread capillary leak, interstitial and alveolar edema, and prothrombotic cascades [27–31] in pulmonary vascular beds. Rapid reversal of these effects may be at least one explanation for the recovery from respiratory failure.

The study was conducted during a time in which the standards of care among hospitalized patients evolved to integrate remdesivir and dexamethasone, which nearly all patients received prior to

infliximab-abda administration. Although clinicopathological outcomes arguably reflect the combination of these agents, in addition to convalescent plasma and supportive antimicrobial therapies, one patient did not receive prior remdesivir or glucocorticoid therapy. The cytokine profile following infliximab-abda therapy in this steroid-naïve patient exemplifies the rapid and sustained reductions from markedly elevated baseline values of IP-10, IL-6, IL-10, CXCL-9, and TNFα (Supplemental Fig. 2) seen in the majority of patients, demonstrating that this response can be independent of steroid therapy. In vitro models demonstrate the lack of impact of glucocorticoids on IP-10 and CXCL-9 secretion induced by IFNγ and TNFα in airway epithelial cells [7]. This suggests that TNFα-blockade may represent a therapeutic strategy that could offer more precise and durable control of the hyperinflammatory cytokine signature of COVID-19 than broad-spectrum anti-inflammatory effects of steroid therapy. Furthermore, IL-6 could not induce IP-10 and CXCL-9 secretion [7], which may infer a limit to the effectiveness of IL-6-directed therapies in controlling the broader hyperinflammatory picture of serious COVID-19 illness. Importantly, serial cytokine profiling from steroid and IL-6-directed randomized clinical trials have not been reported to allow formal assessment of these points [32–34].

The high rate of clinical recovery in our study population and the favorable results of cytokine profiling discussed above, suggests that dual TNFα and IFN-γ targeting, which has been proposed based on preclinical modeling of lethal COVID-19 [1], may not be required. The attenuated yet elevated levels of CXCL-9 contrasted with uniformly diminished IP-10 levels at Day 14 may reflect restoration of physiological IFN-γ-mediated immunosurveillance, rather than persistent pathological signaling [7,22].

In contrast to the lymphopenic subgroup, a strong association between IP-10 and IL12p40 (and not IL12p70) declines was suggested among the small number of non-lymphopenic patients. IL-12 directs the proliferation of activated T lymphocytes toward a Th1 phenotype. The heterodimeric molecule IL-12p70, equates with IL-12 biological activity, whereas IL-12p40 may antagonize IL-12 and inhibit cytotoxic T lymphocyte generation [35]. Increased IL-12 levels derived from macrophages and dendritic cells contribute to the heterogeneity of cytokine storms [36] and restoration of physiological IL12p40:IL-12p70 ratios may recover T-cell function.

Another noteworthy finding was the early and sustained decline in IL-27 (Fig. 2) which has been co-implicated with TNFα in association with disease severity among older patients, but in a cytokine network distinct from TNFα [18]. IL-27 may drive an IFN-γ/TNFα-independent program of immune exhaustion, via potent induction of Tim-3 and IL-10 [37]. In keeping with this potential mechanism of treatment resistance to TNFα-antagonists, two of four patients who died were evaluable with cytokine assays at Day 14; both had rising levels of IL-10, one with a rising IL-27 (Fig. 3).

It should be noted that the primary endpoint of the study, an arbitrary threshold of sustained improvement in oxygenation in patients with severe and critical respiratory failure, did not eventually predict for recovery or survival at 28 days. Furthermore, variable deployment of measures such as proning, airway management, positive airway pressure, and flow rates, independent of estimated Fi02 may further mitigate the predictive value of this endpoint. The secondary endpoints of sustained recovery from severe and critical respiratory failure and 28-day mortality are more definitive outcomes.

Historically, short-term TNF-antagonist therapy has been studied in the critical care setting of septic shock, with a small survival benefit as assessed in meta-analyses [38,39]. Longer term use of TNF-antagonists has been associated with an increased risk of a variety of infections including fungal and mycobacterial illnesses, particularly when combined with steroid therapy [40,41]. The frequency and spectrum of secondary infections and related deaths observed in this study require additional scrutiny in randomized studies of TNF-antagonists in severe and critical COVID-19, with particular caution among patients with preexisting lung disease and/or multiple comorbid factors such as diabetes or prolonged/high doses of steroids, while on MV [42,43]. Herpes simplex reactivation has been associated with prolonged MV and may be routinely underdiagnosed [44,45]. Prospective polymerase chain reaction (PCR)-based evaluation of tracheal aspirates in patients with critical COVID-19 illness on MV for longer than 7 days demonstrated herpesviridae (herpes simplex, cytomegalovirus, or both) reactivation in 18/38 (47%) patients. Patients with herpesviridae reactivation had longer durations of MV (23 vs. 9 days) [44]. Although a retrospective analysis suggested that the use of antiviral therapy improved outcomes among mechanically ventilated patients with evidence of herpes simplex reactivation in the respiratory tract [46], a prospective randomized trial of acyclovir among mechanically ventilated patients with oropharyngeal reactivation of herpes simplex could not demonstrate clinical benefit [47]. The value of routine surveillance is therefore uncertain. Taken together, an association with infliximab-therapy with herpesviridae reactivation in the COVID-19 population cannot be readily inferred. Similarly, estimates of CAPA in COVID-19 have varied widely, from 3–5% to 35% of patients with ARDS with only a small subset of these meeting a stringent tissue-based definition of proven infection [13,43]. Diffuse inflammatory lung injury, chronic lung disease, steroids, antibiotics, lymphopenia, and immune exhaustion may all predispose to colonization and invasive aspergillus infections. Unfortunately, autopsy permission was granted in only one of three infection-related deaths on study and suspected cases of CAPA remained formally unproven. Recommendations for surveillance, prophylaxis, and therapy for CAPA in COVID-19 are evolving and harmonization of practice may generate more reliable outcomes data [13,43]. Altogether three patients on our study had documented bloodstream infections, two with cytomegalovirus viremia and one with a positive serum PCR result for herpes simplex.

A placebo-controlled randomized clinical trial conducted by the National Institutes of Health (ACTIV-1, NCT04593940) features eligibility criteria and a treatment plan similar to this study, integrating an infliximab arm combined with remdesivir, and permitting dexamethasone and convalescent plasma. The principal endpoint of ACTIV-1 is an improvement in time to recovery within 29 days with mortality as a secondary endpoint. Given that dexamethasone is likely to be widely employed in both control and experimental arms, whether TNFα-antagonist monotherapy will offer greater precision, clinical efficacy, and safety over dexamethasone or other cytokine-directed therapeutics in severe and critical COVID-19 respiratory failure may remain an open question. While serial cytokine assays tethered to cellular markers of immune exhaustion can shed additional light on disease heterogeneity, with correlates of response and resistance to TNFα-antagonists, the importance of survival as the optimal principal endpoint of adequately powered trials has been emphasized [34].

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