Therapeutic blockade of granulocyte macrophage colony-stimulating factor (GM-CSF) in COVID-19-associated hyperinflammation: opportunities and challenges

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Abstract:

The COVID-19 pandemic is a global public health crisis, with considerable mortality and morbidity exerting pressure on healthcare resources, including critical care. An overexuberant host inflammatory response, in a subgroup of patients with severe COVID-19 may contribute to the development of acute respiratory distress syndrome (ARDS) and multi-organ failure. Timely therapeutic intervention with immunomodulation in a putative "window of opportunity" in COVID-19 patients with hyperinflammation, may prevent disease progression to ARDS and obviate the need for invasive ventilation. Granulocyte macrophage colony stimulating factor (GM-CSF) is an immunoregulatory cytokine with a pivotal role in initiating and perpetuating inflammatory diseases. GM-CSF has been proposed as a potential target in COVID-19 and blockade of either GM-CSF or its receptor, using monoclonal antibodies are currently being pursued as potential treatment options. GM-CSF signals via the JAK-STAT pathway and induces the production of IL-6 and other proinflammatory cytokines. As such, GM-CSF could serve as an integral link between T-cell-driven acute pulmonary inflammation and an autocrine, self-amplifying cytokine loop leading to monocyte and macrophage activation. This axis has been targeted in the context of cytokine storm syndromes (e.g. cytokine release syndrome post-chimeric antigen receptor (CAR) T-cell therapy) and in chronic inflammatory disorders (e.g. rheumatoid arthritis). Here we review the evidence underpinning the scientific rationale for therapeutic targeting of GM-CSF in hyperinflammation, in ARDS and in the context of COVID-19-asociated hyperinflammation. Given that GM-CSF also plays a key role in homeostasis and host defence, we also discuss potential risks associated with inhibiting GM-CSF in the context of viral infection and the challenges of conducting clinical trials in this setting, highlighting in particular, the need for a patient risk stratification algorithm.

Key messages:

- Immunomodulation in the "window of opportunity" in a sub-group of patients with COVID-19 and hyperinflammation, may reduce progression to ARDS, obviate the need for intubation and may reduce the high mortality.

- Therapeutic blockade of the pro-inflammatory cytokine, GM-CSF has been pursued in trials of chronic inflammatory disease (rheumatoid arthritis) and cytokine storm syndromes (cytokine release syndrome post-CAR T cell therapy)
- There is emerging evidence to support therapeutic targeting of GM-CSF in hyperinflammation, ARDS and hence COVID-19-associated hyperinflammation and indeed there are several companies pursuing this axis in COVID-19.
- Here we discuss the potential risks associated with inhibiting GM-CSF in the context of a viral infection and the challenges of clinical trials in this setting.

Introduction:

As of 28th May 2020, there have been just over 5.5 million confirmed cases and 353 334deaths worldwide from the pandemic COVID-19[1], caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Acute respiratory distress syndrome (ARDS) and multi-organ failure are major causes of mortality in COVID-19[2]. There appear to be two distinct, but overlapping, phases for therapeutic targeting in COVID-19; an initial viral response, followed by a host hyperinflammatory response[3]. We recently recommended screening for virally-driven hyperinflammation in severe COVID-19 and proposed that immunomodulation in this sub-group of patients may reduce the high mortality[4]. Clinical trials of existing, approved immunomodulatory agents (including inhibition of the interleukin -6 (e.g. tocilizumab) and -1 pathways (anakinra)), are either ongoing or about to start in COVID-19. At the time of writing, six companies were planning and/or seeking regulatory approval for clinical trials in COVID-19 using agents which either target the pro-inflammatory cytokine, granulocyte macrophage colony stimulating factor (GM-CSF) or its receptor (Table 1). Moreover, the Food and Drug administration (FDA) has recently approved emergency, compassionate use of an anti-GM-CSF monoclonal antibody (mAb) in COVID-19[5], despite the absence of clinical trial evidence for this approach in this setting. Here we present the accumulating evidence to support the scientific rationale for therapeutic targeting of GM-CSF in hyperinflammation and in ARDS and hence in COVID-19-associated hyperinflammation. We also discuss potential risks associated with targeting GM-CSF in the context of viral infection, and the challenges of conducting clinical trials in this disease setting.

Hyperinflammation in COVID-19

Hyperinflammation describes a spectrum of disorders and the terminology related to these disorders is heterogenous, however collectively the clinical phenomenon has been called a 'cytokine storm syndromes (CSS). Haemophagocytic lymphohistiocytosis (HLH) is characterised by a fulminant and fatal hypercytokinaemia with multiorgan failure, usually manifesting with cytopenias and deranged liver function. When caused by genetic abnormalities it is referred to as primary or familial HLH (fHLH). Secondary HLH (sHLH) is a hyperinflammatory syndrome triggered by infection, rheumatic disorders and malignancy (usually lymphoproliferative disorders). Cytokine storm syndromes can be termed macrophage activation syndrome (MAS) when associated with rheumatic disease and macrophage activation-like syndrome (MALS) in sepsis and cytokine release syndrome (CRS) following chimeric antigen receptor (CAR-) T cell therapy)[3]. In severe COVID-19, a subset of patients appear to exhibit evidence of hyperinflammation and could therefore potentially benefit from immunomodulation.

ARDS occurred in approximately 30% of hospitalised patients with COVID-19 in cohort data from Wuhan, and is associated with high mortality[6]. ARDS is a heterogeneous clinical disorder characterised by refractory hypoxaemia and carries a mortality of 35-55%, despite supportive standard of care, including low tidal volume ventilation. ARDS is defined by the development or worsening of hypoxaemia in the presence of bilateral pulmonary infiltrates and develops most commonly in response to community-acquired pneumonia. Clinical trials of pharmacological agents in ARDS have been disappointing but unbiased latent class analysis of clinical and biomarker characteristics from randomised controlled trial data, identified two distinct ARDS subphenotypes (a hypoinflammatory and hyperinflammatory endotype with distinct clinical characteristics, biomarker profiles, clinical outcomes and treatment responses[7]. Therefore, it is increasingly recognised that a tailored therapeutic approach to individual ARDS patients will be required to improve clinical outcomes.

The clinical presentation of severe COVID-19 appears to be a unique and has been the subject of much discussion. Emerging data suggest that the hyperinflammatory response in COVID-19 does not fit the classical profile of sHLH/CRS; although ferritin levels predict mortality in COVID-19[2], the ranges are lower than observed in sHLH, and the clinical syndrome is lung dominant, often without significant cytopaenias. Of note, lymphopenia is almost universal in severe COVID-19, but the lymphocyte lineage is not classically affected in sHLH and in the context of COVID-19, may therefore be a consequence of a viral driver. There is also

discussion regarding whether patients with COVID-19 pneumonia, present an atypical form of ARDS[8], but it is now increasingly felt that ARDS associated with COVID-19, may not be dissimilar to the phenotype associated with other viral drivers. The management approach for COVID-19-associated ARDS has recently been proposed and is continuously evolving as clinical experiences accumulates[9].

Granulocyte macrophage colony stimulating factor (GM-CSF)

GM-CSF was originally defined as a haemopoietic growth factor due to its ability to form colonies of granulocytes and macrophages *in vitro* by promoting proliferation and differentiation of bone marrow progenitor cells[10]. It later became apparent that GM-CSF could act on mature myeloid cells, such as macrophages and neutrophils, as a pro-survival and/or activating factor with a role in inflammation. Unlike other members of the CSF superfamily of pleiotropic growth factors, macrophage CSF (M-CSF) and granulocyte CSF (G-CSF), GM-CSF does not appear to play a role in steady state myelopoiesis[10]. Instead, GM-CSF is recognised to play a key role in tissue inflammation, with mounting evidence that it contributes to the development of autoimmune and inflammatory diseases, including Th17-driven diseases[11].

GM-CSF is expressed locally in tissues such as the lung, gut and skin but is virtually undetectable in the systemic circulation[12]. Multiple cellular sources of GM-CSF have been described. In the healthy lung, GM-CSF is secreted by alveolar epithelial cells (AEC) type II (Figure 1A) and plays a key role in the maturation and function of alveolar macrophages, including surfactant catabolism; congenital or acquired GM-CSF deficiency may lead to pulmonary alveolar proteinosis (PAP), due to dysregulated surfactant clearance[11]. GM-CSF has an important host defence function in the maintenance of the integrity of the alveolar capillary barrier[13,14], as well as an immunostimulatory protective role in pathogenic clearance in the context of bacterial and virally triggered pneumonia/ARDS[15-17].

There is growing evidence that GM-CSF is produced and acts locally at sites of tissue inflammation. T cells appear to be the most prominent producers in tissue inflammation, but epithelial cells, endothelial cells, fibroblasts, stromal cells, and hematopoietic cells can also all produce GM-CSF, commensurate with a role for this cytokine in integrating tissue-regulated

inflammatory cell infiltration, even prior to T cell migration. Local GM-CSF production rises with inflammation and levels of this cytokine have been shown to be increased in synovial fluid and serum from patients with rheumatoid arthritis (RA), in the cerebrospinal fluid from patients with multiple sclerosis[11] and in the bronchoalveolar lavage (BAL) fluid from patients with ARDS[18]. GM-CSF is pivotal to pro-inflammatory cytokine networks[10] (including the cytokine cascade in HLH[19]) and induces the expression of tumour necrosis factor (TNF), IL-6 and IL-23, as well as promoting the differentiation of Th1/17 cells and polarisation of macrophages to an M1-like phenotype[11].

GM-CSF and neutrophils in hyperinflammation (HLH)

There is emerging evidence for a role for GM-CSF in HLH, based on recombinant administration of G-CSF in patients with HLH and experimental evidence from mouse models. In clinical practice there are concerns that the G-CSF may worsen HLH; indeed, the observation that administration of recombinant G-CSF exacerbated synovitis, supported the rationale to target the GM-CSF pathway in RA. To the best of our knowledge, there have been five reported cases of exacerbation of existing or *de novo* provocation of HLH following G-CSF administration [supplementary information][20-24]. This warrants the cautious use of G-CSF in neutropenic, critically-ill patients with evidence of HLH.

G-CSF, like GM-CSF, plays a role in macrophage and antigen presenting cell activation and can increase neutrophil chemotaxis and migration, but the response kinetics may differ[25]. GM-CSF is considered to be more pro-inflammatory than G-CSF[25]. It is postulated that G-CSF interacts with G-CSF receptors on monocytes, providing continuous stimulation with pharmacologic rather than physiologic concentrations of growth factor[22]. A feedback mechanism may enhance the process with monokines, causing subsequent clonal expansion and activation of T-lymphocytes. Activated T-cells synthesize and secrete IFNγ, GM-CSF and TNF. These factors interact with the GM-CSF receptor and other receptors on the monocyte, providing additional and continuing stimulation of monocytes

Additionally evidence for a role for GM-CSF in HLH comes from murine models of both primary and secondary HLH, where GM-CSF has been shown to be elevated in untreated, active disease and significantly reduced with treatment using JAK inhibition (JAKi)[26].

Moreover, an HLH-like disease was observed in LCMV infected *INFγ-/-Prf1-/-* double knock-out mice, with a cytokine milieu dominated by IL-6 and GM-CSF, similar to human HLH, challenging the dogma that IFN-γ is mandatory for HLH pathogenesis[27]. A humanised mouse model of post-allogeneic stem-cell transplant HLH demonstrated increased GM-CSF levels, in parallel with other cytokines expected to be elevated in HLH, including IL-6, TNF, IFN-ɣ and IL-18[28].

Experimental evidence for neutrophil-mediated tissue injury in the pathobiology of HLH is also emerging. Splenic neutrophils from a mouse model of HLH upregulated triggering receptor expressed on myeloid cells-1 (TREM-1) and increased the production of intracellular TNF, MIP-1α, MIP-1β, and IL-1β. This phenotype was ameliorated with JAK1/2 inhibition (using ruxolitinib), but not IFN-γ inhibition. Interestingly, poorer survival of mice treated with IFN-γ inhibition (compared with ruxolitinib) was rescued by the addition of neutrophil-depleting antibodies, but not anti-IL-6 or anti–TNF antibodies[26,29]. These data support a role for neutrophil activation in the pathobiology of HLH and highlight possible similarities between HLH-mediated organ injury and severe organ injury seen in other critical illnesses, such as sepsis and ARDS[30,31].

GM-CSF in Acute Respiratory Distress Syndrome (ARDS)

There is growing scientific rationale to target GM-CSF in ARDS. The initial injury response or exudative phase of ARDS is characterized by the release of potent pro-inflammatory mediators, including GM-CSF, monocyte chemoattractant protein (MCP) 1, IL-1α, IL-8, and TNF secreted by resident alveolar macrophages leading to the recruitment of neutrophils and monocytes . Neutrophils have been strongly implicated in the development of ARDS[32] by acting as primary effector cells of bystander tissue injury by releasing proteinases, reactive oxygen species, and neutrophil extracellular traps (NETS), and indeed there have been recent reports highlighting the role of NETosis in COVID-19[33,34] . Moreover, the extent, duration and priming status of neutrophils in alveolar airspaces are strong predictors of outcome in ARDS[18]. Alveolar GM-CSF contributes to acute and persistent neutrophilic inflammation by influencing neutrophil function, including promoting the up-regulation of IgA Fc receptor, formyl peptide (FMLP) receptor, CD11b and leukotriene B4 (LTB4) receptor expression; chemotaxis, phagocytosis, the release of LTB4 and arachidonic acid, NADPH oxidase (NOX) 2-mediated superoxide anion generation; as well as by exerting a marked pro-survival effect mediated by

PI3K-dependent inhibition of neutrophil apoptosis[18,35-38]. A recent study further demonstrated that GM-CSF receptor-α blockade inhibited inflammation in response to inhaled lipopolysaccharide (LPS) in a mouse model of acute lung injury[39].

GM-CSF in COVID-19

The case for GM-CSF as a potential therapeutic target in patients with COVID-19-associated hyperinflammation and ARDS is also gaining strength. In COVID-19, a cytokine signature resembling sHLH (including elevated G-CSF, as well as IL -2, -7, IFN-γ-inducible protein 10 (IP10), MCP, macrophage inflammatory protein 1 alpha (MIP1A), and TNF) is associated with disease severity[40]. Although there are no published BAL data, serum levels of GM-CSF and G-CSF are upregulated in COVID-19 patients compared with healthy volunteers, independent of intensive care status[40]. There is also emerging evidence that expansion of GM-CSF expressing immune cells correlates with disease severity in COVID-19[41]. The percentages of GM-CSF-expressing CD4+ T cells (Th1), CD8 positive T cells, natural killer (NK) cells and B cells are significantly higher in the serum of patients with COVID-19, compared with healthy controls and patients with COVID-19 without critical illness[41]. $CD14^{+}CD16^{+}$ inflammatory monocytes (rarely found in healthy controls) are elevated in the peripheral blood of COVID-19 patients and correlate with the extent of a severe pulmonary syndrome in the ICU[41]. In COVID-19 patients, it is plausible that GM-CSF potentially links the severe pulmonary syndrome-initiating capacity of pathogenic $CD4^{+}$Th1cells ($GM\text{-}CSF^{+}IFN\text{-}\gamma^{+}$) with the inflammatory signature of monocytes ($CD14^{+}CD16^{+}$ with high expression of IL-6) and their progeny[41]. GM-CSF could therefore serve as the integral link between Th1-driven acute lung injury and an autocrine loop of monocytes that further secrete GM-CSF and IL-6[41]. An alternative perspective is that rather than being pathogenic and causative of immunopathology, the GM-CSF-positive lymphocytes, could be responding to persistent viral replication and may indicate a potential protective role for GM-CSF in anti-viral immunity in this disease context.

Inhibition of GM-CSF in COVID-19 and non-COVID-19 (hyper)inflammation

Indirect evidence for a role for anti-GM-CSF therapeutic targeting in COVID-19-associated hyperinflammation comes from cytokine release syndrome (CRS) post-chimeric antigen

receptor T (CAR-T) cell therapy. The immunomodulatory agent, tocilizumab (anti-IL6 receptor mAb) is licensed for CRS, which may be associated with neurotoxicity and is directly related to *in vivo* T cell expansion and marked production of the T cell effector cytokines, IL-6, TNF, CCL2 and GM-CSF. Current evidence suggests that serum levels of GM-CSF, ferritin and IL-2 are associated with neurotoxicity[42]. Indeed GM-CSF levels are elevated in the serum and cerebrospinal fluid in children with coronavirus infection and central nervous system manifestations[43]. A proof-of-concept study using a neutralising anti-GM-CSF mAb (lenzilumab) in a xenograft model prevented CRS and enhanced the efficacy of CAR-T cell therapy[44]. Based on these findings a phase II trial of combining anti-GM-CSF mAb (lenzilumab) in the setting of CAR-T cell therapy is being planned. Next generation CAR-T cells in which GM-CSF has been knocked out by CRISPR/Cas9 gene editing are being developed to minimise the risk of CRS[45].

Given its central role in several chronic inflammatory conditions, there has been considerable interest in targeting GM-CSF in these disease contexts Inhibition of GM-CSF or its receptor is currently being investigated in randomised controlled trials in RA[11], including a 24 week phase III head-to-head comparison trial in RA, aimed at inhibiting GM-CSF (otilimab), IL-6 (sarilumab) and the JAK-STAT pathway (tofacitinib) (ClinicalTrials.gov NCT04134728). To the best of our knowledge, there have been no overt safety concerns identified and no reported cases of PAP in the clinical development programmes of any asset targeting GM-CSF or its receptor to date. In the setting of severe COVID-19-associated hyperinflammation, a short treatment duration with an anti-GM-CSF mAb may be sufficient to switch off hyperinflammation, whilst mitigating potential on-target safety concerns that may be associated with long-term GM-CSF blockade. It is also worth highlighting that GM-CSF induces the production of IL-6 and signals via the JAK-STAT pathway[46]. There are clinical trials of JAK inhibitors in COVID-19 (e.g. NCT04362137) and as mentioned previously, the IL-6 pathway is currently the focus of several clinical trials in COVID-19. The interest in the IL-6 axis was probably fuelled by the purported similarities (e.g. elevated CRP) between COVID-19-associated hyperinflammation and CRS post-CAR T cell therapy. Moreover other agents used in sHLH, e.g. IL-1 inhibition with anakinra[47], are also currently being explored in COVID-19. Targeting GM-CSF could present advantages over selective IL-6 blockade, as inhibition of GM-CSF is may impact both hyperinflammation and ARDS and might be less myelosuppressive and hepatotoxic. JAK inhibitors are licensed for chronic inflammatory

conditions (e.g. RA) and myeloproliferative neoplasms, and are being actively investigated in hyperinflammation[48,49]. However, the potential deleterious effects associated with inhibition of multiple cytokines simultaneously, compared with single-cytokine blockade, as well as a potential increased risk of thrombosis requires careful consideration. This might be particularly pertinent in the setting of COVID-19 in light of accumulating evidence of coagulopathy and autopsy findings of pulmonary microthrombi[50].

Challenges associated with immunomodulation, including targeting GM-CSF, in COVID-19

The potential benefits of targeting GM-CSF in the context of a virally-driven condition such as COVID-19, need to be carefully balanced with potential risk associated with its role in tissue homeostasis, including maintenance of alveolar capillary barrier integrity[13], in host defence and epithelial repair as previously described. Rather than blocking GM-CSF, there is therefore an opposing school of thought that the treatment with GM-CSF could have therapeutic potential in ARDS. In a proof-of-concept study, the beneficial effect of inhaled GM-CSF was first demonstrated in six patients with pneumonia-associated ARDS[51]. Indeed there is a clinical trial of administration of inhaled and intravenous recombinant GM-CSF in COVID-19 (NCT04326920). There is evidence that elevated concentrations of GM-CSF in BAL fluid from patients with ARDS positively correlated with survival[18], however in contrast, a subsequent randomised controlled trial of ARDS (n=130) of therapeutic administration of recombinant GM-CSF) did not improve clinical outcomes[52]. In this study, administration of intravenous GM-CSF daily for 14 days, was not associated with adverse clinical outcomes or increased concentrations of systemically measured cytokines, including IL-6, IL-8, TNF or GM-CSF levels in BAL fluid (measured in selected subjects)[52]. These observations, together with studies demonstrating that exogenous administration of GM-CSF does not exacerbate sepsis,[53] may provide a counter-argument for the approach of targeting GM-CSF in ARDS. First, as acknowledged by the investigators of this study, the study included a smaller than anticipated number of patients treated and the timing of treatment initiation may not have been ideal (i.e. during the recovery phase of ARDS). Moreover, interventional studies with an anti-GM-CSF antibody in COVID-19 are aimed at attenuating the immune dysregulation of hyperinflammation before it leads to the development of ARDS and indeed once ARDS is established, it may indeed be too late for this intervention to provide significant clinical benefit.

GM-CSF also plays a highly context-dependent immunoregulatory role and can modulate dendritic cell differentiation to render them "tolerogenic", which in turn leads to increased regulatory T-cell numbers and function[54]. It is also worth highlighting that GM-CSF influences anti-viral/bacterial immunity and host defence[55], so that GM-CSF blockade could potentially compromise T and B cell recovery. Lymphopenia is an established risk factor for secondary bacterial infections and may predict fatality and worse outcomes in COVID-19[2]. In a recent cohort study of 54 non-survivors with confirmed COVID-19, approximately 50% had secondary bacterial infections[6]. Acquired impairment of neutrophil phagocytosis in critical illness predicts nosocomial infections and is reversed by GM-CSF *ex vivo*. However. administration of GM-CSF in a randomised controlled trial in this setting did not improve neutrophil phagocytosis[56]. There is also a theoretical possibility that immunomodulation in COVID-19 may represent a temporary reprieve and enable viral resurgence, from a circulating reservoir of non-cleared virus. Strategies using anti-microbial prophylaxis (e.g. antibiotics and/or antivirals) may be insufficient to mitigate the risk and could promote resistance. Additionally, it may be inappropriate to extrapolate experience (efficacy or safety profiles) of immunomodulation in hyperinflammation secondary to a drug-related trigger (CRS post-CAR T cell therapy) or immunomodulation in chronic inflammatory disorders (e.g. rheumatoid arthritis) to a viral-setting, as a resident pool of virus could serve as a continuous stimulus for cytokinaemia and immunomodulation may potentially impact viral clearance mechanisms. A deeper understanding of the early pathophysiological events in viral (including COVID-19), bacterial or other causes of ARDS will be imperative in our quest to develop novel therapeutic approaches and the role of anti-GM-CSF in these settings.

Furthermore, the pharmacodynamic effects blocking IL-6 (antagonism directly with tocilizumab, or indirectly with JAKi and anti-GM-CSF mAbs), e.g. rapid suppression of CRP and fever, may not only make secondary infection or viral relapse difficult to detect, but could also provide false reassurance of efficacy of the therapeutic agent, as these mechanistic effects may not always correlate with clinically meaningful outcomes.

Randomised controlled trials are the gold-standard to provide evidence for clinical decision-making. However, since COVID-19 is a new disease entity, it presents a number of urgent challenges, around clinical trial design, patient selection and stratification. It is increasingly felt that early intervention prior to the onset of respiratory failure, will likely prevent poor outcomes[57]. Once patients require ventilatory support, the purported "window of opportunity"

for therapeutic intervention might already have been missed so that patients may 'tip into' an accelerated, state when initiation of treatment may be less effective or even futile[47] (Figure 1B). There is an emerging view that the ideal window of opportunity for immunomodulation might be before patients develop severe disease[57,58] and require escalation to invasive mechanical ventilation (intubation). However robust predictive biomarkers for poor outcomes and an in depth characterisation of the host immune response across disease stages in order to minimise the impact of immunomodulatory agents on anti-viral response, are urgently needed. Moreover, non-intubated patients would also present a lower-risk in terms of opportunistic or nosocomial infections, compared with intubated patients, as the latter may have several artificial indwelling catheters (e.g. endotracheal tubes, vascular access lines, urinary catheters) that could act as a nidus for infection.

As mentioned previously, strategies targeted at specific endotypes in ARDS are felt to be essential for optimal clinical outcomes. It is worth commenting that the apparent 'failure' of large clinical trials in critical care, has been attributed to the inclusion of heterogenous, non-stratified patient populations. Indeed the re-analysis of clinical trials in both ARDS (statins[59]) and sepsis (anakinra[60]) have shown potential benefit in specific sub-groups. The identification of COVID-19 patients at highest risk of a poor prognosis, with a modifiable clinical outcome and most likely to benefit from immunomodulation, will minimize exposing COVID-19 patients who could recover on their own, to the potential risks associated with immunosuppression. Stratification of COVID-19 patients for immunomodulation is however extremely challenging in the absence of robust predictive biomarkers to identify those with poor prognosis, that are likely to progress. It is important to maintain a measured clinical and scientific equipoise in the face of a rapidly evolving global pandemic. The bioethical stance of nonmaleficence, needs to be balanced against the risk of withholding potentially life-saving immunomodulatory treatment in a patient population with likely high mortality and limited treatment options. The identification of new therapeutic approaches beyond existing, licenced immumodulatory agents would also address potential issues regarding drug shortages for COVID-19 clinical trials, and also for patients who are dependent on these medications to control chronic conditions. Ongoing and future clinical trials will provide the much needed evidence for the safety and efficacy of these agents in this setting, and their results are eagerly awaited.

Conclusions

COVID-19 is a major global public health crisis, with considerable mortality and morbidity exerting inordinate pressure on healthcare resources, including ICU beds and ventilators. Cross-specialty collaboration and randomised controlled trials will be essential to assess the impact of therapeutic blockade of GM-CSF on both the hyperinflammation and ARDS, as well as host defence and potential risk in COVID-19. Early intervention with immunomodulation in appropriately stratified patients, with careful consideration of the benefit : risk profile, may halt disease progression, obviate the need for mechanical ventilation and reduce mortality in COVID-19.

Figure 1. The role for GM-CSF in homeostasis, viral response and inflammation and a "window of opportunity" in hyperinflammation for optimal treatment intervention

A) GM-CSF plays an important homeostatic role in the maturation and function of alveolar macrophages, which clear and catabolise surfactant, as well as host defence. In response to viral insults, Alveolar epithelial cells (AEC) type II secrete GM-CSF which improves the innate immune responses of myeloid cells, in particular alveolar macrophages. In severe inflammatory states, GM-CSF production is upregulated by AEC type II and monocyte-derived M1-like macrophages, stimulating IL-6 production from CD14+/CD16+ inflammatory monocytes, increasing Th1/17 T cells and driving the recruitment and priming of neutrophils. The resultant, autocrine positive feedback loop of GM-CSF production, further perpetuates the inflammatory milieu.

B) Hyperinflammation can be triggered by an inciting trigger (e.g. SARS-COV2 infection) and can progress from an early, indolent state to a fulminant and fatal hypercytokinaemia. Withholding potentially life-saving immunomodulatory treatment until a patient is intubated, may result in a missed "window of opportunity" for optimal therapeutic intervention.

Table 1 Clinical trials using agents targeting GM-CSF or its receptor in COVID-19

Target	Drug	Company (headquarter)	Phase	ClinicalTrials.gov identifier (if available)	Notes from Press Releases
GM-CSFRα (receptor)	Mavrilumab	Kiniska (U.S.A.)	-	NCT04397497	Prospective, interventional, single-active-arm, single-centre pilot experience of six patients with worsening pulmonary involvement and COVID-19 with biological markers of systemic hyperinflammation status treated with a single IV dose of mavriliumab[61]. All six patients showed early resolution of fever and improvement in oxygenation within 1-3 days and 3/6 patients were discharged within 5 days[61].
GM-CSF (ligand)	Otilimab	GSK	II	NCT04376684	Multi-centre, double-blind randomised, placebo-controlled trial of a single dose of otilimab in 800 patients
	Lenzilumab	Humanigen (U.S.A)	III	NCT04351152	FDA approval for Phase III study[62] FDA approval for emergency compassionate use[5]
	Namilumab	Izana Bioscience (U.K.)	-	-	Two centre compassionate use study planned in Italy[63]
	Gemsilumab	Roivant (Switzerland)	-	-	First patient dosed in the BREATHE trial; an adaptive, randomized, double-blind, placebo-controlled multi-centre trial expected to enrol up to 270 patients with acute lung injury or ARDS[64].
	TJ003234 (TJM2)	I-Mab (China)	-	NCT04341116	FDA IND (investigational new drug application') clearance approved[65]

Search strategy:

Search of the PubMed database, using the search terms "GM-CSF", "haemophagocytic lymphohistiocytosis", "macrophage activation syndrome", "cytokine release syndrome", "COVID-19", "ARDS", "Acute Respiratory Distress Syndrome" for full English-language publications, published until 28th May 2020.

Declarations of Interest:

PM is an MRC-GSK EMINENT (Medical Research Council-GlaxoSmithKline EMINENT) clinical training fellow with project funding outside the submitted work. PM receives co-

funding by the NIHR University College London Hospitals Biomedical Research Centre (UCLH BRC). JCP, JJM and JDI have no conflicts of interest. IBM reports grants and personal fees from GSK, during the conduct of the study. CS reports non-financial support from GlaxoSmithKline, grants from MedImmune, outside the submitted work; and is Chief Investigator of a GSK sponsored clinical trial of Otilimab, an anti-GM-CSF monoclonal antibody, in the setting of severe COVID.

Author contribution statement:

PM and RC drafted the manuscript. All authors contributed to discussion, revised and approved the manuscript. We confirm that all figures and tables were produced specifically for the purpose of this manuscript and have not been published previously.

Appendix: Supplementary Information:

Reported Cases of G-CSF administration exacerbating HLH

1) A 16 year old female with JIA with HLH treated with ciclosporin and 1mcg/kg of G-CSF for neutropenia. She had a relapse of HLH, when the G-CSF dose was increased to 5mcg/kg and an improvement with discontinuation of G-CSF[20].
2) A 75 year old male with exacerbation of his myelodysplastic syndrome (MDS) -related HLH (as evidenced by thrombocytopenia, splenomegaly and deranged liver function tests) after initiation of G-CSF 300mcg/day[21]. Thrombocytopenia improved when G-CSF was withdrawn and deteriorated after a re-challenge (albeit at a lower dose of 300mcg three times/week). A similar pattern was seen after a further withdrawal and re-challenge using G-CSF and GM-CSF.
3) A 71 year old male with known MDS, who developed *de novo* HLH after administration of long-acting G-CSF (pelgrastim) with resolution of haemophagocytosis after cessation of the G-CSF therapy and clearance of the drug[22].
4) A 50 year old male with non-Hodgkin diffuse large B cell lymphoma who developed *de novo* HLH after G-CSF 45MU prophylaxis[23].
5) A 40 year old male with sepsis (possibly due to venomous snake bite) who developed HLH after administration of GM-CSF for severe neutropenia[24].

NB: G-CSF has been used in eight patients with viral associated HLH (VAHS)[66-68], without adverse event, although the dose was very low (100-250mcg/day). GM-CSF is rarely used in clinical practise, but in combination with G-CSF in MDS has been associated with proliferation of histiocytic cells in the bone marrow leading to worsening cytopenia[69].

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