



Early View

Editorial

GM-CSF targeting in COVID-19, an approach based on fragile foundations

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GM-CSF targeting in COVID-19, an approach based on fragile foundations.

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COVID-19 arises as a result of a pathological inflammatory response following infection with the coronavirus SARS-CoV-2. Although the majority of people infected with this virus will experience minimal or mild symptoms, a proportion will go on to develop more severe disease requiring hospitalisation and oxygen therapy. The most severe forms produce acute respiratory failure, necessitating mechanical ventilation or extracorporeal membrane oxygenation (ECMO). The advent of SARS-CoV-2 vaccination has substantially altered the risk-profile of COVID-19 with marked reductions in the severity of illness and hospitalisation. However, for unvaccinated patients and those who do not mount an effective immune response to vaccination, it remains a potentially lethal infection.

Severe COVID-19 is marked by intense immune activation which usually develops days after viral loads have peaked¹. Early transcriptional responses lead to the release of a broad range of immune mediators with a prominent role for type I, II and III interferons². Patients with impaired interferon responses due to factors such as autoantibodies³ or obesity⁴ tend towards a more severe course. A key pathological feature of severe COVID-19 is a macrophage-dominant infiltration of the lungs⁵, and various investigators have noted similarities between the features of severe COVID-19 and secondary hemophagocytic lymphohistiocytosis⁶ where macrophages are thought to play a major causal role⁶. Although incompletely characterised, there is cross-talk between T-lymphocytes and macrophages forming an 'inflammatory network' which appears to underpin severe COVID-19⁷. The early identification of elevations in a broad range of plasma cytokines including Tumour necrosis factor alpha (TNF- α) and interleukins (IL-) IL-1b and IL-6 provided the rationale for successful trials of both pleiotropic immunomodulatory therapy with corticosteroids⁸ and more selective targeting of IL-6⁹. However it is not clear that serum IL-6 levels are predictive of response to IL-6 blockade¹⁰ whilst trials of agents targeting TNF- α ¹¹ and IL-1b¹², as well as those seeking to supplement type I interferons¹³ have not been successful.

Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) plays several critical roles in inflammatory responses. It acts at multiple levels, including on the bone marrow to accelerate emergency granulopoiesis as well as affecting a range of mature immune cells. Its effects include maintaining healthy function of alveolar macrophages¹⁴ and restoration of function in sepsis-induced immune cell failure^{15,16}. GM-CSF acts in immunological niches, so plasma levels are often undetectable even in systemic inflammation, and a failure to detect elevated plasma levels does not automatically invalidate targeting this molecule therapeutically. Elevated levels have been identified in alveolar fluid from patients with acute respiratory distress syndrome, where elevated levels were associated with better outcomes¹⁷, conversely elevated proportions of T-lymphocytes expressing GM-CSF were associated with worse outcomes in sepsis¹⁸. In COVID-19, serum levels are also generally undetectable, but heightened secretion of GM-CSF by T-lymphocytes in the sickest patients has been noted¹⁹. Such observational studies cannot distinguish between a causal role for GM-CSF in driving immunopathology and reactive or even bystander elevation. Animal models of various inflammatory diseases do imply a pathogenic role for GM-CSF²⁰ and multiple anti-GM-CSF therapies are in clinical trials across a range of, mostly autoimmune, conditions²⁰. There are a number of mechanisms whereby GM-CSF may exacerbate or drive pathology in COVID-19, including inhibition of signalling by interferons²¹. Whilst proven

therapies such as tocilizumab and corticosteroids can reduce its secretion^{22,23}, the data from fundamental and translational biology are unclear as to whether GM-CSF is helpful or harmful in COVID-19²⁴. This uncertainty has led to both studies of both inhaled recombinant GM-CSF²⁵ and GM-CSF blockade^{26–30}. The only phase 3 study published to date, the LIVE-AIR trial of Lenzilumab, found a significant improvement in invasive ventilation free survival in the treated group²⁸.

In this edition of the European Respiratory Journal, Patel and colleagues report a two part, phase 3 study of the anti-GM-CSF antibody Otilimab³⁰. They initially included 793 patients in a multi-centre randomised, placebo-controlled trial in adults (age ≥ 18 years) with severe COVID-19 requiring non-invasive or invasive respiratory support with systemic inflammation (C-reactive protein (CRP) or ferritin above normal range). The primary endpoint, proportion of patients alive and free of respiratory failure at day 28, did not differ significantly between the groups (Otilimab 71%, Placebo 67% with a model-adjusted absolute risk reduction of 5.3%, $P=0.09$). However, a pre-defined subgroup analysis did suggest a statistically and clinically significant benefit in older participants (age ≥ 70), where a model-adjusted 19.1% increase in the proportion alive and free of respiratory failure was found following Otilimab treatment, with a reduction in the key secondary outcome of day 60 mortality ($p=0.04$).

To explore this apparent differential effect on age Patel and colleagues went on to conduct part two of the study, also reported in the same paper, restricted to patients over the age of 69. In this second part, 300 older patients were randomised with no benefit of otilimab found (52% of otilimab vs 51% of placebo treated patients reached the primary endpoint, $p=0.86$), and no significant signal to benefit found in day 60 mortality (43% vs 45%, $p=0.69$).

The failure to reproduce the effect seen in the first part's older subgroup, as well as the discrepancy with demonstration of ventilation-free survival benefit in LIVE-AIR²⁸ study beg a number of questions. The first is whether the initial subgroup finding of benefit in older patients was biologically plausible. Age is one the strongest predictors of mortality in COVID-19³¹, so giving a potentially greater modifiable mortality. Chronic inflammation is also seen in older persons, so called "inflammaging"³². This is often accompanied by reduced antiviral responses³², with impaired viral clearance leading to worsening organ failure, in a manner analogous to that recently reported in obesity⁴. However, it is unclear whether GM-CSF is pivotal to these immunosenescent effects and therefore the biological plausibility of the subgroup analysis found in part one is modest at best.

The second question is, why should two monoclonal antibodies targeting the same molecule (Lenzilumab in LIVE-AIR²⁸ and Otilimab in OSCAR³⁰) have apparently different effects on the same disease? The OSCAR trialists demonstrated appropriate target engagement, with Otilimab engaging high proportion of plasma GM-CSF, so the differences are unlikely to be due to failure of pharmacodynamic effect. The divergence may be due to differences in the patients recruited. Whilst OSCAR patients were all receiving more than simple oxygen therapy, in LIVE-AIR over half the patients were on simple oxygen or room air and therefore less severely unwell at the time of enrolment. Interestingly a post-hoc analysis of the LIVE-AIR study suggested that the beneficial effect of Lenzilumab was restricted to patients with lower systemic inflammation (defined by a CRP level of $<150\text{mg/dl}$)³³, however median

levels of CRP did not differ markedly between patients in either part of the OSCAR study and the LIVE-AIR study.

A further plausible explanation for the difference in results is that the LIVE-AIR result²⁸ occurred by chance. Using frequentist statistics with a conventional cut-off of $p < 0.05$ leads, mathematically, to a 1 in 20 chance of a false positive result. An additional method of trial analysis is the fragility index³⁴. The fragility index (FI) indicates how many patients would have had to have a different outcome within the trial setting for the result to have changed. Whilst fragility indices have their limitations and can depend on the statistical method used to calculate them³⁵, a low fragility index indicates that the results are not necessarily robust even if statistically significant results have been reported.

A recent review³⁶ of 47 RCTs in COVID-19 including studies on treatments, vaccines and interventions, found a median FI of the included trials was 4 (1-11), meaning if 4 patients had had different outcomes the studies would have lost statistical significance. The median fragility index of RCTs of pharmacological interventions specifically was lower at 2.5 (1-6) and overall the fragility quotient (FI divided by trial size) of many studies was less than 1% indicating a lack of robustness in individual clinical trials.

The FI for LIVE-AIR is 0²⁸, indicating that the result would be rendered non-significant simply by using a different statistical test (Figure 1 A). We can also use the FI to determine how robust 'neutral' results, such as those reported in the OSCAR trial are. The inverse FI (number of patients needed to give a statistically significant effect by Fisher's exact test) for part one of the OSCAR study was 11 (fragility quotient 2.7%), indicating an improvement in outcome of 2.7% of the cohort would have produced a positive result for Otilimab (Figure 1B). Although the part one subgroup analysis of older patients produced a significant result, it was also a fragile result (Figure 1C). Sub-group analyses should be approached with caution and, as Patel and colleagues rightly did, considered hypothesis generating when the overall result is neutral. Part two of the OSCAR trial was robustly negative – it would have required an increase in positive outcomes of 10% (from 52 to 62%) to bring this aspect of the study into the significant outcome category (Figure 1D). All studies are samples drawn from a population, and therefore prone to error in measurement, the size of that potential error rather than arbitrary probability cut-offs should determine our interpretation of results.

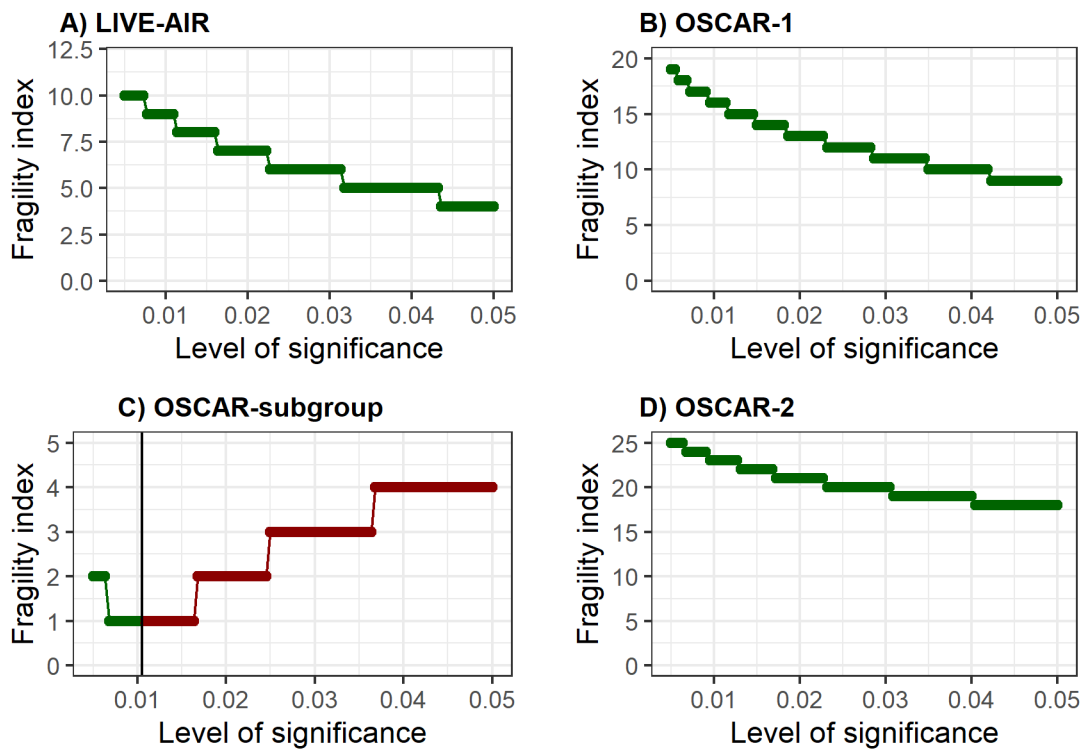


Figure 1: Plots of *fragility index* (y-axis) for each level of significance. A) LIVE-AIR study, B) OSCAR trial part one main cohort C) OSCAR part one - subgroup ≥ 70 years old and D) OSCAR part two. Green shows the areas of non-significance and red significance based on the level indicated on the x-axis based on Fisher's exact method. In C) the vertical line indicates the p-value of the subgroup analysis.

Headline results without formal publication or preprint have been announced for two further large studies of GM-CSF inhibitory strategies, an 807 patient study of GM-CSF receptor blockade with mavrilimumab³⁷ and the ACTIV-5/BET-B study³⁸ which was another trial of Lenizulimab, restricted to patients with CRP <150mg/dl. Both studies were reportedly neutral, although full interpretation of these studies requires more details to be released. The OSCAR trialists should be commended for their transparency in rapidly releasing full details of an industry-sponsored neutral trial in a prominent journal. Rapid publication of trial results has been a notable problem in previous pandemics³⁹.

Although there is a therapeutic rationale in targeting GM-CSF in COVID-19, it is not based on strong mechanistic data from either humans or animal models and relies mostly on inference from other diseases. This is not unusual in COVID-19, given its novelty and the difficulty distinguishing causal from epiphenomenal or consequential effects in observational studies of patients. Although there was an understandable hurry to get therapies to patients, we must ensure that the robustness of both the underlying biological plausibility and trial results themselves are considered when evaluating reports of such therapies. With the current state of evidence there is no justification for the routine use of GM-CSF blockade in COVID-19, and we would suggest a more detailed understanding of its role in the biology of this disease is required before such approaches are trialed again.

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