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TOFACoV-2

TOFAcitinib plus Hydroxycloroquine vs Hydroxycloroquine in patients with early onset SARS-CoV2 (COVID-19) interstitial pneumonia: a multicenter randomized controlled open label trial

EUDRACT: 2020-002035-30

1.0 Background

Multifocal interstitial pneumonia represents the most common cause of admission in intensive care units and death in SARS-CoV2 infections. In our Hospital, similarly to what reported in literature[1,2,] up to 25% of admitted patients with pneumonitis requires mechanical ventilation or oro-tracheal intubation within 5-10 days.

Although information about pathological and pathophysiological features of alveolar-interstitial damage is very limited, few available data, mostly collected on other coronavirus infections with similar clinical behaviour (SARS1, MERS), seem to indicate as primary pathogenic mechanism an intense "cytokine storm" with a consequent inflammatory infiltrate of pulmonary interstitium, macrophage activation, giant cells formation and subsequent extended alveolar damage[3,4,5].

Unfortunately, at present, no effective antiviral treatment is available[6]. The most promising antiviral drug, Remdesivir, because of toxicity and limited availability, at least in Italy, is used only in severe refractory cases as rescue and/or compassionate therapy.

1.1 Hydroxycloroquine in SARS-CoV2 pneumonitis

Although there are no solid data regarding the clinical efficacy of HYQ, based on the results of Gautret' study[7] which showed that viral positivity in respiratory secretions (measured by PCR) is significantly decreased at day 6 in hydroxychloroquine-treated COVID-19 patients (n=26) versus those with supportive care (n=16 controls): 30% positivity versus 87.5%, p<0.001), HYQ has been included in the clinical guidance for patients suspected of/confirmed with COVID-19 in Belgium, France and Spain.

Furthermore, the US Food and Drug Administration (FDA) is allowing the use of the antimalarial drugs hydroxychloroquine to treat coronavirus disease 2019 (COVID-19) since there is no "adequate, approved and available" alternative.

Thus, HYQ has become standard therapy in our Centre as well and its rare toxicity is avoided once the patient is carefully evaluate and vulnerable patients excluded.

1.2 JAK-Inhibitors as promising treatments

Preliminary evidence, however, is accumulating about the efficacy of an aggressive treatment of the corona virus-induced inflammation[8]. Some case series[9] have shown effectiveness of anti-IL6 strategies in reducing the severity of multifocal interstitial pneumonia in patients affected by SARS-CoV2, implying a major role of IL-6 in the pathogenesis of lung damage in these patients and in clinical study recently approved by italian regulatory agency (AIFA)[10], Tocilizumab, which targets IL-6 receptor, is used without concomitant anti-viral therapy.

Furthermore, in this respect it is worth remembering that: i) intracellular signaling, following IL-6 binding to the receptor (IL-6R), occurs mainly via JAK1, which is constitutively bound to the cytoplasmic part of gp130 and activated by gp130 dimerization[11], ii) the generation of knockout mice confirmed that JAK1 is the dominant kinase activated by IL-6 in vivo; iii) gp130 acts as a signaling receptor for additional cytokines such as IL-11, oncostatin M, ciliary neurotrophic factor,

leukemia inhibitory factor, cardiotrophin-like cytokine factor which are considered part of the IL-6 family of cytokines [12].

Moreover, in-vitro studies show that Tofacitinib, both at 5 and 10mg, inhibits IL-6/STAT3 signaling significantly more than Baricitinib 4mg[13]

Thus, based on the above evidence, we believe that blocking JAK1 is clinically rewarding in down-regulating IL-6 driven inflammation in patients with corona-virus infection.

1.3 Tofacitinib

Tofacitinib is a Janus kinase (JAK) inhibitor. JAKs are intracellular enzymes which transmit signals arising from cytokine or growth factor-receptor interactions on the cellular membrane to influence cellular processes of hematopoiesis and immune cell function. Within the signaling pathway, JAKs phosphorylate and activate Signal Transducers and Activators of Transcription (STATs) which modulate intracellular activity including gene expression. Tofacitinib modulates the signaling pathway at the point of JAKs, preventing the phosphorylation and activation of STATs. JAK enzymes transmit cytokine signaling through pairing of JAKs (e.g., JAK1/JAK3, JAK1/JAK2, JAK1/TyK2, JAK2/JAK2). Tofacitinib inhibited the in vitro activities of JAK1/JAK2, JAK1/JAK3, and JAK2/JAK2 combinations with IC50 of 406, 56, and 1377 nM, respectively. However, the relevance of specific JAK combinations to therapeutic effectiveness is not known.

In Italy, Tofacitinib has been approved for the treatment of Rheumatoid Arthritis and Psoriatic Arthritis (5mg bid) and Ulcerative Colitis (up to 10mg bid).

As there is a higher incidence of infections in the elderly and in the diabetic populations in general, caution should be used when treating the elderly and patients with diabetes.

A summary of product characteristics, including details about adverse effects and rules for dose reduction, as approved by European Medicines Agency, is included into Investigator's Brochure.

1.4 Efficacy and safety of Tofacitinib/Hydroxycloroquine combination in the approved indications

Phase 3 studies in patients with Rheumatoid Arthritis have demonstrated that tofacitinib 5 mg and 10 mg twice daily is efficacious and has a consistent safety profile as both monotherapy and in combination with nonbiologic disease modifying antirheumatic drugs (DMARDs) among which hydroxycloquine[14,15,16,17]. The safety profile was confirmed in a post-hoc analysis, in which data from five Phase 3, randomized, double-blind, controlled trials of 6 to 24 months' duration in patients with RA were pooled. All patients had a previous inadequate response to a DMARD. Across these five Phase 3 studies investigating tofacitinib for the treatment of RA, the most common non-serious AEs (excluding infections and laboratory test abnormalities) were headache, hypertension, back pain, abdominal pain, and selected gastrointestinal events. A rate of 10 or more events per 100 patient-years was similar for both the 5 and 10 mg BID doses of tofacitinib with regard to

headache and diarrhea. Overall, the proportions of patients experiencing non-serious, non-infectious AEs were similar for patients receiving tofacitinib as monotherapy or in combination with nonbiologic DMARDs among which hydroxycloroquine18.

More recently these safety data were further confirmed by Cohen SB et al.19 , who report an integrated safety summary of tofacitinib from two phase I, nine phase II, six phase III and two long-term extension studies in adult patients with active RA. Data were pooled for all tofacitinib-treated patients (data cut-off: 31 March 2015). Incidence rates (IRs; patients with event/100 patient-years) and 95% CIs were reported for adverse events (AEs) of interest. 6194 patients received tofacitinib for a total 19 406 patient-years' exposure; median exposure was 3.4 patient-years. IR (95% CI) for serious AEs was 9.4 (9.0 to 9.9); IR for serious infections was 2.7 (2.5 to 3.0) and no difference was detected between the group of patients receiving Constant tofacitinib 5 mg twice daily (N=2342) and those on constant tofacitinib 10 mg twice daily (N=2814). Both groups included a comparable number of patients on hydroxychloroquine and other DMARDs.

In conclusion, safety of Tofacitinib has been established under approved indications in chronic administration20. Increasing in risk of bacterial, viral (herpes zoster) and fungal infections has been well described. In long-term extension of studies investigating the effect of 10mg bid in patients with Ulcerative Colitis and excess of Thromboembolic events and arterial thrombosis appeared. However, these events appear far less likely in short-time administration.

1.5 Tofacitinib 10 mg BID, relative to 5 mg BID, in COVID-19

Efficacy of tofacitinib 10 mg BID, relative to 5 mg BID, on inflammatory response

Tofacitinib has not been evaluated previously for short-term treatment of airway inflammatory response related to COVID-19 or other diseases. In COVID-19 patients with pneumonitis who are at risk for progression to ARDS, rapid onset of anti-inflammatory effects, followed by sustained inhibition of inflammatory mediators over the dosing period is desirable.

Therefore, potential benefits of tofacitinib were assessed based on expected modulation of inflammatory mediators, based on in vitro studies and in vivo characterization of anti-inflammatory effects in RA patients. These data indicate:

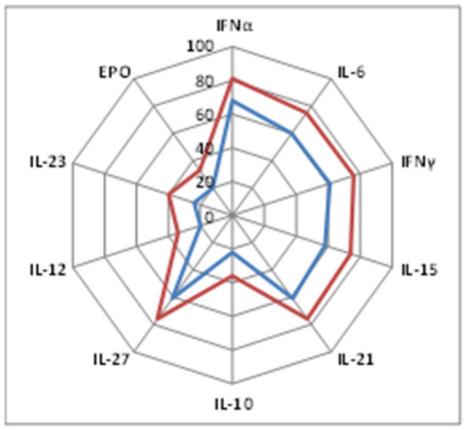
(1) Based on in vitro IC50 values for suppression of inflammatory mediators, the higher systemic exposure of tofacitinib at 10 mg BID, compared to 5 mg BID, is expected to result in greater suppression of inflammatory mediators in vivo.

(2) Dose-response of tofacitinib on markers of systemic inflammation in vivo in RA patients (IP-10, CRP) indicates that 10 mg BID provides substantially higher suppression of these inflammatory markers, and may also provide a faster onset of effect, compared to 5 mg BID.

(1) In vitro studies

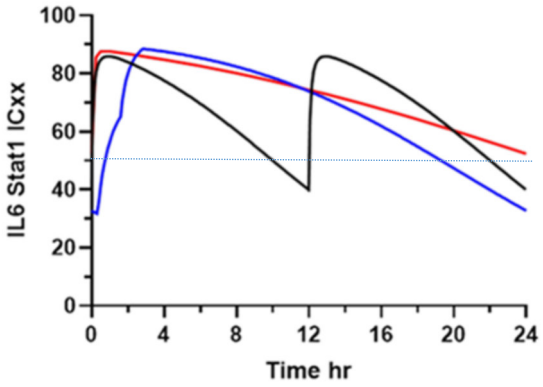
Potency of tofacitinib binding to JAK subtypes and cytokine inhibition were determined in vitro in cell based and whole blood assays21. The expected percent inhibition of various cytokines in humans, at the average plasma tofacitinib concentrations expected at the 5 mg and 10 mg BID dose levels, are shown in the spider plot in Figure 1. At the 10 mg BID dose, approximately 80% suppression of IL-6 may be expected, in addition to substantial inhibition of multiple other cytokines such as IFNγ, IL-15, IL-21, and IL-27. The expected magnitude of cytokine suppression is lower at the 5 mg BID dose, with approximately 60% predicted suppression of IL-6.

Figure 1. Predicted percent inhibition of cytokines in humans by tofacitinib 5 mg BID (blue line) and 10 mg BID (red line) based on in vitro whole blood potency assay and predicted average plasma tofacitinib concentrations



Moreover, evaluation of the time course of predicted IL-6 inhibition over a steady-state dosing interval shows that tofacitinib IR 10 mg BID is expected to provide concentrations above the IC50 value throughout the dosing interval and up to IC85 at peak concentration, indicating sustained pharmacologic activity. Tofacitinib IR 5 mg BID is not expected to provide sustained exposure above the IC50 of IL-6 inhibition over a 24-hour period22(Fig 2).

Figure 2. Inhibitory tofacitinib concentration for a given % inhibition of IL-6 activity (ICxx) over a steady-state dosing interval. Tofacitinib IR 5 mg BID (black line), tofacitinib MR 11 mg QD (blue line), and baricitinib 4 mg QD (red line). The light blue horizontal line represents the IC50 value.



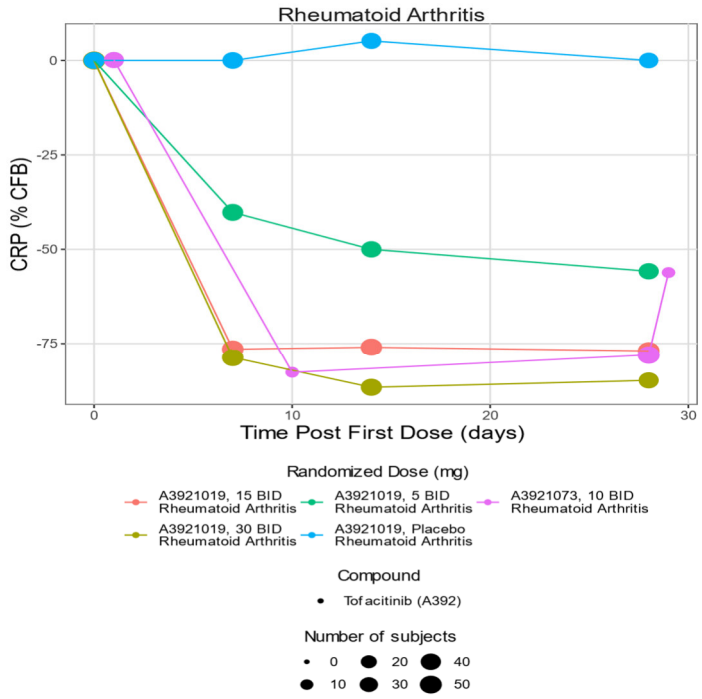
(2) Clinical studies

Biomarkers in the JAK signaling pathway, such as interferon gamma-induced protein 10 (IP-10) and CRP, were measured in multiple clinical studies in RA patients treated with tofacitinib. Both these markers are known to be elevated in COVID-19 patients with inflammatory response.

In Study A3921073 in RA patients, serial IP-10 and CRP measurements were performed early after initiation of treatment and following the end of the treatment phase, to characterize onset and offset of pharmacologic activity of tofacitinib. Onset of pharmacologic activity within the 4 hours after the first dose is suggested by the significant difference from placebo at this time point. This indicates that 10 mg BID may be beneficial in terms of rapid onset of effect

In clinical studies in RA patients, CRP suppression was generally higher at 10 mg BID, compared to 5 mg BID. In Study A3921019, median CRP (% of baseline) was suppressed in a dose-dependent manner, with approximately 50% suppression at 5 mg BID by Day 14, and >75% suppression at higher doses. In Study A3921073, >75% median CRP suppression was observed by Day 10 following tofacitinib 10 mg BID dosing (Figure 3).

Figure 3. Median CRP (% change from baseline) in Studies A3921019 and A3921073 in RA patients



Short-term safety of tofacitinib 10 mg BID, relative to 5 mg BID

In the context of understanding benefit-risk, there are known risks that need to be considered for JAK inhibitors, both as a class and for selective JAK subtypes. The contribution of secondary bacterial infection to COVID-19 related mortality is unknown, and immunomodulation with biologic or targeted synthetic disease-modifying anti-rheumatic drugs (DMARDs) may increase the risk of secondary infections in COVID-19 patients. JAK inhibitors are also known to increase the risk of thromboembolism, therefore use of tofacitinib in this population should be managed in the context of the higher background risk of thromboembolism in COVID-19 disease.

Therefore, thrombosis risk of tofacitinib was assessed in the context of short-term treatment in COVID-19 patients, based on large clinical studies in RA and UC patients. These data indicate:

(1) Short-term exposure to tofacitinib 10 mg BID has not been associated with an increased risk of thrombosis (either arterial or venous) when compared to 5 mg BID.

(2) In pooled RA month 0-3 data, there were no events reported among tofacitinib 5 mg BID or 10 mg BID treated patients who did not have baseline VTE risk factors such as previous VTE, previous heart failure, or age ≥60 years.

(2) No thrombosis events were reported for 10 mg BID patients during the first 14 days of treatment and time to first reported thrombotic event was 72 days and 216 days in RA and UC patients, respectively, treated with 10 mg BID.

Venous or arterial thrombosis

Short-term exposure to tofacitinib 10 mg BID has not been associated with an increased risk of thrombosis (either arterial or venous) when compared to 5 mg BID23, 24. In pooled data from tofacitinib Phase 2 and Phase 3 RA studies, 3 thrombotic events were reported during month 0-3, all events occurring at day 45 (patient receiving tofacitinib 5 mg BID) or later, and no events were reported among tofacitinib 5 mg BID or 10 mg BID treated patients who did not have baseline venous thromboembolic event (VTE) risk factors such as previous VTE, previous heart failure, or ≥60 years of age.

In the clinical development program for UC, no thrombotic events were reported in patients receiving 8 weeks of tofacitinib 10 mg BID induction therapy. In completed studies in RA and UC patients treated with tofacitinib 10 mg BID, there were no events during the first 14 days of treatment and time to first reported thrombotic event was 72 days and 216 days in RA and UC patients, respectively (Table 1).

Table 1. Days of treatment prior to first reported thrombotic event in completed studies in RA and UC patients

	5 mg	**10 mg**	**Pbo**	**MTX**	**ADA**
RA	**45**	**72**	**31**	**25**	**57**
UC	**N/A**	**216**	**3**	**N/A**	**N/A**

Benefit/risk assessment and dose recommendation

Based on the above discussion, there appears to be a meaningful dose-dependent difference in effect on inflammation favoring the higher dose whereas there is no apparent short-term, dose-dependent risk increase for thrombosis. Taken together, in combination with the appropriate risk minimization measures, the benefit/risk is considered more favorable with tofacitinib 10 mg BID relative to 5 mg BID and the higher dose is therefore suggested as appropriate for evaluation in COVID-19 patients. Specifically, the following rationale supports evaluation of 10 mg BID:

At the 10 mg BID dose, plasma tofacitinib concentrations are maintained above the IC50 for IL-6 inhibition throughout a 24-hour dosing interval at steady-state, unlike the lower dose of 5 mg BID. Along with other PD data discussed, the 10 mg BID dose provides better anti-inflammatory effects, than 5 mg BID, for controlling potential increase in cytokines and progression to ARDS.

Tofacitinib 5 and 10 mg BID have been extensively studied in RA, PsA and UC clinical development programs and the short-term and long-term safety profile of tofacitinib 10 mg BID is well characterized.

Tofacitinib 10 mg BID is the approved dose - in the EU and globally - for induction therapy up to 16 weeks in UC patients with potential for longer term use if needed for maintenance of treatment benefit.

Short-term exposure to tofacitinib 10 mg BID has not been associated with an increased risk of thrombosis (either arterial or venous) when compared to 5 mg BID and time to first reported thrombotic event in patients treated with tofacitinib 10 mg BID was 72 days and 216 days in RA and UC patients, respectively.

Protocol risk minimization measures include:

Patients with severe hearth failure (NYHA 3 or 4), history of recurrent DVT or PE and age >65 years are excluded per protocol.

In pooled RA studies, there were no events reported during the first 3 months of tofacitinib 5 mg BID or 10 mg BID treatment in patients who did not have baseline VTE risk factors such as previous VTE, previous heart failure, or age ≥60 years.

All patients will receive Low Molecular Weight Heparin subcutaneously at prophylactic dosage.

Therefore, the benefit/risk of short duration treatment appears more favorable with tofacitinib 10 mg BID relative to 5 mg BID for the control of potential hyperinflammation in COVID-19 and the use of 10 mg BID appears to be justified based on currently available information.

1.6 Potential relevant interactions between Tofacitinib and other drugs in patients affected by COVID 19

Despite this protocol doesn't recommend to use antiviral or antibiotic drugs in COVID-19 patients, investigators have to be aware that adjustment of tofacitinib dose may be required when used in combination with some antiviral or antibiotic drugs. Examples of antiviral drugs that have been evaluated, or are being used, for treatment of COVID-19 patients, and guidance for use of tofacitinib 10 mg BID with each drug, is provided below. However, these examples do not represent a complete list of antiviral drugs used for COVID-19 treatment, and Pfizer will be contacted as needed.

- *Azithromycin:* Tofacitinib dose adjustment is not needed when the antibiotic azithromycin is co-administered, whether alone or in combination with HCQ

- *Remdesivir (not approved in Italy for COVID-19 infection)*: While limited data is available for this nucleoside analog antiviral drug, it is not expected to impact tofacitinib exposure and no dose adjustment for tofacitinib is recommended

- *Favipiravir(not approved in Iatly for COVID-19 infection)*: Available information indicates that favipiravir is not a potent inhibitor or inducer of CYP3A4, and no dose adjustment of tofacitinib is recommended when co-administered

- *Ritonavir/Lopinavir:* Ritonavir and lopinavir are expected to increase tofacitinib exposure due to potent CYP3A4 inhibition. Therefore, when these drugs are co-administered with tofacitinib, individually or in combination, the tofacitinib dose should not exceed 5 mg BID

- *Oseltamivir(not approved in Italy for COVID 19 infection)*: Available information indicates that oseltamivir is extensively metabolized by liver esterases and is not known to cause potent inhibition or induction of CYP3A4. No dose adjustment of tofacitinib is recommended when co-administered
- *Ribavirin(not approved in Italy for COVID 19 infection)*: No dose adjustment of tofacitinib is needed when co-administered with ribavirin.

2.0 Aims

- The main aim of the study (primary outcome) is to verify the effect of adding Tofacitinib to the standard therapy in order to reduce the rate of patients who need mechanical ventilation and/or oro-tracheal intubation.
- Other aims are to verify the safety of TOFA-HYQ combination in these patients and to individuate clinical and/or laboratory factors predictive of good clinical response.

3.0 Study design

Randomized controlled, multicenter, open label, phase 2 pilot study.

The planned study duration is 16 weeks.

3.1 Settings (list of investigators and centers):

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3.2 Population

Inclusion Criteria

- SARS-CoV2 Infection diagnosed by rt-PCR
- CT-scan confirmed interstitial pneumonia
- Hospital admission from less than 24h
- P/F ratio >150 mmHg
- P/F ratio >475 mmHg
- Written Informed Consent

Management of Women in childbirth age

Women in childbirth age must be tested for pregnancy before starting treatment. They will be informed that an effective contraception is mandatory in the 4 weeks following the last dose of the drug.

Exclusion Criteria

- Age <18 ys or >65
- Patients in mechanical ventilation at time of admission
- Severe Hearth failure (NYHA 3 or 4)
- QTc > 470 ms or >500 ms in wide QRS patients
- Severe History of Chronic Ischemic Heart Disease, defined as history of Major Adverse Cardiovascular Event and/or recent (one year) revascularization.
- History of recurrent Deep Venous Thrombosis and Pulmonary Embolism or established thrombophilic conditions (e.g. history of anti-phospholipid antibodies, …)
- Active Bacterial or Fungal Infection
- Hematological cancer
- Metastatic or intractable cancer
- Pre-existent neurodegenerative disease
- Severe Hepatic Impairment,
- History of acute diverticular disease or intestinal perforation
- HBsAg positive and/or HBV-DNA positive patients
- Severe Renal Failure (Creatinine Clearance <30ml/h)
- Active Herpes zoster infection
- Patients with active or latent TB
- Severe anemia (Hb<9g/dl)
- Lymphocyte count below 750/mcl
- Neutrophil count below 1000/mcl
- Platelet count below 50000/mcl
- Pregnancy or Lactation
- History of intolerance to the experimental drugs or excipients
- Degenerative maculopathy or other relevant retinal disease

- Inability to give informed consent (severe transitory or permanent mental impairment, incapacitation)

3.3 Screening and randomization

All patients affected by rt-PCR and CT-scan confirmed COVID-19 pulmonary infection and admitted to the hospital will be consecutively evaluated for inclusion/exclusion criteria and consent.

Reasons for eventual exclusion from the study will be recorded (see screening and randomization sheet)

After obtaining informed consent, eligible patients will be randomized through a centralized randomization.

In details, a data manager not involved in the trial will prepare the permuted block randomization sequence (four patients per block). To minimize allocation bias, a centralized assignment to the treatment will be performed both by web-based platform and by phone. The randomization list will be held concealed for all the investigators.

3.4 Outcome

Primary Outcome

1. Rate of patients needing mechanical ventilation to maintain PaO2/FIO2>150 or, if PaO2 data not available, to maintain SO2>94% with max FiO2 0,5.

Secondary Outcomes

1. Rate of patients needing admission to the intensive care unit for oro-tracheal intubation and/or evidence of Multiple Organ Dysfunction
2. Death
3. Role of some clinical and laboratory factors in predicting outcome (pre-defined list as displayed below)
4. Rate of severe adverse events possibly related to the experimental drugs

Primary Outcome as well as Secondary Outcome#3 will be assessed at day +14, while Secondary Outcomes #1 and#2 will be assessed at day +28

List of factors that will be included in multivariate analysis:

Age, sex, Body Mass Index (BMI), Comorbidities (Diabetes, number of comorbidities), Respiratory Failure at admission defined as PaO2/FiO2<300, Extension of Ct-scan involvement, basal level of serum IL-6, vW-Factor, Thrombomodulin, KL-6 and SP-D (see below)

3.5 Outcome assessment and procedures

Patients will be evaluated at baseline (time 0) and followed for 14 days or until discharge

At baseline and every 24h the following items will be assessed:

CT-scan of the chest (baseline only), Electrocardiogram (baseline and when requested on clinical base), hemodynamic and respiratory parameters (see CRF for details), hemoglobin level, neutrophil, lymphocyte and platelet counts, ALT, arterial blood test (baseline and when requested on clinical base), glycaemia, D-dimer(baseline),pro-calcitonin (baseline), creatinine, CRP, HBsAg (baseline) BNP, CPK and ferritin. At day 7 complete coagulation check will be performed.

At baseline and at day +7 and +14, 7cc of serum will be stored to evaluate the serum levels of: IL-6, Surfactant protein D, KL-6, vWF, Thrombomodulin levels.

Patients who will need mechanical ventilation or ICU transfer within 24h from Hospital admission will be excluded from analysis.

After 14 days from stopping the drug, all patients will be evaluated through direct clinical examination or phone calling (if discharged). Any significant clinical event will be recorded in the CRF.

3.6 Experimental Interventions

Patients will receive Tofacitinib 10mg twice a day per os. Treatment will be started within 12 hours from admission and maintained for 14 days.

Dosage will be reduced to 5mg twice a day in case of moderate hepatic failure (Child-Pugh B)

Hydroxychloroquine will started in the same time at the dose of 200mg three times a day in all patients. The dose will be reduced at 200mg twice a day if body weight is under 50Kg.

3.7 Concomitant treatments

All patients should be treated with Low Molecular Weight Heparin subcutaneously at prophylactic dosage.

In order to reduce risk for herpes zoster re-activation, prophylaxis with acyclovir will be started from day 1 to 14.

Antiviral therapy with ritonavir-lopinavir is not currently used in this condition due to the absence of clinical benefit and its use is not recommended.

3.8 Rescue Therapy

In patients who will need mechanical ventilation, Tofacitinib treatment will be stopped and rescue therapy started according to local protocols.

4.0 Statistics

To test the hypothesis that early administration of tofacitinib will reduce the rate of patients needed of mechanical ventilation from 20% (expected) to ≤5% , 116 patients will be enrolled in the study (power 80%, alpha 0.05, lost to follow-up 3-5%, according to Cochran and Cox, 1957[25]).

All patients will be analyzed in their respective arm of randomization (Intention to treat analysis) if they have taken at least one dose of the drug and have not been mechanically ventilated or intubated within the first 24h.

Continuous variables will be compared using either Student's t-test or Mann-Whitney's U-test, as appropriate.

Categorical variables will be compared using Chi-Square test or Fisher's exact test, as appropriate.

Survival times will be compared using Log-rank test.

Four multivariate logistic regression analyses will be performed using the four outcomes (one primary outcome and three secondary outcomes) as dependent binary variables and the possible prognostic factors (including the arm of randomization) as independent variables.

A significance level alpha=0.05 will be used for all the statistical analyses.

5.0 Experimental Drug

Tofacitinib will be freely supplied by Pfizer in form of 5mg tablet. The commercial packs provided by Pfizer Italy local supply chain will be re-labelled according to GCP Annex XIII label at the trial centers.

6.0 Adverse Events

6.1 Definitions

An adverse event (AE) is any untoward medical occurrence in a study participant administered the medicinal products and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An adverse reaction (AR) is an untoward and unintended response to the investigational medicinal products related to any dose administered, judged by either the investigator or the promoter.

An unexpected adverse reaction (UAR) is an adverse reaction, the nature or severity of which is not consistent with the applicable products information (investigator's brochure).

A Serious Adverse Event (SAE) is untoward medical occurrence or effect that at any dose results in death, risk of death, permanent disability/incapacity, hospitalisation or prolongation of existing hospitalization or need for urgent

medical treatment,or another medically important serious event as judged by the investigator. Further, any unexpected changes in relation to the toxicity profile of the drugs used of grade> 3, as well as adverse event(s) which, although not falling within this definition, are considered unexpected and serious by the Investigator should be reported.

The causality of SAEs (their relationship to all study treatment/procedures) will be assessed by the investigator(s) and communicated to the coordinating centre.

A Suspected Unexpected Serious Adverse Reaction (SUSAR) is an unexpected adverse reaction judged serious by the Investigator and/or Promoter, that is not consistent, either in nature or in severity, with the applicable product information.

Causality assessment between treatment and event will be also reported, according to the rules and definitions stated into Investigators Brochure.

6.2 Regulatory Reporting Requirements for SAEs

Prompt notification by the investigator to the promoter of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study treatment under clinical investigation are met.

The promoter will review all adverse events and issue queries directly to the Investigator reporting the event. The promoter will determine if an event qualifies as a SUSAR.

The promoter has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study treatment under clinical investigation.

The promoter will report all SUSARs to Eudravigilance through the EVCTM, to all

participating Investigators, and manufacturer (Pfizer), within the timelines of the article 17 of the European Directive 2001/20/EC.

Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) and forwarded to investigators as necessary.

6.3 Safety Assessments

Patients will be evaluated for adverse events every day by clinical examination.

Blood examinations will be performed every day. In particular, complete blood counts, ALT, creatinine, C-reactive protein, glycaemia, pro-calcitonin will be checked and reported in the CRF.

6.4 Adverse Events of Special Importance (AESI)

The following adverse events are labelled as AESI

1. Worsening of anaemia, defined as decrease in haemoglobin concentration > 1g/dl in two consecutive determinations
2. Low absolute neutrophil count (< 1000/μl, mild or <200//μl, severe)
3. Low platelet count (<100000/μl, mild or <30000/mcl, severe)
4. Increased ALT (> 3 upper limit of normal (mild) or > 5 (severe))
5. Bacterial infection
6. Herpes zoster infection
7. Any cardiac and vascular event

Investigators have to communicate to PI any AESI within 72h (24h if severe). AESIs will be considered probably drug-related in any case(see: secondary outcome #4 and stopping rule)

6.5 Experimental Drug withdrawal

Experimental drugs will be prematurely stopped:

- If any severe AESI occurs
- If other severe adverse events possibly drug-correlated occurs
- If patient withdraws the consent

7.0 Trial Stopping Rule

The trial will be prematurely stopped if more than three serious drug-related adverse or SUSAR events will be reported.

8.0 Ethics, Quality Assurance and Monitoring

The procedures set out in this study protocol are designed to ensure that the promoter and the Investigators abide by the principles of the Good Clinical Practice guidelines of the International Conference on Harmonization (ICH) and the Declaration of Helsinki in the conduct, evaluation and documentation of this study. The study will be carried out adhering to local legal requirements and the applicable national law, whichever represents the greater protection for the individual. Study protocol, patient information and informed consent will be submitted to the appropriate Ethical Committee for approval. The promoter will inform the appropriate Ethical Committee about any changes in the study protocol which could interfere with the patient's safety. The monitoring activities during pandemia will be primarily or exclusively performed without direct visits in the involved wards.

9.0 Publication Policy

Investigators will publish study results as well as available.

Principal Investigator

Prof Armando Gabrielli

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