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Updated mini-review of the chemistry, pharmacology and validated assays for U.S. FDA approved COVID-19 therapeutic agents

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Abstract:

The fish market in Wuhan, China was the epicenter of Covid-19 pandemic, brought about by the SARS-CoV-2 virus, resulting in significant economic and social worldwide disruption. Although numerous drugs have been indicated for treating the viral infection and/or its symptoms, the U.S. FDA to date has granted approvals for only four therapeutic agents, namely the small-molecules Remdesivir, Baricitinib and two monoclonal antibody combinations amlanivimab/etesevimab and casirivimab/imdevimab]. This review deals with the chemical and pharmacological aspects of these approved therapeutic agents as well as the properties of their reported marketed formulations and biological matrices. Furthermore, it presents a comparison between the cited analytical methodologies for each drug separately. A comprehensive, detailed, mini-overview of Coronavirus authorized therapeutics is also presented.

Key words: *Covid-19, Remdesivir, Baricitinib, Monoclonal antibodies*

1. INTRODUCTION

Coronaviruses [CoVs], or Coronaviridae, are a family of positively enfolded RNA viruses that can infect birds and mammals. Alpha-, Beta-, Gamma- and Delta-CoVs are all known to infect some mammals including humans. However, many CoV infections cause self-controlled symptoms. Infection in humans by two Beta-CoVs, namely SARS-CoV and MERS-CoV resulted in higher than 10 thousand cases with death rates of 37% and 10% for MERS-CoV and SARS-CoV-2 [or "COVID-19"], respectively [1, 2].

In December 2019, atypical pneumonia cases, caused by the previously-unknown Coronavirus, CoV-2, were detected in China [3]. As at May 13, 2022 more than 517,648,631 confirmed cases of COVID-19, including 6,261,708 deaths have been documented due to the CoV-2 virus and its variants [https://covid19.who.int]. Infection is spread through air, on surfaces and incubates with no obvious symptoms during the first 2–14 days post-exposure [4]. Fever, exhaustion, muscle weakness, difficulty in respiration and dry coughing, are the main characteristics of the infection and CoV-2 leads to acute lung injury [5, 6]. In early 2020, the WHO declared Covid-19 to be a pandemic [7].

CoV-2 possesses four essential structural proteins; envelope [E], membrane [M], nucleocapsid [N], and spike [S] proteins; subsidiary [non-structural] proteins; a papain-like protease and three chymotrypsin proteases. The proteases are responsible for the cleavage of viral polypeptides into functional units; and RNA-dependent RNA polymerase [RdRp] which is essential for replication and transcription of the virus [8]. SARS-CoV-2 penetrates living cell by attaching its S-protein to the ACE-2 receptor [9]. Protein-S, PLpro, 3CLpro, RdRp as well as ACE-2 have been targeted in developing antiviral therapeutics [10].

No specific single treatment has yet been developed to treat COVID-19 cases. Researchers have pointed to many therapeutic viral targets in order to develop highly-efficient, least-toxic medications [11]. Mainly however, dealing with infected patients thus far has relied on managing their symptoms. WHO treatment guidelines stress sufficient balanced nutrition, much rest and hydration, in addition to the use of antibiotics [12]. Furthermore, control and prevention by social distancing and elevating immunity have been applied [13, 14].

This presentation addresses FDA-approved drugs which have been reported as having anti-COVID-19 therapeutic effect. Furthermore, their chemistry is reviewed as well as assay methods which are used for Remdesivir, Baricitinib and monoclonal antibodies in their pharmaceutical forms and biological matrices. The

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literature cited herein summarizes the latest updates and lays groundwork for future proceedings.

2. FDA approved COVID-19 therapeutic agents:

2.1. Remdesivir [REMDS]:

REMDS [Veklury®; GS-5734] is a novel antivirulant which was originally developed by Gilead Sciences for the management of Ebola as well as Marburg viral infections [15]. Remdesivir, 2-ethylbutyl [2S]- 2-[[2R,3S,4R,5R]-5-[4-aminopyrrolo [2,1-f] [1,2,4] triazin-7-yl]-5-cyano-3,4-dihydroxyoxolan-2-yl] methoxy-phenoxy phosphoryl] amino] propanoate [Figure 1], is a prodrug of a nucleoside analog that perturbs viral replication. It is an off-white non hygroscopic solid, practically water insoluble, but soluble in ethanol [16].

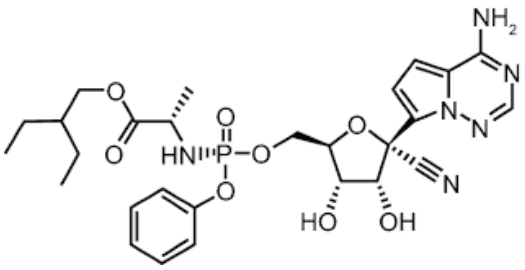

Fig. 1: Chemical structure of REMDS

REMDS is the first and only anti-viral approved by FDA, for hospitalized COVID-19 adults and children over the age of 12 years [weight ≥ 40 kg]. It possesses a complex activation pathway. It metabolizes into [GS-704277] then into [GS 441524], and its major metabolite GS-441524 is instantly changed into the nucleoside triphosphate analog, GS-443902. Overall, it inhibits viral RdRp activity via non-obligate termination of RNA chains, after activation to a triphosphate [17, 18].

The published literature reveals that only LC-MS methods have been applied for REMDS determination in plasma. All of the reported studies focus on the drug's pharmacokinetics and pharmacodynamics. In 2016, Warren et al. [19] developed a LC-MS-MS protocol for a kinetic study of REMDS in uninfected male rhesus monkeys. In 2020, Alvarez et al. [20] determined REMDS and GS-441524 in human plasma using an LC-MS/MS method with the help of a simple protein precipitation [PP] step using a mixture of methanol and zinc sulphate [1.0 M]. A LC-ESI [+]-MS/MS method was also used by Humeniuk et al. [21] to determine plasma REMDS concentrations. Pasupuleti et al. [22] demonstrated a liquid/liquid micro extraction technique coupled with UHPLC/PDA and UHPLC/MS/MS. Recently, Avataneo et al. [23] described UHPLC-ESI[+]-MS/MS, a fast technique [total run time < 2.5 min] to quantify REMDS and GS-441524, in spiked human plasma. The same UPLC system was then employed by Tempestilli et al. [24] for pharmacokinetic evaluation of REMDS and GS 441524 in real patients.

2.2. Baricitinib:

Baricitinib [Olumiant®] is a selective, reversible Janus kinase inhibitor invented by Eli/Lilly Company, originally for treating arthritis and dermatitis[25]. Baricitinib, 2- [1-[ethane sulfonyl]-3-[4-{7H-pyrrolo[2,3-d] pyrimidin-4-yl}-1H-pyrazol-1-yl] azetidin-3-yl] acetonitrile [Figure 2], is an immunomodulatory agent with a significant anti-inflammatory effect [26]. The U.S. FDA has granted EUA for its use simultaneous to REMDS, to treat hospitalized cases [27]. The combination was better than REMDS only in shortening time to recover [28].

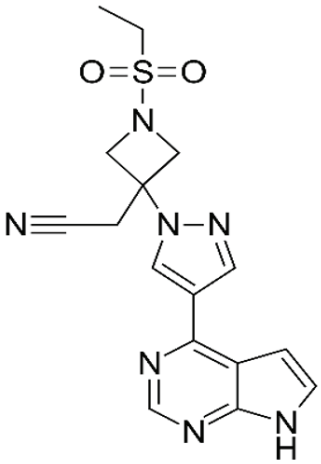

Fig. 2: Chemical structure of Baricitinib

The structure was predicted using artificial intelligence algorithms. Baricitinib inhibits intracellular cytokines that elevate in late cases; including interleukins-2, -6, -10, γ-interferon, and granulocyte macrophage colony-stimulating factor [29-31]. The literature reveals two LC assay methods for its determination in rat plasma, for the drug's pharmacokinetics [32, 33]. The former reported the use of LC/MS/MS for estimation of Baricitinib and Methotrexate [32], whereas the other used UPLC [33]. Recently, a UV-spectroscopic assay was developed for the pure form of the drug and in its dosage form [34]. Furthermore, a RPLC-Diode array detection system was applied for a stability indicating study of Baricitinib [35].

2.3. Monoclonal antibodies:

Monoclonal antibodies are recombinant synthetic proteins that target specific parts of target antigens. They are produced from virus-specific B cells extracted from convalescent plasma or from humanized mice. They show therapeutic efficacy in different viral infections, cancers and different autoimmune diseases [36]. Their antiviral effect is conferred via binding to virus spike proteins thus blocking their binding to ACE2 receptors. This passive immunotherapy is particularly important for outpatient treatment of vulnerable, high-risk individuals or those with mild-moderate infection [37]. As of February 2021, the U.S. FDA has granted Emergency Use Authorization [EUA] for different monoclonal antibodies in COVID-19 treatment. These include combined Bamlanivimab/Etesevimab [38] and combined Casirivimab/Imdevimab [39]. To date, there is no reported methods for analytical quantitation of these monoclonal antibodies.

3. Results and Discussion

Reported Remdesivir assays are limited to biological matrices using a LC-MS technique. Warren et al. [19] determined Remdesivir in the plasma of monkeys with adequate sensitivity, although neither linearity range nor other validation elements were mentioned. Alternatively, Alvarez et al. [20] quantified Remdesivir and its metabolite in human plasma using small plasma volumes and a simple sample preparation procedure. Slightly higher lower limit of quantitation [LLOQ] values were obtained for GS-441524 [5.0 ng/mL]. After the optimization and validation according to European Medicines Agency guidelines, the latter technique was efficiently involved in a kinetic study in a COVID-19 patient after a single dose of Remdesivir [200 mg i.v]. Humeniuk et al.[21] determined REMDS linearly over the range of 4-4,000 ng/mL, and GS-704277 and GS-441524 over 2-2,000 ng/mL.

Pasupuleti et al.[22] monitored the profile of medication in plasma using UPLC/PDA and UPLC/MS/MS in comparison to Avataneo et al. [23] using UPLC-ESI[+]-MS/MS. The latter achieved higher sensitivity for an advanced pharmacokinetic profile during the current worldwide outbreak. The same UHPLC system was then employed by Tempestilli et al. [24] for the pharmacokinetic evaluation of REMDS. Since most studies, including in vivo pharmacokinetics, require a large number of samples to analyze, run time per sample can be very important. The main benefit of reported methodologies is the easy step of preparing the samples, that in turn reduces time of analysis. Although recoveries obtained by LC-MS/MS [20, 21] and UPLC-MS/MS [23, 24] were similar, UHPLC gave significantly better precision.

Both reported LC-MS methods [32, 33] for Baricitinib quantitation were in rat plasma; either in combination with Methotrexate over a concentration range of 0.5–250 ng/mL for both analytes, or as a single component, with 0.2 ng/mL as lower limit of quantitation. A simple spectroscopic A-max method [34] estimated Barictinib over 10-60 µg/mL in laboratory-made tablets. Meanwhile, a reported stability-indicating RP-LC-DAD method [35] was linear at range 10-150µg/mL. No single statement has been reported for simultaneous determination of "Remdesivir and Baricitinib", the FDA approved combination. In the meanwhile, monoclonal antibodies have not been assayed with the aid of analytical techniques.

4. Conclusion:

To sum up, the urgent need for an effective treatment for Covid-19 is of high concern and consideration. However, additional research for possible interactions, and toxicities of the approved medication is complementary for their administration and long-term use.

The present mini-review presents a brief summary of the coronavirus implicated in the SARS-CoV-2 or COVID-19 disease etiology as well as its early onset in China. A short overview is presented of three current FDA approved therapeutics for Covid-19 treatment. The review also summarizes these drugs' different chemical structures, their mechanisms of action and reported assay methods. A comparative summary of reported methods for each drug is separately discussed. This article provides concisely a basic "take-home-message" for the readers seeking information about such an arguing question, "What drugs are being used for Coronavirus SARS-CoV-2??"

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