Therapeutic Antibodies and It's Use in COVID-19: A Review

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Abstract- Proteins, Peptides, Monoclonal antibodies, gene therapy, cell therapy and RNAi (RNA Interference) come under large therapeutic molecules. These are progressively represented as new molecular entities registered through the FDA (Food and Drug Administration). The majority of NME’s (New Molecular Entities) registered to date, are still small molecules. Under antibody-therapeutics, since 2019, in clinical trials over 82 antibody- NMEs (New Molecular Entities) have been approved to treat diseases with hundreds more. The development of antibody-based bio-pharmaceuticals for effective and safe treatment of many diseases like autoimmune, oncology, transplantation and inflammatory disorders has shown noteworthy success in the last three decades. Development and significant rapid growth leading to clinical success began in the 1990s. Over 80 regulatory approvals have demonstrated this fact. Since 2015, it is estimated that more than 100 therapeutic antibodies, such as monoclonal antibodies (mAbs), have entered first-in-human (FIH) studies per year and 700 antibody-based therapeutics are currently in some stage of clinical development.

Keywords - Monoclonal antibodies, Immunoglobulin G, Hybridoma technology, Covid-19, Cancer treatment

INTRODUCTION

Monoclonal antibodies are a combination of various antibodies mainly produced using the hybridoma technique. The main properties of mAbs are binding specificity, unlimited multiplication ability (clones) and homogeneity. Due to the advantages, mAbs are widely used in the diagnosis and treatment of illness like cancer and immunoglobulin disorders [1]. The hybrid myeloma cells, also known as Hybridomas produce long-lived antibodies. The individual cell line also called a “clone” (monoclonal), is used for the selection and sustained production of highly specific antibodies that produce desired binding characteristics to a target antigen. The development of long-lived antibody-producing hybrid myeloma cells in culture, or hybridomas, allowed for the selection and sustained production of highly specific antibodies from an individual cell line or clone (monoclonal) that exhibited the desired binding characteristics to a target antigen. Combining hybridoma screening with recombinant DNA including technology in vitro could produce IgG (immunoglobin G) antibodies with unique specificity including human sequence, leading to the development of mAbs as potential therapeutic candidates. Paul Erlich (in the early 20th century) described these antibodies as a “magic bullet” in search of toxins [1]. Georges Kohler, Cesar Milstein and Niels Kaj Jerne used a mouse x mouse hybridoma and created the monoclonal antibody (mAb) technique in 1975. The US FDA approved the first therapeutic mAb muromonab-CD3 (trade name Orthoclone OKT3) to reduce acute rejection in patients with organ transplant in the year 1992. The side effects of muromonab treatment such as rapid clearance/short half-life and high prevalence for neutralizing anti-drug antibody (ADA) response, were due to its murine sequence and isotype. The discovery of muromonab OKT3 provided the first empirical data on mAb molecules (e.g. mAb comprised murine variable region and human constant regions; nearly 67% human sequence) and sequentially “humanized” or complementary-determining region (CDR)-grafted mAbs (e.g. mAb comprised murine sequence only at antigen-binding CDR regions; > 95% human sequence), and later on fully human mAbs (100% human sequence) greatly improved the clinical pharmacology of mAb therapeutics. For example, the previously marketed therapeutics mAbs, like Rituxan (rituximab) and Remicade (infliximab), were produced as chimeric mAb formats. More than 90% of the approved antibody-based therapies developed during the past decade have been either “humanized” or fully human-sequenced [2]. FDA has approved 119 therapeutic mAbs (including two diagnostic mAb) from 1992-December 31, 2020. From these FDA approved over 10 therapeutic antibodies in 2015, 10 mAbs in 2016, 17 mAbs in 2017, 15 mAbs in 2018 and 16 therapeutic antibodies in 2019. In 2020, FDA approved 13 therapeutic antibodies (during the COVID-19 pandemic). The list of FDAs approved therapeutic antibodies during the year 2020 is as follows:

<table>
<thead>
<tr>
<th>Drug name</th>
<th>Active ingredients</th>
<th>Company</th>
<th>Approval date</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEPEZZ</td>
<td>Teprotumumab-trt</td>
<td>Horizon therapies</td>
<td>January 21, 2020</td>
</tr>
<tr>
<td>VYEPTI</td>
<td>Eptinezumab-jr</td>
<td>Lundbeck</td>
<td>February 21, 2020</td>
</tr>
<tr>
<td>SARCLISA</td>
<td>Isatuximab-irfc</td>
<td>Sanofi Aventis</td>
<td>March 2, 2020</td>
</tr>
<tr>
<td>TRODELVY</td>
<td>Sacituzumabgovitecan-hziy</td>
<td>Immunomedics</td>
<td>April 22, 2020</td>
</tr>
<tr>
<td>DARZALEX FASPRO</td>
<td>Daratumumab, hyaluronidase-fihj</td>
<td>Janssen</td>
<td>May 1, 2020</td>
</tr>
<tr>
<td>UPLIZNA</td>
<td>Inebilizumab-codn</td>
<td>Viela</td>
<td>June 11, 2020</td>
</tr>
</tbody>
</table>

TABLE 1: FDA approved therapeutic antibodies in 2020
<table>
<thead>
<tr>
<th>Drug name</th>
<th>Disease or Condition</th>
<th>ADRs</th>
<th>Mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>PHESGO</td>
<td>Breast cancer (HER2+)</td>
<td>Neutropenia</td>
<td>Anti-HER2; increases permeability</td>
</tr>
<tr>
<td>BLENREP</td>
<td>Multiple myeloma</td>
<td>Thrombocytopenia</td>
<td>Anti-BCMA; microtubule inhibitor; ADC</td>
</tr>
<tr>
<td>ENSPRYNG</td>
<td>NMOSD (AQP4+)</td>
<td>Elevated liver enzymes</td>
<td>Anti-IL6 receptor</td>
</tr>
<tr>
<td>INMAZEB</td>
<td>Zaire ebolavirus infection</td>
<td>Hypersensitivity</td>
<td>Zaire ebolavirus glycoprotein</td>
</tr>
<tr>
<td>DANYELZA</td>
<td>Neuroblastoma</td>
<td>Neurotoxicity; hypertension</td>
<td>Anti-glycolipid GD2, CDC, ADCC</td>
</tr>
<tr>
<td>MARGENZA</td>
<td>Breast cancer</td>
<td>Left ventricular dysfunction</td>
<td>Anti-HER2, ADCC</td>
</tr>
<tr>
<td>RIABNI</td>
<td>NHL, CLL, GPA, MPA</td>
<td>IRR; TLS; PML</td>
<td>Anti-CD20, ADCC, CDC</td>
</tr>
</tbody>
</table>


II. STRUCTURE AND FUNCTIONS
Antibodies belong to the superfamily immunoglobin (ig) of glycoprotein. B Cells secreted these, identifying and neutralizing foreign organisms or antigens. An antibody is made up of two light chains and two heavy chains. These are divided into five isotypes: IgM, IgD, IgG, IgA and IgE; which are based on the type of heavy chains involved. The five types of heavy chains in mammals are: α, δ, ε, γ, μ. These isotypes have differences in immune function and stages of the immune response [3].
Therapeutic antibodies (mAb) belong to the IgGisotype. IgGisotype is further divided into four subclasses (IgG1 –4) based on their amount in human serum, out of which 75% of mAbs are of the IgG1 subclass (most abundant type). The immunoglobulinG antibodies are about 150kDa large molecules made up of four polypeptide chains i.e., two indistinguishable heavy chains (nearly 50kDa) and two indistinguishable light chains (nearly 25kDa). The two heavy chains are linked to each other and one light chain, each by disulfide bonds, forming y-shaped structures with identical halves. The light chain is made up of one constant and variable region each (C_l and V_l), whereas the heavy chains are made up of three constants (C_H1/C_H2/C_H3) and one variable region (V_H). V_L of the light chain gets associated with C_H1 region of the heavy chain to form the antigen-binding domain or fragment of the antibody (Fab), and the regions under the flexible hinge sequence i.e. C_H2 associated with C_H3 form the Fc domain (fragment crystallization). The structure of the four substances of IgG is similar but differs greatly in the length and composition of the C_H1 and C_H2 regions of flexible hinge sequence causing changes in molecular traits like antigen binding, proteolytic stability and Fc function of the antibody. The V_H and V_L regions are comprised of three hyper-variable amino acid sequences, known as CDRs, forming antigen-binding sites of each Fab arm. The interface of each heavy chain and light chain CRDs, along with the more constant segments (framework residues) surrounding them, forming numerous non-covalent interactions creates a highly specific three-dimensional binding structure for a distinct antigenic determinant or epitope [2].

The overall functioning of mAbs is listed as follows [5]:

- Antagonism of suitable receptor or ligand [6].
- Antibody-Dependent cellular cytotoxicity [7].
- Complement-Dependent cytotoxicity [8].
- Agonism of the receptor [9].
- Antibody-dependent cellular phagocytosis [10].
- Apoptosis [12].
- Non-apoptotic programmed cell death [13].

III. METHODS

Hybridoma technology: Hybridoma technology is a well-established method of producing monoclonal antibodies specific to antigens of interest and one of the most important methodological advances in biomedicine during the 1970s. In hybridoma technology we use two different kinds of cells for production of large quantity of the antibody. Hybridoma cell lines are formed via fusion between a short-lived antibody-producing B cell and immortal myeloma cell. The production of mAbs was invented by Cesar Milstein and Georges J.F.Kohler they immortalized antibody-producing cells by fusing them with the tumor cells. The Nobel Assembly of Karolinska institute awarded the Nobel prize in physiology or medicine in 1984 jointly to Neils K. Jerne, George J.F.Kohler and Cesar Milstein for theories concerning “the specificity in development and control of the immune system” and the discovery of the principle for the production of monoclonal antibodies. The myeloma cells lack the enzyme Hypoxanthine GuanosinePhosphoribosylTransferase (HGPT) and are sensitive to HAT media. Whereas spleen B-Lymphocytes contribute the HGPRT gene to the hybrid cell end hence, unfused myeloma cells and spleen cells die in the HAT media. Monoclonal antibodies synthesis requires HAT medium. Initially mice or animal is exposed to antigen the splenocytes are developed/isolated from mice, the immortalized myeloma cells that lack HGPRT gene are fused with B cells using polyethylene glycol. HAT medium is used for incubating the fused cells, nucleotide synthesis does not occur because the aminopterin in myeloma cells die but B-myeloma hybrids doesn’t. Then incubated medium is diluted in such a way that it includes only one cell in each well and check for the presence of the supernatant to know whether an antibody is produced or not. Steps involved in hybridoma technology:

1. Spleen cells are prepared from animals, usually mice which have been immunized with a selected antigen.
2. These cells are fused with myeloma cells maintained in culture in the laboratory.
3. The product of this fusion is referred to as hybridoma.
4. The hybrid obtained are propagated in a highly diluted state so that colonies deriving from single hybrid cells can be isolated.
5. A particular hybridoma can be used in the future, enabling unlimited production of a highly specific antibody [14].
Advancement in hybridoma technology:

Hybridoma technology is an advantageous method due to its ability to be cryopreserved and be used for infinite times along with a large production rate. The advancement of stable cell line production from unstable hybridomas is currently being developed. The Chinese hamster ovary cell line is the most preferred for large-scale mAbs production. The multi-step screening process to identify antigen-specific hybridomas has advanced to robotic screening methods like microarray-based screening technology and Bio-layer interferometry [16].

Limitations:
1. The main limitation of this method is the lack of a suitable fusion partner leading to limiting its applicability to other species. To overcome this problem transgenic mice model H-2kb-tsA58 is discovered. The model is specifically used to isolate mAbs against the filamentous phase.
2. Isolation of mAbs against various membrane proteins. The purified antigens can be generated using methods like the cell-based immunization and screening (CBIS) method for the isolation of mAbs for podoplanin (PDPN) has improved the method [16].

IV. ROLE OF MABS IN COVID-19

Covid 19 also known as SARS-CoV-2 (severe acute respiratory syndrome coronavirus-2) is an airborne disease that first occurred in the month of December 2019 in a seafood market of Wuhan, China. As the name suggests this virus mainly affects the lower respiratory tract along with other body organs [17]. Coronavirus (CoV) belongs to the family “corona viridae”, comprising of four types which are Alpha coronavirus, Beta coronavirus, Gamma corona virus and Delta coronavirus. The first CoV pandemic occurred in the year 2003 known as Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV). Later in the year 2012, a stain of CoV caused Middle East Respiratory Syndrome Coronavirus (MERS-CoV). Covid 19 becomes the third and largest pandemic of these [18]. Humans are affected by Gamma and Delta CoV. SARS-CoV-2 i.e., Covid 19 enters the host by receptor-mediated endocytosis and caused infection by binding to the ACE-2 surface receptors. Monoclonal antibodies bind to the protein spike (S) and prevent the viral attachment to the cells and tag it for destruction [18].

The infection is caused in the following way:
1. The spike (s) protein binds to ACE-2 receptor and enters the cell.
2. Internalisation of virus via. Host trans-membrane protease serine 2 (TMPRSS2)
3. Viral RNA replication via RNA-dependent RNA polymerase
4. Viral particles produced and released [19].

TABLE 2: EUA (emergency use authorization drugs approved by FDA during pandemic to treat corona virus
<table>
<thead>
<tr>
<th>Product name</th>
<th>Mode/source</th>
<th>ROA</th>
<th>Company</th>
<th>Target</th>
<th>Other uses</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bamlanivimab/etesevimab</td>
<td>Human</td>
<td>IV</td>
<td>Eli lilly</td>
<td>Surface spike protein of SARS-COV-2</td>
<td></td>
<td>20</td>
</tr>
<tr>
<td>Casirivimab/imdeviamb</td>
<td>IV or SC</td>
<td>Regeneron pharmaceuticals</td>
<td>SARS-COV-2 spike protein receptor binding domain (RBD)</td>
<td></td>
<td></td>
<td>21</td>
</tr>
<tr>
<td>Sotrovimab</td>
<td>Human</td>
<td>IV</td>
<td>Glaxo smith kline and Vir biotechnology</td>
<td>Epitope on spike protein of SARS-COV-2</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td>Regdanvimab</td>
<td>Human</td>
<td>IV</td>
<td>Celltrion healthcare</td>
<td>SARS-COV-2 spike protein of receptor binding domain</td>
<td></td>
<td>23</td>
</tr>
<tr>
<td>Tixagevimab/cilgavimab</td>
<td>human</td>
<td>IM</td>
<td>Astra zenea</td>
<td>Spike protein of receptor binding domain of SARS-COV-2</td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Bebtelovimab</td>
<td>Human</td>
<td>IM</td>
<td>AbCellera biologics and Eli Lilly</td>
<td>Spike protein receptor binding domain of SARS-COV-2</td>
<td></td>
<td>25</td>
</tr>
<tr>
<td>Tocilizumab</td>
<td>Humanezed</td>
<td>IV</td>
<td>IL-6 receptor</td>
<td>Rheumatoid arthritis, cytokine release syndrome, Idiopathic multicentricCastleman's disease</td>
<td></td>
<td>26</td>
</tr>
<tr>
<td>Baricitinib</td>
<td>Oral</td>
<td>Janus Kinase (JAK1 &amp; JAK2)</td>
<td>Rheumatoid arthritis, atopic dermatitis, alopecia areata juvenile, idiopathic arthritis idiopathic arthritis systemic lupus erythematosus*</td>
<td></td>
<td>27</td>
<td></td>
</tr>
</tbody>
</table>

**TABLE 3:** FDA approved EAU (Emergency Use Authorization) drugs other than mAbs

<table>
<thead>
<tr>
<th>Name</th>
<th>Site of Action</th>
<th>Other uses</th>
<th>ROA</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dexamethasone</td>
<td>GRs (glucocorticoid receptors)</td>
<td>Rheumatic and autoimmune diseases, leukemia, multiple myeloma and Hodgkins and non-hodgkins lymphoma</td>
<td>IV</td>
<td>29</td>
</tr>
<tr>
<td>Budesonide</td>
<td>GRs</td>
<td>Asthma</td>
<td>Inhaler</td>
<td>30</td>
</tr>
<tr>
<td>Nafamostatmesylate</td>
<td>TMPRSS2 (transmembrane protease serine 2)</td>
<td>Pancreatitis, Antiplatelet</td>
<td>DIC, Anticoagulant, Antiplatelet</td>
<td>IV</td>
</tr>
<tr>
<td>Fresenius propoven emulsion (propofol)</td>
<td>2% Upregulation of ACE-2</td>
<td>Anesthetic</td>
<td>IV</td>
<td>32</td>
</tr>
<tr>
<td>Colchicine</td>
<td>NLRP3 inflammasome</td>
<td>Anti-inflammatory, pericarditis</td>
<td>Per-oral</td>
<td>33</td>
</tr>
<tr>
<td>Kineset (Anakinra)</td>
<td>IL-1α and IL-1β (interleukin)</td>
<td>Rheumatoid arthritis, pericarditis, myocarditis</td>
<td>IV</td>
<td>34</td>
</tr>
</tbody>
</table>
V. FDA APPROVED DRUGS

Sotrovimab neutralizes the SARS-COV-2 and other sarbecoviruses [22]. It is developed by Vir Biotechnology in collaboration with Glaxo Smith Kline. It is used for the treatment of covid-19 in adolescents aged grater or equal to 12, adults who do not require oxygen supplementation and who are at high risk to COVID. Sotrovimab received its first full approval on 17 december,2021 [38]. Tocilizumab is a recombinant humanized IL-6 receptor blocking monoclonal antibody which is administered intravenously. It is also used for the treatment of Rheumatoid arthritis, cytokine release syndrome, idiopathic multicentricCastleman's and COVID-19 [26].

Baricitinib is a Janus Kinase JAK1/JAK2 inhibitor. It is an oral medication that inhibits the Janus kinase (JAK) enzymes, Artificial intelligence played a significant role for the identification of baricitinib [27].

V. OTHER APPLICATIONS

1. Diagnostic Testing and Identifying Pathogens: Cancers and infectious diseases i.e., reproductive and respiratory diseases can be diagnosed by mAbs some diseases are as follows: Rabies, Bovine herpes virus, cervine type 1, pneumonia, plum pox virus, HIV, AIDS, dengue, male infertility, etc. The commonly used screening systems used are enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), immune fluorescence and hemolytic plaque assays, immune chromatographic card assay, etc [39].

2. Pregnancy testing: In the latest pregnancy testing the anti-hCG (monoclonal antibodies) present in kithe t binds with the hCG (human chorionic gonadotropin) present in the urine of pregnant women only giving a visible color reaction indicating the positive result of pregnancy [40].

3. Radio Immuno-Detection and Radio Immuno-Therapy: Radio Immuno-Therapy uses radio-nucleotides which have applicability for decay for the therapy. Radio Immuno detection applies to radiolabelled antibodies used for diagnosis purposes in cancer [41].

4. Cancer Treatment through Drugs: Trastuzumab containing ADC, Ado-trastuzumabemtansine was approved by FDA in year 2013, to treat patients with metastatic breast cancer, another drug approved by FDA in the year 2017 i.e., CD19-CD3 BiTEbiatuminomab due to its significanc in acute lymphoblastic leukemia patients. Alemtuzumab (naked mAb) is used to treat chronic lymphocytic leukemia (CLL) and Brentuximabemtansine (chemo-label antibodies drugs conjugates) is used to treat Hodgkin lymphoma and anaplastic large cell lymphoma [42].

5. Virus Disease Treatment: The mabs used in the treatment of the Ebola virus are single antibody mAb114; the mAb cocktails MIL77E (two mAbs combined) and MB-003, ZmAb, ZMappTM (three antibodies combined). [43] Crohn’s disease (an inflammatory bowel disease) caused due to dysregulated immune response to microbiota in the gastrointestinal system. Anti-NKG2D (natural killer group 2D) mAbs are used to treat Crohn’s disease [44].

6. Organ rejection: Monoclonal antibodies are commonly used in induction therapy for renal transplantation, to treat steroid-resistant acute rejection and to allow immunosuppressive minimization and treatment of chronic antibody-mediated rejection [45].

VII. CONCLUSION

Until 2020, there are 119 monoclonal antibodies approved by FDA. There are five methods of production of mAbs: Hybridoma technology, phagedisplay, B-Cell immortalization, transgenic screening and surface display. The most widely used method is Hybridoma technology. Widely used in cancer identification, diagnosis and treatment. The mAbs are widely used in the treatment of cancer, autoimmune diseases, cardiovascular diseases, inflammation and infectious diseases. Some drawbacks of these are: inadequate pharmacokinetics, tissue accessibility as well as impaired interactions with the immune systems. In these areas, additional research is needed. The high production cost limits the wide use of these drugs. These mAbs being large, most of the administered amount remains in blood circulation and only 20% of it act together with the tumor, hence resulting in an elongated serum-half life. The large size of mAbs also causes impaired tissue penetration, especially in the case of solid tumors leading to poor diffusion causing difficulty in treating large tumor masses with mAb therapy. The “binding site binder effect” further limits the penetration of mAbs into the tumor.

Some of the side effects of the use of mAbs are general side effects include fever, nausea, vomiting and skin rashes. Serious side effects are infusion reactions, heart problems, lung problems, skin problems and bleeding. Optimization of antibodies with modified Fc region capable of overcoming some of the limitations mentioned above is being used to develop a new generation. More than 20mAbs of second generation have been approved for clinical use and third generation mAbs are being developed as potentially more efficient mAbs than the conventional mAbs. Development in other various sectors like the selection of high-responder patients, antibody delivery optimization, infusion schemes, antibody pharmacokinetics and biodistribution as well as a better control of the severe side effects generated by some antibody treatments.

REFERENCES:


