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THERAPEUTIC ANTIBODIES AND THEIR ROLE IN TREATMENT OF DISEASES

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***ABSTRACT**: DEVELOPMENT OF THERAPEUTIC ANTIBODIES EXPLODED IN THE 1980'S, AND THE FIRST THERAPEUTIC ANTIBODY, A MOUSE ANTIBODY, WAS LAUNCHED IN 1986 AS AN IMMUNOSUPPRESSIVE AGENT USED DURING ORGAN TRANSPLANTATION. THE UNITED STATES FOOD AND DRUG ADMINISTRATION (US FDA) APPROVED THE FIRST MONOCLONAL ANTIBODY IN 1986, AND ANTIBODY ENGINEERING HAS PROGRESSED SIGNIFICANTLY SINCE THEN DUE TO THEIR EXCELLENT SPECIFICITY, TODAY'S ANTIBODY THERAPIES ARE CAUSING LESS SIDE EFFECTS. AS A RESULT, THERAPEUTIC ANTIBODIES HAVE EMERGED AS THE MOST COMMONLY CREATED NOVEL MEDICINE CLASS IN RECENT YEARS. THE MAJORITY OF THESE LICENSED MABS OR DERIVATIVES ARE HYBRIDOMA-DERIVED OR IMPROVISED ENGINEERED VARIANTS. DESPITE THE RECENT ADVENT OF HIGH THROUGHPUT MAB GENERATION TECHNOLOGIES, HYBRIDOMA REMAINS THE PREFERRED APPROACH DUE TO ITS NATIVE NATURE, WHICH PRESERVES NATURAL COGNATE ANTIBODY PAIRING INFORMATION AND IMMUNE CELL INTRINSIC ACTIVITIES. IN THIS REVIEW ENDEAVOUR HAS BEEN MADE TO DISCUSS THE DEVELOPMENT OF MABS BY HYBRIDOMA TECHNOLOGY AND HOW THEY WERE ISOLATED FROM DIFFERENT SPECIES, THEIR MECHANISM OF ACTION ALSO DISCUSSES AND ABOUT THEIR ROLE IN TREATMENT OF DISEASES AND THEIR FUTURE ASPECTS.*

***KEYWORDS**: MONOCLONAL ANTIBODIES; TARGET ANTIGENS; HYBRIDOMA TECHNOLOGY; B CELLS; HUMANIZED ANTIBODIES*

I. INTRODUCTION

B CELLS CREATE MONOCLONAL ANTIBODIES (MABS) THAT TARGET CERTAIN ANTIGENS. KÖHLER AND MILSTEIN'S HYBRIDOMA TECHNIQUE, INTRODUCED IN 1975, HAS MADE IT POSSIBLE TO OBTAIN PURE MABS IN LARGE QUANTITIES, GREATLY ENHANCING BASIC RESEARCH AND THE POTENTIAL FOR CLINICAL USE [1]. MANY OTHER SCIENTIFIC AND TECHNOLOGICAL ADVANCEMENTS HAVE BEEN ALSO SUCCESSFULLY TRANSLATED MABS TO THE CLINICAL USE. IN 1975, HYBRIDOMA-BASED TECHNOLOGY WAS USED TO GENERATE MABS THAT HAD VERY LITTLE AND ACCEPTABLE BATCH TO BATCH VARIATION AND COULD BE PRODUCED INDEFINITELY. ANTIBODIES CAN BE MADE AGAINST ANY EPITOPE FOUND ON AN ANTIGEN OR IMMUNOGEN. MUROMONAB (OKT3), A MURINE ANTI-CD3 MONOCLONAL ANTIBODY USED TO TREAT ORGAN TRANSPLANT REJECTION, WAS THE FIRST MAB TO BE APPROVED BY THE FDA FOR HUMAN USE [2]. IN 1984, HOWEVER, CHIMERIC ANTIBODIES WERE DEVELOPED IN RESPONSE TO ALLERGIC REACTIONS TO MURINE MABS (IMMUNE REACTIONS TO PROTEINS FROM DIFFERENT SPECIES). THE WHOLE ANTIGEN-SPECIFIC DOMAIN OF A MOUSE ANTIBODY WAS GRAFTED IT ONTO CONSTANT DOMAINS OF A HUMAN ANTIBODY AND USED RECOMBINANT DNA TECHNIQUES, RESULTING IN CHIMERIC MOUSE-HUMAN ANTIBODIES. RITUXIMAB, A MOUSE-HUMAN CHIMERIC MAB AGAINST THE B-CELL LINEAGE MARKER CD20, WAS THE FIRST TO BE APPROVED BY THE FDA IN 1997 FOR THE TREATMENT OF CD20-POSITIVE, B-CELL, LOW-GRADE, OR FOLLICULAR NON-LYMPHOMA HODGKIN'S IN PATIENTS WHO HAD RELAPSED OR REFRACTORY DISEASE. THE CDR GRAFTING METHODOLOGY WAS USED TO HUMANIZE MURINE MABS IN THE MID-1980S [3]. THE DISCOVERY OF BIOSIMILAR MABS HAS REDUCED THE COST OF TREATMENT IN SEVERAL CIRCUMSTANCES.

THE FOOD AND DRUG ADMINISTRATION (FDA), THE EUROPEAN MEDICINES AGENCY (EMA), AND OTHER NATIONAL AGENCIES HAVE APPROVED ANTIBODIES OF ALL TYPES (MURINE,

CHIMERIC, HUMANIZED, AND HUMAN) FOR THE TREATMENT OF A VARIETY OF DISEASES. SINCE THE APPROVAL OF OKT3, THE USE OF MONOCLONAL ANTIBODIES (MABS) HAS STEADILY INCREASED IN ALL FIELDS OF MEDICINE, INCLUDING NEUROLOGY. MANY OF THE MABS UTILIZED IN NEUROLOGY TODAY WERE ORIGINALLY DEVELOPED FOR HEMATOLOGICAL NEOPLASIA OR RHEUMATOLOGICAL DISORDER (E.G., ALEMTUZUMAB, OFATUMUMAB, AND RITUXIMAB, TOCILIZUMAB).

WHILE MABS ARE COMMONLY USED IN BIOCHEMISTRY, MOLECULAR AND CELLULAR BIOLOGY, AND MEDICAL RESEARCH, THEIR MOST IMPORTANT APPLICATION IS AS THERAPEUTIC DRUGS USED IN THE TREATMENT OF HUMAN DISEASES LIKE CANCER, ASTHMA, ARTHRITIS, PSORIASIS, CROHN'S DISEASE, TRANSPLANT REJECTION, MIGRAINE HEADACHES, AND INFECTIOUS DISEASES [4A]. OVER THE LAST DECADE, SIGNIFICANT DEVELOPMENTS IN ANTIBODY ENGINEERING HAVE IMPROVED THE SAFETY AND EFFICACY OF THERAPEUTIC ANTIBODIES. IN THIS REVIEW ITS BEEN DISCUSSED ABOUT THE DEVELOPMENT OF THERAPEUTIC MONOCLONAL ANTIBODIES, HYBRIDOMA TECHNOLOGY, MODE OF ACTION OF THERAPEUTIC ANTIBODIES-THEIR ROLE IN TREATMENT OF DISEASES AND THEIR PROSPECTS.

II. Overview Of Hybridoma Technology

Hybridoma Cells Are Created By Fusing An Immortal Myeloma Cell With A Short-Lived Antibody-Producing B Cell. Each Hybridoma Cell Expresses A Large Amount Of One Purely Specialized Mab And Preferred Hybridoma Clones Can Be Cryopreserved For Long-Term Mab Production. A Host Animal's Innate Ability To Manufacture Functional, Highly Specific, And High Affinity Mabs Is Used In The Hybridoma Production Process [4]. Several Mabs Have Been Developed Utilizing This Approach To Date, And They Are Now Being Utilized To Diagnose, Prevent, And Treat A Variety Of Disorders [4a]

Hybridoma Technique Was Originally Limited To Murine Antigens, But With Advancements In The Area, It Is Now A Well-Established Method For Developing Mabs Against A Wide Range Of Antigens And From A Variety Of Species, Including Humans, Rabbit, Sheep, Goats, Chickens, Mice, Cows, Rats And Guinea Pigs. The Selection Of Animal Species For Mab Isolation Is Influenced By A Number Of Factors, Including The Homologous Availability Of An Appropriate Fusion Partner, The Amount Of Protein Or Antigen Available For Immunization, The Time Required To Obtain An Antibody Response, And, Finally, The Purpose For Which These Mabs Are Required. Mice Are The Most Common Hosts For Mab Production, Followed By Rabbits. In Most Cases, The Inbred BALB/C Mouse Strain Is The Best Choice And Perfectly Suited For Mab Isolation [5]. Due To The Different Evolutionary Link Between The Antigen Giver And The Antibody Production, Chickens Are Also A Chosen Host Of Choice [6].

III. Animal Species Employed In The Production Of Hybridomas Over The Years:

3.1 Mouse

Human And Mouse Antibodies Have Structural Similarities, And That Is Why They Have Such A High Acceptance Rate. The Improved And Simplified Mice Hybridoma Procedure Has Become A More Important Cause For Their Widespread Use In Research And Therapies [7]. The Mice Hybridoma Method Is A Multi-Step Process And Takes Benefit Of A Host Animal's Inherent Ability To Manufacture Highly Specific, High-Affinity, And Fully Functional Monoclonal Antibodies (Mabs). It Leads To The Creation And Refinement Of Specific Immunogenic Antigens (Ag). Following Optimization, A Host Animal Has Been Immunized For Several Weeks With The Ag And Adjuvant. Serum From Immunized Animals Is Tested For Reactivity And Specificity To The Immunizing Antigen, And Animals With High Binding Antibody Titers Are Chosen For Splenocytes Isolation. Within Presence Of Fusogenic Mediators Such As Viruses, Chemicals, And Electric Pulses, Spleen Cells Fuse With Immortalized Myeloma Cells. Sp2/0-Ag 1410 [8] And X63-Ag 8.6539 [9], Both Derived From BALB/C Mice, Are The Most Prevalent Myeloma Fusion Cell Lines. On Hypoxanthine-Aminopterin-Thymidine (HAT) Media, The Fused Cells Are Then Selected. Myeloma Cells Are Sensitive To HAT Media Because They Lack The Hypoxanthine-Guanine Phosphoribosyl Transferase (HGPRT) Gene, Which Is Essential For De Novo Or Salvage Nucleotide Synthesis, Whereas Unfused B Cells Have A Short Life Span. Only Its Hybrid (B Cell-Myeloma) Sustains In This Process Because They Have The Functioning HGPRT Gene From B Cells. Hybrid Cells, On The Other Hand, Retain Both B Cells' Antibody-Secreting Ability And Myeloma Cells' Ability To Grow Indefinitely (Immortality). Fused Or Hybrid Cells Are Then Screened Using The "Limited Dilution Cloning" Method Or Semi-Solid Selective Medium To Choose Only Such Hybridoma That Produce Antibodies With The Desired Specificity. Figure 1 Depicts A Thorough Schematic Illustration Of The Procedures Involved In Hybridoma Formation. Antibody Production Using Mouse Hybridoma

Technology Is Quite Reliable, And It Has Been Used To Find Thousands Of Antibodies For Various Applications. The First Therapeutic Antibody, Created Via Murine Hybridoma Technology And Authorized By The Food And Drug Administration (FDA) In 1985, Could Be Used To Minimize Graft Rejection In Transplant Patients [10]. OKT3 Was The First Monoclonal Antibody (Mab) To Use In Organ Transplantation, And It Has A Decade Of Experience In Both Preventing And Treating Organ Transplant Rejection. OKT3 Acts As An Immunosuppressant By Inhibiting T Cell Function Via Altering CD3 And The T Cell Receptor On The T Cell Surface [11].

3.2 Rabbit

The Scientific Community Requires A New Technology For Producing Mabs With Enhanced Affinity, Specificity, And The Ability To Identify Non-Immunogenic Rodent Epitopes. The Rabbit Immune System Also Shown To Be A Vehicle For Generating Antibodies That Recognized A Wider Range Of Compounds, Including Phosphopeptides, Carbohydrates, And Immunogens Which Are Not Normally Immunogenic In Mice. Rabbit Iggs Are Simpler Than Mouse And Human Antibodies. Igg In Rabbits Has Only One Subclass, The C Gene, And The Bulk Of Light Chains (90–95%) Are Produced From Isotype C1. Isotype L Accounts For Just 5% To 10% Of Total Igg Light Chains. Figure 2. Raybould Et Al. Created The First Stable Rabbit–Mouse Hetero-Hybridoma In 1988 By Fusing Rabbit Spleen B Cells With The Mouse Myeloma Cell Line SP2/0-Ag14 Using Polyethylene Glycol [12].

Under The Name Ranmab, Abcam Patented This Approach To Create Highly Specific Mabs, Which Has Been Studied For The Creation Of Diagnostic And Research Antibodies. Wei Et Al. Recently Developed An Ultrasensitive Ebola Virus Diagnostics Test Using A Carbon Nanoparticle-Labeled Pad With Rabbit Anti-Ebola Virus (EBOV)-VP40 Igg For Quick Detection Of Ebola Virus Lateral Flow Test Strip [13]. Although There Has Been Limited Success In Developing Rabbit Mabs As Therapeutic Agents, The Potential Use Of Rabbit Pabs For The Prophylaxis And Treatment Of Acute Rejection Against T Cells In Organ Transplant Has Sparked Interest In Using Humanized Rabbit Mabs As A Therapeutic Agent [14].

3.3 *Human*

The Most Direct And Successful Technique For The Synthesis Of Natural Therapeutic And Diagnostic Antibodies Is To Use Human Hybridoma Technology, Which Permits The Direct Generation Of Human Antibodies In Their Native Form. It Is Thought To Be The Most Promising And Practical Technological Platform For Therapeutic Mab Isolation. However, The Efficacy Of Human Hybridoma Technology For Therapeutic Reasons Has Been Limited For Years Due To A Number Of Technical Issues, Including A Lack Of Human Fusion Partners (The Majority Of Fusion Partners Available Are From Rodents Or Heteromyelomas). The Usage Of These Mabs For Therapeutic Purposes Is Limited Due To The Fusion Of Human B Cells With Various Fusion Partners. To Address These Obstacles, Other Organizations Have Used The EBV (Epstein-Barr Virus) Transformation Technique To Boost The B Cell Population [15]. B95-8, The Most Widely Used Cell Line, Is A Continuous Cell Line That Produces Significant Levels Of Transforming EBV In Supernatants. Marmoset Blood Leukocytes Were Exposed To EBV Isolated From A Human Leukocyte Cell Line To Create The B95-8 Cell Line. B95-8 Serves As A Source Of Continuous Lymphocytic Cell Lines From Human Donors For EBV. EBV Primarily Interacts To CD21 Receptor-Positive Cells In The Peripheral Blood, And B Lymphocytes In The Peripheral Blood Display These Antigens On Their Surface, Activating Latent Membrane Protein (LMP) 2A And LMP1. Transformed B Cells Cannot Be Grown For Lengthy Periods Of Time Due To Their Limited Antibody-Secreting Capacity And Chromosomal Instability. The Transformation Effectiveness Of EBV To B Cells Is Minimal, Ranging From 0.1 To 1%; However, Adding Cpg, A Toll-Like Receptor (TLR) 9 Agonist, Can Improve The Transformation Efficiency Of B Cells In The Presence Of EBV [16]. These Cpg Patterns Act As Immune Stimulants In Their Unmethylated Form And Are Identified By The Pattern Recognition Receptor (PRR) Toll-Like Receptor 9 (TLR9), Which Is Expressed Constitutively On Immune System Cells Like B Cells. These B Cells Respond To Cpg DNA Stimulation By Proliferating And Differentiating Into Antibody-Producing Cells, Resulting In A Polyclonal Reaction [17]. These Altered B Cells Are Cultured For A Set Amount Of Time In Order To Produce Immortalized Cells, Which Then Fuse With The Fusion Partner To Make Hybridomas. This Cpg Activation Approach Has Been Utilized To Successfully Create Mabs Against The SARS-Cov Coronavirus (SARS-Cov) And HIV. The Most Important Consideration When Creating Hybridoma For Therapeutic Purposes Is That The Final Hybrid Cells Be Free Of EBV And Other Human Viruses.

Due To Significant Variations Between The Human And Rodent Immune Systems, Antibodies Produced By Human Hybridoma Technology Have More Therapeutic Applications Than Those Produced By Rodent Hybridoma Technology [18]. Several Hetero-Myelomas Fusions Have Been Effectively Used To Generate Human-Derived Mabs For Diseases Like As HIV, Chikungunya, And Dengue.

3.4 Chicken

Due To Evolutionary Differences Between Mammalian (Humans And Mice) And Avian (Chicken) Immune Systems, The Avian/Chicken Immune System Detects More Epitopes On Mammalian Proteins As Foreign And Produces A More Robust And Diversified Immune Antibody Repertoire [19]. The Immunological Response Grows In Direct Proportion To The Evolutionary Distance Between The Immunizing Antigen And The Inoculated Animal Because Of The Great Sequence Conservation (> 70%) At The Protein Level, It Is Possible To Create Antibodies In Chicken That Are Difficult Or Even Impossible To Produce In Mammals, Such As Antibodies Against G-Protein-Coupled Receptors (Gpcrs), Which Are Difficult To Produce In Rodents.

Chickens Are Proving To Be Useful Immunization Hosts, Particularly For The Development Of Therapeutic Antibodies For Difficult Targets With Mammalian Sequence Conservation Because Chicken Produces More Antibodies Than Laboratory Rodents, The Use Of Chicken Egg Yolk For Antibody Manufacturing Reduces Animal Use (Ethical Concerns). In 1989, A Successful Attempt Was Made To Create Chicken Hybridomas Against Newcastle Disease Virus (NDV) By Fusing Peripheral Blood Lymphocytes (PBL) With TK- Chicken Myeloma Cells [20]. Amazingly, The Secreted Antibody Hybridomas Were Developed At First, But They Quickly Lost Their Ability To Make Antibody In The Culture. To Address This Problem, Nishinaka S Et Al. Established The R27H4 Fusion Cell Line For The Synthesis Of Chicken Mabs In 1991 [21]. The Novel Cell Line Proved Effective In The Production Of Antibody-Producing Hybridomas With A 6-Month Ability To Secrete Highly Reactive Igg. These Cell Lines Were Refined Further, And Numerous Chicken Hybridomas Were Created Effectively.

The Predominant Avian Antibody Isotype Igy Is Structurally And Functionally Similar To Its Mammalian Counterparts Igg And Ige. Igy Has Four Constant Areas, Whereas Igg Has Three Constant Regions, Due To The Difference In The Number Of Constant Sections In Antibodies. Igy From Chicken Sera Is Transferred To The Embryo Via The Egg Yolk. Antibodies From Egg Igy Have Previously Been Used To Treat Bacterial And Viral Illnesses. Biopharmaceutical Development Could Benefit Greatly From Humanization Of These Antibodies. The Creation Of Transgenic Chicken With Human Immunoglobulin Loci Has Accelerated The Application Of Transgenic Chicken-Derived Mabs For Human Medicinal Purposes. These Transgenic Chickens Produce Antibodies From Immunoglobulin Heavy And Light Chain Loci With Human Variable Regions, As Well As Normal B Cell Development And Immune Responses To Preserved Human Targets That Are Non-Immunogenic In Mice [22]. These Transgenic Chickens Have The Potential To Be Employed In The Generation Of Human Hybridoma-Secreting Antibodies. However, The Lack Of A Reliable Hybridoma Fusion Partner, Like That Of Others, Restricts Its Potential Utility. Various Research Groups Have Been Examining The Display Approach To Overcome The Limits Of Chicken Hybridoma In Recent Years.

IV. Mechanism Of Action

Mabs Can Operate In A Variety Of Ways, Both Directly And Indirectly, And Some Mabs Can Confers Multiple Modes Of Action On A Target [23].

1. Direct Mechanism

Antagonism Of A Soluble Ligand Or Receptor, Cell–Cell Contact Inhibition, Agonism On A Surface Receptor Triggering Certain Signaling Pathways Within The Target Cell, Or Cell Death Are All Examples Of Direct Actions. When An Antibody Attaches To A Soluble Ligand, A Cell-Bound Ligand, Or A Cell Receptor, It Prevents The Ligand From Binding To The Receptor, Interrupting The Downstream Signaling Mediated By That Receptor–Ligand Interaction. The Binding Of Fremanezumab, Galcanezumab, And Eptinezumab To The Calcitonin Gene-Related Peptide (CGRP), Which Prevents It From Signaling Through The CGRP And Amylin-1 Receptors, Is An Example Of This Function [24]. Another Strategy Is To Attach To A Cell Receptor In A Non-Agonistic Manner, As In The Instance Of Erenumab, An Anti-CGRP Receptor Mab, To Prevent Ligand Binding And Activation Of Downstream Signaling Pathways. Finally, Mabs Can Block Cell–Cell Interactions Between A Cell-Bound Ligand And A Cell-Bound Receptor On Another Cell, Such As Natalizumab, Which Blocks Lymphocytic Trans Endothelial Migration By Binding To Lymphocytic VLA-4 (CD49d) And Preventing It From Binding To Endothelial Vascular Cell Adhesion Molecule (VCAM).

Agonistic Mabs Work In The Same Way As The Natural Ligand. The Antibody's Agonist Action Occurs When It Binds To The Receptor In A Way That Replicates The Natural Ligand's Binding, Resulting In Antibody-Mediated Downstream Signaling. Alternatively, Mabs That Bind To Receptors With Agonist Activity, Such As The Tumor Necrosis Factor Related Apoptosis-Inducing Ligand (TRAIL) Receptors, Cause Programmed Cell Death [25].

2. Indirect Or Immune-Mediated Actions

Igg Antibodies Are Classified Into Four Subclasses Based On Changes In Their Constant Regions (Fc): Igg1, Igg2, Igg3, And Igg4. These Fc Regions Are Involved In Binding To Fc Receptors (Fcr), Complement Factor Component 1q (C1q), And The Neonatal Receptor (Fcrn), And As A Result, They Define The Ability Of Various Igg Subclasses To Mediate Effector Functions Such As Phagocytosis, Antibody-Dependent Cell-Mediated Cytotoxicity, And Complement Activation, As Well As Their Half-Life And Capacity For Transplacental And Mucosal Surface Transport [26]. Most Unconjugated Antibodies Include A Human Igg1 Fc, An Isotype That Effectively Activates The Immune System, Allowing Diverse Immune Cells And Chemicals To Be Harnessed To Attack Target Cells. As A Result, Igg1 Mabs May Activate Natural Killer (NK) Cells Via CD16A, Resulting In Antibody-Dependent Cytotoxicity (ADCC), Attach To Macrophage CD16A, CD32A, And CD64 To Boost Antibody-Dependent Phagocytosis (Adph), And Activate The Complement, Resulting In Complement-Dependent Cytotoxicity (CDC). To Activate ADCC, An Antibody's Fc Binding Domain Interacts To A Particular Antigen Expressed On A Target Cell's Surface. NK Cells Are Subsequently Recruited To Lyse The Target Cell By The Antibody. When The C1 Complement Factor Attaches To An Igg1 Or Igg3 Antibody–Antigen Combination, The Complement Cascade Is Activated, Leading To The Production Of Such C5b-9 Membrane Attack Complex (MAC), Which Forms A Water Channel In The Target Cell, Causing It To Lyse [27]. The Majority Of Commercially Available Mabs, Including As Alemtuzumab And Rituximab, Relate To The Igg1 Subclass And Have Been Demonstrated To Cause ADCC And CDC. The Immune-Mediated Method Of Action Of Monoclonal Antibodies (Mabs). Igg2 And Igg4 Subtypes, Have A Lesser Affinity For The Fc Receptor And Are Typically Used To Limit Antigen Action. As In Context Of Erenumab And Fremanezumab, The Igg2 Subclass Is Frequently Chosen To Neutralize Soluble Antigens Without Triggering Host Effector Mechanisms [28]. Similarly, Igg4 Mabs Like Natalizumab And Galcanezumab Are Frequently Used When The Involvement Of Host Effector Systems Is Undesirable.

3. Conjugated Mabs

Conjugated Mabs Are Mabs That Have Been Conjugated To A Medication Or A Radioactive Material. These Mabs Are Now Employed In Oncology To Deliver Drugs To Cancer Cells Directly. They Are Designed To Cause Either A Stop In Cell Proliferation Or Direct Cell Death (Typically Apoptosis), And They Can Deliver Higher Quantities Of Cytotoxic Chemicals Directly To The Target Cells Without Damaging Normal Cells, Minimizing The Risk Of Adverse Responses. Ibritumomab Tiuxetan Is A Radiolabeled Mab Against CD20 (A B Cell Surface Protein) That Is Conjugated With Radioactive Yttrium-90 That Is Used In Radioimmunotherapy, While Ado-Trastuzumab Emtansine (Also Known As TDM-1) Is An Antibody That Attacks The HER2 Protein And Is Conjugated To The Chemotherapeutic Drug DM1 [29]. Despite The Fact That Conjugated Mabs Have No Clinical Or Experimental Applications In Neurology, They May One Day Be Employed To Kill Targets Or Deliver Drugs To Specific Cell Types.

4. Bispecific Monoclonal Antibodies

Bispecific Mabs Are Made To Recognize And Attach To Two Epitopes At The Same Time. Their One-Of-A-Kind Structure Allows Them To Perform An Infinite Number Of New Functions. Combining The Two Discrete Binding Sites In A Single Molecule Result In A Compound Function That Is Location And Time Restricted, Which Cannot Be Obtained By Administering A Mixture Of Two Different Mabs With The Same Specificity. Bispecific Abs Can Drive Effector Cells To Target Cells, Increase Receptor Internalization, Distribute Ligands To Specific Cell Populations, Block Two Routes At The Same Time, Or Encourage Biological Barrier Shuttling. The Latter Is Especially Important In Neurology, Where The Blood-Brain Barrier (BBB) Prevents Mabs From Reaching The CNS. The First Specificity Of A Bispecific Abs Can Be Utilized To Transport It Across The BBB (For Example, Binding To The Transferrin Receptor), While The 2nd Specificity Can Attach To Protein Targets To Hinder Or Stimulate A Process Or Eliminate Brain Tumor Cells [30].

There Are Now Two Bispecific Abs On The Market, And Many More Are In Development. Blinatumomab, For Example, Binds Simultaneously To The CD3 Protein Of T Cells And The CD19 Protein Of Target Neoplastic B Cells In Philadelphia Chromosome-Negative Relapsed Or Refractory Acute Lymphoblastic Leukemia. It Places T Effector Cells In Close Proximity To Attack Neoplastic Cells By Binding To Both Proteins, Facilitating Their Immune-Mediated Lysis. Emicizumab, A Bispecific Ab That Binds Both Coagulation Factors Ixa And X, Is Another Bispecific Ab Authorized In The EU And The US For Hemophilia A [31]. Many More Bispecific Abs Are Being Tested In Clinical Trials For A Variety Of Applications. The Use Of An Adenoviral Vector To Deliver A Bispecific Ab With Such An LDLR-Binding Domain Of Apob To Facilitate Its Move Across The BBB And Promote Alpha Secretase Activity Over Beta Secretase Activity, Favoring The Neuroprotective APP Cleavage By Alpha-Secretase, Has Shown Beneficial Effects In A Mouse Model Of Alzheimer's Disease. Furthermore, Using A Bispecific Ab To Target Both The Angiogenic Factor Angiopoietin-2 (Ang-2) And The Translocator Protein (TSPO), Both Of Which Are Abundantly Expressed In Bevacizumab-Treated Glioblasto-

mas, Resulted In Longer Life In Bevacizumab-Treated Rats. In A Mouse Model With Glioblastoma Xenografts, Another Bispecific Ab Targeting Ang-2 And Vascular Endothelial Growth Factor (VEGF) Was Also Shown To Prolong Longevity, Showing That Bispecific Abs Targeting Suitable Epitopes May Be Advantageous In Neurology [32].

V. Therapeutic Antibodies: Clinical Applications And The Market

1. Therapeutic Antibodies Approved For Disease Treatments

With A Current Market Cap Of $115.2 Billion In 2018, The Mab Industry Has A Robust Pipeline And Is Expected To Develop At A Faster Rate. Despite This High Growth Potential, New Companies Are Unlikely To Take Over Large Portions Of The Trade, Which Is Currently Dominated By Seven Companies: Genentech (30.8 Percent), Abbvie (20%), Johnson & Johnson (13.6 Percent), Bristol-Myers Squibb (6.5 Percent), Merck Sharp & Dohme (5.6 Percent), Novartis (5.5 Percent), Amgen (4.9 Percent), With Other Companies Accounting For The Remaining 13% [33].

In 2018, Many Mabs Drugs Sold For More Than $3 Billion, With Six (Adalimumab, Nivolumab, Pembrolizumab, Trastuzumab, Bevacizumab, And Rituximab) Selling For More Than $6 Billion (Fig. 4).

With Nearly $19.9 Billion In Sales, Adalimumab (Humira) Set A New Record For A Biopharmaceutical Product. Oncology, Immunology, And Hematology Remain The Most Common Medical Uses For Mabs, Which Are Progressively Used For A Wide Range Of Targets [34]. Most Monoclonal Antibodies (Mabs) Have Numerous Clinical Indications, At Least One Of Which Is Cancer-Related (Lymphoma, Myeloma, Neuroblastoma, Sarcoma, Colorectal, Lung, Breast, Ovarian, Head And Neck Cancers, Melanoma, And Glioblastoma). Oncological Disorders Are Hence The Medical Specialty Where Mab Therapies Are Most Readily Available. Furthermore, The Number Of Antibodies Targeting Programmed Cell Death Protein 1 (PD-1, Cemiplimab, Nivolumab, Pembrolizumab), Its Ligand Programmed Death-Ligand 1 (PD-L1, Durvalumab, Avelumab, Atezolizumab), Or Cytotoxic T-Lymphocyte–Associated Antigen 4 (CTLA-4, Ipilimumab) Or Cytotoxic T-Lymphocyte Marketing Approval Have Been Granted [35].

In 2018, Adalimumab (Humira) Was The Best-Selling Medicine In The World. Adalimumab Is A Biological Disease Modifier That Is Administered Subcutaneously For The Treatment Of Rheumatoid Arthritis And Other TNF-Mediated Chronic Debilitating Disorders. It Was First Introduced In The United States By Abbvie In 2002, After Receiving FDA Approval. Adalimumab Has Been Demonstrated To Help People With Moderate To Severe Rheumatoid Arthritis, As Well As Psoriatic Arthritis, Ankylosing Spondylitis, Crohn's Disease, Ulcerative Colitis, Psoriasis, Hidradenitis Suppurativa, Uveitis, And Juvenile Idiopathic Arthritis. It Can Be Taken Alone Or In Conjunction With Disease-Modifying Antirheumatic Medications To Treat Rheumatoid Arthritis.

Immune Checkpoints Play A Crucial Role In Maintaining Self-Tolerance And Regulating Physiologic Immune Responses In Peripheral Tissues. As A Result, The Chemicals That Underpin Checkpoints Have Recently Attracted A Lot Of Attention In Cancer Immunotherapy [36]. Anti-PD-1 Mabs Nivolumab (Opdivo) And Pembrolizumab (Keytruda) Were The Second And Third Best-Selling Mabs In 2018. Nivolumab Is A Human Antibody That Prohibits Activated T Lymphocytes From Attacking Cancer Cells By Blocking A Signal That Ordinarily Prevents Them From Doing So. The PD-1 Receptor Is The Target Of Nivolumab, And The Antibody Prevents PD-1 From Interacting With Its Ligands, PD-L1 And PD-L2, Releasing PD-1 Pathway-Mediated Immune Suppression. Melanoma, Lung Cancer, Head And Neck Cancer, Hodgkin's Lymphoma, And Stomach Cancer Are All Treated With Pembrolizumab, A Humanized Antibody Used In Cancer Immunotherapy. If Cancer Cells Overexpress PD-L1 And Have No Changes In EGFR Or Anaplastic Lymphoma Kinase, Pembrolizumab Is A First-Line Therapy For NSCLC. Patients With NSCLC Diagnosed With Nivolumab And Pembrolizumab (Both Authorized By The US FDA In 2014) Had A Higher Overall Survival Rate Than Those Treated With Docetaxel, The Conventional Second-Line Treatment [37]. In 2018, The US Approved A Total Of 12 Novel Monoclonal Antibodies (Mabs). The Bulk Of These Drugs Were Licensed For Non-Cancer Indications, Which Could Indicate That Antibodies As Therapies For Other Diseases Have A Greater Approval Success Rate. Three Antibodies Have Been Licensed For Migraine Prophylaxis (Erenumab, Galcanezumab, And Fremaezumab), While One (Ibalizumab) Is Used To Treat HIV Infection. Erenumab (Aimovig), Galcanezumab (Emgality), And Fremaezumab (Ajovy), Three Migraine-Prevention Medications, Are Monoclonal Antibodies (Mabs) That Block The Function Of The Calcitonin Gene-Related Peptide (CGRP) Receptor In Migraine Etiology. The Heteromeric Receptor For CGRP Is Made Up Of A G Protein-Coupled Receptor (Calcitonin Receptor-Like Receptor: CALCRL) And A Receptor Activity-Modifying Protein 1. (RAMP1). Galcanezumab And Fremaezumab Are Antibodies That Attach To CGRP And Prevent It From Binding To The Receptor. However, Erenumab Is The Only One Of The Three Antibodies That Interferes With The CGRP Binding Site By Targeting The Extracellular Domains Of Human G Protein-Coupled Receptors CALCRL And RAMP1 [38].

Although Many Mabs Are Being Developed For The Treatment Of Infectious Diseases, The US FDA Has Only Approved Four: Raxibacumab And Obiltoxaximab For The Treatment Of Inhalational Anthrax [39], Palivi-

zumab For The Prevention Of Respiratory Syncytial Virus In High-Risk Infants [40], And Ibalizumab For The Treatment Of HIV Patients [41]. Ibalizumab (Trogarzo) Is A Humanised Igg4 Mab That Is Utilised As A CD4 Domain 2-Directed Post-Attachment HIV-1 Inhibitor. Ibalizumab Has Been Approved By The US Food And Drug Administration (FDA) For Adult HIV Patients Who Have Been Pretreated And Thus Are Resistant To Presently Available Treatments.

VI. Conclusion

Over The Past Few Decades, Monoclonal Antibodies Have Been Approved And Used In Treatment Of Many Diseases Like Cancer, Rheumatoid Arthritis, TNF-Mediated Chronic Debilitating Disorders, Crohn's Disease, Hodgkin's Lymphoma, Etc. In This Review, I Discuss About How The Monoclonal Antibodies Are Produced With The Help Of Hybridoma Technology Which Produces These Designed Humanized, Chimeric And Many More Antibodies That Could Be Utilized To Make A Variety Of Antibody Fragments That Target Novel Antigenic Regions. It's Paved The Way For Therapeutic And Diagnostic Mabs With High Affinity And Specificity For Sites That Aren't Very Immunogenic. Mabs Have A High Level Of Target Selectivity, As Well As A Variety Of Diverse Modes Of Action Given By Modern Molecular Engineering Technologies. These Characteristics Make Monoclonal Antibodies (Mabs) Precise Tools With Limitless Potential To Act On Identified Major Pathogenetic Target.

VII. Future Prospects

Antibody Engineering Techniques Have Progressed Recently, Making It Feasible To Design Antibodies With A Wide Range Of Activities, Such As More Efficient And Long-Lasting Neutralizing Effects, And Agents That Cause Cytotoxicity At Lower Molecule Expression Levels. These Recent Developments, As Well As The Development Of Novel Target Molecules, Have Raised The Prospect Of New Therapeutics. As Antibodies' Functions And Target Molecules Become More Diverse, It's Becoming More Important To Understand How The Target Molecule Works Biologically And What The Biological Response Will Be To The Antibody's Modified Functions. The Toxicological Pathology Linked With These Problems Will Also Need To Be Thoroughly Studied And Investigated.

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