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UNCONJUGATED MONOCLONAL ANTIBODIES AND PROTEASE INHIBITORS IN TREATMENT OF MULTIPLE MYELOMA

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ABSTRACT:

Multiple myeloma (MM) is a malignant plasma cell disorder that affects the bone marrow, leading to various complications such as bone lesions, anaemia, and kidney failure. monoclonal means that those cells resulted from a single "clone" or a mother cell. This is, in fact, the case with most blood cancers where they develop due to a single cell losing control of its proliferating machinery leading to uncontrolled division. The approval of monoclonal antibodies has transformed the treatment landscape for multiple myeloma. These monoclonal antibodies are now integral components of combination regimens and have provided new

avenues for patients who are refractory to traditional therapies.In clinical studies, proteasome inhibitors have improved survival rates, reduced disease burden, and enhanced the quality of life for patients with multiple myeloma.

Key words: Multiple myeloma, malignant, unconjugated, monoclonal antibodies, protease inhibitors.

INTRODUCTION:

Multiple myeloma (MM) is a malignant plasma cell disorder that affects the bone marrow, leading to various complications such as bone lesions, anaemia, and kidney failure. Despite advances in therapy, multiple myeloma remains an incurable disease, and treatment strategies continue to evolve. Recent innovations in immunotherapy and targeted therapy have significantly impacted the management of multiple myeloma, with unconjugated monoclonal antibodies and protease inhibitors emerging as key therapeutic options.

SYMPTOMS:

Some people with multiple myeloma have no symptoms at all. But sometimes multiple myeloma does cause symptoms.

Bone problems

- Bone pain, which can be in any bone, but is most often in the back, the hips, or the skull
- Bone weakness, either all over (osteoporosis), or where there is a tumour
- Broken bones (fractures), sometimes from only a minor stress or injury

Low Blood Counts:

- myeloma and might lead to other symptoms. Anaemia: Having too few red blood cells can cause weakness, a reduced ability to exercise, shortness of breath, and dizziness.
- Leukopenia: Having too few white blood cells can lower resistance to infections such as pneumonia.
- Thrombocytopenia: Having too few blood platelets may cause serious bleeding even with minor scrapes, cuts, or bruises.

High Blood Levels Of Calcium (Hypercalcemia)

- Extreme thirst, leading to drinking a lot of fluids
- Urinating (peeing) a lot
- Dehydration
- Kidney problems, or even kidney failure
- Severe constipation
- Abdominal (belly) pain
- Loss of appetite
- Weakness
- Feeling drowsy
- Confusion
- If the level of calcium gets high enough, a person can even slip into a coma.

Nervous System Symptoms

If myeloma weakens the bones in the spine, they can collapse and press on spinal nerves. This is called spinal cord compression, and it can cause:

- Sudden severe back pain
- Numbness, most often in the legs

Muscle weakness, most often in the legs

This is a medical emergency, so you should contact your doctor right away or go to the emergency room if you have any of these symptoms. If spinal cord compression is not treatedright away, there is a possibility of permanent paralysis.

Nerve Damage

Sometimes, the abnormal proteins produced by myeloma cells can damage nerves. This can lead to weakness and numbness and sometimes a “pins and needles” sensation. This is called peripheral neuropathy.

Hyper viscosity

In some people, large amounts of myeloma protein can cause the blood to “thicken.” This is called hyperviscosity. It can slow blood flow to the brain, which can cause:

- Confusion
- Dizziness
- Symptoms of a stroke, like weakness on one side of the body and slurred speech

People with these symptoms need to call their doctor. Removing the protein from the blood using a procedure called plasmapheresis can rapidly reverse this problem. (Note: This is not something that can be treated with drugs known as "blood thinners.")

Kidney problems

Myeloma protein can damage the kidneys. Early on, this most likely won't cause any symptoms, but signs of kidney damage may be seen on a blood test or a urine test. If the kidneys start to fail, they lose the ability to get rid of excess salt, fluid, and body waste products. This can lead to symptoms such as:

- Weakness
- Shortness of breath
- Itching

Infections

People with myeloma are much more likely to get infections. When someone with myeloma gets an infection, they may be slow to respond to treatment. That person may stay sick for a long time. Pneumonia is a common and serious infection seen in people with myeloma.

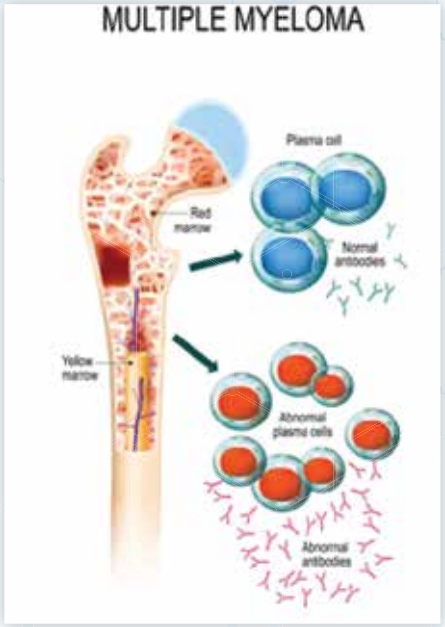
Causes

In 2015, an estimated 124,733 people in the United States were living with this disease. The exact cause of multiple myeloma is not known and no avoidable risk factors have been found. However, certain things appear to make you more likely to develop the disease.

Risk factors for multiple myeloma:

- Age: Over 65
- Gender: Men are slightly more likely to develop myeloma.
- Race: Black people are twice as likely to develop myeloma than white people.
- Radiation exposure
- Family history: If a parent, brother or sister has the disease, your risk is four times higher. However, this is rare.
- Working in oil-related industry: While some studies suggest this, it has not been
- proven.
- Obesity
- Other plasma cell disorders: If you have had one of the following you are at higher risk:
- A precancerous condition called monoclonal gammopathy of undetermined
- significance (MGUS)
- A single tumour of plasma cells (solitary plasmacytoma)
- Smouldering myeloma, a precancerous condition that affects the plasma cells and can turn into multiple myeloma

PATHOPHYSIOLOGY



Multiple myeloma is a cancer of the bone marrow thatresults from several genetic defects that lead to increased division of a certain "line" of cells called plasma cells in the bone marrow. As is the case with many cancers, there is a premalignant stage, which means that such cancer doesn't develop out of the blue but is preceded by a detectable stage in which it was not fully developed. This stage is called MGUS or Monoclonal Gammopathy of Undetermined Significance.

monoclonal means that those cells resulted from a single "clone" or a mother cell. This is, in fact, the case with most blood cancers where they develop due to a single cell losing control of its proliferating machinery leading to uncontrolled division. The term gammopathy refers to the problem arising from plasma cells. Plasma cells are white blood cells of the B lymphocyte type after their activation. They are responsible for the production of antibodies which are proteins that are also termed "gamma globulins".

During the stage of MGUS, mutations accumulate which is another key stage in the development of cancer, we need multiple mutations and alterations in DNA to develop the full-blown uncontrolled disease because we have inherent defence mechanisms that protect us against such conditions. They include repair genes which repair any defects in DNA during cellular division or in response to external factors as well as "suicide" mechanisms that force the cells to kill and eat themselves from within in case of a fatal error in their genetic material.

After the MGUS stage comes the smoldering myeloma stage which is intermediate between MGUS and multiple myeloma. The differentiation between MGUS, smoldering myeloma and multiple myeloma stages is based on certain criteria including the level of a certain protein called the M protein secreted by the abnormal plasma cells of multiple myeloma.

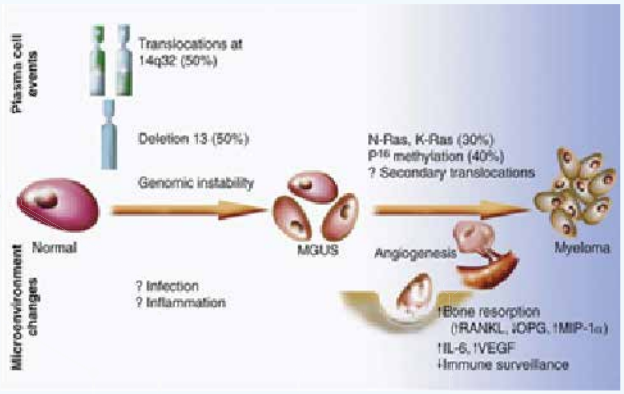
Cells of multiple myeloma are subject to numerous factors that affect their growth and the disease's severity. This usually has to do with what we call a "tumour microenvironment" which includes the multitude of chemical substances secreted from cancer cells that further affect their growth and the pattern of their division. This microenvironment is usually independent of the body's control and therefore the growth of cancer is uncontrollable.

Some of the confirmed associations are those between working in the petrochemical industry, agricultural fertilizers exposure and radiation exposure and the development of multiple myeloma. Other studies point out a possible relation between certain infections or autoimmune diseases and multiple myeloma but they remain largely theoretical

EPIDEMIOLOGY

Multiple myelomas are a less frequent cancer site among both sexes. On a worldwide scale, it is estimated that about 86,000 incident cases occur annually (47,000 males and 39,000 females), accounting for about 0.8% of all new cancer cases. About 63,000 subjects are reported to die from the disease each year (33,000 males and 30,000 females), accounting for 0.9% of all cancer deaths (Parkin et al. 2005). In terms of age-standardized rates, the annual incidence rates amount to 1.7 per 100,000 in males and 1.2 in females, and the mortality rates to 1.2 (males) and 0.9 (females). Among the haematological malignancies, the proportion of multiple myelomas ranges from 15–20% (Devesa et al. 1992; Becker et al. 2007).

Geographically, the frequency is very unevenly distributed globally, with the highest incidence in the industrialised regions of Australia/New Zealand, Europe, and North America. The ethnic comparison within the population of the USA shows an almost doubled occurrence of multiple myeloma among blacks compared to whites, while people of Asian origin, especially Chinese and Japanese, experience a much lower incidence (Coleman et al. 2008; Parkin et al. 2005).



Incidence and mortality seem to be stable in Asian countries and to increase slowly over the decades among whites in the Western countries and blacks in the USA (see Fig. 2.2). The rates and trends for Asian immigrants into the USA resemble those of respective countries of origin (Hirabayashi and Katanoda 2008)

Unconjugated Monoclonal Antibodies in Multiple Myeloma

Monoclonal antibodies (mAbs) are lab-engineered molecules specifically designed to target antigens expressed on the surface of tumour cells. In multiple myeloma, unconjugated monoclonal antibodies, which are not linked to toxic substances, have demonstrated significant promise in targeting myeloma cells, modulating immune responses, and enhancing the body's ability to destroy malignant cells.

Mechanisms of Action:

Unconjugated monoclonal antibodies work through various mechanisms, including:

- **Direct Targeting of Myeloma Cells:** mAbs such as daratumumab target the CD38 protein, which is overexpressed on malignant plasma cells in multiple myeloma. By binding to CD38, daratumumab triggers immune-mediated destruction of myeloma cells through complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and phagocytosis.

- **Immune System Modulation:** In addition to direct cell killing, monoclonal antibodies can modulate the immune microenvironment, enhancing the body's immune response to myeloma. For instance, mAbs can enhance the activity of natural killer (NK) cells and T-cells, which are critical components of the immune system's ability to combat cancer.

- **Prevention of Myeloma Cell Interaction:** mAbs like elotuzumab work by targeting the SLAMF7 receptor, present on both myeloma cells and immune cells. By binding to SLAMF7, these antibodies facilitate the activation of NK cells and other immune cells, which contribute to the destruction of myeloma cells.

Clinical Efficacy:

The approval of monoclonal antibodies has transformed the treatment landscape for multiple myeloma. Key agents include:

- **Daratumumab:** Approved for use as a single agent or in combination with other drugs like lenalidomide or bortezomib, daratumumab has shown significant efficacy in relapsed or refractory myeloma. In clinical trials, it has been associated with improved progression-free survival (PFS) and overall survival (OS) when added to existing treatment regimens.
- **Elotuzumab:** This mAb has demonstrated efficacy when combined with lenalidomide and dexamethasone in patients with relapsed or refractory multiple myeloma, particularly in improving PFS. It works synergistically with other therapies to enhance immune cell activity against myeloma cells.

These monoclonal antibodies are now integral components of combination regimens and have provided new avenues for patients who are refractory to traditional therapies.

Protease Inhibitors in Multiple Myeloma

Proteasome inhibitors (PIs) are another class of drugs that have transformed the treatment of multiple myeloma. The proteasome is a critical cellular machinery involved in degrading damaged or unneeded proteins, and its inhibition disrupts the regulation of cell cycle and apoptosis in malignant cells. Myeloma cells, which are highly dependent on protein turnover for growth, are particularly vulnerable to proteasome inhibition.

Mechanisms of Action:

Proteasome inhibitors, like bortezomib, carfilzomib, and ixazomib, block the proteasome's activity, accumulating misfolded or damaged proteins within the cell. This accumulation induces stress in the myeloma cells, eventually triggering cell death (apoptosis). Additionally, proteasome inhibition can:

- **Disrupt Signalling Pathways:** PIs interfere with key pathways that regulate cell survival, including NF-kB, a transcription factor that is critical for the survival and proliferation of myeloma cells.
- **Enhance Immune Response:** By inducing cellular stress, proteasome inhibitors also promote the activation of immune cells that target myeloma cells. This immune modulation contributes to their anti-myeloma effects.
- **Sensitize Myeloma Cells to Other Therapies:** Proteasome inhibitors can enhance the effectiveness of other treatments, such as immunomodulatory drugs (IMiDs) or monoclonal antibodies, by disrupting protective cellular mechanisms that allow myeloma cells to evade immune surveillance.

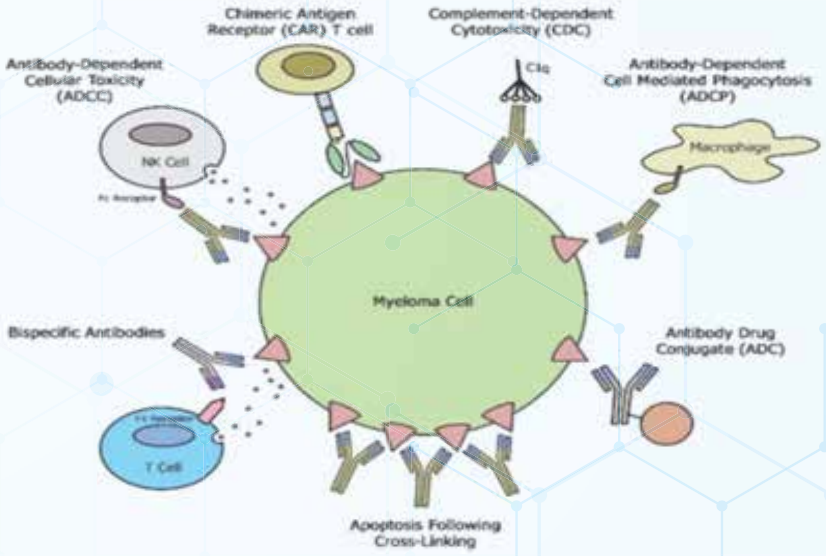
Clinical Efficacy:

Proteasome inhibitors have proven to be among the most effective agents in the treatment of multiple myeloma. Several proteasome inhibitors are approved for use:

- **Bortezomib:** The first proteasome inhibitor approved for myeloma therapy, bortezomib has significantly improved patient outcomes in both newly diagnosed and relapsed/refractory myeloma. It is commonly used in combination with other agents like lenalidomide or dexamethasone.
- **Carfilzomib:** A second-generation proteasome inhibitor, carfilzomib has been shown to be more potent and less neurotoxic than bortezomib. It is often used in patients with relapsed or refractory disease and has demonstrated improved PFS in combination with other agents.

- **Ixazomib:** An oral proteasome inhibitor, ixazomib is the first of its kind approved for use in combination with lenalidomide and dexamethasone for relapsed or refractory multiple myeloma. Its oral administration offers a more convenient treatment option for patients.

In clinical studies, proteasome inhibitors have improved survival rates, reduced disease burden, and enhanced the quality of life for patients with multiple myeloma.



Conclusion

The advent of unconjugated monoclonal antibodies and protease inhibitors has revolutionized the treatment landscape for multiple myeloma. Monoclonal antibodies like daratumumab and elotuzumab enhance immune-mediated destruction of myeloma cells, while proteasome inhibitors such as bortezomib, carfilzomib, and ixazomib disrupt critical cellular processes in myeloma cells, leading to their death.

Together, these therapeutic classes provide synergistic effects when combined with other treatments, offering new hope for patients with relapsed or refractory multiple myeloma. Ongoing research and clinical trials continue to explore novel combinations and next-generation agents, further improving the prognosis and quality of life for patients battling this challenging malignancy.

In the future, the goal remains to achieve deeper remissions, improve survival rates, and ultimately, find a cure for multiple myeloma. The combination of unconjugated monoclonal antibodies, proteasome inhibitors, and other novel agents are poised to play a central role in this effort.

ARTICLE 2

PROTEASE INHIBITORS WITH UNCONJUGATED MONOCLONAL ANTIBODIES: A PROMISING FRONTIER IN THE TREATMENT OF MM (MULTIPLE MYELOMA)

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ABSTRACT:

Use of protease inhibitors (PIs) and monoclonal antibodies has been the therapeutically a promising frontier in the treatment MM (Multiple Myeloma). Proteases precisely control a wide variety of physiological processes and thus are important drug targets. Compared with small-molecule inhibitors, unconjugated monoclonal antibodies (mAbs) are attractive, as they provide required specificity. In the context of carcinogenesis, most extracellular proteases are involved in various processes associated with tumor development and progression by different mechanisms. Thus, they can act as the base for membrane and matrix degradation. Since dysregulated proteolytic activities can contribute to tumor development and metastasis, antagonization of proteases with tailored inhibitors is an encouraging approach. In these aspects, monoclonal antibodies (mAbs) are emerging as attractive alternatives with significant advantages such as high selectivity, long serum half-life, and clear mechanisms of action. Nevertheless, the increasingly common use of unconjugated mAbs has revealed some unanticipated, often clinically significant toxic effects, as well as compromising effective palliative and end-of-life management approaches. Although patients and clinicians'. In these context provides a useful summary and guide for professionals treating patients with malignant diseases. and also discuss the current application and future clinical prospects of this potential combination towards targeted protease-based cancer therapy.

Keywords: Carcinogenesis, Multiple Myeloma, Protease Inhibitor, Unconjugated Monoclonal antibodies, Metastasis.

INTRODUCTION:

Proteases include widely distributed enzymes that are crucial for protein homeostasis and regulate many cellular processes, such as gene expression, differentiation, immunological defense, migra tion and cell death. Recent studies have indicated that the balance between production, activation and inhibition of proteases is often disturbed in malignant tumors, leading to tumor progression and dissemination. (1) Protease regulate important physiological processes and are involved in essential pathogenesis of viruses, and therefore represent attractive targets for pharmaceutical development. Human genome contains 569 proteases of 68 families, belonging to 5 main classes based on their catalytic mechanisms: aspartic, cysteine, threonine, serine, and metalloproteases. Existing in a delicate balance of networks with their endogenous inhibitors and substrates, proteases maintain the body's homeostasis. Dysregulation of proteolysis, however, causes a variety of diseases ranging from cancer, inflammation, and cardiovascular disorders to osteoporosis, neuropathic pain, and degenerative diseases. Notably, it has been estimated that proteases account for 5 to 10% of all drug targets being studied for therapeutic development.

(2,3) Targeting dysregulated protease expression and/or abnormal substrate proteolysis, highly selective inhibition of pathogenic proteases by monoclonal antibodies (mAbs) presents an attractive therapeutic approach for the treatment of diseases including cancer. (4). Many monoclonal antibodies have been approved for treating malignant cancers, inflammatory diseases such as rheumatoid arthritis4, 5 and osteoporosis6. According to a recent survey, about 50 monoclonal antibodies are currently approved for clinical use and approximately 350 monoclonal antibodies are under development. Unlike many small molecule drugs that may target multiple proteins, monoclonal antibodies typically target specific molecules (antigens) associated with diseases. Conceptually, monoclonal antibodies should display higher specificity and have fewer systemic side effects than small molecule drugs. (5)

Modes Of Actions Of Protease Inhibitor:

Proteases are a complex of enzymes that hydrolyze peptide bonds and are responsible for the breakdown of proteins into their individual components.1 Based on their amino acid In the context of cancerogenesis, most extracellular proteases are involved in various processes associated with tumor development and progression by different mechanisms. Thus, they can act as the base for membrane and matrix degradation, inactivation of natural protease inhibitors and chemotherapeutics, cell viability regulation, immune response modulation and inflammatory cell recruitment. (1,6–8) Moreover, proteases localized within the intracellular compartments, including in the cytosol, nucleus, membrane and mitochondria are associated with many signaling pathways through which they can promote: adhesion, proliferation, migration, de-differentiation and epithelial to mesenchymal transition of cancer cells. (8–10) The release of proteases into various cellular compartments and their altered expression can result from different factors, including the activation of membrane receptors (such as the tumor necrosis factor receptor (11) or the generation of reactive oxygen species. (12,13)

Proteases Inhibitors

The invasion and metastasis process of tumor cells is through a lytic machinery that composed of different proteolytic enzymes, named as the proteases. They are subdivided into five categories: matrix metalloproteases (MMPs), cystine proteases, serine proteases, aspartic proteases and threonine proteases (Sanman and Bogyo, 2014). The main classes of proteases that contribute to the lytic processes around tumors are MMPs, cathepsins (cystine proteases), and plasminogen activators (serine proteases). Each class of proteases has natural inhibitors which modulate their activity, such as, tissue inhibitors of metalloproteinase (TIMPs), the cystatins, which inhibit cathepsins, and the plasminogen activator inhibitors. (15)

Common adverse effects of proteases inhibitors

d prolonged inhibition of MMP activities causes aberrant immune responses and other stromal reactions. In addition, cysteine cathepsin inhibitors show potentially broader mechanistic efficacy and less toxicity as compared with the MMPIs (15)

Monoclonal antibodies

Monoclonal antibodies consist of two heavy polypeptide chains and two light polypeptide chains, capable of targeting and binding to specific proteins anywhere in the body. The antigens bound by mAbs can elicit antibody-dependent cellular cytotoxicity or complement mediated cytotoxicity, leading to the blockade of cell membrane receptors and inhibition of intracellular signaling [14]. monoclonal antibodies (mAbs), provide exquisite specificity capable of distinguishing between closely related protease family members .Their stability in serum, potential to cross blood–brain barrier, novel design as prodrugs, and improved effector functions offer significant advantages over small-molecule therapeutics. (2)

Common side effects of mAbs

when combining monoclonal antibodies with protease inhibitors can include: infusion reactions like chills, fever, nausea, vomiting, diarrhea, rash, low blood pressure, headaches, fatigue, muscle aches, and potential allergic reactions; as both medications can independently cause similar side effects, with the added potential for increased severity due to drug interactions depending on the specific medications involved.

Overview of Protease inhibitors with unconjugated mABs for cancer treatment

The exquisite specificity, natural biological functions, and favourable development properties of antibodies make them highly effective agents as drugs. Monoclonal antibodies are particularly strong as inhibitors of systemically accessible targets where trough-level concentrations can sustain full target occupancy. (16) Nowadays Various proteases inhibitors for ex HIV-protease inhibitors (17), Serine protease inhibitors (18) are using experimentally and clinically with different mABs, However, as these inhibitors have an encouraging pharmacokinetic profile and a decisive pharmacodynamic phenotype, they can potentially be used for other proteases and other types of cancer.

Conclusion:

Unconjugated Monoclonal antibodies (mAbs) are a major targeted therapy for malignancies, infectious diseases, autoimmune diseases, transplant rejection and chronic inflammatory diseases. Use of protease inhibitor with mABs can shows various challenges in the development of protease inhibitors, including but not limited to issues of inhibitor specificity, efficiency enhancement, and long-term safety. In response to these challenges, we further explore feasible solutions and strategies, aiming to provide directional guidance for future research.

ARTICLE 3

ANIMAL SPECIES USED FOR PREPARATION AND ISOLATION OF MONOCLONAL \ ANTIBODIES USED FOR THE MANAGEMENT OF CANCER

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ABSTRACT:

Hybridoma technology is one of the most common methods used to produce monoclonal antibodies. In this process, antibody-producing B lymphocytes are isolated from mice after immunizing the mice with specific antigen and are fused with immortal myeloma cell lines to form hybrid cells, called hybridoma cell lines. These hybridoma cells are cultured in a lab to produce monoclonal antibodies, against a specific antigen being cancer cells, will multiply rapidly and indefinitely and will produce large amounts of the desired antibodies. They have to be selected and subsequently cloned by limiting dilution. Supplemental media containing Interleukin-6 (such as briclone) are essential for this step. The production of monoclonal anti-bodies was first invented by Cesar Milstein, Georges J. F. Köhler and Niels Kaj Jerne in 1975. Selection occurs via culturing the newly fused primary hybridoma cells in selective-media, specifically media containing 1x concentration HAT for roughly 10–14 days. After using HAT it is often desirable to use HT containing media. Cloning occurs after identification of positive primary hybridoma cells. Clone by limited dilution.

Keywords: Hybridoma technology, cell lines, HAT, B lymphocytes, Animal species.

INTRODUCTION:

Antibodies are mainly produced for diagnostic and therapeutic applications. Monoclonal antibodies were discovered in 1975. In 1975, Kohler and Milstein discovered a technique called hybridoma technology for the pro duction of monoclonal antibodies. It is one of the mostwidely used techniques in modern research and studies [1]. Kohler and Milstein [2] developed a system in which antibody-producing B cells were fused with immortal cancerous cell lines such as myeloma cells [3] (Fig. 1)

Hybridoma technology produces monoclonal antibodies (mAbs) specific to antigens. These cell lines can also be cryopreserved for a long period of time. Hybridoma technology has resulted in the production of a variety of different monoclonal antibodies with specificity fora specific antigen. Antigen molecules include enzymes, hormones, internal and external structures of bacteria, viruses, and eukaryotic cells. Monoclonal antibodies produced by this method are highly specific antibodies, which are derived from a single parental B cell clone [4].

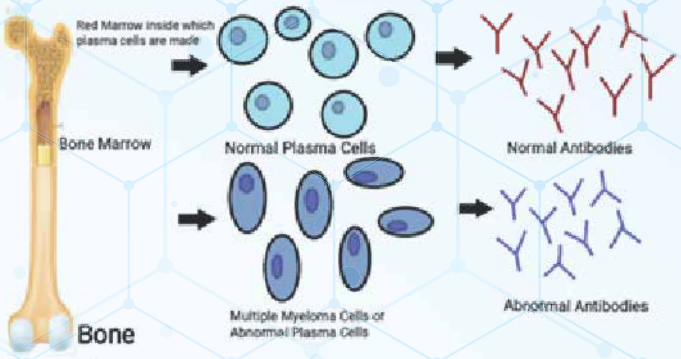

Fig. 1 Formation of myeloma cells

Antibodies are the glycoproteins produced by the B-cells also known as immunoglobulins (Fig.2), which are present in higher eukaryotes. Immunoglobulins are present in either as a soluble form (blood or plasma) or as membrane-bound form (B cell receptors). Antibodies are the major component of the humoral immune system that provides protection against the invading pathogens i.e. viruses and bacteria [5].

An antibody is made up of two structural unit's i.e. heavy and light chain. Generally, each heavy chain has one variable and three constant regions whereas the light chain has one variable and one constant region. The variable region of antibodies is mainly responsible for its interactions with the invading pathogen and antigen recognition. The antigen-antibody recognition mechanism works like a lock and key fashion. Each antibody has a particular paratope (i.e. lock) that binds to a particular antigen (i.e. key). One type of B cell produces one type of antibody against a particular antigen. There are five different types of heavy chains based on the structure of crystallizable fragments (Fc) that is attached to the antigen-binding fragments. On the basis of different Fc region, antibodies are grouped into five different isotypes i.e; IgM, IgG, IgA, IgD, and IgE. Among all the isotypes IgG is the smallest and the most common isotype with the highest therapeutic potential. It makes 70–80% of the total antibodies. IgGs have a longer half-life and are permeable to extravascular spaces [6], [7].

These antibodies are classified into two primary subtypes, monoclonal and polyclonal on the basis of their origin from the lymphocytes [5], [6]. Both polyclonal and mAbs have their advantages and limitations which make them equally suitable for different applications.

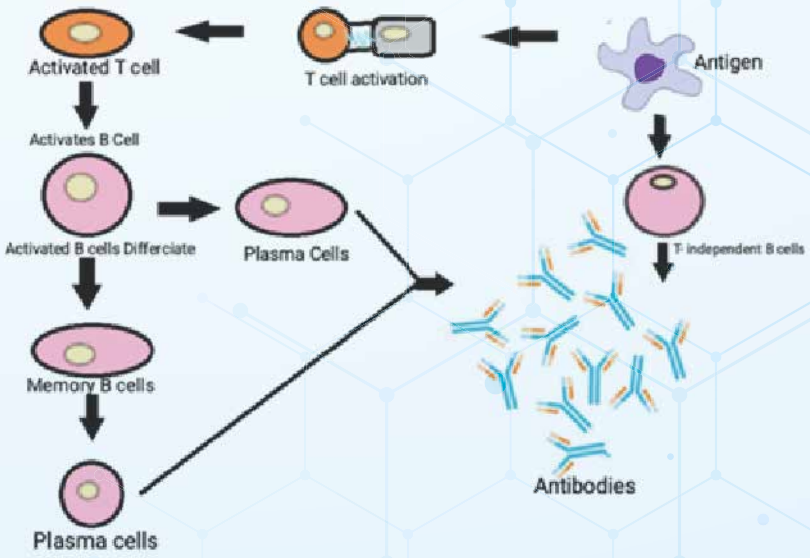

Fig. 2 Activation of B cells and production of antibodies

Polyclonal antibodies (pAbs) are a pool of immunoglobulin molecules that are secreted by different B cell lineages and react against multiple epitopes of a specific antigen. The pAbs are generated by injecting an immunogen into an animal using a prime-boost immunization strategy to produce high titres of antibodies against the particular antigen. After immunization, pAbs can be used directly or in the purified form (through affinity column chromatography to remove other serum protein components). The polyclonal serum is widely used for several decades for the treatment of toxin-mediated bacterial and viral diseases [7]. Emil Adolf von Behring was awarded Nobel Prize in Physiology and Medicine in 1901 for his work on serum therapy, especially its application against diphtheria, Animal serum-derived therapy has been successfully applied for different medical aspects like overdosing of medication, viral disease (like rabies) and as antitoxins in snakebite envenoming [10]. The beneficial effects of pAbs come from its polyclonal nature and biophysical diversity. The poly clonality nature allows targeting multiple sites in a single window of the application and biophysical diversity provides greater stability in environmental changes [11], [12]. Despite having beneficial effects of serum-derived pAbs therapy has several limitations, which need to be evaluated before introducing new interventions. As blood-derived products, intravenous polyclonal immunoglobulins (IVIG) have limited availability, batch-to-batch variability; carry the risk of blood-borne disease transmission and only a small fraction of antibodies from the pool of antibodies binds to the target of interest to exert the desired effect. This sometimes results in low specific activity and relatively needs high doses to observe a desired beneficial clinical effect [13], [14].

Monoclonal Antibodies

Monoclonal antibodies are a homogeneous mixture of antibodies that are monospecific in nature. These anti bodies have affinity and specificity towards one epitope of a selected antigen (monovalent affinity). In the event of the development of monovalent antibodies, the scope of therapeutic and diagnostic applications has expanded encompassing various fields of biotechnology such as molecular biology, toxicology, biochemistry, and medicine. Out of the several techniques developed over years to produce monoclonal antibodies (single lymph cell amplification or by culturing strategies), hybridoma technology is one of the most important and most commonly used [15], The mAbs can be produced against any given epitope present on an antigen or immunogen. Moreover, they can be used to detect, purify and characterize the substance of interest. Since the development of mAb, the scope of antibodies has expanded to various further applications due to their target specificity [17]. This has made mAbs a powerful tool in the fields of biochemistry, molecular biology, and medicine.

Methodology:

A hybridoma, which can be considered as a harry cell, is produced by the injection of a specific antigen into a mouse, procuring the antigen-specific plasma cells (antibody-producing cell) from the mouse's spleen and the subsequent fusion of this cell with a cancerous immune cell called a myeloma cell. The hybrid cell, which is thus produced, can be cloned to produce many identical daughter clones. These daughter clones then secrete the immune cell product.

Since these antibodies come from only one type of cell (the hybridoma cell) they are called monoclonal antibodies. The advantage of this process is that it can combine the qualities of the two different types of cells; the ability to grow continually, and to produce large amounts of pure antibody. HAT medium (Hypoxanthine Aminopetrin Thymidine) is used for preparation of monoclonal antibodies. Laboratory animals (eg. mice) are first exposed to an antigen to which we are interested in isolating an antibody against. Once splenocytes are isolated from the mammal, the B cells are fused with immortalized myeloma cells - which lack the HGPRT (hypoxanthine-guanine phosphoribosyl transferase) gene - using polyethylene glycol or the Sendai virus. Fused cells are incubated in the HAT (Hypoxanthine Aminopetrin Thymidine) medium. Aminopterin in the myeloma cells die, as they cannot produce nucleotides by the de novo or salvage medium blocks the pathway that allows for nucleotide synthesis. Hence, unfused D cell die. Unfused B cells die as they have a short life span. Only theB cell-myeloma hybrids survive, since the HGPRT gene coming from the B cells is functional. These cells produce antibodies (a property of B cells) and are immortal (a property of myeloma cells). [22] The incubated medium is then diluted into multiwell plates to such an extent that each well contains only 1 cell. Then the supernatant in each well can be checked for desired antibody. Since the antibodies in a well are produced by the same B cell, they will be directed towards the same epitope, and are known as monoclonal antibodies.3 Once a hybridoma colony is established, it will continually grow in culture medium like RPMI-1640 (with antibiotics and foetal bovine serum) and produce antibody (Nelson et al., 2000.)[23] The next stage is a rapid primary screening process, which identifies and selects only those hybridomas that produce antibodies of appropriate specificity. The hybridoma culture supernatant, secondaryenzyme labelled conjugate, and chromogenic substrate, is then incubated, and the formation of a colored product indicates a positive hybridoma. Alternatively, immunocytochemical screening can also be used Multiwell plates are used initially to grow the hybridomas and after selection, are changed to larger tissue culture flasks. This maintains the wellbeing of the hybridomas and provides enough cells for cryopreservation and supernatant for subsequent investigations. The culture supernatant can yield 1to 60 ug/ml of monoclonal antibody, which is maintained at 20°C or lower until required By using culture supernatant or a purified immunoglobulin preparation, further analysis of a potential monoclonal antibody producing hybridoma can be made in terms of reactivity, specificity, and cross reactivity.

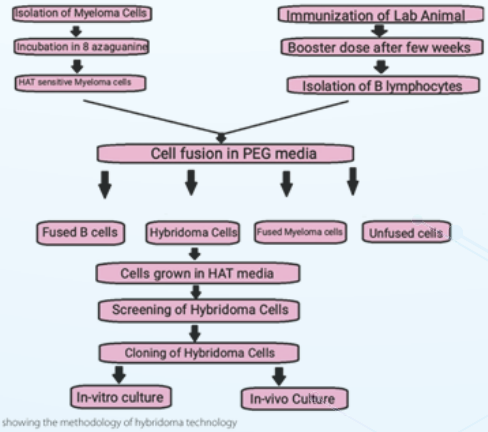

Fig. 3 Flow chart showing the methodology of hybridoma technology

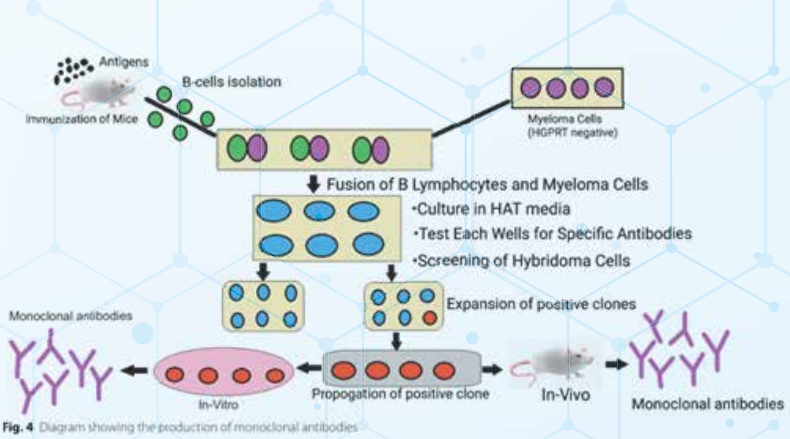

Fig. 4 Diagram showing the production of monoclonal antibodies

Animal Species Used For Hybridoma Development Over The Years:

Mouse: Mouse polyclonal and mAbs held the largest market in 2019 as they are more specific and easier to produce in nature. The structural similarities between human and mice antibodies are the prime reason for their high acceptability rate. Upgradation and simplicity of mice hybridoma process have made it a more prime reason for their high adoption rate in research and therapeutics.

The mice hybridoma technology is a multi-step process that takes advantage of a host animal's natural ability to produce highly specific, high-affinity and fully functional mAbs. It involves the development and optimization of specific immunogenic antigen (Ag). Following the optimization, a host animal is immunized with the Ag along with adjuvant for several weeks. The sera from immunized animals are tested for their reactivity and specificity to the immunizing antigen while the animals with high titres of binding antibodies are selected further for splenocytes isolation . The spleen cells are fused with the immortalised myeloma cells in the presence of fusogenic agents like viruses, chemicals and electric pulses. The most common myeloma fusion cell lines are X63-Ag 8.6539 and Sp2/0-Ag 1410 , with the origin from BALB/c mouse. The fused cells are then selected on hypoxanthine-aminopterin-thymidine (HAT) medium. The myeloma cells are sensitive to HAT medium as they lack hypoxanthine-guanine phosphoribosyltransferase (HGPRT) gene required for nucleotide synthesis by the de novo or salvage pathways while the unfused B cells die as of short life span. In this process, only the hybrid (B cell-myeloma) survives, as they harbour the functional HGPRT gene from the B cells. However, hybrid cells retain the dual properties, antibody secreting property of B cells and continuously growing property (immortality) from myeloma cells. Fused or hybridcells are then screened by "limited dilution cloning" method or with semi solid selective medium to select only those hybridoma that produce antibodies of appropriate specificity. A detailed schematic representation of steps involved in hybridoma production is shown in (Fig.5)

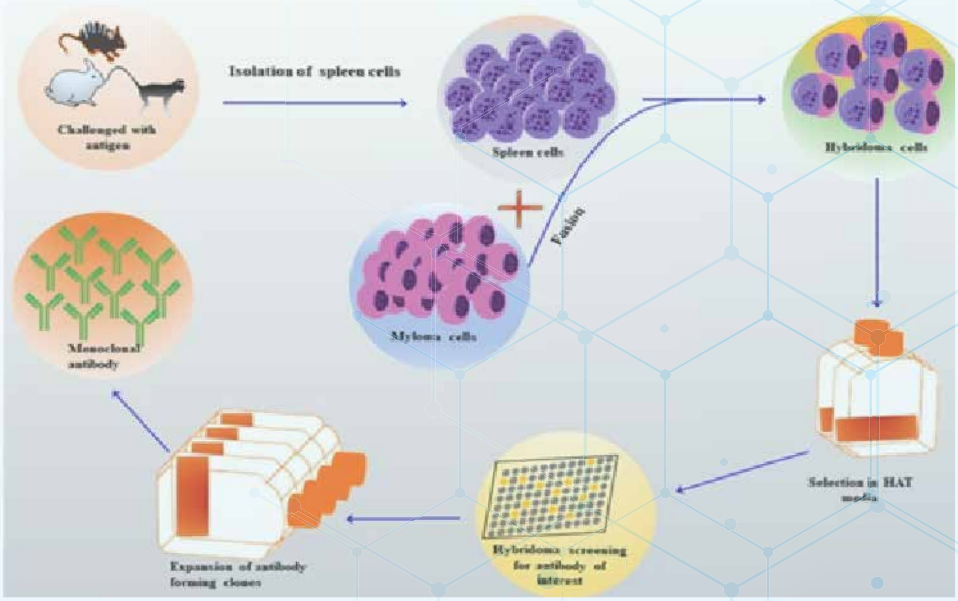

Fig.5. Hybridoma technology used to produce mAbs: Generation of mAb by immunizing laboratory animals with any target antigen. Hybridoma cells generated by the fusion between B-cells from an immunized animal (generally a rat, mouse, rabbit or monkey) and the myeloma cells. Hybrid cells are selected in HAT media and finally, cells secreting desired antibodies are screened.

Table 1. List of FDA approved diagnostic and therapeutic mouse monoclonal antibodies.

EMPTY CELL	TRADE NAME	TARGET	ISOTYPE	COMPANY	FDA / EU EMA APPROVAL YEAR	APPLICATION	TARGET
1	Besilesomab (Scintimun)	NCA-95	Murine IgG1	CIS Bio	2010	Diagnostics	Inflammatory lesions and metastases
2	NeutroSpec® (Fanolesomab)	CD15	Mouse labelled with radioisotope, technetium-99m (99mTc)	Palatin	2004	Diagnostics	appendicitis
3	Ibritumomab tiuxetan (Zevalin)	CD20	Murine IgG1	Biogen Idec	2002	Therapeutics	Treatment for relapsed or refractory, low grade or transformed B cell non-Hodgkin's lymphoma,
4	Tositumomab and iodine 131 tositumomab (Bexxar)	CD20	Murine IgG2a	Corixa and GSK	2003	Therapeutics	Non-Hodgkin lymphoma
5	Arcitumomab (CEA-scan)	carcinoembryonic antigen	Fab' fragment of a murine monoclonal antibody	Immunomedics	1996	Diagnostics	Imaging of colorectal cancers
6	Capromab (ProstaScint)	Prostate Specific Membrane Antigen (PSMA)	Murine IgG1κ	Cytogen	1996	Diagnostics	Prostate adenocarcinoma
7	Nofetumomab (Verluma)	Carcinoma associated antigen	Fab fragment of murine IgG2b	Boehringer Ingelheim, NeoRx	1996	Diagnostics	Small cell lung cancer
8	Satumomab (OncoScint)	tumor-associated glycoprotein (TAG-72)	Murine IgG 1	Cytogen	1992	Diagnostics	Colorectal or ovarian cancer
9	Muromonab-CD3 (Orthoclone OKT3)	CD3	Murine IgG2a	Centocor Ortho Biotech (Johnson & Johnson)	1986	Therapeutics	Reduce acute rejection in patients with organ transplants

Rabbit: The rabbit immunesystem has been documented as a vehicle for developing antibodies with higher affinity and more diverse recognition of many molecules including phospho-peptides, carbohydrates and immunogens that are not otherwise immunogenic in mouse [24]. Antibodies produced in rabbits usually have about 10 to 100 fold greater affinity than those produced by mice. Rabbits generate more diverse and complex immune response towards target antigen as compared to human and mice because of gene conversion and somatic hypermutations phenomenon leads towards more mutations in rabbit antibody repertoire . The gene conversion is responsible for introducing mutations and affinity maturation of variable antibody fragments which takes place in double-stranded rearranged V(D)J DNA segment of antibody gene via homologous recombination [19]. The rabbit IgGs are somewhat simpler than the mouse and human antibodies. Rabbit IgG has only one subclass i.e. Cγ gene and the majority (90–95%) of light chains are derived from isotype Cκ1. Only 5% to 10% of the total IgG light chains are isotype l. (Fig.6) Several efforts were made to generate rabbit mAbs after the development of mouse hybridoma technology in the 1970s. Due to the favourable properties of rabbit antibodies, many scientific groups tried to develop methods for the generation of rabbit hybridomas. This endeavour was significantly complicated by the absence of rabbit myeloma cell lines. Viral transformation of rabbit B cells to generate myeloma-like cell lines also proved to be difficult and rather inefficient. For these reasons, substantial efforts are focused on generating rabbit–mouse hetero-hybridomas. Unfortunately, all hetero-hybridomas generated in the early days of hybridoma technology revealed poor fusion efficiency, genetic instability and impaired functional rabbit heavy- and light-chain pairings. In 1988, Raybould et al. generated the first stable rabbit–mouse hetero-hybridoma by polyethylene glycol-mediated fusion of rabbit spleen B cells with the mouse myeloma cell line SP2/0-Ag14. Even though they observed stable rabbit IgG expression for several months, other groups observed genetic instability and concomitant decrease of mAb secretion.

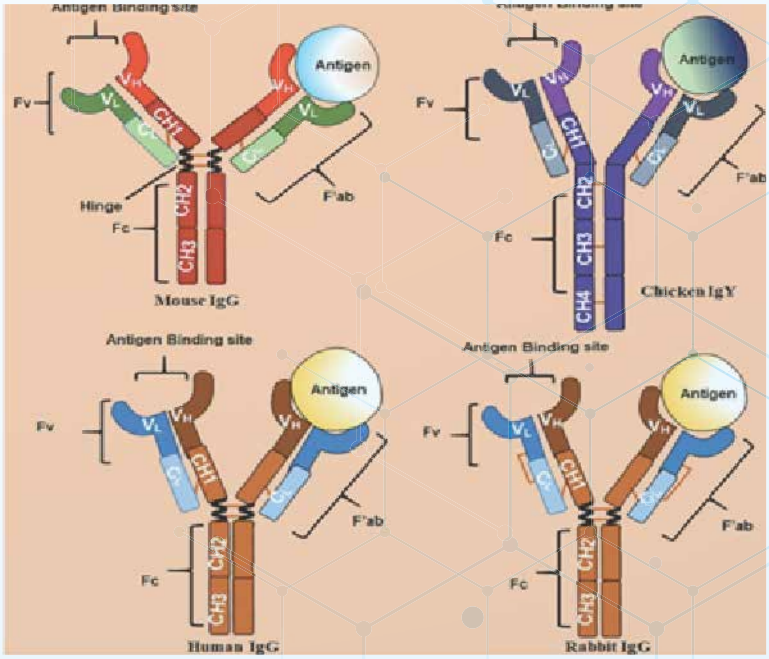

Fig.7. Schematic drawing of natural rabbit, mouse, chicken and human IgG. Generally 150-KDa IgG comprises of two identical κ or λ light chains paired with two identical heavy chains.

Table 2. A list of FDA approved rabbit mAbs that are used in diagnostics.

EMPTY CELL	Device Name	FDA Approval Year	Company	Target	Diagnosis
1	FLEX Monoclonal Rabbit Anti-Human Estrogen Receptor A, Clone EPI, RTU (Dako Onmis)	2017	DAKO DENMARK	Anti-Human Estrogen Receptor α	human breast carcinomas
2	VENTANA PD-L1 (SP263) ASSAY	2017	Ventana Medical Systems, Inc.	anti- Programmed Death-Ligand 1 (PD-L1)	in the assessment of the PD-L1 protein urothelial carcinoma tissue
3	VENTANA PD-L1 (SP142) Assay	2016	Ventana Medical Systems, Inc.	anti- Programmed Death-Ligand 1 (PD-L1)	in the assessment of the PD-L1 protein urothelial carcinoma and non-small cell lung cancer (NSCLC) tissue
4	VENTANA PD-L1(SP142) CDX ASSAY	2016	Ventana Medical Systems, Inc.	anti- Programmed Death-Ligand 1 (PD-L1)	in the assessment of the PD-L1 protein urothelial carcinoma tissue
5	PD-L1 IHC 28-8 PharmDx	2016	Dakoako North America Inc.	anti- Programmed Death-Ligand 1 (PD-L1)	in the assessment of the PD-L1 protein non-squamous non-small cell lung cancer (NSCLC) tissue and melanoma tissue
6	Monoclonal rabbit anti-human estrogen receptor (ER) a, clone epi	2013	Dako North America Inc	Human Estrogen Receptor α	human breast carcinomas
7	Confirm anti-estrogen receptor (SP1) rabbit monoclonal primary antibody	2012	Ventana Medical Systems, Inc	Anti-Human Estrogen Receptor α	human breast carcinomas
8	Confirm anti-progestoron e receptor (1E2) rabbit monoclonal primary antibody	2011	Ventana Medical Systems, Inc	Anti-Progesterone receptor	prognosis, and prediction of hormone therapy for breast carcinoma
9	Ventana anti-helicobacter pylori (SP48) rabbit monoclonal primary antibody	2011	Ventana Medical Systems, Inc	anti-Helicobacter pyloni	in vitro diagnostic against H-. pylori organisms
10	Monoclonal rabbit anti human estrogen receptor alpha antibody clone sp1, model (M3634)	2009	Dako North America Inc.	Anti-Human Estrogen Receptor	Identification of estrogen receptor (ER) expression in normal and neoplastic tissues
11	Ventana medical systems pathway Anti-c-KIT primary antibody	2004	Ventana Medical Systems, Inc	Anti-c-KIT	Gastrointestinal stromal tumors
12	Pathway anti-HCR-2/NCU rabbit monoclonal primary antibody	2000	Ventana Medical Systems, Inc	ANTI-HCR-2/NCU	Assessment of Breast cancer patients for whom HERCEPTIN(R) treatment is being considered

Humanized Antibody

More than 90% of human sequences are present in the humanized protein. Human immunoglobulin G antibodies are completely humanized by fusing the DNA of three CDRs from the mouse variable regions into human immunoglobulin G antibodies [27]. These totally humanized antibodies are made with the help of transgenic mice that have human immunoglobulin; therefore, they include 100% human sequences and are needed in fewer quantities than the antibodies of mice origin [9, 23].

A fusion of human B cells with different fusion partners limits the use of these mAbs for therapeutic applications. Several hetero-myelomas fusion has been successfully employed for the generation of mAbs of human origin for different diseases like HIV , Chikungunya , Dengue etc. The two most common cell lines used for fusion are SHM-D3327 and HMMA 2.5. The SHM-D33; produced by fusing the human myeloma cell line FU-266, clone E-1 (HAT sensitive, 8-azaguanine resistant and resistant to G-418 - an antibiotic similar to gentamicin) with the murine myeloma P3X63Ag8.653 .This cell line has been used as a fusion partner to stabilize the lymphoblastoid cell lines (LCL's)secreting immunoglobulins to produce mAbs against envelope proteins of HIV-1 and parvovirus B19 , . HMMA 2.5 is a human × mouse cell line that was generated by fusing mouse myeloma cell line P3x6Ag8.653 with bone marrow mononuclear cells of a patient with IgA myeloma . Several mAbs have been produced using this cell line as a myeloma fusion partner.

mAb secretion.

Conclusion

Presently, the monoclonal antibodies used are either raised in mice or rats; this poses a risk of dis ease transfer from mice to humans. There is no guarantee that antibodies thus created are entirely virus-free, despite the purification process. Also, there are some immunogenic responses observed against the antibodies of mice origin. Technologically advanced techniques such as genetic engineering helped in reducing some of these limitations. Advanced methods are under development to make lab-produced monoclonal antibodies as human as possible. This review discusses the advantages and challenges associated with monoclonal antibody production, also enlightens the advancement, clinical significance, and future aspects of this technique.

ARTICLE 4

MONOCLONAL ANTIBODIES TO TREAT MULTIPLE MYELOMA: A DREAM COME TRUE

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ABSTRACT:

Immunotherapy is increasingly used in the treatment of multiple myeloma (MM). Monoclonal antibodies (mAbs) are safe and effective ways to elicit immunotherapeutic responses. In 2015, daratumumab has become the first mAb approved by the Food and Drug Administration for clinical use in MM and, in the last 5 years, a lot of clinical and preclinical research has been done to optimize the use of this drug class. Currently, mAbs have already become part of standard-of-care combinations for the treatment of relapsed/refractory MM and very soon they will also be used in the frontline setting. The success of simple mAbs ('naked mAbs') prompted the development of new types of molecules. Antibody–drug conjugates (ADCs) are tumor-targeting mAbs that release a cytotoxic payload into the tumor cells upon antigen binding in order to destroy them. Bispecific antibodies (BiAbs) are mAbs simultaneously targeting a tumor-associated antigen and an immune cell-associated antigen in order to redirect the immune cell cytotoxicity against the tumor cell. These different constructs produced solid preclinical data and promising clinical data in phase I/II trials. The aim of this review article is to summarize all the recent developments in the field, including data on naked mAbs, ADCs and BiAbs.

Keywords: multiple myeloma, immunotherapy, monoclonal antibodies, antibody–drug conjugates, bispecific antibodies

INTRODUCTION:

Multiple myeloma (MM) is the second most common hematologic malignancy, with approximately 5: 100,000 new cases per year in Western countries. Although the introduction of new pharmacologic classes, such as proteasome inhibitors (PIs) or immunomodulatory drugs (IMiDs), has revolutionized treatment in the last decades MM remains an incurable disease and almost all patients relapse after a variable period and become refractory to previously used drugs (relapsed/refractory multiple myeloma, RRMM). In clinical practice, the development and introduction of new drugs with unique mechanisms of action and the combination of different drug classes have increased treatment options for RRMM patients and have improved the depth of response in newly diagnosed (ND) MM patients

In MM, the dysregulation of the immune system plays an important role in the development and progression of the disease. Producing drugs acting on the complex interplay between the immune system and the tumor cells (i.e., immunotherapy) is thus an appealing strategy.

There are different types of immunotherapy cellular immunotherapies usually involve the harvesting of various immune cell populations (e.g., T lymphocytes and natural killer cells) that are properly stimulated or genetically modified ex vivo and then infused into the patients to target tumor cells . A simpler form of immunotherapy relies on the infusion of target-specific antibodies produced from a single clone (monoclonal antibodies, mAbs) that target neoplastic cells and activate the immune system or disrupt a signaling pathway protecting neoplastic cells from immune-cell destruction. In this review, we will focus on mAb-related immunotherapies (Figure 1).

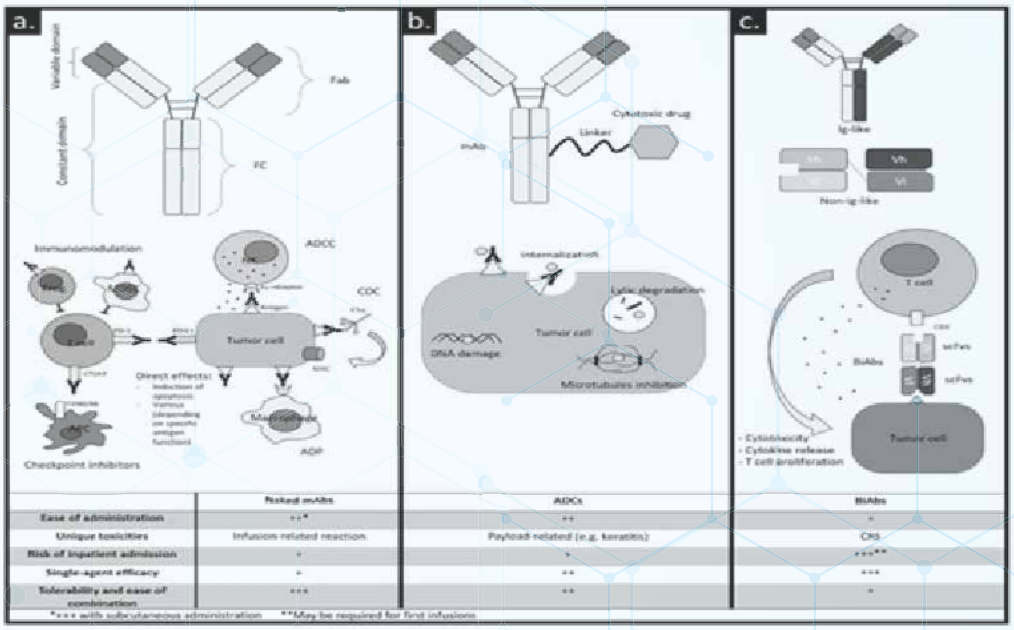

Figure 1. Monoclonal antibodies: types and mechanisms of action. (a) Naked monoclonal antibody (mAb). Antibody-dependent cellular cytotoxicity (ADCC): natural killer cell (NK) binds to the Fc region via the Fc-receptor and releases lytic factors such as perforin and granzymes. Complement-dependent cytotoxicity (CDC): interaction between the Fc region and protein C1q activates the classic complement pathway that results in the formation of membrane attack complex (MAC) and cell lysis. Antibody-dependent phagocytosis (ADP): the binding of macrophages induces activation of phagocytosis. Direct effects: induction of apoptosis directly or through cross-linking; effects depending on specific antigen functions (e.g., inhibition of intracellular signaling, blocking of enzymatic functions). Checkpoint inhibitors: blockade of programmed cell death protein 1 (PD-1) or cytotoxic T-lymphocyte antigen 4 (CTLA-4) preventing immune response suppression. Immunomodulation: interaction of mAb with stroma cells, which inhibit T cell activation restoring the immune response against neoplastic cells. (b) Antibody–drug conjugate. Upon antibody binding to the target, a cytotoxic payload is released in the target cell. (c) Bispecific monoclonal antibodies. Ig-like: two binding sites with different specificity and an Fc region that binds to the Fc-receptor. Non-Ig-like: two different single-chain variable fragments (variable regions comprised only of the variable regions of the heavy and light chains). Bispecific monoclonal antibodies usually bind a tumor antigen and an immune effector antigen (e.g., CD3 on the T-cell surface), in order to activate the immune cells against the neoplastic cell.

Abbreviations: +/++/+++, low/moderate/high; Fab, fragment antigen binding; FC, fragment crystallizable region; Treg, regulatory T cell; MDSC, myeloid-derived suppressor cell; NK, natural killer cell; ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; MAC, membrane attack complex; ADP, antibody-dependent phagocytosis; APC, antigen-presenting cell; ADCs, antibody–drug conjugates; BiAbs, bispecific antibodies; CTLA-4, cytotoxic T-lymphocyte antigen 4; PD-1, programmed cell death protein 1; PD-L1, programmed death ligand 1; di-scFv, bivalent single-chain variable fragment; scFv, single-chain variable fragment; CRS: cytokine release syndrome; Ig, immunoglobulin.

In 1975, Köhler and Milstein developed a technology to produce specific clonal antibodies targeting a desired antigen (mAbs) by fusing mouse MM cell lines with spleen cells from an immunized donor .Starting from this pivotal study, we are now able to produce chimeric, humanized and also totally human mAbs for clinical use.

MAbs without added elements are called “naked” and are composed of two fragment antigen-binding regions (Fab) and a fragment crystallizable region (Fc). The two Fab are responsible for the interaction with the antigen. The Fc is responsible for the interaction with cells that express an Fc receptor (lymphocytes, macrophages, neutrophils and dendritic cells) and with some proteins of the complement system (Figure 1a).

Fc mediates three main mechanisms of immune activation: antibody-dependent cellular cytotoxicity (ADCC) in which effector cells, typically NK cells, are activated and the release of lytic enzymes leads to cytotoxic effects; complement-dependent cytotoxicity (CDC) in which the binding of protein C1q triggers the activation of the classical complement pathway; antibody-dependent phagocytosis (ADP) in which macrophages are activated to phagocytize neoplastic cells. Furthermore, the Fc receptor may induce programmed cell death through the cross-linking of some target antigens. Besides Fc-dependent processes, some mAbs can modulate the enzymatic function of the target cell, ultimately contributing to antineoplastic effects. Naked mAbs can modulate the immune response against tumor cells also without a direct binding to the tumor cell. For instance, the blockade of inhibitory molecules (e.g., PD-1/PD-L1 axis) that usually turn off the immune response can restore the tumor-specific immune function. Moreover, the selective targeting of suppressive cells inhibiting the immune response in the tumor microenvironment (e.g., regulatory T cells (Tregs) and myeloid suppressor-derived cells) can restore the immune function as well structure of naked mAbs can be modified to obtain new mechanisms of action. A naked mAb can be conjugated through a linker to a cytotoxic drug (payload) that is released upon antigen binding into a target cell. In this way, the specific binding of mAbs to the target cell is used to convey a payload directly to the tumor cell. This type of molecule is called an antibody–drug conjugate (ADC) (Figure 1b). Payloads used in ADCs are highly active drugs, usually directed against microtubules or DNA. The binding of the ADC to the antigen induces the internalization of the ADC–antigen complex and the release of the payload via linker cleavage or degradation into lysosomes, reducing systemic adverse effects and achieving a powerful antineoplastic effect. Moreover, the Fc region of the ADC can activate Fc-dependent effects (CDC, ADCC and ADP), contributing to antitumor activity

Recently, mAbs with double specificity (bispecific antibodies, BiAbs, Figure 1c) have been developed.

Typically, targeted antigens are a tumor antigen and a molecule expressed on the immune cell surface (for example CD3 on T-lymphocytes), in order to redirect the immune response against tumor cells. BiAbs promote T cell binding to the tumor cell, activation and tumor cell lysis through the direct stimulation of CD3 and thus bypassing the T cell receptor and antigen presentation. Moreover, T cell dependence on antigen presenting cells costimulation and cytokines production is overcome by reducing the risk of T cell anergy. BiAbs can be classified into two categories according to their similarity to the structure of a normal immunoglobulin (Ig): Ig-like and non-Ig-like. Antibodies in the first class have a structure similar to an Ig, they have an Fc region, and the high molecular weight confers a long half-life because they are not directly excreted by the kidney at the cost of poor tissue penetration. Non-Ig-like molecules do not have an Fc region and they are usually small molecules with a high tissue penetration at the cost of a short half-life because they are directly excreted by the kidney. This issue usually requires a continuous infusion in order to obtain clinically active levels of circulating non-Ig-like BiAbs.

Bispecific T-cell engagers (BiTEs) belong to the non-Ig-like group and they are the first BiAbs to have received regulatory approval for the clinical treatment of hematologic malignancies (blinatumomab in acute lymphoblastic leukemia) [22]. BiTEs are composed of two single-chain variable fragments (scFvs, which are small proteins containing only variable regions acting as binding sites) connected with a peptide linker. The first scFv is directed against a tumor antigen, and the other one against CD3 expressed by T cells. BiTEscolocalize T cells and tumor cells and activate T cells against the tumor cells independently of T cell receptor specificity (Figure 1c).

In the next sections, clinical data on naked mAbs, ADC and BiAbs will be discussed. The clinical results from key trials including mAbs for the treatment of MM are summarized in Table 1 (phase I-II trials) and Table 2 (phase III trials).

Table 1. List of phase I and II trials exploring mAbs in multiple myeloma (MM)

MAb Class	Molecule (Targets)	Study	Treatment	Setting	Toxicities (≥G3)	ORR (MRD Negativity Rate; NGS, Sensitivity 10-5)	PFS	OS
Naked	Daratumumab (anti-CD38)	GEN501 + SIRIUS NCT00574288 NCT01985126	Daratumumab single agent	RRMM	Anemia (17.6%); back pain g3 (2.7%); fatigue g3 (2%)	31.1%	4	20.1
Naked	Daratumumab (anti-CD38)	EQUULEUS [26] NCT01998971	Dara-Poma-dex	RRMM	Neutropenia (77%); fatigue (12%); dyspnea (8%)	60% (6%)	8.8	17.5
Naked	Daratumumab (anti-CD38	GRIFFIN [27] NCT02874742	Dara-VRd vs. VRd	NDMM	Neutropenia (41.4% vs. 21.6%); peripheral neuropathy (7.1% vs. 7.8%); diarrhea (7.1% vs. 3.9%)	99% vs. 91.8% (51% vs. 20.4%)	NR vs. NR	NR vs. NR
Naked	Daratumumab (anti-CD38)	PAVO [28] NCT02519452	Subcutaneous administration of daratumumab single agent	RRMM	Anemia (15.6%); hypertension (8.9%); pneumonia (4.4%); hyponatremia (4.4%); respiratory syncytial virus infection (4.4%); device-related infection (4.4%) Anemia (15.6%); hypertension (8.9%); pneumonia (4.4%); hyponatremia (4.4%); respiratory syncytial virus infection (4.4%); device-related infection (4.4%)	42.2%	NA	NA
Naked	Isatuximab (anti-CD38)	TCD11863 [29] NCT01749969	Isa-Rd	RRMM	Neutropenia (60%); pneumonia (9%); fatigue (7%)	56%	8.5	NR
Naked	Isatuximab (anti-CD38)	TCD11863 [29] NCT01749969	Isa-Rd	RRMM	Neutropenia (60%); pneumonia (9%); fatigue (7%)	56%	8.5	NR

MAb Class	Molecule (Targets)	Study	Treatment	Setting	Toxicities (≥G3)	ORR (MRD Negativity Rate; NGS, Sensitivity 10-5)	PFS	OS
Naked	Isatuximab (anti-CD38)	TCD14079 [30] NCT02283775	Isa-Pd	RRMM	Neutropenia (84%); pneumonia (18%); fatigue (7%); urinary tract infection (7%); traumatic fracture (7%); syncope (7%); dyspnea (7%); hypertension (7%)	62.2% (0%)	17.6	NR
Naked	Isatuximab (anti-CD38)	GMMC-CONCEPT [31] NCT03104842	Isa-KRD	High risk NDMM`	Neutropenia (34%); hypertension (12%); cardiac failure (4%)	100% (40%)	NA	NA
Naked	MOR202	MOR202C101	MOR202+dexamethasone	RRMM	Anemia	28%	8.4	NA
Naked	MOR202 (anti-CD38)	[32] NCT01421186	xamethasone	RRMM	39%); hypertension (11%); bronchitis (6%); pneumonia (6%); hyperglycemia (6%)	28%	8.4	NA
			MOR202-Rd		Lymphopenia (59%); hypophosphatemia (12%); hypertension (12%)	65%	NR	NA
			MOR202-Pd		Neutropenia (71%); pneumonia (24%); hypertension (19%)	48%	17.5	NA
Naked	TAK-079 (anti-CD38)	TAK-079-1501 [33] NCT03439280	TAK-079 single agent	RRMM	Neutropenia (5%); parainfluenza virus infection (5%); diverticulitis (5%)	33%	NR	NA
Naked	Elotuzumab (anti-SLAMF7)	ELOQUENT-3 [34] NCT02654132	Elo-Pd vs. Pd	RRMM	Neutropenia (13% vs. 27%); infections (13% vs. 22%); hyperglycemia (8% vs. 7%)	53% vs. 26%	10.3 vs. 4.7	NA

MAb Class	Molecule (Targets)	Study	Treatment	Setting	Toxicities (≥G3)	ORR (MRD Negativity Rate; NGS, Sensitivity 10-5)	PFS	OS
Naked	Pembrolizumab (anti-PD-1)	KEYNOTE-023 [35] NCT02036502	Pembrolizumab-Rd	RRMM	Neutropenia (27.4%); hyperglycemia (6.5%); pneumonia (6.5%); atrial fibrillation (3.2%); insomnia (3.2%)	44%	7.2	NR
Naked	Pembrolizumab (anti-PD-1)	HP-00061522 [36] NCT02289222	Pembrolizumab-Pd	RRMM	Neutropenia (42%); hyperglycemia (21%); fatigue (15%); pneumonia 15%)	60%	17.4	NR
ADC	Belantamab mafodotin (anti-BCMA, monomethyl auristatin F payload	DREAMM-1 [37,38] NCT02064387	Belamaf single agent	RRMM	Thrombocytopenia (35%); keratopathy (14%); diarrhea (12%)	60%	12	NR
ADC	Belantamab mafodotin (anti-BCMA, monomethyl auristatin F payload)	DREAMM-2 [39,40,41,42] NCT03525678	Belamaf single agent (data on the 2.5 mg/kg cohort are shown)	RRMM	Thrombocytopenia (20%); keratopathy (27%); hypercalcemia (7%)	31%	2.9	14.9
ADC	Belantamab mafodotin (anti-BCMA, monomethyl auristatin F payload)	DREAMM-6 [43] NCT03544281	Belamaf-Vd	RRMM	Thrombocytopenia (61%); keratopathy (56%); hypercalcemia (7%)	78%	NA	NA
BiAbt	AMG 420 (anti-BCMA/anti-CD3)	1351.1 [44] NCT02514239	AMG 420 single agent	RRMM	Infections (24%) neuropathy (5%) CRS (2%)	70%	NA	NA
BiAb	PF-3135 (anti-BCMA/anti-CD3)	C1071001 [45] NCT03269136	PF-3135 single agent	RRMM	Increased liver enzymes (6%) neutropenia (6%), lymphopenia (6%)	0%	NA	NA
BiAb	CC-93269 (anti-BCMA/anti-CD3)	CC-93269-MM-001 [46] NCT03486067	CC-93269 single agent	RRMM	Neutropenia (43%), infections (30%), general physical deterioration (10%)	89% *** (78%) **	NA	NA
BiAbt	Teclistamab (anti-BCMA/anti-CD3)	CR108206 [47] NCT03145181	Teclistamab single agent	RRMM	Neutropenia (48%), infections (21%), neurotoxicity (3%)	67% ***	NA	NA

* Sensitivity 10-6; ** Flow. *** At the maximum tolerated dose (MTD) or at the highest dose tested when the MTD has not yet been reached. Abbreviations: MAb, monoclonal antibody; G, grade; ORR, overall response rate; CRS, cytokine release syndrome; MRD, minimal residual disease; NGS, next-generation sequencing; PFS, progression-free survival; OS, overall survival; RRMM, relapsed/refractory multiple myeloma; NDMM, newly diagnosed multiple myeloma; Dara, daratumumab; P, Poma, pomalidomide; d, dex, dexamethasone; V, bortezomib; R, lenalidomide; NR, not reached; NA, not available; Isa, isatuximab; SLAMF7, signaling lymphocytic activation molecule family 7; Elo, elotuzumab; PD-1, programmed cell death protein 1; BCMA, B-cell maturation antigen; belamaf, belantamabmafodotin; ADC, antibody–drug conjugate; BiAb, bispecific antibody.

* Sensitivity 10-6; ** Flow. *** At the maximum tolerated dose (MTD) or at the highest dose tested when the MTD has not yet been reached. Abbreviations: MAb, monoclonal antibody; G, grade; ORR, overall response rate; CRS, cytokine release syndrome; MRD, minimal residual disease; NGS, next-generation sequencing; PFS, progression-free survival; OS, overall survival; RRMM, relapsed/refractory multiple myeloma; NDMM, newly diagnosed multiple myeloma; Dara, daratumumab; P, Poma, pomalidomide; d, dex, dexamethasone; V, bortezomib; R, lenalidomide; NR, not reached; NA, not available; Isa, isatuximab; SLAMF7, signaling lymphocytic activation molecule family 7; Elo, elotuzumab; PD-1, programmed cell death protein 1; BCMA, B-cell maturation antigen; belamaf, belantamabmafodotin; ADC, antibody–drug conjugate; BiAb, bispecific antibody.

Table 2. List of phase III trials exploring naked mAbs in multiple myeloma (MM)

Molecule (Targets)	Study	Treatment	Setting	Toxicities (≥G3)	ORR (MRD Negativity Rate; NGS, Sensitivity 10-5)	PFS	OS
Daratumumab (anti-CD38)	CASTOR [48] NCT02136134	Dara-Vd vs. Vd	RRMM	Thrombocytopenia (45.7% vs. 32.9%); pneumonia (9.9% vs. 10.1%); hypertension (6.6% vs. 0.8%)	83.8% vs. 63.2% (11.6% vs. 2.4%)	16.7 vs. 7.1	NA
Daratumumab (anti-CD38)	POLLUX [49] NCT02076009	Dara-Rd vs. Rd	RRMM	Neutropenia (55.5% vs. 41.6%); pneumonia (15.2% vs. 10%); diarrhea (9.9% vs. 3.9%);	92.9% vs. 76.4% (30.4% vs. 5.3%)	44.5 vs. 17.5	1-year OS 92.1% vs. 86.8%
Daratumumab (anti-CD38)	CANDOR [50] NCT03158688	Dara-Kd vs. Kd	RRMM	Thrombocytopenia (24% vs. 16%); respiratory tract infection (29% vs. 16%); hypertension (18% vs. 13%)	84% vs. 75% (14% vs. 3%)	NR vs. 15.8	NR vs. NR
Daratumumab (anti-CD38)	ALCYONE [51] NCT02195479	Dara-VMP vs. VMP	NDMM	Neutropenia (39.9% vs. 38.7%); infections (23.1% vs. 14.7%); any infusion-related reaction (4.9% vs. na)	90.9% vs. 73.9% (22.3% vs. 6.2%)	NR vs. 18.1	36-month rate: 78% vs. 67.9%
Daratumumab (anti-CD38)	MAIA [52] NCT02252172	Dara-Rd vs. Rd	NDMM	Neutropenia (50% vs. 35.3%); infections (32.1% vs. 23.3%); fatigue (8% vs. 3.8%)	92.9% vs. 81.3% (24.2% vs. 7.3%)	NR vs. 31.9	NA
Daratumumab (anti-CD38)	CASSIOPEIA [53] NCT02541383	Dara-VTd vs. VTd	NDMM	Neutropenia (28% vs. 15%); stomatitis (13% vs. 16%); peripheral sensory neuropathy (9% vs. 9%)	92.6% vs. 89.9% (64% vs. 44%) *	NA	NA
Daratumumab (anti-CD38)	COLUMBA [54] NCT03277105	Subcutaneous vs. intravenous administration of daratumumab	RRMM	Thrombocytopenia (14% vs. 13%); hypertension (3% vs. 6%); febrile neutropenia (2% vs. 3%); back pain (2% vs. 3%)	41% vs. 37%	5.6 vs. 6.1	NA

Molecule (Targets)	Study	Treatment	Setting	Toxicities (≥G3)	ORR (MRD Negativity Rate; NGS, Sensitivity 10-5)	PFS	OS
Isatuximab (anti-CD38)	ICARIA-MM [55] NCT02990338	Isa-Pd vs. Pd	RRMM	Neutropenia (85% vs. 70%); pneumonia (16% vs. 14%); dyspnea (4% vs. 1%)	60% vs. 35% (5% vs. 0%)	11.5 vs. 6.5	NA
Isatuximab (anti-CD38)	IKEMA [56] NCT03275285	Isa-Kd vs. Kd	RRMM	Respiratory infections (32.2% vs. 23.8%); cardiac failure (4% vs. 4.1%); thrombocytopenia (29.9% vs. 23.8%); neutropenia (19.2% vs. 7.4%) kd, respectively.	86.6% vs. 82.9% (29.6% vs. 13%)	NR vs. 19.2	NA
Elotuzumab (anti-SLAMF7)	ELOQUENT-2 [57] NCT01239797	Elo-Rd vs. Rd	RRMM	Lymphocytopenia (79% vs. 49%); infections (33% vs. 26%); pneumonia (14% vs. 10%)	79% vs. 66%	19.4 vs. 14.9	48 vs. 40

* Flow. Abbreviations: G, grade; ORR, overall response rate; MRD, minimal residual disease; NGS, next-generation sequencing; PFS, progression-free survival; OS, overall survival; RRMM, relapsed/refractory multiple myeloma; NDMM, newly diagnosed multiple myeloma; Dara, daratumumab; d, dex, dexamethasone; V, bortezomib; R, lenalidomide; K, carfilzomib; VMP, bortezomib-melphalan-prednisone; T, thalidomide; P, Poma, pomalidomide; SLAMF7, signaling lymphocytic activation molecule family 7; Elo, elotuzumab; NR, not reached; NA, not available.

2. Naked Monoclonal Antibodies

The first mAb introduced in clinical practice for the treatment of MM was daratumumab, a fully human IgG antibody. Its target is CD38, a multifunctional ectoenzyme with a role in NAD+ metabolism that acts as a receptor [58]. Its high expression on MM cells, Tregs, B reg cells, and myeloid-derived suppressor cells (MDSCs) makes it an ideal antigen to synergistically target MM and revert the immunosuppressive tumor microenvironment. The role of daratumumab in the treatment of MM is well established, both in RRMM patients and, recently, also in NDMM patients. Its efficacy and safety encouraged the development of other mAbs targeting CD38.

Daratumumab was first approved in 2015 by the US Food and Drug Administration (FDA) and in 2016 by the European Medicines Agency (EMA), on the basis of phase I/II studies GEN501 and SIRIUS in RRMM patients who previously received PIs and IMiDs.

The most common toxicity was infusion-related reaction (IRR, 48%), especially with the first dose and mostly of grade (G) 1–2. The combined analysis of the two trials showed an overall response rate (ORR) of 30.4%, with a median overall survival (OS) of 20.5 months.

More important results were obtained by combining daratumumab with other drug classes such as IMiDs and PIs. Combination therapies with bortezomib-dexamethasone (Dara-Vd) and lenalidomide-dexamethasone (Dara-Rd) were evaluated in two phase III studies in RRMM patients: CASTOR and POLLUX . These two studies led to FDA approval in 2016 and EMA approval in 2017 of daratumumab triplets in RRMM patients.

In the CASTOR study, Dara-Vd and Vd were compared. The primary endpoint was progression-free survival (PFS). With a median follow-up of 19.4 months, PFS was 16.7 months in the Dara-Vd arm vs. 7.1 months in the Vd arm . The most common G3-4 adverse events (AEs) were thrombocytopenia (45.7 vs. 32.9) and peripheral neuropathy (4.5 vs. 6.8). Discontinuation of treatment due to AEs was similar (9.5% vs. 9.3%).

In the POLLUX trial, 569 patients treated with a median of 1 prior line of therapy were randomized to Dara-Rd vs. Rd. With a median follow-up of 44.3 months, PFS was 44.5 months in the Dara-Rd arm vs. 17.5 months in the Rd arm. Moreover, the addition of daratumumab to Rd led to an ORR of 92.9% vs. 76.4% in the control arm, with a minimal residual disease (MRD) negativity rate of 30.4% vs. 5.3%. The most common G3-4 AEs were fatigue, diarrhea, thrombocytopenia, neutropenia and lymphopenia.

The third-generation IMiDpomalidomide showed the in vitro ability to upregulate CD38 expression on MM cells and to induce a potential synergistic immunomodulatory effect [Therefore, the association of daratumumab with pomalidomide and dexamethasone (Dara-Pd) was investigated in the phase I/II trial EQUULEUS In this study, Dara-Pd was administered to 103 heavily pretreated RRMM patients (median of prior lines was 4), obtaining an ORR of 60%, with an MRD negativity rate of 6%. Most common AEs included neutropenia (80%), anemia (50%), fatigue (52%), diarrhea (43%) and thrombocytopenia (42%). The most common hematologic G3-4 toxicity was neutropenia (77%), other G3-4 AEs were comparable to those observed with Pd alone A phase III trial (APOLLO) evaluated the efficacy of Dara-PD vs. Pd, and recently a press release announced that the primary endpoint of this trial (PFS) was met Results from the combination of daratumumab with the second-generation PI carfilzomib in the phase III trial CANDOR were recently presented. In this trial, 466 RRMM patients were randomized to receive daratumumab plus carfilzomib and dexamethasone (Dara-Kd) or Kd alone. The ORR was 84% vs. 75%, MRD negativity rate 14% vs. 3%, and PFS not reached (NR) vs. 15.8 months in the Dara-Kd vs. Kd arm respectively. The most frequent G ≥ 3 AEs were thrombocytopenia (24% vs. 16%), respiratory tract infection (29% vs. 16%) and hypertension (18% vs. 13%).

The very good safety profile combined with the synergistic effect with standard-of-care (SOC) combinations in RRMM patients led to exploring the addition of daratumumab to SOC combinations in NDMM patients as well, both in transplant-eligible (TE) and non-transplant-eligible (NTE) patients.

In the phase III trial ALCYONE, 706 NDMM NTE patients were randomized to receive 9 cycles of bortezomib, melphalan and prednisone (VMP) vs. VMP plus daratumumab (Dara-VMP) followed by daratumumab until progression. With a median follow-up of 40.1 months, median PFS was 36.4 vs. 19.3 months, ORR 90.9% vs. 73.9% and the MRD negativity rate 28% vs. 7% in the experimental vs. control arm. A survival advantage of Dara-VMP vs. VMP was also observed (HR 0.60, p = 0.0003). AEs were comparable in both arms, with the exception of G3-4 infections (23.1 vs. 14.7% in the daratumumab vs. control arm).

Another important SOC for NDMM NTE patients is Rd. In the phase III MAIA trial, 737 NDMM NTE patients received Rd with or without daratumumab. With a median follow-up of 28 months, median PFS was NR in the daratumumab group vs. 31.9 months in the Rd group. ORR rates were 92.9% vs. 81.3%, respectively, with a MRD negativity rate of 24.2% vs. 7.3%, thus confirming the greater depth of response achievable in the daratumumab arm. The most common toxicities observed with daratumumab were neutropenia (50% vs. 35.3% in the daratumumab vs. control arm) and pneumonia (13.7% vs. 7.9%), with a low rate of treatment withdrawal in both arms (0.5% vs. 1.4%).

For TE patients, different induction regimens are considered SOC in different countries. The triplet bortezomib, thalidomide and dexamethasone (VTD) is still one of the most used in Europe. In the phase III trial CASSIOPEIA, the efficacy of daratumumab in combination with VTD (Dara-VTD) was evaluated in 1085 NDMM TE patients. The primary endpoint of the study was the rate of the stringent complete response (sCR) 100 days after transplant . This rate was 20% in the VTD arm vs. 29% in the Dara-VTD arm, translating into a significant benefit in PFS (HR 0.47, $p < 0.0001$). The second phase of CASSIOPEIA is ongoing and is evaluating the role of daratumumab in maintenance therapy.

GRIFFIN is another important phase II trial evaluating the efficacy and safety of the addition of daratumumab to the combination of bortezomib, lenalidomide and dexamethasone (Dara-VRd) in NDMM TE patients . With a median follow-up of 22.1 months, the rate of sCR was 62.2% in the daratumumab arm vs. 45.4% in the control arm, with a 24-month PFS of 95.8% vs. 89.8%, respectively. Although there were more cases of neutropenia (41.4% vs. 21.6%), thrombocytopenia (16.2% vs. 8.8%) and infections (90.9% vs. 61.8%) in the daratumumab group, the rate of treatment discontinuation was 15.2% in the Dara-VRd arm vs. 20.6% in the VRd arm. The ongoing phase III trial PERSEUS is evaluating the efficacy of daratumumab combined with VRd in the induction and consolidation phases and with lenalidomide in the maintenance phase The route of administration of daratumumab is intravenous (Dara iv), with IRRs occurring in about half of the treated patients. The median duration of infusion is about 7 h for the first infusion and 4 h for subsequent infusions, with a negative impact on the quality of life of patients, since they need to spend a lot of time receiving infusions in hospital facilities. In the phase Ib trial PAVO, encouraging results were obtained with the subcutaneous administration of daratumumab (Dara sc). These findings were confirmed by the phase III study COLUMBA The sc route of administration was not inferior to the iv route in terms of efficacy (ORR 41% vs. 37%, respectively), while safety was better with the sc route in terms of IRRs (13% vs. 34%). In the PLEIADES study, Dara sc added to SOC regimens showed a clinical activity similar to that of Dara iv-containing regimens, thus further confirming the superiority of the sc route also in combination regimens.

Isatuximab is a chimeric naked mAb targeting CD38 that showed an anti-MM activity similar to that of daratumumab, with a peculiar proapoptotic effect Phase I studies showed its efficacy both as a single agent and in combination therapy

Isatuximab was recently approved by FDA and EMA in combination with Pd, based on the results of the phase III ICARIA-MM trial. In this study, 307 RRMM patients with a median of 3 prior lines of therapy were enrolled to receivePd with or without isatuximab. The ORR was 60% vs. 35% in the isatuximab vs. the control arm. With a median follow-up of 11.6 months, PFS was 11.5 vs. 6.5 months in the isatuximab vs. the control group. As with daratumumab, IRRs with isatuximab were common, mostly observed during the first infusion and rarely severe (3% of IRRs were G3-4).

Another phase III trial enrolled 302 RRMM patients to compare isatuximab-Kd to Kd alone . After a median follow-up of 20.7, PFS was significantly better in the experimental vs. the control arm (HR 0.531, p = 0.0007). The MRD negativity rate was higher in the experimental arm as well (29.6% vs. 13.0%). G ≥ 3 respiratory infections were observed in 32.2% of patients in the isatuximab-Kd arm vs. 23.8% in the Kd arm.

The phase III IMROZ trial will evaluate the efficacy of isatuximab in combination with VRD in NDMM NTE patients]. In the phase Ib GMMC-CONCEPT trial, isatuximab was evaluated in combination with the triplet KRD in high-risk NDMM patients. First data were recently presented at the ASCO 2020 Annual Meeting, showing an encouraging ORR of 100%].

Other two anti-CD38 mAbs, MOR202 and TAK-079, are under investigation. MOR202 is an iv anti-CD38 mAb without CDC activity. Clinically, it showed a lower frequency of IRRs compared to Dara iv. However, the ORR of MOR202 in combination with dexamethasone, lenalidomide or pomalidomide (28%, 65% or 43% respectively) was not higher than that of other anti-CD38 mAbs, despite the limitations of cross-trial comparisons .

Recent data on TAK-079 (mezagitamab) showed an ORR of 33% at the dose of 600 mg sc in heavily pretreated RRMM patients (4 median prior lines of therapy, including patients exposed to other anti-CD38 mAbs). Its advantages are the sc route of administration and a promising safety profile (no IRRs, no significant hematologic toxicity.

Elotuzumab is another naked mAb used in clinical practice. Its molecular target is the surface glycoprotein signaling lymphocytic activation molecule family 7 (SLAMF7), which is mainly expressed by NK and normal or neoplastic plasma cells, promoting their growth and survival and mediating their interaction with the microenvironment. Therefore, the elotuzumab mechanism of action involves a blockade of tumor interactions], growth and survival signals. Moreover, it stimulates NK cells by enhancing their ADCC activity]. In phase I studies, elotuzumab showed no efficacy as a single agent , but encouraging in vitro studies found a potential synergistic effect in combination with IMiDs]. This synergy was clinically evaluated in the ELOQUENT-2 and ELOQUENT-3 trials by adding elotuzumab to Rd and Pd. Positive results from both trials led to the approval of these combinations in RRMM patients.

In NDMM patients, instead, elotuzumab did not show encouraging results. The ELOQUENT-1 trial evaluated the triplet elotuzumab-Rd in NTE NDMM patients, and it was recently announced that the primary endpoint (PFS) was not met

An interesting characteristic of elotuzumab is its safety profile, with the low rate of IRRs and the absence of additional toxicity making it a good option for the treatment of frail patients.

Immune checkpoints shutting down the antineoplastic immune response are important molecules involved in tumorigenesis, and the PD-1/PD-L1 axis is one of the most important pathways working as an immune checkpoint. Many naked mAbs interact with it, blocking PD-1 (nivolumab, pembrolizumab and cepilimab) or PD-L1 (durvalumab and atezolizumab). The importance of these mAbs in cancer immunotherapy is well known, and MM cells and their microenvironment seem to rely on the PD-1/PD-L1 interaction, thus fostering the design of clinical studies with immune

Pembrolizumab as a single agent did not show efficacy, while, in phase I studies, its combination with lenalidomide and pomalidomide showed an ORR of 44% and 60%, respectively Unfortunately, both the KEYNOTE-183 trial (RRMM patients) and the KEYNOTE-185 trial (NDMM NTE patients) were discontinued due to severe toxicities Although these results reduced the interest in checkpoint inhibitors in MM, there are encouraging preclinical data about their use in combination with other mAbs: it seems indeed that the combination with elotuzumab may increase NK peritumoral infiltration and cytokine release. Moreover, the combination with daratumumab showed a synergistic anti-MM effect.

B-cell maturation antigen (BCMA) is another very specific antigen expressed almost exclusively by plasma cells. This surface protein is an interesting target because it promotes survival and growth when interacting with its ligands, BAFF and APRIL. The first naked anti-BCMA mAb was cSG], which showed anti-MM effects, but was not further developed. A phase I trial exploring a humanized, non-fucosylated IgG1 anti-BCMA naked mAb is ongoing Nonetheless, due to its specificity, BCMA is the ideal target for more powerful immunotherapies such as ADCs and BiAbs, as will be discussed in the next section.

3. ADCs

ADCs are a rapidly growing class of immunotherapeutic agents. Different constructs, payloads and target antigens are in preclinical or early clinical investigation for the treatment of MM. Among them, the most promising agent of which we already have clinical data is belantamabmafodotin (belamaf), a humanized anti-BCMA IgG mAb fused to the payload monomethyl auristatin F (MMAF). In preclinical in vitro and in vivo models, belamaf showed anti-MM activity without affecting BCMA-negative cells and the MMAF arrested the cell cycle of malignant plasma cells at the G2/M phase, eventually leading to cell death. Its afucosylated Fc fraction promotes Fc-dependent immune effector functions, mainly ADCP and ADCC.

In a first-in-human phase I trial in heavily pretreated RRMM, single-agent belamaf was tested at different dose levels. The dose level of 3.4 mg/Kg given every 21 days was further tested and expanded to 35 patients. At this dose level, the drug was associated with an ORR of 60% and a PFS of 12 months IRRs were mild and infrequent (29%, mostly G1-2). The 2 main emerging toxicities were thrombocytopenia (63%, 35% of which G ≥ 3) and keratopathy (69% of patients, 14% of which G ≥ 3). Keratopathy is a well-known side effect of MMAF and, though the exact mechanism of toxicity is unknown, it may be due to a non-specific and BCMA-independent uptake of the drug in the basal epithelial layer of the cornea. From a practical standpoint, belamaf infusion lasts 30 min and no inpatient admission is required.

Following these encouraging results, a phase II trial designed for patients refractory to a PI, an IMiD, and an anti-CD38 mAb evaluated both the 3.4 and the 2.5 mg/kg dose levels. Safety and efficacy were comparable between the two dose levels. At 2.5 mg/kg, the ORR was 31% and median PFS was 2.9 months. This study confirmed the frequent occurrence of keratopathy detected on eye examination (72%, any grade). However, few patients experienced ocular symptoms (G3-4 in <5% of patients) and, importantly, keratopathy was always reversible. Indeed, although visual acuity was affected in 18% of treated patients, 82% of them recovered at the current follow-up. Two post-hoc analyses of this trial demonstrated that belamaf was active in patients with high-risk cytogenetics and that renal impairment up to 30 mL/min of the estimated glomerular filtration rate did not impact the efficacy and tolerability of this drug

A trial evaluating the addition of belamaf to standard MM backbone treatments (Rd and Vd) is ongoing in RRMM (DREAMM-6). Recently, data about the first 18 patients treated with belamaf-Vd were presented. Efficacy was good, with a high ORR (78%). However, as expected in a bortezomib-based combination, thrombocytopenia was frequent and severe (G3 17% and G4 44%).
The development of other ADCs was recently reviewed elsewhere

4. BiAbs

BCMA on malignant plasma cells and CD3 on T cells are the two main targets exploited to design anti-MM BiAbs. Other BiAbs targeting different antigens on the plasma cell surface and/or involving different immune effectors have been reviewed elsewhere
AMG 420 is an anti-BCMA BiTE that was tested in a dose-escalation first-in-human study enrolling RRMM patients. At the maximum tolerated dose (400 mcg/die), a very good efficacy was reported (ORR 70%)]. Due to the pharmacokinetics typical of non-Ig-like BiAbs, this drug formulation required a continuous infusion for 4 weeks on therapy followed by 2 weeks off therapy. Infections were frequent (G ≥ 3 24%), and the use of a central venous catheter line to deliver the drug led to central line infections in 12% of patients. Other treatment-emergent AEs were cytokine release syndrome (CRS, 38%, mostly G1-2) and peripheral neuropathy (G 3 5%).
Due to the aforementioned pharmacokinetic issues, AMG 420 has not been further developed. Nonetheless, a study evaluating AMG 701, a half-life extended BiTE not needing continuous infusion, is currently ongoing PF-06863135 (PF-3135) is a humanized Ig-like BiAb that is currently being tested in a dose-escalation study in RRMM Due to its Ig-like structure, PF-3135 is infused once weekly. Results of the first 17 patients showed a minimal response in 1 patient (6%), although the clinical benefit rate (defined as best response ≥ stable disease) was 41% and dose escalation is still ongoing. Three patients (18%) experienced G ≥ 3 AEs and the only non-hematologic AE was an increase in blood liver enzymes in 1 patient.
CC-93269 is an Ig-like BiAb asymmetrically targeting BCMA through two binding sites and targeting CD3 through one binding site. A dose-escalation phase I study in heavily pretreated RRMM patients is ongoing and results of the first 30 patients have been presented. CC-93269 was administered intravenously over 2 h: weekly in cycles 1–3, every other week in cycles 4–6, and every 28 days thereafter. The ORR was 43% throughout the dose cohorts, but it became dose-dependent, reaching 89% at the highest tested dose (10 mg). CRS was mild but frequent (all grades 77%; G ≥ 3 4%). Thus, dexamethasone prophylaxis was implemented in patients treated with doses >6 mg. The main toxicities were neutropenia (G ≥ 3 43%) and infections (G ≥ 3 30%).

Teclistamab is another Ig-like BiAb. Results of the first 78 RRMM patients enrolled in a phase I dose-escalation trial were recently presented. Priming doses followed by weekly iv infusions were administered at different dose levels (0.3–720 mcg/Kg). CRS was common (56%), but all cases were G1 or 2. Neurotoxicity was reported in 8% of treated patients. An ORR of 67% was observed in the 12 patients treated at the highest dose (270 mcg/Kg), while no efficacy data from the 720 mcg/kg cohort are yet available.

5. Future Directions and Conclusions

The introduction of mAbs for the treatment of MM has already changed clinical practice in RRMM patients, leading to better outcomes. Moreover, between 2019 and 2020, daratumumab combinations with Rd and VMP in NTE NDMM patients and with VTd in TE NDMM patients were approved by FDA and EMA. This means that the great majority of NDMM patients will receive an anti-CD38 naked mAb in the near future, due to the higher efficacy of combinations with daratumumab and to its negligible toxicity when added to SOC regimens. In the next years, more and more patients will eventually be exposed or refractory to anti-CD38 naked mAbs after first relapse, thus questioning the current treatment sequencing in RRMM patients. Initial reports of the suboptimal efficacy of retreatment with anti-CD38 mAbs in a small series of patients are beginning to emerge and will require prospective confirmation in a significant number of patients. This issue may be overcome by using different anti-CD38 mAbs with unique mechanisms of action. For example, differently from daratumumab, isatuximab mediates a direct cytotoxic effect against MM cells independently of the presence of Fc-cross-linking agents [. MOR202 does not induce CDC, decreasing the IRR rate at the cost of a reduced single-agent activity. TAK-079 minimally binds to targets with a low density of CD38, leading to an enhanced depletion of high-density CD38+ target cells. However, the predicted efficacy of these mAbs is largely dependent on the mechanisms of resistance to anti-CD38 mAbs. Resistance to ADCC (e.g., fratricidal depletion of CD38+ NK cells), CDC (e.g., upregulation of complement-inhibitory molecules) and ADCP (e.g., upregulation of CD47 inhibiting phagocytosis) have been observed during anti-CD38 mAb treatment and strategies to overcome them are under clinical investigation. Nevertheless, the most relevant issue limiting retreatment with anti-CD38 mAbs is the long-lasting down regulation of CD38 on plasma cell surfaces after anti-CD38 therapy. Even though strategies to reinduce CD38 expression in malignant plasma cells are under clinical investigation changing the target antigen may be a more appealing strategy in RRMM patients who are refractory to anti-CD38 treatment. The new anti-BCMA molecules could find their therapeutic space in this scenario.

Several observations can be made about the comparison among naked mAbs, ADCs and BiAbs. Single-agent activity of naked mAbs is relatively low (ORR around 20–30% in heavily pretreated RRMM patients with anti-CD38 mAbs), while ADCs (ORR up to 60% in RRMM with belamaf) and BiAbs (ORR up to 90% in RRMM with CC-93269) can induce deeper responses. However, naked mAbs are very safe drugs and do not have overlapping toxicities with other MM drugs. As a consequence, they may be easily combined with MM backbone treatments. Moreover, they may be effortlessly administered in outpatient facilities, and their subcutaneous formulations will be available in the future. ADCs have toxicity profiles non-overlapping with IMiDs and PIs and clinical trials exploring ADC-based combination therapies are ongoing. Moreover, they can be easily infused in outpatient facilities as well, although the off-target toxicity of the payload could be an issue (e.g., eye toxicity with belamaf). BiAbs are very effective drugs, but the infection risk makes it difficult to combine them with other MM backbones. Moreover, the strong activation of the immune system and consequent CRS risk may require preventive hospitalization during the first days after treatment or at least a close monitoring of CRS symptoms.

Other types of anti-BCMA immunotherapy, such as CAR T-cell therapy, are currently available However, despite their high efficacy as single agents (up to an ORR of 100%), they are currently not 'off-the-shelf' drugs. Differently from naked mAbs/ADCs/BiAbs, they require specialized centers and inpatient admission for the infusion. Furthermore, CRS/neurotoxicity should be closely monitored and promptly treated.
Currently, the optimal scenario for each of these drugs could depend on both disease risk and patient fitness. For instance, intermediate-fit or frail patients may safely receive naked mAbs or ADCs, but they are unlikely to tolerate BiAbs or CAR T-cell therapy. On the other hand, fit patients who present with high-risk disease or who experienced early relapse after first-line treatment may benefit from BiAbs or CAR T-cell therap]. As with anti-CD38 therapies, the mechanisms of resistance to anti-BCMA agents may shed light on the optimal treatment sequencing. For instance, antigen escape with relapse guided by BCMA-low or BCMA-negative malignant plasma cells has been described during anti-BCMA CAR T-cell treatment, predicting cross-resistance with other anti-BCMA agents

New data from prospective studies will help us understand the best drug to be used in each setting.

In conclusion, therapeutic options in MM are continuously emerging, and mAbs are greatly contributing, and will contribute even more in the future, to improving the outcome of MM patients.

ARTICLE 5

UNCONJUGATED MONOCLONAL ANTIBODIES AND PROTEASE INHIBITORS IN TREATMENT OF MULTIPLE MYELOMA

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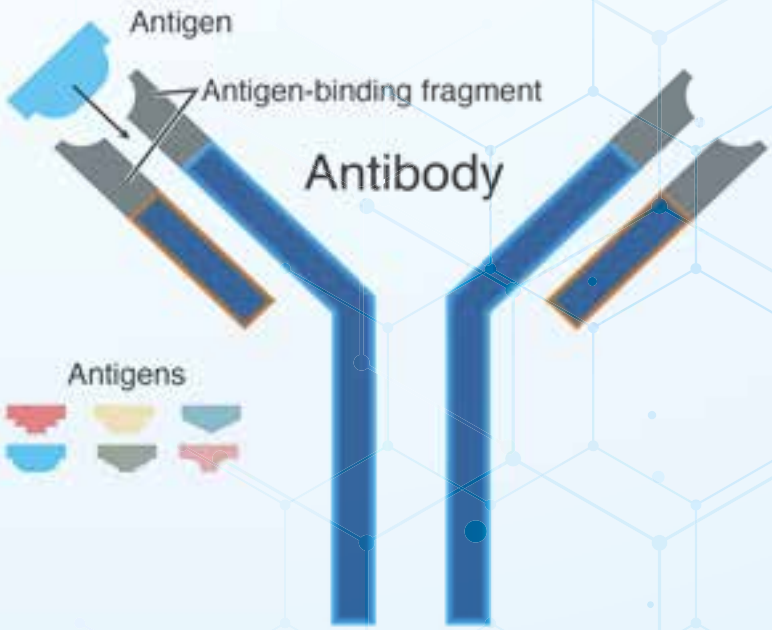
INTRODUCTION:

Monoclonal antibody, antibody produced artificially through genetic engineering and related techniques. Production of monoclonal antibodies was one of the most important techniques of biotechnology to emerge during the last quarter of the 20th century. The method relies on fusing B cells from an immunized animal (typically a mouse) with an immortal myeloma cell line and growing the cells under conditions in which the unfused normal and tumor cells cannot survive. The resultant fused cells that grow out are called hybridomas. hybridoma clones are screened for binding to the antigen of interest, and this single clone with the desired specificity is selected and expanded. The products of these individual clones are monoclonal antibodies.

One of the most important biological catalytic reactions is proteolysis and this is known as proteolytic activity, which has been attributed to a class of enzymes called proteases. Proteolysis is the hydrolysis of peptide bond by attacking the carbonyl group of the peptide. Proteases are of broad enzymes distribution. In human, there are about 990 known protease genes. In addition, about 1605 known protease inhibitor genes have been reported in human.



Use of proteasome inhibitors (PIs) has been the therapeutic backbone of myeloma treatment over the past decade. Many PIs are being developed and evaluated in the preclinical and clinical setting. The first-in-class PI, bortezomib, was approved by the US food and drug administration in 2003. Carfilzomib is a next-generation PI, which selectively and irreversibly inhibits proteasome enzymatic activities in a dose-dependent manner. Ixazomib was the first oral PI to be developed and has a robust efficacy and favorable safety profile in patients with multiple myeloma. These PIs, together with other agents, including alkylators, immunomodulatory drugs, and monoclonal antibodies, have been incorporated into several regimens. This review summarizes the biological effects and the results of clinical trials investigating PI-based combination regimens and novel investigational inhibitors and discusses the future perspective in the treatment of multiple myeloma.

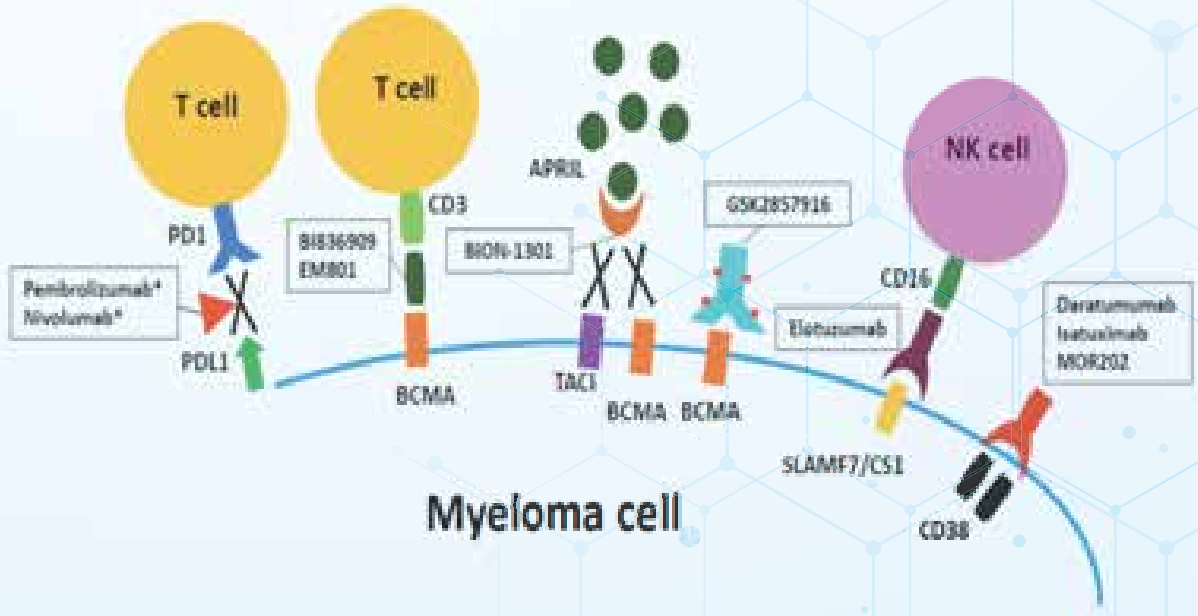
Multiple Myeloma

Multiple myeloma is a cancer that forms in a type of white blood cell called a plasma cell. Healthy plasma cells help fight infections by making proteins called antibodies. Antibodies find and attack germs.

In general, when plasma cells become cancerous and grow out of control, this is called multiple myeloma. The plasma cells make an abnormal antibody (immunoglobulin) known by several different names, including monoclonal immunoglobulin, monoclonal protein (M-protein), M-spike, or paraprotein.

There are, however, other plasma cell disorders that have abnormal plasma cells but do not meet the criteria to be called active multiple myeloma. These other plasma cell disorders include:

- Monoclonal gammopathy of uncertain significance (MGUS)
- Smoldering multiple myeloma (SMM)
- Solitary plasmacytoma
- Light chain amyloidosis



Symptoms

Early in multiple myeloma, there might be no symptoms. When signs and symptoms happen, they can include:

- **Bone pain, especially in the spine, chest or hips.**
- **Nausea.**
- **Constipation.**
- **Loss of appetite.**
- **Mental fogginess or confusion.**
- **Tiredness.**
- **Infections.**
- **Weight loss.**
- **Weakness.**
- **Thirst.**
- **Needing to urinate often.**

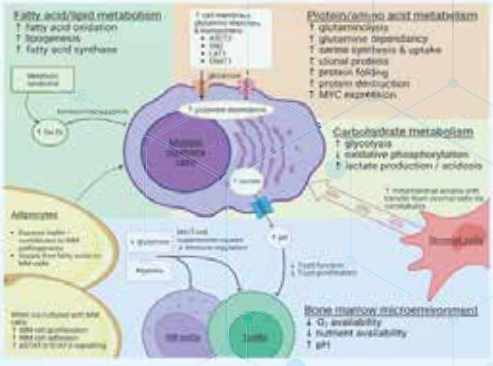
Proteases

Proteases in normal cells are very essential in carrying out imperative biological processes, and can regulate a diversity of different cellular processes such as gene expression, differentiation, and cell death. However, recent studies have indicated that proteases are also implicated in tumor growth and progression, both at primary and metastatic sites

Proteases are essential in carrying out biological processes such as gene expression, differentiation, and cell death. However, for their ability to degrade extracellular matrix and proteins, they are strongly associated with cancer progression. A lot of proteases have been linked with increasing tumor metastasis in different human cancers, suggesting their central functions in the metastatic process. The understanding of the proteolytic network in the tumor microenvironment is rapidly increasing because of a raised interest both in protease and in new techniques that allow for comprehensive analysis of protease activity in physiologically relevant conditions. Generally, well-established proteolytic networks consist of multiple steps of activation, several key nodes through which most signals pass, and inhibitors that can regulate activity of different points in such networks. Having a central role in several signaling pathways proteases represent potential drug targets for a large set of diseases, especially for cancer.

Role of protease inhibitors in Multiple Myeloma

Protease inhibitors are compounds able to block proteases function playing a key role in cancer therapies. However, their design is a complex issue since different types of cancers use different proteases at the fluctuating stages of cancer development and no single inhibitor can be used on all classes of proteases. We focused our attention on protease inhibitors, describing their structure and their mechanism of action.



There are several pharmaceutical strategies aimed to interfere with the proteases using different inhibitors; they may be split on basis of their mechanism of action and molecular class. In fact, protease inhibitors may be proteins, peptides, or small molecules; they are synthetic peptide-like or small molecules and, based on their inhibition process, may be divided into three main classes: reversible inhibitors, irreversible inhibitors, and engineering inhibitors. Some example is proteasome inhibitor used with other medications to treat multiple myeloma. Bortezomib, Carfilzomib , Delanzomib , Ixazomib.

Conclusion

Proteases have long been associated with cancer progression because of their ability to degrade extracellular matrices, which facilitates invasion and metastasis. Conventional chemotherapy uses individual anti-cancer agents or combinations for the treatment of tumor cells, which intervenes primarily with the macromolecular synthesis processes, interfering with DNA synthesis and mitosis and leading to the death of proliferating cancer cells. However, tumor cells often present challenges to chemotherapeutic agents and endow high levels of chemoresistance. Therefore, new therapeutic approaches are urgently needed. The protease inhibitors are promising candidates for anti-cancer agents. Many protease inhibitors have been developed as therapies for diseases such as hypertension, AIDS, adult T-cell leukaemia, malaria, Alzheimer's disease, hepatitis, and diabetes.

ARTICLE 6

UNCONJUGATED MONOCLONAL ANTIBODIES AND PROTEASE INHIBITORS N TREATMENT OF MULTIPLE MYELOMA

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Abstract:

The arsenal of treatments for multiple myeloma has been completely transformed by the recent discovery of monoclonal antibodies (mAbs). There is a lot of excitement for mAbs in this disease because of the efficacy of daratumumab and elotuzumab in relapsed/refractory patients. It is anticipated that combination therapy with additional anti-MM therapeutic modalities and clinical assessment in recently diagnosed patients will significantly alter the disease's natural course. The next quickly coming therapy frontier for multiple myeloma is biopharmaceutical engineering advancements and a strong interest in novel mAb-derivatives, such as antibody drug conjugates and poly-specific antibodies. We provide a thorough overview of the evidence that is currently available as well as the prospects for mAbs and mAb-derivative therapy in multiple myeloma in this study.

Keywords: multiple myeloma, mAbs, combination therapy, clinical assessment, poly-specific antibodies.

Introduction

Multiple Myeloma is a hematologic Malignancy (blood cancer) charaterised by the proliferation of Clonal plasma cells in the bone marrow, leading to production of non functional intact immunoglobulins or immunoglobulin chains. Bone destruction, anaemia, dysfunctioning of many organs. Globally, it is considered as the rare cancer, but currently its incidences are on high.The World Health Organization (WHO) counts it among the lymphoproliferative B-cell diseases. In the WHO classification, multiple myeloma is differentiated from the following plasma cell diseases.

- Monoclonal gammopathy of uncertain significance
- Solitary plasmocytoma of bone
- Systemic light-chain amyloidosis
- POEMS syndrome (polyneuropathy, organomegaly, endocrinopathy, monoclonal plasma cell disease, and skin changes.

MONOCLONAL ANTIBODIES

When a humoral immune response is provoked by an immunogen, such as tetanus toxoid, aplethora of antibodies are produced in an individual against di Verent parts or regions of this foreign substance. These are termed antigenic determinants, or epitopes, which usually com-prise six to eight amino acids. It should be appreciated that most antibodies recognise and interact with a three dimensional shape composed of “discontinuous” residues brought into juxtaposition by the folding of a molecule. Alternatively, antibodies can also recognise linear stretches of amino acids or “continuous” epitopes.

Types of Monoclonal Antibodies Based on Their Function

1. Naked mAbs
2. Conjugated mAbs
3. Bispecific mAbs

Protease inhibitors are a class of molecules that block the activity of proteases, enzymes responsible for breaking down proteins. They are widely used in medicine, particularly in the treatment of viral infections such as HIV and hepatitis C. By inhibiting viral proteases, these drugs prevent the virus from processing essential proteins required for replication, thereby slowing disease progression. Protease inhibitors are also found naturally in certain plants and animals, playing a role in regulating biological processes. In addition to antiviral therapies, they are being studied for their potential in treating cancer and inflammatory diseases.

Types of Protease Inhibitor

1. Antiviral protease inhibitor
2. Serine protease inhibitor
3. Metalloprotease inhibitor
4. Aspartic and Cysteine protease inhibitor

Risk Factors:

While the exact cause of multiple myeloma is unknown, several risk factors have been identified:

- Age: Risk increases with age, particularly after 65+.
- Gender: Men are more likely to develop multiple myeloma than women.
- Ethnicity: African Americans have a higher risk compared to other ethnicities.
- Family History: Having a close relative with multiple myeloma can increase risk.
- Plasma Cell Diseases: Conditions like Monoclonal Gammopathy of Undetermined Significance (MGUS) and solitary plasma cytoma can precede multiple myeloma.
- Obesity: Excessive body weight increases the chances of multiple myeloma.
- Environmental Exposures: direct contact with external environment and harmful chemical increases risk of multiple myeloma.

Treatment

The treatment for multiple myeloma aims to control the disease, relieve symptoms, and prolong survival. While it is not curable, modern treatments have significantly improved outcomes. Here are the main treatment options:

1. Chemotherapy:

- Purpose: Uses powerful drugs to kill cancer cells or stop them from growing and spreading to the different parts of the body.
- Common Drugs: Cyclophosphamide, Melphalan, and others.
- Side Effects: Fatigue, nausea, hair loss, and increased risk of infection.

2. Stem Cell Transplantation:

• Autologous Stem Cell Transplant (ASCT): The most common type, where the patient's own stem cells are used after high-dose chemotherapy to "reset" the bone marrow.

• Allogeneic Stem Cell Transplant: Uses stem cells from a donor (less common due to higher risks of complications).

• Purpose: Helps to restore normal blood cell production in the bone marrow and improve survival.

3. Targeted Therapy:

• Purpose: Focuses on specific abnormalities in myeloma cells to block their growth and survival.

• Common Drugs: Bortezomib (Velcade), Carfilzomib (Kyprolis), and Ixazomib (Ninlaro) target the proteasome, a structure involved in protein degradation.

• Side Effects: Fatigue, low blood cell counts, neuropathy, and gastrointestinal issues.

4. Immunotherapy:

• Purpose: Bodys immune system is use to fight with cancer.

• Common Drugs: Monoclonal antibodies like Daratumumab (Darzalex) and Elotuzumab (Empliciti) help the immune system recognize and destroy myeloma cells.

• Side Effects: Infusion reactions, fatigue, and infections.

5. Corticosteroids:

• Purpose: Help control inflammation and boost the effectiveness of chemotherapy.

• Common Drugs: Dexamethasone and Prednisone.

• Side Effects: Weight gain, osteoporosis, increased risk of infections, mood changes.

6. Radiation Therapy:

• Purpose: Used to target localized areas of myeloma, such as bone lesions causing pain or fractures.

• Side Effects: Skin irritation, fatigue, and nausea.

7. CAR-T Cell Therapy (Chimeric Antigen Receptor T-Cell Therapy):

• Purpose: A cutting-edge immunotherapy where a patient's T-cells are modified to attack myeloma cells.

• Current Status: Approved for some patients with relapsed or refractory multiple myeloma.

• Side Effects: Cytokine release syndrome (CRS), neurotoxicity, and infections.

8. Bisphosphonates and Denosumab (Bone-Targeting Treatments):

• Purpose: Help prevent bone complications like fractures, which are common in multiple myeloma.

• Common Drugs: Zoledronic acid (Zometa) and Denosumab (Xgeva).

• Side Effects: Bone pain, flu-like symptoms, and rare jawbone problems.

9. Maintenance Therapy:

• After initial treatment, patients may receive maintenance therapy (such as low-dose lenalidomide) to prevent relapse.

10. Supportive Care:

• Pain Management: Pain relief through medications and other interventions.

• Blood Transfusions: To treat anemia or low blood cell counts.

• Infection Prevention: Antibiotics and antiviral medications to prevent infections due to weakened immunity.

Conclusion

Monoclonal antibodies and protease inhibitors have significantly improved the treatment for multiple myeloma (MM), offering enhanced efficacy and better patient outcomes. Future advancements focus on optimizing combinations, overcoming resistance mechanisms, and developing next-generation agents to further improve patient outcomes in Multiple Myeloma.

ARTICLE 7

UNCOJUGATED MONOCLONAL ANTIBODIES AND PROTEASE INHIBITORS IN TREATMENT OF MULTIPLE MYELOMA

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Abstract:

Although there have been many advancements in the treatment of Multiple Myeloma (MM), such as Autologous Stem Cell Transplantation (ASCT), immunomodulatory drugs (IMiDs), and proteasome inhibitors (PIs), it remains a common hematologic malignancy with high progression and relapse rates. Monoclonal antibodies (mAbs) have transformed the management of newly diagnosed and relapsed/refractory MM (RRMM) patients in the last few years. The focus of this review is the role of unconjugated mAbs, protease inhibitors, and newer mAb derivatives such as antibody-drug conjugates (ADCs) and bispecific antibodies (bsAbs) in MM therapy.

Daratumumab, an anti-CD38 mAb, has proven to be efficacious in combination therapies with particular patients, relapsed patients, and newly diagnosed patients all showing improvements in Overall Survival (OS) and Progression-Free Survival (PFS). Unconjugated mAbs targeting CD38 and SLAMF7 have shown remarkable clinical efficacy mainly when utilized together. Anti-BCMA mAb belantamab mafodotin is an ADC that has shown promise in anti-MM activity with controllable toxicity. While the toxicity profiles of bispecific antibodies targeting BCMA and CD3, like AMG 420 and CC-93269, may limit their use in combination with other therapies, they have demonstrated great success in inducing deep responses as well.

The treatment strategy of MM has been improved with the addition of mAbs for treatment of the disease, which have certainly made a positive timeless approach even as newer therapies are developed.

Introduction

- Multiple myeloma (MM) is the second most common hematologic malignancy worldwide, representing approximately 1% of all malignancies, with an incidence estimated to be 6 cases per 100,000 persons per year (1). Treatment with autologous stem cell transplantation (ASCT) and combinations of immunomodulatory drugs (IMiDs) and proteasome inhibitors (PIs) have drastically improved treatment outcomes of MM patients. At present, median overall survival (OS) of patients eligible for ASCT is 6–8 years with one-third of patients living more than 10 years. However, despite clear treatment advances, most patients will eventually relapse. Monoclonal antibodies (mAbs) represent a promising group of agents with a unique mechanism of action that in recent years have substantially changed the management of treatment naïve and relapsed/refractory MM (RRMM). Antibodies have an immune-based mechanism, induce durable responses with limited toxicity, and combine well with existing therapies. Furthermore, advances in bio-engineering have enabled the development of a new generation of mAb-derived therapeutics, including antibody-drug conjugates (ADCs) and bispecific antibodies (bsAbs), with the potential to further improve the clinical outcome for patients.

This review will discuss the clinical role of approved and emerging mAbs and mAb-derivatives in the treatment landscape of MM.

UNCONJUGATED MONOCLONAL ANTIBODIES

The U.S. Food and Drug Administration's (FDA) approval of the anti-CD20 mAb rituximab in 1997 led to an exponential interest in the development of mAbs for many malignancies. In the context of MM, antibodies directed against CD38, signaling lymphocytic activation molecule family member 7 (SLAMF7), interleukin-6, B-cell activating factor, CD138, dickkopf 1 (DKK1), and receptor activator of nuclear factor-κB ligand (RANKL) have been evaluated. Of these, anti-CD38 and anti-SLAMF7 are transformative mAbs garnering approval in MM. Clinical trials investigating mAbs are summarized in Table 1

Treatment	Study	Phase	Patients number (Experiment/Control)	Median prior lines of therapies	Overall Response Rate (%)	VGPR or better (%)	CR or better (%)	Median PFS (months)	Median OS (months)
ERd vs Rd	ELOQUENT-2	3	321/325	2	79 vs 66	33 vs 28	4 vs 7	19.4 vs 14.9	43.7 vs 39.6
EVd vs Vd	CA 204-009	2	77/75	1	66 vs 63	37 vs 27	4 vs 4	9.7 vs 6.9	85% vs 74% at 12 mo
EPd vs Pd	ELOQUENT-3	2	60/57	3	53 vs 26	20 vs 9	8 vs 2	10.3 vs 4.7	NA
EPd vs Pd	ELOQUENT-3	2	60/57	3	53 vs 26	20 vs 9	8 vs 2	10.3 vs 4.7	NA
DVd vs Vd	CASTOR	3	251/247	2	84 vs 63	62 vs 29	29 vs 10	16.7 vs 7.1	NA
DRd vs Rd	POLLUX	3	286/283	1	93 vs 76	76 vs 44	55 vs 23	58 % vs 35% at 30 mo	91 % vs 87 % at 12 mo
DMPV vs MPV	ALCYONE	3	350/356	0	91 vs 74	71 vs 50	43 vs 24	72% vs 50% at 18 mo	NA
DVTd vs VTd	CASSIOPEIA	3	543/542	0	93 vs 90	83 vs 78	39 vs 26	93% vs 85% at 18 mo	NA

Treatment	Study	Phase	Patients number (Experiment/Control)	Median prior lines of therapies	Overall Response Rate (%)	VGPR or better (%)	CR or better (%)	Median PFS (months)	Median OS (months)
DRd vs Rd	MAIA	3	368/369	0	93 vs 81	79 vs 53	48 vs 25	NR vs 31.9	NA
Isa-Pd vs Pd	ICARIA - MM	3	154/153	3	60 vs 35	32 vs 9	5 vs 2	11.5 vs 6.5	72% vs 63% at 12 mo

1. Anti-CD38 Antibody Daratumumab

CD38 is a 45-kD, type II transmembrane glycoprotein that associates with cell-surface receptors, regulates cytoplasmic Ca2+ flux, and mediates signal transduction in lymphoid and myeloid cells. CD38 is highly and uniformly expressed on myeloma cells and is expressed at relatively low levels on normal lymphoid and myeloid cells (2). Daratumumab is a human IgG1 antibody that targets CD38 with direct antitumor and immunomodulatory activity (Figure 1). Specifically, daratumumab exerts anti-myeloma activity by multiple mechanism including: i) antibody-dependent cellular cytotoxicity (ADCC) by engaging Fc RII and RIII natural killer (NK) cells; ii) antibody-dependent cellular phagocytosis (ADCP) via macrophages; iii) complement-dependent cytotoxicity (CDC); iv) direct apoptosis mediated by direct cross-linking and inhibiting cellular signaling pathways; and, lastly v) immunomodulation by depletion of CD38-positive regulator immune suppressor cells, which lead to a greater clonal expansion of T effector cells (3). Daratumumab has transformed the MM treatment landscape for both relapsed and newly diagnosed MM (NDMM) patients. Key clinical trials are summarized below.

Current and investigational targets of mAbs in MM (modified from (66)Daratumumab monotherapy for RRMM

Daratumumab (at a dose of 16 milligram per kilogram of body weight) was granted a breakthrough-therapy designation by the FDA in 2015 based on early phase clinical trials that demonstrated an important survival benefit of daratumumab monotherapy in heavily pretreated RRMM. Specifically, the GEN501 trial demonstrated an overall response rate (ORR) of 36%, including two patients with a complete response (CR) and two with a very good partial response (VGPR); 65% of the patients with a response did not have disease progression at 12 months. The main side effect of the antibody was infusion-related reactions, typically at the time of the first infusion (4). Similarly, the SIRIUS trial demonstrated an ORR with 36% with daratumumab with a median PFS was 3.7 months (95%CI 2.8–4.6), 12-month OS was 64.8% (95%CI 51.2–75.5) and, at a subsequent cutoff, median OS was 17·5 months (95%CI 13.7-not estimable. Treatment was well tolerated; fatigue and anemia were the most common side effects encountered (5).

2. ADC

ADCs are a rapidly growing class of immunotherapeutic agents. Different constructs, payloads and target antigens are in preclinical or early clinical investigation for the treatment of MM [6]. Among them, the most promising agent of which we already have clinical data is belantamab mafodotin (belamaf), a humanized anti-BCMA IgG mAb fused to the payload monomethyl auristatin F (MMAF). In preclinical in vitro and in vivo models, belamaf showed anti-MM activity without affecting BCMA-negative cells and the MMAF arrested the cell cycle of malignant plasma cells at the G2/M phase, eventually leading to cell death [7]. Its afucosylated Fc fraction promotes Fc-dependent immune effector functions, mainly ADCP and ADCC [8].

3. BiAbs

BCMA on malignant plasma cells and CD3 on T cells are the two main targets exploited to design anti-MM BiAbs. Other BiAbs targeting different antigens on the plasma cell surface and/or involving different immune effectors have been reviewed elsewhere [9].

AMG 420 is an anti-BCMA BiTE that was tested in a dose-escalation first-in-human study enrolling RRMM patients. At the maximum tolerated dose (400 mcg/die), a very good efficacy was reported (ORR 70%).[10] Due to the pharmacokinetics typical of non-Ig-like BiAbs, this drug formulation required a continuous infusion for 4 weeks on therapy followed by 2 weeks off therapy. Infections were frequent (G ≥ 3 24%), and the use of a central venous catheter line to deliver the drug led to central line infections in 12% of patients. Other treatment-emergent AEs were cytokine release syndrome (CRS, 38%, mostly G1-2) and peripheral neuropathy (G 3 5%).

Due to the aforementioned pharmacokinetic issues, AMG 420 has not been further developed. Nonetheless, a study evaluating AMG 701, a half-life extended BiTE not needing continuous infusion, is currently ongoing [11].

PF-06863135 (PF-3135) is a humanized Ig-like BiAb that is currently being tested in a dose-escalation study in RRMM [12]. Due to its Ig-like structure, PF-3135 is infused once weekly. Results of the first 17 patients showed a minimal response in 1 patient (6%), although the clinical benefit rate (defined as best response ≥ stable disease) was 41% and dose escalation is still ongoing. Three patients (18%) experienced G ≥ 3 AEs and the only non-hematologic AE was an increase in blood liver enzymes in 1 patient.

CC-93269 is an Ig-like BiAb asymmetrically targeting BCMA through two binding sites and targeting CD3 through one binding site [13]. A dose-escalation phase I study in heavily pretreated RRMM patients is ongoing and results of the first 30 patients have been presented. CC-93269 was administered intravenously over 2 h: weekly in cycles 1–3, every other week in cycles 4–6, and every 28 days thereafter. The ORR was 43% throughout the dose cohorts, but it became dose-dependent, reaching 89% at the highest tested dose (10 mg). CRS was mild but frequent (all grades 77%; G ≥ 3 4%). Thus, dexamethasone prophylaxis was implemented in patients treated with doses >6 mg. The main toxicities were neutropenia (G ≥ 3 43%) and infections (G ≥ 3 30%).Teclistamab is another Ig-like BiAb. Results of the first 78 RRMM patients enrolled in a phase I dose-escalation trial were recently presented [14].

4. FUTURE DIRECTION AND CONCLUSION

The introduction of mAbs for the treatment of MM has already changed clinical practice in RRMM patients, leading to better outcomes. Moreover, between 2019 and 2020, daratumumab combinations with Rd and VMP in NTE NDMM patients and with VTd in TE NDMM patients were approved by FDA and EMA. This means that the great majority of NDMM patients will receive an anti-CD38 naked mAb in the near future, due to the higher efficacy of combinations with daratumumab and to its negligible toxicity when added to SOC regimens. In the next years, more and more patients will eventually be exposed or refractory to anti-CD38 naked mAbs after first relapse, thus questioning the current treatment sequencing in RRMM patients. Initial reports of the suboptimal efficacy of retreatment with anti-CD38 mAbs in a small series of patients are beginning to emerge [15] and will require prospective confirmation in a significant number of patients. This issue may be overcome by using different anti-CD38 mAbs with unique mechanisms of action [16]. TAK-079 minimally binds to targets with a low density of CD38, leading to an enhanced depletion of high-density CD38+ target cells [17]. However, the predicted efficacy of these mAbs is largely dependent on the mechanisms of resistance to anti-CD38 mAbs. Resistance to ADCC (e.g., fratricidal depletion of CD38+ NK cells), CDC (e.g., upregulation of complement-inhibitory molecules) and ADCP (e.g., upregulation of CD47 inhibiting phagocytosis) have been observed during anti-CD38 mAb treatment and strategies to overcome them are under clinical investigation [18]. Nevertheless, the most relevant issue limiting retreatment with anti-CD38 mAbs is the long-lasting downregulation of CD38 on plasma cell surfaces after anti-CD38 therapy [16]. Even though strategies to reinduce CD38 expression in malignant plasma cells are under clinical investigation [19,20], changing the target antigen may be a more appealing strategy in RRMM patients who are refractory to anti-CD38 treatment. The new anti-BCMA molecules could find their therapeutic space in this scenario.

Several observations can be made about the comparison among naked mAbs, ADCs and BiAbs. Single-agent activity of naked mAbs is relatively low (ORR around 20–30% in heavily pretreated RRMM patients with anti-CD38 mAbs), while ADCs (ORR up to 60% in RRMM with belamaf) and BiAbs (ORR up to 90% in RRMM with CC-93269) can induce deeper responses. However, naked mAbs are very safe drugs and do not have overlapping toxicities with other MM drugs. As a consequence, they may be easily combined with MM backbone treatments. Moreover, they may be effortlessly administered in outpatient facilities, and their subcutaneous formulations will be available in the future. ADCs have toxicity profiles non-overlapping with IMiDs and PIs and clinical trials exploring ADC-based combination therapies are ongoing. Moreover, they can be easily infused in outpatient facilities as well, although the off-target toxicity of the payload could be an issue (e.g., eye toxicity with belamaf). BiAbs are very effective drugs, but the infection risk makes it difficult to combine them with other MM backbones. Moreover, the strong activation of the immune system and consequent CRS risk may require preventive hospitalization during the first days after treatment or at least a close monitoring of CRS symptoms.

Other types of anti-BCMA immunotherapy, such as CAR T-cell therapy, are currently available However, despite their high efficacy as single agents (up to an ORR of 100%) [21], they are currently not ‘off-the-shelf’ drugs. Differently from naked mAbs/ADCs/BiAbs, they require specialized centers and inpatient admission for the infusion. Furthermore, CRS/neurotoxicity should be closely monitored and promptly treated.

Currently, the optimal scenario for each of these drugs could depend on both disease risk and patient fitness. For instance, intermediate-fit or frail patients may safely receive naked mAbs or ADCs, but they are unlikely to tolerate BiAbs or CAR T-cell therapy. On the other hand, fit patients who present with high-risk disease or who experienced early relapse after first-line treatment may benefit from BiAbs or CAR T-cell therapy.

ARTICLE 8

A COMPREHENSIVE REVIEW ARTICLE: UNCONJUGATED MONOCLONAL ANTIBODIES AND PROTEASES INHIBITORS IN TREATMENT OF MULTIPLE MYELOMA AND THEIR MANAGEMENT

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Abstract:

Multiple myeloma (MM) is a hematologic malignancy that is currently incurable. Immunological changes in lymphocytes and myeloid cells are the hallmark of this illness. In the bone marrow, multiple myeloma (MM), a hematological malignancy, is typified by the growth of cancerous plasma cells. To put it simply, this review gives information about the etiology of multiple myeloma and the management of the disease. Conventional chemotherapy is used as the first-line treatment; however, many patients have a relapsed form of MM that may develop into a resistant disorder. New monoclonal antibodies (Mab) like elotuzumab, isatuximab, and daratumumab are used in the new therapeutic frontiers. New immunotherapies based on chimeric antigen receptor (CAR) T cell treatment and contemporary bispecific antibodies have been studied in addition to monoclonal antibodies.. The treatment of multiple myeloma has been completely transformed by the recent discovery of monoclonal antibodies (mAbs). There is a lot of excitement for mAbs in this disease because of the efficacy of daratumumab and elotuzumab in relapsed/refractory patients. It is anticipated that combination therapy with additional anti-MM therapeutic modalities and clinical assessment in recently diagnosed patients will significantly alter the disease's natural course. The novel authorized antibody targets for MM now utilized in clinical practice—CD38 (daratumumab and isatuximab), SLAM7 (elotuzumab), and BCMA (belantamab mafodotin)—are the main focus of this review. Despite the fact that the condition is currently incurable, the goal for the future is to identify the most effective therapy combination based on the results of all existing medications.

Keywords: Myoclonoal Antibodies, Cancer, Myeloma, Proteases enzyme,
CD38 (Daratumumab), SLAM7 (elotuzumab).

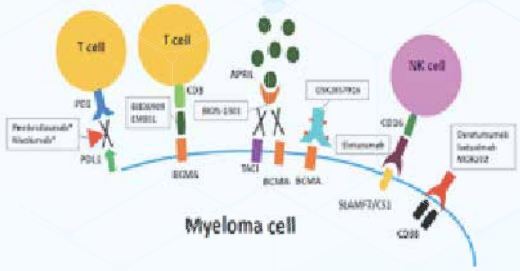
Introduction

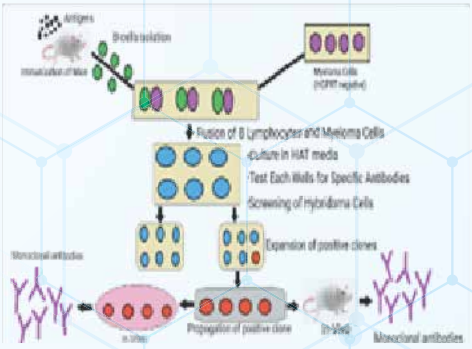
- The hallmark of multiple myeloma (MM), a hematological malignancy, is the accumulation of plasma cells in the bone marrow (BM) through clonal development. With a slightly greater prevalence in males than in women, MM is the second most common blood malignancy, primarily affecting those aged 60 to 65. Clinically, the disease can manifest as either silent or aggressive; kidney failure or elevated blood calcium levels due to malignant cell invasion of the bones are common symptoms. Increased calcium levels can lead to weakness and mental disorientation because they disrupt central nervous system activities. Despite the numerous therapeutic options that are currently available, MM is still an incurable condition, and after a varied amount of time, the patient stops responding to the previously prescribed treatment. Myeloid cells and lymphocytes are two immune groups that are affected by the immunological changes that define the illness.

A promising class of drugs with a distinct mode of action, monoclonal antibodies (mAbs) have significantly altered the treatment of MM (RRMM) and treatment-naïve patients in recent years. Antibodies work well with current treatments, produce long-lasting effects with little harm, and have an immune-based mechanism.

Unconjugated Monoclonal Antibodies:

Interest in creating mAbs for a variety of cancers has skyrocketed since the U.S. Food and Drug Administration (FDA) approved the anti-CD20 mAb rituximab in 1997. Antibodies against CD38, B-cell activating factor, CD138, dickkopf 1 (DKK1), interleukin-6, signaling lymphocytic activation molecule family member 7 (SLAMF7), and receptor activator of nuclear factor-κB ligand (RANKL) have all been assessed in relation to MM. Among these, transformative mAbs that are being approved in MM are anti-CD38 and anti-SLAMF7. The immune system produces antibodies, which are proteins, in reaction to an antigenic stimuli. An antigen invasion triggers the immune response, which includes the development and multiplication of B lymphocytes in memory cells and plasma cells, which are in charge of generating a large number of soluble antibodies against the antigen. The antibodies have a high level of specificity; they can identify foreign chemicals and negate their effects.





1. Anti-CD38 Antibody

Daratumumab- The 45-kD type II transmembrane glycoprotein CD38 promotes signal transduction in lymphoid and myeloid cells, controls cytoplasmic Ca2+ flux, and binds to cell-surface receptors. Normal lymphoid and myeloid cells express CD38 at relatively modest levels, whereas myeloma cells express it evenly and at high levels. Human IgG1 antibody daratumumab directly targets CD38 and has immunomodulatory and anticancer effects. Daratumumab specifically works against myeloma through a number of mechanisms, such as

- Antibody-dependent cellular cytotoxicity (ADCC), which involves interacting with Fc RII and RIII natural killer (NK) cells.
- Antibody-dependent cellular phagocytosis (ADCP), which involves macrophages; ;
- Complement-dependent cytotoxicity (CDC).
- Direct apoptosis caused by blocking cellular signaling pathways and direct cross-linking
- Immunomodulation by depletion of CD38-positive regulator immune suppressor cells, which lead to a greater clonal expansion of T effector cells. Daratumumab has transformed the MM treatment landscape for both relapsed and newly diagnosed MM (NDMM) patients. Key clinical trials are summarized below.

Daratumumab monotherapy for RRMM:

Based on early phase clinical trials showing a significant survival benefit of daratumumab monotherapy in heavily pretreated RRMM, the FDA designated daratumumab (at a dose of 16 mg per kilogram of body weight) as a breakthrough treatment in 2015. With two patients experiencing a complete response (CR) and two experiencing a very good partial response (VGPR), the GEN501 trial specifically showed an overall response rate (ORR) of 36%; at 12 months, 65% of the patients who experienced a response did not see disease progression. Infusion-related responses were the antibody's primary adverse impact, usually occurring during the initial infusion.

Daratumumab combinations for RRMM:

Daratumumab in combination with pomalidomide/ dexamethasone (Pd),lenalidomide/ dexamethasone (Rd), bortezomib/dexamethasone (Vd), and, probably soon, carfilzomib/ dexamethasone (Kd) has been shown to provide enough benefit in RRMM to warrant FDA clearance. The POLLUX experiment, a phase 3 trial, assessed daratumumab with Rd (DRd) and showed a 63% decreased risk of disease progression or mortality compared to Rd alone. Following a median follow-up of 44.3 months, the daratumumab group's median PFS was 44.5 months, whereas the Rd group's was 17.5 months. Daratumumab with Vd also produced a noticeably longer PFS in the CASTOR study than Vd alone (12-month PFS rate was 60.7% in the daratumumab group versus 26.9% in the control group).

2. Anti-SLAMF 7

Elotuzumab Elotuzumab is a humanized IgG1 MAb aimed against the cell surface glycoprotein CS1 (also known as SLAMF7, CRACC, CD2 subset-1 or CD319). Hematopoietic stem cells and other cell lineages exhibit nearly low expression of this surface antigen, but MM cells, normal plasma cells, NK cells, and a subset of CD8+ T lymphocytes exhibit high expression.

When treating MM in adult patients who have had at least one previous course of therapy, in conjunction with lenalidomide and dexamethasone.

For the treatment of adult patients with relapsed and refractory MM who have had at least two lines of therapy (including lenalidomide and a proteasome inhibitor) and whose disease has progressed, in conjunction with pomalidomide and dexamethasone.

Mechanism: The surface glycoprotein SLAMF7 (signaling the lymphocytic activating molecule family 7), which is mostly produced by NK and normal or malignant plasma cells and promotes growth and survival, is the molecular target of elotuzumab.Elotuzumab works by preventing the connections that allow cancer cells to proliferate and prolong their lives. Additionally, it increases the ADCC activity of NK cells, stimulating them.

Elotuzumab increases the anti-myeloma activity of NK cells in vitro by directly activating them via SLAMF7 and Fc receptors. The protein SLAMF7, which is found on myeloma cells, interacts with Fc receptors on particular immune system cells to induce cell death.

Isatuximab

Like daratumumab, isatuximab is a naked chimeric monoclonal antibody that targets CD38. It also has proapoptotic activity via the lysosomal cell death route and the caspase-dependent apoptotic pathway. The FDA and EMA both authorized itatixumab in March and June of 2020, respectively: In conjunction with dexamethasone and pomalidomide for the treatment of adult patients with refractory and relapsed MM who have undergone at least two prior treatments, such as lenalidomide and a proteasome inhibitor, and whose illness progressed during the most recent therapy;

Mechanism:

An IgG1-derived monoclonal antibody called isatuximab attaches itself to a particular extracellular CD38 receptor epitope. In vitro, isatuximab induces apoptosis by an Fc-independent mechanism and operates via IgG Fc-dependent pathways, including as ADCC, ADCP, and CDC. Moreover, it can stimulate NK cells even when target tumor cells that are CD38-positive are not present.
Isatuximab was approved following a number of phase 1 trials that assessed its safety.
and effectiveness as a monotherapy as well as in combination.

Table no.- 1: Monoclonal antibodies used in the treatment of myeloma.

Target	Name of the Antibody	Anti-Myeloma Mechanism	Immunomodulatory Effects
CD38	Daratumumab	CDC, ADCC, ADCP, induction of apoptosis when cross-linked, enzymatic modulation	1. Deletion of CD38+ Tregs and Bregs 2.Expansion of CD8+ cytotoxic T cells and CD4+ helper T cells
CD38	Isatuximab	ADCC, CDC, ADCP, direct cell death via lysosome-mediated and apoptotic pathway	1. Augmentation of NK and CD8+ T effector cell-mediated anti-tumor immune responses . 2.Reduction of Foxp3 and IL10 in Tregs. 3.Restoration of proliferation and function of naive T cells
SLAMF7/CS1	Elotuzumab	ADCC	Activation of NK cells
PD1	Pembrolizumab	Induction of apoptosis	Activation and proliferation of T cells
BCMA	BI 836909	Potent induction of apoptosis	BCMA- induced T-cell activation and cytokine release
APRIL	BION-1301	Blockage of APRIL-induced growth and survival, induction of apoptosis	Decreased expression of PD-1, TGF-βand IL-1 genes)

CDC, complement-dependent cytotoxicity; ADCC, antibody-dependent cell-mediated cytotoxicity; ADCP, antibody-dependent cellular phagocytosis. IMID, immune modulatory drugs; NK, natural killer cell; SLAMF, signaling lymphocytic activation molecule F7; BCMA, B-cell maturation antigen; APRIL, proliferation-inducing ligand.

Conclusion:

AThe creation of monoclonal antibodies that specifically target multiple myeloma antigens is a significant step in bettering immunotherapies that work for multiple myeloma patients. Monoclonal antibodies can have immunomodulatory effects on immune cells in the bone marrow microenvironment through a variety of mechanisms mediated by FcR-expressing effector cells (ADCC, CDC, or ADCP). These mechanisms include reducing the number and function of immunosuppressive cells and reviving the immune effect or cells' ability to kill tumors. As demonstrated by daratumumab in recent large phase 3 clinical studies, such unique immunomodulatory effects may further result in deeper clinical responses and increased efficacy. Monoclonal antibodies are an effective treatment option, even for patients with relapsed and refractory multiple myeloma who have received extensive pretreatment, according to prior clinical trials.

ARTICLE 9

UUNCONJUGATED MONOCLONAL ANTIBODIES AND PROTEASE INHIBITORS: A TARGETED APPROACH IN MULTIPLE MYELOMA TREATMENT

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Abstract:

Multiple myeloma (MM) is a malignant plasma cell disorder that leads to bone marrow failure, immune dysfunction, and organ damage. The advent of targeted therapies, including unconjugated monoclonal antibodies (mAbs) and proteasome inhibitors (PIs), has significantly improved survival and quality of life for MM patients. Unconjugated mAbs work by specifically targeting antigens on myeloma cells, while PIs interfere with protein degradation pathways critical to tumor cell survival. This article explores the mechanisms, clinical applications, and emerging research on these key therapeutic agents in MM treatment.

Introduction

Multiple myeloma (MM) accounts for approximately 10% of hematologic malignancies and primarily affects older adults. Historically, treatment options were limited to chemotherapy and corticosteroids, but survival rates remained poor due to frequent relapses.

The development of novel targeted therapies, particularly monoclonal antibodies (mAbs) and proteasome inhibitors (PIs), has transformed the treatment landscape. These agents have been integrated into first-line therapies, maintenance regimens, and relapse settings, providing substantial improvements in progression-free survival (PFS) and overall survival (OS).

In this review, we focus on unconjugated monoclonal antibodies and protease inhibitors, discussing their mechanisms, efficacy, and future directions in MM therapy.

Unconjugated Monoclonal Antibodies in Multiple Myeloma

Monoclonal antibodies (mAbs) have gained prominence in MM treatment due to their ability to selectively target tumor-associated antigens with minimal off-target toxicity. Unlike conjugated antibodies that deliver cytotoxic payloads, unconjugated mAbs exert their effects through direct immune-mediated mechanisms such as:

- Antibody-Dependent Cellular Cytotoxicity (ADCC) – Recruiting natural killer (NK) cells and macrophages to destroy myeloma cells.
- Complement-Dependent Cytotoxicity (CDC) – Activating the complement system to lyse cancer cells.
- Direct Apoptosis Induction – Triggering cell death pathways without additional immune activation.

Key Unconjugated Monoclonal Antibodies in MM

1. Daratumumab (Anti-CD38 mAb)

- Target: CD38, a glycoprotein highly expressed on myeloma cells.
- Mechanism: Induces ADCC, CDC, and apoptosis. It also depletes immunosuppressive regulatory cells, enhancing T-cell function.
- Clinical Use: Approved for newly diagnosed and relapsed/refractory MM (RRMM), often combined with lenalidomide-dexamethasone (DRd) or bortezomib-dexamethasone (DVd).
- Efficacy: In the MAIA trial, DRd improved median PFS to 56.7 months versus 34.4 months in the control group.

2. Isatuximab (Anti-CD38 mAb)

- Target: CD38, similar to daratumumab but with distinct binding properties.
- Mechanism: Triggers immune-mediated cytotoxicity and modulates the tumor microenvironment.
- Clinical Use: Approved for RRMM in combination with pomalidomide-dexamethasone (IsaPd).
- Efficacy: The ICARIA-MM trial showed IsaPd reduced disease progression by 40% compared to pomalidomide-dexamethasone alone.

3. Elotuzumab (Anti-SLAMF7 mAb)

- Target: SLAMF7, a receptor found on MM cells and NK cells.
- Mechanism: Enhances NK cell activation and MM cell recognition.
- Clinical Use: Approved for RRMM in combination with lenalidomide-dexamethasone (ERd) or pomalidomide-dexamethasone (EPd).
- Efficacy: The ELOQUENT-2 trial demonstrated a 30% reduction in disease progression with ERd versus Rd alone.

Advantages and Challenges of Unconjugated Monoclonal Antibodies

Advantages:

- Highly selective targeting of MM cells.
- Reduced systemic toxicity compared to chemotherapy.
- Long-term efficacy in maintenance therapy.

Challenges:

- Risk of infusion-related reactions (IRRs), especially with daratumumab.
- Resistance mechanisms such as antigen downregulation.
- Requires combination therapy for optimal efficacy.

Proteasome Inhibitors in Multiple Myeloma Treatment

Proteasome inhibitors (PIs) are a cornerstone of MM therapy, targeting the ubiquitin-proteasome system to block protein degradation. This leads to accumulation of misfolded proteins, causing endoplasmic reticulum stress and apoptosis in MM cells.

Key Proteasome Inhibitors in MM

1. Bortezomib (First-Generation PI)

- Mechanism: Reversibly inhibits the 26S proteasome, preventing protein degradation.
- Clinical Use: First-line therapy in combinations like VRd (bortezomib, lenalidomide, dexamethasone).
- Efficacy: Studies show a PFS of ~43 months when combined with lenalidomide-dexamethasone.
- Side Effects: Peripheral neuropathy (20-30% incidence).

2. Carfilzomib (Second-Generation PI)

- Mechanism: Irreversibly inhibits the proteasome with greater selectivity than bortezomib.
- Clinical Use: Used in KRd (carfilzomib, lenalidomide, dexamethasone) for newly diagnosed and RRMM.
- Efficacy: The ASPIRE trial showed a PFS of 26.3 months with KRd versus 17.6 months with Rd alone.
- Side Effects: Lower neuropathy risk but higher cardiovascular toxicity (hypertension, heart failure).

3. Ixazomib (Oral PI)

- Mechanism: First oral PI, offering convenience with once-weekly dosing.
- Clinical Use: Approved in IRd (ixazomib, lenalidomide, dexamethasone) for RRMM.
- Efficacy: The TOURMALINE-MM1 trial demonstrated a 35% improvement in PFS compared to placebo.

Advantages and Challenges of Proteasome Inhibitors

Advantages:

- Effective in newly diagnosed and relapsed settings.
- Synergistic effects when combined with mAbs or IMiDs.
- Oral options improve patient convenience.

Challenges:

- Risk of drug resistance due to mutations in the proteasome.
- Cumulative toxicities, including neuropathy (bortezomib) and cardiovascular effects (carfilzomib).

Combination Therapies and Future Directions

Combination regimens integrating mAbs, PIs, and immunomodulatory drugs (IMiDs) are now the standard of care for MM. Future research focuses on:

- Next-generation monoclonal antibodies targeting alternative MM antigens.
- Dual-acting proteasome inhibitors with enhanced specificity.
- CAR-T cell therapy and bispecific antibodies to improve long-term responses.

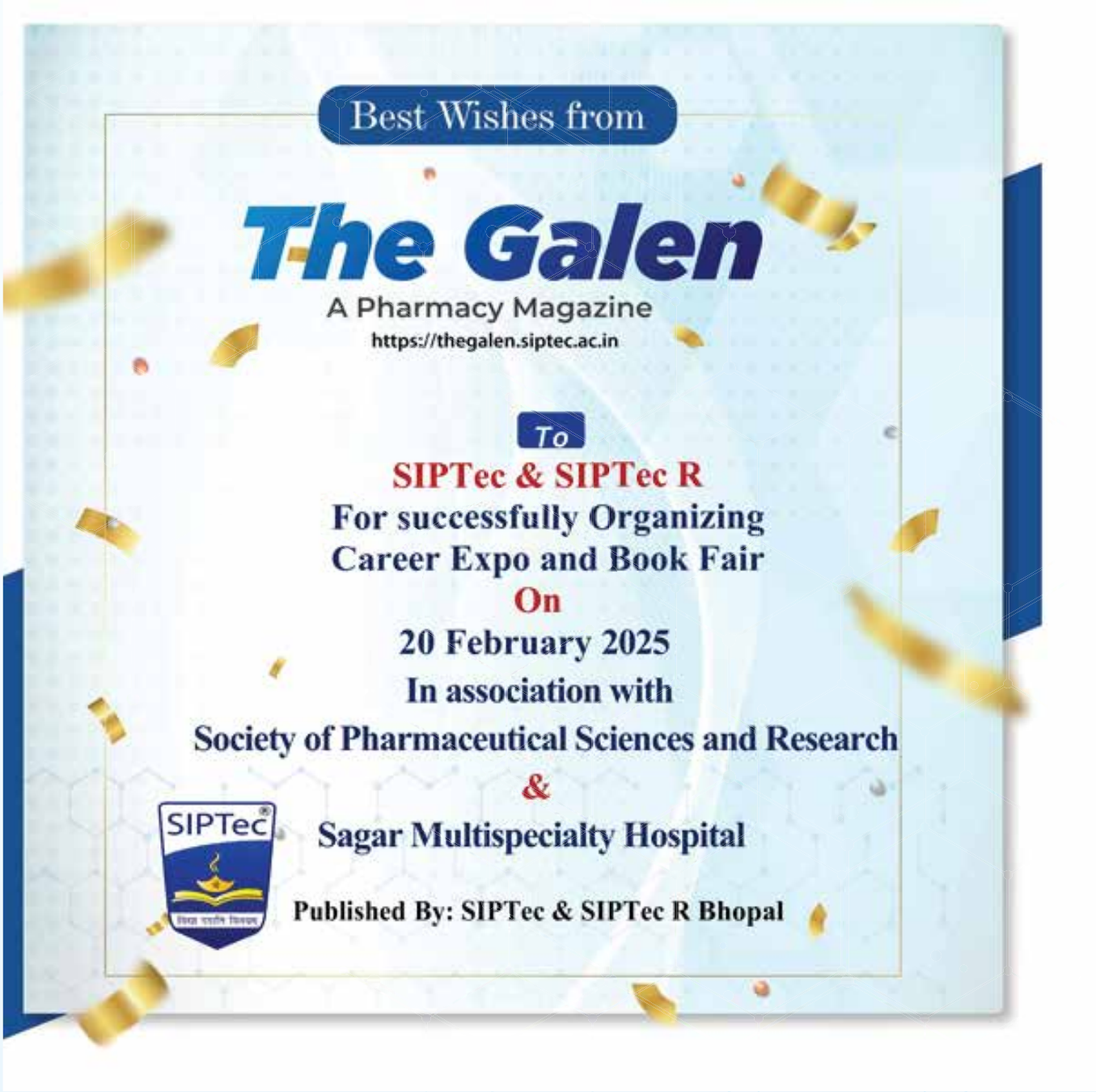
Conclusion

Unconjugated monoclonal antibodies and proteasome inhibitors have revolutionized multiple myeloma treatment, improving patient outcomes and redefining treatment strategies. As research progresses, novel drug combinations and resistance mechanisms will shape the next era of MM therapy, offering hope for prolonged remission and potential cures.

About Sagar Group, SIPTec And SIPTec-R

Sagar Group came into existence in the year 1983 under the visionary leadership of Chairman **Shri Sudhir Kumar Agrawal.** Over the years, it has now transformed into one of the largest corporate houses and business conglomerates of Central India. In its journey of over three decades, the group has successfully ventured into the fields of education, real estate, production, and manufacturing, **Employing 5000+ people** and impacting the lives of more than two lakh people every day. Sagar Group has been felicitated with the IBC24 Excellence Award 2017 for its contribution to Madhya Pradesh's Industrial Development and Incredible Societal Development. **Agrawal Builders** have established their presence as one of the leading Real Estate giants with over 40 years of rich experience in building state-of-the-art residential projects. **Sagar Manufacturers Pvt Ltd** has pledged to use the best fibers to produce superior quality yarns with world-class production technology. In a short span of time, the company has achieved an installed capacity of 2,00,000 spindles and is exporting its products to over 20+ countries. **Sagar Nutriments Pvt Ltd** is Sagar Group's recent venture in food processing premium quality basmati rice. Sagar Group has earned a lot of praise across the nation for empowering the youth of Madhya Pradesh with bright careers and lives. **The group provides world-class school and technical education** under **Sagar Group of Institutions to 20,000+ students with 2000+ dedicated faculties.** The group imparts schooling through the chain of **Sagar Public Schools (SPS)** to nurture young minds. Today, SPS is considered the most preferred brand for holistic education and Indian Value System, **featuring amongst the Top 100 schools in India with its campuses at Saket Nagar, Gandhi Nagar, Rohit Nagar, Ratibad, Katara Extension, and Dwarka Dham. Sagar Institutes (SISTec)** are engaged in providing the best technical education in the fields of **engineering, pharmacy, and management. Sagar Institute of Pharmacy and Technology (SIPTec)** is the premier institution known for its high standards in teaching and research in pharmaceutical sciences. **SIPTec** was established in 2008. The Institute is also registered under CCSEA. Today, within a short span of 15 years, the institute has gained a reputation of being one of the top Pharmacy Colleges in MP that provides total pharmaceutical education comprising B.Pharm. and M.Pharm. (Pharmaceutics & Pharmaceutical Chemistry). Sagar Institute of Pharmaceutical Technology and Research (SIPTec-R) is one of the Best Pharmacy Colleges in Bhopal, Madhya Pradesh with all Modern Facilities and Lab Equipments. It is the premier pharmacy college of Sagar Group of Institutions which was established in the year 2023 under the aegis of Shri Agrawal Educational & Cultural Society with the main objective of imparting quality pharmacy education. Since its inception, the institute is imparting quality education to undergraduates. The B. Pharm course is duly approved by PCI, New Delhi, DTE, Govt. Of M.P. and affiliated to RGPV, Bhopal.

Media Coverage of Recent Conferences by This Magazine



Annual Day Celebration Sagar Therafest

Sagar Institute of Pharmacy and Technology (SIPTec) and Sagar Institute of Pharmaceutical Technology and Research (SIPTec-R) Bhopal celebrated its Annual Day, Sagar Therafest on 14 April 2025, with harmony and joy. The function began with a warm welcome speech followed by the lighting of the ceremonial lamp. Students showcased their talent through various cultural programs.

The Principal SIPTec Dr. Kuldeep Ganju and Principal SIPTec-R Dr. Jitendra Bajaj, welcomed the gathering and presented Academic Toppers of both Colleges.

Next Theme: "Artificial Intelligence in Pharmaceutical Technology and Drug Delivery Design"

Deadline for article submissions: 30 June 2025

https://thegalen.siptec.ac.in/