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REVIEW OPEN ACCESS

The Investigation of the Effect of Antibody Recruiting Molecules on Various Antigenic Markers (Cancer, Bacteria, Viruses): A Literature Review

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Introduction: Antibody recruiting molecules (ARMs) are small molecules with low molecular weight that guide endogenous antibodies towards both cancer and infectious cells, they facilitate the process of immune-mediated clearance. ARMs have two specific regions; a Target Binding Terminus interacts with disease biomarkers and the Antibody Binding Terminus, associated with endogenous antibodies. These modules are linked together by a tunable linker region bridging the endogenous antibody and the infected cell. ARMS can be used for a broad range of therapeutic applications, especially for its use against cancer, bacterial, and viral infections. ARMs serve a new potential treatment option over traditional therapies.
Methods: To conduct our research, specific search terms were created, and relevant articles were screened on Covidence using an inclusion/exclusion criteria. The CASP and CRAAP checklist will be used for the quality assessment of the utilized sources.
Results: ARMs treatment is a novel pathway which can treat a wide range of diseases from cancer, bacteria, to viruses. ARMs clearly represent promising alternatives in antitumor immunotherapy over traditional methods.
Discussion: One hurdle of using ARMs is that its effect on individuals might differ based on antibody concentrations, their affinities, isotypes etc. Due to the non-specific nature of ARMs, there's a selectivity issue regarding binding to specific biomarkers or antigens. The use of non-covalent ARMs to target the highly expressed receptors on the tumor can sometimes lead to endocytosis during the binding process before the recruitment of antibodies. This can be potentially solved by adding covalent linkages in the ARMs molecular construct. This paper analyzes the limitations of utilizing ARMs as an effective means for immunotherapy and proposes potential avenues of improvement for greater efficacy.
Conclusion: This paper will potentially advance pharmaceutical and immunotherapeutic interventions available for numerous cancers and infectious diseases.

Keywords: antibody recruiting molecules; antibody; cancer; immunotherapy; pathogen; monoclonal antibody; virus; immune engager

Introduction

The development of synthetic immunotherapies represents an emerging field of research with tremendous possibilities in the treatment of human diseases [1]. One specific area of focus within this field is the development of antibody recruiting molecules (ARMs). ARMs are bifunctional, synthetic, low molecular-weight species capable of enhancing antibody binding to biological agents such as cancer cells, bacteria, or viruses. This ability to increase the recruitment of endogenous or exogenous antibodies to diseased sites aids in immune-mediated clearance [1]. ARMs are considered bifunctional because of their ability to simultaneously associate with antibodies and target antigens [1]. They are defined by two specific functional domains. The first domain is the Target Binding Terminus (TBT) which interacts with cancer or pathogen specific biomarkers. The second domain is the Antibody Binding Terminus (ABT), which is responsible for associating with endogenous or exogenous antibodies, which are linked together by a tunable linker region that bridges the antibody and the infected cell. With the help of these molecules, antibodies can form ternary complexes with infected cells [2]. ARMs are highly flexible with respect to their abilities in targeting a variety of diseased biomarkers, making them a potential novel immunotherapeutic intervention for future research in the field of immunology. This paper aims to explore how effective ARMs are against various antigenic markers.

Currently Available Cancer Treatments and Their Limitations

Cancer is one of the leading causes of death worldwide. There are a wide variety of traditional cancer treatments that are available such as chemotherapy, surgery, and radiotherapy [3]. Unfortunately, these current treatment interventions are associated with severe side-effects [1]. Chemotherapy utilizes chemical agents to destroy proliferating cancer cells [4]. It has numerous side effects, including acute and chronic toxicity [4]. Some of these short-term symptoms include hair loss, nausea, and fatigue, while some long-term conditions include infection, heart problems, and nerve damage [4]. The intensity of these side effects can range from mild to life threatening [4]. Radiotherapy is another cancer treatment that uses high powered energy sources to destroy cancer cells and shrink solid tumors. A major drawback is that the emitted radiation can damage surrounding healthy tissues that are distant from the site of the tumor [3]. The final major form of cancer treatment is the use of surgery [3]. However, the surgical techniques pose a huge risk to the patients such as internal bleeding [3].

Currently Available Treatments for Infectious Diseases and Their Limitations

The World Health Organization (WHO) has estimated that the infectious agents (bacteria and viruses) account for an estimated 15 million of global deaths each year [1]. Vaccines are among the most successful strategies for fighting infections. Limitations of current vaccines include difficulties in production, variable levels of immunostimulation and high costs [1].

Vaccine-based strategies have been successful against various viral diseases, except some viral pathogens such as Human Immunodeficiency deficiency virus (HIV) and Herpes [1]. Available antiviral agents function by inhibiting enzymes such as reverse transcriptase, polymerase, protease, integrase, primase, and neuraminidase. However, the application of antiviral agents is limited due to resistance development, low efficacy, and a high rate of spontaneous mutation [1]. Monoclonal antibodies (mAbs) that target viruses have experienced only moderate levels of success [1]. Currently, viral pathogens are being targeted by harnessing the endogenous immune response [1].

Traditionally, bacterial pathogens have been treated with antibiotics but the major disadvantage is that bacteria are continually developing novel mechanisms to become resistant to these drugs, making treatment through antibiotics problematic [1]. mAb based therapies are a novel therapeutic intervention that has shown tremendous preclinical success. Therefore, the use of ARMs with mAbs as an immunotherapeutic drug serves as a possible way to target infectious agents. There has been significant interest in developing mAbs for treating drug-resistant bacteria as these agents could exploit mechanisms distinct from conventional antibiotics, making bacteria less likely to induce cross resistance [1].

Methods

The first step towards conducting the literature review was to use the keywords mentioned above to conduct a search in Google Scholar, which were then added to an online screening tool called Covidence. Initially a total of 196 articles were added to Covidence from google scholar. There were no duplicates. The screening was completed independently by two of the authors (Das and Ghai) with the third author (Patel) resolving any discrepancies. Any articles that were published before the year 2000, written in a language other than English or lacked scientific data (editorial, conference abstract, trial description) or relevant information (such as, ARMs and/or its therapeutic use in cancer and pathogen treatment) were excluded. In the first round of screening, the two reviewers (Das and Ghai) screened the abstracts of the articles to exclude any non eligible articles. This step resulted in the exclusion of 68 articles, leaving 128 articles, out of the previously added 196 articles, for further screening. In the second step, the full body texts of the remaining articles were screened to determine their eligibility in terms of the presence of relevant information. After the thorough screening, only 18 articles were left that were used to extract information and data needed to conduct this review. This was followed by the Currency, Relevance, Authority, Accuracy, and Purpose (CRAAP) test, which allowed us to evaluate, assess and eliminate bias from the reviewed article with the help of a list of questions [5].

Results

The Application of ARMs in the Field of Cancer Treatment

Recently, biological agents and cellular immunotherapies have emerged as popular alternatives for cancer treatments, due to their high specificity. They have potential to address the plethora of problems that are associated with traditional cancer therapies such as the toxicity of chemotherapeutic agents on normal healthy cells [1]. ARMs may represent promising alternatives for cancer patients, and have the potential to both complement and improve anti-cancer effects [1]. ARMs are easy to administer and are nonimmunogenic, thus have the potential to overcome obstacles found during clinical trials [6].

Barbas et al. exemplified the benefits of combining small molecules with antibody proteins for cancer applications [7]. They exploited the catalytic mAb 38C2 which was conjugated with numerous β-diketone-containing ARMs that can recognize various cancer cell-surface targets such as integrins. These antigenic biomarkers are highly overexpressed in ovarian, breast, cervical, and melanoma cancers [7]. The cp38C2 conjugate ARM has demonstrated remarkable efficacy in human M21 melanoma cells, exhibiting complement dependent cytotoxicity (CDC) and generating a 81% reduction in

tumor growth after a 42 day span [7]. This experiment demonstrates that -diketone-containing ARM can be a potential treatment that is suitable for cancer.

Murelli et al found that targeted CDC of prostate cancer cells (LNCaP cells) can be performed upon formation of ternary complexes between ARMs targeting prostate cancer (ARM-Ps), LNCaP cells, and 2,4-dinitrophenol (DNP) [8]. The ARM-P can target the prostate specific membrane antigen (PSMA) that is highly overexpressed on different cancers [8]. Linker length has significant effects on the binding affinity. The longer the linker length is, the better it is in preventing excessive dehydration of the protein-protein interface [8]. As the ARMs linker sizes were increased from ARM-P4 to ARM-P12, the affinity decreased, but the number of ternary complexes increased [8]. However, ARM-P8, containing 8 oxyethylene, was determined to possess optimal linker length. ARM-P8 effectively induces antibody dependent cellular cytotoxicity (ADCC) in the presence of anti-DNP [8]. This demonstrates that the linker length of the ARM is an important factor in determining the efficacy of ARM-Ps with respect to cancer treatment.

So far, there are 30 antibodies which have been approved by the Food and Drug Administration (FDA) to be utilized in cancer treatment [9]. Each cancer type has unique cell surface protein targets including proteins such as CD22, integrin receptors, folate receptors, LHRH receptors, uPAR, and VEGF [1]. There is still ongoing research on approximately 570 ARMs to test its efficacy against cancerous tumors [9]. In the last 25 years, the number of antibody-based cancer treatments that have progressed from preclinical and clinical trials to the approval stage has increased and the projected market value for therapeutic antibodies will be $300 billion by 20259.

The Application of ARMs in the Field of Infectious Disease: Bacteria

The use of antibiotics to treat infectious disease has been slowly losing its effectiveness due to the development of bacterial cross resistance [10]. To combat this rising global issue, mAbs therapies have demonstrated substantial preclinical success in treating various infectious diseases [11]. Since there are no clinically approved mAbs that target bacterial pathogens, there is a critical need to develop novel therapeutics.

Bednarski et al, designed an ARM capable of directing anti-avidin antibodies to E. *coli* [12]. They utilized biotin and conjugated it to the C-glycoside group of mannose, which is a known ligand of bacterial mannose receptors [12]. Furthermore, ARMs for bacterial clearance could mediate complement and macrophage dependent cytotoxicity [12]. The multivalency of avidin enhances the binding affinity of the C-glycoside ligand to the mannose receptor [12]. This concept was further expanded in a study where bacteria was targeted via an ARMs therapeutic capable of redirecting endogenous anti-a-Gal antibodies (naturally abundant antibodies found in the human body) to E. *coli* [13]. This ARM derivative contained a poly-mannose, which is a polysaccharide formed from the sugar unit called mannose, at the TBT and a poly-a-Gal at the ABT [13]. They found that the binding of ARMs to the receptor led to the inhibition of agglutination [13].

Most research within this field is based on the study of gram-negative bacteria, but recently gram-positive bacteria have been receiving greater attention [14]. ARMs targeting pathogenic gram-positive bacteria were developed by utilizing the antibiotic vancomycin [14]. The ARM construct was made of a polyvalent polymer containing fluorescein (fluorescent tracer molecule) at the ABT and vancomycin at the TBT [14]. These ARMs were able to redirect anti-fluorescein antibodies to the surface of gram-positive bacteria such as S. *aureus* and S. *pneumoniae*. The researchers determined that this ARM polymer could mediate phagocytosis of opsonized bacteria in the presence of anti-fluorescein antibodies [14].

Despite tremendous development in this area, some barriers must be overcome before it can be approved for clinical trials [15]. The above mentioned interventions studied up to this point did not show any optimal activity and lacked specificity [15]. By exploiting an understanding of biological systems, future efforts to expand knowledge on anti-bacterial ARMs have the potential to prevent, diagnose, and treat bacterial diseases.

The Application of ARMs in the Field of Infectious Diseases: Virus

Many novel monoclonal antibody-based drugs are under development for treatment of viral disorders [1]. The current limitation of vaccine-based therapeutics is based on the development of spontaneous mutations and resistance of different viral phages, leading to the insufficient efficacy of current vaccine-based strategies [1]. Naicker et al. utilized chemo-enzymatic synthesis to prepare a bifunctional molecule that is designed to redirect endogenous anti-a-Gal antibodies to the HIV virus [16]. The ARM construct contains a a-Gal trisaccharide epitope at the ABT and is linked to the peptide T-20 at the TBT [16]. The N-terminal tagged T20 acts as an HIV cell fusion inhibitor [16]. The bifunctional glycopeptide could recognize and bind anti-a-Gal IgG and IgM antibodies in human serum and direct these antibodies to HIV [16]. This allows αGal-T20 to perform antibody mediated cytotoxicity or antibody dependent complement mediated lysis of HIV-infected cells [16]. Recently, Valhne et al. synthesized ARMs that were derived by linking α-Gal to a series of oligopeptides obtained from the gp120 binding region of T-cells (CD4) [17]. These ARMs could direct endogenous a-gal antibodies to both immobilized and cell surface expressed gp120 [17]. Glycopeptide derived ARMs are also known to mediate immune responses to chronically HIV IIIB/LAV infected ACH2 cells in human serum and isolated natural killer cells via an ADCC mechanism [17]. Thus, taking

advantage of innate immune responses could be beneficial as HIV causes cellular and humoral immune responses to be compromised.

Spiegel's laboratory designed ARM targeting HIV (ARM-H) which redirects anti-DNP antibodies, which are present in the human bloodstream in a high concentration, to HIV gp120 env gene product [18]. The Env glycoprotein is a complex between gp120 and membrane protein gp41 and is expressed on the surface of both HIV and the infected cells [18]. The gp120 component of Env is responsible for mediating viral entry into host cells by binding to CD4 protein [18]. The study demonstrates that ternary complexes between ARM-H, anti-DNP antibodies and Env expressing cells can lead to complement dependent destruction of these cells [18] . ARM-H can outcompete CD4 protein and binds to gp120 on HIV. Consequently, HIV cannot bind to human CD4 and fails to invade human T cells [18]. The entire termolecular complex is crucial as ARM-H alone is not toxic to HIV infected cells [18]. This approach was shown to increase the survival rate by 48% which highlights that ARMs treatment can significantly delay disease onset and increase the survival rate. Some evidence for viral suppression was obtained but it fell short of the desired outcome [18].

Currently, the efficacy of ARMs treatment of H5N1 influenza virus, respiratory syncytial virus (RSV), hepatitis C virus (HCV), Marburg virus, severe acute respiratory syndrome (SARS) virus, dengue virus, and Ebola virus is under clinical trials to test for their neutralizing activity and target specificities [9]. While a few methods have been tested for using ARMs in viral treatment, this area remains underexplored until further research is done to ensure its efficacy in treatment of other types of viruses.

Discussion

Limitations of Current Research on ARMs

While ARMs seem to be a promising intervention, it is still a relatively new field of research and has to overcome many obstacles before it can be approved for commercial use. Most of the ARMs used for cancer treatment target receptors overexpressed on the cancer cells with specific ligands [6]. However, the interaction between these ARMs and some receptors can result in endocytosis before the ARMs can even recruit antibodies to carry out ADCC [6]. The majority of the current ARM treatments are non-covalent in nature, and ultimately lead to a multitude of limitations that makes it very difficult to determine experimental conditions for optimal efficiency. The formation and stability of the ternary complex is highly dependent on the concentration and dissociation constants of each component of the complex [6]. To overcome this issue, some researchers have attempted to use membrane covalent bonds that allow multivalency of antibody ligands on the cell surface [6]. While multivalent structures have shown to have high avidity, more research is needed to create the suitable TBT that can exhibit high selective expression on the target cell and thus decrease the chances of off-target binding [2]. There is also a risk of immune-complex formation in the bloodstream through binding of multiple endogenous antibodies to the multivalent ARMs and hence, more studies are needed to determine the optimal strategies for in vivo application [2]. For effective immune activation through multivalent interactions, multimeric antigen presentation is crucial [6]. However, there is a huge diversity in antibody specificity and isotype distribution in humans. There is a limited variety of ARMs that can recruit a diverse range of antibodies with high affinity [6]. There is the potential that some uncertainty is associated with the efficiency of ARMs within a large population, due to the physiological differences between individuals. The effect of different ARMs might vary between individuals as functions of antibody concentrations, affinities, isotype and subisotype distributions [1].

Potential Ways to Improve the Efficacy of ARMs

The potential applications of ARMs technology serves great promise for a plethora of disease targeting interventions [1]. Despite the significant progress, various obstacles remain before it can be utilized in the clinical setting [1]. Different ABT and TBT types mentioned above have advantages and disadvantages. One of the demerits of this approach is inter-individual variation. A potential treatment for those with a low antibody abundance is to assemble an ARMs construct that possesses both the TBT and effector cell activating modules, which elicits cancer cell phagocytosis [6]. While most TBT motifs are characterized with relatively specific ligand-receptor systems, ARMs can be improved by strategies that enable unbiased targeting of biomarkers [1]. Other ways to increase the efficacy of ARMS is to improve receptor-binding profiles, decreasing molecular weight, and enhancing oral bioavailability to patients [1].

Non-covalent ARMs aim to directly target membrane features of cancerous tumors such as ovarian, breast, and melanoma to form a ternary complex [6]. The problem with this strategy is that internalization may occur during the binding process with non-covalent ARMs, and their rapid *in vivo* clearance leads to major obstacles during therapeutic applications [6]. A potential solution to this problem is the introduction of covalent labelling of the cancer cell membrane [6]. These improvements to ARMs may help with the binding affinity of the TBT to the target and the ABT to recruit antibodies efficiently due to longer and highly proximal ternary interactions [6]. The linker region between the ABT and the TBT in ARMs construction represents another important parameter to be optimized to increase ARM binding affinity [6].

In contrast to monovalent ARMs, multivalent ARMs could provide a selective advantage by increasing the efficiency of antibody recruitment [2]. Multivalent ARMs consist of multiple TBT and ABT, making them unique

with respect to the number of binding modules present in the molecule [2]. The presence of multivalent interactions aids in the conversion of low binding affinities into increased binding avidities [2]. These ARMs classes could potentially be beneficial to patients with lower concentrations of circulating endogenous antibodies [2]. Despite this spectacular schematic design, more studies are needed because these benefits have mainly been demonstrated *in vitro*. *In vivo* testing is a future prospective step that can embrace the full potential and elucidate any challenges that require further molecular engineering [2].

Conclusions

Through the advancement of synthetic and biophysical chemistry, researchers have constructed ARMs against a broad variety of structurally unrelated disease-relevant targets [1]. Due to the modular nature of ARMs, they are able to form ternary complexes with multiple macromolecular species. ARMs treatment is a novel pathway which can treat a wide range of diseases from cancer, bacteria, to viruses. ARMs clearly represent promising alternatives in antitumor immunotherapy over traditional methods. While all the above-mentioned approaches have been proved to show tremendous potential, they still have limitations in areas such as non-covalency, stability of ternary complexes, and risk of immune complex formation. Thus, advances in clinical trials is a next milestone required to gain success in this field. Introducing ARMs to existing therapy regimens will reduce the high costs and unfavourable side-effects of present treatments, hence providing considerable advantages to patients. Thus, the application of ARMs has created novel treatment possibilities for both cancer and infectious disease for future development.

List of Abbreviations Used

RM: antibody recruiting molecule
ARMs: antibody recruiting molecules
ABT: antibody binding terminus
TBT: target binding terminus
mAb: monoclonal antibody
mAbs: monoclonal antibodies
FDA: Food and Drug Administration
WHO: World Health Organization
HIV: human immunodeficiency virus
CDC: complement dependent cytotoxicity
ADCC: antibody dependent cellular cytotoxicity
DNP: dinitrophenol
RSV: respiratory syncytial virus
HCV: hepatitis C virus
SARS: severe acute respiratory syndrome
CRAAP: currency, relevance, authority, accuracy, and purpose
CASP: critical appraisal skills programme

Conflicts of Interest

The author(s) declare that they have no conflicts of interest.

Ethics Approval and/or Participant Consent

Since this study did not utilize human participants, consent was not needed. Ultimately, research ethics board (REB) approval was not required.

Authors' Contributions

AD: made contributions to the design of the study, collected and analysed data, drafted the manuscript, revised the manuscript critically, and gave final approval of the version to be published.
TG: made contributions to the design of the study, collected and analysed data, drafted the manuscript, revised the manuscript critically, and gave final approval of the version to be published.
RP: made contributions to the design of the study, collected and analysed data, drafted the manuscript, revised the manuscript critically, and gave final approval of the version to be published.

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