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DUAL AND MULTI-SPECIFIC ANTIBODIES AS IMMUNE CELL ENGAGERS REDEFINING CHECKPOINT BLOCKADE IN ONCOLOGY

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ABSTRACT

Immunotherapy using the immune system to destroy malignant cells has transformed the field of oncology, and immune checkpoint inhibitors (ICIs) are a breakthrough regarding clinical success. Nevertheless, the traditional monospecific antibodies are restricted by the heterogeneity of tumors, resistance to treatment, and immune-associated adverse effects. Next-generation therapeutics that overcome these barriers and target multiple antigens or immune checkpoints have been developed as dual and multi-specific antibodies. Over and above restoring the activity of T- cells, these engineered molecules expand cytotoxic responses, increase the recruitment of natural killer (NK) cells, and reduce tumor immune escape. Preclinical research as well as clinical trials reveal their effectiveness in hematologic malignancies and solid tumors and better response durations and manageable toxicities. They also have the potential to be combined with more sophisticated modalities like CAR-T therapy and combinatoric immunotherapies, which makes them a disruptive category in the realm of modern oncology. The following review discusses the design, action mechanisms, therapeutic purposes, and clinical developments of dual and multi-specific antibodies and how they could transform the concept of immune checkpoint blockade and create new horizons in cancer immunotherapy.

Keywords: Cancer immunotherapy, Immune checkpoint inhibitors (ICIs), Dual-specific antibodies, Multi-specific antibodies, T-cell engagers (BiTEs)

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1. INTRODUCTION

Cancer remains one of the main causes of morbidity and mortality in the world, and it becomes an ongoing problem, even in the face of the considerable development of surgery, chemotherapy, radiotherapy, and targeted therapies [1]. In the last ten years, immunotherapy developed as a revolutionary treatment in cancer therapy radically changing the paradigm of therapy by directly attacking cells with cancer to use the own immune system of the patient to recognize and eliminate malignant cells. Immune checkpoint blockade (ICB) is among the alternative immunotherapeutic approaches that have shown an exceptional clinical response, through the inhibitory pathways, including programmed cell death protein-1 (PD-1), programmed death-ligand 1 (PD-L1), and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), to stimulate the exhausted T cells to generate a strong anti-tumor response. Nevertheless, the traditional monospecific checkpoint inhibitors are restricted by the inconsistent response rates, acquired resistance, and immune-adverse events despite these successes because tumors often use multiple immune evasion mechanisms simultaneously, and single-agent therapies are inadequate to provide full anti-tumor immunity. To compensate these shortcomings, new generation therapeutic antibodies have been engineered, which are able to interact with multiple immune pathways simultaneously. Dual and multi-specific antibodies are developed to effect interaction with two or more antigens at a time, allowing specific immune cell interaction, higher cytotoxicity and overcoming tumor heterogeneity and immune escape [2]. These bispecific antibodies, trispecific antibodies, and bispecific T-cell engagers (BiTEs) have major benefits compared to conventional monospecific therapies: they have the potential to simultaneously block multiple inhibitory checkpoints, recruit and activate cytotoxic immune cells, and achieve better pharmacokinetic properties, potentially minimizing systemic toxicity and other adverse reactions and enabling more customized therapeutic treatment. Preclinical research and ongoing clinical trials have shown that they are effective in hematologic malignancies as well as in solid tumours with increased T-cell activation, improved tumour cell killing and potential therapeutic indices. Moreover, dual and multi-specific antibodies provide a platform within which multi-dimensional immune modulation can be achieved as a transition point between traditional checkpoint blockade and novel immunotherapeutic approaches, such as CAR-T therapy and combination therapy. Recent developments in synthetic biology and structure-guided antibody engineering have pushed

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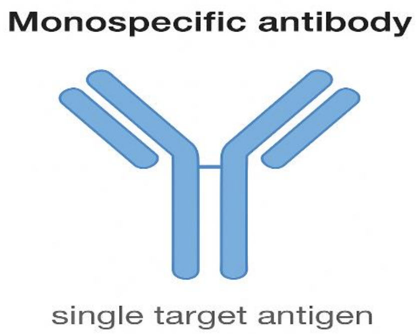
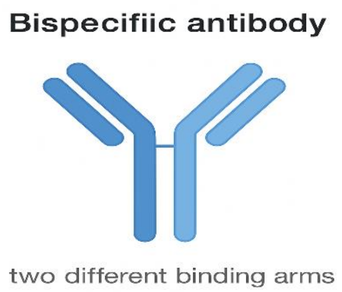
cancer immunotherapy beyond the current manuscript's traditional scope. Novel designs such as tetravalent antibodies, checkpoint-fusion proteins, and immune cell engagers integrating AI-guided epitope mapping now represent the frontier of research. These innovations are redefining immune checkpoint blockade, enabling more precise, potent, and safer cancer therapeutics.

As the future of immune therapy, due to their innovative architecture, mechanistic flexibility and expanded clinical interests, dual and multi-specific antibodies are rewriting the history of immune checkpoint blockade in cancer therapy, providing new opportunities to overcome resistance, improve therapeutic efficacy and define the future of cancer immunotherapy, which this review seeks to critically analyse by discussing their mechanisms of action, applications in cancer therapy, clinical studies, problems and outlook.





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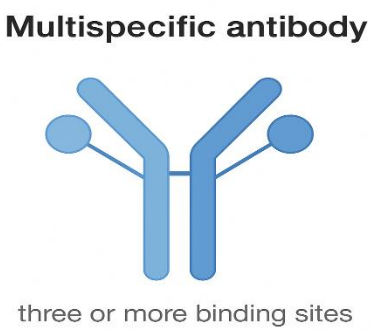

Figure 1: Evolution of Antibody Design in Cancer Immunotherapy (a) Monospecific antibody – single target antigen. (b) Bispecific antibody – two different binding arms. (c) Multi specific antibody – three or more binding sites connecting immune cells and tumor antigens.

Figure 1 illustrates the progressive evolution of antibody engineering in cancer immunotherapy, highlighting the increasing structural and functional complexity from monospecific to multi specific formats. The **monospecific antibody** (Figure 1a) represents the classical immunoglobulin structure, designed to recognize and bind a *single target antigen* on tumor cells. Although they can be useful in selectively binding to a single molecular epitope, a common problem with these antibodies is that they cannot overcome tumor heterogeneity or counter the alternative immune escape mechanisms. To address these shortcomings, the bispecific antibody (Figure 1b) includes two separate antigen-binding arms and thus allows the simultaneous recognition of two different targets (usually one on immune effector cells (e.g. CD3 on T cells) and the other on tumor-associated antigens). Such a dual interaction facilitates immune cell recruitment and tumor-specific cytotoxicity and greatly improves the therapeutic effectiveness and specificity. The multispecific antibody (Figure 1c) is the next-generation novelty, as it has three or more binding sites and is able to bind to immune cells and two or more tumor antigens or checkpoints simultaneously. This architecture can be used to obtain increased immune activation, better tumor detection, and less immune evasion. Collectively, the figure demonstrates how advances in antibody architecture—from monospecific to

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bispecific and multispecific—have redefined immune checkpoint blockade, enabling a more comprehensive, synergistic, and effective strategy for cancer immunotherapy.

Table 1: Dual and multi-specific antibodies key studies and findings

Author	**Year**	**Title**	**Research Study**	**Key Findings**
Trabolsi, A., Arumov, A., & Schatz, J. H. [3]	2019	Bispecific antibodies that activate the T cells in cancer treatment.	Oncol Therapy Review T-cell engaging bispecific antibodies (BiTEs).	BiTEs target T-cells to tumor cells, facilitating targeted lysis; spot mechanisms, clinical potential and limitations in solid and hematologic malignancies.
Müller, D., & Kontermann, R. E. [4]	2010	Bispecific cancer-immunotherapy antibodies: State of affairs.	The bispecific antibody formats and applications in cancer Review.	Overviews bispecific antibody engineering, design opportunities and possibilities, and challenges in stability and specificity, highlighting immunotherapy.
Dahlén, E., Veitonmäki, N., & Norlén, P. [5]	2018	Bispecific cancer immunotherapy antibodies.	In-depth discussion about bispecific antibodies.	Reports clinical progress, mechanism of action, and clinical use; presents advantages in immune cell-engaging and tumor-targeting.
Rader, C. [6]	2020	Cancer immunotherapy using bispecific antibodies.	Bispecific antibody development and uses.	Emphasizes novel formats, mechanisms of action and preclinical/clinical

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				research; highlights superior anti-tumor activity over monospecific antibodies.
Fenis, A., Demaria, O., Gauthier, L., Vivier, E., & Narni-Mancinelli, E. [7]	2024	New immune cell engagers for cancer immunotherapy	Review on novel immune cell-engaging therapies	Discusses the concept of innovative bispecific and multi-specific T-cell and NK cell targets; highlights next-generation designs and therapeutic opportunities in the cancer field.
Fucà, G., Spagnoletti, A., Ambrosini, M., De Braud, F., & Di Nicola, M. [8]	2021	Immune cell engagers in solid tumors: promises and challenges of the next generation immunotherapy	Review of immune cell-engaging antibodies in solid tumors	Reports on issues related to solid tumor targeting, microenvironment obstacles in tumors and approaches to effective multi-specific antibodies.

1.1.Background on Immunotherapy in Cancer

Immunotherapy has transformed the treatment of cancer by no longer attacking the tumor cells directly but rather using the body immune system to detect and destroy the malignant cells as a paradigm shift in the field of oncology. The role of the immune system in cancer surveillance had been historically known by observation, as immunocompromised persons were found to have increased cancer rates, and that some tumors were capable of spontaneously regressing, demonstrating the possibility of immune mediated tumor control. Early approaches to cancer immunotherapy were cytokine therapy (interleukin-2, interferon-alpha) to achieve a general stimulation of immune responses, and cancer vaccines to produce tumor-specific immunity. Nonetheless, the methodologies were usually constrained by the low efficacy and systemic toxicity [9]. The identification of immune checkpoints - regulatory mechanisms such as PD-1/PD-L1 and CTLA-4 that control self-tolerance, but are used by tumors to elude immune

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surveillance, revolutionized immunotherapy by offering the specific molecular targets with which to release anti-tumor immunity. Clinical trials of immune checkpoint inhibitors (ICIs) of these pathways produced astounding clinical response in multiple malignancies, such as melanoma, lung cancer, and renal cell carcinoma, confirming the idea that immune response regulation might result in long-term tumor control. However, with these achievements, several patients exhibit low response or develop resistance, and thus more advanced methods are required to activate the immune systems and address the system weaknesses inherent in standard immunotherapies by integrating the advantages of dual and multi-specific antibodies that are capable of targeting multiple immune system work simultaneously, improving immune activation, tumor targeting, and overcoming individual immunotherapy weaknesses.

1.2.Limitations of Conventional Monospecific Antibodies

Although traditional monospecific antibodies are efficient at attacking single tumor-associated antigens or immune checkpoints, they have a number of major limitations which limit their therapeutic advantages. The heterogeneity of tumors usually undermines their action because not every cancer cell expresses the targeted antigen homogeneously as a result some tumor populations can avoid treatment. Also, the resistance to tumors may occur due to the loss of antigens or the engagement of alternative immune-suppressive mechanism, which leads to decreased long-term efficacy [10]. The responses of patients are also extremely diverse because of the variations in the microenvironment of tumors and genetic variations whereas immune-related adverse events may occur as a result of off-target immune activation. Moreover, monospecific antibodies activate only one immune pathway, which does not allow them to fully activate immune cells and to overcome multifaceted tumor immune evasion mechanisms, which suggests the necessity of more versatile therapeutic options.

- **Limited Efficacy Against Tumor Heterogeneity**: Monospecific antibodies react with one antigen and it might not be evenly expressed in all tumor cells. The heterogeneity of the tumors usually creates subpopulations that are not acknowledged, making it impossible to fully eliminate the tumor and leading to possible recurrence of the disease.
- **Acquired Resistance and Immune Escape**: Mono specific therapy may be resisted by tumors via loss of the antigen, resistance in the pathway being targeted or via overexpression of other immune checkpoints. This evasion of adaptive immune

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response makes it less effective over time and usually requires combination or sequential therapy.

- **Variable Response Rates Among Patients**: Mono species antibodies do not work in all patients because some of them have variations in tumor microenvironment, immune cells infiltration, or genetic and epigenetic differences. Consequently, this leads to inpredictable clinical outcomes and is difficult to stratify patients.
- **Immune-Related Adverse Events (irAEs)**: Mono specific antibodies have the potential to induce off-target immune responses by modulating immune checkpoints and result in autoimmune-like toxicities to various organs, including the skin, liver, lungs, and the gastrointestinal tract. These unwanted occurrences may decrease dosing and patient tolerability.
- **Insufficient Engagement of Multiple Immune Pathways**: The monospecific antibodies have only one mechanism, usually by blocking one checkpoint or pathway. This dispensation denies full activation of the immune system whereby tumors can use the redundant inhibitory pathways to suppress immune responses and minimize the overall therapeutic effect.

1.3.Failure of Monospecific Antibodies and the Rise of Mult specific Designs

Monospecific antibodies used in the past were hindered by failure to deal with tumor heterogeneity and immune escape. Tumors more often than not express different or changeable antigens and one-target method of treatment usually fails to eliminate resistant clones thereby causing relapse and a low effect in the long term. Also, the presence of compensatory immune checkpoints (e.g., TIM-3, LAG-3) in case of one pathway (e.g. PD-1) being blocked by tumors limits response persistence and leads to immune evasion.

In order to address these deficiencies, researchers started developing multi Sspecific antibodies that may interact with more than one antigen or checkpoint at a time. This technology essentially rewrote the natural law of antibody monospecificity, which was obtained by sophisticated molecular engineering methods, including knobs-into-holes, CrossMab and dual-variable domain (DVD-Ig) technology. These constructs allow an antibody molecule to express multiple domains of antigen binding, and each is bound to a different epitope or receptor. This enables the simultaneous blockade of several inhibitory pathways or the simultaneous recruitment of various effector cells.

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Table 2: Multi specific and Polyclonal Antibodies

Parameter	Multi specific Antibodies	Polyclonal Antibodies
Origin	Genetically engineered in laboratories using recombinant DNA and protein engineering technologies.	Naturally produced by different B-cell clones in response to an antigen.
Composition	Single, well-defined molecular entity with multiple engineered binding domains.	Heterogeneous mixture of immunoglobulins recognizing multiple epitopes.
Target Recognition	Binds specifically to two or more predetermined antigens or epitopes.	Recognizes a broad range of epitopes on the same or different antigens.
Reproducibility	High reproducibility due to controlled molecular design and production.	Low reproducibility as production varies among animals and batches.
Stoichiometry and Structure	Precisely designed stoichiometry and binding configuration.	Undefined mixture with variable antibody proportions.
Pharmacokinetics	Predictable and consistent pharmacokinetic behavior.	Variable pharmacokinetics due to antibody diversity.
Clinical Applicability	Suitable for targeted, scalable, and reproducible clinical use.	Limited clinical use due to batch variability and low specificity.

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Reference	[Chen et al., 2021]	[Chen et al., 2021]

1.4.Emergence of Dual and Multi-Specific Antibodies

Development of dual and multi-specific antibodies is a major breakthrough in cancer immunotherapy, the aim of which is to provide a remedy to the shortcomings of the traditional monospecific antibodies by combining various immune targets together at once. In contrast to monospecific antibodies, which are engineered to bind to one antigen or checkpoint, dual and multi-specific antibodies have been engineered to bind to two or more antigens or immune checkpoints on tumor cells or immune effector cells [11]. This versatile design enables the fine-tuning of the immune system, improving tumor recognition and cytotoxicity and reducing immune survival. The activation of several inhibitory checkpoints (PD-1 and CTLA-4) simultaneously can be considered one of the main mechanisms that inhibit the activation of alternative immune-suppressive responses in tumors, which can then enhance T-cell activation and maintain anti-tumor responses. Besides disrupting checkpoint signaling, such antibodies may physically connect immune effector cells, e.g. T cells or natural killer (NK) cells, to tumor cells via bispecific and trispecific interactions, allowing specific cytotoxicity even in tumors with mixed antigen expression. These antibodies can also be customized to particular clinical requirements, such as the selective targeting of tumor-associated antigens, dual immune cell recruitment, or combination of checkpoint inhibition and direct tumor killing based on the modular architecture of these antibodies. In general, the principle of targeting multiple immune targets simultaneously offers a wide-ranging and powerful solution to tumor resistance, improved treatment efficacy, and increased cancer susceptible to immunotherapy, making dual and multi-specific antibodies a breakthrough in the future of the modern treatment of cancer.

2. METHODS / LITERATURE SEARCH

Literature search was done to find pertinent literature on any studies dealing with dual and multi-specific antibodies as immune cell engagers in oncology. To cover the preclinical and clinical research in detail, several credible databases were searched, such as PubMed, Scopus, Web of Science, and ClinicalTrials.gov. A set of keywords and phrases was searched (e.g., dual-specific antibodies, bispecific T-cell engagers, multi-specific antibodies, immune checkpoint blockade, and cancer immunotherapy) and Boolean operators were used to filter and maximize

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the search results. The articles were included in case they were preclinical study, clinical trials, or peer-reviewed reviews published less than 10 years with the specific focus on antibody-based immune cell engagement and checkpoint blockade in cancer treatment [12]. They have filtered out the studies that were not related to oncology and the ones that had not been published in peer-reviewed journals like an editorial or a conference abstract. This methodological strategy made the review capture all high-quality, current and applicable evidence in the area of cancer immunotherapy.

2.1.Databases Searched

In order to collect the appropriate literature on dual and multi-specific antibodies as immune cell engenders in oncology, the extensive search of various reputable databases of biomedical and clinical research was performed. These included:

- PubMed: This is the primary source of biomedical literature which gives access to research articles, clinical studies, and reviews related to the research topic of cancer immunotherapy.
- Scopus: A massive abstract and citation database of peer-reviewed scholarly literature in science, technology, and medicine which assists in uncovering extensive research pertinence.
- Web of Science: It will be used to find high-impact studies and cross-disciplinary references and allow considering the immunotherapies based on antibodies in a much broader context.
- Clinical Trials.gov: A critical site that can be used to locate current and completed clinical trials in the area of bispecific and multi-specific antibodies in the treatment of cancer.

This multi-database strategy provided a comprehensive and extensive coverage of pre-clinical research studies and clinical research studies.

2.2.Search Strategy and Keywords

A literature search was conducted through an integration of particular keywords and phrases that were used to represent the essence of the review. The search was maximized by using the following terms, whether individually or in combination: “dual-specific antibodies”

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- “bispecific T-cell engagers”
- “multi-specific antibodies”
- “immune checkpoint blockade”
- “cancer immunotherapy”

Boolean operators (AND, OR) and MeSH terms were applied where appropriate to refine searches and retrieve the most relevant literature. This strategy allowed for identification of studies focusing on both molecular mechanisms and clinical applications of immune cell-engaging antibodies.

2.3.Inclusion Criteria

In order to achieve the quality and relevance of the literature involved in this review, the following inclusion criteria were used:

- Study Types: Preclinical studies, clinical trials, and peer-reviewed review articles.
- Time Frame: Articles published within the last 10 years (2015–2025) to capture the most current advancements in the field.
- Relevance: The focus of research to be taken is specifically on dual or multiple specific antibodies and their application in immune cell interactions and checkpoint inhibition in cancer biology.

These were the criteria that aimed to narrow down the review to up-to-date and quality evidence that was applicable in cancer immunotherapy.

2.4.Exclusion Criteria

Articles were not included in the review when they outlined any of the following conditions:

- Studies outside the oncology field, such as studies on infectious diseases or autoimmune disorders, were eliminated to keep oncology immunotherapy in focus.
- To guarantee reliability and scientific rigor, non-peer-reviewed sources (conference abstracts, editorials, commentaries, and non-indexed articles) were not included.

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By applying these exclusion criteria, the review maintained a high standard of evidence and relevance, ensuring that only significant studies were considered.

3. MECHANISM

Dual and multi-specific antibodies operate by simultaneously combining their immune effector cell-based functions with multiple immunologic checkpoints, which increase anti-tumor responses. One of the main mechanisms is the activation of T-cells through bispecific T-cell engagers (BiTEs), which connect situations with cytotoxic T lymphocytes to tumor-associated antigens, and induce the targeted lysis of tumor cells. Simultaneously, approaches to the natural killer (NK) cell interaction recruit the natural cytotoxic capacity of NK cells, which boosts immune-mediated tumor clearance. Moreover, the concept of dual or multispecific checkpoint inhibition can be used to block the inhibitory pathway(s), i.e., PD-1/CTLA-4 or PD-L1/TIGIT simultaneously (bypassing adaptive resistance mechanisms that tend to limit monospecific therapies) [13]. These agents are characterized by increased cytotoxicity, decreased potential of tumors to immune escape, and may be used in combination with other immunotherapies, which give them a multifaceted, potent method of cancer therapy compared to traditional monospecific antibodies.

3.1.Immune Cell Engaging Mechanism

Dual and multi-specific antibodies are specifically created to make use of the innate immune system of the body so as to select and kill tumor cells selectively, a major step up to the traditional monospecific therapy. One of them is T-cell activation using bispecific T-cell engagers (BiTEs), a special type of molecules that have two binding domains: one binds the CD3 receptor on cytotoxic T lymphocytes, and the other one binds a certain antigen on the surface of tumor cells. T-cell activation, proliferation and the release of cytotoxic molecules that cause specific lysis of tumor cells are triggered by this physical bridging of T-cells and cancer cells. It is interesting to note that this process is possible without classical antigen presentation, and this enables efficient immune systems even in the case of tumors that avoid natural immune recognition. Besides T-cells, dual and multi-specific antibodies can also activate natural killer (NK) cells, which are important components of the innate immune system and they can kill tumor cells without being sensitized. These antibodies can activate NK cell-activating receptors, including CD16, and tumor-associated antigens, which causes NK cell

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cytotoxic activation, thereby increasing the overall response of the anti-tumor immune response [14]. Together, this twofold approach of engagement enhances immune-mediated tumor clearance and offers a potent and well-rounded approach, which reinforces adaptive and innate immune responses to cancer.

3.2.Dual/Multi specific Checkpoint Inhibition

To avoid immune-surveillance, cancer cells have evolved advanced strategies that mainly use inhibitory checkpoint receptors like PD-1/PD-L1 and CTLA-4, which restrain the activation of T-cells and the anti-tumor response. Dual or multispecific antibodies provide an effective approach to defeat this immune escape by blocking more than one checkpoint molecule at a time, e.g., PD-1/CTLA-4 or PD-L1/TIGIT. These antibodies can activate T-cell proliferation and revitalize T-cell cytotoxic action on tumor cells by the simultaneous inhibition of these inhibitory signals, thereby restoring T-cell functionality. The multi-target nature of these is especially beneficial, since tumors tend to employ a redundant or compensatory checkpoint pathway to avoid the effects of single-agent therapy; as such, concurrent inhibition would minimize the chance of adaptive resistance. Moreover, dual or multispecific blockade of checkpoints only enhances the direct anti-tumor immune response but may also alter the immunological microenvironment in the tumor microenvironment to be more conducive to the other immune-mediated effectors and can potentially be used to synergize with other immunotherapies. Together, this approach is a big improvement to the past monospecific checkpoint inhibitors as it offers a more holistic, robust, and sustained stimulation of the immune system to fight cancer.

3.3.Advantages over Monospecific Antibodies

Dual and multi-specific antibodies have a number of therapeutic benefits compared to the monospecific antibodies:

- Improved cytotoxicity: These agents create a stronger immune-mediated tumor killing by more effectively stimulating immune effector cells and inhibiting tumor-surveillance checkpoints.
- Immuno-resistance of the tumor: The ability to attack many different pathways at once diminishes the chances of tumor cell immune escape as is the case with single-target antibodies.

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- Synergistic effect with other immunotherapies: These antibodies may be used with other immunotherapeutic approaches, like cytokine therapies or vaccines, to generate a compounded anti-tumor effect, which enhances the overall effectiveness of treatment.

4. CLASSIFICATION OF DUAL AND MULTI-SPECIFIC ANTIBODIES

The different categories of dual and multi-specific antibodies are defined in terms of structural design, specificity of their target, and the mechanism of action as a representation of the various approaches to improving anti-tumor immunity. The most mature of these classes are bispecific antibodies (BiTE) like Blinatumomab that combines cytotoxic T-cells and tumor-associated antigens to target tumor cells. These molecules are designed to achieve proximity of immune effector cells with cancer cells to induce powerful immune responses without conventional antigen presentation [15]. Outside of bispecific format, tri-species and multi-species antibodies are being developed as the next generation of therapeutic platforms, and they have the capability to respond to multiple immune pathways at once. Such complex molecules can either carry multiple antigen-binding sites to engage different immune cells, alter tumor microenvironment effects, or engage multiple tumor antigens, to increase efficacy and decrease immune escape. The design of such multi-specific formats is usually based on the optimization of linker sequences, binding affinities, and space orientation of the binding domains to result in the maximum activation of immune response and stability and safety. Also, dual antibodies specific to checkpoints are made to inhibit other checkpoint molecules, e.g., both PD-1/PD-L1 to inhibit an additional checkpoint, overcoming redundancy in immune evasion. Together this classification highlights the versatility of dual and multi-specific antibodies, which are not only customized to interact with immune effector cells, but to influence inhibitory pathways, providing a multi-pronged strategy to cancer immunotherapy.

4.1.Bispecific Antibodies (BiTEs)

Bispecific antibodies or BiTEs are synthetic immunotherapeutic compounds that identify two different antigens, usually on a tumor cell and the other on an immune effector cell, in most cases a T lymphocyte. One of the notable representatives of this category is Blinatumomab which is connected with CD3 on cytotoxic T-cells and CD19 on the B-cell malignancies [16]. Physically connecting T-cells and tumor cells, BiTEs allow the development of an immunological synapse that provokes the activation of T-cells, their growth, and discharge of

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cytotoxic factors like perforin and granzymes that cause tumor cells to undergo apoptosis. This process enables T-cells to switch the cytotoxic effect of BiTEs against tumor cells, regardless of major histocompatibility complex (MHC) presentation, and therefore an effective anti-tumor response can be directed to tumor cells when the standard antigen presentation process is defective. BiTE design is designed to target and bind to the targets with high specificity and binding affinity to improve targeting of malignant cells with the lowest off-target effects. They have a relatively smaller size than the conventional antibodies, which enables them to penetrate tissues better, which is part of why they are effective clinically. On the whole, BiTEs are a novel form of immunotherapy, which means a direct connection between immune effector cells and cancer cells to obtain effective, specific destruction of tumor cells.

4.2. Tri-specific and Multi-specific Antibodies

Tri- and multi-specific antibodies are the future of engineered immunotherapeutics, which are engineered to interact with multiple targets concurrently to increase anti-tumor efficacy. These novel formats further the principle of bispecific antibodies by adding three or more antigen-binding domains, which enables them to bind multiple tumor-associated antigens, immune effector cells or immunomodulatory receptors at once. In this way, they are able to attract heterogeneous immune cells that include T-cells, natural killer (NK) cells, and macrophages, in addition to regulating the tumor microenvironment to overcome immune suppression. The action of the mechanisms consists of coordinated activation of immune: one binding domain can bring cytotoxic T-cells closer to tumor cells, another can block inhibitory checkpoint molecules, and a third one can target a secondary tumor antigen or co-stimulatory receptor, and its interactions generate synergistic anti-tumor effects. The design approach of these antibodies aims at maximizing the spatial orientation and binding affinity of each domain, structural stability, minimizing immunogenicity as well as maximizing efficacy [17]. Also, these multi-specific molecules can be engineered to be flexible and have precise control over simultaneous engagement of multiple targets by careful design of the linkers and scaffold structures. In general, tri-specific and multi-specific antibodies represent a flexible and very powerful strategy in immunotherapy which overcomes the shortcomings of monospecific and bispecific antibodies by integrating a variety of therapeutic functions into a single molecular unit.

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4.3.Checkpoint-Specific Dual Antibodies

Checkpoint-specific dual antibodies Checkpoint-specific dual antibodies are a special type of multi-targeted immunotherapeutics that inhibits multiple tumor-induced immunosuppressive immune checkpoint pathways more effectively than single-agent therapies. One way this can be done is to hit PD-1/PD-L1 with another inhibitory checkpoint molecule like CTLA-4, TIGIT or LAG-3. The simultaneous inhibition of these signaling pathways by dual antibodies renews T-cells activating, proliferating and cytotoxic capacities, which are frequently inhibited in the tumor microenvironment. This dual blockade does not only improve anti-tumor immunity, but it also overcomes the redundancy and compensatory mechanisms, which tumors use to overcome single checkpoint blockade, and thus increases the chances of adaptive resistance. These antibodies are designed, mechanistically, to be highly specific and affine to both but then be able to effectively engage simultaneously without significant off-target effects. Also, these antibodies can streamline the treatment regimens due to their ability to incorporate several checkpoint inhibitory activities in a single molecule, may enhance the pharmacokinetics, and have synergistic therapeutic effects when used with other immunotherapies. In general, the concept of checkpoint-specific dual antibodies is a perspective of stronger and longer-lasting immune activation in cancer immunotherapy.

5. PRECLINICAL AND CLINICAL APPLICATIONS

In both preclinical and clinical research, dual and multi-specific antibodies have already shown considerable promise, as they can be the next-generation of cancer immunotherapies. These antibodies are tested in preclinical assays in vitro by cytotoxicity tests to determine how they induce the lysis of tumor cells by either T-cell or NK-cell interaction, and in animal models to test their tumor growth inhibitory effects, immune activating effects, and their overall efficacy in vivo [18]. These basic researches furnish important details on how they work, which are their best doses, and safety data. As part of the shift to clinical trials, a number of dual and multi-specific antibodies are being evaluated in a number of trials (Phase I to Phase III) to evaluate their safety, tolerability, pharmacokinetics and anti-tumor efficacy in patients. Initial clinical findings have demonstrated positive responses in terms of objective tumor response and manageable toxicity rates, especially in blood cell-based malignancies, i.e. B-cell leukemias and lymphomas. Besides, they are also being tested in solid tumors, where their capacity to activate various immune mechanisms and evade tumor immune defense provides a

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competitive edge over traditional therapies. All the above preclinical and clinical data highlight the potential of dual and multi-specific antibodies to enable the development of drugs with broad therapeutic applicability in a wide range of cancer indications and warrant their further development and implementation in contemporary oncology treatment regimens.

5.1.Preclinical Studies

Preclinical research is the most important first step in designing dual and multi-specific antibodies, and its basis gives the needed basis to enable safe and efficient transfer of the results to clinical studies in humans. An in vitro cytotoxicity testing is a key ingredient at this phase, wherein the researcher can determine the efficiency with which these antibodies can be utilized in inducing a targeted cell death in tumor cells under controlled laboratory settings. These tests are frequently aimed at indirectly measuring the cytotoxic ability of T-cells or natural killer (NK) cells redirected by the antibodies on cancer cells to provide detailed information on the strength, specificity and how this antibody interacts with immune effector cells. In addition to these in vitro experiments, animal models are used to test the antibodies in a complex living system, test their efficacy in vivo, pharmacokinetics, biodistribution and safety concerns [19]. Models that have often been used are xenograft models where human tumor cells are transplanted into immunocompromised mice and syngeneic tumor models where immune interactions in immunocompromised hosts can be studied. Such in vivo experiments allow researchers to see tumor growth suppression, invasion of immune cells at tumor sites, and possible off-target or side effects and provide a proof-of-concept to use in therapy. In addition, preclinical analyses play a vital role in the optimization of the dosing schedules, optimization of the molecular design, and prediction of the possible toxicities, all of which are used to design future clinical trials. Preclinical studies represent a prerequisite step in the development pipeline by offering all-inclusive mechanistic and safety information, thus dual and multi-specific antibodies can have a strong scientific and translational foundation before exposure to humans.

5.2.Clinical Trials

Successful completion of preclinical studies is followed by the clinical trials which are designed, multi-phase studies, intended to determine the safety, tolerability, pharmacokinetics, and therapeutic efficacy of the preclinical studies on human patients. Clinical trials are

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commonly classified under Phase I, Phase II and Phase III clinical trials with various objectives and designs [20].

- Phase I trials are the initial phase in human testing and they mainly aim at determining a safe range of dosage. These are small group studies designed to determine dose-limiting toxicities (DLTs), to observe adverse effects and to note the earliest signs of clinical efficacy. The results of Phase I trials are important to determine the maximum tolerated dose (MTD) and guide the design of further trials.
- Phase II trials increase the population of the study, to test the therapeutic effects more stringently. Such trials determine the efficacy of the antibody in greater numbers of patients with certain types of cancer, frequently both hematologic malignancies and solid tumors. The outcome measures usually contain objective response rate (ORR), progression free survival (PFS) and overall survival (OS).
- Phase III trials: The new therapy is compared with current standard of care therapies using a large and diverse population of patients. Such trials are conclusive in terms of clinical benefit, long-term safety and tolerability and in many cases, form the foundation of regulatory approval.

Today, there are several clinical trials of dual and multi-specific antibodies, each of which have different indications. Initial optimization has demonstrated encouraging anti-tumor activity, and suggestive objective response and prolonged remissions, with acceptable toxicity levels compared to standard immunotherapies.

Table 3 : Clinical Trial Phases and Objectives for Dual/Multi-Specific Antibodies

Phase	Primary Objective	Patient Cohort Size	Key Endpoints	Examples of Data Collected
Phase I	Safety, tolerability, dose-limiting toxicity	20–80	Maximum tolerated dose (MTD), adverse events	Pharmacokinetics, initial anti-tumor activity
Phase II	Efficacy, continued safety	100–200	Objective response rate (ORR),	Biomarker responses, immune activation

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			progression-free survival (PFS)	
Phase III	Comparison to standard therapy, long-term safety	300–1000+	Overall survival (OS), PFS, quality of life	Long-term adverse events, durable responses

Table 4: Examples of Dual and Multi-Specific Antibodies in Clinical Trials

Antibody	**Target(s)**	**Indication**	**Phase**	**Observed Outcome**
Blinatumomab	CD3 × CD19	B-cell acute lymphoblastic leukemia	III	High remission rate, manageable toxicity
XmAb14045	CD3 × IL-3Rα	Acute myeloid leukemia	II	Promising cytotoxicity and tumor regression
MGD013	PD-1 × LAG-3	Solid tumors	I/II	Restoration of T-cell function, partial responses
AFM13	CD16 × CD30	Hodgkin lymphoma	II	Enhanced NK-cell activity, durable responses

All in all, clinical trials with dual and multi-specific antibodies are providing some promising results in terms of efficacy and safety in a range of hematologic and solid malignancies. Their capacity to activate more than one immune pathway or checkpoint inhibition simultaneously makes them an innovation of the next generation of therapeutic approach in cancer treatment.

Current Potentials and Challenges in Multispecific Antibody Research

Dual and multispecific antibodies hold immense promise in revolutionizing cancer immunotherapy. Their ability to activate multiple immune pathways and target heterogeneous tumors positions them as next-generation therapeutics capable of producing durable responses and reduced immune escape. There have been impressive clinical results in hematologic malignancies (e.g., Blinatumomab) and promising early results in solid tumors.

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Nevertheless, there are still a number of challenges. One of the biggest concerns is manufacturing complexity because the complex molecular design needs to be precisely folded, stabilized and expressed. Safety concerns include off-target toxicity and cytokine release syndrome (CRS), which require a strategy of affinity tuning and dosing. Additionally, immunosuppressive tumor microenvironment remains a limiting factor to efficacy in solid tumors, suggesting the utility of optimized delivery systems and combination regimens. Other barriers that have to be overcome to expand clinical translation include cost of production, manufacturability on large-scale basis, and regulatory pathways. Next-generation studies will combine multi specific antibodies and CAR-T therapy oncolytic viruses and nanoparticle delivery systems, and hence, the prospects of personalized and combinatoric immunotherapy are paved.

6. CONCLUSION

Dual and multi-specific antibodies are a paradigm of the immunotherapy of cancer since they address the shortcomings of monospecific checkpoint inhibitors. They have the ability to respond to multiple immune pathways simultaneously to provide increased recognition of tumor, enhanced cytotoxic activity, and reduced probability of immune evasion. Clinical trials are already demonstrating promising outcomes in hematological and solid tumors, so the agents can be used as significant components of the standard of care in the future. Nevertheless, structural stability, lowered immunogenicity and scale-up are problematic. As their design innovates and they are used to increase their clinical applications, dual and multi-specific antibodies will change the future of oncology and provide an effective, versatile, and longlasting approach to cancer management and patient outcomes.

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