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Will the use of nanobodies in camel bodily fluids to treat various cancers Be successful?

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Abstract: Cancer is a major health issue and one of the world's top causes of mortality. Innovative methods are needed to aid in the detection and treatment of different types of cancer. The use of nanobodies generated from camels as cancer therapy techniques has gained popularity recently. Nanotechnology, which uses nanobodies, is a novel and fascinating subject that holds promise for scientists hoping to progress a number of scientific domains, including medicine and oncology. Nano bodies are small biologics that have a large surface area that permits deep tissue penetration and the spread of cancer cells. They also have exceptional stability at high pH and temperature. The current research highlights the Single-domain antibodies, such as the camelid variable region of the heavy chain and the antigen-binding variable domains of the shark immunoglobulin new antigen receptor are the smallest antigen recognition domains (approximately 15 kDa) and differ from conventional antibodies in certain ways. The majority of antibodies, which are molecules that patrol our tissues and blood for invaders, are quite heavy for proteins. However, sharks, camels, and their close cousins produce smaller, simpler antibodies. Since their discovery in the late 1980s, scientists have discovered that these antibodies are extremely potent; they have the ability to attach to molecules' hidden components and pierce tissues more deeply, which increases their potential as treatments for many cancer kinds.

Keywords: pH and temperature, oncology, nanobodies, and nanoossible use of nanobodies that are naturally secreted in the body fluids of camels, Single-domain.

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INTRODUCTION

An overview of cancer and nanobodies Despite the quick advancements in molecular biology, cancer remains one of the world's top causes of death [1]. Over 14.1 million cancer cases and over 8.2 million cancer-related deaths were reported in 2012, according to data from the International Agency for Research on Cancer (IARC)'s GLOBOCAN, an online database that provides global cancer statistics and estimates of incidence and mortality in 185 countries for 36 types of cancer. By 2030, it is estimated that there would be over 13 million cancer-related deaths and 21.7 million cancer cases [1]. Early detection and timely execution of therapies are key to successfully halting the course of cancer in affected people.

An overview of cancer and nanobodies in Introduction

Cancer is one of the world's top causes of mortality, despite the tremendous advancements in molecular biology [1]. The International Agency for Research on Cancer (IARC) reports that over 14.1 million cancer cases and over 8.2 million cancer-related deaths occurred in 2012, according to data generated by the GLOBOCAN, an online database that provides global cancer statistics and estimates of incidence and mortality in 185 countries for 36 types of cancer. Over 13 million cancer-related deaths and 21.7 million cancer diagnoses are expected to occur by 2030 [1]. Early detection and timely application of treatments are key to successful interventions of cancer progression in impacted patients.

Distinguished features of the nanobodies and nanoparticles

Nanoparticles are small molecules with a diameter of about 200 nm. A range of nanoparticle-based delivery modalities, such as magnetic, polymeric, and inorganic nanoparticles, have been developed as a result of numerous pharmaceutical companies' aspirations to develop effective drug delivery techniques [5]. To increase the drug-conjugated nanoparticles'

permeability, transport, and penetration into the target tissues, the targeting moieties have been designed to attach to the drug cargo. These targeted compounds that shield the nanoparticles include poly-ethylene glycol (PEG) molecules and nanobodies [6]. The aggregation of the drug-carrying nanoparticles into the sick tissues was improved by decorating them with nanobodies [7]. Nanobodies can be bioengineered by monomerizing the dimeric variable domains of conventional human or mouse antibodies.

As an alternative, it is possible to extract and separate nanobodies from the blood of inoculated camels and identify them as single variable domain on a heavy chain (VHH) antibodies or nanobodies [6, 8]. Camel blood-derived VHH, or heavy chain antibodies (HCAbs), are single domains with a single amino acid chain and are less lipophilic. These features provide VHH some benefits over traditional antibodies, which are typically 10 times bigger (around 150 kDa) and comprise two amino acid chains. Small size (15–74 kDa) and large surface area, high stability and solubility, high binding affinity and detection of various epitopes, rapid tissue internalization, ease of production and manipulation, and low immunogenic reactions are some of the distinctive structural and functional characteristics of nanobodies [21]. As a result, equipment, transportation and automobiles, and medicine and drug development [22]. engineering and construction, common consumer goods, microscopes and scientific Numerous studies have confirmed the potential of bio-engineered nanobodies as innovative therapeutic agents for a wide range of illnesses. For example, it has been demonstrated that hemagglutinin influenza A H5N1-specific nanobodies inhibit virus multiplication in infected mice, lowering morbidity and mortality [23]. In vitro, nanobodies mitigate cytopathic effects in fibroblasts by targeting the binding region of virulence factors, such as Clostridium difficile's toxin A and toxin B [24]. Nanobodies have also been shown to be an effective target for gastrointestinal tract diseases, such as inflammatory bowel disease and colon cancer [16].

Antithrombotic drug candidate (ALX-0081), regarded as an antithrombotic, is another outstanding treatment based on nanobodies that showed total inhibition of platelet adhesion. And nanobody-encapsulated cancer treatments based on viruses. ALX-0081 was effectively employed as a Von Will brand factor target in a clinical trial to lower the risk of thrombosis in patients suffering from acute coronary syndrome [4]. Because of their capacity to bind tumor antigens, such as Human Epidermal Growth Factor Receptor 2 (HER2), nanobodies have also been employed in photo thermal therapy. For example, cleft-gold nanoparticles absorb light energy and produce heat that kills cancer cells. Therefore, after being exposed to a laser beam in an experimental setting, cancerous cells can be photo thermally killed [25].

Furthermore, bundling the viral vectors with nanobodies will guarantee tumor-specific targeting because viral-based cancer therapies can be integrated with most cells while only proliferating within cancer cells. This would be a useful tool for successfully addressing metastatic cancer [26].

Abbreviations

A549: Lung cancer cell line
BGI: Beijing genomics institute
C. bactrianus: Camelus bactrianus
C. dromedaries: Camelus dromedarius
CM: Camel milk
DDS: Drug delivery systems
HCV: Hepatitis C virus
IARC: International agency for research on cancer
KACST: King Abdul-Aziz city for science and technology
N/A: Not applicable
NCGT: National center for genomic technology
PMF: Prophet medicine fraction
PRP: Peptidoglycan recognition protein
SA: Saudi Arabia
TNF: Tumor necrosis factor-A
VHH: Variable domain of HC antibodies
le beau: Camel electronic marketing
ALX-0081: Antithrombotic drug candidate
(HCT-116): human colon cancer cell line
(IL-4): interleukin
PMF: Prophet Medicine Fraction

Nanobodies (VHH) application

As seen in figure 1, Nano antibodies are widely used in the fields of medicine and drug development, engineering and construction, microscopy and scientific applications, transportation and automobiles, common consumer goods, and the environment and agriculture.

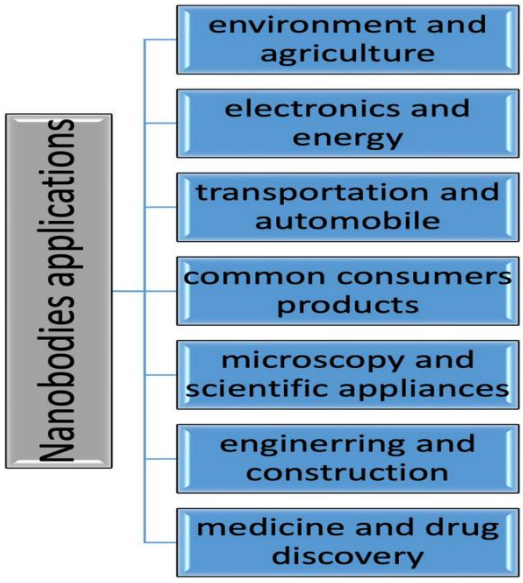
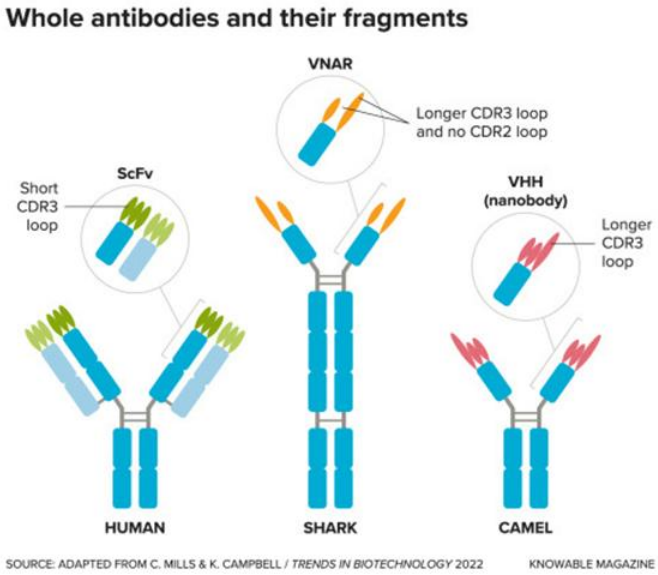


Figure 1: Nanobodies Application



https://www.discovermagazine.com/the-sciences/small-wonders-the-antibodies-from-camels-and-sharks-that-could-change

Figure 2: Human, shark, and camel whole antibodies and their fragments

Full-size antibodies, like those of humans (left), usually contain both light and heavy protein chains (dark and light blue, respectively), according to Knowable Magazine. Sharks, camels, and their cousins produce antibodies with only heavy chains (middle and right) in addition to these common antibodies. The variable domains—fragments at the antibody tips, represented by circles—stick to any portion of toxins or pathogens that the body recognizes as alien. The Complementarity determining regions CDR3 loop is an extra-long fingerlike extension found on the variable domains of sharks (VNARs, middle) and camels (VHHs, or nanobodies, right). This extension can reach into crevices and nooks that a conventional antibody fragment ScFv (left) cannot reach.

According to LeBeau, sharks employ immunoglobulin novel antigen receptors (IgNARs), which are single-chain antibodies that are even smaller and more stable than those found in camelids. Common to sharks, rays, and other cartilaginous fish, this system developed entirely on its own, indicating that it offers some benefit. Perhaps in both situations, as suggested by Brooks' research, the resilience of the smaller molecules provides some defense against hostile conditions, such as high body temperatures for camelids or high urea concentrations for sharks and their relatives, who keep urea in their blood to counteract the high salt concentrations in seawater.

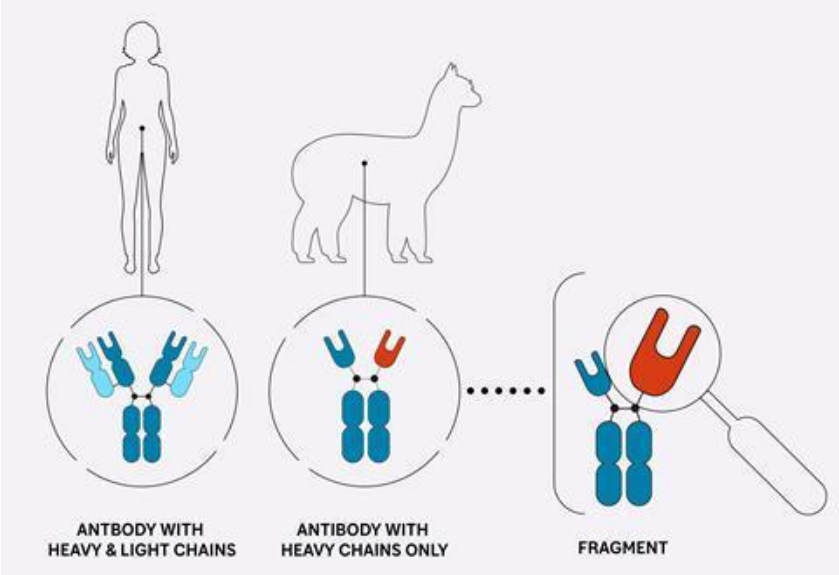


Figure 3: Camelids have no light chains on some of their antibodies

Cancer treatment with nanobodies and nanoparticles

Since targeted distribution lessens the harm that non-selective medications inflict, it represents a significant advancement in cancer treatment. Through the use of multipurpose Nano carriers, nanoparticles enable the controlled release of medications inside cancer cells. As a result, several researchers have recently become interested in the therapeutic potential of nanobodies as vehicles for delivering drug-loaded nanoparticles to tumors [27, 28]. The medicine is concentrated within the target malignant cells using the nanoparticle-based method, which also shields the nearby healthy cells and tissues from harmful chemicals. Modified nanoparticles have been successfully created by a number of researchers as a possible target for anticancer medications [29]. For instance, nanobodies expressing excellent specificity for the cancer marker Mucin-1 have been effectively coupled with nanoparticles [30]. Breast and colon cancers are associated with overexpression of mucin-1 [31].

This specification allows the killing of only cancerous cells and shields healthy tissue from the toxicities associated with cancer treatment. A "killer gene," which is expressed in the target cells and results in cell death, has been engineered into polymer-based nanoparticles. The promotor gene for Mucin-1 regulated the expression of the killer gene [30]. Only malignant cells can be killed thanks to this specificity, which also protects healthy tissue from the toxicities of cancer treatment.

Nanobodies based on camels

It is important to remember that every camelid naturally produces a unique set of antibodies that circulate in their blood. Conventional antibodies typically consist of two identical heavy chains and two identical light chains connected by disulphide bonds formed by non-covalent interactions [40]. Conversely, as their name suggests, heavy chain antibodies (HCAbs) are unique molecules that exhibit distinctive characteristics due to the absence of light chains and the presence of a heavy-chain homodimer [16]. These HCAbs have lower immunogenic reactivity than conventional antibodies and are easy to separate and clone from the serum of an immunized camelid [8].

Some camels have a single antigen binding site, which is known as the variable domain of heavy chain of HCAbs (VHH) or nanobodies [35, 41]. To increase the effectiveness of the large-size conventional antibodies, the protein-engineering strategy has employed a single-domain unit approach.

Single-domain antibodies in Nanobodies have greater resistance. Instead of having the typical first constant domain (CH1), HCAbs in C. By further improving the structure of single-domain antibodies to make them more gastrointestinal tract permeable and hence appropriate for local per-oral applications, this resistance can be raised [17]. Additionally, single-domain antibodies have a very short plasma retention time and can be eliminated by the kidneys into the urine because their molecular mass is below the renal threshold [20]. Additionally, their lack of the FC fragment, which was implicated in the activation of the complement system, results in reduced cytotoxicity. Furthermore, Rapid cellular internalization combined with excellent specificity and affinity for the targeted antigen enables the nanobodies to function more quickly and effectively than traditional antibodies [19, 42].

It is a difficult goal to develop a treatment instrument that targets tumor cells specifically while ignoring normal cells. Certain receptors or biomarkers are overexpressed in cancer cells, which can serve as an

alluring location to improve drug delivery and accumulation in the afflicted tissues [25].

Clinical medicine and biological research have made extensive use of conventional monoclonal antibodies, which are based on a ligand-receptor or antibody-antigen paradigm. In order to treat different types of cancer, many vehicles have been developed to transport medications to their therapeutic location [6, 46]. Furthermore, typical big molecular weight (150 kDa) antibodies have poor permeability, extravasation, and tissue penetration, resulting in an uneven distribution of the drug within the targeted tissue [13].

Similar to this, traditional nanoparticles showed a slower diffusion pattern and significantly impeded the delivery of drugs to the tumors, which is linked to less than ideal treatment outcomes [14, 15]. As a result, tumor cells are not exposed to comparable medication concentrations, and when they are, the concentration falls short of the therapeutic threshold that causes cellular death, which lowers the effectiveness of treatment [11,12,13]. Consequently, a large number of cancer patients showed resistance to the antibody-based strategy, which was associated with poor outcomes and therapeutic failure.

To boost the efficacy of the antibody-based treatment, it is recommended that these nanoparticles have a size of 5–200 nm. In actuality, nanoparticles less than 5 nm are easily eliminated from the bloodstream, but those larger than 200 nm would become lodged in organs such as the liver and spleen that have reticuloendothelial systems. Accordingly, it was suggested that the ideal size of the used nanoparticles be less than 100 nm and possess the ability to be hydrophilic [6, 49].

They are regarded as the tiny, entire antigen-binding entities that are found naturally in the form of nanobodies since the heavy chain antibodies (HCAbs) camelid antibodies exhibited a molecular mass of around 95 kDa and the VHH domain showed a mass of about 12–14 kDa [8]. In order to overcome the challenges currently faced by the nontherapeutic modality, the current trend in drug delivery establishment has shifted to the use of smaller molecules with high specificities, such as camelid nanobodies [46].

Disease screening and prevention, diagnosis, treatment, and follow-up have all improved as a result of the use of camelid-derived nanobodies in many fields of Nano medicine and monotherapy. In fact, aside from their small size, camelid-based nanobodies are originally manufactured and have remarkable qualities such as a high sensitivity and specificity spectrum, a higher safety level, water solubility, and bio stability. These characteristics enable nanobodies to play a major role in the advancement of pharmaceutical companies and the management of a range of illnesses.

The tumors an excellent illustration of how well nanobodies interact in delivering the drug cargo to the intended tissues was provided by a microenvironment. Nanobodies can overcome the limits of traditional antibody-based therapy in the treatment of solid tumors because of their smaller size and greater penetrating capacity. The impact of camelid-based nanobodies on the treatment of many illnesses, including lung and breast malignancies, infectious disorders, and inflammatory ailments, has been investigated in a number of research, including experimental preclinical investigations and clinical trials [46].

Using the biological secretions of camels to treat a variety of illnesses

The biological properties and components of camel milk because it contains a variety of proteins, lipids, oligosaccharides (lactose), nucleotides

Vital amino acids, vitamins, and minerals, camel milk has a high nutritional value [50, 51]. Vitamins C and E, lactic acid bacteria (LAB), caseins (α, β, and κ isoforms), and a variety of whey acidic proteins, including lysozyme, alpha-lactalbumin, immunoglobulin, lactoferrin, and lacto peroxidase, are undoubtedly abundant in camel milk [52]. Phospholipids from camel milk, including phosphatidyl-ethanolamine (PE), phosphatidyl-choline (PC), lysophosphatidylcholine (LPC), and phosphatidylinositol (PI), have also been found [53].

Strong probiotics, antioxidants, antimicrobials, and anti-inflammatory properties are provided by milk proteins, especially lactoferrin [57]. Lysozyme and immunoglobulins found in camel milk have antimicrobial and anti-inflammatory properties [37]. The structural characteristics of milk proteins, particularly β-caseins, and their higher content of antioxidant amino acids were thought to be responsible for the antioxidant's effect [58]. Crucially, these milk proteins were discovered to reduce the burden of oxidative stress and the generation of harmful free oxygen radicals that interact with the microenvironment of cancer, hence inhibiting the growth of cancer cells [59]. Furthermore, Korashy *et al.* proposed that camel milk had a potent apoptotic effect on breast and liver cancer cells [57]. Additionally, the evidence for the active camel whey component (TR35). Evidence suggests that active camel whey fraction (TR35) confer anti-tumor effects in non-small cell lung cancer (NSCLC) [6].

This was achieved by stimulating the death of cancer cells in vitro and preventing the growth of tumors in vivo through the phosphorylation of c-Jun N-terminal kinases (JNK), the suppression of P38, and the phosphorylation of the transcription factor Signal transducer and activator of transcription 3 (STAT3) [39]. Numerous in vitro and in vivo studies have confirmed the tumor-fighting ability of camel milk [59-63].

Exposure of different cancer cell lines such as breast cancer (BT-474), laryngeal (HE-p2), and human hepatoma (HepG2) cells to lyophilized camel milk blocked the growth and proliferation of these cells [61]. Moreover, treated the Michigan Cancer Foundation-7, breast cancer cells cell line, Michigan Cancer Foundation-7 (MCF-7) and the colorectal cancer cells HCT 116 with the commercial camel milk induced cancer cell autophagy (Macro autophagy) manifested by cell membrane deformity, intracellular vacuoles formation, elevated autophagosomes engulf cytoplasmic components (LC3-II/LC3-I) ratio, and formation of the auto phagosomes [61].

Vitamin E and C [64], casein, lactoferrins, lacto peroxidase, fatty acids, various ions and metals, and immunoglobulins [65] are among the biological components of camel milk that have been endowed with the capacity to provide therapeutic benefits. The majority of the camel milk's antitumor genic effects were thought to be delivered by lactoferrin [59, 61, 62, 66]. The main iron-binding glycoprotein in camel milk, lactoferrin, has antioxidant properties and has been shown to inhibit colon cancer cell proliferation in vitro human colon cancer cell line (HCT-116) and shield DNA from damage [59].

Additionally, a high content of vitamin C in camel milk has been shown to provide protection against mutagenesis and clastogenic impacts [67]. In order to prevent the development of cancer, selenium, zinc, and casein [68, 69] are essential for removing some of the genotoxic effects of toxic substances and guaranteeing proper DNA and RNA synthesis [68]. In fact, HepG2 and HeLa cells undergo cellular death and the apoptotic pathway is triggered when camel milk containing casein containing α-lactalbumin is consumed [70]. However, more research was needed to determine the precise active ingredients in camel milk that specifically decrease tumors and the molecular processes that follow.

Additionally, unique immunoglobulins known as VHH antibodies or nanobodies were expressed in camel milk [20]. In contrast to human IgGs, these immunoglobulins are tiny, which allows them to penetrate tissues to localize and operate inside cells [53]. Furthermore, a single-domain antibody (sdAb) known as Nano bodies (VHH) can readily target tumor tissues and metastatic loci due to its physicochemical properties [71-73]. The structural configurations of the secreted nanobodies were revealed by Cortes-Retamozo et al.'s investigation of nanobodies in camel milk. They showed that nanobodies are less hydrophilic than the camel variable domain of HCAbs (VHH) domains and are naturally occurring single-domain antigen-binding units.

They also have strong selectivity for solid tumors and don't include immunogenic fragments [71]. These characteristics have been proposed as therapeutic or diagnostic tools and aid in the development of stable and effective antibody constructions with superior target selectivity against cancer cells [71]. According to individual studies, naturally occurring nanobodies in camels' biological fluids, such as milk and urine, have shown promise as therapeutic agents for the treatment of a number of illnesses, including peptic ulcers, inflammatory and infectious diseases, various cancers, and chronic hepatitis and hepatitis C virus infection (HCV) [60, 74-76].

The biological properties of camel urine components

Although camel urine is a waste product, it has been used as a base for a number of therapeutic mediators. According to a study by Abdul Qader *et al.*, camel urine contains over 32 proteins, including thyroxine-binding globulin (T3 and T4) thyroid hormones, vitamin D-binding protein, serum albumin, alpha-1B-glycoprotein, alpha-1-acid glycoprotein, and serotransferrin. The anti-inflammatory and anti-infectious properties of camel urine may be explained by these proteins [1]. According to in vitro research, camel urine and milk may cause malignant cells to undergo apoptosis and may prevent mutagenesis and the growth of mutant cells [2]. Romli *et al.* examined how camel urine containing nanobodies affected 4T1 breast cancer cells both in vitro and in vivo.

They discovered that 4T1 cancer cells' proliferation was suppressed and their ability to spread was limited when exposed to pure camel urine in vitro. Mice injected with 4T1 cells and treated with camel urine demonstrated a substantial reduction in tumor size in the treated group when compared to the control group in a double-blind evaluation [3]. The therapeutic Alhaider AA was also demonstrated in two separate investigations by Evers et al. [4] and Alebie *et al.* [5]. Survey of the camel urine proteome by shotgun proteomics employing a multiple database search technique.

Additionally, camel secretions, such as milk and urine, have shown promising outcomes as a treatment strategy for lung cancer [85], breast cancer [57], and hepatoma [70] (Fig. 4). In vitro tests conducted in 2006 by Khorshid and Moshref showed that lyophilized camel urine might stop the growth of tumor cells in a variety of cancer cell lines, including leukemia [85], colon cancer, lung cancer, and hepatocellular carcinoma 2 (HEPG2) [86]. Individual reports that showed cellular cytotoxicity and proliferative inhibitory effects after camel urine stimulation of various cancer cells further assessed the cytotoxic effect of camel urine [87,88].

Lyophilized camel urine was used to stimulate ten different types of cancer cells, which were then split into two groups at the conclusion of the treatment. The first set of cells, which included the DAOY, MED-4, MED-13, and MDA-MB-231 breast cancer cell lines, demonstrated more than 50% cellular death, particularly in the MDA-MB-231 breast cancer cells, where 80% of

the cells underwent cellular apoptosis. In contrast, MCF 10A, HFSN-1, U2OS, MCF-7, MED-8, LoVo, and HCT-116 showed either a negligible or insensitive response to the camel urine treatment [88].

Gader and Alhaider recently confirmed that camel urine could decrease tumor angiogenesis [76]. Furthermore, by introducing camel milk and urine into murine sponge cells, Alhaider et al. found inhibiting inflammatory angiogenesis.

This was accomplished by the reduction of numerous cytokines, macrophage recruitment, vascular endothelial growth factor (VEGF) expression, transforming growth factor beta (TGF-beta), and important components of fibro vascular tissue [63]. A study by Cyplal further demonstrated camel urine's anticancer properties by demonstrating that it significantly suppresses the transcription of the gene encoding a carcinogen-activating enzyme [88]. Furthermore, it has been observed that camel urine has antitumor genic effects by lowering the expression of several cytokines that promote tumor growth, including IL-4, IL6, and IL-10 [88,89].

Curiously, another proposed mechanism by which camel urine enhanced tumor cytotoxicity while shielding normal cells from reactive oxygen radicles generated by chemotherapeutic agents was the upregulation of chemo protective gene expression, such as Nicotinamide adenine dinucleotide (NAD) (P)H dehydrogenase [quinone] 1 (Nqo1) and glutathione S-transferase A1 (Gsta1) [82]. All of these results confirmed that camel biological secretions in milk and urine had a powerful therapeutic potential to stop the spread of cancer cells.

It is still unclear exactly what the anticancer ingredients in camel milk and urine do. The presence of the iron-binding lactoferrin component was mostly responsible for the tumorigenic suppression capacity shown by camel urine [62, 90-92].

Benzene propanoic acid derivatives, fatty acid derivatives, amino acid derivatives, sugars, prostaglandins, erythritol, melibiose, and canavanine are among the many metabolites that were shown to have a possible role in the biological effects of camel urine [98]. One anti-metabolite of L-arginine derivatives with a hazardous characteristic is canavanine. Canavanine, which makes up 2% of the total components of camel urine, has been shown to have tumor-suppressive properties [99]. As a result, camel pee offers a potentially effective therapeutic option for treating various cancers [89].

Additionally, camel urine has been shown to include a variety of chemical and inorganic substances, crystals, nanobody, and nanoparticles, including Prophet Medicine Fraction (PMF) and PM701. Many components, including calcium oxalate, cysteine, uric acid crystals, ammonium urate, calcium phosphate, benzoic acids, glycine, alanine, and arginine, are found in PMF crystals. Furthermore, ions such as Cs, Rb, K, Ca, Cd, Y, Eu, Th, and Zn have large concentrations of PMF [82]. Crucially, the PMF compositions are crucial in generating cytotoxic effects against several cancer cell types by enhancing the permeability of cancer cell membranes, which permits lysis and killing of the cells [82].

Zn which is presents in the camel urine as ZnO this when bound to the nanoparticle produces a distinctive metal oxide nanomaterial featured with cytotoxic effects [100].

In order to produce this biological impact, cellular oxidative stress is generated [101, 102], and the membrane of cancer cells is deformed and ruptured [103]. By raising their pH level and ignoring normal cells, other PMF elements like Cs and Rb can efficiently target cancer cells and stem cells [82, 104]. Through its amino acid constituents, camel urine PMF has been shown to alter the growth functions of both healthy and malignant cells [105]. While glycine and cysteine enhance PMF antioxidant capacity, hence fortifying the immune system, tyrosine promotes PMF targeting to the cancer cells [106].

The use of nanobodies produced from camels in nano-oncology

The scientific discipline of nano-oncology, which integrates biochemistry, engineering, and medicine, aids in the development of tumor screening, diagnostic, and treatment strategies [109]. The diagnostic or imaging agent must localize selectively into the afflicted area in order to produce an accurate cancer diagnosis, particularly in the early stages of the disease. Because of their small size, short half-life in circulation, and reduced background noise, nanobodies offered a more accurate imaging tool with better tumor targeting and retention [110,111].

Additionally, utilizing the positron emission tomography-computed tomography scan (PET/CT) imaging technology and Positron Emission Tomography, nanobodies bound with a diagnostic isotope such 18F were successfully used to detect the expression of human epidermal growth factor receptor 2 (HER2) in cases of breast cancer [112, 113]. A monoclonal anti-HER2 antibody called trastuzumab has demonstrated positive outcomes in individuals with breast cancer who overexpress human epidermal growth factor receptor 2) (HER2) receptors. On the other hand, instances with breast cancer with low or variable HER2 expression showed a partial trastuzumab response. Before trastuzumab is administered to patients with breast cancer, anti-HER2-specific 5F7GGC Nb nanobodies that are radio iodinated

with131I IB-Mal-D-GEEEK were created to measure HER2 expression.

Both in vitro and in mouse models, these attached nanobodies showed promising effects in targeting HER2 tumors, encouraging receptor internalization and subsequent signal suppression [114].

In addition to the detection of human epidermal growth factor receptor 2 (HER2), nanobodies have been produced to target other growth factor receptors that are overexpressed in a number of cancers, including chemokine receptor type 7 (CXCR7), vascular epidermal growth factor receptor 2 (VEGFR2), c-Met, hereditary gingival fibromatosis (HGF), and epidermal growth factor receptor 1 (EGFR1) [115,116,117,118]. Anti-HGF nanobodies (1E2-Alb8 and 6E10-Alb8) labeled with positron emitter zirconium-89 were created to be employed for in vivo detection of HGF expression in aggressive cancer types where HGF and its receptors, hepatocyte growth factor receptor (c-Met), were abundantly expressed.

Furthermore, when compared to the control group, these designated nanobodies inhibited the growth of tumors in the treated animals, demonstrating therapeutic effects [116]. The capacity of a system made up of nanobodies linked to polyethylene glycol (PEG-liposomes) to identify the EGFR was investigated both in vitro and in vivo. It was discovered that this system had an antagonistic effect on the expression of the epidermal growth factor receptor (EGFR), which led to the internalization and downregulation of receptor expression and, ultimately, the prevention of tumor cell proliferation [119]. Another type of manufactured nano body that targets EGFR expression is the nano body-based targeting module (Nb-based TM).

This result is accomplished by causing T lymphocytes to be selectively recruited to cancer cells that overexpress EGFR, and then eliminating the tumor cells both in vitro and in vivo [120].

The goal of the smart medication therapy was accomplished by this nanobody-based drug delivery system (DDS) [121]. In a similar vein, animal research has also employed nanobodies to target the pulmonary surfactant protein A (SPA), which is linked to airway disorders.

The development of Nb6 and Nb17 nanobodies revealed their quick accumulation in the pulmonary tissues. Alongside this impact, there was minimal accumulation in the liver and spleen and quick removal from the blood [122]. Additionally, research was done on the potential of nanobodies as therapeutic agents for several brain tumors, including glioma and glioblastoma [123]. Anti-EGFR nanobodies inhibited the development and multiplication of tumor cells in vitro [124]. Additionally, both in vitro and in vivo, a combination modality comprising an immune conjugate targeting tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), an anti-EGFR nanobody, and a pro-apoptotic EGFR-specific nanobody demonstrated a strong inhibitory effect on tumor growth and aggressiveness [123].

Since several nanobodies have been evaluated in preclinical settings and many more are undergoing clinical trials, the use of nanobodies in medical decisions has advanced significantly [111, 125, 126]. The aerosol or inhaler method of administration is mostly beneficial for respiratory diseases because it guarantees medication accumulation in the lungs and prevents systemic side effects [127]. Because of their distinct biological characteristics, nanobodies make a great medication delivery system for the lungs.

A 42 kDa trivalent Nano body inhaler called ALX-0171 is presently undergoing clinical trials to combat the human respiratory syncytial virus (RSV) and reduce infection duration [128]. The safety and tolerability of ALX-0171 in the people under examination were proven in a phase I clinical trial [127]. The first 28 kDa bivalent nanobody (ALX-0681) licensed by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for the treatment of thrombotic thrombocytopenic purpura (TTP) is Caplacizumab. Platelet clumping, microvascular thrombosis, and obstruction are caused by autoantibodies that target the von Willebrand factor in TTP, an autoimmune hematological illness.

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Camel milk's effects on different signaling pathway regulation

The biological secretions of camels have been found to provide therapeutic effects in the treatment of a variety of medical diseases, as previously described. However, more research is required to determine the exact subcellular molecular mechanisms that these secretions regulate. Camel milk has been proposed as a supplemental treatment for type 1 diabetes and has been shown to produce a hypoglycemic impact in both human and animal models [130,131,132]. To determine the mechanism by which camel milk regulates insulin

The human embryonic kidney 293 (HEK293) cells, which expressed transiently human insulin receptors (hIR), were stimulated with camel milk to elicit its

glycemic action. By quantifying the strength of the physical contact between hIR and insulin receptor signaling proteins (IRS1) and growth factor receptor-bound protein 2 (Grb2), the bioluminescence resonance energy transfer (BERT) assay was utilized to investigate the activation of insulin signaling. The strength of BERT signals between hIR and Grb2 but not IRS1 was found to be significantly increased only by concurrent administration of insulin and camel milk. Additionally, it was discovered that camel milk enhanced ERK1/2 but not Akt activation, which is a downstream signaling pathway for insulin [133,134].

Furthermore, the administration of camel milk reduced the pulmonary recruitment of oxidative stress factors and several cytokines, including tumor necrosis factor (TNF), interleukin-1 beta (IL-1b), and interlukin-10 (IL-10). As an endogenous modulator of leukocytes remarkably, it was hypothesized that the regulation of the Mitogen-activated protein kinase (MAPK) signaling cascade was responsible for the reported therapeutic effects of camel milk [137]. Similar to rheumatoid arthritis (RA) in humans, the anti-inflammatory properties of camel milk were further confirmed in an additional animal model that had both arthritis and air pouch edema.

A chronic inflammatory state that results in damaging lesions in the bones and cartilages of the small joints, especially the hands, is the hallmark of RA, an autoimmune disease [138].

A common immunosuppressive medication for a number of autoimmune disorders is cyclosporine. Kidney damage that might lead to renal failure is one of the most dangerous adverse effects of this medication. It was suggested that camel milk, an anti-inflammatory and antioxidant mediator, might be used as a natural remedy to lessen the negative effects of taking cyclosporine's. In vivo the levels of biomarkers associated with kidney impairment, including serum creatinine, blood urea nitrogen (BUN), and Biomarker Kidney Injury Molecule-1 (KIM-1), were significantly decreased after three weeks of camel milk administration.

Additionally, a significant decrease in a number of inflammatory cytokines and degradation signals, including MCP-1, IL-8, TNF, MMP-2, and MMP-9, is linked to camel milk treatment.

In addition to its capacity to decrease p38/ERK/JNK MAPK signaling, camel milk inhibited the expression of NF-κBp65, p-NF-κBp65, and p-IκBα proteins, thereby blocking the activation of the NF-κB pathway. The oxidative stress on the kidneys was reduced by blocking certain pathways and increasing glutathione antioxidant and antioxidant activity. As a result, camel milk can be utilized as a natural therapy to prevent kidney damage caused by cytotoxic medications [105].

Another camel's biological excretion, camel urine, has also been shown to inhibit tumors and enhance the effects of doxorubicin treatment on breast cancer cells. By causing DNA damage and death in cancer cells, reversing emergency medical technician (EMT) indicators, and recovering cell adhesion expression (E-cadherin epithelial marker), this anticancer effect was achieved. [106].

Proteomics by nanotechnology

After the camel genome sequencing project was finished in 2003, the data it generated allowed scientists to employ proteomics to examine and understand every cellular activity that genomics alone was unable to explain. In addition to just investigating the complete proteome, a proteomics investigation provides useful information on the post-translational modifications, localizations, structures, and interactions of all proteins expressed by an organism [107]. Additionally, proteomics has a variety of clinical uses, such as assisting researchers in assessing the efficacy and safety of therapeutic therapies [108,109]. investigating the mechanisms behind medication reactions, identifying novel therapeutic targets, comprehending the causes of illness, and identifying novel biomarkers for the early diagnosis of disease [110,111].

Nano proteomics has been applied to the identification of biomarkers in two primary domains: Nano porous materials and nanostructured surfaces. To help researchers overcome the difficulties of identifying low-abundance proteins, a number of nanomaterials have been produced, such as carbon nanotubes, quantum dots, gold nanoparticles, and nanowires [113, 114]. A sensitive and reliable analytical platform for high-throughput screening of low-abundance biomarkers is offered by Nano proteomics [112]. Notably, biomarkers linked to a number of human diseases, such as cardiovascular disorders [116] and autoimmune diseases [115], have been found using Nano proteomics.

Thus, the ability to apply Nano proteomics to enable safe and timely cancer diagnosis is the primary focus of cancer researchers. As a result, a number of assessments have been carried out on specific biomarkers in different cancer types, such as prostate and breast malignancies [117, 118]. To ensure their safety in biological applications, more study is necessary to address the compatibility and toxicity of nanotechnology, which continue to be health concerns [119].

Camel nanobodies' remarkable pharmacokinetic and physicochemical characteristics are in line with those required for cancer treatment and offer advantages over traditional antibody technology in drug delivery, immunotherapy, and diagnostics. Compared to normal antibodies, camel-heavy antibodies have greater

affinities, with some even reaching up to 100 pM affinity constants [120, 121].

This promotes connections between proteins and can serve as a solid foundation for detecting intracellular signaling and cancer biomarkers. Chrome bodies that can be utilized in single-molecule localization using super-resolution imaging techniques can also be created by bonding heavy-chain antibodies with fluorescent dyes [122,123,124]. A more stable disulphide bond is formed at the hydrophobic area when cysteines are present at amino acid positions 54 and 78 [125, 126]. Furthermore, the melting temperatures of nanobodies are between 67 and 78 degrees Celsius, they can refold once thermal unfolding has been observed, and they have functional activity up to 90 degrees Celsius [18].

Because of their durability, nanobodies (heavy-chain antibodies) are ideal for pharmacological formulations [129], tumor targeting [8], and antibody engineering [127, 128]. Furthermore, nanobodies' ability to recognize recessed antigenic domains has been ascribed to both their small size and the extended CDR3 loop's ability to quickly penetrate epitopes [121, 130]. Therefore, it is possible to effectively use nanobodies to target specific enzymes, transmembrane proteins, or even signaling pathways in specific tumor cells. In order to identify the carcinoembryonic antigen, Cortez-Retamozo et al. (2004) employed nanobodies linked to the β-lactamase enzyme to target tumor cells [131].

An administered non-toxic pro-drug is changed by the enzyme into a toxic substance that is more abundant in the targeted tumor cells. Nanobody conjugates like these have a lot of potential for cancer immunotherapy. It is widely known that cell-surface protein conjugates that target the receptor for epidermal growth factor can inhibit the growth factor by binding to its receptor; solid tumors have been cured using this strategy [94]. Similarly, malignant tumors may be cured by using nanobodies that target the tumor necrosis factor-α (TNF) [132].

Additionally, studies have been conducted to investigate and use the synergistic potential of bioinformatic tools and nanoparticles for cancer treatment [133].

By suggesting that protein corona is created when proteins bind to nanoparticles, Arvizo and colleagues (2012) suggested that an integrated bioinformatics, proteomics, and nanotechnology approach may be utilized to identify novel therapeutic targets for cancer [133]. Using designed surface-functionalized gold nanoparticles (AuNPs) on corona, proteins were discovered that shed light on the onset and progression of ovarian cancer. They came to the conclusion that a promising treatment strategy for a number of illnesses, including ovarian cancer, is protein corona modification surrounding nanoparticles. With the development of animal biotechnologies, such as transgenesis and camel cloning, the creation of targeted nanobodies released from genetically modified camels would be a unique way to cure tumors.

Recent studies have demonstrated that stem cell-delivered anti-EGFR nanobodies may be used to deliver anti-EGFR treatments to brain tumors. When paired with cytotoxic compounds, they suppressed tumor cells, significantly improving the therapeutic success [77].

Exosomes

Exosomes are special nanoparticle drug carriers with significant nanoparticle characteristics. They are nano-sized extracellular membrane vesicles with a size range of 50 to 200 nm [43, 134]. The potential of exosome biomimetic nanoparticles to combine natural and synthetic ingredients to provide a more effective drug delivery system is favorable. However, they are still constrained by the synthesis process, which is evident in the protein.

Integrity on the surfaces of the exosomes, which impairs their ability to function [82]. Therefore, natural sources of exosomes from biological fluids, like milk, are crucial, particularly if large-scale synthesis is our goal [138, 135].

Exosomes from camel milk may be crucial in preventing the formation of breast cancer cells, according to preliminary research [137]. They might also alter the immunotoxicity and oxidative stress brought on by cyclophosphamide in mammals. Exosomes carry biomolecules like bioactive lipids, a specialized functional proteome, nucleic acids (including DNA, microRNA, and ncRNA), metabolites, and signaling molecules that can be transported over long distances while being protected by a lipid bilayer-enclosed structure [136]. These effects are the result of the significance of these biomolecules.

Additionally, EL-Kattawy *et al.* discovered that exosomes generated from camel colostrum, which are rich in milk protein, exhibited exceptional apoptotic effects on liver cancer cells while leaving normal cells unharmed. By increasing the expression levels of both Bax and caspase3 and decreasing the levels of Bcl2, these carcinogenic suppressive effects are achieved. Alongside this, there was a decrease in the production of angiogenic-related proteins including VEGF and inflammatory mediators such TNFα, NFkB, TGFβ1, and Cox2 [43].

Final thoughts and viewpoints

Why antibodies against camelids?

Camelid antibodies have excellent solubility, great thermostability, and tight monomeric behavior. They are also highly specific. Additionally, because of their small size, genetic engineering is simple, which lowers the cost of production overall.

Since the discovery of naturally occurring camelid nanobodies, numerous research fields have been developed, including medical, biotechnology, engineering, and economics. Recently, nanobodies have become appealing and effective methods for both therapeutic and diagnostic purposes, especially in the field of cancer. Fortunately, in addition to their capacity to penetrate biological barriers like the blood-brain barrier, nanobodies also display a number of biophysical and biochemical properties. Additionally, they exhibit the ability to reach solid organs such as the liver, lungs, lymph nodes, and brain with ease. Nanobodies have been included into multipotent constructions or conjugated with chemotherapeutic medicines to create extremely selective and effective molecules due to their flexible manufacturing and assembly capabilities.

Because of these characteristics, nanobody-based therapies are effective and potent means of delivering the medication just to the targeted tissues. However, because of their small size, nanobodies still showed significant drawbacks, such as the potential for renal toxicity and quick renal clearance. Coupling nanobodies with serum albumin can change this limitation by extending their retention period in the bloodstream, but doing so will inevitably lessen their diffusion and penetration advantages. To determine their precise molecular mechanisms for modifying neoplastic signaling pathways and to evaluate their safety profile in preclinical experimental studies prior to their inclusion in any clinical trials, more research is essential.

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