R E V I E W

Wolf in Sheep's Clothing: Taming Cancer's Resistance with Human Serum Albumin?

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Abstract: Human serum albumin (HSA) has emerged as a promising carrier for nanodrug delivery, offering unique structural properties that can be engineered to overcome key challenges in cancer treatment, especially resistance to chemotherapy. This review focuses on the cellular uptake of albumin-based nanoparticles and the modifications that enhance their ability to bypass resistance mechanisms, particularly multidrug resistance type 1 (MDR1), by improving targeting to cancer cells. In our unique approach, we integrate the chemical properties of albumin, its interactions with cancer cells, and surface modifications of albumin-based delivery systems that enable to bypass resistance mechanisms, particularly those related to MDR1, and precisely target receptors on cancer cells to improve treatment efficacy. We discuss that while well-established albumin receptors such as gp60 and gp18/30 are crucial for cellular uptake and transcytosis, their biology remains underexplored, limiting their translational potential. Additionally, we explore the potential of emerging targets, such as cluster of differentiation 44 (CD44), cluster of differentiation (CD36) and transferrin receptor TfR1, as well as the advantages of using dimeric forms of albumin (dHSA) to further enhance delivery to resistant cancer cells. Drawing from clinical examples, including the success of albumin-bound paclitaxel (Abraxane) and new formulations like Pazenir and Fyarro (for Sirolimus), we identify gaps in current knowledge and propose strategies to optimize albumin-based systems. In conclusion, albumin-based nanoparticles, when tailored with appropriate modifications, have the potential to bypass multidrug resistance and improve the targeting of cancer cells. By enhancing albumin's ability to efficiently deliver therapeutic agents, these carriers represent a promising approach to addressing one of oncology's most persistent challenges, with substantial potential to improve cancer treatment outcomes.

Keywords: HSA, drug delivery system, drug resistance, nanomedicine, gp60, HSA dimer, pazenir, fyarro, abraxane

Introduction

Human serum albumin (HSA) is among the most extensively studied carriers in cancer therapy because of its unique characteristics, such as biocompatibility and biodegradability.[1] Albumin is also actively scavenged by cancer cells due to its high capacity for fatty acid binding and its glutamine content, which makes up approximately 10% of its amino acid composition. This makes albumin a valuable source for fueling the Krebs cycle and lipid biosynthesis, as well as providing nitrogen necessary for amino acid and nucleotide synthesis.[2–7] Interestingly, hypoalbuminemia in cancer is frequently associated with cancer chemoresistance due to reduced drug transportation (reviewed in).[8]

Albumin structure is amenable to modifications, also allows for the efficient design of active targeting strategies, specifically directed at particular receptors. Thus, it enables both passive and active (via receptor-mediated pathways) targeting of cancer cells.

Passive targeting results from the prolonged circulation time of human serum albumin (HSA). The growth of cancerous tissue causes local swelling (due to a reduction in lymphatic flow), increasing the accumulation of albumin

in the interstitial space. This accumulation is caused by a significant increase in vascular permeability called the enhanced permeability and retention (EPR) effect, reported by Maeda and Matsumura in 1986. Hoogenboezem and Duvall provided a comprehensive review of the relationship between the EPR effect and albumin accumulation in tumor tissues, highlighting its role as a drug carrier in oncology.[9] Despite passive drug targeting, clinical evidence suggests that tumor accumulation of organic compounds, including albumin-based nanoparticles, typically only reaches less than 1% of the administered dose, and satisfactory outcomes are seldom achieved solely through the enhanced permeability and retention effect strategy.[10,11]

This is because mere accumulation at the target site is insufficient for effective drug delivery. The issue of drug penetration into tumor tissue remains complex and is dependent on factors such as the tumor's vascularization. Furthermore, a critical question is – is it possible for the nanoparticle or drug to enter the cancer cell to excerpt therapeutic effect? To precisely address this question and tackle it effectively, it is essential to understand how a cancer cell internalizes a given particle and whether the process could be enhanced or bypassed. Targeting and uptake can be improved by incorporating ligands into the nanoparticle that are known to effectively bind to receptors on the cancer cell surface. On the other hand, resistance mechanisms can actively expel the particle from the cell, preventing it from interfering with the cancer cell's life pathways.

It is estimated that therapy resistance is responsible for almost 90% of deaths caused by cancer.[12] The resistance can be pre-existing, also described as primary, innate or intrinsic. This means that the patient is characterized with a certain quality of cancer cells or tissues that causes therapy to be ineffective. Also, in terms of chemotherapy, such resistance is present before the treatment. On the other hand, due to the extraordinary plasticity, cancer cells can get resistant during the treatment. Such resistance is called acquired, secondary or extrinsic.[13] One of the factors that can emerge as a result of both primary and secondary resistance is perturbation of cell influx/efflux mechanisms. In 1976, Juliano and Ling reported a specific membrane protein of 170 kDa, which was the reason for colchicine resistance in cell culture.[14] They called it P-glycoprotein (P-gp, multiple drug resistance type 1 – MDR1). MDR1 belongs to the ATP-binding cassette (ABC) transporters, responsible for the distribution, absorption and secretion of unspecific substrate repertoire.[15,16] Once MDR1 is expressed on a cancer cell, its nonspecificity enables cross-resistance to various drugs, a phenomenon known as multidrug resistance. MDR1 is not only a concern confined to cytostatic therapy; evidence has demonstrated that certain targeted therapeutics, such as tyrosine kinase inhibitors, are able to interact with MDR pumps.[16] Using nanoparticles that prevent expulsion through MDR, enable drug release within the cancer cell and are readily internalized by the cancer cell, like albumin, a seemingly essential nutrient, may serve as a "wolf in sheep's clothing"—a strategy that deceives cancer cells and significantly hinders the development of drug resistance.

This review focuses on the unique molecular features of human serum albumin that make it a versatile carrier for drug delivery, particularly in overcoming cancer cell resistance. While much has been written about the general effectiveness and methods for synthesizing albumin-based delivery systems,[9,11,17–20] our review takes an interdisciplinary approach, integrating insights from chemistry, cell biology, and translational medicine. We highlight how specific chemical modifications to albumin can improve its ability to bypass resistance mechanisms, especially those related to MDR1 (Figure 1). By examining the interplay between albumin's chemical properties, its cellular interactions, and the potential for manipulating these to overcome specific oncological barriers, we emphasize the translational relevance of this work. Rather than focusing solely on how the carrier reaches cancer cells, we investigate how the choice of receptor can influence the success of overcoming resistance mechanisms. Drawing on key studies, including the clinically used formulation Abraxane and new formulations – Pazenir and Fyarro, we identify areas where current knowledge is lacking and propose strategies to enhance the therapeutic impact of albumin-based drug delivery systems. Our goal is to shed light on how albumin can be optimized to break through cancer resistance and improve treatment outcomes. Although various sources of albumin are commonly used (as reviewed extensively in[21]), our attention will be given to human origin, as there is some evidence that the source of albumin carrier might influence the risk of allergic reactions,[20] which is highly important for potential use in immunotherapy.[9]
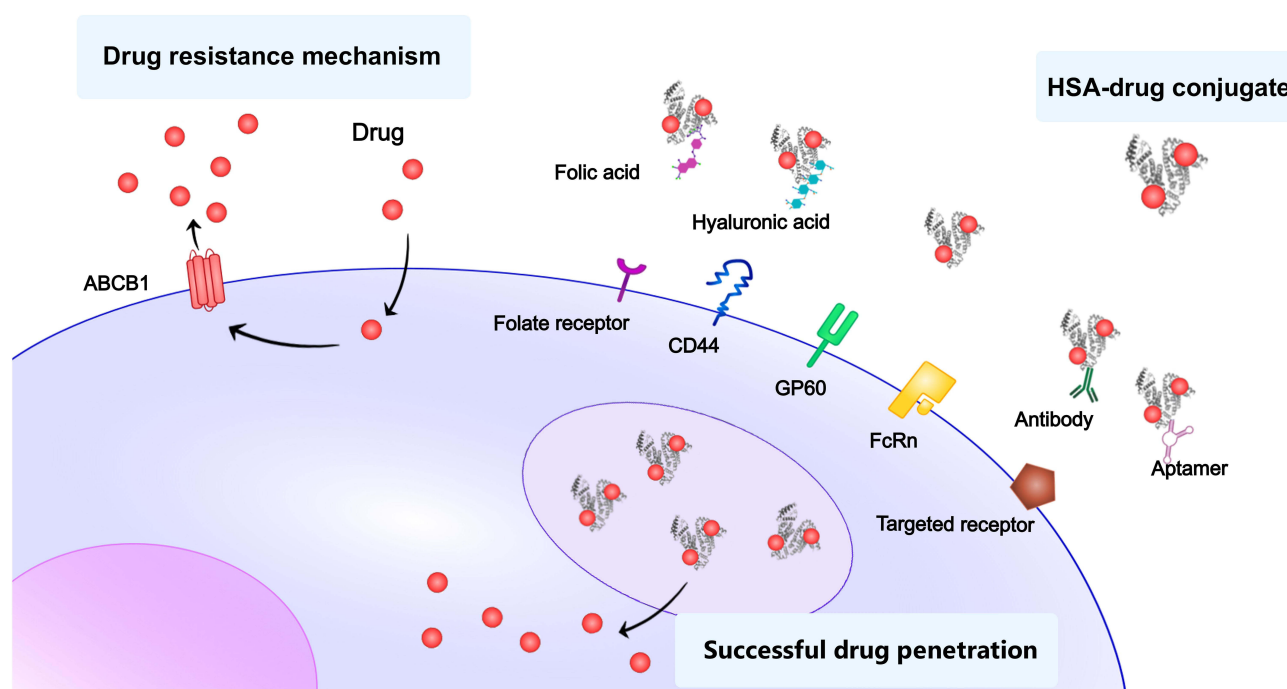
Figure 1 Human serum albumin as a modifiable carrier to target cancer cells. Folate receptor, gp60 (glycoprotein 60), CD44 (cluster of differentiation 44), and FcRn (neonatal Fc receptor) are involved in the uptake and transport of albumin-based carriers.

Basic Properties of Human Serum Albumin
Human Serum Albumin Function

Human albumin is the most abundant, with an average concentration of 42 grams per liter (equivalent to 632 μM)[22] and a half-life of 19 days, which allows it to maintain a prolonged presence in circulation.[23] During its life of 27 days, an average albumin molecule makes about 15000 trips around the circulatory system plus about 15 trips through extra-vascular spaces.[24,25] Approximately 120 grams of albumin are present within blood vessels, and due to its relatively low molecular weight compared to other plasma proteins, it plays a crucial role in maintaining about 80% of the colloid osmotic pressure. This function facilitates the movement of fluids between tissues and the bloodstream, helping to retain fluid within the blood vessels.[19,26,27]

The highly organized structure and multiple binding sites of HSA enable it to transport various compounds, including ions, fatty acids, hormones, and other molecules, serving as a high-capacity reservoir that stabilizes the concentration of free ligands. Albumin is indeed a crucial protein in the human body functions primarily as a carrier protein, which is vital for transporting various substances in the bloodstream and controlling their active concentration, making them an important part of drug delivery.[28–30] The transport of metabolites involves uptake by albumin at one organ and release to the cells of another. However, the failure of protein therapy in clinical trials has been attributed to poor knowledge of protein pharmacokinetics, as well as the uncontrolled spread of proteins when they are administered systemically.

Furthermore, HSA extends beyond its traditional functions in transport, osmotic regulation, and detoxification, playing a crucial role in cellular defense through its significant antioxidant properties. HSA is central to combating oxidative stress, a process closely linked to various diseases and aging. It contributes to cellular antioxidant defense by scavenging free radicals, binding metals, participating in redox reactions, and preventing lipid peroxidation.[31–33] Additionally, HSA serves as a biomarker for several diseases, including cancer, rheumatoid arthritis, ischemia, and obesity, and is clinically used to treat conditions such as blood loss, hemorrhage, chronic and acute liver failure, and hypoalbuminemia.[34]

Human Serum Albumin Structure

The liver synthesizes HSA, a non-glycosylated protein chain of 585 amino acids with a molar mass of 66,439 Daltons.[35] Human serum albumin and its derivatives, including recombinant HSA (rHSA) and the albumin dimer (dHSA), have well-known primary and secondary chemical structures. These molecules have a globular shape and are relatively stable under normal physiological conditions.

The crystalline structure of HSA is predominantly composed of α-helix formations, which make up 69% of its overall structure. The sequence of albumins is characterized by a unique arrangement of subunits linked by disulfide bonds. This configuration includes 35 cysteine residues that are integral in forming 17 disulfide bridges, which are crucial for maintaining the molecule's spherical stability.[31,33]

The molecule is organized into three repeating domains: I (residues 1 to 195), II (residues 196 to 383), and III (residues 384 to 585), each displaying a similar structural motif (Figure 2).

Across these three domains, a total of ten helices are arranged in antiparallel, presenting a surface of hydrophobic residues oriented toward the interior of each domain. These helices are further grouped into two subdomains: Subdomain A consists of six helices (h1 to h6), whereas Subdomain B contains four helices (h7 to h10).[5,30,36,37]

The amino acid composition of albumins is distinguished by a low abundance of tryptophan, glycine, and methionine, along with a high concentration of cystine and charged amino acids, such as aspartic acid, glutamic acid, lysine, and arginine.[25] The albumin molecule is highly polar, possessing approximately 100 negative and 82 positive charges, with the net negative charge being most pronounced in the N-terminal domain and lowest in the C-terminal domain, resulting in an asymmetric charge distribution. Human albumin's high solubility and stability are attributed to its overall net charge of −15 at neutral pH and the numerous disulfide bonds within its structure. Therefore, developing an HSA-based delivery system requires determining the electrokinetic charge of HSA, as this information is crucial for understanding drug adsorption mechanisms and ensuring optimal functionality. It is important to note that both electrokinetic and surface charges should be understood as net (averaged) values representative of the entire molecule, forming regions of positive and negative charges across a broad pH range. Various techniques are employed to estimate or predict a protein's isoelectric point (IEP), with one approach involving computational methods that consider factors such as crystallographic structure, electrostatic interactions, and amino acid properties to determine the IEP of human serum albumin, which ranges from 5.67 to 5.9.[38,39] When dispersed in aqueous solutions, HSA has a positive electrokinetic charge at pH values below 5 because of specific amino acid residues, such as cysteine (35), tyrosine (19), lysine (59), and arginine (23). However, when the pH of these solutions reaches 5, the electrokinetic charge of the molecule becomes negative, primarily because of the presence of glutamic acid (59) and aspartic acid (41) residues.[40] This shift establishes the molecule's isoelectric point at 5.1.[41] Depending on the methodology used, the isoelectric point values for human albumin can range from 4.7 to 5.8.[38,40,42,43] The differences observed when comparing experimental and computational methods for determining IEP can be attributed to variations in amino acid composition, the presence of fatty acids, or potential errors in measurement techniques.[44–46]
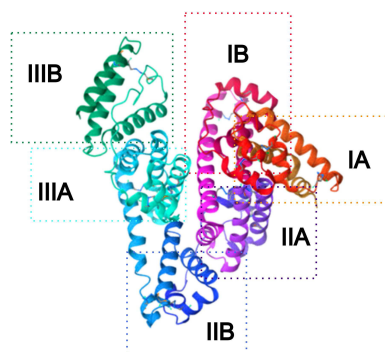

Figure 2 Modular domain structure of the HSA molecule. The panel illustrates the structure of the HSA molecule, with its subdomains distinctly color-coded. The molecule is organized into three repeating domains, each exhibiting a similar structural motif: Domain I (IA: residues 1–112, IB: residues 112–195), Domain II (IIA: residues 196–303, IIB: residues 303–383), and Domain III (IIIA: residues 384–500, IIIB: residues 500–585).

Ligands Binding and Functional Sites

A defining characteristic of albumin is its ability to reversibly bind a wide range of endogenous and exogenous ligands. Most of these interactions rely on hydrophobic bonds that affect albumin's structural flexibility, enabling it to adapt its shape to accommodate various ligands, which likely explains its extensive binding capacity. The majority of ligands bind reversibly, with typical association constants (Ka) ranging from 10^4 to 10^6 M^{-1}.

Studying the pH-dependent conformational isomerization of HSA offers valuable insights into its properties and interactions with various molecules, including drugs. These pH-induced changes in HSA are particularly significant in pharmacology and biochemistry, as shifts in pH lead to notable alterations in protein shape and structure. The abundance of acidic and basic amino acid residues in HSA allows it to undergo reversible conformational changes across different pH values (Figure 3).
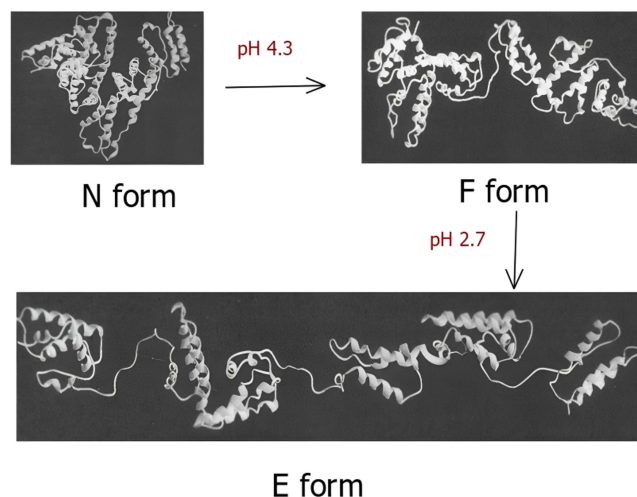

Figure 3 Proposed configurations and interrelation of the N, F, and E isomeric forms of HSA by Carter and Ho (1994) and Peters (1985).

At pH values below 2.7, HSA takes on an elongated structure known as the "E-form." Interestingly, between pH 2.7 and 4.3, HSA adopts a different form, termed the "F-form", which is characterized by increased viscosity, reduced solubility, and a decrease in the alpha-helix content. From pH 4.3 to 8.0, HSA primarily exists in the "N-form", recognized by its heart-shaped structure. When the pH exceeds 8.0, HSA transitions to the "B-form", which is characterized by a reduced alpha-helix content and a greater affinity for certain ligands than the "N-form".[30,34] The spherical arrangement of domains in HSA gives each HSA monomer allosteric properties, enabling it to interact effectively with a diverse array of ligands. This allosteric nature allows HSA to undergo conformational changes, which alter its binding sites and enhance its ability to bind and transport various molecules efficiently. This structural flexibility and adaptability in ligand interactions make HSA essential for transporting substances within the bloodstream.

Tryptophan in the HSA Structure

The tryptophan residue in HSA (Figure 4) is particularly noteworthy because of its strategic position near the hydrophobic cavity of subdomain IIA, the principal active binding region of HSA (eg aspirin, warfarin, diazepam, ibuprofen).[25] This area also contains two binding sites, Sudlow I and II, known for their significant binding affinities classified based on studies on molecular interactions with fluorescent probes. The binding locus of large heterocyclic compounds possessing a negative charge was identified as Site I, with Site II showing a preference for small aromatic carboxylic acids. Consequently, the tryptophan residue is often utilized in spectroscopic studies and is regarded as a valuable tool in this area of research.[36,47,48]
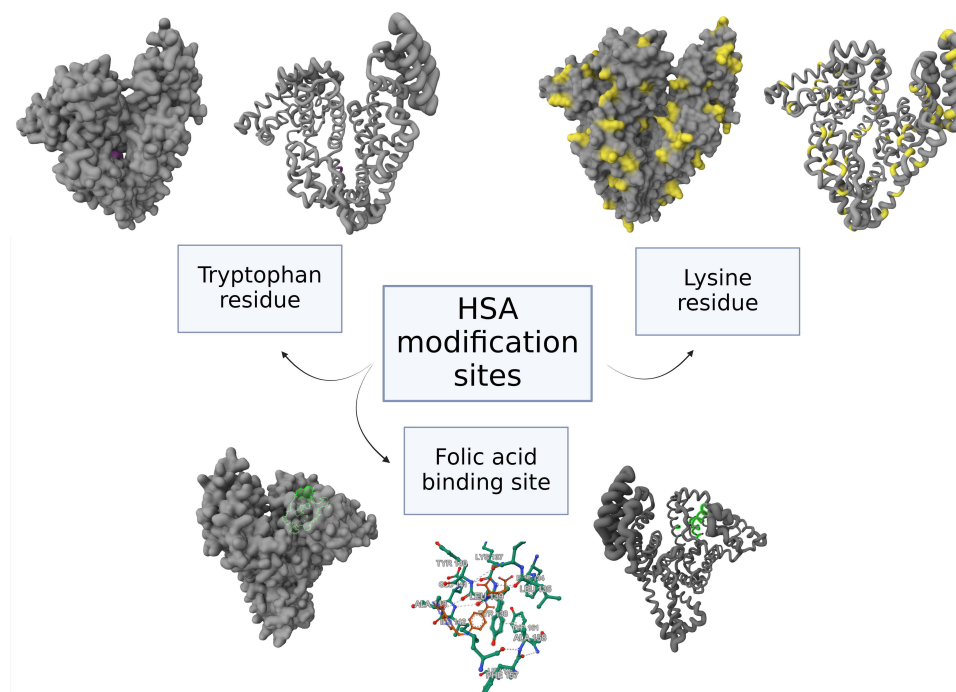
Figure 4 Modification and ligand binding sites on human serum albumin molecules: Tryptophan Residue 214 (Purple), Lysine Residues and N-terminal Primary Amine (Yellow) as Potential mPEG/PEG-NHS Binding Sites, and FA Binding Site (Light Green) with Structural Illustration (Orange) and Labeled Surrounding Amino Acids.[37]

Lysines in the HSA Structure

HSA contains 59 lysine residues with free N-termini (primary amino groups) that have become a key focus for structural modifications of the protein (Figure 4). Lys-199 interacts with drugs of Sudlow's Site I, corresponds to the hydrophobic pocket in subdomain IIA, and his Site II to the pocket within IIIA. The reactivity of Lys-199 may be attributed to its unusually low pKa of 7.9,[24,25] which can be rationalized based on its close interaction with His-242. These residues readily react with N-hydroxysuccinimide (NHS) esters and propionaldehydes, allowing significant alterations to the protein structure without causing denaturation. One common modification involves conjugating polyethylene glycol (PEG) or polyethylene glycol monomethyl ether (mPEG) through reactions between the primary amines of lysine residues and NHS esters or propionaldehydes.[49,50]

Folic Acid Binding Site in the HSA Structure

Human albumin has a binding site for folic acid (FA) located within domain I (Figure 4). The ligand fits into the protein's structure through noncovalent interactions, primarily hydrogen bonds. Structural analysis revealed that folic acid binds to HSA through a combination of hydrophilic and hydrophobic contacts, with a total binding constant in the range of 10^4 to 10^5 M^{-1}.[51] In the folic acid-HSA complex, folic acid is surrounded by Ile-142, Leu-185, Tyr-138, and Trp-161.[51] These findings indicate that HSA can effectively bind and transport folic acid, highlighting its potential for further exploration in the context of folic acid utilization in complex with HSA.

Albumin in Cancer

Despite more than 30 years since identification of albumin receptors, and numerous studies and scientific reviews on their expression and function,[1,9,19,34,52,53] the precise mechanisms by which they operate remain poorly understood.[54,55] This knowledge is however crucial to identify cancer types that respond efficiently to albumin-based drug delivery systems and also to improve efficacy of existing therapies, also in the context of drug resistance.[56]

Mechanisms of Albumin Uptake in Healthy and Cancer Cells

Albumin is not merely a passive protein circulating in bodily fluids, it plays a dynamic role in cellular physiology, tailored to the needs of the environment. Albumin uptake operates through two general mechanisms: endocytosis and micropinocytosis. Endocytosis is specific to substrate-receptor binding, which involves frequently auxillary role of clathrin or caveolin-1 (CAV1). Clathrin-mediated endocytosis operates as a stochastic process, suggesting a more random and ubiquitous mechanism for internalizing molecules, whereas CAV1-dependent endocytosis is spatially restricted to lipid raft regions, indicating a more organized and compartmentalized pathway. This distinction highlights the potential for differential targeting strategies in drug delivery, depending on the desired endocytic route and cellular localization.[57,58] Albumin uptake in healthy cells is primarily mediated by caveolin-1 (CAV-1), however clathrin-mediated endocytosis and macropinocytosis-like processes have been described in pulmonary epithelium or skeletal muscle primary cells.[59,60] Macropinocytosis is not specific to the substrate, but it involves cytoskeleton movement it is actin-dependent. This process enables sampling of large volumes of extracellular fluid and is considered a preferred mechanism for albumin scavenging by cancer cells.[61] It is a form of nutrient sensing and may facilitate survival in nutrient-poor environments.[2,4,7]

Cancer patients often experience hypoalbuminemia, a condition associated with cachexia.[62] This reduction in albumin levels is thought to stem, at least in part, from the disruption of albumin recycling mechanisms that normally maintain its balance in bodily fluids. Under physiological conditions, this recycling is orchestrated by glycoprotein 60 (gp60), the neonatal fragment crystallizable (Fc) receptor (FcRn) and the cubulin/megalin complex in kidneys. However, in the tumor microenvironment, these mechanisms face significant challenges. Driven by the demands of unchecked growth, tumor cells scavenge albumin from their environment. They employ micropinocytosis and upregulate receptor-mediated pathways to access essential nutrients, such as fatty acids and iron. Notably, receptors like glycoprotein 18/30 (gp18/30), Cluster of Differentiation 36 (CD36) or Transferrin receptor protein 1 (TfR1) play key roles in this process, making them attractive targets for therapeutic strategies (Table 1). A deeper understanding of these pathways is essential to justify modifications that circumvent resistance mechanisms, paving the way for more effective interventions.

gp60

The first and most described receptor responsible for native albumin binding is gp60, also known as albondin. It serves as a key mediator of native albumin transcytosis across endothelial cells, which is essential for maintaining vascular oncotic pressure and delivering nutrients bound to albumin to tissues where they are needed, including tumor microenvironment[63] (Table 1). Binding of albumin to gp60 initiates a CAV-1-dependent formation of caveolae – a structure that facilitates transcellular transport (Figures 5 and 6).[64] CAV-1 correlates with a favorable response to Abraxane, suggesting that gp60 might be involved in Abraxane uptake, it is unclear whether gp60 would be able to recognize albumin-based nano carriers.[64] Despite its importance, research on gp60 remains limited by the lack of specific tools to study its mechanisms in detail.[56,65] Several sources reference a 1984 paper by Schnitzer et al, describing a sequence of gp60 derived from bovine leukemia virus envelope proteins,[66] later identified as the gp60 albumin receptor.[53] However, up to date this sequence is not deposited in any database. Some groups have reported the use of antibodies for immunofluorescent labeling directed against gp60/110 isolated from human herpesvirus 6,[56,67] but it is not suggested by the antibody producer that it can be used to visualize human gp60. Another discrepancy in the literature concerns the localization of the gp60 receptor. While it is widely accepted that gp60 receptors are located on endothelial cells, some sources, perhaps erroneously, report their presence on the surface of cancer cells.[68] This possibility cannot be entirely excluded, given the lack of appropriate investigative tools and limited data on gp60 expression. Understanding the nuances of gp60-mediated albumin transcytosis could reveal new therapeutic targets, particularly in diseases where albumin transport is co-opted for pathological purposes.

Neonatal Fc Receptors

Albumin recycling is possible because of FcRn receptors, that are widely expressed in various tissues.[69–75] In mice, FcRn deficiency caused rapid albumin turnover and 50% reduction in the half-life in blood serum.[76,77] This effect was irrespective of increased albumin production in those animals.[26,76]

FcRn-mediated albumin recycling is an elegant, pH-dependent mechanism (Figure 5) Figure 3. FcRn does not interact with albumin at neutral pH (pH 7.0), but binds it efficiently at acidic pH (6.5–5.5). When albumin is internalized either

Table 1 The Ability of Selected Albumin-Recognizing Receptors to Improve Cancer Cell's Targetability and Nanoparticle Design

| Receptor	Function	Albumin-Based Ligand	Role in Cancer	Therapeutic Applications	Localization
FcRn	Albumin recycling, extending serum half-life	Native albumin; nanoparticles	Reduced expression in many cancers, associated with poor prognosis	Target for prolonging the half-life of albumin-based drugs; research into increasing expression for therapeutic benefits	Cell surface; Endosomes
gp60 (Albondin)	Albumin transcytosis through endothelial cells	Native albumin; nanoparticles	Overexpressed in cancers, promoting albumin accumulation in the TME	Therapeutic target of albumin-coated/conjugated nanoparticle; potential biomarker for predicting treatment efficacy; limited by the lack of available research tools	Endothelial cell surface; Cancer cell surface?
gp18/gp30	Degradation of modified albumin	Modified or damaged albumin (eg, ischemia-modified albumin)	Important in cancer cell metabolism under hypoxia and oxidative stress conditions	Potential target for cancer therapy design, but limited by the lack of available research tools	Endothelial cell surface; Cancer cell surface?
CD36	Uptake of long-chain fatty acids, lipoproteins, and albumin	Albumin (including oxidized, glycated forms)	Overexpressed in cancers, facilitates albumin transcytosis and nutrient delivery	Potential target for cancer therapies; research into CD36 inhibition	Cancer cell surface
TfR1	Promotes cancer cell proliferation, survival, and neoangiogenesis	Transferrin, Heme-albumin	Overexpressed in multiple cancers, including pancreatic, colorectal, lung, and bladder cancers	Research into targeted therapies with anti-TfR1 antibodies; potential use of HSA-heme in drug delivery	Cancer cell surface
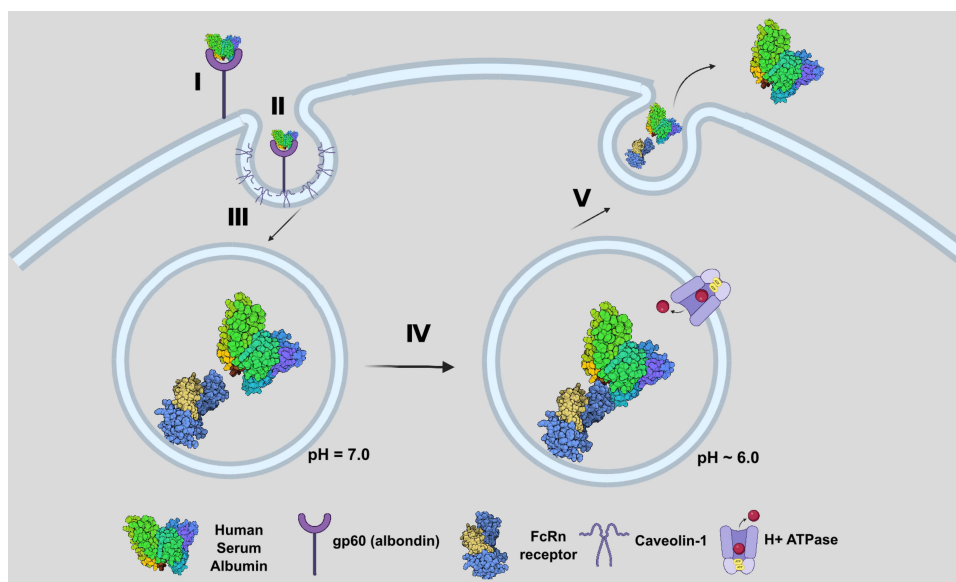
Figure 5 Gp60 recycles undamaged albumin. Albumin is recognized by gp60 glycoprotein receptors on the cell surface (I). After binding, albumin is internalized via caveolin-1-mediated endocytosis (II) and becomes entrapped in an endosome (III). Because pH changes due to H+ ATPase activity, the FcRn receptor located in the endosome subsequently recognizes albumin (IV), which prevents it from further lysosomal degradation. Endosomes merge with the cellular membrane, and the pH increases, resulting in albumin release (V).
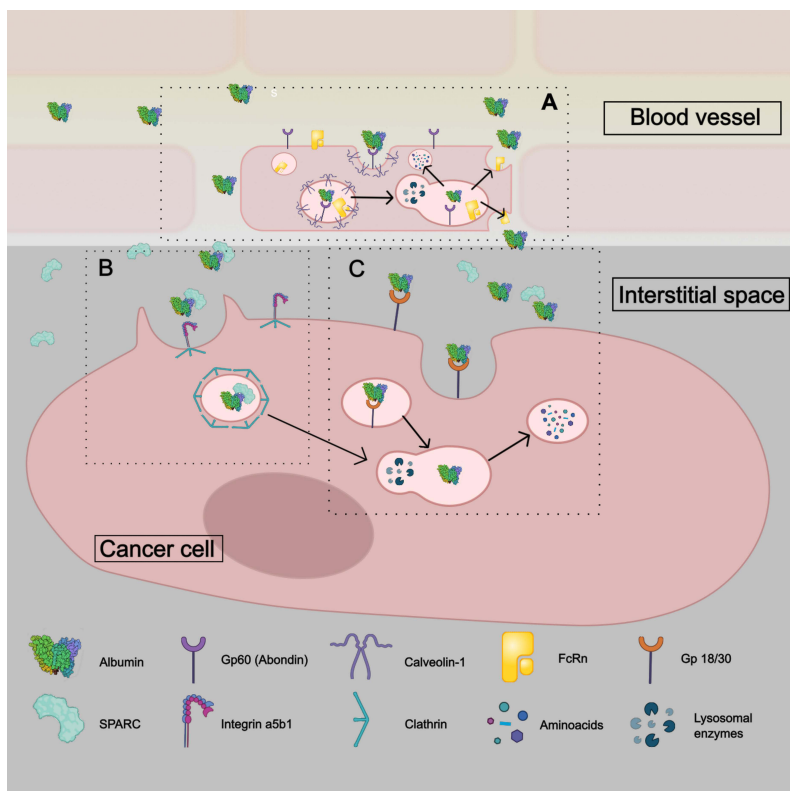

Figure 6 Pathways of albumin acquisition by cancer cells. Albumin or albumin-based nanoparticles present in the bloodstream (**A**) can enter the tumor interstitial space through the leaky vasculature or via transcytosis mediated by the gp60/caveolin-1/FcRn pathway. (**B**) Alternatively, albumin may be internalized by macropinocytosis, where the invagination of the cell membrane encloses both albumin and the FcRn receptor within an endosome. This process protects albumin from degradation, allowing it to be released into the extracellular milieu. A detailed description of this process is provided in Figure 5. In the interstitial space, albumin or albumin-based nanoparticles can interact with the SPARC (secreted protein acidic and rich in cysteine) protein and be endocytosed in a clathrin-dependent manner, potentially entering the lysosomal degradation pathway. (**C**) An additional pathway involves the gp18/30 system, where albumin or albumin-based nanoparticles are internalized and degraded within the lysosomal compartment. This process is further detailed in Figure 7.

by gp60-receptor or by micropinocytosis, it enters a cell with an endosome at neutral pH. When pH within endosome decreases, it activates FcRn to bind albumin. This event prevents albumin degradation in the lysosome. The endosome then releases its contents into the serum, where the pH is approximately 7.0–7.4. The binding affinity between albumin and FcRn decreases again, allowing the release of albumin back into the extracellular space.[27,77,78] Notably, the tumor extracellular microenvironment is characterized by intense acidification, with pH values ranging between 6.4 and 7.0.[79] While this acidic milieu theoretically may influence the release of albumin into the extracellular space, this aspect remains unexplored in the literature. The literature is also inconsistent in the meaning of the FcRn expression level in cancer. There is a significant reduction in FcRn expression, associated with poor prognosis in many cancer types.[80,81] Swiercz et al has also demonstrated that increased FcRn expression prevents albumin scavenging and instead promotes albumin recycling, which reduces glutamine levels in cancer cells and decreases the number of metastases.[80] In contrast, Larsen et al reported that FcRn overexpression in human cancer cells promotes albumin recruitment and cellular growth in breast and colorectal cancer cell lines. As proof of principle, human FcRn knockout in a mouse xenograft significantly decreased the tumor volume. Additionally, the authors reported that FcRn was significantly overexpressed at the protein level in eight out of ten investigated cancer types, including breast, cervical, pancreatic, lung, colorectal, head and neck and renal cancer. In the discussion, the authors underline the need for further research in terms of albumin acquisition by cancer cells in the context of future targeted albumin-based therapies.[82] The evidence points to a deeper story, where the interaction between FcRn and cancer emerges as a pivotal factor in shaping the success of albumin-based therapies. This discrepancy hints that the therapeutic outcome may depend significantly on FcRn expression levels, varying across cancer types and individual patients.

SPARC

Research conducted from the late 1990s revealed that secreted protein acidic and rich in cysteine (SPARC, osteonectin, BM-40) is highly expressed in stromal tissue and various advanced cancers.[83–85] Indeed, SPARC plays a significant role in neovascularization,[53,86,87] metastasis, and cancer cell survival, underscoring its critical contribution to tumor progression.[53,88–90] SPARC regulates cancer interactions with the extracellular matrix (ECM) and has a strong affinity for albumin, which can lead to albumin accumulation in inflamed and tumorous tissues.[91] SPARC-bound albumin facilitates the gp60/CAV-1-mediated transcytosis and simultaneously protects and directs albumin to cancer cells (Figure 6). Main SPARC-albumin receptor on the cancer cell surface is α5β1 integrin complex.[60,92] The internalization of SPARC depends on clathrin, and the subsequent fate of albumin remains uncertain.[60,93] SPARC regulates transport from early endosomes to late endosomes, suggesting that albumin enters the lysosomal pathway.[3,52,60,94] However, some studies report that albumin may become trapped in endolysosomal or autophagosomal compartments.[95] Interestingly, Abraxane appears to be rescued from lysosomal degradation by SPARC, which inhibits the cytotoxic effect of Abraxane but not paclitaxel.[65]

gp18/gp30

In contrast to gp60, gp18/30 are membrane receptors that bind modified or damaged albumin,[96–98] for instance ischemia-modified albumin (IMA).[99] These receptors were identified via agarose gel electrophoresis[98] and first described by Schnitzer JE & Bravo J in 1986.[98] After receptor recognition, albumin is internalized through macropinocytosis and directed to the lysosomal degradation pathway, making it a key route used by cancer cells for albumin scavenging (Figure 7). Damaged vasculature and the pressure exerted by the growing tumor on surrounding tissues, leads to inadequate nutrient supply through blood vessels. As a result, tissues resort to alternative means for essential amino acids to suffice cellular anabolism. It is unknown to what extent are those receptors involved in HSA nanoparticles intake, however Abraxane, initially hypothesized to be internalized through the gp60/CAV-1 system, is actually transported via the denatured albumin transport system involving gp18/30.[65] The pathway of nanoparticle internalization is critical, as it determines the intracellular fate of the cargo. While degradation before reaching the molecular target can reduce therapeutic efficacy, this is not the case for Abraxane. Transport via gp18/30 still allows for efficient delivery of paclitaxel, outperforming administration without a carrier. This mechanism also holds significance for overcoming treatment resistance, a topic explored further in the subsequent sections.
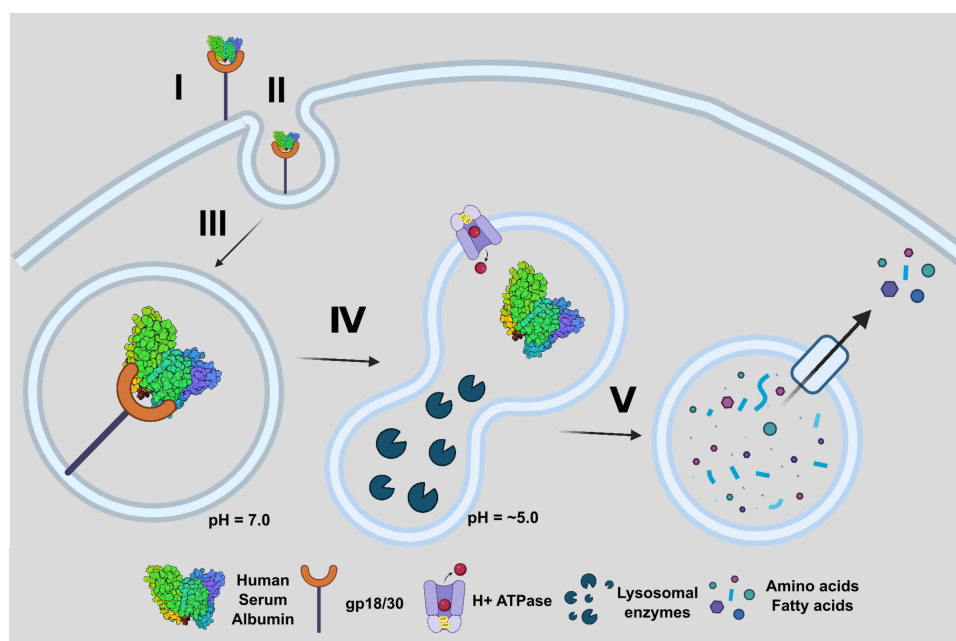
Figure 7 Gp18/30 recognizes damaged albumin and targets it for degradation. Damaged albumin is recognized by glycoprotein receptors on the cell surface, specifically by gp18/30 (I). Upon binding, albumin is internalized via clathrin-mediated endocytosis (II) and becomes entrapped in an endosome (III). The activity of H+ ATPase leads to a reduction in pH (IV). The endosome subsequently merges with the lysosome, and degradation enzymes are released (IV). Albumin is digested into amino acids, and its fatty acid cargo is salvaged which can be further used by cancer cells (V).

CD36

The interaction between albumin and the cluster of differentiation 36 (CD36) transmembrane receptor is well-documented in the literature, yet therapeutic approaches targeting this specific interaction remain largely unexplored. Cancer cells, notably, often overexpress CD36, a receptor essential for the uptake of long-chain fatty acids and lipoproteins, and which plays a pivotal role in immunological recognition, inflammation, cell adhesion, and apoptosis.[100,101] Albumin, both in its native and modified forms, serves as one of its primary ligands.[102] A series of experiments conducted by Raheel et al shed light on the functional relevance of this interaction, demonstrating that CD36 mediates albumin transcytosis across the dermis, and, to a lesser extent, the pulmonary microvascular endothelium. Interestingly, they found that this process of transcytosis could be saturable and exhibited variability depending on the tissue type, suggesting the potential for targeting this pathway in therapeutic applications.[54]

Transferrin Receptor Protein 1

TfR1 plays a key role in cancer cell survival through NF-κB activation,[103] stimulation of neoangiogenesis[104] and altered energy metabolism, leading to its overexpression in various cancers.[103,105,106] Despite efforts to use anti-TfR1 antibodies for targeted drug delivery, safety concerns have hindered their success.[107] TfR1 can also recognizes heme-albumin, facilitating the transport of nutrients or drugs into cells.[108] HSA binds heme at two stites: one unaffected by fatty acids.[109–111] The role of HSA in heme scavenging and delivery has been extensively reviewed.[112] Brell et al demonstrated that TfR1 recognizes heme-albumin in Abraxane, promoting endocytosis and proliferation as efficiently as iron-loaded transferrin. Inhibition of TfR1 with the monoclonal antibody mAb 5–528, educed apoptotic cells by 25%, likely due to impaired endocytosis and reduced cytotoxicity.[108] The potential of heme-albumin in targeting tumors compared to unmodified albumin remains to be explored. Additionally, HSA-based nanoparticles may spontaneously incorporate hemin, enabling TfR1-mediated uptake and improving drug delivery in cancer therapy.

Bae et al proposed another potential application of increased TfR expression on cancer cell surfaces. In their study, they developed doxorubicin-loaded nanoparticles functionalized with transferrin using sulfosuccinimidyl-4-(N-maleimidomethyl)cyclohexane-1-carboxylate (Sulfo-SMCC). The transferrin modification aimed to enhance receptor-mediated

endocytosis and successfully overcame multidrug resistance in MCF-7/ADR cells, which is significant given that this resistance is typically mediated by MDR1. This highlights the critical importance of targeting TfR1 as a viable strategy for bypassing MDR1-associated resistance and improving therapeutic efficacy in drug-resistant cancers.[113]

Albumin and Multi Drug Resistance in Cancer

Cancers possess the ability to expel harmful substances from their cells by inducing multidrug resistance (MDR) systems, such as MDR1. When albumin delivers toxic drugs to cancer cells, it raises the question of whether tumors counteract this by upregulating MDR1. Insights into this question come from years of research on Abraxane, the first albumin-based drug approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). A related and equally critical question is whether albumin can circumvent an already active MDR1 system, effectively delivering the drug despite the presence of functional efflux pumps. These issues will be addressed in this section.

Resistance to Abraxane

Abraxane (ABI-007, nab-paclitaxel) is a 130 nm albumin-bound paclitaxel formulation used for various solid tumors.[114] It is twice as effective as unbound paclitaxel, eliminates toxic solvents, and allows for higher dosing.[114–116] Paclitaxel is a well-known substrate for MDR1.[117] In a Phase 3 clinical trial (NCT00046514), Abraxane was administered to patients with metastatic breast cancer who had likely developed taxane resistance likely due to MDR1 induction. The study demonstrated that Abraxane effectively bypassed cross-resistance, even in patients heavily pretreated with solvent-based paclitaxel or docetaxel.[118]

The potential of Abraxane to circumvent multidrug resistance (MDR) was further investigated in vitro (Table 2). Zhao et al established a non-small cell lung cancer cell line resistant to Abraxane (A549/Abr) and used RNA-Seq to compare its transcriptome with that of the parental line. They reported significant *MDR1* overexpression in A549/Abr cells, a common mechanism of resistance to unbound paclitaxel, and confirmed that MDR1 contributes to resistance by reversing the phenotype with verapamil, an MDR1 inhibitor.[119] In another study, Zhao et al analyzed global protein expression changes between treatment-naïve and treatment-resistant A549/Abr cells.[120] These authors reported *MDR1* overexpression and suggested that resistance arises from the release of paclitaxel from albumin, as MDR1 cannot export the large albumin–paclitaxel complex. This study identified additional proteins related to lipid metabolism, the cell cycle, the cytoskeleton and apoptosis processes, including novel resistance markers such as the E3-ubiquitin ligase ring finger protein 139 (RNF139) and an enzyme involved in cholesterol synthesis, 3-hydroxy-3-methylglutaryl-CoA synthase 1 (HMGCS1).[120] Similarly, Vallo et al reported that MDR1 overexpression in urothelial cell lines caused resistance to nab-paclitaxel.[121] While Abraxane remains pioneering, analogous systems have been developed for the controlled delivery of various chemotherapeutics. However, the MDR1 substrate repertoire accommodates a variety of hydrophobic substances. Kong et al tested gemcitabine-loaded HSA nanoparticles in pancreatic cancer cells resistant to gemcitabine due to MDR1 and MRP1 overexpression. The nanoparticles inhibited proliferation and induced apoptosis in these resistant cells.[122] The authors proposed that this effect was likely due to ATP consumption during albumin degradation, which limits ATP availability for ATP-dependent ABC transporters such as MDR1 or MRP1.

Onafuye et al developed HSA-based nanoparticles encapsulating doxorubicin and tested them in neuroblastoma cells resistant to vincristine and doxorubicin respectively. The doxorubicin-loaded nanoparticles were effective in MDR1-expressing cell lines, particularly in vincristine-resistant cells. In doxorubicin-resistant cells, the effect was present but less pronounced, possibly because the released drug faces a resistance mechanism similar to that of doxorubicin in solution.[123] This highlights the possibility that multiple resistance mechanisms may be involved depending on the specific chemotherapeutic agent used.

Yuan et al investigated triple-negative breast cancer, which is characterized by a high percentage of cancer stem cells (CSCs), which are key drivers of tumor progression and metastasis.[124] CSCs are chemoresistant due to mechanisms such as MDR1 overexpression and the activity of detoxifying enzymes, such as aldehyde hydroxygenase (ALDH). The authors reported that while Taxol and Abraxane had similar efficacy in reducing tumor size, Abraxane significantly reduced the frequency of CSCs by 3–9-fold and was more effectively taken up by ALDH+ cells than Taxol.[125] Although

Table 2 HSA-Based Nanoparticles in MDR1-Expressing Cancer Cell Lines and Their Outcomes

Cell Line	Source of Resistance	Observations	Conclusion	Ref
A549 (non-small cell lung cancer)	Abraxane	A549/Abr exhibited a significant overexpression of *ABCB1* mRNA. Resistance to Abraxane was mainly mediated by overexpression of ABCB1 and other identified proteins, related to lipid metabolism, cell cycle, cytoskeleton and apoptosis processes	The cause of MDR1 induction is the release of Paclitaxel from albumin, as the albumin itself is too large to be exported by ABCB1. The origin of Abraxane resistance is rather complex	[105,106]
T24 and TCC-SUP (human transitional cell Carcinoma)	Gemcitabine and Vinblastine	Urothelial cell lines with ABCB1 expression were similarly resistant to nab-paclitaxel and paclitaxel. Doxorubine-loaded HSA nanoparticles overcame the MDR1-mediated resistance	The interaction of paclitaxel and albumin might be different than in the case of doxorubicin-loaded nanoparticles because of the use of glutaraldehyde as a cross-linker	[107]
BxPC3 and PANC1 (pancreatic cancer)	Lentivirus-mediated overexpression of MDR1 and MRP1	The gemcitabine-loaded human serum albumin nanoparticle inhibited proliferation and induced apoptosis in resistant cell line	MDR1 and MRP1 are ATP-dependent therefore this effect was possibly a result of ATP consumption during albumin degradation, which would competitively limit the available cellular ATP	[108]
UKF-NB-3 (neuroblastoma)	Vincristine and Paclitaxel	Doxorubicin-loaded HSA-based nanoparticle caused cytotoxicity in ABCB1-expressing cell lines. The effect was more pronounced in cells resistant to vincristine than in those resistant to doxorubicin	ABCB1 expression is a primary doxorubicin resistance mechanism. The drug is released from the nanoparticle, thus being subjected to the same resistance mechanism as doxorubicin alone in solution.	[109]
CSCs ALDH+ (triple-negative breast cancer patients) and SUM149 (human breast inflammatory carcinoma)	Taxol/Abraxane	Taxol and Abraxane showed similar drug concentration within the tumor and tumor shrinking effects. Abraxane significantly reduced the frequency of CSCs by 3-9-fold and was efficiently taken up by ALDH+ cells compared to Taxol	Utilizing albumin as a carrier may enhance chemotherapy outcomes, even in cases of drug resistance	[111]
MCF-7/ADR (human breast tumor)	Adriamycin	HSA-based nanoparticle, decorated with EGFR-targeting antibody, Cetuximab, delivering doxorubicin and short interfering RNA targeting MDR1 (siMDR1) inhibited MDR1 expression in vitro and in vivo without noticeable off target toxicity and caused enhanced intracellular delivery of doxorubicin	Due to the active and passive targeting effect, nanoparticles exhibited an enhanced tumor targeting effect and tumor growth inhibition after in vivo administration.	[115]
EC109 (human esophageal cancer)	Taxol	The folate-conjugated HSA nanoparticles coencapsulating paclitaxel and the chemosensitizer-2 exhibited improved target specificity and caused less damage to noncancerous tissues	The folic acid receptor is likely involved in the nanoparticle uptake, which limits the action of P-glycoprotein (P-gp)	[116]

multidrug resistance has not been specifically studied, the use of albumin as a carrier may enhance chemotherapy outcomes, even in cases of drug resistance.

Overall, despite conflicting evidence, albumin-based delivery systems show promise in facilitating drug transport, potentially overcoming multidrug resistance independent of active MDR1. There are possible specific modifications that would allow for active MDR1 targeting.

HSA-Based Drug Delivery in Presence of Active MDR1

Cancer cells often overexpress MDR1, reducing the efficacy of therapy by decreasing the bioavailability of therapeutics.[126] Specific allosteric MDR1 inhibitors (reviewed in[127]), have been developed, but clinical trials have shown that they can cause significant toxic side effects due to MDR1's role in regulating the distribution of various substances in the body.[128] Therefore, enhancing drug delivery into cells may be more beneficial than inhibiting MDR1 directly (Table 2).

Yang et al developed HSA-based nanoparticles decorated with the EGFR-targeting antibody cetuximab to deliver short interfering RNA (siRNA) targeting MDR1 (siMDR1) and doxorubicin to breast cancer tissues. This nanoparticle successfully reversed multidrug resistance in vitro and in vivo with minimal off-target toxicity.[129] Similarly, Liu et al coencapsulated paclitaxel and chemosensitizer-2 in folate-conjugated HSA nanoparticles. This approach aimed to reduce multidrug resistance and improve antitumor efficiency. The nanoparticles showed enhanced target specificity and reduced damage to noncancerous tissues, suggesting that folic acid receptor-mediated uptake limited the action of MDR1 (folic acid as a target for HSA modification is further described in Section 4.3).[130]

PEG, which is commonly used as an excipient in pharmaceuticals, can be efficiently conjugated to HSA and HSA-core nanoparticles through specific binding sites on albumin. This involves the use of functional groups, such as the -NHS ester of PEG, which bind to primary amines on the free N-termini of lysine residues within the albumin structure. This modification enables HSA nanoparticles to enhance drug delivery and inhibit MDR1 activity, improving drug bioavailability.[131,132] Hodaei et al demonstrated that the effectiveness of PEG depends on its molecular weight.[133] While the exact mechanism is not fully understood, it may be related to changes in membrane fluidity[134,135] and direct inhibition of MDR1 through alkyl and unsaturated C–C bonds in PEG.[136]

HSA Utilization in Receptor-Based Targeting Strategies

The intrinsic properties of albumin as a drug carrier offer a unique advantage in bypassing MDR1-mediated drug resistance by facilitating efficient cargo delivery to cancer cells. Yet, the challenge of multidrug resistance extends beyond simple transport—it demands strategies that simultaneously enhance membrane translocation and therapeutic specificity. By addressing these dual requirements, it becomes possible to disrupt the mechanisms that enable cancer cells to expel therapeutic agents. HSA-based nanotherapeutics employ passive targeting, mainly EPR effect, and active targeting strategies. Due to its large molecular size, passive diffusion of albumin is not feasible; instead, tissue accumulation occurs primarily through endocytosis.[137] Active targeting involves functionalizing the surface of albumin nanoparticles with ligands that bind to receptors overexpressed on cancer cells (Figure 1). This approach enhances specific targeting of affected tissues, increasing the uptake of drug-loaded nanoparticles by cancer cells while reducing systemic exposure and toxicity. Thanks to albumin's inherent properties and ease of modification, ligands can be conjugated to the protein, facilitating the internalization of nanoparticles and their cargo via endocytosis (Figure 8).

In pharmaceutical applications, albumin serves as a crucial carrier for drugs through two primary mechanisms: albumin–drug conjugation and drug encapsulation within albumin nanoparticles. Albumin–drug conjugates include albumin-fusion proteins, drugs coupled to exogenous albumin, and prodrugs designed to bind efficiently to endogenous serum albumin. While modified albumin molecules can enhance the pharmacokinetic profiles of drugs, nanoparticle formulations improve their stability and solubility. Fan et al proposed a preclinical optimization strategy that correlates a drug's binding affinity to albumin with its serum half-life, influencing dosing regimens and therapeutic effectiveness.[138]

Protein modification techniques can enhance drug delivery precision and reduce chemotherapy side effects. By chemically modifying the hydroxyl, amine, and carboxyl groups on HSA, selective binding to cancer cell receptors can be achieved. Strategies targeting tumor-specific markers, such as transferrin receptors, aptamers, targeting peptides, CD44, folate receptors, and others, have been explored in nanocarrier systems.[139] Despite promising reports, only a few
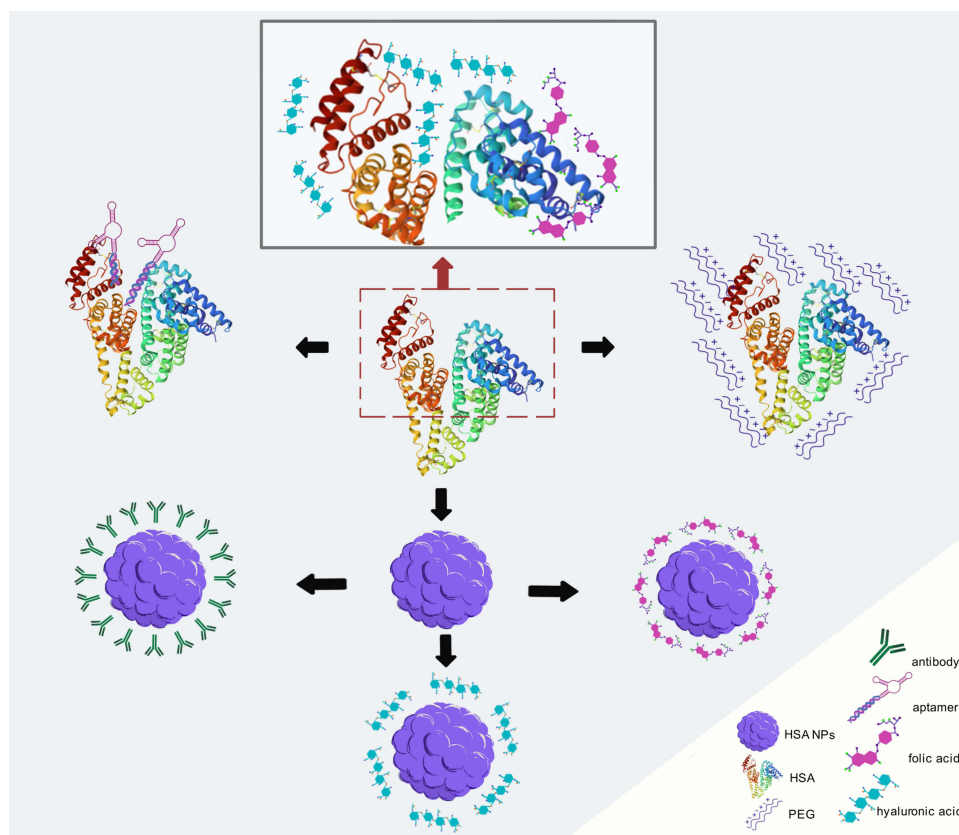
Figure 8 Utilization of human serum albumin as a versatile drug carrier.

of these modifications have been widely implemented (Table 3). Advances in albumin modification, as described in recent studies, represent a critical step toward achieving this objective (Figure 8). In this section, we delve into the innovative modifications of albumin that not only improve drug delivery efficiency but also hold potential to overcome resistance mechanisms, paving the way for more effective cancer therapies.

Aptamers

Aptamers are short single-stranded oligonucleotides capable of selectively binding to tumor tissue, offering advantages over monoclonal antibodies, such as lower cost, reduced immunogenicity, and easier high-throughput screening and chemical synthesis.[152] However, their small size (approximately 20 kDa) leads to rapid renal clearance, limiting their clinical use. Conjugating aptamers with larger molecules, such as albumin, extends their circulation time in the bloodstream.[153] Although aptamers have shown potential to improve cancer therapy (as reviewed in[154–156]), human serum albumin–aptamer conjugates have yet to be explored in this context. There have been attempts to enhance immunotherapy with bovine serum albumin conjugated with PD-L1[157,158] or CTLA-4 aptamers.[159] In non-cancer applications, Zhang et al reported the double conjugation of a newly designed bone anabolic aptamer targeting sclerostin, which exhibits high binding affinity for human serum albumin, thereby extending the system's half-life.[153] These findings, along with results from aptamer-conjugated BSA nanoparticles, underscore the potential of albumin as an aptamer carrier. This research holds significant promise and is crucial for advancing translational oncology.

Hyaluronic Acid (HA)

HA is an unbranched glycosaminoglycan that is an important component of the extracellular matrix. Its synthesis increases in proliferating cells, which indicates its role in the metabolism of cancer cells.[160] The HA receptor CD44 is present to varying degrees on multiple cells in the body. It plays a role in cell adhesion and migration, inhibits apoptotic

Table 3 Examples of Cancer-Specific Modifications of Albumin

Cancer Type	Receptor	Transported Drug	Nanoparticle	Outcome	Reference
Human non-small-cell lung cancer	CD44	Erlotinib	HSA nanoparticles loaded with erlotinib and coated with hyaluronic acid via the electrostatic deposition	In vitro, Erlotinib release was slow and steady, without noticeable burst effects. In vivo, the formulation exhibited significant antiproliferative effects, suppressed tumor growth, and prevented relapse for 30 days. Hyaluronic acid enhanced the uptake of ERT-NPs by A549 cells and improved bioavailability. No substantial differences in pharmacokinetic parameters were observed between ERT-HSA NPs and HA-modified nanoparticles.	[140]
MCF7 and MDA-MB-231 breast cancer cells	CD44	Doxorubicin	Hyaluronic acid-human serum albumin conjugate that was further organized into redox-crosslinked nanoparticles (HAlbNPs) were loaded with doxorubicin	The results showed a selective 2- to 8-fold increase in DOX efficacy against cancer cells, with no additional toxic effects on healthy mammalian cells. DOX-loaded nanoparticles targeted multiple aspects of metastasis, including colony formation, cell motility, and inhibition of metalloproteinases.	[141]
CD44-enriched Murine melanoma B16F10 cell line, MCF7 human breast cancer cells	CD44	All trans retinoic acid (ATRA)	ATRA encapsulated within a BSA-based nanoparticle functionalized with HA (HA-eNPs)	HA-eNPs exhibited prolonged release of ATRA and increased cytotoxicity. HA functionalization enhanced binding affinity to CD44-enriched B16F10 cells. In vivo, the nanoparticles successfully accumulated in the lungs of tumor-bearing mice, resulting in significant inhibition of metastasis.	[142]
HeLa cells (cervical cancer) overexpressing the folate receptor and human non-small-cell lung cancer A549 cells (FR-negative)	Folate receptor	Cabazitaxel	FA-functionalized HSA nanoparticles loaded with cabazitaxel FA-NPs-CTX	FA-NPs-CTX exhibited increased cytotoxicity against HeLa cells overexpressing the folate receptor (FR) compared to CTX-loaded human serum albumin. In a HeLa xenograft mouse model, it showed enhanced tumor accumulation, which resulted in inhibited tumor growth and reduced systemic toxicity. The formulation improved the safety and tolerability of cabazitaxel.	[143]
Human non-small-cell lung cancer A549, lung adenocarcinoma lesions induced by urethane	Folate receptor	Actionin	Folate conjugated actionin-loaded HSA nanoparticles	The formulation increased the cytotoxic effect of the drug compared to its free form and enhanced anticancer effects both in vitro and in vivo. Additionally, in vivo nanoformulated actionin demonstrated anti-cancerous effects in low dose frequency	[144]

MCF7 human breast cancer cells	Folate receptor	Hydroxycamptothecin (HCPT)	Folate-decorated nano-hydroxycamptothecin-loaded HSA	The FA-HSA-HCPT-NPs showed slow release, high tumor inhibitory rate and increased targeting properties. In vivo, the toxicity was reduced to free HCPT.	[145]
MCF7 human breast cancer cells	Folate receptor	5-fluorouracil and curcumin	Folic acid-decorated HSA-coated citrate-functionalized Fe3O4 codelivering 5-fluorouracil and curcumin	Magnetic field increased cellular uptake in C-MNP-HSA-FA nanoparticles and subsequent cytotoxicity	[146]
HeLa cervical cancer cell line	Folate receptor	Doxorubicin, nanorods	Folic acid (FA) conjugated to HSA, loaded with doxorubicin, and supplemented with gold nanorods for photothermal therapy (HSA-AuNR-Dox NP)	HSA-AuNR-Dox NP sensitized HeLa cells to irradiation, enhancing synergy between chemotherapy and photothermal therapy. Drug release was pH- and light irradiation-dependent. FA functionalization increased internalization. Cell toxicity was further enhanced when Au NR-DOX-HSA-FA nanoparticles were irradiated with NIR light. This dual approach could be useful for drug-resistant cells, where hyperthermia may complement cell death.	[147]
HER2+ Breast cancer	HER2-receptor	Methotrexate	TMAB-coated methotrexate-HSA conjugate nanoparticle (TMAB MTX-HSA)	Enhanced cytotoxicity and cellular uptake in HER2-positive cells compared to non-targeted MTX-HSA NPs and free MTX; excessive TMAB density reduces effectiveness due to steric hindrance; optimized antibody density crucial for targeting and therapeutic efficacy.	[148]
EGFR+ Breast cancer	EGFR receptor	Docetaxel (DTX)	Conjugation of short chain fragment variable (scFv) of anti EGFR antibody (scFv-EGFR) to DTX-HSA-immunonanoparticles	Enhanced accumulation and cytotoxicity in EGFR-positive breast cancer cells compared to non-targeted NPs and marketed formulations; improved pharmacokinetics and prolonged circulation suggest promise for breast cancer therapy.	[149]

(*Continued*)

Table 3 (Continued).

Cancer Type	Receptor	Transported Drug	Nanoparticle	Outcome	Reference
Esophageal cancer cells KYSE150, KYSE520, Eca109	EGFR receptor	Lidamycin (apoprotein) (LDP) or enediyne chromophore (AE)	Fusion protein of fragment variable (Fv) of anti-EGFR antibody, (LDP) or (AE) and the third domain of human serum albumin (D3) (Fv-LDP-D3 or Fv-LDP-D3-AE)	ScFv-LDP-D3 binds EGFR-overexpressing esophageal cancer cells, inhibits EGFR phosphorylation, and downregulates IMPDH2 expression. Enhanced macropinocytosis, tumor-specific accumulation (26-day retention), and apoptosis induction were observed. Fv-LDP-D3-AE, with lidamycin's enediyne chromophore, exhibited potent cytotoxicity, DNA damage, G2/M arrest, and mitochondrial damage. Both agents inhibited tumor growth in esophageal cancer xenografts, indicating their promise for targeted esophageal cancer therapy.	[150]
–	PD1 receptor	PD1-inhibiting nanobody	Fusion protein MY2935 consisting of HSA and anti PD-1 nanobody	Prolonged half-life; stimulates the immune function through PD-1	[151]

pathways, promotes cell proliferation, and may also enhance early angiogenesis in tumors (detailed in[161,162]). While present in normal tissue, CD44 is overexpressed in multiple tumors and has been demonstrated to be correlated with increased aggressiveness and a greater risk of metastasis.[163] Spadea et al reported that cancer cell lines that express standard CD44 (the shortest CD44 isoform[164]), which is present in high quantities on the membrane of cancer cells, can efficiently take up HA. Furthermore, owing to heightened receptor activity, the uptake of HA by malignant cells surpasses that of noncancerous cells, as evidenced by various studies.[164–166] This phenomenon may enhance drug accumulation within cancerous tissues relative to healthy tissues. Importantly, CD44 is also a molecular marker specific for cancer stem cells (CSCs). Moreover, hyaluronic acid is a nontoxic, biocompatible, and biodegradable polymer, which makes it an excellent candidate for use as a targeting ligand in delivering drugs to tumors, including HSA-based delivery systems (Table 3). Shen Y et al synthesized spherical albumin nanoparticles loaded with erlotinib and coated them with hyaluronic acid via the electrostatic deposition technique. This approach enhanced drug uptake and anticancer effects in the lung cancer cell line A549, which was further confirmed in experiments using a mouse model.[140] Brindisi M et al designed a similar system in which they synthesized a hyaluronic acid-human serum albumin conjugate that was further organized into redox-crosslinked nanoparticles (HAlbNPs). The HA-HSA NPs were loaded with doxorubicin (DOX). The authors tested several breast cancer cell lines with various CD44 receptor expressions and evaluated cancer cell viability and the main molecular drug resistance and metastasis pathways. The results demonstrated a selective, 2- to 8-fold increase in the efficacy of DOX against cancer cells, without an increased toxic effect on healthy mammal cells.[141] HA demonstrates excellent targeting capabilities for CSCs, as demonstrated by Li et al, through the utilization of all-trans retinoic acid encapsulated within a BSA-based nanoparticle functionalized with HA (HA-eNPs). These HA-eNPs exhibited heightened binding affinity to the CD44-enriched B16F10 cell population and effectively accumulated in the lungs of tumor-bearing mice, leading to significant inhibition of metastasis.[142]

Folic Acids

Folates play crucial roles in cell growth, survival and proliferation.[167] Because of their hydrophilic properties, folates rely on folate receptors (FRs) for the cellular internalization through receptor-mediated transcytosis.[168,169] Tumor cells require folates for essential processes, a vulnerability exploited by widely used anticancer drugs such as folate antagonists.[170] FOLR1 and FOLR2 folate receptor isoforms are overexpressed in many types of carcinomas, including ovarian, endometrial, kidney, lung, and brain carcinomas; mesothelioma; and myeloid leukemia.[171,172] This heightened expression could also be utilized in nanoparticle design (Table 3). Ulbrich et al demonstrated that, in general, cancer cells exhibit greater binding and uptake of HSA nanoparticles conjugated with folic acid than noncancer cells do.[173] Sun et al demonstrated that FA-functionalized HSA nanoparticles loaded with cabazitaxel (FA-NPs-CTX), compared with CTX-loaded human serum albumin alone, induced greater cytotoxic effects against HeLa cells overexpressing the folate receptor (FR). Moreover, in a HeLa xenograft mouse model, researchers reported a significant increase in the accumulation of FA-NPs-CTX at the tumor site, leading to inhibited tumor growth and reduced toxicity. Importantly, FA conjugation enhanced the safety and tolerability of cabazitaxel.[143] In another study, Ahlawat et al reported that the functionalization of HSA nanoparticles with folic acid enhanced the targeting of hydroxycaptothecin (HCPT) in lung adenocarcinoma and increased the cytotoxic effect of the drug (actinonin) encapsulated within the nanoparticles compared with that of the free drug.[144] Wang et al reported a similar enhanced anticancer effect. The authors developed a folate-decorated HSA nanoparticle loaded with nano-HCPT. Although the drug loading efficacy was 7.8%, compared with the free drug, the nanoparticles presented enhanced anticancer effects both in vitro and in vivo. More HCPT was delivered to the tumor tissue, resulting in an increased tumor inhibitory rate.[145] FA-decorated nanoparticles can also enhance the delivery of magnetic nanoparticles that contain iron oxide. Hiremath et al designed human serum albumin-coated citrate-functionalized iron oxide decorated with folic acid that also delivers two anticancerous compounds, 5-fluorouracil and curcumin. When a magnetic field was present, the uptake was further enhanced, especially in FA-decorated nanoparticles, increasing the sensitivity of cancer cell lines.[146] An intriguing application was presented by Encinas-Basurto et al. The authors introduced a nanoparticle comprising folic acid (FA) conjugated to human serum albumin (HSA), loaded with doxorubicin, and supplemented with gold nanorods for photothermal therapy. This

formulation sensitized HeLa cells to irradiation, facilitating synergy between chemotherapeutic and photothermal therapies.[147]

Antibodies

Monoclonal antibodies (mAbs) have transformed cancer therapy by selectively targeting receptors overexpressed on tumor cells. Examples such as trastuzumab (anti-HER2) and bevacizumab (anti-VEGF) illustrate their role in improving drug delivery.[174] However, their potential expands further when integrated with nanocarriers (Table 3). Taheri et al synthesized trastuzumab-decorated methotrexate-albumin nanoparticles (TMAB-MTX-HSA NPs), demonstrating that antibody density on the nanoparticle surface significantly influences cytotoxicity.[148] Notably, a moderate density of trastuzumab yielded superior cytotoxic effects compared with high-density configurations, suggesting that overcrowded antibodies may hinder receptor-ligand interactions. This underscores the need for precise control in nanoparticle engineering.

Overcoming Size Limitations with Recombinant Antibodies

Nanoparticle size plays a critical role in cellular uptake and therapeutic efficacy. Ideally, particles ranging from 30 to 50 nm can bypass lysosomal degradation via caveolin-mediated endocytosis.[175,176] Larger particles, however, risk lysosomal disposal when taken up through macropinocytosis or phagocytosis.[177,178] Recombinant antibody derivatives such as single-chain variable fragments (scFvs), diabodies, and nanobodies address this challenge by offering targeted binding capabilities with reduced particle size.[179,180]

In an innovative study, Yewale et al developed docetaxel-loaded HSA nanoparticles conjugated with scFvs targeting EGFR-overexpressing breast cancer cells.[149] The resulting immunonanoparticles (approximately 160 nm) exhibited enhanced uptake, prolonged circulation, and greater cytotoxicity compared with unconjugated formulations. These results highlight the potential of scFvs to balance specificity and size in antibody-nanoparticle systems.

Further advancements integrate scFvs with albumin to create multifunctional systems. Wang et al combined an EGFR-targeting scFv with domain 3 of HSA and the LDP apoprotein of lidamycin, leveraging the macropinocytosis pathway upregulated in Ras-driven cancers like pancreatic carcinoma.[181,182] This bifunctional system not only enhanced drug internalization but also synergistically inhibited EGFR signaling while promoting apoptosis and cell cycle arrest.

Similarly, Xian et al developed a fusion protein of anti-PD-1 nanobody (Nb97) and HSA to extend the half-life of immune checkpoint inhibitors while maintaining PD-1/PD-L1 pathway inhibition.[151] This approach demonstrates how HSA can act as both a drug carrier and a stabilizing agent for therapeutic molecules prone to rapid renal clearance.

HSA Dimers

Some studies have demonstrated not only the utility of HSA monomers but also the use of HSA dimers as effective carriers for enhancing the pharmacokinetic profiles of low-molecular-weight drugs and increasing their accumulation within tumor tissues through the EPR phenomenon. Human serum albumin dimers are two HSA molecules linked together and have gained attention in recent years as potential drug carriers for various therapeutic applications.

The increased uptake of albumin dimer-based drug delivery systems in solid tumors is facilitated by the pathophysiology of the tumor tissue. The accumulation of HSA dimers is attributed not only to transcytosis initiated by the binding of albumin to 60-kDa glycoprotein (gp60) and SPARC but also to the presence of these proteins in various types of tumor stroma. Therefore, the endogenous albumin transport system presents significant potential as an effective drug delivery approach for addressing resistant forms of cancer.

Ishima et al[183] demonstrated the potential of poly-nitric oxide (NO)-conjugated human serum albumin (Poly-SNO-HSA) as a drug delivery system (DDS) capable of accumulating NO in tumors. However, they noted the need for improved stability of poly-SNO-HSA in circulation and emphasized the importance of evaluating its optimal molecular size for maximizing the EPR effect. This study employed two murine tumor models: one with Colon 26 (C26) cells, which originate from mouse colon cancer and exhibit a pronounced endogenous EPR effect, and the other with B16 cells, which are derived from mouse melanoma and demonstrate a comparatively smaller EPR effect. By dimerizing with albumin, they achieved a tenfold increase in antitumor activity, enhancing NO delivery in Colon 26 tumor-bearing mice.

Their research focused on substantial accumulation of albumin in tumors, leveraging the S-nitrosated human serum albumin dimer (SNO-HSA dimer) to increase the EPR effect. They aimed to develop a DDS strategy utilizing the endogenous albumin transport (EAT) system to target tumor cells, underscoring its potential for future advancements. Furthermore, the same group investigated the effectiveness of PEGylated liposomal doxorubicin (Doxil), a typical example of a stealth liposome approved for medical usage.[184] In this study, the antitumor efficacy of Doxil® was enhanced when Doxil® was combined with the S-nitrosated form of HSA dimer in the treatment of solid tumors in B16-bearing mice with poorly permeable vasculature, which corresponded with increased survival rates and diminished tumor sizes. To validate the tumor response, the doxorubicin levels in the tumor were quantified. The concentrations of doxorubicin in the tumor increased by more than threefold in the C26 (murine colon 26 adenocarcinoma) and sixfold in the B16 (murine melanoma) tumor-bearing models. These findings suggest that albumin dimers have the potential in enhancing the EPR effect in highly resistant cancer, likely due to their ability to utilize the natural albumin transport mechanism. Consequently, the endogenous albumin transport system appears to be a promising drug delivery strategy for combating refractory cancers.[184] In 2017, Ryo Kinoshita et al studied an albumin dimer incorporating the chemotherapeutic agent doxorubicin (HSA-d-DOX) as a next-generation drug delivery system for tumor treatment, employing state-of-the-art nab (nanoparticle albumin-bound) technology.[185] The results showed that HSA-dimers-DOX demonstrated greater cytotoxicity than HSA-monomers-DOX did in vitro in SUIzo Tumor-2 (SUIT2) cells. Furthermore, comparative analysis revealed that the HSA dimer significantly improved tumor migration and cellular uptake abilities compared with its counterpart, the HSA monomer, which was synthesized via conventional nab techniques. Notably, HSA-d-DOX efficiently delivered DOX to a human pancreatic tumor model (SUIT2) and effectively inhibited tumor growth without causing severe side effects. Consequently, the dimerization of HSA has emerged as a multifaceted strategy, facilitating the solubilization of previously insoluble drugs and serving as an active targeting carrier, which is particularly valuable in low vascular permeability or in the context of intractable pancreatic tumors.[67] HSA-d, a dimer of HSA, has a greater tumor distribution than the HSA monomer does, making it a more effective drug carrier for antitumor therapy.[186] Recent research suggests that dimeric albumin formulations can more efficiently target anticancer drugs to tumor tissues, such as chemically synthesized dimers created using a 1.6-bis(maleimido)hexane (BMH) spacer at the Cys-34 residue.[187–189]

Managing Pharmacokinetics

Throughout its 27-day lifespan, an average albumin molecule completes approximately 15,000 circulatory trips and around 15 journeys through extravascular spaces.[24] The use of biomacromolecule-based albumin carriers has been shown to improve the pharmacokinetics of payloads and reduce systemic toxicity and immunogenicity. Albumin, with its long half-life, serves as an effective biomimetic drug delivery carrier, enhancing solubility, stability, and targeted delivery to tissues. Its natural affinity for tumor cells and EPR effect enable preferential accumulation of anticancer drugs in tumor tissues. The strategy for controlling the EPR may apply not only to liposome preparations, such as Doxil®, and albumin-binding drugs, such as Abraxane® but also to micelle preparations and antibodies, such as OPDIVO®, which has recently received prominent attention. Albumin-based systems also reduce the likelihood of adverse side effects by controlling the release and distribution of the drug. Moreover, the reduced immunogenicity associated with albumin-based drugs is particularly advantageous for avoiding unwanted immune responses, which can further complicate cancer treatment. Overall, managing the pharmacokinetics of albumin-based anticancer drugs is crucial for addressing the challenges posed by the complex microenvironment of refractory tumors. By leveraging the unique properties of albumin, these drug delivery systems enhance the efficacy and safety of anticancer therapies.

There are three approved drugs formulated as albumin-bound particles: Abraxane, Pazenir, and Fyarro (Table 4). Owing to the unique pharmacokinetics of these formulations, dosing adjustments may be required on the basis of patient-specific factors such as body weight, renal and hepatic function, and overall health. Regular monitoring for efficacy and side effects is essential, as these drugs have different toxicity profiles than nonalbumin-bound versions do. Additionally, potential drug interactions should be carefully considered, particularly with respect to medications that could influence the binding or metabolism of albumin-bound drugs.

Abraxane and Pazenir

Both Abraxane and Pazenir contain human serum albumin–paclitaxel nanoparticles of approximately 130 nm in size, where paclitaxel is present in a noncrystalline, amorphous state (Table 4). Upon intravenous administration, the nanoparticles dissociate rapidly into soluble, albumin-bound paclitaxel complexes approximately 10 nm in size. As described in Section 2, albumin is known to facilitate endothelial caveolar transcytosis of plasma components, and in vitro studies have shown that albumin in Abraxane improves the transport of paclitaxel across endothelial cells. It is hypothesized that this enhanced transendothelial caveolar transport is driven by gp-60, leading to increased paclitaxel accumulation in the tumor area due to the albumin-binding SPARC proteins.[190]

Pharmacokinetics of Abraxane

The pharmacokinetics of total paclitaxel have been determined in clinical studies. Following intravenous administration of Abraxane, paclitaxel concentrations in plasma declined in a biphasic manner, with the initial rapid decline representing distribution to the peripheral compartment and the slower second phase representing drug elimination (Table 5). The terminal half-life was approximately 27 hours. Drug exposure (area under the curve, AUC) was proportional to the dose over 80–375 mg/m^2, and the pharmacokinetics of paclitaxel were independent of the duration of Abraxane administration. The mean total clearance was 15 L/hr/m^2. Abraxane is characterized by extensive extravascular distribution and/or tissue binding of paclitaxel.

Between 89% and 98% of the drug is bound to human serum proteins, and the presence of cimetidine, ranitidine, dexamethasone, or diphenhydramine does not affect the protein binding of paclitaxel.

Table 4 Regulatory Approvals and Therapeutic Applications of Albumin-Based Anticancer Therapies for Improved Efficacy and Safety

Drug	Formulation	Indication and Approval	References
Abraxane	100 mg of paclitaxel (bound to human albumin) and approximately 900 mg of human albumin (containing sodium caprylate and sodium acetyltryptophanate	**FDA**: 2005 for treatment of breast cancer after failure of combination chemotherapy for metastatic disease or relapse within 6 months of adjuvant chemotherapy 2012 first-line treatment of locally advanced or metastatic non-small cell lung cancer, in combination with carboplatin, in patients who are not candidates for curative surgery or radiation therapy 2013 first-line treatment of patients with metastatic adenocarcinoma of the pancreas, in combination with gemcitabine **EMA**: 2008: metastatic breast cancer, metastatic adenocarcinoma of the pancreas I combination with gemcitabine, first line treatment in non-small cell lung cancer in combination with carboplatin for patient ineligible for surgery or radiotherapy	[190–194]
Pazenir	Generic medicine and is bioequivalent to the reference medicine Abraxane; 100 mg of paclitaxel formulated as albumin bound nanoparticles (approximately 130 nm in size). The other ingredient is human albumin (containing sodium caprylate and N-acetyl-DL-tryptophan).	**FDA**: unapproved **EMA**: 2019 same indications as Abraxane	[195–197]
Fyarro	100 mg sirolimus in lyophilized powder, formulated as albumin-bound particles and approximately 850 mg of human albumin in single-dose vial for reconstitution	**FDA**: adult patients with locally advanced unresectable or metastatic malignant perivascular epithelioid cell tumor (PEComa). **EMA**: unapproved	[198, 199]

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Table 5 Comparison of Pharmacokinetics Between Chemotherapeutic-Loaded HSA Nanoparticles and Chemotherapeutic Agent Alone: Emphasizing the Role of HSA Nanoparticles

Drug	Generic Name	Dosage Form	Pharmacokinetics	Ref.
Abraxane	Paclitaxel, formulated as protein-bound particles	Powder for dispersion (injection)	Half-life is approximately 27 hours	[190–194]
			Drug exposure (area under the curve, AUC) was proportional to the dose 80–375 mg/m^2	
			Mean total clearance was 15 L/hr/m^2	
			Extensive extravascular distribution and/or tissue binding of paclitaxel	
			89% - 98% of the drug is bound to human serum proteins	
			Metabolized primarily to 6α-hydroxypaclitaxel by CYP2C8, secondarily to two minor metabolites, 3′-p-hydroxypaclitaxel and 6 α, 3′-p-dihydroxypaclitaxel, by CYP3A4	
			Mean values for cumulative urinary recovery of unchanged drugs (4%) indicated extensive nonrenal clearance	
			Less than 1% of the total administered dose was excreted in the urine as the metabolites 6 α-hydroxypaclitaxel and 3′-p-hydroxypaclitaxel	
Pazenir	Paclitaxel, formulated as albumin bound nanoparticles	Powder for dispersion (injection)	Half-life ranges between 13 and 27 hours	[195–197]
			Plasma clearance of paclitaxel is between 13 and 30 L/h/m²	
			94% of the drug is bound to human serum proteins	
			Total volume of distribution: ca. 1741 L	
			Metabolized primarily to 6α-hydroxypaclitaxel and secondarily to two minor metabolites, 3′-p-hydroxypaclitaxel and 6 α-3′-p-dihydroxypaclitaxel is catalyzed by CYP2C8, CYP3A4	
			Average cumulative urinary excretion of unchanged active substances accounted for 4% of the total administered dose	
			Less than 1% excreted as the metabolites 6α-hydroxypaclitaxel and 3′-p-hydroxypaclitaxel, indicating extensive nonrenal clearance	
			Primarily eliminated through hepatic metabolism and biliary excretion	

(*Continued*)

Table 5 (Continued).

Drug	Generic Name	Dosage Form	Pharmacokinetics	Ref.
Fyarro	Sirolimus formulatedas protein-bound particles	Suspension or powder for dispersion (injection)	Half-life is approximately 59 hours	[198,199]
			Estimated drug exposure (AUC) was proportional to the dose of 2590 ng/mL (30% of patients with advanced solid tumors), and 22100 ng/mL (50% of patients with advanced solid tumors)	
			Metabolized by CYP3A4	
			99% of the drug is bound to human serum proteins	
			Primarily eliminated through feces (91%) and urine (2%)	
Paclitaxel	Paclitaxel	Suspension/solution (injection)	Half-life ranges from 12 to 33 hours	[200–202]
			Total clearance is between 11.6 and 24.0 L/hr/m^2	
			Volume of distribution: 121–638 L/m^2	
			The pharmacokinetics of paclitaxel exhibit non-linearity (concentration dependence), with systemic exposure increasing disproportionately with higher doses	
			88% - 99% of the drug is bound to human serum proteins	
			Maximum concentrations ranged - from 761 to 2860 ng/mL	
			Mean drug exposure (AUC) - was proportional to the dose 2609–9428 ng/mL	
			Clearance is between 11 and 38 L/h/m^2	
			Less than 10% of the dose is excreted in the urine as unchanged drug; the major elimination pathway is metabolism followed by biliary excretion	
			CYP2C8 and CYP3A4 are involved in the formation of 6 alpha-hydroxypaclitaxel and 3′-para dihydroxypaclitaxel and 6α −3′p-dihydroxypaclitaxel	
Rapamune	Sirolimus	Oral solution or coated tablets	Half-life ranges from 62±16 hours to 7 days	[203, 204]
			92% of the drug is bound to human serum proteins	
			Metabolized by CYP3A4	
			Total clearance for solution is 194±78 L/h/m^2; for tablet is 230±67 L/h/m^2	
			Primarily eliminated through feces (91.1%) and urine (2.2%)	

Abraxane is metabolized primarily to 6α-hydroxypaclitaxel by CYP2C8 and to two minor metabolites, 3′-p-hydroxypaclitaxel and 6 α, 3′-p-dihydroxypaclitaxel, by CYP3A4. The pharmacokinetics of paclitaxel may be altered in vivo as a result of interactions with compounds that are substrates, inducers, or inhibitors of CYP2C8 and/or CYP3A4. The metabolism of paclitaxel is catalyzed by CYP2C8 and CYP3A4. In the absence of formal clinical studies on drug interactions, caution is advised when administering Abraxane alongside medications known to inhibit (eg, ketoconazole and other imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or induce (eg, rifampicin, carbamazepine, phenytoin, efavirenz, and nevirapine) the enzymes CYP2C8 or CYP3A4. The mean values for cumulative urinary recovery of unchanged drugs (4%) indicated extensive nonrenal clearance. Less than 1% of the total administered dose was excreted in the urine as the metabolites 6 α-hydroxypaclitaxel and 3′-p-hydroxypaclitaxel.[190,191]

Pharmacokinetics of Pazenir

The plasma clearance of paclitaxel administered as Pazenir was 43% greater than that of solvent-based paclitaxel injections, with a 53% increase in volume of distribution. The mean terminal half-life ranges between 13 and 27 hours, and the average plasma clearance of paclitaxel is between 13 and 30 L/h/m² (Table 5). No evidence of paclitaxel accumulation was observed over multiple treatment cycles.[195]

After Pazenir is administered to patients with solid tumors, paclitaxel is evenly distributed between blood cells and plasma, with a high plasma protein binding rate of 94%. Population pharmacokinetic analysis estimated the total volume of distribution to be approximately 1741 L, suggesting significant extravascular distribution and/or extensive tissue binding of paclitaxel.[195]

Pazenir is metabolized primarily to 6α-hydroxypaclitaxel and to two minor metabolites, 3′-p-hydroxypaclitaxel and 6 α-3′-p-dihydroxypaclitaxel. The formation of these hydroxylated metabolites is catalyzed by CYP2C8, CYP3A4, and both the CYP2C8 and the CYP3A4 isoenzymes, respectively.[195] The average cumulative urinary excretion of unchanged active substances accounted for 4% of the total administered dose, with less than 1% excreted as the metabolites 6α-hydroxypaclitaxel and 3′-p-hydroxypaclitaxel, indicating extensive nonrenal clearance. Pazenir is primarily eliminated through hepatic metabolism and biliary excretion.[195]

Caution is advised when administering paclitaxel alongside medications known to inhibit CYP2C8 or CYP3A4 (eg, ketoconazole and other imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, clopidogrel, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir), as increased exposure to paclitaxel may increase its toxicity. Coadministration of paclitaxel with medicines known to induce CYP2C8 or CYP3A4 (eg, rifampicin, carbamazepine, phenytoin, efavirenz, nevirapine) is not recommended, as it may reduce paclitaxel exposure and compromise its efficacy, since paclitaxel clearance is driven primarily by CYP2C8- and CYP3A4-mediated metabolism, followed by biliary excretion.[195]

Fyarro

Fyarro is a nanoparticle albumin-bound formulation of sirolimus, also known as nab-sirolimus. The active ingredient, sirolimus, inhibits the mammalian target of rapamycin (mTOR) pathway, which is vital for cell growth and proliferation. By blocking this pathway, sirolimus helps slow or halt the growth of cancer cells. The protein binding ability of sirolimus is greater than 99%, and sirolimus primarily attaches to serum albumin in vitro. Additionally, sirolimus has a mean elimination half-life of approximately 59 hours, is metabolized by CYP3A4, and is primarily eliminated through feces (91%) and urine (2%) (Table 5). Fyarro's active ingredient, sirolimus, is also a substrate for P-gp.[198] CYP3A4 and/or P-gp inhibitors may increase sirolimus concentrations, increasing the risk of Fyarro-related adverse reactions, whereas CYP3A4 and/or P-gp inducers may lower sirolimus levels, potentially reducing Fyarro's effectiveness. To avoid these effects, the concomitant use of Fyarro with strong CYP3A4 and/or P-gp inhibitors or inducers, as well as grapefruit or grapefruit juice (a strong CYP3A4 inhibitor), should be avoided. If Fyarro is used with a moderate or weak CYP3A4 inhibitor, a dosage reduction is recommended. Additionally, using moderate or weak CYP3A4 inducers with Fyarro may decrease sirolimus effectiveness.[198,205,206]

Conclusion

Albumin is a well-studied carrier in cancer therapy, serving as an alternative energy source for cancer cells that adapt to its uptake. Its physical structure can be modified without denaturation, for instance, through pH manipulation or lysine modification with NHS ester, enabling the design of novel drug delivery systems. Such modifications support a "wolf in sheep's clothing" strategy for effective therapy.

The cellular uptake of HSA-based nanotherapeutics, though promising, requires further investigation. While gp60-mediated uptake and its interplay with SPARC enhance HSA accumulation in cancer cells, the underlying mechanisms remain unclear, as discussed in Section 2.2. A key challenge is the lack of detailed genetic and molecular information on gp60 and gp18/30 receptors, limiting therapeutic advancements targeting these pathways. Emerging albumin receptors, such as CD36 and transferrin, present additional targets for HSA modifications.

Overcoming multidrug resistance (MDR1) remains critical, particularly regarding resistance to Abraxane, linked to paclitaxel release from albumin. HSA-based nanoparticles, however, have demonstrated efficacy in MDR1-resistant cell lines. Strategies such as active targeting of overexpressed cancer cell surface proteins—including transferrin receptors, CD44, folic acid receptors, and specific antibodies—offer potential solutions. Dimeric HSA has shown enhanced cellular uptake and migratory properties, further advancing therapeutic opportunities.

Drugs like Abraxane, Pazenir, and Fyarro require careful pharmacokinetic optimization to balance efficacy and minimize adverse effects, although little is known about Pazenir's effectiveness in drug-resistant cancers. HSA-based nanoparticles hold significant promise for overcoming resistance, but understanding uptake mechanisms in specific cancer types remains vital to developing multi-receptor-targeting delivery systems for improved outcomes Overall, we believe that HSA-based nanoparticles show potential for bypassing resistance, but a deeper understanding of uptake mechanisms in specific cancer types is crucial for designing delivery systems targeting multiple receptors for enhanced efficacy. Overall, we believe that HSA-based nanoparticles show potential for bypassing resistance, but a deeper understanding of uptake mechanisms in specific cancer types is crucial for designing delivery systems targeting multiple receptors for enhanced efficacy.

Abbreviations

(ABC) proteins, ATP-binding cassette; ABCB1, ABC-binding protein 1 (MDR1); Akt, Protein kinase B; BCRP (ABCG2), Breast Cancer Resistance Protein; CAV1, caveolin-1; CD36, cluster of differentiation 36,; CD44, cluster of differentiation 44; CSCs, cancer stem cells; CTX, Cabazitaxel; CYP2C8, Cytochrome P450 2C8; CYP3A4, Cytochrome P450 3A4; Db, diabodies; DDS, drug delivery system; dHSA, human serum albumin dimer; DTX, docetaxel; EGFR, epidermal growth factor receptor; EMA, European Medicines Agency; EPR, enhanced permeability and retention; FA, folic acid; FcRn, neonatal fragment crystallizable (Fc) receptor; FDA, US Food and Drug Administration; FR, Folate Receptor; gp18/30, glycoprotein 18/30; gp60, glycoprotein 60; HA, Hyaluronic acid; HCPT, hydroxycaptothecin; HER2, Receptor tyrosine-protein kinase erbB-2; HSA, human serum albumin; MAPK, mitogen-activated protein kinase; MDR1, multidrug resistance type 1; MRP (ABCC), multiple drug resistance protein; mTOR, mammalian target of rapamycin; MTX, Methotrexate; Nb, nanobodies; NF-κB, Nuclear factor kappa-light chain enhancer of activated B cells; NPs, nanoparticles; OVA, ovalbumin; PEG, polyethylene glycol; PgP, glycoprotein P (MDR1); PI3Ks, Phosphoinositide 3-kinase; rHSA, recombinant human serum albumin; scFv, single-chain variable fragment; SPARC, secreted protein acidic and rich in cysteine; TfR1, transferrin receptor 1, CD71; TMAB, trastuzumab; TME, tumor microenvironment; VEGF, Vascular endothelial growth factor.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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