Clinical impact of serum proteins on drug delivery

Felix Kratz a,⁎, Bakheet Elsadek b

a Tumor Biology Center, Division of Macromolecular Prodrugs, Breisacher Strasse 117, 79106 Freiburg, Germany
b Department of Biochemistry, Faculty of Pharmacy, Al-Azhar University, Assiut Branch, 71524 Assiut, Egypt

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A B S T R A C T

Among serum proteins albumin and transferrin have attracted the most interest as drug carriers in the past two decades. Prior to that, their potential use was overshadowed by the advent of monoclonal antibodies that was initiated by Milstein and Köhler in 1975. Meanwhile intensive pursuit of exploiting transferrin, but above all albumin as an exogenous or endogenous carrier protein for treating various diseases, primarily cancer, rheumatoid arthritis, diabetes and hepatitis has resulted in several marketed products and numerous clinical trials. While the use of transferrin has clinically been primarily restricted to immunotoxins, albumin-based drug delivery systems ranging from albumin drug nanoparticles, albumin fusion protein, prodrugs and peptide derivatives that bind covalently to albumin as well as physically binding antibody fragments and therapeutically active peptides are in advanced clinical trials or approved products. For treating diabetes, Levemir® and Victozin® that are myristic acid derivatives of human insulin or glucagon-like peptide 1 (GLP-1) act as long-acting peptides by binding to the fatty acid binding sites on circulating albumin to control glucose levels. Levemir® from Novo Nordisk has already developed into a blockbuster since its market approval in 2004. Abraxane®, an albumin paclitaxel nanoparticle as a water-soluble galenic formulation avoiding the use of cremophor/ethanol, transports paclitaxel through passive targeting as an albumin paclitaxel complex to the tumor site and is superior to conventional Taxol® against metastatic breast cancer. INNO-206, an albumin-binding doxorubicin prodrug that also accumulates in solid tumors due to the enhanced permeability and retention (EPR) effect but releases the parent drug through acid cleavage, either intra- or extracellularly, is entering phase II studies against sarcoma. An expanding field is the use of albumin-binding antibody moieties which do not contain the fragment crystallizable (Fc) portion of, conventional immunoglobulin G (IgG) but are comprised of monovalent or bivalent light and/or heavy chains and incorporate an additional albumin-binding peptide or antibody domain. The most advanced antibody of this kind is ATN-103 (Ozoralizumab), a trivalent albumin-binding nanobody that neutralizes the pro-inflammatory tumor necrosis factor alpha (TNF-α) as a causative agent for exacerbating rheumatoid arthritis. ATN-103 is currently in multi-center phase II trials against this debilitating disease. In summary, because albumin as the most abundant circulating protein cannot only be used to improve the pharmacokinetic profile of therapeutically relevant peptides and the targeting moiety of antibodies but also for peptide-based targeting as well as low-molecular weight drugs to inflamed or malignant tissue, it is anticipated that R&D efforts of academia and the pharmaceutical industry in this field of drug delivery will prosper.

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⁎ Corresponding author at: Division of Macromolecular Prodrugs, Tumor Biology Center, Breisacher Strasse 117, D-79106 Freiburg, Germany. Tel.: +49 761 2062930; fax: +49 761 2062965.
E-mail address: kratz@tumorbio.uni-freiburg.de (F. Kratz).
URL: http://www.tumorbio.uni-freiburg.de/04_forschung/04_02_05.html (F. Kratz).

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1. Introduction

Any drug, whether applied orally, intravenously, sublingual, subcutaneous or intramuscularly, is transported by the blood and its first encounter is not only with the various cellular components and low-molecular weight compounds, but also with a multitude of plasma proteins. The complexity of the human plasma proteome is immense comprised of approximately 100,000 proteins whose concentrations span over 12 orders of magnitude. The major components can be separated by conventional cellulose acetate or PAGE electrophoresis and are shown in Fig. 1.

Albumin is by far the most abundant protein with a concentration of ~35–50 mg/mL. The amounts of other major plasma proteins are much lower (see Fig. 1), e.g. with concentrations of transferrin in the range of 2.5–3.5 mg/mL. Further separation and identification of all plasma proteins and peptides is a formidable task, and indeed the high abundant plasma protein components (primarily albumin and immunoglobulins) have to be removed prior to the identification of plasma proteins by 2D gel electrophoresis in the low nano- or picomolar range followed by subsequent 2D electrophoresis. In this way approximately 600–1000 peptides and proteins can be separated (see for example http://www.biocompare.com).

Drugs can bind to several blood components, such as albumin, α1-acid glycoprotein, lipoproteins and immunoglobulins, but due to the large excess and small size of albumin this protein is predominantly involved. The extent of plasma protein binding for basically all drugs is stated on index of medicines or patient instruction leaflets and is generally determined by equilibrium dialysis with the protein-fraction bound corresponding to the same or similar value if pure human serum albumin is used. Intuitively, this value is taken as being predictive for the pharmacokinetic characteristics of the drugs in question by drug developers and clinicians and is also taken into account when analyzing drug-drug interactions and disease drug interactions leading to increased free plasma levels with concomitant drug exposure and unforeseen side effects or loss of activity. However, this can be considered as a general misconception, and changes in plasma protein binding have little clinical relevance for most drugs [1, 2].

The reasons – although the unbound drug is regarded as the therapeutically active fraction that exerts its effect on the molecular target within cells and tissues – are threefold: (1) The half-life, clearance and tissue distribution of the vast majority of low-molecular weight drugs are so fast (in the order of minutes or at the most a few hours) that the fraction of the drug that is actually protein-bound in the plasma is very small or sometimes negligible; (2) The binding constants of the drug for albumin are generally low (Kd ~ 10^6 M) so the drug displays a very rapid pharmacokinetic-pharmacodynamic equilibration time; (3) As a consequence, protein-binding of a drug measured in an isolated non-dynamic system outside of the body can but often does not correlate with other pharmacokinetic parameters such as plasma half-life, clearance, area under the curve (AUC), or volume of distribution. We have no intention to propagate that drug-drug interactions be ignored, these obviously are present and certainly belong to an important field of research of their own, but protein-binding as routinely determined and stated for commonly prescribed drugs is a poor predictor for the displacement of drugs from their protein-bound state and rarely alters a patient’s overall exposure to a drug [1]. Indeed, renal or hepatic elimination or the metabolism of the drug may influence the toxicity profile of a drug to a much larger extent than the small fraction that is protein-bound.

When moving to the area of drug delivery, plasma protein-binding becomes important when the binding constants are higher, in the order of 10^3–10^5 M, because in these cases a major fraction of the administered drug is bound to plasma proteins, primarily albumin. This is true for endogenous ligands such as fatty acids or bilirubin which both bind tightly to albumin and are transported in the albumin-bound form in the body with the fraction of free ligand being less important. Scientists in drug design have used these insights to develop drugs with high binding affinity for albumin to either improve the pharmacokinetic profile and bioavailability of peptides and antibody moieties or to exploit the targeting property of albumin for inflamed or malignant tissue. Although human serum albumin (66.5 kDa) is a transport protein per se trafficking fatty acids, metal ions (Ca^{2+}, Zn^{2+}, Cu^{2+}), bilirubin and high binding drugs within the body, historically other transport proteins such as transferrin and the low density lipoprotein (LDL) as a source of supplying the cells with iron (III) and cholesterol, respectively, in the body first attracted the attention of scientists involved in drug delivery or diagnostic applications.

The transfer of iron(III) and ferritin between very different cell types such as the intestine epithelium, where iron enters the body from the diet, the liver, where it is stored as ferritin (a protein containing iron–phosphate–hydroxide complexes), the developing erythroid cells, which require it for globin synthesis, and cells which need iron for cell growth including tumor cells. LDL is the principal carrier of cholesterol to tissues. 

Fig. 1. Overview of the major human plasma proteins that can be identified by simple electrophoretic techniques.
All proteins have been elucidated by X-ray structure analysis (Fig. 2A–C):

Iron-free transferrin is called apotransferrin and can bind two equivalents of iron(III). Diferric transferrin binds to the specific receptor for transferrin (a 190 kDa glycoprotein) located on the cell surface [6] and is then rapidly internalized in nonlysosomal vesicles or recycled. The acidic environment of these vesicles causes iron to dissociate from the protein while apotransferrin remains bound to the receptor.

The approximate three-dimensional shape of HSA can be described as a heart shaped macromolecule and is one of the smallest proteins present in blood plasma. Both size and abundance explain the fact that so many metabolic compounds and therapeutic drugs are transported by this protein. The numerous binding sites for metabolic substrates and therapeutic drugs have been extensively studied and reviewed [7–9]. HSA has a half-life of ~19 days in humans.

LDL is a much larger protein lipid system with a spherical particle size of approximately 220 Å consisting of an outer coat and inner core of hydrophobic lipids (about 1500 molecules of cholesteryl esters and about 300 molecules of triacylglycerol) — see Fig. 2C. LDL is one of the five major groups of lipoproteins (chylomicrons, VLDL, LDL, and HDL) that enable lipids like cholesterol and triglycerides to be transported.

**Fig. 2.** A–C: Structures of rabbit serum Fe(III) transferrin (PDB: 1JNF) [3], structure of human serum albumin (PDB: 1BM0) [4], structure of low-density lipoprotein (LDL) (modified according to [5]).

**Fig. 3.** Targeting strategies in gene or drug delivery using transferrin (Tf) constructs directed against the transferrin receptor (A) Conjugates with oligonucleotides; (B) Immunotoxins with transferrin; (C) Oligonucleotides or drugs encapsulated in immunoliposomes with transferrin constructs directed against the transferrin receptor linked to the surface of the liposome through a PEG linker; (D) Structure of a lipoplex where transferrin is conjugated to a cationized polymer, such as poly-L-lysine or polyethyleneimine (PEI) and negatively charged oligonucleotides are complexed with the polymer.

Adapted from [11].
transported within the water-based bloodstream. The coat of human LDL is comprised of phosphatidylcholine, sphingomyelin, unesterified cholesterol and a single protein termed apolipoprotein B (MW 514 kDa). This protein is responsible for the specific binding to cell surface LDL receptors where LDL is internalized by endocytosis and then transported to lysosomes in which the cholesteryl esters are hydrolyzed making free cholesterol available for cell membrane and steroid synthesis (for a review see [10]).

Although all proteins have been investigated as drug carriers, no LDL drug delivery systems have entered the clinical setting and only a few transferrin drug conjugates have entered clinical trials as drug conjugates or immunotoxins; the major clinical and commercial impact has undoubtedly been with albumin-based drug delivery systems. Consequently, this review will focus on the clinical trials and applications with these two proteins, above all with albumin. The major indications have been oncology, diabetes, rheumatoid arthritis and hepatic C.

2. Transferrin and albumin as drug carriers — overview of technologies

An emphasis with respect to transferrin-based drug delivery has been on gene delivery and the development of immunotoxins. Other drug delivery systems comprise transferrin conjugates, protamine or cationized nanoparticles, e.g. with poly-lysine or polyethylenimine, or immunoliposomes, and the general structure of these systems is depicted in Fig. 3.

Since the pioneering work of E. Wagner in Vienna, a multitude of studies have been reported that use these systems for delivering oligonucleotides or siRNA to cells, either using transferrin or antibody constructs directed against the transferrin receptor. Several in vitro and some in vivo proof of concepts have been obtained preclinically, and we refer to comprehensive reviews that cover this field [11–13]. However, only one transferrin-based immunotoxin designed for treating glioblastoma, TransMID®, has reached an advanced stage of clinical evaluation (see Section 4.2).

In contrast, over the past decades albumin has played an increasing role as a drug carrier in the clinical setting. It has emerged as a versatile carrier for therapeutic and diagnostic agents, primarily for diagnosing and treating diabetes, cancer, rheumatoid arthritis and viral diseases. Principally, the four main technologies that use albumin as a drug carrier are summarized in Fig. 4. Drugs, prodrugs or polypeptides can either be bound physically or covalently through a ligand or protein-binding group to HSA (top left and right). More complex systems are characterized by the attachment of numerous targeting ligands and prodrugs bound to the protein surface or nanobodies or bispecific antibodies that bind physically or are fused with albumin replacing the Fc fragment of immunoglobulin G (bottom right). Finally, nanoparticles, micellar structures or microbubbles with lipophilic drugs or diagnostic agents can be prepared as water-soluble suitable galenic formulations for intravenous injection (bottom left).

Fig. 4. Albumin, a versatile drug carrier for developing drug-, peptide- or antibody-based drugs as conjugates, complexes or nanoparticles.

Fig. 5. Gallium-scintgramme of a Morbus Hodgkin patient using 67Ga-citrate (arrows show tumor localization around the breast bone).
The pertinent developments in these different areas of applications that have progressed commercially and clinically and are continually expanding the field of transferrin and albumin-based drug delivery concepts are described in the following chapters. Other promising applications which are currently in preclinical development including those which are focused on ligands such as peptides or sugar moieties bound to human serum albumin are not described in this review, and we refer to several original and review articles on this topic [14–19].

3. Diagnostic applications of radioactive labeling of transferrin or albumin

3.1. Ga\(^{67}\)(III)- and \(^{68}\)Ga(III)-citrate for the detection of lymphoma

Ga\(^{67}\)(III)- and \(^{68}\)Ga(III)-citrate are useful radiopharmaceuticals to detect tumors, especially for patients with lymphoma, to stage the extent of the disease and are also very sensitive to detect and locate infections and inflammatory foci [20].

Pioneering work by Edwards and Hayes in 1969 had shown that gallium-68 is concentrated in the lymph nodes in Hodgkin’s disease [21], and the medical literature is meanwhile rich on reports of the use of \(^{67}\)Ga(III)- and \(^{68}\)Ga(III)-compounds to detect many types of malignancies in humans, mostly Hodgkin and non Hodgkin lymphomas, but also lung cancer, malignant melanoma, breast carcinoma and Ewing’s sarcoma. (reviewed in [22]).

When gallium citrate is administered parenterally in a readily dissolvable form, it binds quickly to plasma transferrin at the iron(III) binding sites. Further biodistribution processes are then mainly governed by transferrin, even if not in the same way as for iron(III) ions. At the cellular level, iron uptake is mediated by the specific receptor for transferrin located on the cell surface (a 190 kDa glycoprotein). The distribution of the transferrin receptor in different cell types, the modulation of its expression and its molecular recognition properties appear to be critical factors with respect to the physiology of iron metabolism and to the use of transferrin and its derivatives in medicine. Several investigators have determined the number of transferrin receptors on a variety of human cell types, and their numbers per cell range from around 40,000–2,800,000 in tumor cells as compared to approximately 45,000–400,000 present on reticulocytes [23].

As an example, a radioscintigraphic picture of a Morbus Hodgkin patient treated with \(^{67}\)Ga-citrate which binds rapidly to apotransferrin after intravenous administration is shown in Fig. 6. Tumor nodes are seen at the breast bone and collar-bone.

A very recent analysis of 99 patients with diffuse large B-cell lymphoma that were followed from 1996 to 2007 has shown that both 2-[fluorine-18]fluor-2-deoxy-o-glucose-emission tomography (PET) and \(^{67}\)Ga(III)-citrate response after chemotherapy are powerful prognostic factors for this disease [24]. Five-year in-field control was 95% with a negative PET/gallium scan versus 71% with a positive scan (P < 0.01). Five-year event-free survival (EFS; 83% versus 65%, P = 0.04) and overall survival (89% versus 73%, P = 0.04) were also significantly better when the post-chemotherapy PET/gallium was negative demonstrating that a positive PET/gallium scan after chemotherapy is associated with an increased risk of local failure and death.

3.2. \(^{99m}\)Tc-aggregated albumin for diagnostic use in nuclear medicine

In the past decades, the growth of nuclear medicine has been due mainly to the availability of technetium-99 m (\(^{99m}\)Tc) radiopharmaceuticals. This single isotope is a metastable nuclear isomer with a half-life of 6 h and is used in over 80% of all diagnostic procedures.

In 2010, approximately, 20 million diagnostic nuclear medical procedures were carried out with \(^{99m}\)Tc radiopharmaceuticals with this number estimated to grow at a rate of around 15% per annum [25].

For transporting \(^{99m}\)Tc to its required location, a variety of complexes with tailor-made properties have been developed that allow imaging of several body organs such as bones, heart, liver, kidneys and lungs. \(^{99m}\)Tc-aggregated albumin, a gamma-emitting radionuclide imaging agent, represents an injectable radiopharmaceutical that has been in use in nuclear medicine in almost 30 years [26]. It consists of a sterile aqueous suspension of \(^{99m}\)Tc labeled to pathogen free human serum albumin aggregated particles in the pH range of 3.8 to 8.0. Although the precise structures of the aggregated albumin complexes are currently unknown, several kits for preparing these formulations are available from a number of different manufacturers including; Bracco Imaging s.P.a, CIS-US, Draximage, Pharmalucence, Inc., Nycomed Amersham and Mallinckrodt. These kits have several trade names such as \(^{99m}\)Tc-Albures®, \(^{99m}\)Tc-Nanocoll®, \(^{99m}\)Tc-Human Serum Albumin®, \(^{99m}\)Tc-99m-Microalbumin® and Technetium-99m Albumin Colloid®, and they differ essentially in the amount of human serum albumin, SnCl\(_2\), particle size and preservatives.

In the clinical settings, \(^{99m}\)Tc-aggregated albumin such as Nanocoll®, Albures®, Pulmolite® and Draximage® which differ primarily in their size (from a diameter of a few nm up to 1000 nm) have found various applications. For instance, they have been used for bone marrow scanning, inflammation scanning in areas other than the abdomen, perfusion lung imaging to assess the presence of pulmonary emboli, and isotope venography to identify lower extremity venous thrombosis. It has also been used diagnostically for various
indications including cardiac function tests [26], lymphoscintigraphy [27], sentinel node detection in breast cancer [28, 35], stage I non-small cell lung carcinoma (NSCLC) [29], esophageal squamous cell carcinoma [30], and other solid tumors [31], leg edema [32], protein-losing gastroenteropathy assessment [33] and rheumatoid arthritis [34].

$^{99m}$Tc-aggregated albumin is administered by different routes (intravenously, interarterially, subcutaneously, intradermally, intratumorally) and at different doses (range 10–200 MBq) depending on the diagnostic aim. An example for the use of Nanocoll® that has an average diameter of 8 nm is sentinel lymph node detection, and a scintigramme is illustrated in Fig. 6.

Although, $^{99m}$Tc-aggregated albumin is still in use, its use has gradually decreased and is being replaced by other diagnostic techniques to detect solid tumors.

4. Therapeutic applications in oncology

There is a large body of evidence available which demonstrates that tumors in animals and humans sequester transferrin and albumin to cover their high demand for iron(III) and amino acids when they are fast growing. Thus, anemia due to transferrin depletion as well as hypoalbuminemia is a characteristic feature of cancer patients that is especially prominent in patients with high tumor burden.

Fig. 7 illustrates depletion of albumin as well as of transferrin in a recent study of 260 anemic tumor patients which clearly shows that the serum levels for both proteins in for the majority of patients fall below the norm range of healthy individuals.

Such cases are generally associated with cachexia, and albumin infusions are used to compensate for the overall albumin loss. As an explanation for the high albumin turnover in tumors, Stehle et al. have proposed that plasma proteins such as albumin are major energy and nutrition sources for tumor growth (reviewed in [37]) due to an excessive plasma protein catabolism by the tumor itself and an active metabolic role of the liver which seem to be important factors for the genesis of cachexia.

4.1. Clinical trials of gallium(III) nitrate and a ruthenium(III) complex for treating lymphoma and solid tumors

From the mid eighties onwards, two gallium(III) salts, i.e. GaCl$_3$ and Ga(NO$_3$)$_3$, underwent a number of clinical trials: Gallium trinitrate has shown antitumor activity in pretreated patients with malignant lymphoma, bladder carcinoma as well as small cell lung carcinoma and is also effective in the treatment of cancer-related hypocalcaemia [38, 39]. Clinical trials with gallium trichloride in combination with cisplatin and etoposide have shown that gallium chloride potentiates the therapeutic effect of cisplatin and etoposide [40]. Gallium salts very likely exert their antitumor effect by binding to apotransferrin in the blood circulation and subsequently interfere with the transferrin cell cycle of tumor cells.

In a further approach with anticancer metal complexes which exploit apotransferrin, transferrin as well as albumin as drug carriers, the anticancer ruthenium(III) complex trans-indazolium-[tetrachloro(bisindazole)ruthenate(III)]$^+$ ([HInd][RuInd$_2$Cl$_4$]) — Fig. 8 developed by Keppler and co-workers exhibited high in vivo antitumor activity.
Kratz et al. have demonstrated a fast and specific binding of this complex to human serum transferrin, apotransferrin, and human serum albumin [42, 43]. These protein adducts of HInd[RuInd₂Cl₄] retain the antitumor efficacy of the original complex indicating that transferrin and albumin play an important role in the biodistribution of this highly active complex [44]. Through soaking experiments with the related apolactoferrin, the binding sites of [trans-HInd(RuInd₂Cl₄)] could be elucidated by X-ray structure analysis (see Fig. 9).

The interactions between HInd[RuInd₂Cl₄] and human serum albumin have also been investigated through UV–Vis, circular dichroism (CD), fluorescence spectroscopy and inductively coupled plasma-atomic emission spectroscopy (ICP(AES)) method suggesting binding in the domain IIA binding pocket [45].

Due to an insufficient aqueous solubility of the original complex for preparing a galenic formulation, an analogous ruthenium(III) indazole complex has been developed in which the indazolium ion has been replaced by sodium trans-Na[RuInd₂Cl₄] and the indazolium ion re-introduced when preparing the metal complex for intravenous administration, and in this form it has been investigated in a pivotal clinical trial. Seven patients with various types of solid tumors were treated intravenously with escalating doses of the reconstituted ruthenium complex (25–600 mg total dose) on a twice weekly schedule for 3 weeks. Unfortunately, the increasing volume that had to be administered due to the water-solubility of the ruthenium complex did not allow dose-limiting toxicities to be established at the employed doses. The pharmacokinetic properties were characterized by a small volume of distribution, low clearance and long half-life in accordance with significant binding to albumin and transferrin [46]. Meanwhile galenic issues have been solved, and the company Niiki-Pharma is conducting a phase I study with this ruthenium(II) complex (re-named NKP-133) in patients with metastatic solid tumors — see http://www.niikipharma.com.

4.2. Transferrin immunotoxin TransMid® for local administration of brain tumors

Because conventional chemotherapy is not very effective in the treatment of brain tumors, and healthy brain cells generally do not divide and express only small amounts of transferrin receptors, researches focused on the development of a conjugate consisting of transferrin that is bound to the highly potent diphtheria toxin through a lysine cross-linker and a thioester, that was nicknamed Tf-CRM107 (later TransMID® in clinical trials), and its structure is shown in Fig. 10.

Once bound to glioma cells, it is taken up by transferrin receptor-mediated endocytosis, and the thioester is cleaved and releases the toxin which kills the malignant glioblastoma cells [47], Convection-enhanced delivery (CED), a method for the delivery of large molecules to brain tissue via continuous interstitial microinfusion, has permitted direct administration of toxins to brain tumors or to surrounding brain tissue infiltrated by tumor cells. In preclinical studies, TF-CRM107 showed an apparent activity in the picomolar range and was able to induce complete remissions in glioblastoma models such as subcutaneously growing U251 gliomas in nude mice after intratumoral administration of TF-CRM107. Subsequently, TF-CRM107 was studied in a...
nanoparticles (see min are passed through a jet under high pressure to form nab-paclitaxel colloidal suspension in which a lipophilic drug and human serum albumin®) at American Bioscience for use as an intravenously administered free 130-nanometer albumin bound paclitaxel (nab-paclitaxel, Abraxane®, for treating solid tumors

4.3. Abraxane®, an albumin paclitaxel nanoparticle, for treating solid tumors

Neil Desai and co-workers developed a novel formulation of solvent free 130-nanometer albumin bound paclitaxel (nab-paclitaxel, Abraxane®) at American Bioscience for use as an intravenously administered colloidal suspension in which a lipophilic drug and human serum albumin are passed through a jet under high pressure to form nab-paclitaxel nanoparticles (see Fig. 12).

Based on the pivotal phase III clinical trial results, nab-paclitaxel was approved in the United States by US Food and Drug Administration (FDA) in January 2005 and in Europe by the European Medicines Agency (EMEA) in January 2008 for use in patients with metastatic breast cancer who have failed combination chemotherapy or relapsed within 6 months of adjuvant therapy where prior therapy included an anthracycline.

One of the main advantages of Abraxane® is its dramatically improved water-solubility and therefore avoids the use of conventionally used CremophorEL. Being potentially toxic, CremophorEL solvent is responsible for the major serious side effects and dose-limiting toxicities associated with taxane-based therapy including the risk of hypersensitivity reactions and neuropathies, and also impairs drug delivery to the tumor limiting the clinical effectiveness of Taxol®.

In a randomized phase III study in 460 patients with metastatic breast cancer, Abraxane® at a higher maximum tolerated dose of 260 mg/m² taxol equivalent compared to Taxol®/CremophorEL at 175 mg/m² in a three-weekly schedule, demonstrated a statistically significant higher response rates (33% versus 19%, P = 0.001), longer time to tumor progression (23.0 versus 16.9 weeks, P = 0.006), and increased survival in the subset of patients receiving second-line or greater treatment (65.0 versus 55.7 weeks, P = 0.024). These higher doses were generally well tolerated over a shorter infusion time (30 min intravenous infusion) without the need for special infusion sets or pre-medications with corticosteroids to avoid hypersensitivity reactions. These encouraging results were also seen in a comparison of Abraxane® with Taxotere® (polysorbate-based docetaxel) in a randomized phase II clinical trial of first-line treatment of 300 patients with metastatic breast cancer [51] at lower doses on a weekly schedule, either at 100 mg/m² or 150 mg/m² taxol equivalents compared with Taxotere® at 100 mg/m² on a 3-weekly schedule. Progression-free survival was increased by approximately 5 months and both doses of Abraxane® very associated with an improved safety and toxicity profile compared with docetaxel. Subsequently, American Bioscience have extended the evaluation of Abraxane® to other tumor indication including NSCLC [52], pancreatic cancer [53], melanoma, and head and neck cancer [54], often in combination with conventional chemotherapy.

One of the most interesting aspects of Abraxane® is the detailed insights into its mode of action that has been obtained preclinically as well as clinically. Abraxane®, although stable as a nanoparticle in its galenic formulation, dissolves rapidly after intravenous infusion
resulting in soluble albumin-bound paclitaxel complexes having a size basically comparable to that of endogenous albumin [55]. The albumin-paclitaxel complexes accumulate in the tumor through the well-established EPR effect of solid tumors, but a further albumin transport pathway mediated by the 60-kDa glycoprotein gp60, also known as albonidin, located on the endothelial cell surface seems to be responsible for tumor uptake and above all for the even tumor distribution of Abraxane® and the subsequent release of paclitaxel [56–58].

The albumin-paclitaxel complexes bind to gp60 with a high affinity in the nanomolar range inducing gp60 clustering and transcytosis carrying both gp60-bound and fluid phase albumin through the tumor endothelium subsequently releasing paclitaxel into the subendothelial space (Fig. 13).

Upon entering the tumor interstitium, the accumulation of albumin is possibly facilitated by SPARC (Secreted Protein, Acidic and Rich in Cysteine), a 43 kDa secreted matricellular glycoprotein with high binding affinity to albumin and significant homology to gp60 [59, 60]. Over-expression of SPARC as a key modulator of extracellular matrix proliferation, and cell migration is associated with increased tumor invasion, metastasis, and poor prognosis in multiple tumor types [61], and albumin-binding and subsequent uptake by tumor cells has been demonstrated in several in vitro and in vivo tumor models [61–63]. Clinical studies strongly suggest that SPARC is an important part of the mechanism of action of Abraxane® contributing to its improved clinical efficacy versus Taxol®/CremophorEL. In a recently reported phase I/II study in patients with metastatic pancreatic cancer treated with the combination of Abraxane® of 125 mg/m² paclitaxel equivalents and gemcitabine at 1000 mg/m² a median overall survival of 12.2 months was achieved [53]. A sub-analysis of the overall SPARC levels in 36 of the 44 evaluable patients showed that higher SPARC

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**Fig. 13.** Uptake of albumin-paclitaxel nanoparticles is presumably mediated by the EPR effect and the gp60 transcytosis pathway and subsequent binding to SPARC (Secreted Protein, Acidic and Rich in Cysteine) in the tumor extracellular matrix. Illustration provided with kind permission of Neil Desai.

**Fig. 14.** Product pipeline showing different development stages for current nab technology-based drugs.
expression correlated with improved overall survival (n = 20; median survival 13.6 months, P = 0.02) compared to patients with low SPARC levels (n = 16; median survival 8.1 months).

In a retrospective analysis of 16 head and neck cancer patients, this trend for SPARC-positive patients was confirmed [54]. Based on these observations, the role of SPARC as a predictive biomarker for the clinical response to Abraxane® is currently being investigated in several clinical trials. The gp60 receptor most likely also plays an important role for the even tumor tissue distribution and deep tumor penetration of albumin-binding bispecific antibodies and nanobodies. In 2010, sales for Abraxane® were approximately US$ 430 Mio, and the albumin-based paclitaxel nanoparticle has meanwhile been acquired by Celgene, who now market Abraxane®.

The nab technology platform is focused on encapsulating highly lipophilic drugs and a number of anticancer drug nanoparticles are in different stages of preclinical and clinical development such as with docetaxel, the mTOR inhibitor rapamycin, the heat-shock protein inhibitor Hsp90, the tubulin polymerization/topoisomerase I dual inhibitor 5404, and a novel taxane CY196 (an overview is shown in Fig. 14):

4.4. Clinical trials with a methotrexate albumin conjugate MTX-HSA

The first drug conjugate with human serum albumin (HSA) that was evaluated in phase I/II clinical studies was a methotrexate albumin conjugate (MTX-HSA). MTX-HSA was synthesized by directly coupling the drug to lysine residues of HSA. It was found that the drug-loading ratio significantly determined the tumor targeting properties of MTX-albumin conjugates in rats. Sinn and co-workers have convincingly emphasized that the drug loading rate determines the tumor targeting properties of MTX albumin conjugates in rats. In a systematic study, in which the tumor uptake of MTX-albumin conjugates loaded with 1, 5, 7, 10 or 20 molecules of MTX was compared in Walker-256 carcinoma bearing rats, only loading rates of close to one equivalent of MTX per molecule of albumin offered optimal tumor uptake [68]. A noteworthy finding of this study was that two patients with renal cell carcinoma and one patient with mesothelioma responded to MTX-HSA therapy (one partial response, two minor responses). However, it was not possible to confirm these results in a subsequent phase II study in 17 patients with metastatic renal carcinoma sponsored by Klinger Pharma, Germany (now Fujisawa) in which no objective response was seen [67].

Another phase II study with MTX-HSA in combination with cisplatin was conducted for the first line treatment of patients with advanced bladder cancer [68]. Treatment was started with a loading dose of 110 mg/m² of MTX-HSA followed by a weekly dose of 40 mg/m². Cisplatin was given monthly at a dose of 75 mg/m². One complete and one partial remission were observed in seven evaluable patients. However, there is currently no indication that the clinical assessment of MTX-HSA is being further pursued.

4.5. INNO-206, an albumin-binding prodrug of doxorubicin

Over the past years, Kratz and co-workers have investigated a targeting strategy that is based on two features: (a) in situ binding of a thiol-binding prodrug to the cysteine-34 position of circulating albumin after intravenous administration with subsequent accumulation of the drug-albumin conjugate in the tumor due to passive targeting; (b) release of the albumin-bound drug at the tumor site due to the incorporation of a cleavable bond between the drug and the carrier (see Fig. 15) [69, 70].

The first and most advanced prototype of these types of prodrugs is the (6-maleimidocaproyl)hydrazone derivative of doxorubicin (DOXO-EMCH, now INNO-206, Fig. 16), an acid-sensitive prodrug of doxorubicin that is rapidly and selectively bound to the cysteine-34 position of endogenous albumin [71].

INNO-206 contains an acid-sensitive hydrazone linker allowing doxorubicin to be released either extracellularly in the slightly acidic environment often present in tumor tissue or intracellularly in acidic endosomal or lysosomal compartments after cellular uptake of the albumin conjugate by the tumor cell. INNO-206 emerged as a clinical candidate due to a high water-solubility, a high plasma stability in its albumin-bound form, its profound acid-sensitive properties, and superior efficacy in meanwhile nine murine tumor models and a favorable toxicity profile including significantly reduced cardiotoxicity [72, 73].

In a phase I study, INNO-206 showed a good safety profile at doses up to 260 mg/m² doxorubicin equivalents [74]. Although not the primary end point of the phase I study, INNO-206 was able to induce tumor regressions in breast cancer, small cell lung cancer and sarcoma. Scale-up of active pharmaceutical ingredient as well as a suitable galenic formulation for large-scale lyophilization has been completed, and a currently ongoing abbreviated safety study has confirmed excellent tolerability at 260 mg/m² doxorubicin equivalents for INNO-206. Phase II studies will be carried out in sarcoma in 2011 and gastric cancer and possibly further indications (see http://www.cytRX.com).

Inspired by the translational research with DOXO-EMCH, a broad spectrum of albumin-binding pipeline prodrugs has been developed.
by Kratz and co-workers in the past 10 years. These prodrugs consist of anticancer drugs such as daunorubicin, camptothecin, paclitaxel, 5-fluorouracil, methotrexate, and platinum(II) analogs and contain either an acid-sensitive linker or a peptide bond that is cleaved by a tumor-associated proteases such as matrix metalloproteases 2 and 9, cathepsin B, urokinase, plasmin, or unidentified proteases.

A further extension of the in situ albumin technology has been the development of novel albumin-binding prodrugs that combine passive and active targeting or act as dual acting or synergistically acting prodrugs by attaching either an additional receptor- or antigen-recognizing ligand or two different drugs to the maleimide-bearing linker.

4.6. MM-111, an albumin fusion antibody targeting epidermal growth factor receptors

Using its understanding of the epidermal growth factors network, Merrimack Pharmaceuticals, Inc. has developed an albumin fusion protein that targets the epidermal growth factors ErbB2 (HER2) and ErbB3 (HER3). To date, four variants of the ErbB family of receptor tyrosine kinases are known: EGFR, ErbB2 (HER2), ErbB3 (HER3), and ErbB4 (HER4). They are a part of a complex molecular network whose activation is commonly linked with cancer and can be associated with disease recurrence and poor prognosis for the patient. Over-expression or amplification of the epidermal growth factors is known to occur in several cancers including lung, colon, breast, stomach, head and neck cancer in addition to other types of cancer.

Commercially available IgG antibodies targeting HER2 or EGFR are Herceptin® (trastuzumab) and Erbitux® (cetuximab) that are used to treat breast and colorectal cancer, respectively. HER2 is over-expressed in ~25% of breast cancer patients, and primarily causes breast cells to reproduce uncontrollably. The original studies of trastuzumab showed that it improved survival in late-stage metastatic breast cancer, but there is controversy over whether trastuzumab is effective in the adjuvant or in earlier stage breast cancer. Treatment with Herceptin® is mostly associated with the formation of resistance at some stage and is also associated with cardiac dysfunction in 2–7% of cases and annual costs can be as much as US$ 100,000. Thus there is a need to develop new antibody systems that overcome these disadvantages.

MM-111 represents the first bispecific antibody targeting two receptors on the same cell to enter clinical development. Structurally, MM-111 has two antibody arms fused to human serum albumin that replaces the Fc fragment of an IgG; a targeting arm that binds to ErbB2 with high affinity and a therapeutic arm that binds to ErbB3 (see Fig. 17).

ErbB3 is an enzyme that has a neuregulin binding domain but not an active kinase domain and forms heterodimers with other EGF receptor family members such as ErbB2. Heterodimerization leads to the activation of pathways which lead to cell proliferation or differentiation. Over-expression of ErbB3 has been reported in numerous cancers, including prostate, bladder, and breast tumors. The bispecific format of MM-111 could be an optimal approach of inhibiting the enhancement of cell proliferation of ErbB3 in ErbB2-overexpressing tumors. Preclinical proof of concepts have been obtained in ErbB2-positive tumors and in addition the combination of trastuzumab and MM-11 in a ErbB-2 positive breast cancer xenograft model has resulted in complete tumor regressions.

In June 2009, MM-111 entered clinical development for a Phase I/II trial, and Merrimack Pharmaceuticals are currently enrolling patients with tumors that generally over-express ErbB2 in a phase I study. The Phase II portion of the study will be restricted to
breast cancer patients that solely over-express ErbB2 (see http://www.merrimackpharma.com).

5. Therapeutic applications in diabetes

5.1. Levemir® and Victoza® for treating diabetes

In 2010, there were approximately 285 million cases of type 1 and 2 diabetes which is equivalent to ~6.4% of the world population making it one of the most common diseases (for a comparison: ~12 Mio cases of cancer in 2010). Diabetes mellitus is caused by a lack of insulin production in the islets of Langerhans in the pancreas (type 1 diabetes) or insulin resistance (type 2 diabetes).

Until the recent introduction of basal insulin analogs, Neutral Protamine Hagedorn (NPH) insulin has been the most frequently used basal insulin, usually administered in the evening. It is characterized by peaks in plasma insulin concentrations 5 to 10 h after administration, increased risk of hypoglycemia during the night, and duration of action of approximately 12 to 18 h that may contribute to hyperglycemia in the morning. Common differences in crystal size and inadequate resuspension make absorption kinetics and dosing precision with NPH insulin variable and result in unpredictable glucose levels [78]. Therefore, it was important to develop new basal insulins to minimize these concerns.

The introduction of basal insulin analogs has resulted in a series of clinical trials that provide information on the most effective way of using these insulins in the treatment of type 1 and 2 diabetes mellitus. For instance, insulin detemir (Levemir®), one of the basal insulin analogs, has been recently developed by Novo Nordisk and provides an effective therapeutic option for patients with type 1 and type 2 diabetes mellitus. After its approval in 2004 in Europe and reaching sales well over US$ 500 Mio in 2010, Levemir® has opened a new therapeutic area of the glucagon-like peptide 1 (GLP-1) for treating diabetes mellitus. After its approval in 2004 in Europe and reaching sales ~0.66 Mio US$ by 2011. Its bene
dict of endogenous or exogenous albumin [81, 85]. Structurally, Levemir® is based on a very simple principle: the C-terminal amino acid threo
dine threono-nine in recombinant human insulin, produced by Escherichia Coli, is replaced by a lysine moiety, and myristic acid is then covalently
ned to its cysteic acid group (see Fig. 18). After the subcutaneous injection, the half-life of Levemir® is extended from 4 to 6 min for native human insulin to 5–7 h for Leve
dr for most patients, especially patients with juvenile diabetes (type 1 diabetes), one subcutaneous injection per day with Levemir® FlexPen® is sufficient to normalize the blood glucose level [79]. Additionally, in contrast to competing products, Levemir® is the only long-acting insulin that remains soluble both before and after injection and does not form microprecipitates or crystals unlike insulin glargine (Sanofi-Aventis) and NPH insulin (Novo Nordisk).

In a similar strategy, the scientists at Novo Nordisk used the same principle to improve the pharmacokinetic profile of the glucagon-like peptide 1 (GLP-1) for treating diabetes mellitus. The biologically ac
ctive forms, GLP-1(7–37) and GLP-1(7–36)NH2, are produced after cleavage of proglucagon in the gut and stimulate the insulin secretion in pancreatic cells in a glucose-dependent manner. GLP-1(7–37) has a half-life of 1.5–2 min due to degradation by ubiquitous enzymes. Lir
glutide (Victoza®) is an albumin-binding derivative of GLP-1 in which this peptide hormone is derivatized with myristic acid at the N-terminal position of a glutamic acid introduced at the ε-amino position of lysine in the GLP-1 peptide sequence. In contrast to GLP-1, liraglutide is stable against metabolic degradation due to albumin
binding and has a plasma half-life of ~15 h after subcutaneous administration making it suitable for once daily administration.

Subsequent to its approval in 2009 in Europe and in 2010 in the USA for treating patients with type 2 diabetes mellitus, Victoza® achieved sales reaching ~0.66 Mio US$ by 2011. Its benefits for treating type 2 diabetes mellitus lie in that it targets beta cells, allowing for increased and prolonged insulin secretion concomitantly regulating blood sugar levels, it lowers weight and only has mild and transient side effects, mainly gastrointestinal disorders [80].

5.2. CJC-1134-PC, an albumin-modified exendin-4 conjugate for treating type 2 diabetes

As mentioned above, GLP-1 is a member of the incretin group of gastrointestinal hormones that cause an increase in the released amount of insulin from the beta cells of the islets of Langerhans after eating, even before blood glucose levels become elevated. It regulates glucose homeostasis through multiple mechanisms including direct actions on the endocrine pancreas and indirect activation of the central nervous system to regulate gastric emptying, satiety, and body weight [81].

Other analogous injectable GLP-1 receptor agonists have recently been introduced into clinical practice in order to increase insulin release from the beta cells and have shown a good safety profile as new antidiabetic agents [82, 83]. However, the therapeutic efficacies of these GLP-1 analogs are restricted mainly by their rapid clearance from the body or their cleavage by peptidases and as a consequence a very short serum half-life of ~5 min to 1 h, which mean that they must be frequently administered to maintain therapeutic levels [84].

Therefore, there is a substantial interest in developing more potent GLP-1 receptor agonists with sustained activity. As an option, ConjuChem, Inc. developed a thiol-binding derivative of GLP-1 (abbreviated CJC-1131) that binds rapidly and selectively to the cysteine-34 position of endogenous or exogenous albumin [81, 85]. Structurally, CJC-1131 is a maleimide derivative of GLP-1 with the structure; HAECTFTSDVSYLEGQA [N-c-(γ-Glu(N-ε-exadecanoyl)]-EFIAWLV-Lys34-GR-lys-[2-2-(2-maleimidopropio-amido)ethoxy] ethoxy)acetamide. Interestingly, it was active in experimental diabe
tes models [85] and was generally well tolerated in rats, beagle dogs and humans with no signs of immunogenicity [86].

However, ConjuChem, Inc. decided to continue their clinical development with an ex vivo synthesized albumin conjugate of the peptide exendin-4–4, i.e., CJC-1134-PC (PC-DAc™; exendin-4), possibly due to formulation issues with CJC-1131 or owing to the fact that exendin-4 is more potent at lowering glucose concentrations than human GLP-1. In a phase I/II trial, CJC-1134-PC, as a long-acting antidiabetic albu
mun conjugate, demonstrated a good tolerability profile and positive ef
cacy on glucose reduction in a once-a-week dosing system. However, it needs to be mentioned that Novo Nordisk already has an albumin
binding derivative of a myristic derivatized GLP-1 (Victoza®) approved (see above Section 5.1. Levemir® and Victoza® for treating diabetes) so further development of this product is facing a competitive commercial situation and in addition the manufacturing company has recently been reduced to a very small company, ConjuChem, Biotechnologies, Inc.

Fig. 19. Schematic illustration of the trivalent nanobody ATN-103 comprising two VH peptides with affinity for TNF-α and one VH for albumin.
6. Therapeutic applications for treating rheumatoid arthritis

The strategy of binding peptides, e.g. cytokines, various antibody fragments, to albumin is meanwhile being extensively explored for the design of a new generation of albumin-binding antibodies and therapeutically active peptides [87]. One very promising application is an improved treatment of rheumatoid arthritis with nanobodies as described in the following section.

6.1. ATN-103, an albumin-binding nanobody directed against pro-inflammatory TNF-α

The advent of the novel antibody-based technology by the Belgian pharmaceutical company Ablynx using albumin-binding nanobodies (VHH) led to the development of unique formulations that have already reached clinical phase II studies for the treatment of rheumatoid arthritis. A trivalent antibody consisting of two anti-tumor necrosis factor-α (TNF-α) nanobodies (TR2) and one albumin-binding nanobody (AB1), named ATN-103 (now Ozoralizumab) with a MW~45 kDa (see Fig. 19) was successfully developed preclinically.

TNF-α is one of key mediators of the inflammatory response and has served as the molecular target for developing three approved immunoglobulins, Enbrel® (etanercept), Remicade® (infliximab), and Humira® (adalimumab), for treating inflammatory diseases such as rheumatoid arthritis where they are used alone in late-stages of this severe disease or in combination with methotrexate [88].

In a phase I study, ATN-103 showed good tolerability and subsequently was licensed to Pfizer who have completed the recruitment of two phase II studies in 48 patients in Japan and the USA with subcutaneous administration of Ozoralizumab every 4 weeks for 16 weeks in patients with rheumatoid arthritis. In addition, open label extension of a phase II study in 260 patients with rheumatoid arthritis is ongoing to ensure long term safety of the novel albumin-binding nanobody (see http://www.ablynx.com).

Ozoralizumab addresses an extremely large market (US$ 16.9 billion for all TNF-α inhibitors in 2008) and could have substantial advantages over the three conventional and only partially humanized antibodies Remicade®, Enbrel®, and Humira® due to ease of manufacturing and reduced cost, a better efficacy due to an albumin-mediated targeting to inflamed joints and a reduction of side effects compared to the currently approved antibodies that can include serious and sometimes fatal blood disorders, serious infections, lymphoma and solid tissue cancers, reports of serious liver injury, reactivation of hepatitis B, reactivation of tuberculosis, drug-induced lupus, and demyelinating central nervous system disorders.

7. Therapeutic applications for treating hepatitis C with the albumin fusion protein, Albuferon

Human Genome Science have developed a broadly applicable albumin fusion protein technology in which a therapeutically active protein or peptide can be genetically fuse to recombinant human albumin (see Fig. 20).

The lead compound of this technology has been Albuferon® “Albinterferon-α-2b”, a fusion protein of albumin and interferon-α-2b (INF-α-2b) for the treatment of hepatitis C infection which affects approximately 180 million people worldwide [89]. INF-α-2b has a molecular weight of ~19 kDa and despite its antiviral properties has a half-life in humans in the range of 2 to 3 h requiring frequent injections (daily or three times weekly). By genetically fusing recombinant INF-α-2b with recombinant HSA a fusion protein with a molecular weight of 85.7 kDa (Fig. 21) is generated that has been developed by Human Genome Sciences in collaboration with Novartis as a long-acting interferon for the treatment of chronic hepatitis C and has been evaluated in several phase III trials of which most have been completed.

Several phase III programs have compared the efficacy, safety and impact on health-related quality of life of Albuferon-α-2b versus Pegassys® (INFα-2b pegylated with a branched 40 kDa poly(ethylene)glycol) representing the current standard of care in combination with the antiviral nucleoside Ribavirin [89-91]. Recently, two phase III clinical trials demonstrated comparable efficacy of Albuferon-α-2b given every 2 weeks to weekly Pegassys®, both in combination with Ribavirin. However, the most promising schedule seems to be a subcutaneous injection of Albuferon-α-2b every 4 weeks resulting in a lower frequency of adverse events and improved quality of life [89].

Another phase III study was conducted to assess the efficacy/safety of Albuferon-α-2b in patients with chronic hepatitis C virus genotype 2/3 [90]. In this study, 933 patients were randomized to open-label subcutaneous treatment with Pegassys® (180 μg once a week) or Albuferon-α-2b (900 or 1200 μg every 2 weeks) for 24 weeks, each administered with oral Ribavirin 800 mg/day. As a main conclusion, this study showed that Albuferon-α-2b was not inferior to Pegassys® in patients with chronic hepatitis C virus genotype 2 or 3 and Albuferon-α-2b 900 μg administered every 2 weeks provides an alternative efficacious treatment option for these patients [90].
A further recently completed phase III trial study including 1331 patients with chronic hepatitis C virus genotype 1, revealed that Albinterferon-α-2b was not inferior to Pegasys® in these patients and treatment with Albinterferon-α-2b at 900 μg every 2 weeks showed comparable efficacy with a similar toxicity profile but a higher discontinuation rate [91].

For patients infected with chronic hepatitis C Virus genotype 1, the current standard of care is a once-weekly dose of Pegasys® combined with daily Ribavirin for 48 weeks [91]. Although Albinterferon-α-2b has, on the whole, shown high antiviral activity, a good tolerability profile, is not considered to be inferior to the current standard of care with Pegasys® and daily Ribavirin, Novartis have discontinued the regulatory process for obtaining market approval (personal communication from Novartis).

8. Conclusions and perspectives

Serum proteins, especially transferrin and primarily albumin, are playing an increasing role in the design of new pharmaceuticals and two albumin-based drug delivery systems, the albumin-paclitaxel nanoparticle Abraxane® in oncology and Levemir®, an albumin-binding human insulin for treating diabetes, have established themselves as blockbusters. Inspection of Table 1 reveals that several other transferrin-based or albumin-binding drugs are marketed or in clinical trials, and there is a historical experience with a number of discontinued clinical investigations. The main reasons for focusing primarily on transferrin and albumin are three-fold:

1) Both serum proteins with molecular weights of 78 kDa and 66.5 kDa lie above the renal threshold, have a long circulation time, and can thus accumulate in inflamed and malignant tissue due to the enhanced permeation and retention effect.

2) Three important receptors dictate the biodistribution as well as cellular uptake of these two proteins: (a) the transferrin receptor that is over-expressed in malignant cells and takes up Fe(III)-transferrin through receptor-endocytosis, (b) the gp60 receptor on endothelial cells that is responsible for transcytosis of albumin and aids in transporting this protein into the tumor interstitium against the efflux induced by the interstitial fluid pressure of solid tumors, and (c) the neonatal Fc receptor (FcRn) which binds IgGs but also serum...
albumin is responsible for the long-half life of both proteins in the body. FcRn is a cell membrane bound receptor which is found on endothelial cells, but also on cells of various organs such as in the kidneys, liver and intestine. The binding of serum albumin to FcRn was discovered and elucidated by Anderson and co-workers who showed that similar to IgG, serum albumin was bound in a pH dependent manner [92, 93].

As shown schematically in Fig. 22 for an endothelial cell, human serum albumin is continuously taken up by fluid-phase endocytosis forming endosomes where the pH value drops to ~pH 6.0 and HSA serum albumin is continuously taken up by

3) The high abundance, multiple binding sites, and very long half-life of serum albumin make it a versatile and ideal endogenous serum protein for improving the pharmacokinetic properties of therapeutically active peptides or small-sized antibody moieties. Albumin-binding cannot only help to reduce a loss and the costs of the often valuable peptide, but also to improve its uptake into the affected tissue as well its compliance.

It is more than likely that the pharmaceutical, clinical and commercial use of albumin and transferrin will be fully explored in the coming decade and the field may expand to further indications besides solid tumors, diabetes, rheumatoid arthritis and viral diseases. In addition, there are a number of transport proteins in the blood whose potential as drug carriers has not been investigated in sufficient detail and could open new avenues for drug delivery including lipoproteins, haptoglobin, serum proteins for hormones and vitamins such as retinol-binding protein, vitamin D transport protein, transcobalamins I and II as natural transport proteins of vitamin B12, thyroxin-binding globulin, thyroid hormone serum transport proteins or the copper transport carrier protein ceruloplasmin among others.

References


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