Expert Opinion

Native albumin for targeted drug delivery

Elena Neumann†, Eva Frei, Dorothee Funk, Matthias D Becker, Hans-Hermann Schrenk, Ulf Müller-Ladner & Christoph Fiehn
†*Justus-Liebig-University Gießen, Department of Internal Medicine and Rheumatology, Kerckhoff-Klinik, 61231 Bad Nauheim, Germany*

Importance in the field: Activated cells metabolize albumin to cover their increased need for amino acids and energy. In inflamed, diseased and malignant tissue, extravasation of macromolecules into the tissue is upregulated. Drug carriers such as albumin have been used to target specifically diseased and malignant cells, resulting in higher efficacy of treatment and reduced side effects.

Areas covered in this review: Owing to its advantageous biochemical and pharmacological properties, albumin has been regarded as an interesting candidate as a drug carrier. Covalent coupling to albumin carries drugs specifically to tumors and sites of inflammation, leading to reduced side effects as long as the native structure of albumin is unchanged. In this review, the means of coupling drugs to native albumin as well as exemplary studies for the use of albumin as drug carrier are summarized and discussed.

What the reader will gain: An overview of the state-of-the-art using albumin as drug carrier for specific accumulation in tumors and inflammatory cells using the advantageous properties of native albumin is given in this review.

Take home message: Native albumin is an effective drug carrier to sites of inflammation or malignancy.

Keywords: albumin, arthritis, drug delivery, human serum albumin, inflammation, tumor

Expert Opin. Drug Deliv. (2010) 7(8):915-925

1. Introduction

Activated cells such as tumor or inflammatory cells metabolize albumin to cover their increased need for amino acids and energy. Therefore, albumin has been regarded an interesting candidate as a drug carrier. Serum albumin makes up half of the protein in human blood plasma. The reference range for human serum albumin (HSA) concentrations in serum is 42 – 54 g/l. It has a blood half-life of ~ 20 days. Albumin is produced in the liver and the molecular mass of the monomeric protein is 67 kDa. The human albumin gene located on chromosome 4 is 16,961 nucleotides long from the putative 'cap' site to the first poly(A) addition site. It is split into 15 exons, which are symmetrically placed within the 3 domains that are thought to have evolved by triplication of a single primordial domain [1]. The tertiary structure of the albumin molecule is formed by eight S–S bonds, which are formed directly on translation of its mRNA. Albumin has one free cysteine residue, which is important for the scope of this review and will be dwelt on later. Albumin is a multifunctional protein that is involved in the maintenance of colloid oncotic pressure, balances the pH of plasma due to the highly abundant amino acids with functional groups such as 16.8% acidic amino acids (Asp, Glu), 16.9% basic amino acids (Lys, His, Arg) and the transports of hydrophobic molecules such as thyroid and other hormones and fatty acids.

Inflammation and tumors share several characteristics and many tumors need the presence of activated macrophages and mast cells to develop into invasive

10.1517/17425247.2010.498474 © 2010 Informa UK Ltd ISSN 1742-5247

Article highlights.

- In this review, the advantages of native albumin as drug carrier to sites of disease and malignancy are presented.
- Options for coupling compounds to human serum albumin are shown, highlighting several examples.
- High versus low loading rations are compared for their effectiveness.
- *ex vivo*/*in vivo* loading approaches are discussed, showing several examples for both approaches.
- The use of different spacers between albumin and drug are summarized to achieve specific cleavage of the drug from albumin.
- Several examples for *in vitro* and *in vivo* applications of albumin conjugates as well as a proof of concept with examples for treatment as well as diagnostic uses are given in more detail to emphasize the key messages.

This box summarizes key points contained in the article.

carcinomas [2]. Several mechanisms are common features in inflammation and in tumors. These include angiogenesis and its humoral regulation by growth factors such as VEGF. Moreover, in inflamed and in malignant tissues extravasation of macromolecules into the tissue is upregulated in a similar way. For albumin, the increased extravasation helps to satisfy the high energy and nitrogen demand of the activated cells. It has been shown that in chronic inflammatory diseases such as rheumatoid arthritis (RA) – very similar to tumors – the extravasation of albumin is increased approximately sixfold [3]. Moreover, patients with RA have an increased catabolism of albumin that can lead to hypalbuminemia [4]. It was hypothesized that this may be owing to increased degradation of albumin in the inflamed joints [5]. The similarities of albumin metabolism in RA as well as other inflammatory diseases and tumors prompted the development of albumin as a carrier molecule for drugs into tumors and inflamed tissue. Covalent coupling to albumin would carry drugs specifically to tumors and the sites of inflammation, leading to a higher efficiency and reduced side effects. The native structure of albumin should not, however, be changed by the conjugation reaction. Using albumin as carrier, lower concentrations of the drug-albumin conjugate in comparison with the original drug would be required. To reach this goal it has been shown that changes of the molecular structure can be avoided by coupling the drug to albumin in a molar ratio of unity [6]. The target properties and long half-life of albumin are advantageous for its use as carrier for therapeutic drugs and diagnostic agents [7].

In this review, the means of coupling therapeutic and diagnostic agents to albumin as well as some exemplary studies for the use of albumin as drug carrier for therapeutic and diagnostic purposes are summarized. The review focuses on albumin conjugates in which the native structure of albumin is retained. Applications in which albumin is heavily glycosylated to form neo-glycoproteins to target, for example, liver diseases, are not covered in detail as these conjugates have lost the albumin-specific properties.

2. Human serum albumin as carrier

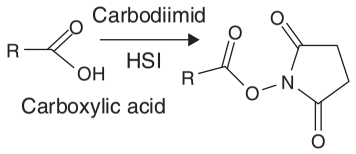
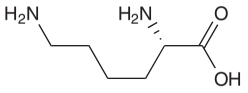
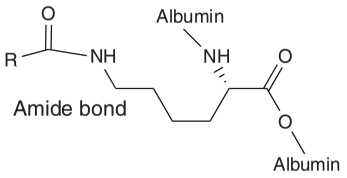
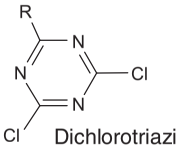
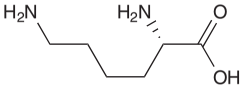
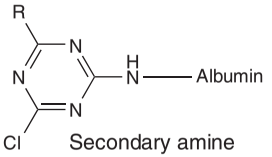
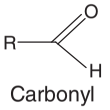
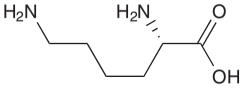
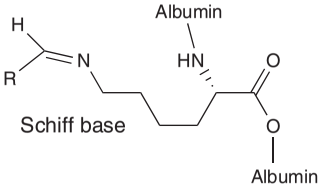
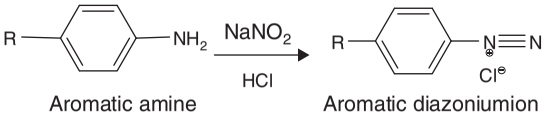
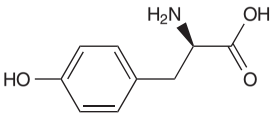
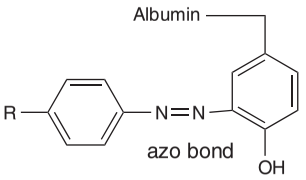
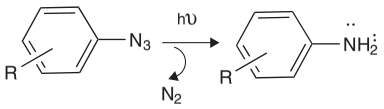
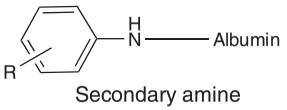
Serum albumin is a protein with many functional groups that are accessible on the hydrophilic surface of the molecule, and also in the hydrophobic binding pockets to which hydrophobic molecules such as fatty acids and many drugs bind [1]. Therefore, reactions with side chain amino, hydroxyl, carboxy or carbonyl groups are possible. A selection of such reactions and the structures of the products with albumin are summarized in **Table 1**. Contrary to all other plasma proteins, albumin contains a cysteine at position 34 (Cys34), which is moderately accessible to chemical reactions. Moreover, albumin is stable against denaturing agents and solvents at moderate concentrations. These properties have resulted in different approaches to generating covalent albumin drug conjugates. These approaches include modifications targeting specific receptors such as the $\alpha_v\beta_3$ integrins by decorating albumin with RGD peptides to deliver siRNAs to cells expressing these integrins. This approach was effective *in vitro* with heavily modified albumin [8], and also with albumin carrying an auristatin derivative. In the latter approach, the authors found the cellular handling to be equally important as the cellular uptake of the conjugates [9], a point that will be considered later in this review where the cleavability of linkers between drug and albumin is discussed. Folates bound to albumin to target the folate receptor in addition to paclitaxel aiming to eliminate the target cells have been shown to be effective *in vitro* as well [10]. Such conjugates with heavily modified albumin are effective *in vitro* but usually have only moderate effects *in vivo* [11,12]. In these studies, the authors observed that albumin loaded with 10 mol of dexamethason per mol of albumin resulted in cell-specific targeting of Kupffer cells as well as sinusoidal epithelia cells in fibrotic liver but had no effect on the fibrotic process.

Many approaches used highly loaded albumin with and without specifically targeting molecules to take advantage of the enhanced permeability and retention (EPR) effect of macromolecules observed by Maeda *et al.* [13] and Jain [14] in tumors. In this review, only a selection of such attempts is presented as the main scope of the review is to summarize approaches using the native properties of albumin for drug delivery.

3. High versus low loading ratios and native versus modified albumin

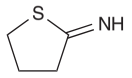
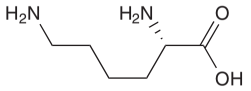
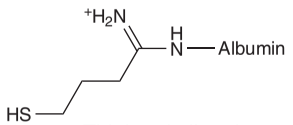
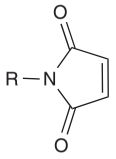
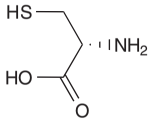
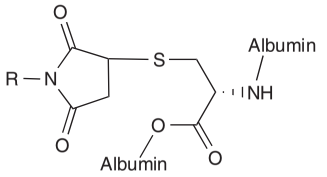
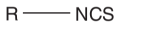
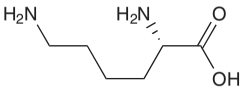
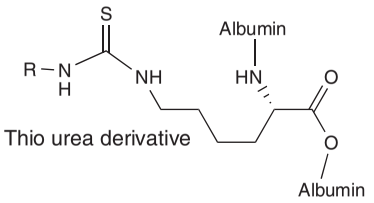
Early work by Chu *et al.* [15] showed that bovine serum albumin (BSA) with up to 20 mol methotrexate (MTX) per mol BSA was effective in MTX-transport-deficient cells *in vitro*. However, no *in vivo* data have been reported. Similar attempts by Fiume *et al.* (reviewed in [16]) with corticosteroid hemisuccinates linked to HSA at molar ratios between

Table 1. Reactions with which drugs or diagnostic agents (R in the table) can be covalently linked to serum albumin.

Functional group in drug R	Amino acid in HSA	Bond formed with HSA in conjugate	Cleavable*	Ref.‡
 Carboxylic acid	 ε-amino group of lysine	 Amide bond	±	[6]
 Dichlorotriazine	 ε-amino group of lysine	 Secondary amine	-	[47]
 Carbonyl	 ε-amino group of lysine	 Schiff base	+	[52]
 Aromatic amine / Aromatic diazoniumion	 Tyrosine	 azo bond	+	[53]
 Benzylic azide	Electrophilic atoms	 Secondary amine	±	[54]

*Cleavable by enzymes or by acid hydrolysis intracellularly or in the extracellular space.
‡Key references for the use of the conjugates.
HSA: Human serum albumin.

Table 1. Reactions with which drugs or diagnostic agents (R in the table) can be covalently linked to serum albumin (continued).

Functional group in drug R	Amino acid in HSA	Bond formed with HSA in conjugate	Cleavable*	Ref.‡
 2-iminothiolane	 ε-amino group of lysine	 Thiolated albumin	-	[9]
 Maleimide	 SH group of cysteine	 Thioether	±	[21]
 Isothiocyanate	 ε-amino group of lysine	 Thio urea derivative	?	[55]

*Cleavable by enzymes or by acid hydrolysis intracellularly or in the extracellular space.

‡Key references for the use of the conjugates.

HSA: Human serum albumin.

10 and 19 were effective *in vitro*. In these experiments a higher and selective uptake of the conjugate in comparison with the drug itself was observed in macrophages. Thereafter, an attempt was made to develop the compound to target inflamed liver cells. Stehle *et al.* [6] showed that a high loading ratio (**Figure 1**) automatically leads to accumulation in the liver, where modified albumin is taken up by scavenger receptors and degraded by the reticuloendothelial system. Therefore, the observed effect is due to the denatured protein and not a consequence of specific targeting.

The only albumin conjugates shown to be effective and non-immunogenic in experimental animals and patients were conjugates with a molar loading ratio close to unity. In this case albumin retains its native state and has a long half-life in circulation [17,18]. Therefore, aspects of covalently linking drugs to native albumin presented in this review are:

- *ex vivo*/*in vivo* loading of albumin
- variations in spacers between albumin and drug.

Possible direct conjugation reactions, the target amino acid in albumin and the probability of enzymatic or hydrolytic cleavage are shown in **Table 1**.

The aim of drug conjugation to albumin is to achieve a drug-albumin conjugate that is physiologically indistinguishable from native albumin. It has to be stable in circulation but must be cleaved at the site of inflammation, or in the tumor, either in the extracellular space or in the cells in lysosomes. The most commonly used method to link a drug to albumin is an amide bond with the ε-amino group of lysine. This bond is chemically and enzymatically very stable. It has been shown that in the case of methotrexate linked to albumin by a lysine in albumin (MTX-HSA), methotrexate is liberated from MTX-HSA in cell culture [19] and in patients [20]. However, it is not possible yet to elucidate the enzyme responsible for the reaction.

4. *ex vivo*/*in vivo* loading of albumin

The advantages of synthesizing and standardizing a drug that binds to endogenous albumin on infusion as opposed to *ex vivo* conjugation of human serum albumin of blood donors is quite evident and delineated in a review [16]. Kratz's group addressed this problem by developing an approach that uses the free cysteine Cys34 of endogenous albumin for the *in vivo* binding of drugs. MTX or other drugs such as platin derivatives or doxorubicin are linked by a peptide spacer to maleimide, which then binds to the SH group of Cys34 in albumin after parenteral administration of the drug. This cysteine makes up most of the free thiol groups in plasma, one-third of the molecules are usually bound as mixed disulfides, and in the other two-thirds of albumin molecules the thiol is in its reduced state. Owing to its amino acid environment, the pKa value of this thiol is ~ pH 5 as opposed to the normal pH 8 – 9 of cysteine SH. This results in the S^- form under physiological conditions and therefore a reactive partner for the maleimide [1].

In a recent publication [21] the albumin-binding prodrug of methotrexate AWO54 with the formula 6-maleimidocaproic acid (EMC)-D-Ala-Phe-Lys-Lys-MTX was introduced (**Figure 4**). It was designed to bind specifically to the cysteine 34 position of endogenous albumin (for reaction, see **Table 1**). In addition, the conjugate was cleavable *in vitro* by proteases such as cathepsin B and plasmin, which are present at the site of inflammation, leading to albumin and MTX-Lys. AWO54 was tested *in vitro* and *in vivo* for its effectiveness at reducing inflammation and joint destruction in rheumatoid arthritis, as will be discussed later in this review [21].

The property of albumin to avidly bind hydrophobic molecules has been used successfully by Abraxis Bioscience, who produce albumin nanoparticles with paclitaxel (nab-paclitaxel ABI-007), a highly insoluble but effective taxol used to treat a large variety of cancers. The formulation of paclitaxel associated with albumin eliminates the use of Cremophor as solvent and thus the pronounced side effects induced by this agent. Abraxane® (Abraxis Bioscience, Los Angeles, USA) has meanwhile been approved by the FDA [22] and the EMEA [23] for the treatment of metastatic breast cancer.

Albumin-bound peptides (for a review on these, see [16]) can be generated either by covalent binding of these to albumin or by transgenic means, creating fusion proteins. In both cases the aim is to prolong the very short half-lifes of the peptides, caused by their rapid clearance in the kidneys. The peptide-HSA fusion protein with the C terminus of activated recombinant Factor VII [24] fused to albumin is biologically active, as was an albumin fusion protein with interleukin-2 in mice, in which this compound induced the proliferation of T cells and inhibited liver tumor growth [25]. IFN-α_{2b} fused to albumin was effective against chronic hepatitis C in animals as well as in Phase II clinical trials (reviewed in [26]). All authors describe a long plasma half-life of their product as the main advantage over the peptides used otherwise, without loss of biological activity. The release mechanism of these peptides in a biologically active state has not, however, been investigated. If the liberation takes place by lysosomal enzymes that degrade albumin after its uptake, then the peptides are degraded as well. By contrast, if the peptide is set free by extracellular enzymes, then the mechanism of their pharmacologic activity is unknown.

5. Variations in spacers between albumin and drug

The quest for *a cleavable linker,* stable in blood but hydrolyzed at the target site, is one of the main issues in the development of drug-albumin conjugates. Conjugates with carboxylic ester bonds between the highly abundant glutamic and aspartic acid side chains in albumin [1] are readily synthesized. However, they have the disadvantage of being unstable in plasma. Esmaeili *et al.* found 50% of a succinic ester derivative of docetaxel to be hydrolyzed from albumin in 24 h [27]. Similar

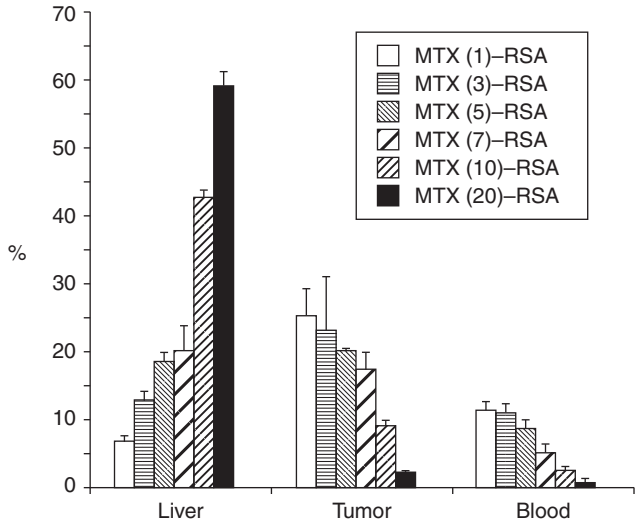


Figure 1. Distribution of radioactive methotrexate-albumin conjugates with different loading ratios. The distribution of ^{111}In-DTPA-labeled methotrexate-rat serum albumin conjugates in different rat organs is shown. The ratio of drug-to-albumin ranged from 1 to 20, radioactivity was assessed 24 h after intravenous administration to rats.
Used with permission from [6].

values were obtained by Tanaka *et al.* In this approach mitomycin C was linked to albumin that had been heavily modified by reaction with glutarate [28]. Owing to the relatively rapid hydrolysis of albumin-drug conjugates in plasma the advantage of targeting the drug to the tumor or the site of inflammation is lost and side effects similar to those obtained with the free drug are to be expected. Therefore, carboxylic esters are not shown in **Table 1**, which presents stable drug-albumin conjugates that may specifically be cleaved at the target site. Kratz and co-workers constructed conjugates with spacers cleavable by matrix metalloproteases, cathepsin B or plasmin (reviewed in [16]). The product of such enzymatic cleavage is always a drug with the last amino acid linked to it as amide, such as MTX-Lys. The biological activity of MTX-Lys can be shown in a murine arthritis model [21]. The cleavage step of MTX-Lys from albumin most probably takes place in the lysosomal compartment of the target cell or in the interstitial space by secreted enzymes in the malignant or inflammatory tissue.

6. *In vitro*/*in vivo* evidence for the effectiveness of albumin as drug carrier

The best analyzed HSA conjugate is albumin-coupled methotrexate (MTX-HSA) [18]. It has a molar loading ratio of unity and has been shown not to be immunogenic after long-term administration to patients. Studies in cellular systems with radiolabeled MTX-HSA have shown the release of MTX from the conjugate and the formation of polyglutamates of MTX up to a chain length of three extra glutamic acid residues [19]. MTX resistance based on decreased MTX uptake could be overcome by MTX-HSA, and thymidylate synthase was inhibited, an indication for intracellular liberation of MTX. Cell cycle arrest could be shown as proof of a biologically effective MTX release [29]. The uptake by endocytosis into a variety of cells could be shown with fluorescently labeled HSA as well as its lysosomal localization [7,30], where degradation of albumin by lysosomal enzymes occurs [19].

MTX-HSA was effective against a panel of xenografted human tumors in severe combined immune-deficient (SCID) mice, which were not MTX sensitive [31], as well as against transplantable rat tumors [32]. As MTX is not normally used in the treatment of carcinoma and solid tumors, the tumor targeting achieved by coupling to albumin opens up new treatment perspectives. This was indeed at least partly verified by clinical studies [18,33].

MTX-HSA was also tested in a murine graft versus host disease rat model, in which it was found to prevent very effectively the onset of graft versus host disease without causing toxicity [34].

MTX-HSA prevented the onset of arthritis in a collagen-induced arthritis mouse model and a combination with free MTX was found to be synergistic [35,36]. In the SCID mouse model of rheumatoid arthritis, in which normal human cartilage is transplanted together with synovial fibroblasts from rheumatoid arthritis patients, MTX-HSA decreased cartilage degradation and synovial fibroblast invasion [37]. As an example for a chronic inflammatory disease, the use of albumin as a drug carrier for rheumatoid arthritis is discussed in detail later in this review.

The antiproliferative capacity of MTX is limited. Therefore, the more potent anti-folate aminopterin was coupled to albumin in order to increase the antitumor efficiency. Indeed, *in vitro* and particularly *in vivo* data with rats showed an improved antitumor effect of aminopterin-HSA [38]. However, in the graft versus host model the therapeutic window of this compound was demonstrated to be narrow and toxicity occurred [34].

Albumin conjugates with maleimido derivatives of carboplatin or oxaliplatin were as effective in cisplatin-resistant cells as in the parental cell line. An endocytotic uptake mechanism is the reason for the observed activity. This was shown by inhibition of the uptake using bafilomycin A1. This finding points to an uptake of the albumin conjugates by clathrin-coated pits [39].

The efficiency of the prodrug concept of derivatizing drugs with acid or enzymatically cleavable linkers attached to a maleimido group to react with endogenous thiols has been shown in tumor-bearing rats using camptothecin and doxorubicin derivatives. In these studies the prodrug was more effective than camptothecin in a xenograft model [40,41]. The same group developed a prodrug of doxorubicin with an acid-sensitive linker (INNO-206), which is in clinical trial, and was found to be effective against anthracycline-sensitive tumors [42].

7. Proof of concept for albumin-based drug delivery in a chronic inflammatory disease: rheumatoid arthritis

The model of murine collagen-induced arthritis (CIA) is a commonly used animal model for human RA [43]. Similar to the human disease, mice with CIA experience arthritis of small and larger joints, which are histologically characterized by severe synovitis. After a relatively short time the joints show severe destruction of cartilage and bone. Owing to the similarity to human RA, albumin was expected to accumulate in the inflamed joints, where it would extravasate and be taken up by metabolically active cells of the inflamed synovium [3-5]. Indeed, 3 h after intravenous injection, aminofluoresceine-labeled human serum albumin (AFL-HSA) is detectable by laseroptical imaging in the inflamed but not in the non-inflamed joints of the same paw (**Figure 2**).

The distribution of radioactively labeled albumin (^{111}In-DTPA-HSA) was compared with that of radioactive MTX (^{3}H-MTX) in CIA mice. The albumin conjugates showed a six- to sevenfold higher accumulation in inflamed in comparison with non-inflamed paws [36]. By contrast, although free MTX accumulated also in the inflamed paw, the difference to the healthy leg was much smaller than in the case of albumin (**Figure 3**). MTX covalently coupled to albumin in a molar ratio of unity (MTX-HSA) was used to treat CIA mice. Different doses of MTX-HSA or MTX were administered to mice after immunization with bovine collagen. MTX-HSA was significantly more effective at suppressing arthritis than comparable doses of free MTX [36]. At least a fivefold higher dose of MTX was required to achieve the levels of suppression seen with MTX-HSA. Further experiments showed that MTX and MTX-HSA acted synergistically and that maximum levels of arthritis suppression were reached when both drugs were combined [35].

The effect of MTX-HSA is not restricted to murine models of arthritis. The human SCID mouse model of RA was used to detect albumin uptake by effector cells of cartilage destruction in the synovial pannus and to test therapeutic efficiency of albumin-coupled drugs *in vivo*. In this model human synovial fibroblasts from RA patients are co-implanted subcutaneously with cartilage in mice with severe combined immune deficiency [44-46]. After a few days the implanted RA synovial fibroblasts autonomously invade the cartilage and induce its degradation. Twenty-four hours after intravenous injection of AFL-HSA the fluorescence signal of the albumin conjugate can be detected laseroptically in sections of the inflammatory pannus, which invades the cartilage [36]. Moreover, the fluorescence can be localized in the cytoplasm of cells, demonstrating the cellular uptake of the conjugate. This confirmed previous findings from *in vitro* experiments with cultured RA synovial fibroblasts in which fluorescence-labeled albumin is taken up into the lysosomes of the cells [36]. Treatment of the SCID mice with MTX-HSA significantly inhibited RA synovial fibroblast invasion and reduced cartilage degradation, comparable to the effects elicited by MTX [37].

Synovial fibroblasts are not the only human cell types that are able to take up albumin. FACS analyses of mononuclear cells from healthy donors and RA patients incubated with AFL-HSA showed its uptake primarily into monocytes. AFL-HSA was also taken up by granulocytes, and B and T lymphocytes. A significant difference in AFL-HSA uptake between RA patients and normal controls was observed, however, only in T lymphocytes [35].

As described above, studies in animal models of arthritis show a good effectiveness of MTX-HSA owing to its accumulation in areas of synovial inflammation. Synthetic conjugates of drugs with human serum albumin have, however, the disadvantage of intravenous administration of donor albumin, which is increasingly subjected to regulatory restrictions. Therefore, a compound that would bind to endogenous albumin as a carrier for immunomodulatory drugs to the sites of inflammation would be preferable; this is the case for AWO54 (EMC-D-Ala-Phe-Lys-Lys-MTX) [21], as discussed above.

After intravenous injection of AWO54 a superior therapeutic effect to MTX in the treatment of mice with CIA was achieved. To obtain a similar effect AWO54 had to be given at only ~ 20% of the dose of free MTX. The efficacy of the drug was tested in different stages of CIA in the mice: whereas MTX lost its efficacy in later stages of the disease, that is, after onset of RA, AWO54 was active, reducing disease activity in arthritic mice even in later disease stages. In conclusion, the use of albumin-binding prodrugs such as AWO54 is a way to use endogenous albumin to deliver drugs such as MTX to the site of inflammation and to increase the efficiency of the anti-arthritic drugs even in later stages of the disease.

8. Albumin-labeled drugs for diagnostic use

The use of fluorescence-labeled albumin for intraoperative fluorescence staining of malignant brain tumors has been described recently [47]. After the observation of a very specific uptake of AFL-HSA into rat glioblastoma [7], this compound was tested as an agent to guide tumor resection in brain surgery by intravital staining of brain tumor tissue. Patients received intravenous injections of AFL-HSA 1 – 4 days before surgery. Intraoperatively, an argon laser was used to guide the extent of tumor resection by the surgeon. All glioblastoma in the 10 patients investigated accumulated AFL-HSA. Agreement between fluorescence – or no fluorescence – and histology was 83% [47]. It was concluded that albumin is a suitable carrier system for selective intraoperative imaging of glioblastoma as an aid for neurosurgeons. The pharmacokinetic values for the HSA conjugate agree very favorably with those published for native HSA in humans, and therefore emphasize the usefulness of conjugates in which the native structure of albumin is conserved. A further example for a diagnostic use of albumin conjugates, an albumin conjugate decorated with

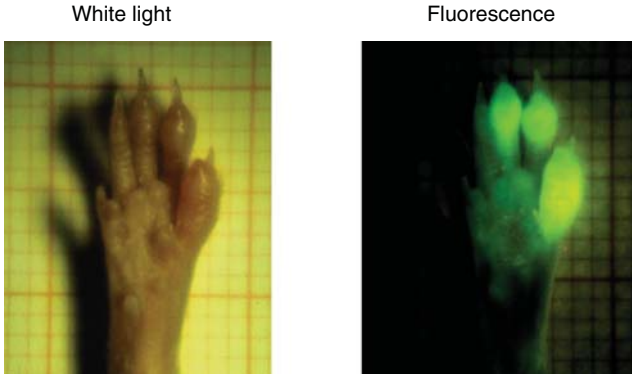


Figure 2. Uptake of fluorescent albumin into arthritic joints *in vivo*. Uptake of human serum albumin labeled with aminofluorescein (AFL-HSA) by inflamed toes of a mouse with collagen-induced arthritis determined by laseroptical imaging. The pictures show images under white and laser light of an inflamed paw with three arthritic toes, 3 h after intravenous injection of AFL-HSA. Only toes affected by arthritis showed strong fluorescence when illuminated at 488 nm, demonstrating the high rate of albumin accumulation in inflamed toes.
Used with permission from [36].

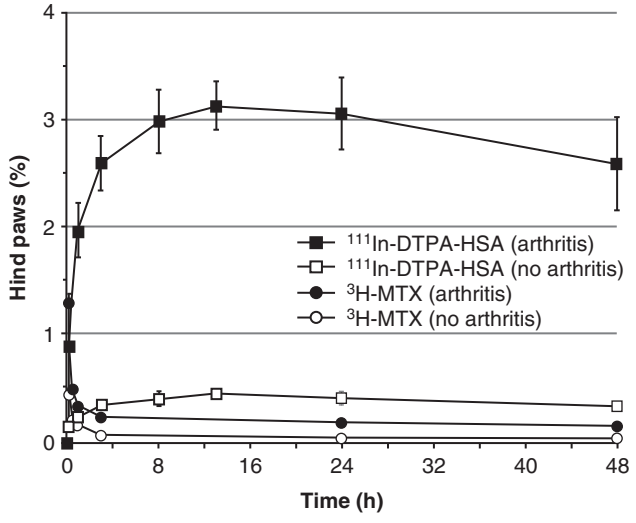


Figure 3. Comparison of uptake of radioactively labeled albumin and methotrexate *in vivo*. Uptake of radiolabeled human serum albumin (^{111}In-DTPA-HSA) and radiolabeled methotrexate (^{3}H-MTX) in hind paws of mice with and without collagen induced arthritis after intravenous injection of the compounds. Significant albumin amounts accumulated in arthritic hind paws, exceeding uptake into non-inflamed paws six- to sevenfold. By contrast, the difference in uptake of radiolabeled methotrexate into arthritic and healthy hind paws was found to be significantly less.
Used with permission from [36].

RGD peptides targeting cells expressing $\alpha_v\beta_3$ and linked with dyes emitting near-infrared fluorescence, was used successfully for *in vivo* imaging of vasculature and tumor tissue and confirmed to be dependent on integrin binding of the conjugate [48].

For the diagnosis of muscle dystrophy in dystrophin-deficient mice, a model for Duchenne muscular dystrophy, gadolinum was complexed to diethylenetriamine penta-acetic acid bound to albumin (Gd-DTPA-HSA) and the distribution in mice observed by MRI. Gd-DTPA-HSA accumulated only in damaged fibers. This could be confirmed by histology and using anti-HSA antibodies [49].

In an early compassion at use study with 23 patients with bronchogenic tumors using ^{111}In-DTPA-HSA and single-photon emission tomography (SPEC), albumin uptake into the tumors of 8 patients with adenocarcinoma and squamous cell carcinoma was seen, but no uptake into small cell carcinoma could be observed [50].

Comparable techniques have not been tested in inflammatory diseases so far. Preliminary data from animal models show, however, that fluorescence-labeled albumin might also be helpful in inflammatory diseases. Studies were performed in the animal model of endotoxin-induced uveitis, which is based on the induction of an autoimmune uveitis 24 h after intraperitoneal injection of endotoxin. This model has a lot of similarities to human uveitis and the inflammation can be visualized by intravital microscopy [51]. When fluorescence-labeled albumin is given by intravenous injection it accumulates in the inflamed eye. Using a laser technique, the fluorescence can be visualized and intravital microscopy can be used to distinguish anatomic sites of the eye, which show increased albumin uptake. **Figure 5** shows the macroscopic perspective of endotoxin-induced uveitis after intravenous injection of fluorescence-labeled albumin under white and laser light. Intravital microscopy of the vessels of the iris in the inflamed eye in these mice showed an increased uptake of albumin in the inflamed vessels.

The model of endotoxin-induced uveitis shows that imaging techniques using fluorescence-labeled albumin can be used to detect not only tumors but also autoimmune inflammation and to distinguish clearly between inflamed and non-inflamed anatomic structures.

9. Conclusion

Conjugating drugs to native albumin is a promising approach for drug delivery that has already found its way into clinical trials. The design of albumin-coupled drugs leading to the site-specific delivery of a physiologically active drug in the human organism remains, however, challenging. Mechanisms of *in vivo* loading as well as the optimization of specific cleavage sites within the construct are being developed. Evidence from *in vitro* experiments, as well as from animal models of different diseases, is promising for the therapeutic and diagnostic development of albumin as drug carrier.

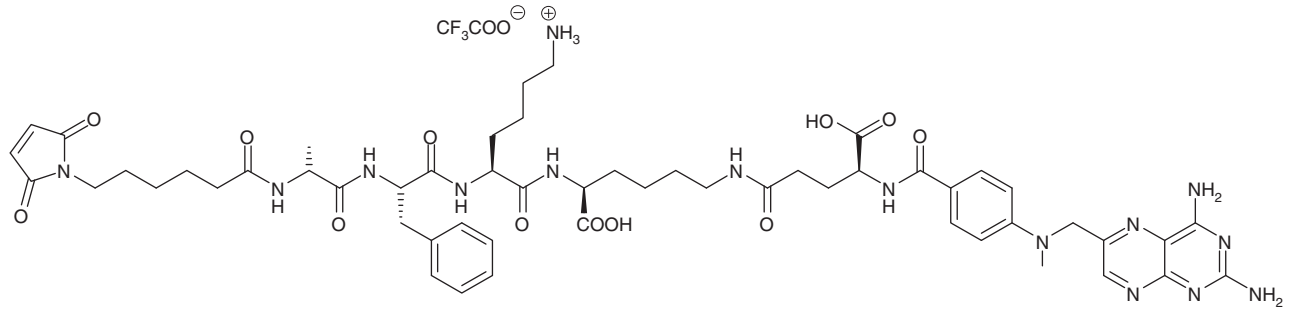


Figure 4. Structure of AWO54. AWO54 is a prodrug of methotrexate with the formula 6-maleimidocaproic acid-D-Ala-Phe-Lys-Lys-MTX that consists of methotrexate, a lysine spacer, and an enzymatically cleavable peptide linker bearing the albumin-binding maleimido group (EMC).
Used with permission from [21].

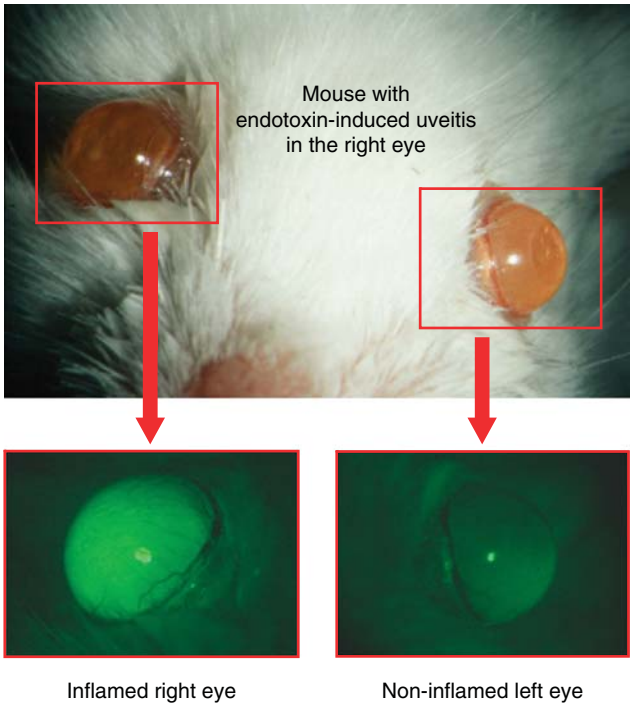


Figure 5. Albumin uptake into an inflamed eye. White light and fluorescent images of a BALB/c mouse with endotoxin-induced uveitis are shown 3 h after intraocular administration of endotoxin. Aminofluorescein-labeled albumin was administered simultaneously intravenously. Only the inflamed right eye showed a strong green fluorescence signal.

10. Expert opinion

Albumin-based drug delivery is a promising approach to increase the local effectiveness of the drug by specifically targeting cells in inflamed or tumor tissue. Present data show that the required systemic drug concentration of HSA-drug conjugates can be lower than that of the low-molecular-mass drug or an enhanced effect can be obtained with the same dose. This goal can be achieved owing to the favourable biochemical and pharmacological properties of albumin conjugates as long as the albumin molecule retains its native state. This prerequisite for synthetic albumin conjugates is, on the one hand, a disadvantage for the conjugation of drugs with low potency because a molar loading ratio of one reduces the effective dose per cell; on the other hand, specific targeting using very effective but toxic drugs conjugated to albumin will reduce their systemic toxicity and the ensuing side effects.

The specific cleavage of the drug from the HSA conjugate at the site of inflammation or tumor is, however, still a biochemical challenge. In addition, intravenous administration is compulsory for both approaches: the synthetic drug-HSA conjugates, as well as the prodrug administered to bind covalently to HSA in the blood.

The goal of continuing and future research is to target specifically diseased areas (e.g., tumors, inflammation, as well as sites of tissue destruction). To decrease side effects further and reduce the required dose are other important issues for the development of HSA-coupled drugs. Fluorescence-guided surgery and the use of fluorescent HSA conjugates for diagnostic purposes are very promising applications now under investigation.

To achieve these goals, *in vivo* loading of a prodrug with a specifically designed spacer and/or cleavage sites to optimize liberation of the drug from the albumin carrier for therapeutic applications is now being investigated. However, for diagnostic purposes, *ex vivo* coupled fluorescent HSA is most probably more suitable.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

1. Peters TJ. All about albumin. Biochemistry, genetics, and medical applications 1996. Academic Press, New York; 1996
2. Coussens LM, Werb Z. Inflammatory cells and cancer: think different! J Exp Med 2001;193:F23-6
3. Levick JR. Permeability of rheumatoid and normal human synovium to specific plasma proteins. Arthritis Rheum 1981;24:1550-60
4. Ballantyne FC, Fleck A, Dick WC. Albumin metabolism in rheumatoid arthritis. Ann Rheum Dis 1971;30:265-70
5. Niwa Y, Iio A, Niwa G, et al. Serum albumin metabolism in rheumatic diseases: relationship to corticosteroids and peptic ulcer. J Clin Lab Immunol 1990;31:11-6
6. Stehle G, Sinn H, Wunder A, et al. The loading rate determines tumor targeting properties of methotrexate-albumin conjugates in rats. Anticancer Drugs 1997;8:677-85
7. Kremer P, Wunder A, Sinn H, et al. Laser-induced fluorescence detection of malignant gliomas using fluorescein-labeled serum albumin: experimental and preliminary clinical results. Neurol Res 2000;22:481-9
8. Kang H, Alam MR, Dixit V, et al. Cellular delivery and biological activity of antisense oligonucleotides conjugated to a targeted protein carrier. Bioconjug Chem 2008;19:2182-8
9. Temming K, Meyer DL, Zabinski R, et al. Improved efficacy of alphavbeta3-targeted albumin conjugates by conjugation of a novel auristatin derivative. Mol Pharm 2007;4:686-94
10. Dosio F, Arpicco S, Stella B, et al. Folate-mediated targeting of albumin conjugates of paclitaxel obtained through a heterogeneous phase system. Int J Pharm 2009;382:117-23
11. Melgert BN, Weert B, Schellekens H, et al. The pharmacokinetic and biological activity profile of dexamethasone targeted to sinusoidal endothelial and Kupffer cells. J Drug Target 2003;11:1-10
12. Melgert BN, Olinga P, Jack VK, et al. Dexamethasone coupled to albumin is selectively taken up by rat nonparenchymal liver cells and attenuates LPS-induced activation of hepatic cells. J Hepatol 2000;32:603-11
13. Maeda H, Wu J, Sawa T, et al. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 2000;65:271-84
14. Jain RK. Delivery of molecular and cellular medicine to solid tumors. J Control Release 1998;53:49-67
15. Chu BC, Fan CC, Howell SB. Activity of free and carrier-bound methotrexate against transport-deficient and high dihydrofolate dehydrogenase-containing methotrexate-resistant L1210 cells. J Natl Cancer Inst 1981;66:121-4
16. Kratz F. Albumin as a drug carrier: design of prodrugs, drug conjugates and nanoparticles. J Control Release 2008;132:171-83
17. Wunder A, Stehle G, Sinn H, et al. Enhanced albumin uptake by rat tumors. Int J Oncol 1997;11:497-507
18. Hartung G, Stehle G, Sinn H, et al. Phase I trial of methotrexate-albumin in a weekly intravenous bolus regimen in cancer patients. Phase I Study Group of the Association for Medical Oncology of the German Cancer Society. Clin Cancer Res 1999;5:753-9
19. Weigand M, Hartung G, Roboz J, et al. Mode of action of methotrexate-albumin in a human T-cell leukemia line and activity against an MTX-resistant clone. Anticancer Drug Des 2001;16:227-37
20. Hartung G, Heeger T, Bertsch T, et al. Adaption and clinical evaluation of a homogenous enzyme multiplied immunoassay technique (EMIT) for drug monitoring of a methotrexate-albumin conjugate (MTX-HSA) in humans. Onkologie 2000;23:352-7
21. Fiehn C, Kratz F, Sass G, et al. Targeted drug delivery by in vivo coupling to endogenous albumin: an albumin-binding prodrug of methotrexate (MTX) is better than MTX in the treatment of murine collagen-induced arthritis. Ann Rheum Dis 2008;67:1188-91
22. Gradishar WJ. Albumin-bound paclitaxel: a next-generation taxane. Expert Opin Pharmacother 2006;7:1041-53
23. EMEA. Available from: http://www.ema.europa.euhumandocs/Humans/EPAR/abraxane/abraxane.htm
24. Schulte S. Use of albumin fusion technology to prolong the half-life of recombinant factor VIIa. Thromb Res 2008;122(Suppl 4):S14-19
25. Melder RJ, Osborn BL, Riccobene T, et al. Pharmacokinetics and in vitro and in vivo anti-tumor response of an interleukin-2-human serum albumin fusion protein in mice. Cancer Immunol Immunother 2005;54:535-47
26. Rustgi VK. Albinterferon alfa-2b, a novel fusion protein of human albumin and human interferon alfa-2b, for chronic hepatitis C. Curr Med Res Opin 2009;25:991-1002
27. Esmaeili F, Dinarvand R, Ghahremani MH, et al. Docetaxel-albumin conjugates: preparation, in vitro evaluation and biodistribution studies. J Pharm Sci 2009;98:2718-30
28. Tanaka T, Shiramoto S, Miyashita M, et al. Tumor targeting based on the effect of enhanced permeability and retention (EPR) and the mechanism of receptor-mediated endocytosis (RME). Int J Pharm 2004;277:39-61
29. Wosikowski K, Biedermann E, Rattel B, et al. In vitro and in vivo antitumor activity of methotrexate conjugated to human serum albumin in human cancer cells. Clin Cancer Res 2003;9:1917-26
30. Eschen N, Bauder-Wust U, Frei E, et al. Aminopterin-human serum albumin conjugate (AP-HSA): uptake and cytotoxic effects in tumor cell lines. Int J Clin Pharmacol Ther 2002;40:564-6
31. Burger AM, Hartung G, Stehle G, et al. Pre-clinical evaluation of a methotrexate-albumin conjugate (MTX-HSA) in human tumor xenografts in vivo. Int J Cancer 2001;92:718-24
32. Wunder A, Stehle G, Schrenk HH, et al. Antitumor activity of methotrexate-albumin conjugates in rats bearing a Walker-256 carcinoma. Int J Cancer 1998;76:884-90
33. Vis AN, van der Gaast A, van Rhijn BW, et al. A phase II trial of methotrexate-human serum albumin (MTX-HSA) in patients with metastatic

renal cell carcinoma who progressed under immunotherapy. Cancer Chemother Pharmacol 2002;49:342-5

34. Wolff D, Frei E, Hofmeister N, et al. Methotrexate-albumin and aminopterin-albumin effectively prevent experimental acute graft-versus-host disease. Transplantation 2006;82:527-33
35. Fiehn C, Muller-Ladner U, Gay S, et al. Albumin-coupled methotrexate (MTX-HSA) is a new anti-arthritic drug which acts synergistically to MTX. Rheumatology (Oxford) 2004;43:1097-105
36. Wunder A, Muller-Ladner U, Stelzer EH, et al. Albumin-based drug delivery as novel therapeutic approach for rheumatoid arthritis. J Immunol 2003;170:4793-801
37. Fiehn C, Neumann E, Wunder A, et al. Methotrexate (MTX) and albumin coupled with MTX (MTX-HSA) suppress synovial fibroblast invasion and cartilage degradation in vivo. Ann Rheum Dis 2004;63:884-6
38. Kremer P, Hartung G, Bauder-Wust U, et al. Efficacy and tolerability of an aminopterin-albumin conjugate in tumor-bearing rats. Anticancer Drugs 2002;13:615-23
39. Garmann D, Warnecke A, Kalayda GV, et al. Cellular accumulation and cytotoxicity of macromolecular platinum complexes in cisplatin-resistant tumor cells. J Control Release 2008;131:100-6
40. Schmid B, Chung DE, Warnecke A, et al. Albumin-binding prodrugs of camptothecin and doxorubicin with an Ala-Leu-Ala-Leu-linker that are cleaved by cathepsin B: synthesis and antitumor efficacy. Bioconjug Chem 2007;18:702-16
41. Schmid B, Warnecke A, Fichtner I, et al. Development of albumin-binding camptothecin prodrugs using a Peptide positional scanning library. Bioconjug Chem 2007;18:1786-99
42. Unger C, Haring B, Medinger M, et al. Phase I and pharmacokinetic study of the (6-maleimidocaproyl)hydrazone derivative of doxorubicin. Clin Cancer Res 2007;13:4858-66
43. Wooley PH. Collagen-induced arthritis in the mouse. Methods Enzymol 1988;162:361-73
44. Neumann E, Judex M, Kullmann F, et al. Inhibition of cartilage destruction by double gene transfer of IL-1Ra and IL-10 involves the activin pathway. Gene Ther 2002;9:1508-19
45. Muller-Ladner U, Kriegsmann J, Franklin BN, et al. Synovial fibroblasts of patients with rheumatoid arthritis attach to and invade normal human cartilage when engrafted into SCID mice. Am J Pathol 1996;149:1607-15
46. Lefevre S, Knedla A, Tennie C, et al. Synovial fibroblasts spread rheumatoid arthritis to unaffected joints. Nat Med 2009;15:1414-20
47. Kremer P, Mahmoudreza F, Ding R, et al. Intraoperative fluorescence staining of malignant brain tumors using 5-aminofluorescein-labeled albumin. Neurosurgery 2009;64:53-60; discussion 60-51
48. Chen K, Xie J, Chen X. RGD-human serum albumin conjugates as efficient tumor targeting probes. Mol Imaging 2009;8:65-73
49. Amthor H, Egelhof T, McKinnell I, et al. Albumin targeting of damaged muscle fibres in the mdx mouse can be monitored by MRI. Neuromuscul Disord 2004;14:791-6
50. Clorius JH, Sinn H, Manke HG, et al. Serum albumin (SA) accumulation by bronchogenic tumours: a tracer technique may help with patient selection for SA-delivered chemotherapy. Eur J Nucl Med 1995;22:989-96
51. Becker MD, Nobiling R, Planck SR, Rosenbaum JT. Digital video-imaging of leukocyte migration in the iris: intravital microscopy in a physiological model during the onset of endotoxin-induced uveitis. J Immunol Methods 2000;240:23-37
52. Simi CK, Abraham TE. Nanocomposite based on modified TiO2-BSA for functional applications. Colloids Surf B Biointerfaces 2009;71:319-24
53. Tracey BM, Shuker DE. Characterization of azo coupling adducts of benzenediazonium ions with aromatic amino acids in peptides and proteins. Chem Res Toxicol 1997;10:1378-86
54. Hanstein WG, Hatefi Y, Kiefer H. Radiochemical synthesis and photochemical properties of the uncoupler 2-azido-4-nitrophenol, a versatile photoaffinity labeling reagent. Biochemistry 1979;18:1019-25
55. Murtaza Z, Herman P, Lakowicz JR. Synthesis and spectral characterization of a long-lifetime osmium (II) metal-ligand complex: a conjugatable red dye for applications in biophysics. Biophys Chem 1999;80:143-51

Affiliation

Elena Neumann[†1], Eva Frei[2], Dorothee Funk[2], Matthias D Becker[3], Hans-Hermann Schrenk[4], Ulf Müller-Ladner[1] & Christoph Fiehn[5]
[†]Author for correspondence
[1]Justus-Liebig-University Gießen, Department of Internal Medicine and Rheumatology, Kerckhoff-Klinik Bad Nauheim, Benekestr. 2-8, 61231 Bad Nauheim, Germany
Tel: +49 6032 996 2801;
Fax: +49 6032 996 2809;
E-mail: e.neumann@kerckhoff-klinik.de
[2]National Center for Tumor Diseases and German Cancer Research Center, Division of Molecular Toxicology, Preventive Oncology, Im Neuenheimer Feld 581, 69120 Heidelberg, Germany
[3]Triemli Hospital, Department of Ophthalmology, Birmensdorferstrasse 497, 8063 Zürich, Switzerland
[4]National Center for Tumor Diseases and German Cancer Research Center, Division of Molecular Toxicology, Functional Proteome Analysis, Im Neuenheimer Feld 581, 69120 Heidelberg, Germany
[5]Centre of Rheumatic Diseases, 76530 Baden-Baden, Rotenbachtalstraße 5, Germany