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Review article

Antibody-drug conjugates- stability and formulation

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ABSTRACT

The number of antibody-drug conjugates (ADCs) on the market is expected to multiply in the upcoming years. The main reason: this novel drug delivery system combines the benefits of the selectivity of the antibody and the potency of the cytotoxic agent. The interplay of the antibody, linker and payload, however, calls for a stable and unique formulation. In this review, the literature on the stability of marketed ADCs and the respective formulations are summarized and used as a basis to give general formulation considerations for ADCs. Whereas the same excipients are used as in antibody formulations, specific focus is on the ionic strength and concentrations of the excipients of the ADC. Further, a short outline of the analytical toolbox to characterize ADC formulations is included.

What are antibody-drug conjugates?

The use of antibodies in cancer treatment has led to a tremendous increase in therapeutic possibilities [1–3]. Antibodies demonstrate little off-targeted toxicity due to their selective binding of the antigen or antigen-positive cells, respectively. However, antibodies are often not potent enough to efficiently combat a tumor [4]. Antibody-drug conjugates (ADCs) combine the benefits of an antibody and a cytotoxic agent, which enhances the efficacy and selectivity for tumor cell killing [5]. Once the antibody specifically binds to the target receptor of a cell, the ADC may be internalized and the cytotoxic agent released, leading to cell death (Fig. 1) [6–8]. ADCs can thus be considered as advanced drug delivery systems [9]. This concept had already been discussed in the 1970s, but initial trials in the 1980s had a lack of success [4,10] with b96-doxorubicin [11] and desacetylvinblastine [12] as payloads.

Since the approval of Mylotarg® (Gemtuzumab ozogamicin) in 2000 and later Adcetris® (brentuximab vedotin), Kadcyla® (trastuzumab emtansine), and most recently Bespona® (inotuzumab ozogamicin), research has flourished [4,13,14]. Sales are expected to multiply, as the market was valued at USD 1.3 Bn in 2015 and is predicted to increase up to USD 29.3 Bn by 2022 [15,16]. Currently, there are at least 80 ADCs in clinical development, of which most are still in the pilot stages [17–20]. Of the nine ADCs in advanced trials, the FDA has rewarded seven of them with Breakthrough Therapy Designation [20,21]. Different factors challenge the success of this payload delivery system, e.g. payload internalization as an intact ADC, expression of the target antigen on tumor tissue, extra- as well as intracellular linker stability and the correct payload for the tumor to be addressed [22,23].

With respect to antigen binding, the ADC should act like the parent antibody [9]. Conjugation may change the pharmacokinetic and thus pharmacological properties of the antibodies, for example the mean half-life of trastuzumab is reduced from 28.5 to 6 days upon conjugation with emtansine [2,24]. The pharmacokinetics of an ADC are mainly determined by the antibody, but it is also influenced by the linker, payload, as well as the conjugation itself [25]. Bender et al. have demonstrated faster ADC clearance compared to the mAb clearance because of deconjugation which involves antibody degradation [26]. Only 1.56% of the administered payload reaches the target cells if each step in the ADC mechanism is 50% efficient [27]. The actual uptake is assumed to be even lower [28]. A compromise for the number of drugs per antibody is typically needed for the highest possible cytotoxicity and the highest possible stability of the antibody [9]. A number of two to four drugs per antibody is considered to generate the best therapeutic window [9,29,30]. Moreover, larger drug payloads (e.g. bacterial exotoxins) may interfere with antigen/Fc receptor binding [31]. Binding to the receptor may be decreased as it has been observed for larger PEG moieties [32].

Abbreviations: ADC, antibody-drug conjugates; DAR, drug-to-antibody ratio; EDTA, Ethylenediaminetetraacetic acid; FDA, Food and Drug Administration; EMA, European Medicines Agency; mAb, monoclonal antibody; SMCC, succinimidyl4-(N-maleimidomethyl)cyclohexan-1-carboxylate; TRIS, tris(hydroxymethyl)aminomethane

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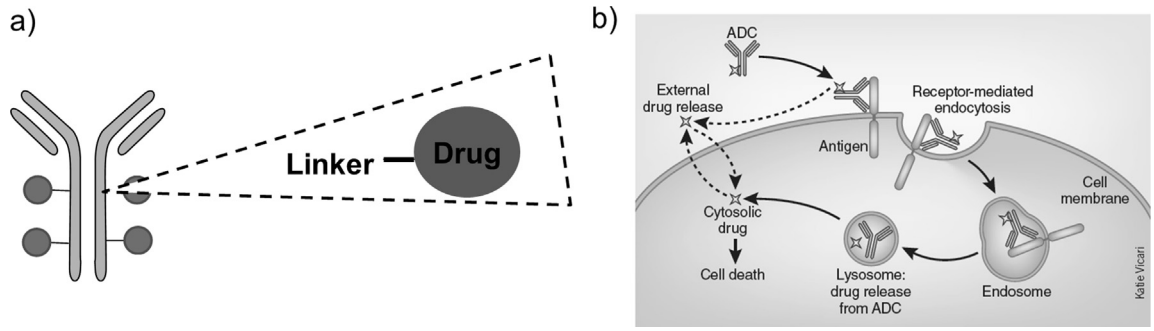

Fig. 1. (a) Basic concept of an ADC, adapted from [27]. (b) Primary mechanism of action [6]. Solid lines depict cell death through receptor-mediated endocytosis and dashed lines through external drug release.

1. Specific components of the ADC

During the development of a clinically efficient monoclonal antibody (mAb) or ADC to a market product, a stable formulation needs to be developed. Proteins are susceptible to chemical and physical degradation [33–35]. Drug conjugation introduces more complexity and instability aspects to the antibody. In the following, stability considerations for each component and the whole ADC molecule will be discussed.

1.1. Antibody component

MAbs can be characterized quite extensively, although Rituxan® as the first commercially available mAb was only approved in 1997 [36–42]. So far, only full IgG molecules have been utilized in ADC development because of their accumulation at the tumor and long circulatory half-life [27,43]. Other antibody formats are conceivable for ADCs, but have not been tested in the clinics. These can include fragments, e.g. single-chain Fv fragments or minibodies [43]. For solid tumors for example, the use of diabodies and minibodies might increase tumor penetration [44]. Furthermore, a bispecific ADC targeting HER2 and CD63 has been tested in preclinical studies [45]. Following the general trend for mAbs, most ADCs are based on humanized or fully human sequences [46]. Moreover, the antibodies used in ADCs should typically be internalized upon target binding as most payloads are devised to be released intracellularly [47,48]. Preclinically, Gébleux et al. have shown that ADCs with non-internalizing antibodies can also display potent anti-cancer activity [49].

It is vital for the product that the high immunoaffinity of the parent mAb is retained after conjugation [9]. For conjugation, various functional groups of the antibody have been used. These are the interchain cysteine residues (thiols), amines (lysines), alcohols, aldehydes and azides [50]. Free sulfhydryls may decrease mAb stability, but the reduction of interchain cysteines does not negatively affect antibody stability and was one of the first used conjugation methods [50,51]. Cysteine-linked ADCs are heterogeneous in terms of drug load and conjugation site, as zero to eight drugs per antibody are possible [52]. In order to improve homogeneity, reactive thiol groups that do not alter IgG functions have been introduced through cysteine substitutions in the primary structure, which have been termed ThioMabs [24,53,54].

Modification of the surface lysine residues by addition of the linker neutralizes positive charge on these residues [55]. Up to eight conjugated drugs per antibody have been reported to result from lysine conjugation, although theoretically a higher number is possible [52]. In a lysine-conjugated ADC, almost 50% of the lysines were partially modified, especially in regions of high flexibility and solvent accessibility [55,56]. Hence, lysine-linked ADCs are more heterogeneous, but deviate less from the naïve mAb compared to the cysteine-linked ADCs [57]. In order to enable a better control of drug load and specificity for lysine-coupled ADCs, research is conducted to only target the lysines that are most solvent-accessible and reactive [58]. Further techniques such as enzymatic conjugation, conjugation by using non-natural amino acids [59–61], e.g. p-acetylphenylalanine [62] and site-specific conjugation via additional glycans [63] are under development [64].

1.2. Linker component

The stability of the linker in the context of process chemistry, plasma circulation and product storage is of crucial importance for the ADC [9,57,65,66]. Furthermore, the payload must be inert against endogenous reactive molecules and has to be released at the target [31]. The choice of linker also affects antigen and Fc receptor binding as well as thermal stability [32].

The different linkers have been nicely reviewed in literature [9,50,65,67,68]. In short, the linkers can be divided into chemically-labile, enzyme-labile and non-cleavable. The chemically labile linkers are cleaved through pH-dependent mechanisms, implying that they are susceptible to the acidic pH of the lysosome. An example is the hydrazone linker used in Mylotarg® and Besponsa® (Fig. 2) [69,70]. At pH 7.2, the linker is relatively stable ($t_{1/2} > 60$ h) compared to pH 5.0 ($t_{1/2}$ 3 h) [48]. As acidic conditions can also be found in the body outside of the lysosome, nonspecific drug release can occur [69]. One of the reasons for the temporary withdrawal of Mylotarg® was its instability in plasma [69]. Linker stability is increased using enzyme-labile linkers, which are cleaved by lysosomal proteases. This linkage has been successfully implemented for Adcetris ® using a valine-citrulline dipeptide linker [48]. ADCs with non-cleavable linkers are internalized, upon which the antibody is degraded. For non-cleavable linkers, the non-targeted release of the free drug is reduced, which decreases systemic toxicity [71]. An example for an effective usage is Kadcyla ® with the succinimidyl 4-(N-maleimidomethyl)cyclohexan-1-carboxylate (SMCC) linker. The drug and linker together act as the potent cytotoxic [9,47,72]. The optimal choice of linker also depends on the target antigen, which should be located close to the cell-surface to enable binding of the ADC [52]. New forms of less hydrophobic linkers, which enable conjugation of a higher number of hydrophobic payloads per antibody are also discussed in literature [73]. Stability of the linker itself is not part of this review and the reader is referred to other publications [9,65,68].

The choice of linker defines the drug-to-antibody ratio (DAR), drug load distribution and stability of the linkage, which are vital to product quality [74]. Further, the analysis of residual or dissociated free drug is important for toxicity and safety assessment [75]. As an example, the frequently used thio-succinimide linker can undergo retro and exchange reactions at physiological pH and temperature if other thiols are in the vicinity and its hydrophobicity may increase protein aggregation [74,76–78].

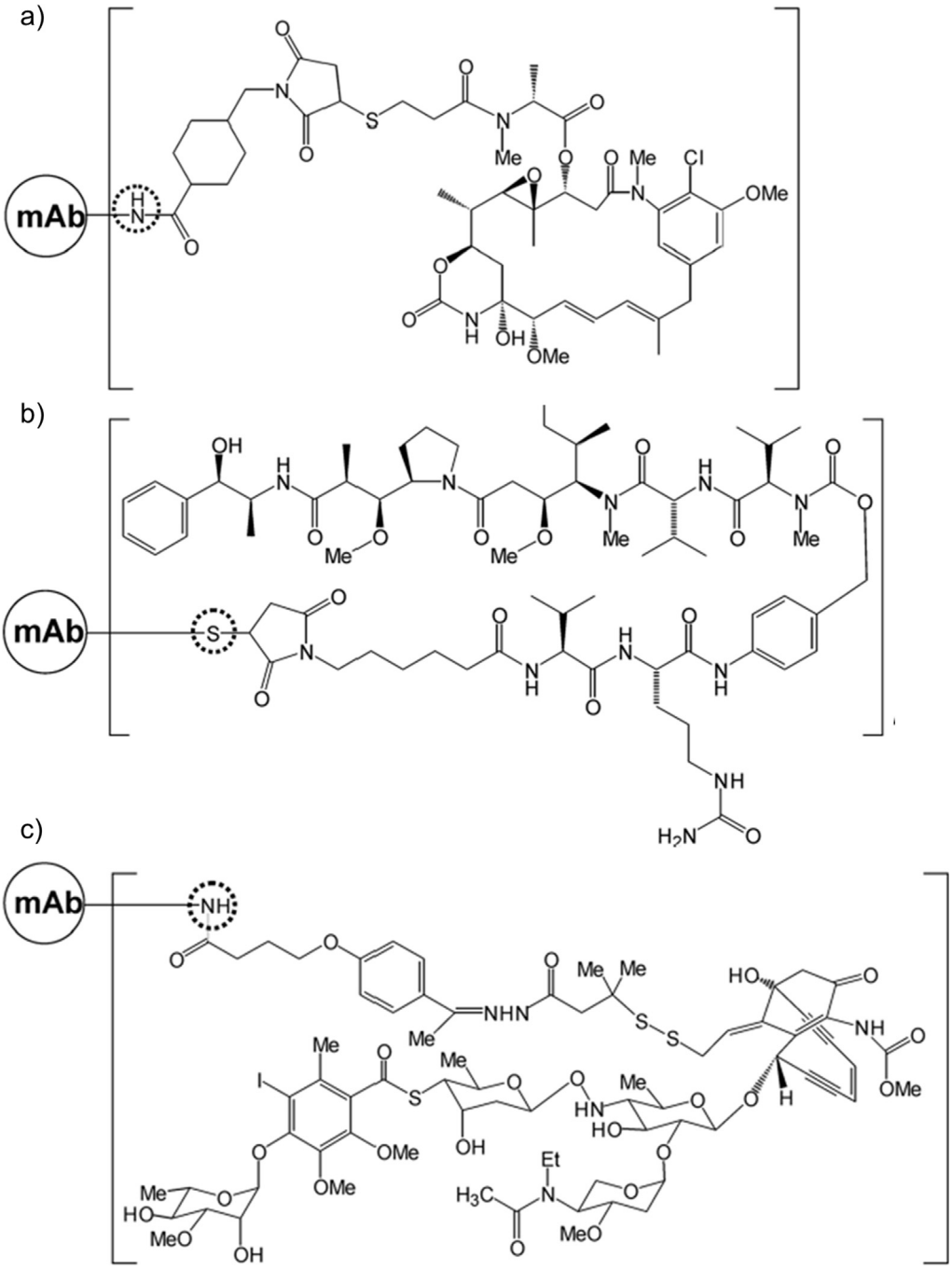

Fig. 2. Chemical structures of the three linkages and payloads on the market: SMCC (succinimidyl 4-(*N*-maleimidomethyl)cyclohexance-1-carboxylate) linked emtansine (a), valine-citrulline linked vedotion (b) and hydrazone linked ozogamicin (c) [13].

1.3. Payload component

Drugs that are chosen as payloads for ADCs are highly cell toxic and are released from the ADC in their potent forms [68]. The payload and this final metabolite form determine the toxicity of an ADC molecule [79]. In the first era of ADCs, the released payload molecule differed from the free drug, which led to a reduced potency of the ADC [11,66]. Current ADC payloads fall into two categories: DNA-targeting payloads and payloads targeting tubulin. DNA-targeting payloads that have been used in clinical trials are calicheamicin, pyrrolobenzodiazepine dimers, doucarmaycin, and indolinobenzodiazepine dimers. Payloads that target tubulin are anti-mitotic, examples are auristatins and maytansinoids [46]. Because of the low percentage of ADC that actually reaches the target, the potency of the released drug must be high enough to kill the tumor cell ($> 10^6$ molecules/cell) [9,14].

The payloads are typically too toxic for traditional chemotherapy and often have little selectivity for tumor cells [9,14,50,80,81]. For example, DNA alkylating agents such as duocarmycins [82] and pyrrolobenzodiazepines [83–85] have been reintroduced into research development and new highly potent drugs are currently evaluated [80,86,87]. Typical physicochemical properties of payloads based on emtansine and vedotin (Fig. 2) are a molecular weight of about 700–750 Da, an H donor and acceptor sum of about 15 and a log P of approx. four. As described by McCombs et al., drugs are suitable as an ADC payload, if the solubility, amenability to conjugation and stability are high enough. For example, whereas lipophilicity is beneficial to pass

cell membranes, conjugation in aqueous buffers is also necessary [69]. Hydrophobicity may also impact the stability of the ADC (please s. 1.4) and the use of hydrophilic linkers may reduce the hydrophobic impact on the ADC itself [73]. Moreover, the drugs must have a functional group that enables conjugation, but which does not affect potency [69]. As described, maytansinoids are used as an ADC payload. The challenge of adding a functional group to enable linkage led to studies on the moiety which position could be modified and with which functional group. Finally, a defined position was found adequate to be able to add a new ester side chain that contains a terminal thiol group [14,88]. Other than a terminal thiol groups, prodrugs have also been considered, for example chloromethyl, carbamoyl, peptidyl and carbonate derivatives [14].

1.4. ADC considerations

ADC characteristics are influenced by the DAR, as it determines the efficacy and stability of the ADC [75]. If the chosen DAR is too low, cytotoxicity is insufficient. If it is selected too high, the ADC might be recognized by the immune system and it is also more prone to aggregate [9]. A DAR of two to four is considered to be optimal [29]. For a specific ADC, the optimal DAR needs to be determined experimentally to determine the stability, in vivo efficacy and pharmacokinetics. For example, Sun et al. described that whereas the potency increased with increasing DAR, only ADCs with DAR over 6 showed faster clearance [89]. Hamblett et al. found a clear correlation between the DAR and plasma clearance [29]. Coupling itself may induce structural changes of the antibody molecule, which in turn could affect other biophysical characteristics [90]. Conformational and colloidal instability can induce protein aggregation, which may lead to a loss of efficiency and immunogenicity [42,91].

Ross and Wolfe recently reviewed the physical and chemical stability of ADCs [92]. Upon cysteine linking of payloads, protein conformation is not considerably altered, but the conformational energy of unfolding is decreased, and a more hydrophobic local surface is created. Correspondingly, the melting temperatures can be decreased and aggregate formation is triggered [93–95]. Molecules with higher DARs are more prone for aggregation [31,93] and a higher DAR species in a mixture determines the stability of the ADC as a whole [94]. In a recent publication, mAb destabilization was directly linked to payload hydrophobicity of surrogate ADCs with a DAR of 8 [96]. The thermal stability of a cysteine-linked ADC was considerably decreased with a higher DAR and also with an increasing ionic strength [95]. The melting temperature of the C_H2 region was more influenced by conjugation compared to the Fab/C_H3 domain even, if the antibody was solely conjugated in the Fab region [93,95].

Less literature is available on the stability of lysine-linked ADCs. Upon conjugation, the positive charge of a lysine is removed and multiple isoforms are generated [97]. The melting temperatures of an ADC and its antibody-linker intermediate were decreased and the percentages of higher molecular weight species were increased after 7 days of storage at 40 °C. Wakankar et al. also showed that conjugation influenced the C_H2 domain the most, which can be explained by the high flexibility of the C_H2 domain and increased likeliness of conjugation [55]. Interestingly, the addition of the linker alone increased aggregation more than the addition of both linker and payload. The unconjugated linker may react with side chains of nucleophilic amino acids leading to aggregation [55]. The hydrophobic payload and uneven charge distributions increase the aggregation propensity of lysine-linked ADCs [98]. The colloidal stability has been shown to be decreased for Kadcyla® compared to Herceptin® [99]. The stability of the Fab domain was less decreased by conjugation via lysines compared to thiol conjugates [32]. The effect of pH, temperature, agitation and freeze/thaw cycles on the stability of trastuzumab and the corresponding lysine-linked ADC (T-DM1) was recently compared by Mohamed et al. [100]. Degradation of T-DM1 was increased compared to the parent mAb upon thermal and mechanical stressing, as well as pH stress [100]. The comparison of Kadcyla® and a biosimilar candidate displayed a comparable thermal stability and aggregation behavior although the DARs slightly differed [101].

The chemical stability of ADCs depends on the parent mAb, the linker-payload instabilities and the site of conjugation on the antibody [31]. For example, the thioether succinimide linkage can oxidize in a mild aqueous environment followed by sulfoxide elimination [31,102].

For the conjugation process, the different physical and chemical properties of the antibody, linker and payload must be considered. For example, antibodies are more stable in buffered, aqueous solutions whereas the payloads often have limited aqueous solubility [103,104]. The necessary use of organic solvents during conjugation can destabilize the antibody [105,106]. Furthermore, the mAb should be supplied in a buffer at a pH that is compatible with the conjugation step. Side reactions during coupling may destabilize the antibody, e.g. succinimidyl esters that react not only with lysines but also cysteine and tyrosine [107,108]. Moreover, buffers with primary amines, e.g. histidine, cannot be used for lysine-based coupling. For the patient's safety, the clearance of unconjugated drug and residual organic solvent is also indispensable [109].

2. Formulation considerations for ADCs

The structural diversity of proteins calls for the development of a unique and specific formulation for each product, which can be very time-consuming [110–112]. For ADCs, the optimal formulation does not only depend on mAb stability, but must also consider the chemical stability of the linker and payload [31,109]. A compilation of the formulations used for ADCs and immunoconjugates on the market in September 2018 is given in Table 1 and Table 2, respectively. The current ADC market was used as a basis for the formulation considerations below.

2.1. Key factors for the product

The concentration of the marketed ADCs is below 20 mg/mL (Table 1). This is in marked contrast to the concentrations above 50 mg/mL that are broadly discussed in literature for traditional mAb therapeutics [113–116]. The high selectivity and efficacy of ADCs for tumor cell killing as well as the i.v. application avoids the necessity to use high concentrations [5]. Lower concentrations reduce the risk of aggregation, especially for a mAb carrying a hydrophobic payload. This is an important advantage since the solubility of an ADC may be decreased compared to the parent mAb because of a loss of net surface charges and the lipophilicity of the payload [97].

The ADCs on the market are given as infusions. In the infusion bag, the ADC concentration is low, increasing the risk of drug loss due to adsorption onto plastic, which might by enhanced by the hydrophobic payload [57]. Dilution of the drug also decreases the level of stabilizer, which may induce aggregation and particle formation [117,118]. In 0.9% NaCl solution electrostatic charge shielding may enhance attractive protein-protein interactions, leading to a possibly reduced solubility of high DAR species or an increased aggregation tendency [57]. Both 0.9% NaCl and 5% dextrose infusion solutions are slightly acidic and depending on dilution factor, pH and buffer capacity of the ADC formulation, a pH shift changing the charge state of the ADC molecules may occur, although ADC formulations are typically also slightly acidic [119,120].

Formulations with low ionic strength have shown to decrease aggregation and fragmentation for ADCs [95]. Especially for lysine-linked ADCs, surface charges are unevenly distributed. In traditional mAbs, molecules with an inconsistent charge distribution are expected to be more prone for intermolecular attractions [121]. The DAR distribution and high DAR species strongly influence the stability and formulation composition of the ADC [31,94]. Furthermore, the choice of the

Table 1
Formulations of approved ADCs.

Antibody product	Company	Generic name; description	Delivery route	Pharmaceutical form; storage	Formulation concentration	Dilution media	Diluted concentration[a]	Buffer components	Excipients	Stage[b]	Source
Mylotarg™	Pfizer	Gemtuzumab ozogamicin; humanized IgG4; anti-CD33	IV, infusion	lyophilized, 2–8 °C	1 mg/mL	NaCl	0.065 mg/mL	Na phosphate	NaCl, sucrose, dextran 40	A	EMA
Kadcyla™	Roche	trastuzumab emtansine; humanized IgG1; anti-HER2	IV, infusion	lyophilized, 2–8 °C	20 mg/mL	NaCl	1.008 mg/mL	succinic acid	NaOH, sucrose, polysorbate 20	A	EMA
Adcetris™	Takeda	brentuximab vedotin; recombinant chimerized IgG1, anti-CD30	IV, infusion	lyophilized, 2–8 °C	5 mg/mL	NaCl, dextrose, Ringer-Lactate	0.84 mg/mL	citrate	a,a-trehalose dihydrate, polysorbate 80	A	EMA
Besponsa™	Pfizer	inotuzumab ozogamicin, humanized IgG4; anti-CD22	IV, infusion	lyophilized, 2–8 °C	0.25 mg/mL	NaCl	0.009 mg/mL	TRIS	sucrose, polysorbate 80, sodium chloride	A	EMA

Abbreviations: EMA = European Medicines Agency; TRIS = tris(hydroxymethyl)aminomethane.
[a] Calculations based on 70 kg, 175 m patient with the highest applicable dose and dilution into infusion bag with the lowest volume allowed.
[b] A: approved, DA: disapproved.

Table 2
Formulations of immunoconjugates.

Antibody product	Company	Generic name; description	Delivery route	Pharmaceutical form; storage	Formulation concentration	Dilution media	Diluted concentration[a]	Buffer components	Excipients	Stage[b]	Source
Bexxar™	GSK	Tositumomab + Iodine 1131	IV, injection	frozen solution	14 mg/mL	NaCl	0.7 mg/mL	ascorbic acid	maltose, povidone, sodium chloride, pH 7.0	DA	FDA
Zevalin™	Spectrum	Ibritumomab tiuxetan	IV, infusion	solution, 2–8 °C	1.6 mg/mL	–	0.2 mg/mL	– added later: sodium acetate	sodium chloride albumin, sodium chloride, sodium phosphate, pentetic acid, potassium phosphate, potassium chloride	A	FDA
Ontak™	Seragen	denileukin diftitox	IV, infusion	frozen solution	150 mcg/mL	NaCl	15 mcg/mL	citric acid	EDTA, polysorbate 20, pH 6.9–7.2	A	FDA

Abbreviations: EDTA = Ethylenediaminetetraacetic acid; EMA = European Medicines Agency; FDA = Food and Drug Administration.
[a] Calculations based on 70 kg, 175 m patient with the highest applicable dose and dilution into infusion bag with the lowest volume allowed.
[b] A: approved, DA: disapprove.

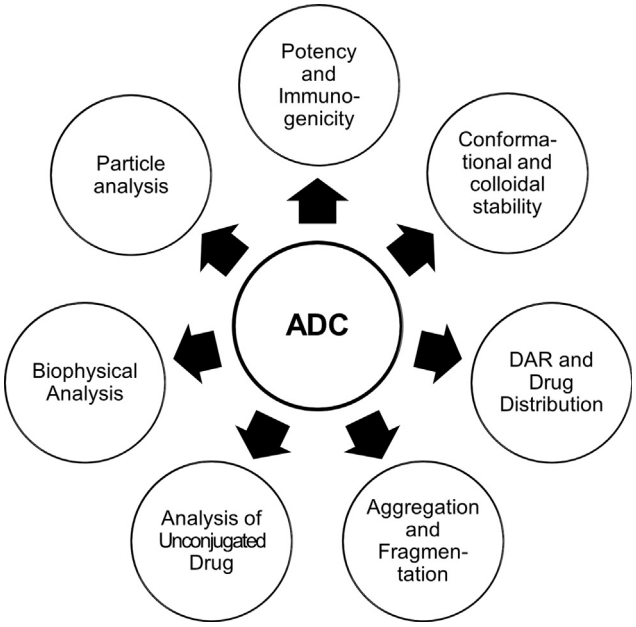

Fig. 3. Analytical methods necessary to characterize an ADC (antibody-drug conjugate).

appropriate pH is not only influenced by the IEP of the ADC but also by the linker e.g. hydrolysis of the thiol-succinimide linker is stimulated by an increasing pH [74]. The hydrolytic degradation of the linker during storage may be decreased with a freeze-dried formulation [74].

For biopharmaceuticals liquid formulations are preferred and about 2/3 of the marketed products in Japan and the US in 2013 were solutions [122]. Reasons for the preference of liquid solutions are the lower costs and the higher convenience comparable to lyophilizates [123,124]. However, the product might face stability problems [34,125]. Hitherto, all approved ADCs are lyophilized and formulated in a buffer with sugar and surfactant (Table 1). Only the immunoconjugates come as liquid or frozen solution (Table 2). So far, no publication has focused on the stability of ADCs in the liquid compared to the lyophilized state. Clearly, chemical degradation and especially the risk for free drug formation is strongly reduced by lyophilization [57,126]. For example, the thio-succinimide linkage in Adcetris® and Kadcyla® can be degraded by succinimide hydrolysis or a retro-Michael reaction [74,76–78]. The hydrazone linkage employed in Besponsa® and Mylotarg® is very labile [127] and is slowly hydrolyzed in plasma [77]. Interestingly, in contrast to traditional mAb therapeutics, ADCs can be light sensitive, and Mylotarg® and Besponsa® come in amber glass vials [128,129].

2.2. ADC formulation components

ADC formulations are based on the same excipients as a mAb formulation [31,74]. The choice of buffer depends on the target pH, the instability profile of the ADC and whether a liquid or lyophilized form is of interest. For highly concentrated mAb solutions, buffer-free formulations are recently discussed [130–132], but this might not be an option for the labile ADCs. As ADCs are admixed to infusions for applications, e.g. 0.9% sodium chloride or 5% dextrose solutions (Table 1), isotonicity of the ADC formulation is unnecessary. Still, tonicity agents such as sodium chloride, mannitol or sucrose are added to the ADC formulations. Sugars, specifically sucrose and trehalose, function as cryo- and lyoprotectants for the lyophilized ADCs [57,123,133,134]. The concentration of sugar may need to be up to 5% (w/v) during freezing, whereas a weight ratio of sugar to protein of at least 1:1 is necessary for the dried state [126]. With the next generation of site-specific ADCs, therapeutic doses may increase and DARs may be lower, sugars may not be needed [57]. Surfactants, specifically polysorbate 20 or 80, are used in mAb as well as in ADC formulations. Polysorbates may degrade, contain impurities, are an inhomogeneous mixture and are challenging to characterize [135–137]. But, their positive effects prevail as the surfactants stabilize the protein against accumulation and aggregation at interfaces and additionally may increase wettability during reconstitution [138]. Higher polysorbate concentrations may be required for ADCs compared to mAbs due to the heterogeneity and hydrophobicity of the drug substance [74]. To reduce the hydrophobic influence of the payload, the use of a low ionic strength buffer with a surfactant is suggested to [57,94,95,98]. ADCs show more pronounced aggregate formation and a lower T_m at higher ionic strength and independent of the type of salt [95].

3. Analytics for ADCs

The market approval for an ADC relies on a proven stability and maintained biological activity during long-term storage and possible stresses the protein may encounter [111]. The analytical toolbox employed for traditional mAbs also applies to ADCs (Fig. 3) [75,139]. However, not all methods can be transferred to an ADC without modifications, e.g. if the payload shows UV absorbance or the sample is highly heterogeneous. Especially for ADCs obtained via lysine and cysteine conjugation chemistries, the resulting drug substance is highly heterogeneous. As this may impact drug safety and efficacy, the target product profile typically contains DAR and degree of homogeneity specifications to control product quality and ensure consistency within different batches [140]. More distinct ADC analytics are still scarce and under development [98,141,142]. In the following some of the analytics with special considerations for ADCs will be shortly described.

The DAR can be measured by UV/Vis spectroscopy, but only if the absorption maxima differ between payload and antibody. Other methods to determine the DAR, as well as the drug load distribution and free drug are hydrophobic interaction chromatography and LC-ESI-MS [143,144]. Peptide mapping can be used to identify specific protein modifications that may result from conjugation and degradation [75,141]. Unbound drug is traditionally determined using RP-HPLC, but CE and ELISA have also been used. Some characterization techniques require adaptions for ADCs, e.g. in SEC, stronger interactions with the column due to the hydrophobic payload may have to be reduced by the addition of an organic solvent [145]. Moreover, the extrinsic fluorescent dye SYPRO® Orange, used to determine the T_m in dynamic scanning fluorimetry [146] can interact with the hydrophobic payloads, preventing its applicability for ADCs.

4. Conclusion

Various approaches to predict the stability and to find the optimal formulation can be adopted from conventional antibody concepts for ADCs. However, ADCs are not only antibodies, but also have two small molecules, i.e. the linker and the payload, attached to the antibody which need to be considered. Up to date only four ADCs have been approved by FDA or EMA and the publicly accessible knowledge on the stability and formulation is very limited. We therefore recommend all formulation scientists to carefully develop a formulation for an ADC and critically monitor the stability during all stages of development and processing. For example, the instability of the linker and payload in the liquid state may inhibit the analysis of the aimed lyophilized ADC as a liquid for accelerated conditions. This calls for new high-throughput stability indicating methods for ADCs. At the same time, computational modelling approaches present highly valuable tools in early ADC candidate selection and advanced understanding.

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