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ANTIBODY-DRUG CONJUGATES, COMBINATIONS OF LARGE AND SMALL THERAPEUTIC MOLECULES

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Antibody-drug conjugates are a promising therapeutic class of cytostatic agents. They consist of a monoclonal antibody, usually of the IgG type, to which one or more molecules of a cytotoxic drug are conjugated via a suitable linker (covalent chain). The conjugated form of the drug has significantly lower toxicity than the free form, which cannot be used alone in therapy. The Czech State Institute for Drug Control database has so far registered 9 antibody-drug conjugates for use in oncology. Given the vast number of possible combinations of antibody-drug pairs, a boom in sales is expected by 2025. In the blood circulation, the antibody-drug conjugate specifically targets the antigen expressed on the surface of the tumor cell. The linker between the antibody and the drug must be stable in the blood circulation environment and only be completely degraded in the target tumor cell after internalisation of the conjugate or remain bound to the drug after degradation of the protein part of the conjugate (the so-called cleavable or non-cleavable linkers). Subsequently, the drug causes apoptosis of the tumor cell by various mechanisms. The drugs used in the 2nd generation antibody-drug conjugates were up to 1,000 times more toxic than the chemotherapeutic drug doxorubicin and were mainly auristatin and maytansine derivatives. Less toxic drugs based on camptothecins, amanitins, etc. are also being tested in the current 3rd generation of conjugates under development. The linker drug is attached to the antibody by various bioconjugation methods. Here, a range of synthetic chemistry techniques are applied, and bioconjugation can be either non-specific or specific. In particular, the peripheral amino acids of the antibody – cysteine, lysine, histidine, tyrosine, glutamine and reduced disulfide bridges between the two heavy chains or between the heavy and light chains – are conjugated. For a specific conjugation, e.g. glycoengineering techniques based on N-glycosylation of antibody on asparagine (N297) have been developed. Conjugation techniques, as well as the synthesis of "naked" human antibodies are the subject of classified "know-how" of a number of commercial progressive companies and laboratories.

Keywords: antibody-drug conjugates, cancerostatics, linker, bioconjugation

Content

1. Introduction

In 2022, Rádl et al.[1] published a paper on: Will the drugs of the future be small molecules or biologics? The answer to this question is the belief of the authors of this paper that a reasonable balance between the two types will dominate the drug market in the future. One can certainly agree with this, but the above paper does not cover the very current therapeutic group of anticancer drugs, which are precisely those that synergistically combine large and small therapeutic molecules: antibody-drug conjugates (ADCs). The present paper can be seen as a loose continuation of the theme of the future direction of drug development.

The proportion of newly approved therapeutic molecules each year containing either only small or only large ones (biologics) has been stable over the last decade. According to FDA statistics[2], approximately two-thirds are small molecules and one-third are biologics (Fig. 1). However, the market is currently seeing a dynamic rise in anticancer ADCs, in which one large molecule is combined with small molecules (payload) into a single entity.

If we disregard the theoretical concepts and considerations of P. Ehrlich, G. Mathé and others about future directions in cancer treatment many decades ago[3],

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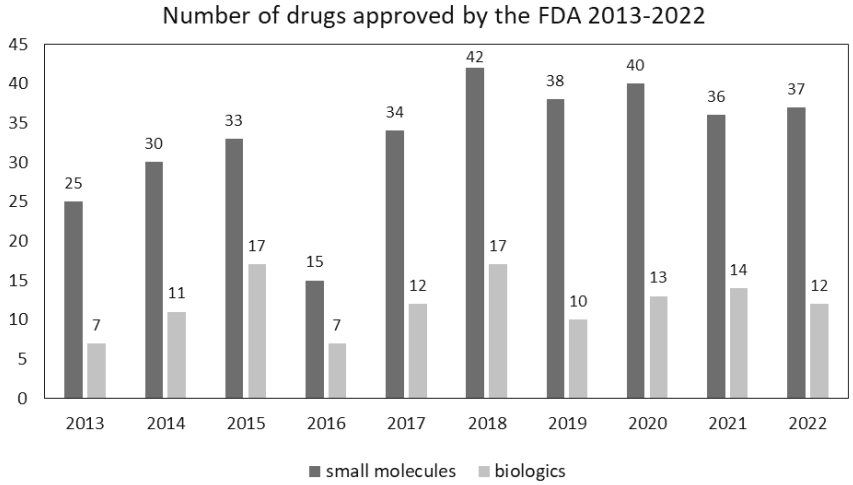


Fig. 1. **Ratio between therapeutic small and large molecules (biologics) approved by the FDA over the last decade**

the history of ADCs begins in 2000 when Mylotarg was launched by Wyeth company for the treatment of leukemia. However, it was voluntarily withdrawn in 2010 in the US by Pfizer (who bought Wyeth in 2009) because it had not shown therapeutic benefit compared to chemotherapy (small molecule treatment). Nevertheless, after review and a change in dosage regimen, Mylotarg was re-registered by the FDA in 2017 and by the EMA in 2018 (ref.[4]).

The fact that the issue is highly attractive is evidenced by the upcoming special issue of Molecules[5] devoted exclusively to ADCs and planned for publication in 2023.

The Czech State Institute for Drug Control (SÚKL)[6] lists in its database a total of 9 registred ADCs products for now (Table I).

Historically, ADC preparations have been divided into three generations, with each generation differing in the types of antibodies used, the types of small molecules used (payload), and the method of their conjugation on the antibody part[7].

The formulations of ADCs are generally liquid dosage forms for injection or infusion. It is evident that there is a huge number of possible combinations of antibody-drug pairs and far from all of them have been synthesized and therapeutically tested against possible targets. And this is undoubtedly a challenge and opportunity for pharmaceutical and biotechnology research. The development of next-generation ADCs is moving towards greater homogeneity, stability and efficacy[8].

Table I
Antibody-drug conjugate preparations registered in the SUKL database[6a]

Preparation	Antibody-drug conjugate	Treatment
Adcetris	brentuximab-vedotin	lymphocyte cancer
Besponsa	inotuzumab-ozogamicin	lymphoblastic leukaemia
Blenrep	belantamab-mafodotin	bone marrow sarcomas
Enhert	trastuzumab-deruxtecan	breast cancer
Kadcyla	trastuzumab-emtansine	breast cancer
Mylotarg	gemtuzumab-ozogamicin	myeloid leukaemia
Padcev	enfortumab-vedotin	bladder cancer
Polivy	polatuzumab-vedotin	diffuse large B-lymphocyte tumor
Trodelvy	sacituzumab-govitecan	breast cancer

[a] Not all products registered by the FDA (which has approved 12 so far) are also registered in Europe (EMA)

2. Molecular structure of antibody-drug conjugates

As the name implies, the ADCs contain a large recombinant monoclonal antibody (mAb) molecule. Currently, chimeric antibodies containing variable regions of non-human origin and constant regions of human origin are generally taking a back seat. This type of antibody is used, for example, in Adcetris[9]. Most current conjugates are humanized antibodies (containing only hypervariable regions from the animal), and the development is moving towards fully humanized antibodies. These modifications result in significantly lower immunogenicity of the antibodies used, longer biological half-life and improved effector functions[10,11]. These antibodies, especially the IgG isotypes, are conjugated at various sites on the light or heavy chain to one or more small molecules of cytotoxic drug (M_r = 300–1000), Fig 2. This drug is sometimes referred to in therapy as a warhead or payload. The conjugated form of the drug has significantly lower toxicity than the free form, which cannot be used alone in therapy. The covalent link (conjugation) between the antibody and the drug is referred to as a linker, which is a molecular chain of varying length and functionally optimized[12]. The structurally important parameters of ADCs, each of which affects the final efficacy, are:

- amino acid sequences in mAb,
- post-translational modification of mAb,
- drug-linker conjugation positions on mAb,
- linker structure and its stability,
- average loading of mAb with drug (drug-antibody ratio, DAR value).

These parameters can be determined by studying the molecular 3D structure of the ADCs. Currently, two major experimental methods are available: single crystal X-ray diffraction (SCXRD) and mass spectrometry (MS). However, the size of the conjugates studied is a problem with both methods. Therefore, structural studies are sometimes limited to mAb fragments, namely F_{ab} and F_c (see Fig. 2). Fragmentation does not, however, imply a limitation in the interpretation of the structure of the conjugate under study.

The molecular structure of ADCs can best be studied by SCXRD[13], and possibly by other diffraction techniques (neutron or electron diffraction[14]) if a suitable single crystal can be obtained. However, this is not at all easy and represents a major limitation of diffraction methods. Only a few structures of whole antibodies can be found in the PDB database[15], but thousands of drug conjugates with the F_{ab} fragment can be found because fragments crystallize more easily. The second method is represented by various mass spectrometry techniques, such as ESI/LC/MS or CXL/MS, which are probably the best for determining the binding site of a drug on an antibody and which are also commercially offered[16]. A review article on current LC/MS strategies for solving the structure of ADCs is the work of Xiaoyu et al.[17]. Other potential methods under consideration include NMR spectroscopy in solution[18] and cryo-electron microscopy[19], but these methods are usually used in tandem with MS because their resolution is generally lower[20].

An illustration of a structural study of a trastuzumab-emtansine conjugate (Kadcyla[21]) by LC-MS is provided by Chen et al.[22] (Fig. 3). Trastuzumab contains 88 lysine molecules and 4 N-terminal groups that can be modified by conjugation steps. The resulting conjugates consist of subpopulations that differ in the number of drugs attached as well as the location of their linkers on trastuzumab. The linker consists of a stable thioether bridge referred to as MCC. The covalently attached drug is called mertansine

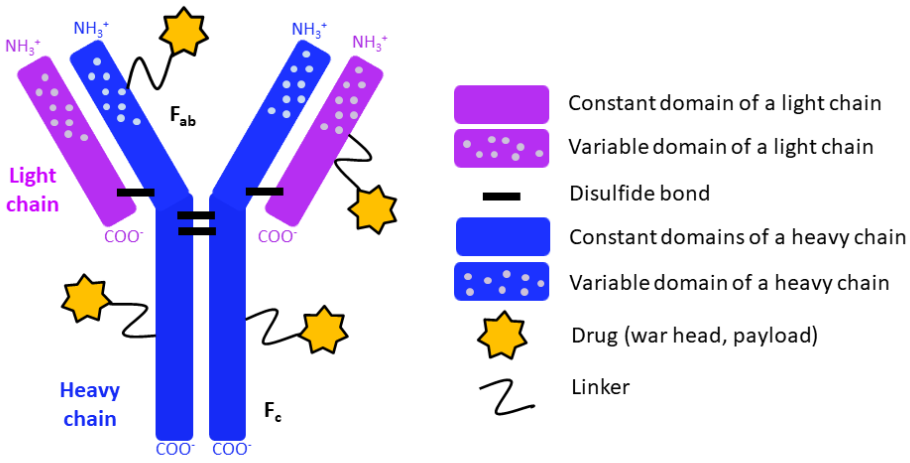


Fig. 2. **Scheme of the molecular structure of antibody-drug conjugation**

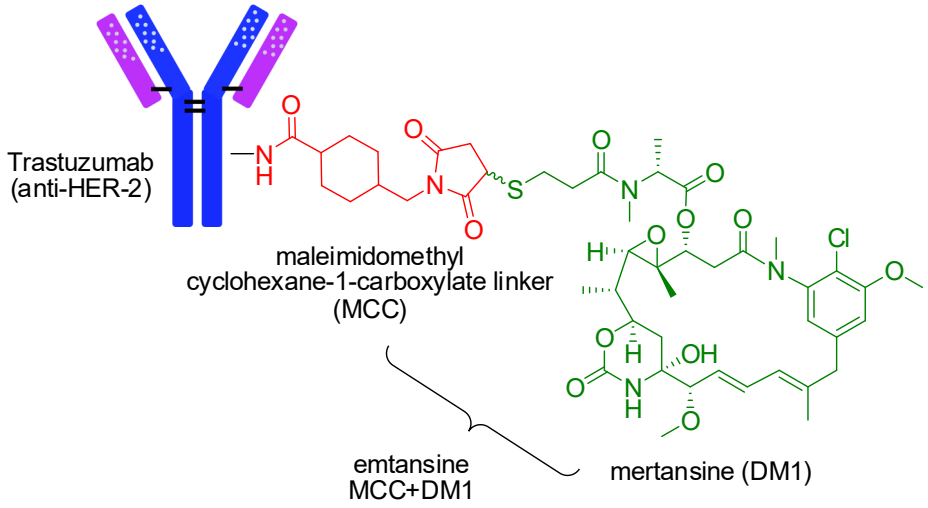


Fig. 3. **Trastuzumab-emtansine (Kadcyla)**

(DM1). The term emtansine refers to the MCC-DM1 complex, i.e. the linker-drug. In Kadcyla, there is an average of ~3.5 drug molecules per trastuzumab molecule (DAR value). Kadcyla is marketed by Roche and was approved by both the FDA and EMA in 2013.

2.1. Linker

A linker is a binding connector between an antibody and a drug (Fig. 4). After intravenous administration of an ADC, the linker must remain intact in the blood circulation environment (enzyme action). Otherwise, unwanted release of cytotoxic drug in non-target tissue would occur. Only in the target tumor cell will the linker either release the drug (deconjugate) or remain bound to the drug. Linkers are therefore divided into cleavable and non-cleavable. Linker design significantly influences the pharmacokinetics and pharmacodynamics, therapeutic parameters and selectivity of the ADCs[23] .

Cleavable linkers are designed to be stable in the extracellular and unstable in the intracellular environment of the tumor cell. Sensitive stability parameters are pH, redox potential or the presence of specific lysosomal enzymes. The most common types of cleavable linkers[23] are:

– hydrazone linker (the hydrazone bond is cleavable by hydrolysis in the acidic intracellular environment of endosomes (pH 5–6) and lysosomes (pH 4.8)).
– disulfide linker (disulfide bond is cleavable by hydrolysis at higher concentrations of intracellular glutathione (1–10 mmol l^{-1})).
– dipeptide linker (this linker is cleaved by cathepsin B, a lysosomal protease that is overproduced in some types of cancer cells). Val-Cit and Val-Ala are the most successfully cleaved linkers.

Another type of cleavable linker, which is less frequently used than the linkers mentioned above, is the β-glucuronide linker, which is recognized and hydrolyzed by β-glucuronidase.

An example of a non-cleavable linker is a substituted stable thioether chain that resists proteolytic degradation, which provides higher stability of the entire ADC. However, non-cleavable linkers must be designed so that they do not reduce the effect of the attached drug or appropriately modify its properties. After the degradation of the protein part of the ADC, i.e. the antibody, at least one amino acid, usually lysine or cysteine, is retained on the resulting drug-linker complex[23]. One of the advantages of non-cleavable linkers is the possibility of influencing the final properties of the small molecule (drug), such as their hydrophilicity, etc.[24]. Finally, it should be emphasized that at present it is not possible to make a clear judgment on which types of linkers are more suitable for ACDs, whether cleavable or non-cleavable.

2.2. Drug

The requirements placed on a small cytotoxic molecule – a drug – are high and it is difficult to fill them all without fail. These include sufficient efficacy and water solubility, the ability to bypass mechanisms causing multidrug resistance, low immunogenicity and the ability to form a suitable chemical bond (handle) for attachment to the linker. During the development of ADCs, chemotherapeutic drugs based on doxorubicin, methotrexate, plant Vinca alkaloids (vincristine, vinblastine, vinorelbine), etc., have been shown to be unsuitable. The second generation mainly used derivatives derived from two groups of antimitotic agents: auristatins and maytansines[12].

Auristatins[25] are complex analogues of dolastatin 10. This pentapeptide is a metabolite of the sea slug, the sea hare (*Dolabella auricularia*), from which it can be isolated. Individual derivatives are distinguished by letters,

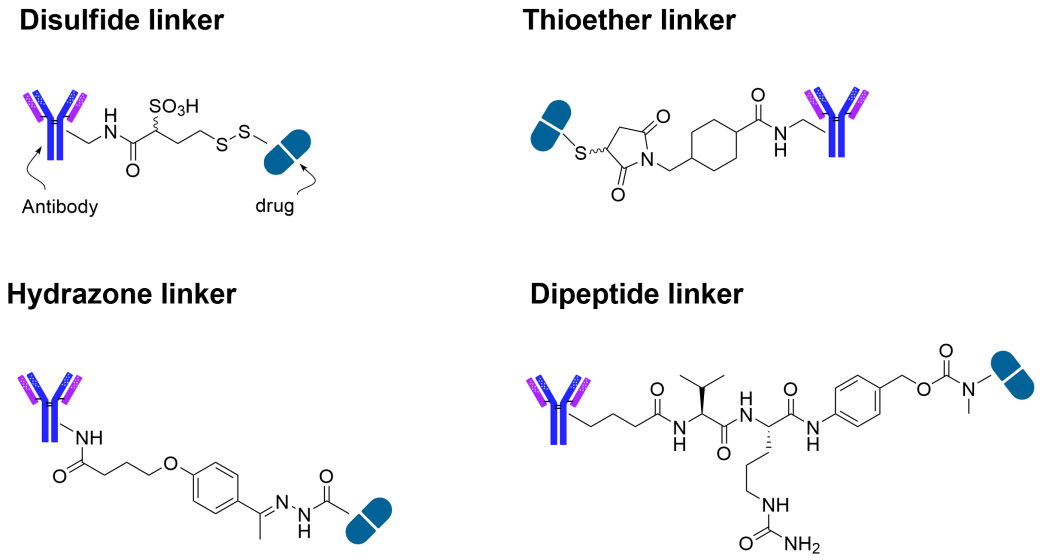


Fig. 4. **Cleavable and non-cleavable linkers**

e.g. auristatin E, auristatin F, etc. (Fig. 5). In addition, there are also their derivatives, e.g. monomethylauristatin E, monomethylauristatin F, etc. The range of these substances is therefore wide. Auristatins belong to the destabilizers of microtubules because their binding disrupts the necessary balance between constant addition and removal of tubulin units, thus stopping the ability of cancer cells to divide. This eventually leads to apoptosis. These agents are 100 to 1000 times more toxic than doxorubicin.

Maytansins[25,26] (see Fig. 3), or maytansin derivatives, are substances derived from the macrolide maytansin, which can be isolated from the bark of the African shrub *Maytenus ovatus*, but also from some mosses and microorganisms. Maytansine inhibits tubulin polymerization, leading to mitotic block and apoptosis. Thus, the mechanism of action of maytansins is similar to that of auristatins, as is their toxicity relative to doxorubicin (200 to 1000 times more toxic). Clinical trials with maytansins or auristatins alone have shown that they sometimes completely lack a therapeutic window due to their high systemic toxicity, i.e. the minimum therapeutic concentration is also the minimum toxic concentration.

Drugs that act by mechanisms other than as tubulin inhibitors are calicheamicins or duocarmycins, classified as bacterial anticancer antibiotics. In both cases, these are DNA-damaging agents. An example of an ADC containing this type of drug is the gemtuzumab-ozogamicin (Mylotarg)[27,28] mentioned above.

Current research focuses on less toxic drugs than tubulin inhibitors. A promising group are the camptothecins (camptothecin and its derivatives topotecan, irinotecan and belotecan). These topoisomerase inhibitors are about as toxic as doxorubicin. An example of a product containing this type of the agent is the recently approved sacituzumab-govitecan (Trodelvy)[29], see Tab. I.

In the long term perspective, all drugs that affect the cell cycle by some mechanism and lead to apoptosis will undoubtedly be investigated. These include topoisomerase inhibitors, transcription inhibitors, Bcl-xl inhibitors, tyrosine kinase inhibitors, HSP90 inhibitors, translation inhibi-

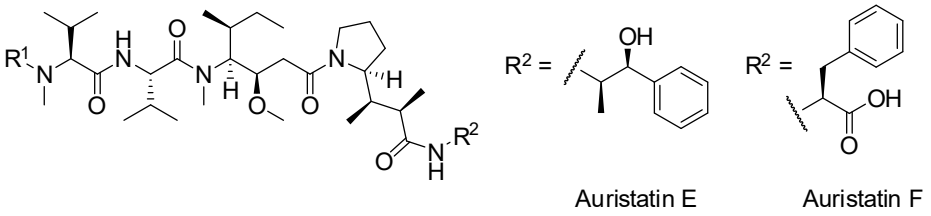


Fig. 5. **Auristatins**[25]

tors, proteasome inhibitors, drugs affecting mitochondrial metabolism, etc.[30]. For example amanitins (contained in the green fly agaric mushroom), pyrrolobenzodiazepines, indolinobenzodiazepines[31], thailanstatin, carmaficins[32] etc. are being tested.

For completeness, it can be added that antibodies can also be used to target radionuclides. An example is Zevalin (ibritumomab-tiuxetan)[33], which represents the first approved radioimmunotherapy. In this case, a mouse antibody targeting the CD20 antigen present on B-lymphocytes is used. The radionuclide used in this case is yttrium 90 [^{90}Y]. Unlike drug conjugates, radionuclide conjugates do not require internalization into the cell and kill both the target (tumor) cell to which the conjugate has bound and the cells in its vicinity[33].

3. Mechanism of therapeutic action

In order to understand the mechanism of action of ADCs, it is necessary to realize that the antibody plays a much more important role than it might seem at first glance and is not just a simple carrier of the drug to the target tumor cell. The conjugate synergistically combines the cytotoxic small molecule properties of the drug with important antibody properties such as selective binding to the appropriate antigen. At the same time, stability, pharmacokinetics or the ability to overcome drug resistance mechanisms can be suitably influenced in this way. ADCs are therefore an excellent combination of immunotherapy and chemotherapy.

In terms of the mechanism of action, ADCs specifically target the antigen produced on the surface of tumor cells in the blood circulation. The target antigen of the antibody must be highly expressed in the tumor, be readily accessible on the surface of the tumor cell, and finally have strong internalization properties (allowing the conjugate to enter the cell easily). Current therapies target eight different antigens and receptors (see their division in the SÚKL database)[6]. After internalization, the antibody is degraded and the drug acts in the tumor cell either free or in conjunction with a non-cleavable linker.

As an illustrative example of the therapeutic effect of a free antibody in contrast to the mechanism of action of an ADC, a pair consisting of a single humanized antibody used to treat HER2-positive breast cancer and HER2-positive metastatic gastric cancer (Herceptin, trastuzumab)[34] and a product containing a similar antibody, but this time conjugated to a cytostatic drug (Kadcyla, trastuzumab-emtansine), see Fig. 6, can serve. Even with Kadcyla, the main indication is for the treatment of HER-2 positive breast cancer[21]. These cancers are characterized by overproduction of a receptor involved in a number of signaling pathways that influence cell proliferation and apoptosis[35].

From a therapeutic point of view, it is important that there is no known extracellular ligand for the HER-2 receptor that is responsible for its activation. This occurs

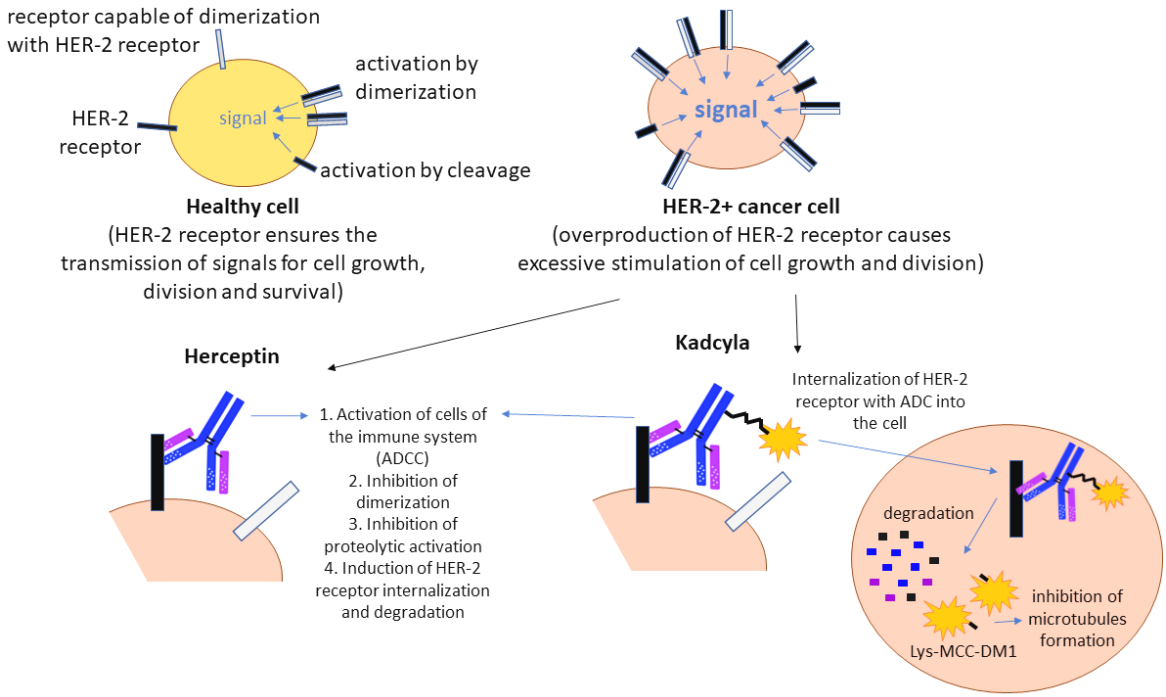


Fig. 6. **Comparison of the mechanism of action of Herceptin and Kadcyla**

during its dimerisation with another member of this receptor family, and it is the ligands of these receptors that induce the dimerisation. The second possible pathway leading to HER-2 receptor activation is cleavage of its extracellular domain. The action of Herceptin[34], which specifically binds to the extracellular domain of the HER-2 receptor, prevents the possibility of its dimerisation and thus suppresses the signals that activate proliferation and inhibit apoptosis. Activation of the HER-2 receptor by cleavage of the extracellular domain is also inhibited. However, this is not the end of the function of the antibody (trastuzumab) in Herceptin. The F_c fragment of trastuzumab is able to mediate antibody-dependent cellular cytotoxicity (ADCC), the activation of which leads to the destruction of the tumor cell. Another presumed mechanism of action of Herceptin is its effect on HER-2 receptor internalization and degradation[34,36].

In Kadcyla[21], the primary function of the antibody is its binding to the HER-2 receptor, but it is also capable of mediating all of the therapeutic effects mentioned above for Herceptin. Upon binding of the ADC to the HER-2 receptor, the entire ADC is internalized by endocytosis into the cell interior, followed by proteolytic degradation of the antibody in the lysosome. Degradation of this protein part of the whole ADC leads to the release of cytotoxic products – catabolites (primarily lysine-MCC-DM1 complexes), which inhibit microtubule formation (prevent tubulin polymerization) and thus cause apoptosis of the tumor cell. The present antibody allows the targeted action of the cytotoxic agent only in tumor cells that overproduce the HER-2 receptor. Due to its low permeability, the lysine-MCC-DM1 catabolite does not exhibit undesirable side effects.

The comparison of the mechanism of action of these two therapeutics, i.e. Herceptin and Kadcyla, is illustrated in Figure 6. It clearly demonstrates the basic difference in action, whereas Herceptin does not enter the cancer cell, the conjugate present in the Kadcyla does.

4. Preparation of antibody-drug conjugates

ADCs involve three variables – antibody, linker and drug and there is clearly no universal strategy for their preparation. During the development of the manufacturing process, a number of issues need to be addressed, for example:

How to choose the right antibody? Where and how to attach the linker to the antibody? What kind of linker to use? How many molecules of drug to attach? How to link the linker and the drug? Do I attach the linker to the drug first and then conjugate it to the mAb or conjugate the linker and mAb first and then attach the drug to the linker? What is the optimal DAR value?

In addition, it is clear that for each ADC preparation the answers will be different. In the following lines, examples of possible strategies for the synthesis of ADC preparations will be outlined.

4.1. Biosynthesis of the protein part of the conjugate

Before the preparation of an ADC can proceed, it is necessary to produce a "naked" monoclonal antibody, which requires considerable knowledge of modern technologies. A detailed description of all the methods currently used in the preparation of chimeric, humanized or fully human antibodies would exceed the scope of this paper, so we refer to the relevant literature[37]. For an idea, we will mention at least one of the possible approaches, namely the combination of hybridoma technology and genetic engineering. At the beginning of the process, it is necessary to immunize a laboratory animal, usually a mouse, with a suitable antigen. This is followed by the fusion of spleen cells, which are capable of producing antibodies, with tumor cells, giving the resulting hybridoma the ability to divide endlessly. This stage of antibody development is completed by the selection of a hybridoma producing an antibody of the desired specificity. At this stage, it is still a fully mouse-derived antibody. Using sequencing, it is then possible to determine the order of the nucleotides in the sequence encoding this mouse antibody[11].

The next stage takes advantage of the fact that the complete nucleotide sequence of the human genome is known[38]. Using genetic engineering methods, it is then possible to prepare a combined nucleotide sequence containing both parts of mouse origin, derived from the sequence obtained by hybridoma technology, and parts of human origin[39]. The sequence thus obtained is inserted into cells suitable for production. The cells most commonly used are Chinese hamster ovary cells (CHO cells)[40].

It is possible to obtain chimeric or humanized antibodies by the above mentioned method. For the preparation of fully humanised antibodies, it was necessary to develop even more sophisticated methods, including, for example, the phage display method or production in mice whose genome has been altered so that they are not capable of producing mouse antibodies, but only human antibodies[41].

4.2. Bioconjugation

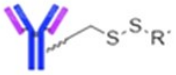
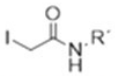
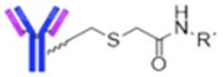
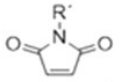
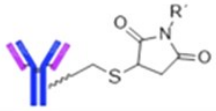
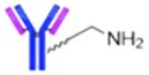
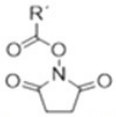
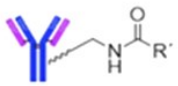
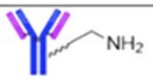
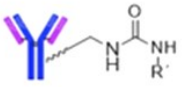
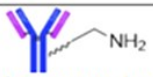
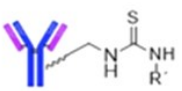
The subsequent process is bioconjugation (abbreviated as conjugation), which is a chemical strategy that allows the covalent bonding of two molecules, at least one of which is a biomolecule (in the case of ADC, a monoclonal antibody). The development of these methods has been ongoing for approximately two decades and new techniques are still being introduced that allow precise control of the number of small molecules introduced into the conjugate as well as the sites at which binding occurs[42,32]. Many strategic variants and a diverse range of synthetic chemistry tools are currently available. The main approaches used include classical chemical synthesis strategies and modern methods of biological catalysis using specifically acting enzymes[43].

The biosynthesis of ADCs faces a number of specific challenges related, among others, to the selectivity of the process. Chemoselective modification of one or more sites on the antibody is thus the subject of the "know-how" of a number of development and service biotechnology laboratories that offer their services on the web: Seattle Genetics, Genentech, ImmunoGen, Regeneron, Abzena, Arco Biosystems, Wuxibiologics, Lonza molecules, Spirogen, Synthon, Medchemexpres, etc.

Attractive sites for bioconjugation on the antibody are amino acids located on the external surface of the protein molecule, especially amino acids with an ionizable group in the side chain, such as lysine, cysteine, histidine or tyrosine. The most commonly modified groups are: $-NH_2$, -NH, -SH, -COOH, $-NC(NH_2)_2$ etc. In lysine, for example, the nucleophilic group $-NH_2$ can react with an electrophilic *N*-hydroxysuccinimide reagent to form an amide (Table II)[44]. In this case, the conjugation is nonspecific because there are several dozen lysine molecules in the antibody (it is reported that the average antibody contains about 80 lysines), and of these, about 10 are accessible for modification reactions. At the same time, it cannot be guaranteed that the lysine molecules necessary for antigen-antibody interaction will not be modified. Nevertheless, this methodology is one of the most widely used and has been used, for example, in the preparation of Besponsa, Mylotarg or Kadcyla[43,45].

Another synthetic strategy targets the four disulfide bridges formed by thiol groups in the side chains of cysteine residues in the IgG1 antibody, with two connecting the light and heavy chains and two located in the hinge region of the heavy chains (see Fig. 2). Reduction of these four bridges, e.g. by tris(2-carboxyethyl)phosphine (TCEP), generates eight thiol groups that are able to react with e.g. a maleimide linker (Table II), allowing the binding of up to eight drug molecules (max. DAR = 8). This technology has been used, for example, in the production of Adcetris[43]. An even more advanced approach is the production

Table II
Modification of the side chains of external cysteines and lysines in the antibody[44]

Functional group in the amino acid side chain	Reagent	Product
 sulfhydryl (thiol) group, Cys	 disulfide	
 sulfhydryl (thiol) group, Cys	 iodoacetamide	
 sulfhydryl (thiol) group, Cys	 maleimide	
 amino group, Lys	 *N*-hydroxysuccinimide-activated ester	
 amino group, Lys	O=C=N-R' isocyanate	
 amino group, Lys	S=C=N-R' isothiocyanate	

of antibodies, the sequence of which is genetically modified with a new cysteine that can be targeted to produce ACDs (THIOMAB)[43,46].

Since all monoclonal antibodies are N-glycosylated either on asparagine (N297) or in its immediate vicinity (i.e., it is a placement on the F_c fragment), the so-called glycoengineering technique allows conjugation with the drug on the carbohydrate portion of the antibody[47]. In this case, it is a position-specific conjugation and its advantages are obvious – the possibility of controlling the DAR and the heterogeneity of the resulting product. It is during these processes that the highly specific catalytic capabilities of enzymes are often exploited[43]. Direct conjugation of the drug linked to the linker to the antibody can be chosen or a so-called bioorthogonal bond can be synthesized first, which is then functionalized with the drug. Common examples of bioorthogonal reactions are ketone and aldehyde modification reactions[44]. In this scenario, a ketone or aldehyde functional group is linked to the protein using an aminooxy group ($H_2N–O–$) or a carbohydrazide group ($–C(=O)–NH–NH_2$) to form stable oxime or hydrazone bonds.

In the preceding paragraphs, only some of the possible bioconjugation methods have been presented. For a more comprehensive list and a more detailed description, we recommend the comprehensive publications[32,43].

To conclude the description of ADC preparation technology, it is important to note that the process does not end with the synthesis itself. The resulting product must be appropriately purified to meet the requirements for pharmaceutical products. Due to the different properties of the different ADC preparations, very few general techniques have been developed for purification of the products of bioconjugation reactions. Different chromatographic techniques (gel permeation chromatography, affinity or ion-exchange chromatography, hydrophobic interaction chromatography, etc.) are used, usually in combination with tangential filtration. In many cases, however, purification methods unique to the chosen bioconjugation reaction need to be developed[32,40,48].

5. Conclusion

ADCs represent a "boom" in cancer treatment. About 200 new ADCs are currently in development and sales are expected to quadruple by 2025 (ref.[49]). However, this does not mean the end of chemotherapeutics and other successful cancer drugs widely used in current therapy. The aim is to target the treatment as precisely as possible only to tumor cells and avoid damaging healthy cells, i.e. to limit very unpleasant side effects. And in this respect, ADCs are a significant step forward. That is why there is consideration of their future use in other indications (atherosclerosis, bacteremia, etc.)[50]. Development in the near future will show whether the hopes placed in ADCs will be fulfilled.

List of abbreviations

ADC	Antibody-Drug Conjugate
ADCC	Antibody Dependent Cellular Cytotoxicity
API	Active Pharmaceutical Ingredient
Bcl-xl	transmembrane protein
CD20	B-lymphocyte antigen CD20
CXL-MS	Chemical Crosslinking-Mass Spectrometry
DAR	Drug Antibody Ratio
DM1	maytansine derivative
ECI-LC-MS	Electrospray-Liquid Chromatography-Mass Spectrometry
EMA	European Medicines Agency
FDA	Food and Drug Administration
F_{ab}	fragment antigen-binding region
F_c	fragment crystallizable region
HER-2	Human Epidermal Growth Factor Receptor 2
HSP90	Heat shock protein 90
mAb	monoclonal antibody
MCC	4-[*N*-maleimidomethyl]cyclohexane-1--carboxylate
NCL	Native Chemical Ligation
PDB	Protein Data Bank
SCXRD	Single Crystal X-Ray Diffraction
SÚKL	State Institute for Drug Control
TCEP	tris(2-carboxyethyl) phosphine
Val-Cit	valine-citrulline
Val-Ala	valin-alanin

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