Antibody-drug conjugates: the new generation of biotechnological therapies against cancer

Guadalupe Melgarejo-Rubio,¹ Sonia M. Pérez-Tapia,² Emilio Medina-Rivero² and Marco A. Velasco-Velázquez^{1*}

¹Universidad Nacional Autónoma de México, Faculty of Medicine, Pharmacology Department; ²Instituto Politécnico Nacional, National School of Biological Sciences, Unit of Development and Research in Bioprocesses. Mexico City, Mexico

Abstract

Therapeutic antibodies are recombinant proteins used in the treatment of cancer. There is a new generation of monoclonal antibodies with activity against cancer cells, known as antibody-drug conjugates. These molecules are made up of three elements: a monoclonal antibody, a highly potent cytotoxic drug, and a chemical linker that binds them together. The antibody recognizes tumor antigens, thereby allowing targeted delivery of the cytotoxic agent to cancer cells. After recognizing its antigen, the antibody-drug conjugate is endocytosed by the target cells, where the protein fraction is degraded into lysosomes, releasing the cytotoxic drug. This article reviews antibody-drug conjugates general characteristics and describes the clinical evidence of efficacy and safety of the first four approved by regulatory agencies in the United States and Europe.

KEY WORDS: Antibody-drug conjugates. Gemtuzumab ozogamicin. Brentuximab vedotin. Trastuzumab emtansine. Inotuzumab ozogamicin.

Anticuerpos conjugados a fármaco: la nueva generación de terapias biotecnológicas contra el cáncer

Resumen

Los anticuerpos terapéuticos son proteínas recombinantes empleadas en el tratamiento del cáncer. Existe una nueva generación de anticuerpos monoclonales con actividad contra las células cancerosas, conocidos como anticuerpos conjugados a fármacos. Estas moléculas están integradas por tres elementos: un anticuerpo monoclonal, un fármaco citotóxico con alta potencia y un enlazador químico que los une. El anticuerpo reconoce antígenos tumorales, por lo que permite la entrega dirigida del agente citotóxico hacia las células cancerosas. Tras el reconocimiento de su antígeno, el anticuerpo conjugado a fármaco es endocitado por las células blanco, donde se induce la degradación lisosomal de la fracción proteica y se libera el fármaco citotóxico. En el presente artículo se revisan las características generales de los anticuerpos conjugados a fármacos y se describe la evidencia clínica de la eficacia y seguridad de los primeros cuatro aprobados por las agencias reguladoras de Estados Unidos y Europa.

PALABRAS CLAVE: Anticuerpos conjugados a fármacos. Gemtuzumab ozogamicina. Brentuximab vedotina. Trastuzumab emtansina. Inotuzumab ozogamicina.

Correspondence:

*Marco A. Velasco-Velázquez
E-mail: marcovelasco@unam.mx

Date of reception: 03-10-2019

Date of acceptance: 23-01-2020

DOI: 10.24875/GMM.M20000388

Gac Med Mex. 2020;156:228-235

Contents available at PubMed

www.gacetamedicademexico.com

0016-3813/© 2020 Academia Nacional de Medicina de México, A.C.. Published by Permanyer. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).

Therapeutic antibodies overview

Antibodies are excellent agents that with high specificity inhibit specific molecules. Clinical use of an antibody was first carried out in 1986, after the development of the technology to produce monoclonal antibodies. It was an anti-CD3 murine antibody (muromonab), the use of which, although effective in reducing transplant rejection, was limited by its rapid elimination, poor capability to induce effector functions in the immune system and due to the development of antibodies against the drug.¹

To avoid these problems, chimeric, humanized or totally human antibodies have been generated and, by means of protein engineering, the solubility, aggregation and stability profiles have been improved in order to scale up their production. Currently, there are recombinant antibodies that can be therapeutically used. These products, generically known as therapeutic antibodies (TA), are used in the treatment of cancer and other chronic-degenerative or infectious diseases. Owing to the incidence of diseases that can be treated with TAs, as well as to TAs effectiveness and positioning in the market, these agents constitute the fastest growing segment among biotech products, with sales that annually grow by 5.3 %.² Only in 2018, the Food and Drug Administration (FDA) approved 11 new TAs.³

TAs are immunoglobulins G

All TAs on the market are immunoglobulins G (IgG) that have stability in the circulation, good effector capacity and adequate physicochemical properties for their formulation and administration.⁴ IgGs are glycoproteins composed of two light chains and two heavy chains (Fig. 1A). The light chain has two immunoglobulin domains: one variable (V_L) and one constant (C_L) at N-terminus. The heavy chain has four immunoglobulin domains, one variable (V_H) and three constant (C_{H1} , C_{H2} , and C_{H3}) at N-terminus. The structure of the antibody is stabilized by disulfide bonds that link light to heavy chains and heavy chains to each other, which confers flexibility and a “Y” shape with two identical antigen-binding fragments (Fab). Each Fab is made up of the variable domains of a heavy and a light chain (Fig. 1B). Hyper-variability of the Fab sequences can generate an almost infinite diversity of antibodies with different specificity.⁵

The fragment crystallizable region (Fc region) is made up only of heavy chains and plays an effector role by binding to specific receptors (FcR) expressed on cells

Figure 1. A: Representation of the structure of an IgG: in red, the light chains; in gray, the heavy chains; in green, the disulfide bridges that bind them. The image was generated with Protein Data Bank information (PDB ID: 1IGT). B: Interaction of an antigen-binding fragment (Fab) with an antigen (green). In this region is where the specificity of the antibody lies. Image generated based on PDB information (ID: 5KEL). C: Interaction of the Fc region with a FcR fragment (yellow). Other sites within the Fc region can mediate the interaction with Cq1 or FcR. Image generated based on PDB information (ID: 1DN2).

of the immune system, the kidney or placenta (Fig. 1C), or by activating the complement. In humans, there are four IgG subclasses with different heavy chains ($\gamma 1$, $\gamma 2$, $\gamma 3$, and $\gamma 4$), which despite being highly similar (90 % identical), each one has a unique profile with regard to:

- Responsiveness to the antigen.
- Antibody-dependent cytotoxicity activation.
- Complement-dependent cytotoxicity activation.
- Antibody-dependent phagocytosis induction.
- Half-life time.
- Placental transfer.⁶

Fc region glycosylation induces changes in the quaternary structure, which affect its functions; therefore, it is crucial knowing the glycosylation patterns of the different subclasses and their relationship with the antibody functional properties.⁷ Most TAs used in neoplasms are of the IgG1 isotype, due to its higher capability to induce antibody-dependent cytotoxicity or complement-dependent cytotoxicity in cells that express the target antigen.⁴

Conjugated antibodies, a new generation of antineoplastic drugs

Antibody-drug conjugates (ADCs) are monoclonal antibodies targeted against tumor antigens, which deliver

Figure 2. A: ADC consists of three essential elements for its functioning: an antibody (red), a cytotoxic drug (gray) and a linker that binds both (blue). As the conjugation site (green), lysines, cysteines or modified amino acids can be used. B: ADCs pharmacokinetics and pharmacodynamics are complex and involve bringing the conjugate to the tumor (1), binding to the tumor antigen (2), incorporation of the complex by endocytosis (3), lysosomal hydrolysis of proteins (4), release of the cytotoxic drug (5), interaction of the drug with its intracellular target, either DNA (6a) or tubulin (6b), and cell death induction (7).

cytotoxic payloads to cancer cells; hence, ADCs are considered prodrugs.⁸ ADCs have three central elements: the monoclonal antibody carrier, the cytotoxic drug and the chemical linker that allows the conjugation of both (Fig. 2A). The antibody allows antigen-specific recognition in cancer cells. After binding, the ADC-antigen complex is incorporated into the cell by endocytosis and its lysosomal degradation is induced. Consequently, the cytotoxic payload is released inside the cell and triggers cell death by interacting with its target (Fig. 2B). This mechanism takes advantage of the specificity of the antibody to deposit the cytotoxic payload on tumor tissue,⁸ thus reducing systemic toxicity and possible resistance.

Antigen and antibody characteristics

The target tumor antigen directs ADC biodistribution, and it must therefore be overexpressed in the cells or in the tumor microenvironment and be present at very low levels or absent in healthy tissues.⁹ Cell surface proteins are the most widely used targets owing to their accessibility. For example, the efficacy of anti-HER2 TAs is associated with high receptor expression, since up to two million molecules have been found on cancer cells surface.¹⁰ However, in B-cell lymphomas, it is enough for 30,000 molecules per CD19 antigen cell to be expressed for an ADC to exert its effect.¹¹

Cells that lack the antigen of interest on their surface may be exposed to the cytotoxic drug if they are close to those that are recognized by the ADC. This mechanism, known as the “innocent bystander effect”, is relevant when the antibody identifies tumor vasculature

or stromal antigens or when antigen expression is not homogeneous in solid tumors.¹²

After selecting the antigen, identifying the antibody's optimal isotype is required, which influences on ADC efficacy, pharmacokinetics and therapeutic index. Most ADCs that have been clinically tested are of the IgG1 isotype.⁹ As previously mentioned, IgG1s induce antibody-dependent or complement-mediated cytotoxicity; therefore, whether or not maintaining these functions is desired should be evaluated. In general, the antibodies used to generate ADCs should not trigger effector functions.⁹ However, when the antibody to be conjugated has therapeutic activity by itself, it may be relevant to retain these functions. The IgG2 and IgG4 isotypes are inefficient to stimulate secondary immune responses.¹³ IgG2s allow conjugating a larger number of cytotoxic molecules, since they have four disulfide bridges in the hinge region that can be reduced for conjugation, whereas IgG1 and IgG4 only have two.¹⁴ Although comparative studies have shown that ADCs made up of IgG1 or IgG2 antibodies have similar tolerability and toxicity profiles,¹⁵ IgG2 hinge regions remain more difficult to reduce. Therefore, the design of IgG1 mutated in the Fc region has been chosen in order to attenuate its effector function,¹⁵ while preserving its ability to bind to FcR in order to optimize its half-life.

Chemical linker characteristics

The linker is a fundamental part of an ADC since it must allow the release of the drug exclusively at the

site of action. If the drug is released into the circulation, systemic toxicity increases and ADC efficacy decreases. Ideally, the linker should not interfere with the chemotherapeutic agent cytotoxicity, since the cytotoxic agent-linker by-product is what generates the innocent bystander effect.¹⁶ In addition, the linker may serve to counteract resistance to the cytotoxic drug, since hydrophilic linkers generate metabolites that are not substrates for efflux or drug-expulsion active pumps, such as P-glycoprotein (P-gp).¹⁷

Two main types of linkers are currently used in the development of ADCs: cleavable and non-cleavable.¹⁷ Cleavable linkers are stable for as long as they are circulating and efficiently release the drug after the ADC is endocytosed by the tumor cell. Non-cleavable linkers remain bound to an amino acid of the antibody after its lysosomal degradation. This type of linker is unsuitable for inducing the innocent bystander effect, since the amino acid-linker-cytotoxic agent complex does not spread outside the tumor cell.¹⁶

Both types of linkers take advantage of lysines or cysteines reactivity in the antibody to form covalent bonds. Conjugation to lysines is efficient, but it generates multiple antibody species with differences in the number of cytotoxic drug molecules and their localization.¹⁸ In addition, since many reactive lysines are found in the C_H2 domain, conjugation increases ADC aggregation.¹⁹ Conjugation to cysteines requires for them to be in their reduced form and, thus, if those that form interchain disulfide bridges are employed, linkers with two reactive groups must be used in order to allow the bridge to regenerate. This strategy allows better control of the number of molecules of the conjugated cytotoxic drug, but still, more than 100 ADC different species can be generated.¹⁸

The diversity of species affects ADC's stability, pharmacokinetics and pharmacodynamics.²⁰ For this reason, technologies have been developed that seek to increase the proportion of a particular species, such as site-specific conjugation, incorporation of new cysteine residues or non-natural amino acid residues or enzymatic conjugation.¹⁸

Cytotoxic drugs characteristics

The drug conjugated to the antibody is responsible for exerting the cytotoxic effect on tumor cells; therefore, it must meet several characteristics (recently, Yaghoubi et al. carried out a review²¹):

- It must have a mean inhibitory concentration below the nanomolar range, since only 1 to 2 % of

the cytotoxic agent reaches its intracellular target.

- Covalent binding to the chemical linker must not interfere with its activity.
- It must be poorly sensitive to P-gp-mediated transport in order to avoid the generation of resistance.
- It must have physicochemical properties that allow its formulation for intravenous administration.
- It must be stable within the pH range existing in the lysosome.

It is difficult to find molecules that meet all these characteristics, which is why most ADCs assessed in humans use agents from three families: calicheamicins, auristatins or maytansinoids.

Calicheamicin is an antitumor agent isolated from the actinomycete *Micromonospora echinospora*. Calicheamicin γ 11 is approximately 1000 times more potent than doxorubicin for inducing cytotoxicity. N-acetyl- γ -calicheamicin, a modified analog, is used in ADCs. Calicheamicins induce cell death by binding to DNA minor groove, preferably to the TCCT/AGGA sequence,²² forming diradical species that cause DNA strand scission.²³ These compounds are hydrophobic and, therefore, few molecules can be conjugated to the antibody without causing aggregation.⁹

Auristatins are synthetic analogues of dolastatin 10, an antimetabolic isolated from the sea hare *Dolabella auricularia* and subsequently from cyanobacteria. Auristatins generate microtubule continuous and excessive growth by binding to the β subunit of tubulin dimers and preventing guanosine triphosphate (GTP) hydrolysis; consequently, sister chromatid separation and mitosis are blocked.²⁴ While mean inhibitory concentrations of other drugs that inhibit tubulin polymerization, such as vincristine or vinblastine, are in the range of 10^{-8} to 10^{-9} M, auristatins have average inhibitory concentrations ranging from 10^{-10} to 10^{-12} M.²⁵ Monomethyl auristatin E is a synthetic molecule with optimized physicochemical properties, which is why it has been used in the development of multiple ADCs.²⁶⁻²⁹ When monomethyl auristatin E is conjugated to the antibody by cleavable linkers, the product is hydrophobic enough to induce the innocent bystander effect on neighboring cells.³⁰

Maytansinoids are cytotoxic substances derived from maytansine, a macrolide antibiotic isolated from the *Maytenus ovatus* shrub. They bind to the tubulin located at the ends of microtubules, favoring their depolymerization and leading the cell to apoptosis.³¹ Maytansinoids have been shown to be 100 to

1000-fold more potent in vitro than other cytotoxic agents;³² emtansine and mertansine are characterized by a substituent that contains a thiol group, which facilitates their conjugation to linkers.⁸ These compounds have good solubility and stability in aqueous solution, but can promote ADC aggregation or limit antigen binding, especially at high conjugation ratios.²¹

Other cytotoxic agents in ADCs used in clinical trials include doxorubicin,³³ pyrrolobenzodiazepines,³⁴ indolinobenzodiazepines,³⁵ camptothecin derivatives,³⁶ duocarmycins³⁷, and tubulins.³⁸

Available ADCs

Up to May 2019, four ADCs had received marketing approval by the FDA and the European Medicines Agency (EMA); each one is described below.

Gemtuzumab ozogamicin

Gemtuzumab ozogamicin is a humanized anti-CD33 IgG4 conjugated to N-acetyl- γ -calicheamicin by a bifunctional linker.^{39,40} CD33 is a cell-adhesion molecule that belongs to the sialic acid-binding lectin superfamily,⁴¹ which is expressed in myeloid cells and in approximately 85 to 90 % of patients with acute myeloid leukemia.

In 2000, gemtuzumab ozogamicin was the first ADC to be approved by the FDA for the treatment of patients with relapsed acute CD33+ myeloid leukemia, aged 60 years or older and who were not candidates to other chemotherapies. However, in the post-marketing period, its lack of efficacy and its association with serious side effects and premature death were demonstrated: gemtuzumab ozogamicin addition to standard induction chemotherapy (daunorubicin + cytarabine) did not improve the complete response rate or relapse-free survival,⁴² increased mortality from 1.4 to 5.5 %, ⁴² and the incidence of hepatic veno-occlusive disease was approximately 10 %.⁴³ For these reasons, the drug was withdrawn from the market in 2010.

Various hypotheses have been proposed to explain the failure of the product: poor stability of the chemical linker, heterogeneity in the amount of drug molecules bound to the antibody, and susceptibility of the cytotoxic agent to be carried by transporters.^{9,17} Given that it is poorly immunogenic, the relationship of anti-ADC antibodies with gemtuzumab ozogamicin efficacy and safety was not identified.⁴⁴

Subsequent trials demonstrated that lower and more frequent doses increased gemtuzumab ozogamicin

efficacy and safety. In adults with newly diagnosed acute myeloid leukemia, the addition of ADC to chemotherapy increased 2-year event-free survival, as well as overall survival.⁴⁰ As monotherapy, it favors obtaining complete responses in children with refractory or recurrent acute myeloid leukemia.³⁹ These results contributed to gemtuzumab approval in 2017 by the FDA for the treatment of patients with CD33+ acute myeloid leukemia during adulthood and in children who relapse or who do not respond to primary treatment.⁴⁵ Months later, it was approved by the EMA.⁴⁶ Side effects, although less serious, are still reported.^{40,45}

Brentuximab vedotin

Brentuximab vedotin is a chimeric anti-CD30 IgG1 conjugated to monomethyl auristatin E by a cathepsin B-sensitive valine-citrulline cleavable linker.⁴⁷⁻⁴⁹ CD30 belongs to the tumor necrosis factor receptor family and normally it is expressed in lymphoid tissues and overexpressed in neoplastic cells of patients with Hodgkin's lymphoma, anaplastic large cell lymphoma or cutaneous T-cell lymphoma.⁵⁰

In patients with Hodgkin's lymphoma who relapse and who fail to respond to autologous stem cell transplantation, brentuximab vedotin monotherapy improves complete response rates, as well as 5-year progression-free survival and overall survival.⁴⁷⁻⁴⁹ In patients with previously untreated Hodgkin's lymphoma, the combination of brentuximab vedotin with doxorubicin, vinblastine, and dacarbazine is equally efficacious and less toxic than adding a fourth chemotherapeutic agent to the therapeutic regimen.^{51,52} Brentuximab vedotin has demonstrated benefits when used as monotherapy in patients with anaplastic large cell lymphoma⁵³ or cutaneous T-cell lymphoma,⁵⁴ as it significantly increases overall response rate and progression-free survival.

Brentuximab vedotin induces adverse reactions, especially in patients with impaired liver or kidney function,⁵⁵ or who take drugs that inhibit CYP3A4, since monomethyl auristatin E is metabolized by this cytochrome P450 isoform.⁵⁶

Trastuzumab emtansine

Trastuzumab emtansine is a humanized anti-HER2 IgG1 conjugated to emtansine via a non-cleavable thioether linker bound to lysine residues.^{57,58} HER2 is overexpressed in a subgroup of breast tumors and,

for this reason, trastuzumab was developed as a TA.⁵⁹ Trastuzumab pharmacokinetics, pharmacodynamics, and adverse reactions are well known.⁵⁹ Trastuzumab emtansine retains the mechanisms of action of the unconjugated antibody, in addition to inducing emtansine cytotoxicity.⁶⁰

In patients with previously-treated locally advanced, unresectable or metastatic HER2+ breast cancer, trastuzumab emtansine is more effective than the combination of capecitabine + lapatinib⁵⁷ or a third-line treatment,⁵⁸ since it increases progression-free survival and median overall survival. In addition, adverse reactions were better tolerated in the groups treated with the ADC.^{57,58} In contrast, in patients with treatment naïve HER2+ advanced breast cancer, trastuzumab emtansine is not more efficacious than trastuzumab combined with a taxane,⁶¹ and the approval for its use is therefore limited to previously-treated patients.

There are reports of development of resistance to this ADC. This may be due to the following:⁶²

- Mechanisms of resistance to trastuzumab.
- ADC inefficient internalization.
- Reduced lysosomal degradation.
- Action of recycling endosomes that allow the HER2-ADC complex to return to the membrane.

Although 5 % of patients receiving trastuzumab emtansine develop antibodies against this agent,⁴⁴ this does not impact on its efficacy.

Trastuzumab emtansine has been observed to induce multiple adverse responses; thrombocytopenia is the most serious and the one that motivates dose limitations.⁶³ Like trastuzumab, it can induce left ventricular dysfunction or liver failure.⁶³ These effects are increased by the consumption of drugs that interfere with emtansine metabolism.⁶²

Inotuzumab ozogamicin

Inotuzumab ozogamicin is a humanized anti-CD22 IgG4 conjugated to N-acetyl-γ-calicheamicin via the same bifunctional linker used in gemtuzumab ozogamicin.⁶⁴⁻⁶⁶ CD22 is a transmembrane glycoprotein of the sialic acid-binding lectins superfamily⁴¹ that is overexpressed in more than 90 % of patients with acute lymphoblastic leukemia.⁶⁷ Inotuzumab ozogamicin is used as monotherapy for the treatment of adults with relapsing or refractory CD22+ acute lymphoblastic leukemia. In these patients, the use of the ADC significantly increases the rate of patients with complete remission, 2-year overall survival, and the quality of life.⁶⁴⁻⁶⁶

The linker in inotuzumab ozogamicin is stable in systemic circulation,⁶⁸ even though it is attributed the toxicity of other ADCs. Inotuzumab ozogamicin induces adverse effects that can be mitigated with preventive measures and signs and symptoms constant monitoring.⁶⁹ Although 3 % of patients receiving inotuzumab ozogamicin generate antibodies against the product, the impact of this reaction on the agent's efficacy and safety has not yet been determined.⁴⁴

Conclusions and perspectives

Although ADCs represent a breakthrough in oncology, they have various limitations; for example, even when an ADC is indicated for one type of tumor, its usefulness is limited to specific patient subgroups. Therefore, analyzing the effect of ADCs in different populations and types of tumors that express the target antigen is required. An example of this type of analysis is the one related to trastuzumab emtansine efficacy in lung cancer.⁷⁰ In addition, the mechanisms by means of which resistance to ADCs is developed should be studied in order to propose strategies to reverse it. Cytotoxic drugs efflux is an important factor in resistance and, therefore, combining the ADC with inhibitors of the transporters that allow this mechanism has been proposed,⁷¹ or generating new agents that are not transported.²¹ More research on the frequency whereby anti-ADC antibodies are developed and their role in the development of resistance is also necessary.⁴⁴

ADC safety still needs to be improved. Adverse effects are generally caused by induction of healthy cell death. This cytotoxicity might be due to target antigen expression on the cells, to uptake of the conjugate by the cells independently of the antigen or to the cytotoxic drug being released to the circulation by target cells that processed the ADC.

It is to be expected that new ADCs that are more effective and safer will be developed in the near future, since there is sufficient information regarding what the nature of the selected antigen should be, what properties the antibody should have, which are the essential pharmacological and physicochemical properties of the cytotoxic agent and how the linker should work. ADCs will increase survival opportunities and quality of life for cancer patients.

Acknowledgements

We thank Dr. Luis Córdoba Bahena for the critical review of the manuscript and the generation of the figures.

Conflict of interests

None

Funding

Funding from Consejo Nacional de Ciencia y Tecnología (project A1-S-18285) was received for this project, as well as support from Laboratorio Nacional para Servicios Especializados de Investigación, Desarrollo e Innovación para Farmoquímicos y Biotecnológicos (LANSEIDI-FarBiotec-CONACyT).

Ethical disclosures

The authors declare that no experiments were performed on humans or animals for this research.

Confidentiality of data. The authors declare that no patient data appear in this article.

References

- Liu JKH. The history of monoclonal antibody development – Progress, remaining challenges, and future innovations. *Ann Med Surg (Lond)*. 2014;3:113-116.
- Elvin JG, Coustou RG, van der-Walle CF. Therapeutic antibodies: Market considerations, disease targets and bioprocessing. *Int J Pharm*. 2013;440:83-98.
- Mullard A. 2018 FDA drug approvals. *Nat Rev Drug Discov*. 2019;18:85-89.
- Goulet DR, Atkins WM. Considerations for the design of antibody-based therapeutics. *J Pharm Sci*. 2020;109:74-103.
- Backhaus O. Generation of antibody diversity. En: Bödicke T, editor. *Antibody engineering*. México: InTech; 2018. DOI: 10.5772/65238
- Vidarsson G, Dekkers G, Rispens T. IgG subclasses and allotypes: from structure to effector functions. *Front Immunol*. 2014;5:520.
- Cobb BA. The history of IgG glycosylation and where we are now. *Glycobiology*. 2020;30(4):202-213.
- Dan N, Setua S, Kashyap VK, Khan S, Jaggi M, Yallapu MM, et al. Antibody-drug conjugates for cancer therapy: chemistry to clinical implications. *Pharmaceuticals (Basel)*. 2018;11(2). pii: E32
- Beck A, Goetsch L, Dumontet C, Corvaia N. Strategies and challenges for the next generation of antibody-drug conjugates. *Nat Rev Drug Discov*. 2017;16:315-337.
- Horton J. Her2 and trastuzumab in breast cancer. *Cancer Control*. 2001;8:103-110.
- Blanc V, Bousseau A, Caron A, Carrez C, Lutz RJ, Lambert JM. SAR3419: An anti-CD19-Maytansinoid Immunoconjugate for the treatment of B-cell malignancies. *Clin Cancer Res*. 2011;17:6448-6458.
- Damelin M, Zhong W, Myers J, Sapra P. Evolving strategies for target selection for antibody-drug conjugates. *Pharm Res*. 2015;32:3494-3507.
- Kretschmer A, Schwanbeck R, Valerius T, Rösner T. Antibody isotypes for tumor immunotherapy. *Transfus Med Hemother*. 2017;44:320-326.
- Wiggins B, Liu-Shin L, Yamaguchi H, Rathnaswamy G. Characterization of cysteine-linked conjugation profiles of immunoglobulin G1 and immunoglobulin G2 antibody-drug conjugates. *J Pharm Sci*. 2015;104:1362-1372.
- McDonagh CF, Kim KM, Turcott E, Brown LL, Westendorf L, Feist T, et al. Engineered anti-CD70 antibody-drug conjugate with increased therapeutic index. *Mol Cancer Ther*. 2008;7:2913-2923.
- Kovtun YV, Goldmacher VS. Cell killing by antibody-drug conjugates. *Cancer Lett*. 2007;255:232-240.
- Tsuchikama K, An Z. Antibody-drug conjugates: recent advances in conjugation and linker chemistries. *Protein Cell*. 2018;9:33-46.
- Panowski S, Bhakta S, Raab H, Polakis P, Junutula JR. Site-specific antibody drug conjugates for cancer therapy. *MAbs*. 2014;6:34-45.
- Wakankar AA, Feeney MB, Rivera J, Chen Y, Kim M, Sharma VK, et al. Physicochemical stability of the antibody-drug conjugate Trastuzumab-DM1: changes due to modification and conjugation processes. *Bioconjug Chem*. 2010;21:1588-1595.
- Boylan NJ, Zhou W, Proos RJ, Tolbert TJ, Wolfe JL, Laurence JS. Conjugation site heterogeneity causes variable electrostatic properties in Fc conjugates. *Bioconjug Chem*. 2013;24:1008-1016.
- Yaghoubi S, Karimi MH, Lotfinia M, Gharibi T, MahiBirjand M, Kavi E, et al. Potential drugs used in the antibody-drug conjugate (ADC) architecture for cancer therapy. *J Cell Physiol*. 2020;235:31-64.
- Ellestad GA. Structural and conformational features relevant to the anti-tumor activity of calicheamicin γ 1I. *Chirality*. 2011;23:660-671.
- Zein N, Sinha AM, McGahren WJ, Ellestad GA. Calicheamicin gamma 1I: an antitumor antibiotic that cleaves double-stranded DNA site specifically. *Science*. 1988;240:1198-1201.
- Chen H, Lin Z, Arnst K, Miller D, Li W. Tubulin inhibitor-based antibody-drug conjugates for cancer therapy. *Molecules*. 2017;22:1281.
- Bai R, Pettit GR, Hamel E. Dolastatin 10, a powerful cytostatic peptide derived from a marine animal. Inhibition of tubulin polymerization mediated through the vinca alkaloid binding domain. *Biochem Pharmacol*. 1990;39:1941-1949.
- Li Z, Wang M, Yu D, Luo W, Fang J, Huang C, et al. Monomethyl auristatin E-conjugated anti-EGFR antibody inhibits the growth of human EGFR-positive non-small cell lung cancer. *Cancer Chemother Pharmacol*. 2019;84:61-72.
- Lütje S, Gerrits D, Molkenboer-Kuennen JD, Herrmann K, Fracasso G, Colombatti M, et al. Characterization of site-specifically conjugated monomethyl auristatin E- and duocarmycin-based anti-PSMA antibody-drug conjugates for treatment of PSMA-expressing tumors. *J Nucl Med*. 2018;59:494-501.
- Bourillon L, Bourcier C, Gaborit N, Garambois V, Liès E, Zampieri A, et al. An auristatin-based antibody-drug conjugate targeting HER3 enhances the radiation response in pancreatic cancer. *Int J Cancer*. 2019;145:1838-1851.
- Tilly H, Morschhauser F, Bartlett NL, Mehta A, Salles G, Haioun C, et al. Polatuzumab vedotin in combination with immunochemotherapy in patients with previously untreated diffuse large B-cell lymphoma: an open-label, non-randomised, phase 1b-2 study. *Lancet Oncol*. 2019;20:998-1010.
- Okeley NM, Miyamoto JB, Zhang X, Sanderson RJ, Benjamin DR, Sievers EL, et al. Intracellular activation of SGN-35, a potent anti-CD30 antibody-drug conjugate. *Clin Cancer Res*. 2010;16:888-897.
- Lopus M, Orudjev E, Wilson L, Wilhelm S, Widdison W, Chari R, et al. Maytansine and cellular metabolites of antibody-maytansinoid conjugates strongly suppress microtubule dynamics by binding to microtubules. *Mol Cancer Ther*. 2010;9:2689-2699.
- Widdison WC, Wilhelm SD, Cavanagh EE, Whiteman KR, Leece BA, Kovtun Y, et al. Semisynthetic maytansine analogues for the targeted treatment of cancer. *J Med Chem*. 2006;49:4392-4408.
- Govindan SV, Cardillo TM, Sharkey RM, Tat F, Gold DV, Goldenberg DM. Milatuzumab-SN-38 conjugates for the treatment of CD74+ cancers. *Mol Cancer Ther*. 2013;12:968-978.
- Mantaj J, Jackson PJM, Rahman KM, Thurston DE. From anthramycin to pyrrolobenzodiazepine (PBD)-containing antibody-drug conjugates (ADCs). *Angew Chem Int Ed Engl*. 2017;56:462-488.
- Miller ML, Fishkin NE, Li W, Whiteman KR, Kovtun Y, Reid EE, et al. A New class of antibody-drug conjugates with potent DNA alkylating activity. *Mol Cancer Ther*. 2016;15:1870-1878.
- Nabada T, Masuda T, Naito H, Yoshida M, Ashida S, Morita K, et al. Novel antibody drug conjugates containing exatecan derivative-based cytotoxic payloads. *Bioorg Med Chem Lett*. 2016;26:1542-1545.
- Elgersma RC, Coumans RGE, Huijbregts T, Menge WMPB, Joosten JAF, Spijker HJ, et al. Design, synthesis, and evaluation of linker-duocarmycin payloads: toward selection of HER2-targeting antibody-drug conjugate SYD985. *Mol Pharm*. 2015;12:1813-1835.
- Li JY, Perry SR, Muñoz-Medina V, Wang X, Wetzel LK, Rebelatto MC, et al. A biparatopic HER2-targeting antibody-drug conjugate induces tumor regression in primary models refractory to or ineligible for HER2-targeted therapy. *Cancer Cell*. 2016;29:117-129.
- Zwaan CM, Reinhardt D, Zimmerman M, Hasle H, Stary J, Stark B, et al. Salvage treatment for children with refractory first or second relapse of acute myeloid leukaemia with gemtuzumab ozogamicin: Results of a phase II study. *Br J Haematol*. 2010;148:768-776.
- Catarine S, Pautas C, Terré C, Raffoux E, Bordessoule D, Bastie JN, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): A randomised, open-label, phase 3 study. *Lancet*. 2012;379:1508-1516.
- Läubli H, Varki A. Sialic acid-binding immunoglobulin-like lectins (Siglecs) detect self-associated molecular patterns to regulate immune responses. *Crit Mol Life Sci*. 2020;77:593-605.
- Petersdorf SH, Kopecky KJ, Slovak M, Willman C, Nevill T, Brandwein J, et al. A phase 3 study of gemtuzumab ozogamicin during induction and postconsolidation therapy in younger patients with acute myeloid leukemia. *Blood*. 2013;121:4854-4860.
- Tack DK, Letendre L, Kamath PS, Tefferi A. Development of hepatic veno-occlusive disease after mylotarg infusion for relapsed acute myeloid leukemia. *Bone Marrow Transplant*. 2001;28:895-897.

44. Carrasco-Triguero M, Dere RC, Milojic-Blair M, Saad OM, Nazzal D, Hong K, et al. Immunogenicity of antibody-drug conjugates: observations across eight molecules in eleven clinical trials. *Bioanalysis*. 2019;11:1555-1568.
45. Appelbaum FR, Bernstein ID. Gemtuzumab ozogamicin for acute myeloid leukemia. *Blood*. 2017;130:2373-2376.
46. Ali S, Dunmore H-M, Karres D, Hay JL, Salmonsson T, Gisselbrecht C, et al. The EMA review of mylotarg (gemtuzumab ozogamicin) for the treatment of acute myeloid leukemia. *Oncologist*. 2019;24:e171-e179.
47. Younes A, Gopal AK, Smith SE, Ansell SM, Rosenblatt JD, Savage KJ, et al. Results of a pivotal phase II study of brentuximab vedotin for patients with relapsed or refractory Hodgkin's lymphoma. *J Clin Oncol*. 2012;30:2183-2189.
48. Chen R, Gopal AK, Smith SE, Ansell SM, Rosenblatt JD, Savage KJ, et al. Five-year survival and durability results of brentuximab vedotin in patients with relapsed or refractory Hodgkin lymphoma. *Blood*. 2018;128:1562-1566.
49. Moskowitz CH, Nademanee A, Masszi T, Agura E, Holowiecki J, Abidi MH, et al. Brentuximab vedotin as consolidation therapy after autologous stem-cell transplantation in patients with Hodgkin's lymphoma at risk of relapse or progression (AETHERA): a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet*. 2015;385:1853-1862.
50. Chiarle R, Podda A, Prolla G, Gong J, Thorbecke GJ, Inghirami G. CD30 in normal and neoplastic cells. *Clin Immunol*. 1999;90:157-164.
51. Younes A, Connors JM, Park SI, Fanale M, O'Meara MM, Hunder NN, et al. Brentuximab vedotin combined with ABVD or AVD for patients with newly diagnosed Hodgkin's lymphoma: a phase 1, open-label, dose-escalation study. *Lancet Oncol*. 2013;14:1348-1356.
52. Connors JM, Jurczak W, Straus DJ, Ansell SM, Kim WS, Gallamini A, et al. Brentuximab vedotin with chemotherapy for stage III or IV Hodgkin's lymphoma. *N Engl J Med*. 2018;378:331-344.
53. Pro B, Advani R, Brice P, Bartlett NL, Rosenblatt JD, Illidge T, et al. Five-year results of brentuximab vedotin in patients with relapsed or refractory systemic anaplastic large cell lymphoma. *Blood*. 2017;130:2709-2717.
54. Prince HM, Kim YH, Horwitz SM, Dummer R, Scarisbrick J, Quaglini P, et al. Brentuximab vedotin or physician's choice in CD30-positive cutaneous T-cell lymphoma (ALCANZA): an international, open-label, randomised, phase 3, multicentre trial. *Lancet*. 2017;390:555-566.
55. Oak E, Bartlett NL. A safety evaluation of brentuximab vedotin for the treatment of Hodgkin lymphoma. *Expert Opin Drug Saf*. 2016;15:875-882.
56. Han TH, Gopal AK, Ramchandren R, Goy A, Chen R, Matous JV, et al. CYP3A-mediated drug-drug interaction potential and excretion of brentuximab vedotin, an antibody-drug conjugate, in patients with CD30-positive hematologic malignancies. *J Clin Pharmacol*. 2013;53:866-877.
57. Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med*. 2012;367:1783-1791.
58. Krop IE, Kim SB, González-Martín A, LoRusso PM, Ferrero J-M, Smitt M, et al. Trastuzumab emtansine versus treatment of physician's choice for pretreated HER2-positive advanced breast cancer (TH3RESA): a randomised, open-label, phase 3 trial. *Lancet Oncol*. 2014;15:689-699.
59. Lambertini M, Pondé NF, Solinas C, de Azambuja E. Adjuvant trastuzumab: a 10-year overview of its benefit. *Expert Rev Anticancer Ther*. 2017;17:61-74.
60. Junttila TT, Li G, Parsons K, Phillips GL, Sliwkowski MX. Trastuzumab-DM1 (T-DM1) retains all the mechanisms of action of trastuzumab and efficiently inhibits growth of lapatinib insensitive breast cancer. *Breast Cancer Res Treat*. 2011;128:347-356.
61. Perez EA, Barrios C, Eiermann W, Toi M, Im YH, Conte P, et al. Trastuzumab emtansine with or without pertuzumab versus trastuzumab plus taxane for human epidermal growth factor receptor 2-positive, advanced breast cancer: primary results from the phase III MARIANNE study. *J Clin Oncol*. 2017;35:141-148.
62. Barok M, Joensuu H, Isola J. Trastuzumab emtansine: Mechanisms of action and drug resistance. *Breast Cancer Res*. 2014;16:209.
63. Kowalczyk L, Bartsch R, Singer CF, Farr A. Adverse events of trastuzumab emtansine (T-DM1) in the treatment of HER2-positive breast cancer patients. *Breast Care (Basel)*. 2017;12:401-408.
64. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab ozogamicin versus standard therapy for acute lymphoblastic leukemia. *N Engl J Med*. 2016;375:740-753.
65. Kantarjian HM, DeAngelo DJ, Stelljes M, Liedtke M, Stock W, Gökbüget N, et al. Inotuzumab ozogamicin versus standard of care in relapsed or refractory acute lymphoblastic leukemia: Final report and long-term survival follow-up from the randomized, phase 3 INO-VATE study. *Cancer*. 2019;125:2474-2487.
66. Su Y, van Oostrum I, Vandendries E, Welch V, Loberiza FR. Hospitalization for patients in the United States (US) and European Union (EU) treated with inotuzumab ozogamicin (InO) vs standard of care (SOC) for relapsed/refractory acute lymphoblastic leukemia (R/R ALL) in a global phase 3 trial. *J Clin Oncol*. 2017;35:e18500-e18500.
67. Shah NN, Stevenson MS, Yuan CM, Richards K, Delbrook C, Kreitman RJ, et al. Characterization of CD22 expression in acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2015;62:964-969.
68. Boghaert ER, Khandke KM, Sridharan L, Dougher M, DiJoseph JF, Kunz A, et al. Determination of pharmacokinetic values of calicheamicin-antibody conjugates in mice by plasmon resonance analysis of small (5 microl) blood samples. *Cancer Chemother Pharmacol*. 2008;61:1027-1035.
69. Kebriaei P, Cutler C, de Lima M, Giralt S, Lee SJ, Marks D, et al. Management of important adverse events associated with inotuzumab ozogamicin: expert panel review. *Bone Marrow Transplant*. 2018;53:449-456.
70. Ohashi K, Hotta K, Hirata T, Aoe K, Kozuki T, Ninomiya K, et al. Trastuzumab emtansine in HER2+ recurrent metastatic non-small-cell lung cancer: study protocol. *Clin Lung Cancer*. 2017;18:92-95.
71. Chang CH, Wang Y, Zalath M, Liu D, Cardillo TM, Goldenberg DM. Combining ABCG2 inhibitors with IMMU-132, an anti-Trop-2 antibody conjugate of SN-38, overcomes resistance to SN-38 in breast and gastric cancers. *Mol Cancer Ther*. 2016;15:1910-1919.