Emerging Role of Antibody Drug Conjugates (ADCs) in Therapeutics: An Appraisal

Raja Chakraverty¹, *, Tatini Debnath²

¹Department of Pharmaceutical Technology, Bengal School of Technology (A College of Pharmacy) Hooghly, West Bengal, India
²Department of Quality Control, Abate Medicaments, Barasat, West Bengal, India

Email address: rchakraborty20@yahoo.com (R. Chakraverty)
*Corresponding author

To cite this article:
Raja Chakraverty, Tatini Debnath. Emerging Role of Antibody Drug Conjugates (ADCs) in Therapeutics: An Appraisal. European Journal of Clinical and Biomedical Sciences. Vol. 3, No. 1, 2017, pp. 7-12. doi: 10.11648/j.ejcbs.20170301.12

Received: October 15, 2016; Accepted: January 12, 2017; Published: February 9, 2017

Abstract: Antibody drug conjugates intends to pursue the monoclonal antibodies (mAbs) as the potent source of delivering cytotoxic drugs to more specific site which binds selectively to the antigen expressing tumor cells. Inspite of the facts, various other safety profile must be considered while designing and optimizing ADC such as selecting congruous target antigen and method of conjugation. Each and every component of the ADC i.e antibody, linker and the drug should be optimized to the extent of desirable targeted therapy which will ameliorate as well as enhance tolerability. The past decade had witnessed advances in newer cancer treatments with extremely selective small molecules targeting the specific genetic abnormality causing the disease. The approach of traditional cytotoxic agents in the treatment of cancer, unlike the target specificity, they affect both healthy as well as cancer cells. In order to build a powerful and more specific cytotoxic agent with target oriented mAb’s designing attributes would lead to pertinent and potential breakthrough in cancer treatments. Therefore ADC’s were developed with the intention that antibody would target the specific antigen of the tumor wherein the drug attached to it would induce its cytotoxicity. Development of new techniques and methods in implementing new generation ADC’s in the past decades incorporated non-immunogenic monoclonal antibodies comprising linkers having equitable stability and distinctly potent cytotoxic agents. Newer challenges although remain but comprehensive clinical accomplishment is generating increased interest in this therapeutic class of drugs.

Keywords: ADC, Monoclonal Antibodies, Bioconjugation, Linker

1. Introduction

Bioconjugation strategies offer promising therapeutic benefits by synergy of those of the individual components [1-2]. The bioconjugation method approaches towards numerous medical necessities hence it plays an important role in the emerging therapeutic field. Simply bioconjugates are the covalent crosslinking of a semisynthetic/synthetic chemotypes such as drugs, oligomers, peptides etc to a biomolecule like macromolecular proteins, polysaccharides or nucleotides [2-3]. The bioconjugates are attributed towards transpiring class of medicines and are gaining more importance in the biopharmaceutical field across the globe. Bioconjugates like Antibody Drug Conjugates (ADCs) in targeted oncology research is found to improve the durability of anti-tumor responses in cancer patients [4-13].

Promising preliminary data of the Antibody Drug Conjugates in the research pipeline and in clinical trial stages help to move forward cancer therapy from traditional chemotherapy to target anti-cancer treatment modalities [7-8, 14-19]. The increased growth of ADC’s were proportionally higher as corroborated in the last two decades. The clinical approval of Adcetris (brentuximab vedotin) in 2011 and Kadcyla (ado-trastuzumab emtansine) in 2013 has sparked development of novel ADCs [14]. In addition, towards the improvement of safe and effective immuno-oncology drugs like ADCs, a plethora of payloads with specific molecular mechanisms of cytotoxic activity have been considered...
during past years [8, 12]. The progress of preclinical and clinical research of ADCs driven by the higher ADCs exposure levels, development of site-specific conjugation biotechnology, combined with improvements in linker chemistries [12].

The majority of ADCs in clinical pipeline are conjugation of IgG antibodies with bioactive tubulin-targeting antimitic agents like derivatives of maytansine, auristatins etc [14]. Maytansinoids and auristatins bind with the vinca binding domain of tubulin and arrest the microtubule dynamics in the G2/M phase [20-21]. Although, emerging clinical experience with ADCs containing DNA-targeting payloads, explore the pivotal requirements needed for successful ADC design [14]. In order to evaluate the impact of different mechanism-of-action payloads on various immune cells more exploration of ADC research is needed.

This review article intends to exemplify the proposed challenges, mechanism of action, therapeutic efficacy and safety level parameters which are in association with the judicious use of ADC’s thereby enhancing the improvement in the upcoming treatment methods in medical science. This developmental process which targets the payloads would be prospective enough to bridge the gap in future findings.

2. Study Methodology
More than 50 relevant articles from indexed journals were extensively reviewed based on the keywords- Bioconjugation, Antibody Drug Conjugation, site-specific conjugation, ADCs cytotoxic payload, etc. from PubMed central and Google Scholar with special emphasis on articles published after 2014. Recent developments in ADCs design, ADCs in the research pipeline, various targeting antibodies were carefully studied and comprehensively noted down to come to the novel findings.

3. Antibody Drug Conjugate and Its Design
Previously, anti-cancer agent conjugated with chimeric or murine antibody fails to address enough localization in the target cancerous cells thereby leading to immunogenicity [22-23]. In order to avoid off-target toxicity, the antibody should be designed in such a way where the antibody is selective and have better binding affinity to that of target antigen with high expression at the tumor site but low expression on normal tissue [23-28]. The knowledge arises from the recent development of chemistry and biology is able to address the designing problem of ADCs [24].

4. Antibody Selection for Antibody Drug Conjugates
Various points including affinity, specificity, pharmacokinetics (PK) should be considered before selecting the mAbs [22]. Currently researchers like to select human or humanized IgG1 mAbs with a few chimerics in the development of ADCs as it show acceptable PK properties [29]. Brentuximab vedotin is one of the approved ADC is effective in Anaplastic Large Cell Lymphoma and Hodgkin’s lymphoma, It comprises a chimeric IgG1 (cAC10) against CD30 receptors on cancer cells. T-DM1 is an ADC that contains humanized anti-HER2 IgG1 antibody trastuzumab. [23] Comparing in vivo efficacy between IgG1 and IgG2 conjugates was identified for anti-CD70 which is an IgG isotype while conjugating with the payload mono methyl auristatin phenylalanine (MMAF). [30] In addition, IgG2 and IgG4 (hinge stabilized) are also used in clinical development i.e., AGS-16M8F (anti-ENPP3 IgG2-MMAF) and inotuzumab ozogamicin (anti-CD22 IgG4-calicheamicin) [26, 27].

Therefore, the isotype of the antibody plays a key role in substantiating the stability factor of the ADC pertaining to the strategy of conjugation and payload attachment.

5. Cytotoxic Payloads Delivery
As very small amount of the injected dose gets localized to the tumor site, payloads should active in the picomolar or better potencies range, i.e. cytotoxic payload should be highly potent to the tumor cells [22]. Scientists generally favour to choose natural product or derivatives of natural product as ADC payload [31].

In order to get more insight into the mechanism of action of cytotoxic payload there are mainly two types of mechanism:

(1) Microtubules inhibitor: Auristatins (monomethyl auristatin E, MMAE; monomethyl auristatin F, MMAF), Maytansines (DM1, DM4)
(2) DNA binding alkylating agent: Calicheamicins, Duocarmycins
(3) RNA polymerase II inhibitor: the mushroom amatoxins (cyclic octapeptide analogs)
(4) DNA crosslinker: Pyrrolobenzodiazepines (PBDs)
(5) Other: Epothilones, Anthracyclins.

Table 1. Requirements to design an ideal ADC.

| Antibody | Drug | Linker |
|----------|------|--------|
| Should target a well-characterized antigen | High potency (in picomolar range) | Must remain stable at systemic circulation |
| High expression at tumor site | Neither low drug loading (potency factor) nor high drug loading (problematic pharmacokinetics) | Rapidly cleave after the ADC finds its intended target antigen |
| Low expression at normal tissue | Selectivity towards target cells | |
| Low immunogenicity | |

Therefore, the isotype of the antibody plays a key role in substantiating the stability factor of the ADC pertaining to the strategy of conjugation and payload attachment.
Table 2. List of some cytotoxic payloads with their properties.

| Category                              | Auristatins                                 | Calicheamicins                          | Duocarmycins                         | Maytansinoids                           |
|---------------------------------------|---------------------------------------------|-----------------------------------------|--------------------------------------|----------------------------------------|
| isolated from                         | Indian Ocean sea hare Dolabella auricularia. [32] | soil microorganism M. echinosophora calcehensis. [35-37] | Streptomyces sp. Bacteria. [39] | African shurb Maytenus ovatus and subsequently in the soil microorganism Nocardia sp. [42-44]. |
| MOA                                   | Inhibit tubulin polymerization. Metaphase arrest. The third amino acid (dolaisoleucine) was the most important for cytotoxicity. [33, 34] | Generation of highly-reactive 1,4-benzenoid diradical, that causes cleavage of DNA double strand and finally cell death. [37] | Duocarmycins derivatives bind to AT-rich regions of the DNA minor groove, which results in irreversible alkylation, and ultimately lead to cell death. [40] | Bind to the same site on tubulin as the vinca alkaloids. [45-46] |
| Remarks                               | Various conjugated antibody for this payloads are anti-CD79b (Genentech/Roche), anti-PSMA (Progenics Pharmaceuticals, Inc.), anti-AGS-16 (Agensys, Inc.) anti-GCC (Millenium/Takeda Co.), anti-5T4 (Pfizer, Inc.), and anti-EGFR (Abbvie, Inc.). | Linker: 1. a hydrazone cleavage site (AcBut) 2. without (DMA) Both react with Lys on the monoclonal antibody through an N-succinimidyl ester functionality. [38] | Prodrug of duocarmycin analog with conjugated human anti-CD70 antibody are currently in Phase I clinical trial. [41] | DM1 payload forms ADC: ado-trastuzumab emtansine (T-DM1) |

The clinically successful antibody drug conjugates currently under evaluations are briefly enumerated:

Table 3. Current Antibody Drug Conjugates in Clinical Evaluation and targeted approaches [51].

| Candidate                          | Drug       | Antigen          | Lead Indicator | Developer                  |
|------------------------------------|------------|------------------|----------------|---------------------------|
| Phase III                          |            |                  |                |                           |
| Inotuzumab ozogamicin (CMC-544)    | Calicheamicin | CD22             | ALL            | Pfizer                    |
| Gemtuzumab ozogamicin (CMA-676)    | Calicheamicin | CD33             | AML            | Pfizer                    |
| Phase II                           |            |                  |                |                           |
| SAR3419                            | DM4        | CD19             | B-Cell Malignancies | Sanofi/immunogen          |
| RG7593                             | MMAE       | CD22             | B-Cell Malignancies | Roche/Gentech/Seattle Genetics |
| RG7596                             | MMAE       | CD79b            | B-Cell Malignancies | Roche/Gentech/Seattle Genetics |
| Glembatubumab vedotin (CDX-011)    | MMAE       | GPNMB            | Breast Cancer, Melanoma | Cellix Therapeutics/Seattle Genetics |
| PSMA-ADC                           | MMAE       | PSMA             | Prostate Cancer | Progenics Pharma/Seattle Genetics |
| Phase I                            |            |                  |                |                           |
| Lorvotuzumab mertansine            | DM1        | CD56             | SCLC           | ImmunoGen                 |
| IMGN529                            | DM1        | CD37             | B-Cell Malignancies | ImmunoGen                 |
| IMGN853                            | DM4        | FRα              | Solid Tumors   | ImmunoGen                 |
| IMGN289                            | DM1        | EGFR             | Solid Tumors   | ImmunoGen                 |
| SAR5666658                         | DM4        | CA6              | Solid Tumors   | Sanofi/immunoGen          |
| BT-062                             | DM4        | CD138            | Multiple Myeloma | Biotest/ImmunoGen         |
| BAY 94-9343                        | DM4        | Mesothelines     | Solid Tumors   | Bayer/ImmunoGen           |
| AMG595                             | DM1        | EGFRvIII         | Gliomas        | Amgen/ImmunoGen           |
| AMG 172                            | DM1        | CD27L            | ccRCC          | Amgen/ImmunoGen           |
| SGN-CD19A                          | MMAF       | CD19             | NHL, ALL       | Seattle Genetics          |
| AGS-22ME                           | MMAE       | Nectin 4         | Solid Tumors   | Astellas Pharma/seattle Genetics |
| RG7450                             | MMAE       | STEAP1           | Prostate Cancer | Roche/Genentech/Seattle Genetics |
| RG7458                             | MMAE       | MUC16            | Ovarian Cancer | Roche/Genentech/Seattle Genetics |
| RG7599                             | MMAE       | NaPi2b           | NSCLC, Ovarian Cancer | Roche/Genentech/Seattle Genetics |
| MLN0264                            | MMAE       | GCC              | GI Malignancies | Takeda/Seattle Genetics    |
| SGN-CD33A                          | PBD        | CD33             | AML            | Seattle Genetics          |
| MDX-1203                           | Duocarmycin | CD70             | NHL, RCC       | Bristol-Myers Squibb      |
| Labetuzumab- SN-38                 | SN-38      | CD66e            | CRC            | Immunomedics               |
| IMMU-132                           | SN-38      | Trop-2           | Epithelial Cancers | Immunomedics               |
| Milatuzumab Doxorubicin            | Doxorubicin | CD74             | Multiple Myeloma | Immunomedics               |
| RG7598, RG7600, RG7636             | Undisclosed | Undisclosed      | Various        | Roche/Genentech/Seattle Genetics |
6. ADC Payloads

Numerous other cytotoxic payloads also draw the attraction in this regards, molecules like pyrrolbenzodiazepines derivatives (PBDs) doxorubicin, centanamycin (indolecarboxamide), etc [47].

Doxorubicin binds to topoisomerase, via intercalation it inhibit DNA replication and poses cellular cytotoxicity. For CD74-positive multiple myeloma, milatuzumab-doxorubicin conjugate is presently in Phase I/II clinical trials [48].

The pyrrolbenzodiazepines containing ADC’s (SGN-CD33A and SGN-CD70A) are some of the evident payloads which binds covalently to discontinuous sequences in the subsidiary groove of DNA manifesting the potential chemotherapeutic activity and they are isolated from Streptomycin species. These PBD-containing ADCs are also under clinical pipeline [22].

In order to explore novel cytotoxic payloads, researches are being investigated worldwide. Identification of novel antitumor chemotype leads to future development of ADCs.

7. Conclusion

The multiple linker payloads screening on multiple sites of an antibody is very challenging. There has been a growing interest to screen ADCs with the best combination of site and payload. Previous mentioned combinatorial problem may be addressed by high-throughput methodologies. Very recently Puthenveetil et al. [49] published an article, in which they introduce a method to screen multiple linker payloads, sites or their combinations in-parallel in 96-well plate. The authors reported solid-phase, site-specific conjugation methodology of linker-payloads to antibodies and Fab fragment. Interestingly the purification of ADCs with typical size-exclusion or hydrophobic interaction chromatography is also avoided by this novel method.

Very recently a novel ADC ASG-15ME (Product name, AGS15E, conjugation of SLITRK6- specific human gamma 2 antibodies (Iγ2) with MMAE via a protease-cleavable linker) is developed by Morrison et al [50]. The proposed ADC were found to be effective for the treatment of advanced urothelial cancer as suggested by the evident in vitro and in vivo cytotoxicity after conjugation to Monomethyl Auristatin E and this AGS15E entered into the Clinical Trials Phase I. SLITRK6, belongs to the transmembrane SLITRK proteins family that having conserved leucine-rich repeat domains, is a bladder tumor antigen containing an open reading frame gene of 841 amino acids and. High expression of SLITRK6 in advanced transitional cell bladder cancer and in addition of a lesser expression in lung, breast and glioblastoma epithelial tumors were first time reported by Morrison et al. Waiting for the success of ASG-15ME in all phases of clinical trials that may leads to clinical development of ASG-15ME as a new treatment for advanced bladder cancer.

On the other hand, a new type of stable, pharmacokinetically safe antibody drug conjugate was designed in the lab of Yusuke Ogitani with having excellent anti-tumor activity[51] In rats and cynomolgus monkey the designed payload DS-8201a has got admissible safety profile. It contains novel topoisomerase 1 inhibitor namely exatecan mesylate (DX-8951F) as a payload with antibody that targeting epidermal growth factor receptor HER2. Belonging to the family of transmembrane receptor, HER2 is highly over expressed in breast, bladder, cervical, colorectal, esophageal, gastric, head and neck, liver, lung, ovarian and salivary gland cancer cell lines. Generally the drug-to-antibody ratio (DAR) of ADCs ranges from 2 to 4. The rapid clearance of the ADC is observed in vivo if the DAR is increased and also off-target toxicity results due to high exposure of the released drug in plasma. All though 8 molecules of THE DRUG is conjugated with each antibody in case of DS-8201a, but interestingly the above mention problems were not observed. Above all, DS-8201a is promising to answer T-DM1 refractory breast cancer, IHC 1+ and 2+/FISH negative patients and HER2-positive patients.

References

[1]  N. Stephanopoulos and M. B. Francis, Nat. Chem. Biol., 2011, 7, 876–883.
[2]  Qi-Ying Hu, F. Bertib and R. Adamo, Chem. Soc. Rev., 2016, DOI: 10.1039/c4cs00388h
[3]  C. H. Fu and K. M. Sakamoto, Exp. Opin. Pharmacother., 2007, 8, 1977–1984.
[4]  P. Agarwal and C. R. Bertozzi, Bioconjugate Chem., 2015, 26, 176–192.
[5]  G. Li, S. Wang, X. Xue, X. Qu and H. Liu, Drug Discov. Ther., 2013, 7, 178–184.
[6]  R. V. J. Chari, M. L. Miller and W. C. Widdison, Angew. Chem., Int. Ed., 2014, 53, 3796–3827.
[7]  P. Strop, K. Delaria, D. Foletti, J. M. Witt, A. Hasa-Moreno, K. Poulsen, et al., Nat. Biotechnol., 2015, 33, 694–696.
[8]  F. Tian, Y. Lu, A. Manibusan, A. Sellers, H. Tran, Y. Sun, et al., Proc. Natl. Acad. Sci. U. S. A., 2014, 111, 1766–1771.
[9]  J. R. Junutula, K. M. Flagella, R. A. Graham, K. L. Parsons, E. Ha, H. Raab, et al., Clin. Cancer Res., 2010, 16, 4769–4778.
[10]  P. Strop, S. H. Liu, M. Dorywalska, K. Delaria, R. G. Dushin, T. T. Tran, et al., Chem. Biol., 2013, 20, 161–167.
[11]  T. V. Jerjian, A. E. Glode, L. A. Thompson, C. L. O’Bryant, Pharmacother., 2016, 36, 99116. H. P. Gerber, P. Sapra, F. Loganzo, C. May, Biochem. Pharmacol., 2015, http://dx.doi.org/10.1016/j.bcp.2015.12.008.
[12]  S. C. Alley, D. R. Benjamin, S. C. Jeffrey, N. M. Okeley, S. Meyer, R. J. Sanderson, and P. D. Senter, Bioconj. Drug., 2008, 19, 759-765.
[13]  B. Goeij, and J. M. Lambert, Curr. Opinion in Immunol., 2016, 40, 14–23.
[14]  J. R. Junutula, H. Raab, S. Clark, S. Bhakta, D. D. Leipold, S. Weir, et al., Nat. Biotechnol., 2008, 26, 925–932.
15] A. Beck, P. Senter, and R. Chari, World Antibody Drug Conjugate Summit Europe: February 21-23, 2011; Frankfurt, Germany. Mabs 3, 331-337.

16] I. Melero, D. M. Berman, M. A. Aznar, A. J. Korman, J. L. Gracia, and J. Haanen, Nat. Rev. Cancer., 2015, 15, 457–472.

17] R. P. Lyon, T. D. Bovee, S. O. Doronina, P. J. Burke, J. H. Hunter, H. D. Neff-LaFord, et al., Nat. Biotechnol., 2015, 35, 733–735.

18] K. M. Mahoney, P. D. Rennert, and G. J. Freeman, Nat. Rev. Drug Discov., 2015, 14, 561–584.

19] C. Dumontet, and M. A. Jordan, Nat. Rev. Drug Discov., 2010, 9, 790-803.

20] E. Oroudjev, M. Lopus, L. Wilson, C. Audette, C. Provenzano, H. Erickson, Y. Kvtun, R. Chari, and M. A. Jordan, Mol. Cancer Ther., 2010, 9, 2700-2713.

21] Eunhee G. Kim and Kristine M. Kim, Strategies and Advancement in Antibody-Drug Conjugate Optimization for Targeted Cancer Therapeutics, Biomol Ther 23 (6), 493-509 (2015).

22] Satish K. Singh, Donna L. Luisi, Roger H. Pak, Antibody-Drug Conjugates: Design, Formulation and Physicochemical Stability, Pharm Res, 2015, DOI 10.1007/s11095-015-1704-4.

23] Panowsk S, Bhakta S, Raab H, Polakis P, Junutula JR. Site-specific antibody drug conjugates for cancer therapy. mAbs. 2014; 6 (1): 34–45.

24] Arias JL. Drug delivery strategies in targeting cancer: current concepts and future developments. Curr Drug Targets. 2011;12(8):1094–5.

25] Goldmacher VS, Kvtun YV. Antibody-drug conjugates: using monoclonal antibodies for delivery of cytotoxic payloads to cancer cells. Ther Deliv 2011; 2 (3): 397–416.

26] Basile L, Pignatello R, Passirani C. Active targeting strategies for anticancer drug nanocarriers. Curr Drug Deliv. 2012; 9 (3): 255–68.

27] Salfeld JG. ISotype selection in antibody engineering. Nat Biotechnol. 2007; 25 (12): 1369–72.

28] McDonagh, C. F., Kim, K. M., Turcott, E., Brown, L. L., Westendorf, L., Feist, T., Sussman, D., Stone, J., Anderson, M., Miyamoto, J., Lyon, R., Alley, S. C., Gerber, H. P. and Carter, P. J. (2008) Engineered anti-CD70 antibody-drug conjugate with increased therapeutic index. Mol. Cancer Ther. 7, 2913-2923.

29] Gerber HP, Koehn FE, Abraham RT. The antibody-drug conjugate: an enabling modality for natural product-based cancer therapeutics. Nat Prod Rep. 2015; 30 (5): 625–39.

30] Bai RL, Pettit GR, Hamel E. Structure-activity studies with chiral isomers and with segments of the antimitotic marine peptide dolastatin 10. Biochem Pharmacol. 1990; 40 (8): 1859–64.

31] Doronina SO, Toki BE, Torgov MY, Mendelsohn BA, Cerveny CG, ChaceDF, et al. Development of potent monoclonal antibody auristatin conjugates for cancer therapy. Nat Biotechnol. 2003; 21 (7): 778–84.

32] Bai R, Roach MC, Jayaram SK, Barkocy J, Pettit GR, Lduena RF, et al. Differential effects of active isomers, segments, and analogs of dolastatin 10 on ligand interactions with tubulin. Correlation with cytotoxicity. Biochem Pharmacol. 1993; 45 (7): 1503–15.

33] Smith AL, Nicolaou KC. The enediyne antibiotics. J Med Chem. 1996; 39 (11): 2103–17.

34] Hinman LM, Hamann PR, Wallace R, Menendez AT, Durr FE, Ueslasic J. Preparation and characterization of monoclonal antibody conjugates of the calicheamicins: a novel and potent family of antitumor antibiotics. Cancer Res. 1993; 53 (14): 3336–42.

35] Dosio F, Brusa P, Cattel L. Immunotoxins and anticancer drug conjugate assemblies: the role of the linkage between components. Toxins. 2011; 3 (7): 848–83.

36] Hamann PR, Hinman LM, Beyer CF, Lindh D, Ueslasic J, Flowers DA, et al. Anti-CD33 antibody-calicheamicin conjugate for treatment of acute myeloid leukemia. Choice of linker. Bioconjug Chem. 2002; 13 (1): 40–6.

37] Takahashi I, Takahashi K, Ichimura M, Morimoto M, Asano K, Kawamoto I, et al. Duocarmycin A, a new antitumor antibiotic from Streptomyces. J Antibiot. 1988; 41 (12): 1915–7.

38] Wolfe AL, Duncan KK, Lajiness JP, Zhu K, Duerfeldt AS, Boger DL. A fundamental relationship between hydrophobic properties and biological activity for the duocarmycin class of DNA alkylating antitumor drugs: hydrophobic-binding-driven bonding. J Med Chem. 2013; 56 (17): 6845–57.

39] Boger DL. The duocarmycins: synthetic and mechanistic studies. Acc Chem Res. 1995; 28 (1): 20–9.

40] Kupchan SM, Komoda Y, Court WA, Thomas GJ, Smith RM, Kartm A, et al. Maytansine, a novel antileukemic ansa macroclide from Maytenuus ovatus. J Am Chem Soc. 1972; 94 (4): 1354–6.

41] Widdison WC, Wilhelm SD, Cavanagh EE, Whiteman KR, Lecce BA, Kvtun Y, et al. Semisynthetic maytansine analogues for the targeted treatment of cancer. J Med Chem. 2006; 49 (14): 4392–408.

42] Remillard S, Rebhun LI, Howie GA, Kupchan SM. Antimitotic activity of the potent tumor inhibitor maytansine. Science. 1975; 189 (4207): 1002–5.

43] Kupchan SM, Snedan AT, Branfman AR, Howie GA, Rebhun LI, Melvor WE, et al. Structural requirements for antileukemic activity among the naturally occurring and semisynthetic maytansinoids. J Med Chem. 1978; 21 (1): 31–7.

44] Higashide E, Asai M, Otsui K, Tamida S, Kozai Y, Hasegawa T, et al. Ansamitocin, a group of novel maytansinoid antibiotics with antitumour properties from Nocadaria. Nature. 1977; 270 (5639): 721–2.

45] Beck, A., Senter, P. and Chari, R. (2011) World Antibody Drug Conjugate Summit Europe: February 21-23, 2011, Frankfurt, Germany. Mabs 3, 331-337.

46] Sapra, P., Stein, R., Pickett, J., Qu, Z., Govindan, S. V., Cardillo, T. M., Hansen, H. J., Horak, I. D., Griffiths, G. L. and Goldenberg, D. M. (2005) Anti-CD74 antibody-doxorubicin conjugate, IMMU-110, in ahuman multiple myeloma xenograft and in monkeys. Clin. Cancer Res. 11, 5257-5264.
[48] Sujiet Puthenveetil, Sylvia Musto, Frank Loganzo, L. Nathan Tumey, Christopher J. O'Donnell, and Edmund Idris Graziani. Development of solid-phase site-specific conjugation and its application towards generation of dual labeled antibody and Fab drug conjugates. 2016. DOI: 10.1021/acs.bioconjchem.6b00054.

[49] Kendall Morrison, Pia M. Challita-Eid, Arthur Raitano, Zili An, Peng Yang, Joseph D. Abad, Wendy Liu, Dawn Ratay Lorrie, Josh T. Snyder, Linnette Capo, Alla Verlinsky, Hector Aviña, Fernando Doñate, Ingrid B. J. Joseph, Daniel S. Pereira, Karen Morrison, David R. Stover. 2016, DOI: 10.1158/1535-7163.MCT-15-0570.

[50] Yusuke Ogitani, Tetsuo Aida, Katsunobu Hagihara, et al. DS-8201a, a novel HER2-targeting ADC with a novel DNA topoisomerase I inhibitor, demonstrates a promising anti-tumor efficacy with differentiation from T-DM1. Clin Cancer Res. doi:10.1158/1078-0432.CCR-15-2822.

[51] Antibody Drug Conjugates Currently in Clinical Evaluations: Angew. Chem. Int. Ed.,2014,53,3796.