Expert Opinion on Investigational Drugs

Update

Oncologic, Endocrine & Metabolic

The development of antibody delivery systems to target cancer with highly potent maytansinoids

Changnian Liu & Ravi VJ Chari

Improving the tumour selectivity of cytotoxic drugs through conjugation to tumour-reactive monoclonal antibodies may lead to novel, more potent agents for cancer therapy. The maytansinoid drugs are 100- to 1000-fold more cytotoxic *in vitro* than current clinical anticancer drugs. We recently demonstrated that conjugation of maytansinoid drugs to monoclonal antibodies renders them highly efficacious against cancers of breast and colon in both *in vitro* and in *in vivo* tumour models. Antibody-maytansinoids represent a new generation of immunoconjugates that may yet fulfil the promise of effective cancer therapy through antibody targeting of cytotoxic agents.

Keywords: *antibody, cancer therapy, immunoconjugates, maytansinoids*

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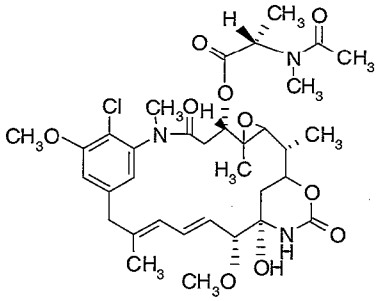
1. Antibody-drug conjugates and cancer therapy

Cancer chemotherapy could be greatly improved by utilising agents with enhanced potency and cancer specificity. The development of conjugates between potent cytotoxic agents and monoclonal antibodies with tumour reactivity has now advanced to a stage that antibody-drug conjugates look very promising as novel anticancer agents with the above characteristics.

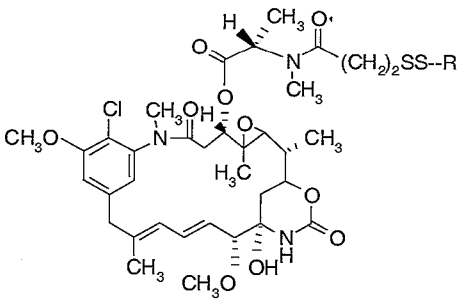
The early development of monoclonal antibody-drug conjugates focused on the use of well-established clinical anticancer agents, such as doxorubicin, methotrexate, vinblastine, mitomycin C, and melphalan, in conjugated form [1-3]. However, evaluation of *in vitro* cytotoxicity revealed that most of these conjugates were not potent enough to be clinically useful. Drug levels achieved inside target cells were too low, and only marginal antigen-specific killing of cultured tumour cells was observed. Not surprisingly, therefore, in tumour xenograft animal models, therapeutic effects with these conjugates were observed only when the treatments were commenced before the tumours were well-established [4] or when extremely large doses were used [5]. For example, it has been recently reported that an immunoconjugate, prepared with the monoclonal antibody BR96 and doxorubicin, cured athymic mice bearing human tumour xenografts [5]. However, this effect was only achieved at the maximum tolerated dose (MTD) of the immunoconjugate (a doxorubicin dose of 20 mg/kg/d x 3).

A few antibody-drug conjugates have been further evaluated in humans [6-8]. In general, no significant anticancer effects have been observed with these agents in clinical trials. Indeed, the peak circulating serum concentrations of conjugate were typically in the range of their *in vitro* IC_{50} values (inhibitory concentration resulting in 50% cell death) values and, thus, capable of eliminating at best only about 50% of tumour cells. Lack of clinical success with these early antibody-drug conjugates suggests that it was not possible to achieve the intratumoural and intracellular concentrations of drugs sufficient to kill large numbers of cancer cells.

Figure 1: Structural representation of maytansine (**1**), DM1 (**2**), and antibody-DM1 (**3**).



1 Maytansine



2 DM1: R= CH_3
3 Ab-DM1: R=Ab

Possible reasons for these outcomes may be:

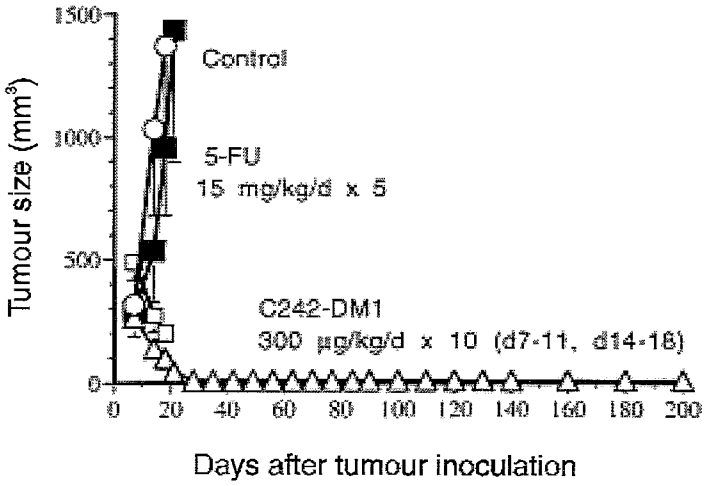
- lack of cytotoxic potency - the majority of commonly used anticancer drugs are only moderately cytotoxic at clinically achievable concentrations in antibody- drug conjugate form (large numbers of drug molecules have to be internalised to cause cell death);
- tumour cells only express limited numbers of target antigens, which restricts the amount of drug delivered;
- poor penetration of immunoconjugates into tumours, and inefficient internalisation of antigen-antibody complexes;
- inefficient release of the active drug from the antibody inside target cells.

We reasoned that immunoconjugates must be composed of drugs possessing much higher potency than the currently used anticancer agents if therapeutic levels of conjugates at the tumour sites are to be achieved in patients. We have recently reported antibody conjugates with the maytansinoid drug DM1 [9,10], a sulfydryl-containing derivative of maytansine (Takeda, Osaka) (**Figure 1**). Maytansinoids effect cell killing by interfering with the formation of microtubules and depolymerisation of already formed microtubules [11]. They are 100- to 1000-fold more cytotoxic than chemotherapeutic drugs such as doxorubicin, methotrexate, and *Vinca* alkaloids. DM1 is linked to the antibody *via* a novel disulfide linker which allows for rapid release of the fully active drug inside the target cells. Disulfide linkers are superior to other linkers in that they are more stable during storage and in serum, but are still able to release the active drugs from the conjugates inside target cells efficiently [12,13].

2. *In vitro* cytotoxicity and specificity of antibody-maytansinoid conjugates

Antibody-maytansinoid conjugates were assessed for *in vitro* cytotoxicity against human cancer cell lines using a clonogenic assay [14]. The disulfide-linked maytansinoid immunoconjugates exhibited high antigen-specific cytotoxicity [10,11]. The C242-maytansinoid conjugate (C242-DM1) prepared with the monoclonal antibody C242 (Pharmacia Oncology, Lund, Sweden), which recognises the CanAg antigen [15] expressed on all human colorectal cancers, killed antigen-positive COLO 205 cells with an IC_{50} value of 3.2×10^{-11} M (3.5 pg/ml), with > 99.999% of the cells killed at a conjugate concentration of 4.5×10^{-9} M (3.3 ng/ml) (all concentrations refer to DM1; one microgram of DM1 corresponds to 54 µg of C242-DM1 conjugate). In contrast, the conjugate was 1100-fold less cytotoxic towards antigen-negative A-375 melanoma cells ($IC_{50} = 3.6 \times 10^{-8}$ M, 26.5 ng/ml), demonstrating that the cytotoxicity effect of C242-DM1 is antigen-specific. Both cell lines were found to be equally sensitive to unconjugated maytansinoid ($IC_{50} = 4 \times 10^{-11}$ M). Similar results were observed with TA.1-DM1 conjugate prepared with the monoclonal antibody TA.1, which binds to the HER-2/neu oncogene protein expressed on the surface of human breast cancer cells [16]. The TA.1-DM1 conjugate was highly cytotoxic to antigen-positive SK-BR-3 breast cancer cells ($IC_{50} = 1.6 \times 10^{-11}$ M). The conjugate was at least 1000-fold less cytotoxic to antigen-negative human oral epidermoid carcinoma KB cells ($IC_{50} > 2 \times 10^{-8}$ M) [10].

Figure 2: Anti-tumour activity of C242-DM1 against large COLO 205 tumours (mean tumour size = 260 mm^3). Each mouse was inoculated with 5 x 10^6 COLO 205 cells and treatments were started on day 7 after tumour inoculation. Each group contained 8 animals.



3. C242-DM1 in treatment of human colon cancer xenografts in severe combined immunodeficient mice

Animals bearing COLO 205 colon tumours (homogeneous antigen expression) were treated either with

five daily injections of C242-DM1 at a dose of 300 µg/kg/d, or with an equivalent dose of the isotype-matched non-binding conjugate N901-DM1, or with a mixture consisting of corresponding amounts of C242 antibody and unconjugated DM1 [10]. Treatment with C242-DM1 completely eliminated all tumours within two weeks of the initiation of therapy, and all eight animals were tumour-free for 200 days (duration of the experiment). In contrast, very little antitumour activity was observed in animals treated with non-targeted conjugate or with the mixture of antibody and free DM1. In a dose-response study, C242-DM1 eliminated COLO 205 tumours in all 8 animals at a dose as low as 225 µg/kg/d x 5, which is 59% of the maximum tolerated dose (MTD = 380 µg/kg/d x 5) [10].

These results encouraged us to evaluate the therapeutic efficacy of C242-DM1 in mice bearing larger (average size 260 mm^3) subcutaneous COLO 205 xenografts **(Figure 2)**. Animals received two courses of 5-day treatment with C242-DM1 (300 µg/kg/d) or, for comparison, treatment with 5-fluorouracil (5-FU), the standard chemotherapeutic drug used for the treatment of colorectal cancer. C242-DM1 again cured all animals rendering them tumour-free for greater than 200 days (duration of the experiment) without intolerable toxicities. This therapeutic effect on large tumours is especially remarkable in view of the finding that 5-FU at its MTD (15 mg/kg/d x 5) only slightly (by about 5 days) delayed tumour growth [10].

C242-DM1 was then evaluated against established colon tumour xenografts from the LoVo and HT-29 colon cancer cell lines which express the CanAg antigen heterogeneously on only 20 - 30 % of their cells [10]. Animals bearing LoVo tumour xenografts were treated with either one or two courses of C242-DM1 (300 µg/kg/d x 5). Remarkably, complete tumour regressions lasting 5 weeks were observed in all animals treated with one course of C242-DM1. The period of complete regression could be prolonged to 9 weeks by a second course of treatment with C242-DM1 initiated 21 days after the start of the first course, suggesting that using multiple cycles of this immunoconjugate for treatment of colorectal cancer may be a feasible clinical regimen. Similar effects were obtained in the HT-29 colon tumour model [10].

4. Conclusion

The use of antibody-drug conjugates for the treatment of cancers, i.e., the selective delivery of cytotoxic drugs to tumour cells, seems to be a promising approach [17]. However, no such agent has yet demonstrated significant antitumour activity in the clinical setting. Antibody-maytansinoid conjugates represent a new generation of immunoconjugates that may fulfil the promise of effective cancer therapy through antibody targeting of cytotoxic drugs. From the preclinical data,

C242-DM1 stands out as a promising new candidate for clinical evaluation against colorectal cancer. The conjugates made using humanised antibodies [18] will allow patients to be treated with several courses of targeted chemotherapy, potentially increasing clinical benefit substantially.

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