Abstract No. 661

Proteomics and Selecting the Right Combination of Target and Toxin for Antibody-Drug-Conjugate (ADC) Development.

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Abstract

With the successes of Kadcyla and Adcetris, and the emerging data from early phase trials of many other ADCs our understanding of the critical attributes for target selection and ADC development has improved significantly. Clinical experience indicates that the dose-limiting toxicities observed are predominately off-target and thus are similar across ADCs using the same linker and drug combinations and independent of the target antigen. Furthermore, the maximum tolerated doses (MTDs) in humans, monkeys, and mice for many ADCs seem to be very similar regardless of the protein being targeted. Thus, the therapeutic index of new ADCs using the current drug/linker technologies (tubulin and DNA targeting toxins) will be driven predominately by efficacy, specifically the ability to achieve antitumor activity at exposure levels below the MTD. Therefore, the focus of future target selection should be on efficacy within the therapeutic window rather than on antigen-dependent (i.e. on-target) toxicity arising from normal tissue expression.

We have selected targets and toxins for ADC development based on the likelihood of robust potency against cancer cells. The properties favored are : 1) high expression and high prevalence in selected malignancies, 2) normal tissue expression profiles known to be tolerable for ADCs (e.g. her2neu, gpnmb, nectin4), 3) targets enabling rapid intracellular delivery of ADC and release of active toxin, 4) clinical indications sensitive to the toxin of choice.

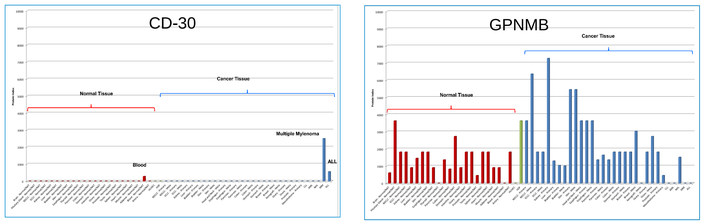
This process and its success are demonstrated here through the development of two ADCs. Firstly, Ox-1476 (ADC-1, ADC-4) for triple negative breast cancer (TNBC). The target was selected based on consistent high expression in TNBC, the MAb selected from a large screen of greater than 1000 candidates based on activity as an actual ADC, and the toxin selected based on the positive experience with maytansine in breast cancer (Kadcyla) and the preclinical data presented here. The Ox-1476 (ADC-1, ADC-4) shows greater and broader activity than Kadcyla in vitro and an initial toxicity profile consistent with pursuing clinical development. Secondly, Ox-1425 (ADC-2) is being developed for small cell lung cancer (SCLC). The target and MAb were selected by the same processes but for Ox-1425 (ADC-2) the toxin screen resulted in the selection of a DNA alkylating agent.

Our proteomics database has provided a unique data set of candidate cell surface targets for the development of antibodies compatible with the current state of the art ADC payloads. The selected targets show high and prevalent expression in cancers, are in protein families shown to internalize efficiently, and have normal tissue expression profiles similar to other proteins already being targeted by clinical stage ADCs. The preclinical safety and activity data shown here is supportive of our strategy for selecting ADC targets.

Introduction

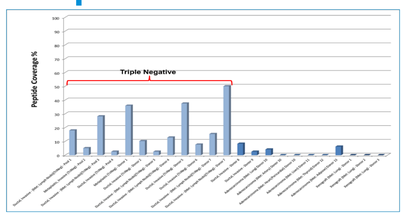
Here we present 2 case studies in which we evaluated RNA/protein expression, *in vitro* cytotoxicity, and *in vivo* anti-tumor activity in order to define a set of criteria for choosing the most effective target, linker, and toxin combination for developing an antibody-drug conjugate (ADC) as a therapeutic to treat a particular type of cancer. We highlight Target 1, a protein with high expression and prevalence in TNBC. The ADCs directed against Target 1 demonstrate cytotoxic activity both *in vitro* and *in vivo* but these data are not always concordant, and show the value of empirical evaluation of a toxin. In addition, we show Target 2, a protein with high expression and prevalence in SCLC. ADCs directed against Target 2 were composed of two different toxin classes. Empirical assessment provided further granularity to identify a specific member of the toxin class which demonstrated superior efficacy for this target and indication.

Proteomics profiles for clinical ADC targets



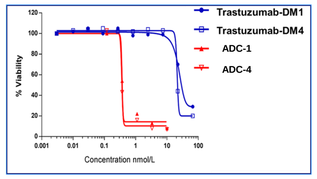
Proteomics analysis demonstrated expression profiles for two targets of ADCs that have been recently evaluated in the clinic. Differential tumor vs normal target expression may not necessarily predict efficacy of the ADC, as the maximum tolerated dose (MTD) is similar for both broadly and specifically expressed targets e.g. the MTD for an ADC directed against the CD30 antigen with a highly restricted expression profile was 1.8 mg/kg (1), the same as for an ADC directed against a more broadly expressed antigen like GPNMB (2).

Proteomics identifies Target-1 expression in TNBC



Target-1 is expressed in breast cancers, and the proteomics profile indicates significantly higher expression of the target in triple negative breast cancer donors.

Comparison of T-DM1/4 and ADC-1, ADC-4 in TNBC



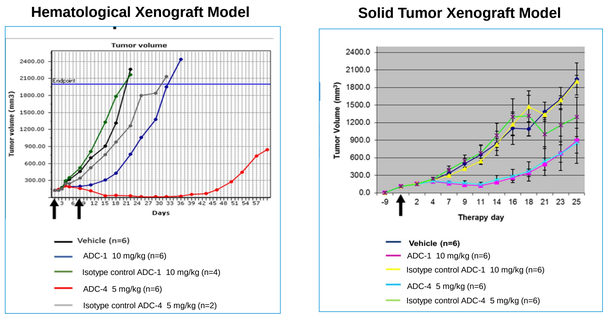
***In vitro* activity of trastuzumab-DM1 conjugates versus free DM1 (IC_{50}, nmol/L DM1 equivalents)**

	trastuzumab-SPP-DM1	trastuzumab-MCC-DM1	free L-DM1
SK-BR-3	0.24	0.22	1.00
BT-474	2.71	4.26	4.52
MCF7	>100	>100	2.92
MDA-MB-468	18.37	41.37	0.59

Cells were treated with trastuzumab-DM1 conjugates or L-DM1 as free drug for 72 h; IC_{50} values were determined from four-parameter curve fitting.

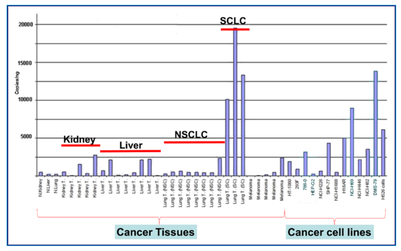
Antibody-1 (Ab-1) targets a protein which is highly expressed in TNBC, and the maytansinoid conjugates (ADC-1 and ADC-4) directed against this target exhibit robust *in vitro* cytotoxicity in the TNBC cell line, MDA-MB-468. In contrast, an ADC using the same maytansinoid drug payloads but conjugated to an antibody directed against a different target antigen, Her-2/Neu (Trastuzumab-DM1/DM4), did not exhibit significant *in vitro* cytotoxicity activity in the TNBC cell line where Her-2/Neu is not expressed (3). Furthermore, the Her2-targeted ADC T-DM1 (Kadcyla™) has exhibited *in vitro* cytotoxicity in cell lines (SKBR-3, BT-474, HCC1954, KPL-4) that express the target and efficacy in patients with Her2-positive metastatic breast cancer (4, 5-6). ADC-1 could target breast cancers not suitable for treatment with Kadcyla™.

ADC-1 and ADC-4 exhibit potent anti-tumor activity *in vivo*



The *in vivo* anti-tumor activity of ADC-1 and ADC-4 were evaluated in subcutaneous xenograft models of hematological malignancies and solid tumors. A single dose or 2 doses of ADC-1 and ADC-4 were administered to mice after tumors were established. Treatment was well tolerated and mice showed no clinical signs of toxicity. In the hematological tumor model, both ADCs exhibited significant anti-tumor activity compared to the controls, and ADC-4 was clearly more active, eliminating 5/6 tumors completely. Interestingly, ADC-1 and ADC-4 were similarly effective in *in vitro* cytotoxicity assays on the same tumor cell line (data not shown). In contrast, in the solid tumor xenograft model, while ADC-1 and ADC-4 both exhibited significant anti-tumor activity compared to the controls, there was no difference in the degree of activity between the two ADCs. However, ADC-4 demonstrated more activity in *in vitro* cytotoxicity assays performed with this solid tumor cell line (data not shown). Thus the relative activity of the ADCs differed based on the cancer model and based on the study type. These data indicate the importance of empirically optimizing the payload for the specific cancer indication.

Target-2 mRNA is highly expressed in SCLC



Q-RT-PCR data demonstrate the RNA expression profile of Target-2 in clinical cancer samples and cancer cell lines. Target-2 is highly expressed and highly prevalent in SCLC.

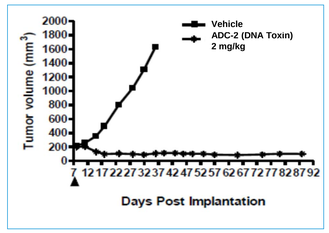
Comparing payloads for ADC-2 *in vitro* cytotoxicity in SCLC

Model	H526	DMS79	H69	H1048	H522	DM533	SHP77
DNA T1	✓	✓	✓	✓	=	=	=
DNA T2	✓	✓	✓	✓	=	=	=
DNA T3	✓	✓	✓	✓	✓	✓	=
DNA T4	✓	✓	✓	✓	✓	✓	✓
MTI T1	✓	=	✓	=	✓	=	X
MTI T2	✓	=	=	=	X	X	X
MTI T3	✓	=	=	=	X	X	X
MTI T4	✓	=	=	=	X	X	X

✓ Robust cytotoxic activity
= Modest cytotoxic activity
X No cytotoxic activity

Many *in vitro* experiments were performed to define the appropriate and most active antibody-linker-drug combination for SCLC; the results are summarized in the table above. It is clear that in SCLC cell lines, DNA-alkylating agents exhibited superior *in vitro* cytotoxicity to microtubule inhibitors (MTIs) when conjugated to the same antibody. The majority of the lung cancer cell lines evaluated were killed by each of the 4 DNA-binding agents tested and one of them was active in all 7 of the cell lines. The poor *in vitro* cytotoxicity data with MTIs in SCLC cell lines correlates with lack of clinical activity for MTIs in SCLC treatment to date. We propose that the DNA-alkylating class of drugs may be a more appropriate choice than MTIs as the payload for ADCs intended for treatment of SCLC.

ADC-2 exhibits potent *in vivo* anti-tumor activity in a SCLC xenograft



The *in vitro* cytotoxicity studies informed the design of our *in vivo* activity studies by identifying the drug class which was most active in SCLC cell lines. Thus, *in vivo* anti-tumor activity of an ADC carrying a DNA-alkylating agent (ADC-2) was evaluated in a SCLC xenograft model. A single dose of ADC-2 was administered to mice after tumors were established, and resulted in durable, long term tumor regression compared to the vehicle control. These data support the importance of empirically optimizing the target, linker and drug for the specific indication.

Conclusions

- High target expression in cancer tissues combined with low expression in normal tissues is likely not sufficient to ensure clinical efficacy of an ADC; empirical optimization of target, linker, drug, and indication is critical.
- Case study 1: ADCs comprised of the same antibody with different payloads demonstrate different activities which are not always concordant across assays and model systems, underscoring the need to empirically test each ADC across multiple assays both *in vitro* and *in vivo*.
- Case study 2: Experimental identification of the right toxin class and the specific class member that works best for a particular target and indication is clearly illustrated by this example and could be the difference between achieving clinical benefit or not in patients with high unmet medical needs.

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