A novel antibody drug conjugate linker enabling production of ADCs with high drug to antibody ratios and fast payload release for improved efficacy.

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ABSTRACT #4882

SUMMARY

We developed a novel drug linker technology, Multilink™, that enables the generation of stable and potent antibody drug conjugates (ADCs). It is selectively recognized and cleaved by the carboxydipeptidase activity of Cathepsin B, shown to be overexpressed in cancer cells. The technology allows a fast and specific cleavage, which results in efficient drug release in the tumor. Different payloads can be attached to antibodies, and the drug to antibody ratio (DAR) can be modulated up to DAR16, resulting in customized cytotoxic activity. Here we present the characteristics of Multilink™ using Trastuzumab as the targeting antibody (targeting Human epidermal growth factor receptor 2 (Her2)), testing different payloads and different DARs.

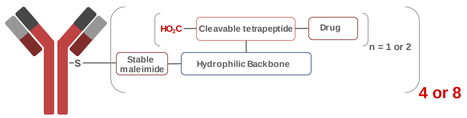
Trastuzumab based Multilink™ ADCs (T-Multilink™ ADCs) were generated and first tested *in vitro*. Kinetic release experiments show that the payload could be cleaved and released within minutes (Figure 1). In cellular assays, ADCs were tested for their activity and proved highly potent, especially in the Jimt-1 Her2 low expressing cells. Increasing the DAR from 8 to 16 correlates with improved activity (Figure 2), while the aggregation levels remained low (Figure 3).

We then tested the molecules in a breast cancer model expressing low levels of Her2. A Trastuzumab Multilink™ ADC with mertansine (DM1) at DAR8 showed superior activity, including complete tumor regressions, as compared to T-DM1 and T-DXd. In the same model, a Multilink™ ADC with Auristatin F (AF) was even more active and able to induce durable complete regression in all mice (Figure 6). Both compounds were well tolerated. In addition, the Trastuzumab Multilink™ ADCs showed total antibody PK profiles comparable to T-DM1 and T-DXd (Figure 5). Plasma stability was also assessed in vitro for T-Multilink™ DM1 ADC and stability was good after incubation in human and murine serum (Figure 4).

In conclusion, we are able to produce stable and high DAR ADCs with improved efficacy using the Multilink™ technology.

INTRODUCTION

- Multilink™ technology is a peptidic linker specifically recognized and cleaved by carboxydipeptidase activity of Cathepsin B.
- Different kind of payloads can be conjugated to Multilink™, such as DM1 and AF.
- It allows multiple drug conjugation, with up to 8 linkers per antibody, and 1 to 2 payloads per linker.



MATERIAL AND METHODS

These studies were conducted in accordance with institutional guidelines and NCRI Guidelines for the welfare and use of animals in cancer research[1].

ADC preparation. The linker Multilink™ was prepared by standard chemistry methods and convergent strategies. The payloads AF or DM1 were conjugated to Multilink™ using peptidic coupling in solution. The resulting drug-linker was then conjugated to the monoclonal antibody Trastuzumab using the classic cysteine conjugation resulting from reduction of interchain disulfide bonds.

Control antibodies: T-DM1 (Kadcyla™ from Roche) and T-DXd (Enhertu® from Daiichi Sankyo) were purchased in a local pharmacy as powder and prepared as per manufacturer instructions.

Release kinetics by Cathepsin B-induced cleavage: The drug release kinetic was evaluated using an *in vitro* enzymatic cleavage assay with recombinant human Cathepsin B (BioTechne AG, #953-CY-010). The release kinetics were normalized with cleavage of Cys-MC-Val-Cit-PABC-MMAF (IBIOsource, #S10001) as reference.

In vitro cytotoxic assay (performed in CROs): Cells were plated and serial dilutions of drugs were added to the cells. After 72 hours of incubation, CellTiter-Glo (Promega kit) were added to each well and the plates were incubated for the appropriate amount of time before reading of luminescence. Relative IC50 were calculated using GraphPad Prism.

Pharmacokinetic (PK) study (performed in CROs): Three mice per compound were injected intravenously with 5mg/kg of each compound. Blood was collected at the defined timepoints (5min, 1h, 6h, 24h, 48h, 72h) and total antibody was measured using a MSD ligand binding assay with coating Anti-human IgG (Fc specific) antibody, detection Biotin-SP-conjugated affinipure goat anti-human IgG Fc fragment specific, and revealed with Enzyme Sulfo-TAG streptavidin.

In vivo mouse xenograft studies (performed in CRO): Briefly, the mice were inoculated subcutaneously with Jimt-1 Her2 low breast cancer cells. When tumors reached an average size of 110mm3, mice were randomized in a groups of 8 mice and treatment started. Two intravenous injections of 5mg/kg were performed 1 week apart. Vehicle is PBS. Tumor volume and body weights were recorded twice a week.

RESULTS

Kinetic release

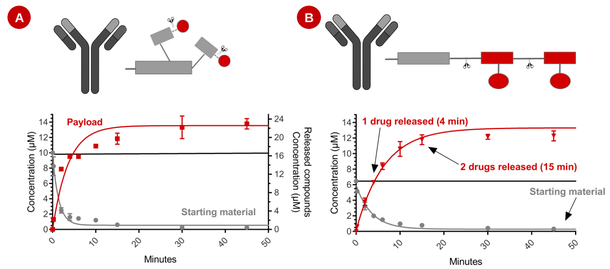
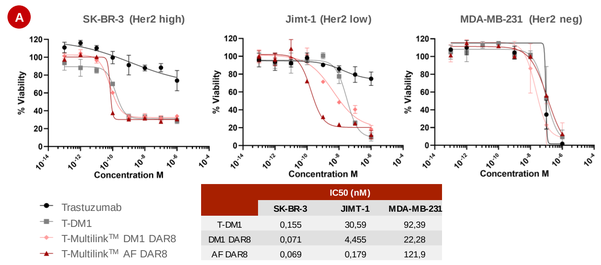


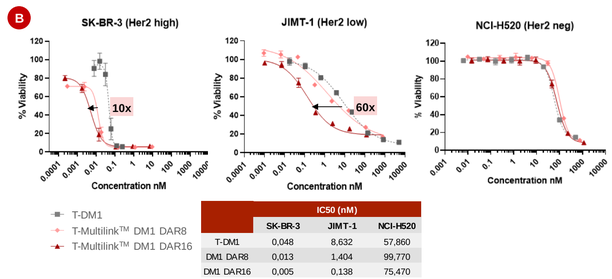
Figure 1 : Release kinetics by Cathepsin B cleavage on two types of linkers. (A) Simultaneous cleavage, T1/2 = 0.98 min (B) Sequential cleavage. The red lines represent the payload release, the grey lines represent the decay of starting material in presence of Cathepsin B and the black lines represent the control without Cathepsin B

In vitro cytotoxicity

T-Multilink™ ADCs show high *in vitro* cytotoxicity especially in low Her2 expressing cells (Jimt-1). Increasing the DAR correlates with an improved *in vitro* activity.



IC50 (nM)	SK-BR-3	JIMT-1	MDA-MB-231
T-DM1	0,155	30,59	92,39
DM1 DAR8	0,071	4,455	22,28
AF DAR8	0,069	0,179	121,9



IC50 (nM)	SK-BR-3	JIMT-1	NCI-H520
T-DM1	0,048	8,632	57,860
DM1 DAR8	0,013	1,404	99,770
DM1 DAR16	0,005	0,138	75,470

Figure 2: In vitro cytotoxic activity. Cell lines were incubated with serial dilution of Trastuzumab Multilink™ (T-Multilink™) ADCs, as indicated, or T-DM1 and cytotoxicity was measured after 72h using CellTiter-Glo. The graphs represent the percent of viability and IC50 are indicated in the tables. (A) SK-BR3, Jimt-1 and MDA-MB-231 cell lines were used (B) SK-BR3, Jimt-1 and NCI-H520 were used

Percentage of aggregation

Aggregation propensity of ADCs with hydrophobic payloads is a key issue for ADC therapeutic applications. Here we show low aggregation even at high DAR.

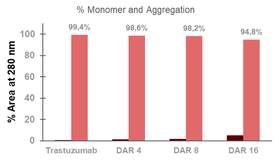


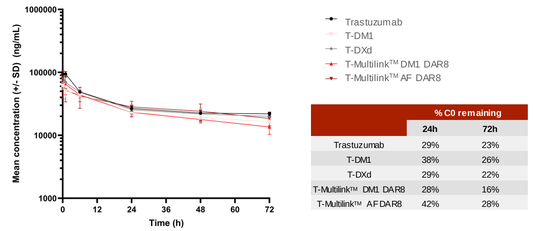
Figure 3 : Aggregation level. T-Mutlilink™ ADCs were prepared with DM1 at different DAR. Aggregation level was tested by Size Exclusion Chromatography analysis

In vitro stability in serum

Figure 4: In vitro serum stability: T-Mutilink™ ADC DM1 DAR8 was tested, along with control ADC (control Vedotin on Trastuzumab T-VC-PABC-MMAE DAR4) in (A) human and (B) mouse IgG depleted sera. ADCs were incubated in serum for up to 96h and the average DAR was determined by HIC analysis.

PK profile

T-Multilink™ ADCs pharmacokinetic properties were tested in Swiss mice. T-Multilink™ PK profiles-are comparable to the naked antibody Trastuzumab as well as T-DM1 and T-DXd.



	% C0 remaining	
	24h	72h
Trastuzumab	29%	23%
T-DM1	38%	26%
T-DXd	29%	22%
T-Multilink™ DM1 DAR8	28%	16%
T-Multilink™ AF DAR8	42%	28%

Figure 5: Pharmacokinetics. Three animals per group were injected intravenously with 5mg/kg of indicated compounds. Plasma was collected after 5min, 1h, 6h, 24h, 48h and 72h. The percentage of concentration as compared to concentration at time 0 (% C0) was calculated for each compound at 24h and 72h.

In vivo efficacy in Her2 low breast cancer model

Her2 positive breast cancers with a low Her2 expression are difficult to treat. Here we used the Jimt-1 breast cancer xenograft model with a moderate Her2 expression to test activity of the different ADCs. T-Multilink™ ADC with DM1 at DAR8 show good activity, slightly better than T-DXd, while T-Multilink™ ADC with AF at DAR8 showed the best efficacy, inducing complete regressions in all mice. All treatments were well tolerated.

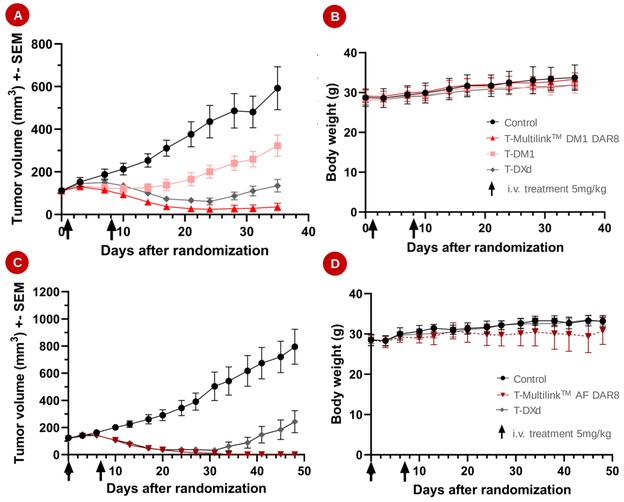


Figure 6 : In vivo efficacy: T-Multilink™ ADCs with DM1 or AF were tested in the Jimt-1 breast cancer xenograft model. (A) T-Multilink™ DM1 DAR8 shows 78% tumor regression with 3/8 mice have transient complete regression (CR), while T-DXd treatment induced a regression of the tumor of 45% and 1/8 transient CR (B) Relative mean body weight during treatment of (A) (C) T-Multilink™ AF DAR8 shows a complete regression up to day 48 post 1st treatment with 8/8 mice showing CR, while T-DXd treated mice showed 72% regression and 3/8 mice had transient CR. (D) Relative mean body weight during treatment of (C)

CONCLUSIONS

- Trastuzumab based Multilink™ ADCs were successfully generated with DM1 and Auristatin F as payloads, and are active in vitro
- An increase of drug to antibody ratio (DAR) was shown to improve activity in vitro
- The compounds display low aggregation
- The pharmacokinetic profile of Trastuzumab Multilink™ ADCs were not altered as compared to naked Trastuzumab and the compounds are stable in serum
- In vivo, both DM1-based and AF-based Trastuzumab Multilink™ DAR8 ADCs were very potent, and showed improved activity as compared to newly approved T-DXd.
- Overall, we show that the Mutlilink technology allows generation of very potent ADCs with favorable drug-like properties

REFERENCES

(1) Workman et al., British Journal of Cancer (2010) 102, 1555 – 1577

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