Development of alphalex™-toxin low pH targeting conjugates for the treatment of solid tumors

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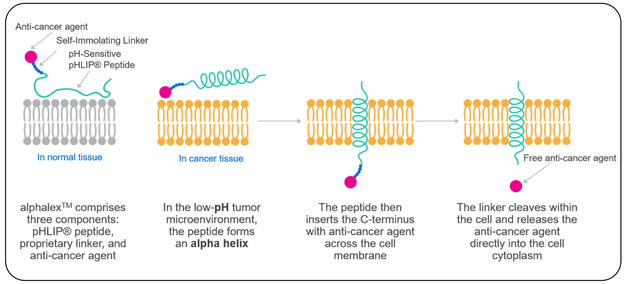
ABSTRACT

Maytansines are high potency microtubule targeting compounds that have an extremely narrow therapeutic window. Unacceptable dose limiting systemic toxicity has limited the therapeutic potential of these potent anti-oncogenic compounds. Targeting maytansines to the tumor is the only current feasible method of reaching the clinical potential of such toxic molecules. To date trastuzumab-DM1 (Kadcyla®) remains the only approved antibody-maytansinoid conjugate on the market. Most preclinical maytansinoid conjugates to date face the same issues encountered by Kadcyla® – tumor restriction by target antigen and the potential for off target release of payload.

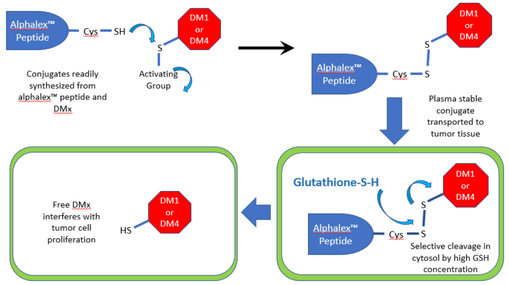
alphalex™ is a tumor targeting technology consisting of a unique variant of a pH-Low Insertion Peptide (pHLIP®; References 1-3), a cleavable small molecule linker and an anti-cancer agent warhead. alphalex™ thereby allows for antigen independent targeting of the tumor and enables intracellular delivery of the warhead by leveraging the low pH microenvironment of the tumor, a universal feature common to all tumors due to the Warburg effect.

Here we demonstrate the ability to conjugate the maytansinoids DM1 and DM4 to alphalex™ via both direct and linker-mediated conjugation.

alphalex™ Enables Antigen-Independent Tumor Targeting



alphalex™ – DMx Design and Enablement



The alphalex™ peptide is designed to allow easy, chemoselective derivatization by inclusion of cysteine at the C-terminus of the peptide. Maytansine derivatives DM4 and DM1 are covalently conjugated via a dithiane bond to the alphalex™ peptide by thiol activation of either species and reaction with the complimentary thiol. DM1, DM4 and the alphalex™ offer different steric and electronic environments close to the dithiane bond that affords differing cleavage rates for the cytotoxic payload release when exposed to the glutathione-rich environment of the tumor cell cytosol.

Characterization of Tubulin Binding by alphalex™ – DM4 (CBX-13)

Figure 1. Analysis of the effect of unconjugated DM4 and CBX-13 on in vitro tubulin polymerization. CBX-13 inhibits in vitro tubulin polymerization similarly to unconjugated DM4.

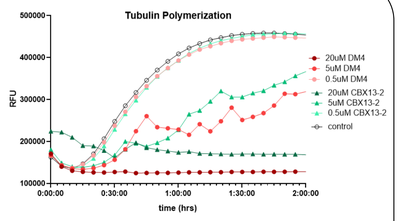
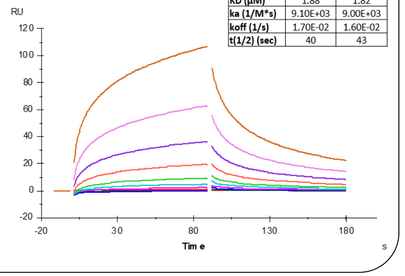


Figure 2. Kinetic analysis of CBX-13 binding to β-tubulin in vitro as determined by via Biacore surface plasmon resonance. CBX-13 is able to bind to β-tubulin with a similar KD as unconjugated DM4 (3.55uM) and slower on/off rates relative to unconjugated DM4.

	DM4 Conjugate	
	N=1	N=2
KD (μM)	1.88	1.82
ka (1/M*s)	9.10E+03	9.00E+03
koff (1/s)	1.70E-02	1.60E-02
t(1/2) (sec)	40	43



alphalex™ – DM4 (CBX-13) Induces Durable and Potent Anti-Tumor Activity

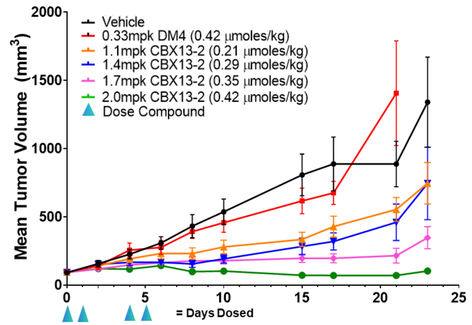


Figure 3. Efficacy of dosing either unconjugated DM4 or a dose response of CBX-13 in nude mice bearing HCT116 colorectal cancer flank tumors. Animals were dosed QDx4 with a two day interval between the 2nd and 3rd doses. Data are expressed as means ± SEM.

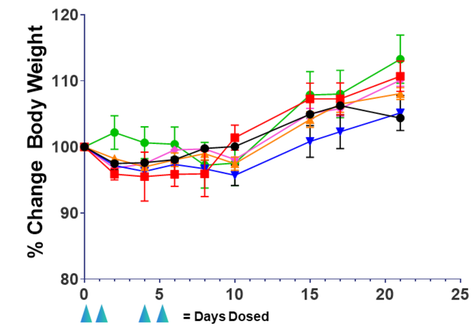


Figure 4. Percentage change in body weight relative to day 0 from the DM4 efficacy study in Figure 3. Data are expressed as means ± SEM.

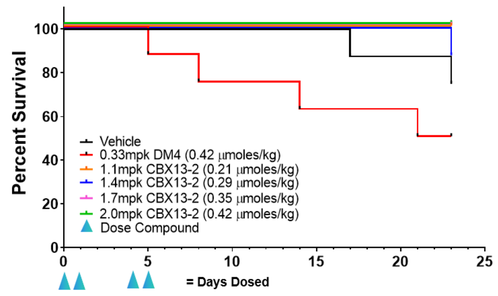


Figure 5. Kaplan-Meier plot of surviving animals from the DM4 efficacy study in Figure 3. Animals were removed from the study due to either death, tumor size exceeding 2000mm³ or due to loss of >20% body weight. Unconjugated DM4 induced the spontaneous death of half of the DM4 group of animals during the post-dosing period.

alphalex™ – DM4 (CBX-13) Inhibits Lung Metastases

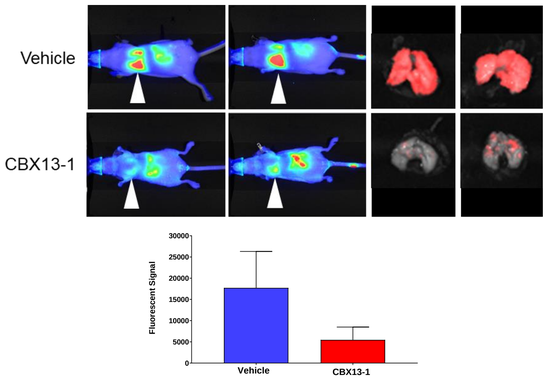


Figure 6. Top: Ventral view and extracted lungs of nude mice inoculated with 4T1-RFP fluorescent TNBC cells via tail vein injection and imaged with the Pearl Trilogy analyzer 11 days after inoculation and after 3 doses of CBX-13. Arrows indicate lung metastases. Bottom: Quantification of fluorescent signal from extracted lungs of 4T1-RFP inoculated mice after 3 doses of CBX-13.

CONCLUSIONS

- **We have demonstrated the ability to conjugate maytansinoids to our alphalex™ platform and demonstrated their exquisitely potent and long-lasting anti-tumor activity in HER2-negative xenograft models that would otherwise be un-targetable by competing therapies.**
- **We have demonstrated that CBX-13 safely delivers amounts of maytansinoid *in vivo* that otherwise result in systemic toxicity and death when dosed as unconjugated warhead.**
- **Based on the SAR of this first generation of maytansinoid conjugates we are further optimizing our alphalex™ – maytansinoid conjugation strategy with the goal of moving forward with IND-enabling studies in the near future.**

REFERENCES

[1] Rather than targeting a specific antigen, alphalex™ includes a pHLIP® peptide. pHLIP® peptides are a family of pH-Low Insertion Peptides that target acidic cell surfaces. pHLIP® was developed at Yale University and the University of Rhode Island, and is exclusively licensed to pHLIP, Inc

[2]Wyatt LC, Lewis JS, Andreev OA, Reshetnyak YK, Engleman DM. Applications of pHLIP Technology for Cancer Imaging and Therapy. Trends Biotechnol. 2017. Jul; 35(7):653-664.

[3]Wyatt LC, Moshnikova A, Crawford T, Engleman DM, Andreev OA, Reshetnyak YK. Peptides of pHLIP family for targeted intracellular and extracellular delivery of cargo molecules to tumors. Proc Natl Acad Sci USA. 2018 Mar 20;115(12):E2811-2818.