Development of an alphalex™-auristatin low pH targeting conjugate for the treatment of solid tumors

#1764

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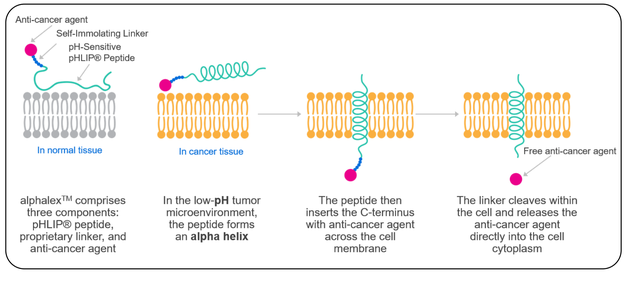
Abstract

Auristatins such as monomethyl auristatin E (MMAE) are a class of high potency microtubule targeting compounds that have an extremely narrow therapeutic window. Targeting potent auristatins to the tumor is the only feasible method of unlocking the clinical potential of such toxic molecules. While there are currently four marketed ADCs featuring auristatins, these ADCs face the same fundamental issues of tumor restriction by target antigen and the potential for off target release of payload.

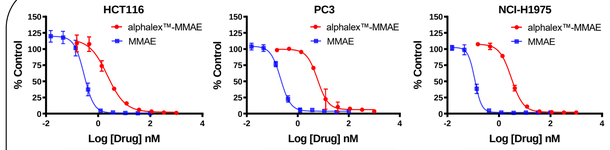
alphalex™ is a tumor targeting technology consisting of a unique variant of a family of pH-Low Insertion Peptides (pHLIP®) that target acidic cell surfaces (references 1-2), a cleavable self-immolating linker, and an anti-cancer agent warhead. This technology allows for antigen-independent targeting 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 report the preclinical efficacy, safety, and antigen-independent tumor-targeting properties of alphalex™ conjugated to MMAE. We demonstrate the ability of alphalex™-MMAE to display potent *in vitro* and *in vivo* efficacy in colorectal, non-small cell lung, and prostate carcinoma lines. We further show that alphalex™-MMAE efficiently and safely delivers efficacious levels of MMAE selectively to tumor and demonstrates extreme plasma stability, with 0.05% warhead release over 24h in the rat. Based on the excellent preclinical safety and efficacy profile of alphalex™-MMAE, Cybrexa will move forward with the goal of initiating IND-enabling studies in 2022.

alphalex™ Enables Antigen-Independent Tumor Targeting



Potent *In Vitro* Activity of alphalex™-MMAE in Multiple Tumor Types



	IC50
alphalex-MMAE	2.278
MMAE	0.2882

	IC50
alphalex-MMAE	5.950
MMAE	0.2123

	IC50
alphalex-MMAE	3.041
MMAE	0.1163

Figure 1. Growth delay of HCT116 colorectal, PC3 prostate, and NCI-H1975 NSCLC cell lines *in vitro* after four-day incubation with the indicated concentrations of alphalex™-MMAE or unconjugated MMAE.

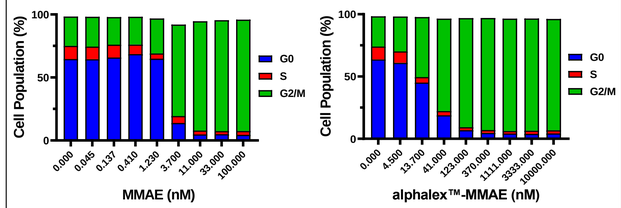


Figure 2. Cell cycle analysis of HCT116 colorectal cells *in vitro* after 24-hour incubation with MMAE warhead (left) or alphalex™-MMAE (right). Cells display dose responsive accumulation in G2/M, with an IC50 of 2.6nM (MMAE) or 19.6nM (alphalex™-MMAE).

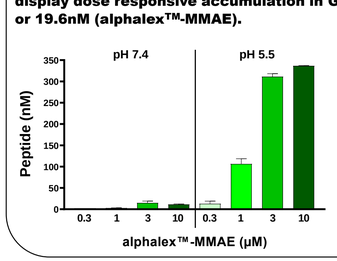


Figure 3. Levels of peptide present in HCT116 cells as assessed by ELISA after 1-hour incubation with the indicated concentrations of alphalex™-MMAE in either neutral or pH 5.5 cell culture media.

alphalex™-MMAE Displays Specific Release of MMAE Payload in Tumor

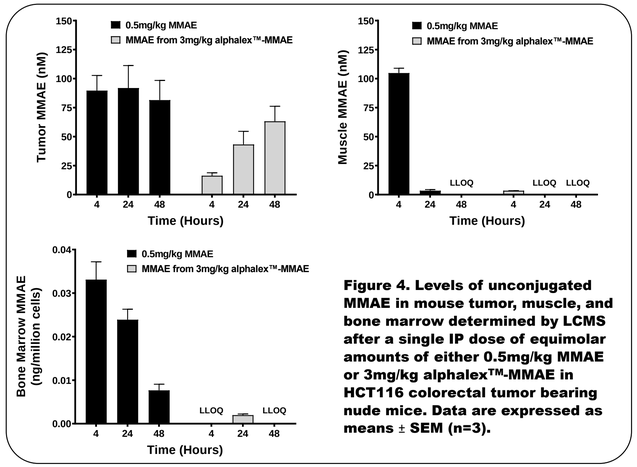


Figure 4. Levels of unconjugated MMAE in mouse tumor, muscle, and bone marrow determined by LCMS after a single IP dose of equimolar amounts of either 0.5mg/kg MMAE or 3mg/kg alphalex™-MMAE in HCT116 colorectal tumor bearing nude mice. Data are expressed as means ± SEM (n=3).

alphalex™-MMAE Is Efficacious With Minimal Dosing Paradigms

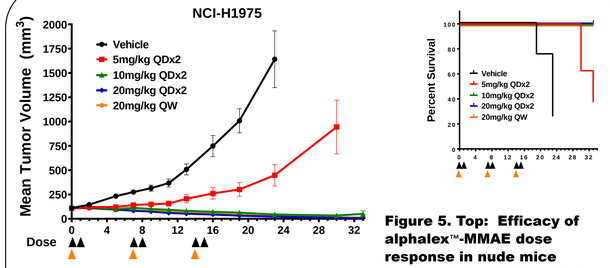


Figure 5. Top: Efficacy of alphalex™-MMAE dose response in nude mice bearing NCI-H1975 NSCLC flank tumors. Animals were dosed IP either QDx2/week or QW for three weeks. Data are expressed as means ± SEM (n=8). Inset displays Kaplan Meier survival curve.

Bottom: Changes in body weight of nude mice on study. Data are expressed as means ± SEM (n=8).

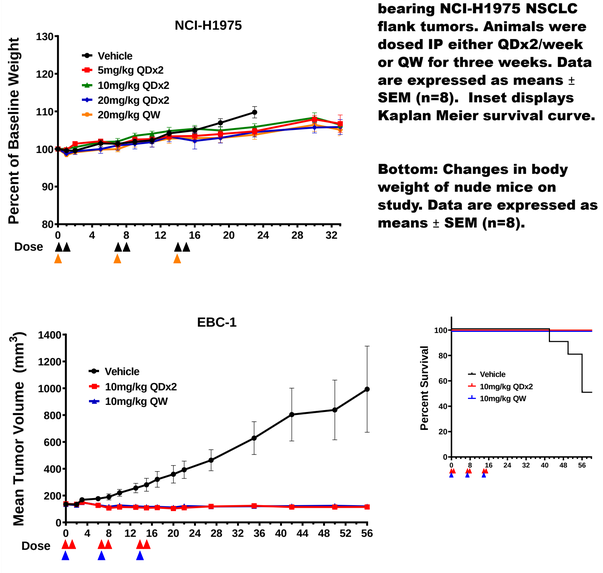


Figure 6. Efficacy of dosing 10mg/kg alphalex™-MMAE with different dosing paradigms in nude mice bearing EBC-1 NSCLC flank tumors. Animals were dosed IP either QDx2/week or QW for three weeks. Data are expressed as means ± SEM (n=10). Inset displays Kaplan Meier survival curve.

alphalex™-MMAE Displays Superior *In Vivo* Plasma Stability

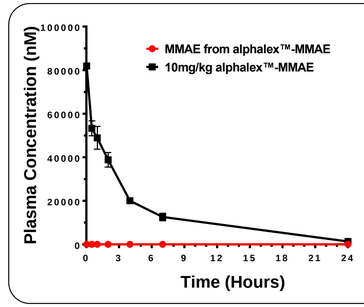


Figure 7. Levels of intact conjugate (black) and unconjugated MMAE (red) in plasma after a single bolus 10mg/kg IV dose of alphalex™-MMAE in the rat. alphalex™-MMAE displays release of just 0.5% warhead in circulation over 24-hours in the rat. Data are expressed as means ± SEM (n=3).

Durable and Potent *In Vivo* Anti-Tumor Activity in Multiple Tumor Types

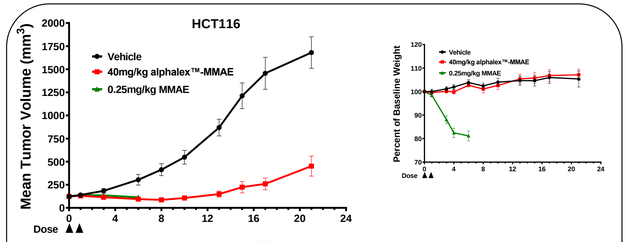


Figure 8. Efficacy of alphalex™-MMAE versus unconjugated MMAE warhead in nude mice bearing HCT116 colorectal flank tumors. Animals were dosed IP QDx2. Data are expressed as means ± SEM (n=5). Inset displays changes in body weight.

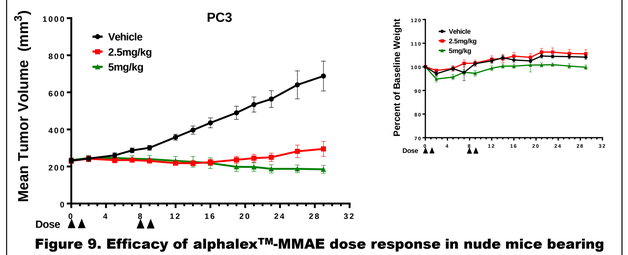


Figure 9. Efficacy of alphalex™-MMAE dose response in nude mice bearing PC3 prostate flank tumors. Animals were dosed IP QDx2/week for 2 weeks. Data are expressed as means ± SEM (n=8). Inset displays changes in body weight.

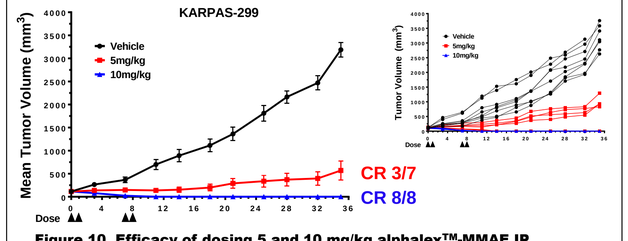


Figure 10. Efficacy of dosing 5 and 10 mg/kg alphalex™-MMAE IP QDx2/week for two weeks in SCID mice bearing KARPAS-299 Non-Hodgkin's lymphoma flank tumors. Data are expressed as means ± SEM (n=8). Inset displays spider plots of change in tumor volume. CR=complete response

Conclusions

- **alphalex™-MMAE displays exquisite plasma stability and tumor targeting ability *in vivo*.**
- **alphalex™-MMAE is safe and displays potent, antigen independent anti-tumor activity in multiple preclinical models.**
- **Cybrexa plans to rapidly move forward with the clinical development of alphalex™-MMAE.**

References

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

2. 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. 115(12):E2811-2818.