Characterization of Antigen Agnostic Tumor Selective Delivery and Immunomodulatory Activities of Auristatin and Maytansinoid alphalex™ Peptide-Drug Conjugates

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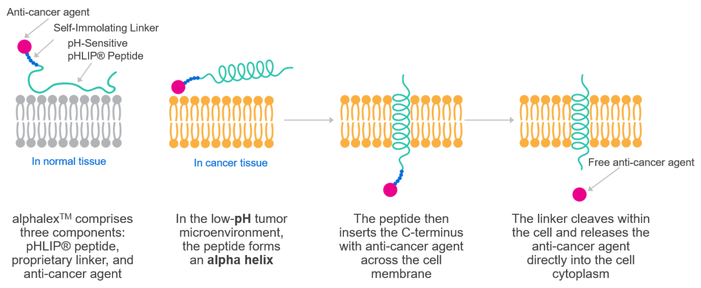
ABSTRACT

The alphalex™ series of tumor targeting, antigen agnostic peptide-drug conjugates was developed to address the gaps left by antibody drug conjugates (ADCs). The alphalex™ is based on a unique variant of the pH Low Insertion Peptide (pHLIP®)[1-3]. Unlike an ADC, pH-based targeting by the alphalex™ platform allows for universal targeting of solid tumors and avoids the pitfalls of antigen-based restrictions and toxicities, leading to safer delivery of cytotoxics.



We characterized the efficacy, safety, and immunomodulatory activities of a series of microtubule-targeting alphalex™ conjugates bearing auristatin and maytansinoid payloads in the HCT116 colorectal, B16-F10 melanoma, and Renca renal mouse tumor models and in the rat 13762 syngeneic breast tumor model. We additionally characterized the impact of conjugate treatment on T- and B-cell recognition of tumor *in vivo* and *ex vivo*.

Conjugates bearing microtubule inhibiting payloads produced complete tumor suppression in the HCT116 human colorectal model as single agent and demonstrated synergy with doxorubicin and anti-PD-L1 in the B16-F10 melanoma lung metastasis model and Renca syngeneic kidney flank model, respectively. Activity was further extended to the rat 13762 syngeneic breast flank model. *Ex vivo* characterization demonstrated both tumor specific delivery of the payload as well as immunomodulatory activities to induce anti-tumor immune recognition through immunogenic cell death, resulting in rejection of tumor rechallenge and splenocyte release of IFNɣ, IL-2, and tumor-binding IgG in response to exposure to tumor cells.

CONCLUSIONS

- These results demonstrate that the alphalex™ safely delivered efficacious levels of microtubule inhibiting payloads in a tumor-selective manner to a variety of HER2 null models.
- Selective delivery to tumor and avoidance of delivery to healthy immune cells led to immunogenic cell death of the tumor and subsequent Th1 and B-cell anti-tumor immune responses.
- Antigen agnostic delivery of auristatin and maytansinoid payloads is poised to potentially serve as second line therapy as single agent or in combination with immunotherapy post treatment with a TOP1 inhibitor.

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.

DISCLOSURES AND CONTACTS

- S. Gayle, Q. Zhang, C. Hagen, T. Paradis, L. Tylaska and V. Paralkar are employees of Cybrexa Therapeutics
- For additional information contact sophia.gayle@cybrexa.com or vishwas.paralkar@cybrexa.com.

RESULTS

Stable Conjugate Allows for Specific Tumor Targeting

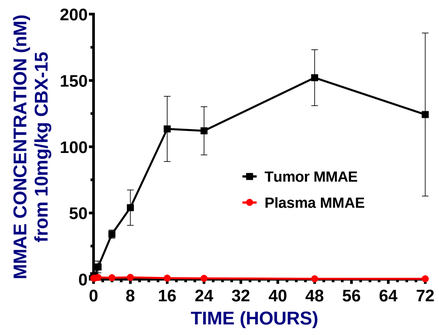


Figure 1. LCMS quantitation of released MMAE in tumor and plasma of HCT116 flank tumor-bearing nude mice after a single i.p. dose of 10 mg/kg CBX-15 (n=3).

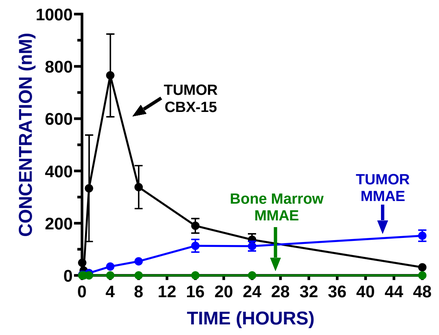


Figure 2. LCMS quantitation of CBX-15 conjugate in tumor and released MMAE in tumor and bone marrow of HCT116 flank tumor-bearing nude mice after a single i.p. dose of 10 mg/kg CBX-15 (n=3).

alphalex™ – MMAE (CBX-15) Induces Durable and Potent Anti-Tumor Activity

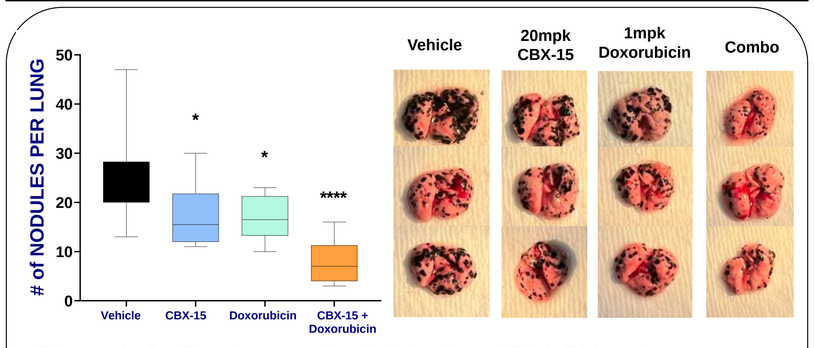


Figure 3. On Day 1 post tail vein injection of B16-F10 melanoma cells, C57BL/6J mice (n=8) were dosed with 20 mg/kg CBX-15 i.p. QDx2/week x two weeks and/or a single dose of 1mg/kg doxorubicin i.p. On Day 14, mice were sacrificed and melanoma lung nodules and surface area assessed from pictures of cleaned lungs using ImageJ software. Image shows representative samples of lungs bearing black B16-F10 metastases. Box and whiskers plot depicts quantitation of manually counted lung metastases. *p<0.5 ****p<0.0001

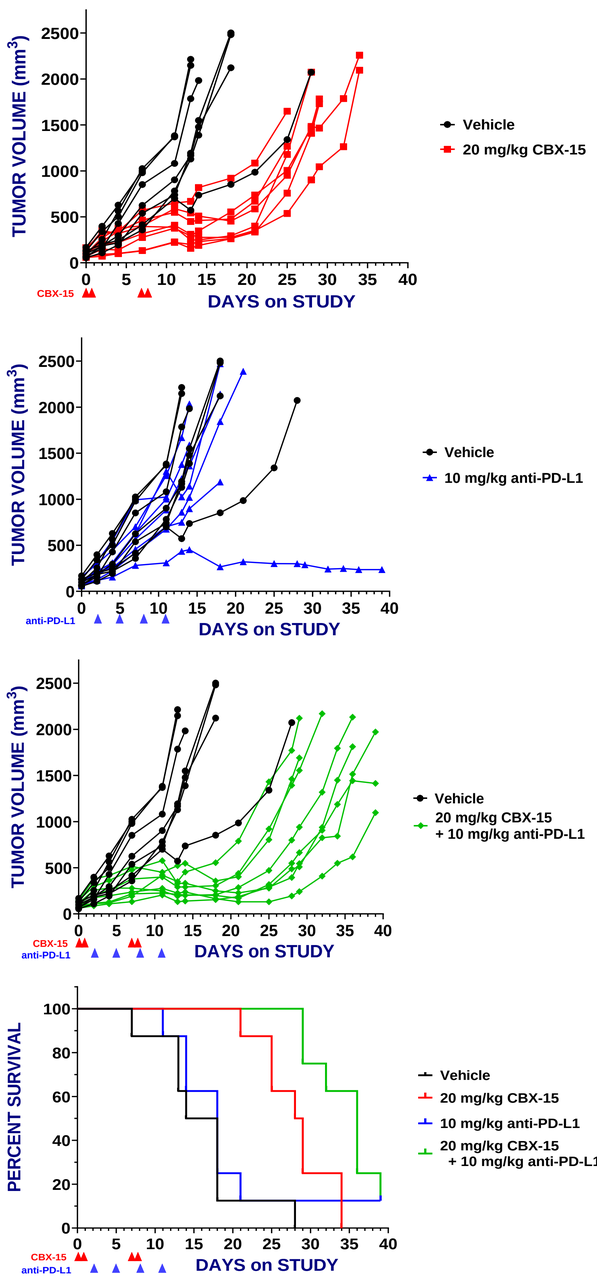


Figure 4. Efficacy and survival of twice weekly i.p. doses of 20 mg/kg CBX-15 and 10 mg/kg anti-PD-L1 for a period of two weeks in Balb/c mice bearing Renca syngeneic kidney flank tumors (n=8).

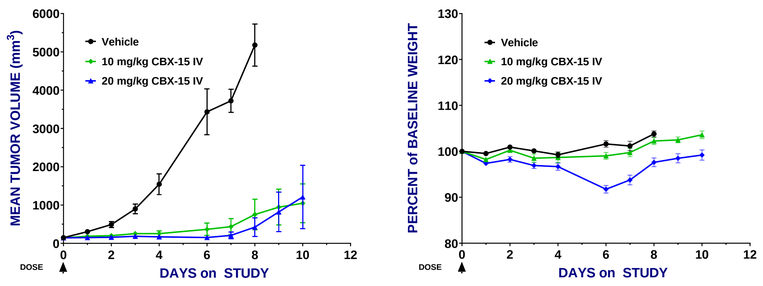


Figure 5. Efficacy and safety of a single i.v. bolus dose of CBX-15 in Fischer rats bearing 13762 syngeneic breast flank tumors (n=6).

alphalex™ – DM4 Inhibits Microtubule Polymerization and Tumor Growth

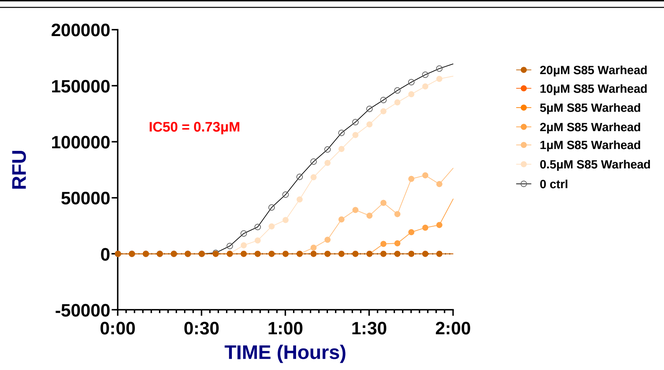


Figure 6. *In vitro* inhibition of microtubule polymerization by S85 DM4 warhead as assessed by incorporation of fluorescent reporter into microtubules during polymerization in the presence of drug.

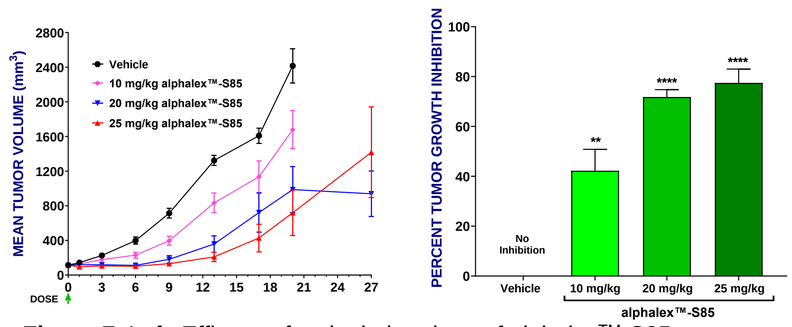


Figure 7. Left: Efficacy of a single i.p. dose of alphalex™-S85 conjugate in nude mice bearing HCT116 xenografts (n=8).
Right: Quantitation of maximal tumor growth inhibition from single dose of alphalex™-S85 (n=8).

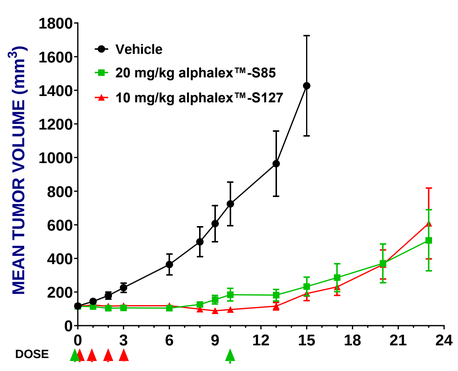


Figure 8. Efficacy of alphalex™-S85 dosed i.p. on Days 0 and 10 relative to efficacy of alphalex™-S127 dosed i.p. QDx4 in nude mice bearing HCT116 xenografts (n=8).

Anti-Tumor Immunomodulatory Activities in the Rat Syngeneic Model

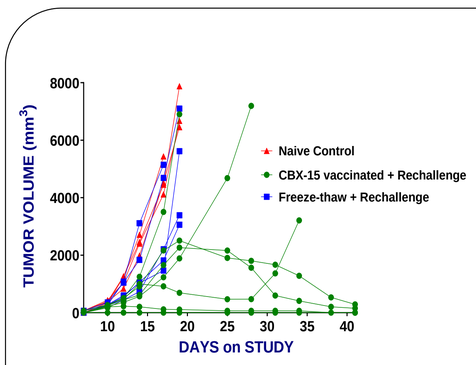


Figure 9. Live tumor cell challenge of rats vaccinated with 13762 cells killed *in vitro* by CBX-15 treatment (n=8) or by freeze thawing (n=5). Rats were injected with killed cells on the left flank twice, with two weeks between each injection. Seven days after the last vaccination, rats were injected with live cells on the opposite flank, including a naïve control group (n=4).

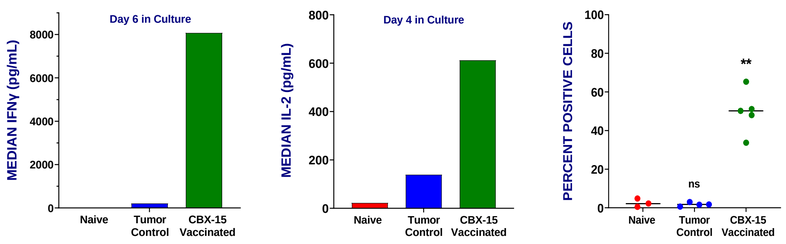


Figure 10. Left and middle: Splenocyte single cell suspensions generated 40 days post live tumor rechallenge were tested for T-cell cytokine production by ELISA after co-culture with mitotically inactivated 13762 cells. Right: Plasma from vaccinated rats was co-incubated with live 13762 tumor cells and cells assessed for rat IgG binding by FACS. **p<0.01