Evaluation of antigen-agnostic anti-tumor activity and immunological memory induced by CBX-15 (alphalex™-MMAE) in the rat syngeneic breast cancer model

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Abstract

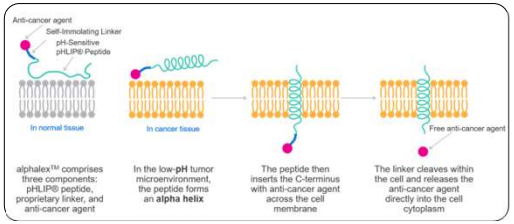
CBX-15 is a peptide-drug conjugate consisting of alphalex™–monomethyl auristatin E (MMAE). The alphalex™ is a unique variant of pH-Low Insertion Peptide[1] (pHLIP®) designed to target the low pH microenvironment of cancer cells, a universal feature of all rapidly growing tumors. The peptide of CBX-15 forms an alpha helix only in low pH conditions, resulting in unidirectional insertion of the peptide and delivery of MMAE across the cancer cell membrane, and avoidance of delivery to healthy tissues, including immune cells.

Efficacy and anti-tumor immunological memory induced by CBX-15 was evaluated in Fischer 344 rats bearing syngeneic 13762 mammary adenocarcinoma tumors. The development of anti-tumor immunological memory was examined in CBX-15-cured animals by *in vivo/ ex vivo* rechallenge with live tumor cells and subsequent assessment of tumor rejection, cytokine release by T-cells, tumor immune cell infiltration, and memory T-cell composition of bone marrow.

CBX-15 rapidly regressed rat tumors, resulting in complete responses while sparing healthy tissues such as bone marrow. Cured rats rejected live tumor rechallenge and exhibited a doubling of bone marrow-resident CD4 T-cells 58 days post-dose. Splenocytes and lymph node suspensions derived from cured rats demonstrated formation of a Th1-mediated IFNγ response when exposed *ex vivo* to tumor cells. The ability of CBX-15 to induce immunogenic cell death was established by vaccinating syngeneic animals with CBX-15 treated tumor cells and subsequent tumor challenge, which demonstrated anti-tumor immunity induced by CBX-15.

These preclinical data demonstrate the anti-tumor efficacy of CBX-15 in the rat as well as the ability of CBX-15 to enhance tumor immunogenicity leading to tumor immune memory through immunogenic cell death by utilizing a universal pH-based tumor targeting mechanism.

alphalex™ Enables Antigen-Independent Tumor Targeting



Single Agent Efficacy of CBX-15 in the Rat

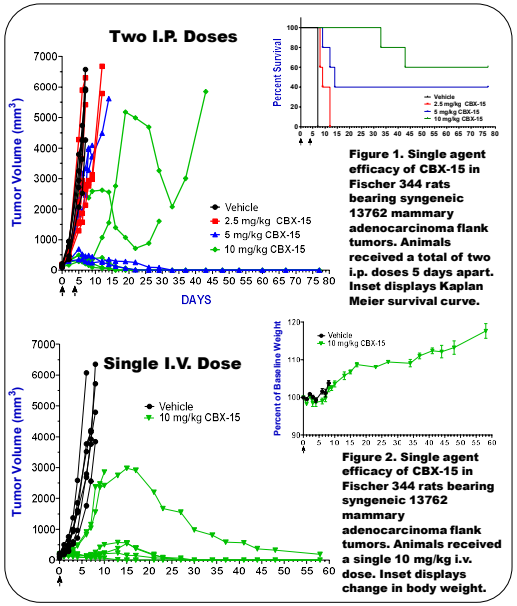


Figure 1. Single agent efficacy of CBX-15 in Fischer 344 rats bearing syngeneic 13762 mammary adenocarcinoma flank tumors. Animals received a total of two i.p. doses 5 days apart. Inset displays Kaplan Meier survival curve.

Figure 2. Single agent efficacy of CBX-15 in Fischer 344 rats bearing syngeneic 13762 mammary adenocarcinoma flank tumors. Animals received a single 10 mg/kg i.v. dose. Inset displays change in body weight.

CBX-15 Enhancement of Tumor Immunogenicity

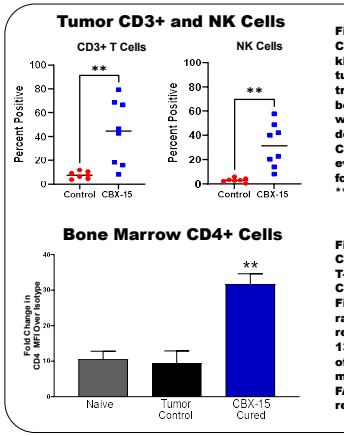


Figure 3. Infiltration of CD3+ T-cells and natural killer cells into 13762 tumors after CBX-15 treatment. Fischer 344 rats bearing 13762 flank tumors were given a 20 mg/kg i.p. dose of vehicle (n=7) or CBX-15 (n=8). Tumors were evaluated *ex vivo* by FACs four days post dose. **p<0.01

Figure 4. Assessment of CD4+ bone marrow resident T-cells in CBX-15 cured rats. Cured rats from the study in Figure 2 (n=5) and control rats (n=5) were rechallenged with live 13762 tumor cells on day 48 of the study, and bone marrow was assessed by FACS 10 days post rechallenge. **p<0.01

Induction of Anti-Tumor Immunological Memory After CBX-15 Induced Cure

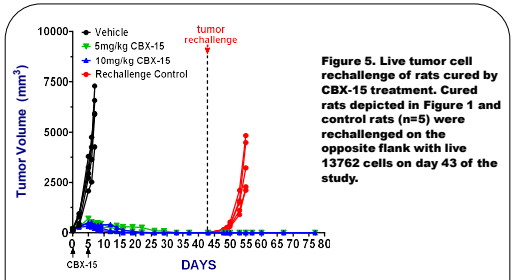


Figure 5. Live tumor cell rechallenge of rats cured by CBX-15 treatment. Cured rats depicted in Figure 1 and control rats (n=5) were rechallenged on the opposite flank with live 13762 cells on day 43 of the study.

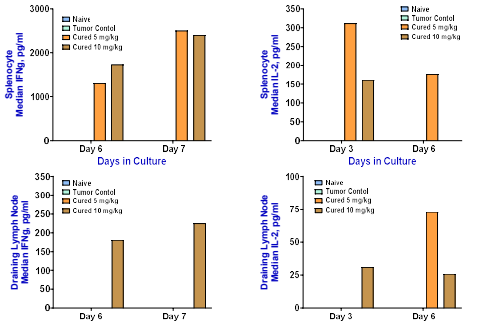


Figure 6. Induction of Th1 cytokines released from splenocytes and lymph nodes of cured rats upon *ex vivo* exposure to 13762 tumor cells (n=5 rats per group). CBX-15 cured rats were rechallenged 43 days after the start of CBX-15 therapy and immune organs were harvested 35 days post rechallenge. Splenocyte and draining lymph node single cells suspensions were tested for cytokine production by ELISA after co-culture with mitotically inactivated 13762 cells.

Rescue of Tumor-Induced Suppression of Dendritic Cell Maturation

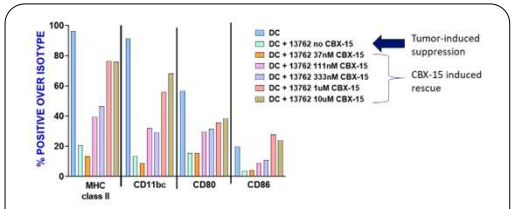


Figure 7. Assessment of the effect of 13762 co-culture on bone marrow derived DC maturation. 13762 cells were treated with a dose response of CBX-15 for 48-hours and washed. DCs were differentiated with GM-CSF and IL4 for 9 days and incubated with washed 13762 cells for 24-hours. Markers of DC differentiation specifically on DC cells were assessed by FACS.

Induction of Anti-Tumor Immunological Memory After CBX-15 Vaccination

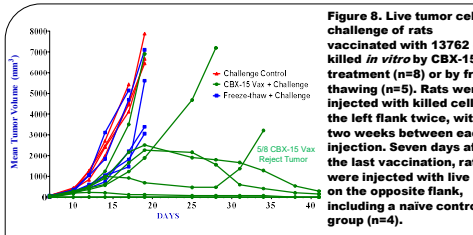


Figure 8. Live tumor cell challenge of rats vaccinated with 13762 cells killed *in vitro* by CBX-15 treatment (n=8) or by freeze thawing (n=5). Rats were injected with killed cells on the left flank twice, with two weeks between each injection. Seven days after the last vaccination, rats were injected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

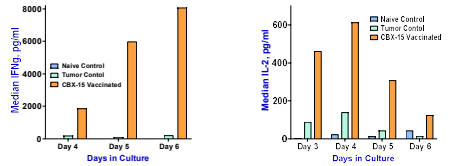


Figure 9. Induction of a Th1 response in splenocytes isolated from tumor protected vaccinated and control rats upon *ex vivo* exposure to 13762 tumor cells. The tumor vaccinated animals demonstrated strong Th1 cytokine immune memory response 40 days post live tumor challenge Splenocyte single cell suspensions were tested for cytokine production by ELISA after co-culture with mitotically inactivated 13762 cells.

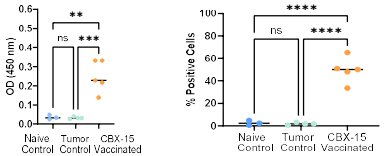


Figure 10. Quantitation of tumor cell-binding circulating IgG in plasma of vaccination-cured rats and naive and tumor-bearing controls. Left: Plasma from vaccinated rats on day 40 after the live tumor challenge was incubated on plates coated with 13762 tumor cell lysate and probed for binding of anti-rat IgG. 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 ***p<0.001 ****p<0.0001

Conclusions

- **CBX-15 is safe and highly efficacious in the rat 13762 triple negative breast cancer model as an i.p. or i.v. dose.**
- **CBX-15 treatment induces immune recognition of the tumor and long-term anti-tumor immunological memory as evidenced by TIL infiltration after treatment, long term enhancement of bone marrow resident T cells, and *ex vivo* recognition of tumor cells by T-cells of rats cured by either vaccination by CBX-15-killed cells or by direct CBX-15 treatment.**
- **Cybrexa plans to rapidly move forward with the clinical development of CBX-15.**

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.