The oncolytic peptide LTX-315 enhances T cell clonality and induces synergy with CTLA-4 blockade

REKDAL Ø.[1], CAMILIO K.[1], YUSKO E.[2], VIGNALI M.[2], SANDERS C.[2], BENZENO S.[2], NESTVOLD J.[3], SAUNDERS A.[1], YAMAZAKI T.[4], ZITVOGEL L.[4], SVEINBJØRNSSON B.[1,5]

[1]Lytix Biopharma, Oslo, Norway, [2]Adaptive Biotech, Seattle, United States, [3]University of Oslo, Oslo, Norway, [4]Gustave Roussy Cancer Campus, Villejuif, France, [5]University of Tromsø, Tromsø, Norway

Lytix Biopharma

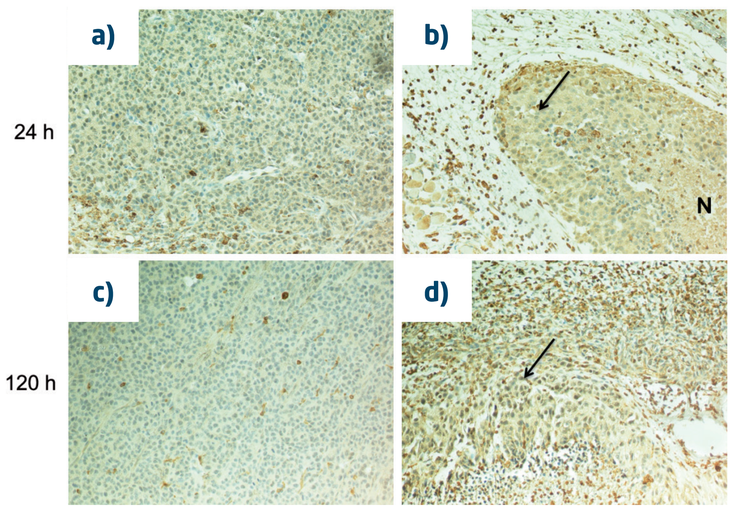
Background

- LTX-315 induces complete regression and adaptive tumor-specific immune responses in several rodent tumor models [1]
- In some animal models LTX-315 has demonstrated abscopal effects, i.e. effects on non-treated lesions.
- LTX-315 targets and disintegrates the mitochondrial membrane and subsequently other cytoplasmic membranes resulting in the release of DAMPs (Damage-Associated Molecular Pattern molecules) such as ATP, cytochrome C and HMGB1 [1-5].
- Multi-domain proteins from the BCL-2 family seem to be partially involved in LTX-315-mediated killing [5].
- The membranolytic activity of LTX-315 is expected to induce release of a broad spectra of tumor antigens.

Aim

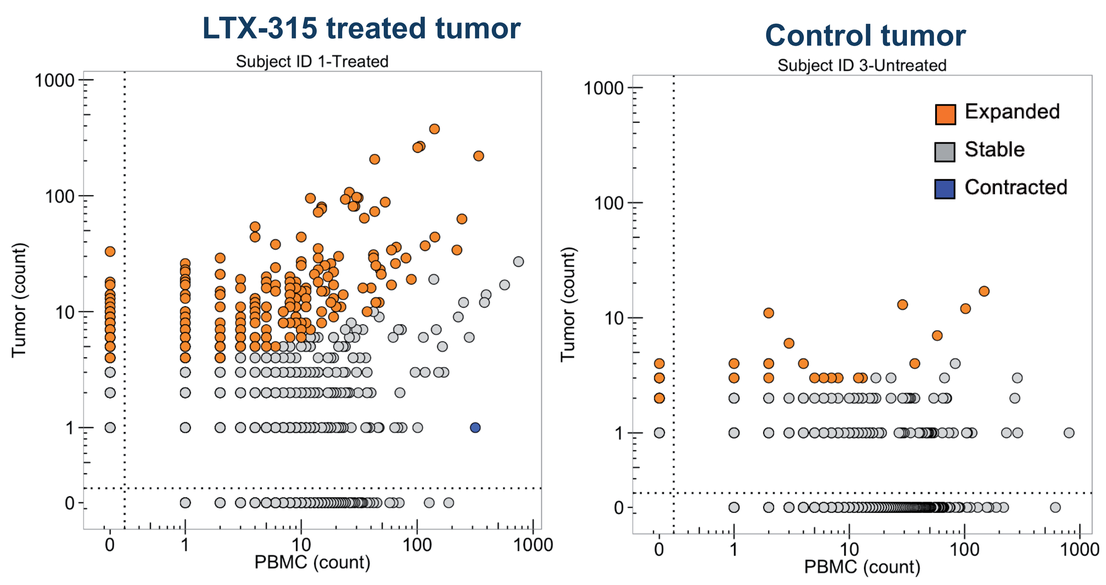
- Investigate whether LTX-315 enhances T-cell inflammation in the tumor microenvironment and thereby expands the proportion of responders to checkpoint inhibitors.

Fig. 2 LTX-315 induced T-cell infiltration in treated B16 melanomas



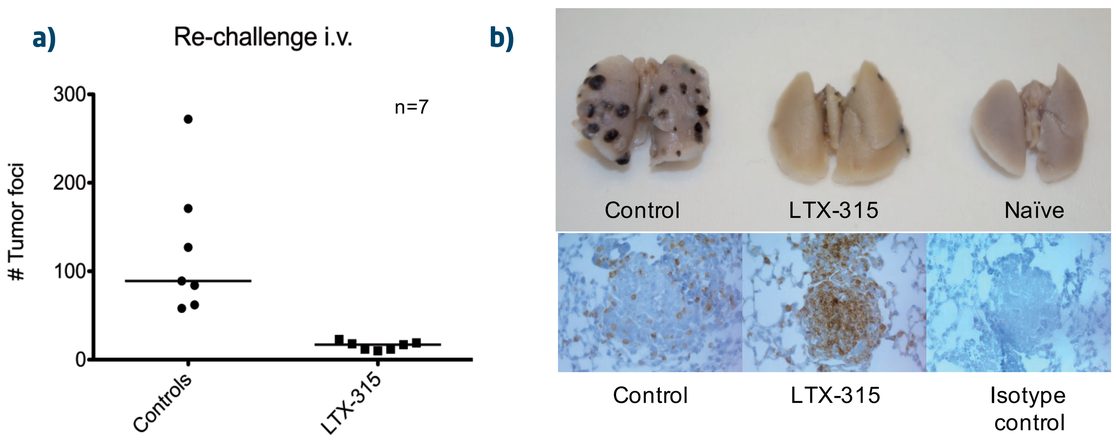
B16 tumors were surgically excised 24h and 120h post-injection with vehicle (**a** and **c**) or LTX-315 (**b** and **d**). Tumors injected with LTX-315 exhibited tumor tissue necrosis **(N)**. Immunolabeling with anti-CD3 showed many of the infiltrating immune cells to be CD3+ T cells (**b** and **d**), compared to low- or non-infiltrated control tumors (**a** and **c**).

Fig. 3 LTX-315 increases the T-cell clonality



Adaptive's TCR sequencing platform (immunoSEQ) was used to characterize mmTCRB chains in 10 tissue samples and 10 blood samples from mice that was treated i.t. with either saline or LTX-315 for two consecutive days.

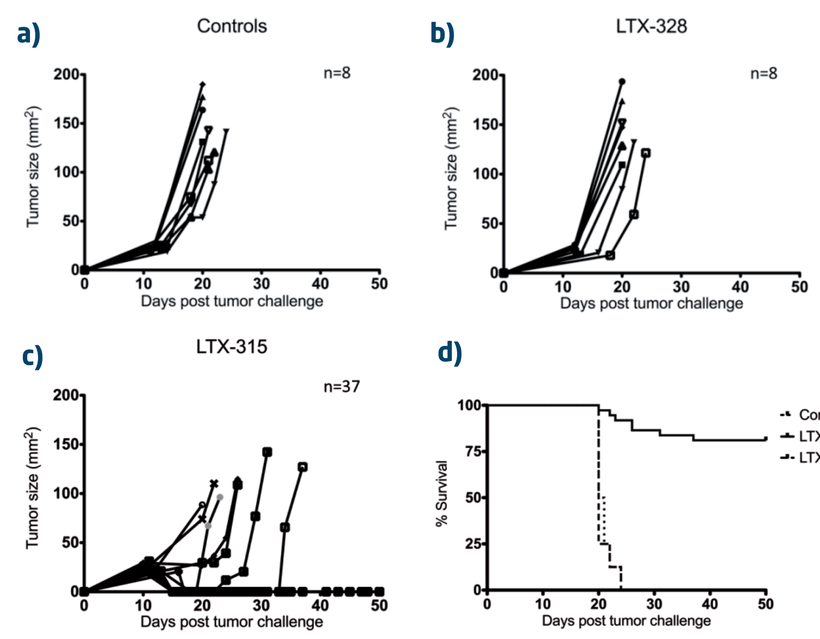
Fig. 4 LTX-315-treatment of B16 melanomas inhibits lung tumor foci formation in the B16 metastasis model



Animals were re-challenged intravenously with 2 x 10^5 viable B16F1 cells. The tumor foci of animals previously cured by LTX-315 were highly infiltrated by $CD3^+$ T cells compared to control animals **(a)**. A digital image illustrates representative lungs from the different groups re-challenged intravenously **(b)**.

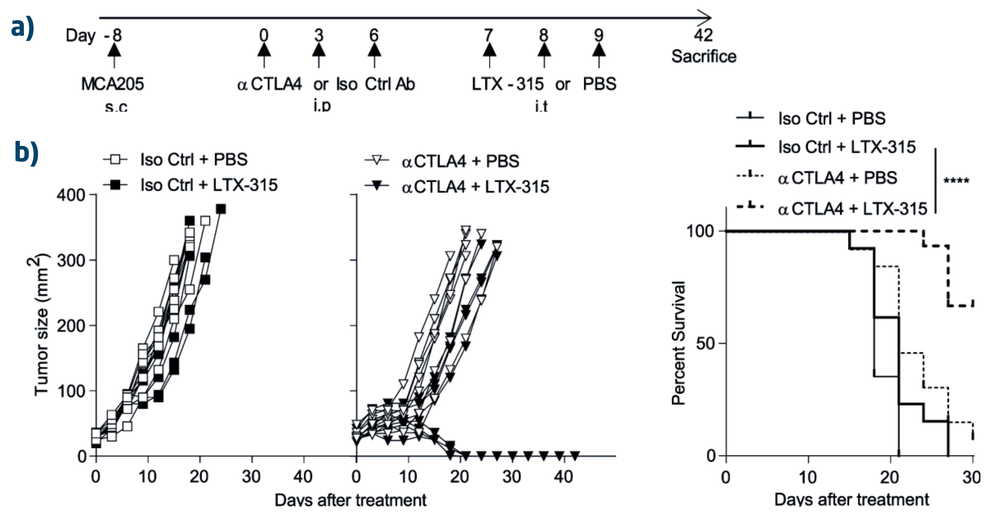
Results

Fig. 1 LTX-315 induces complete regression of B16F1 tumors



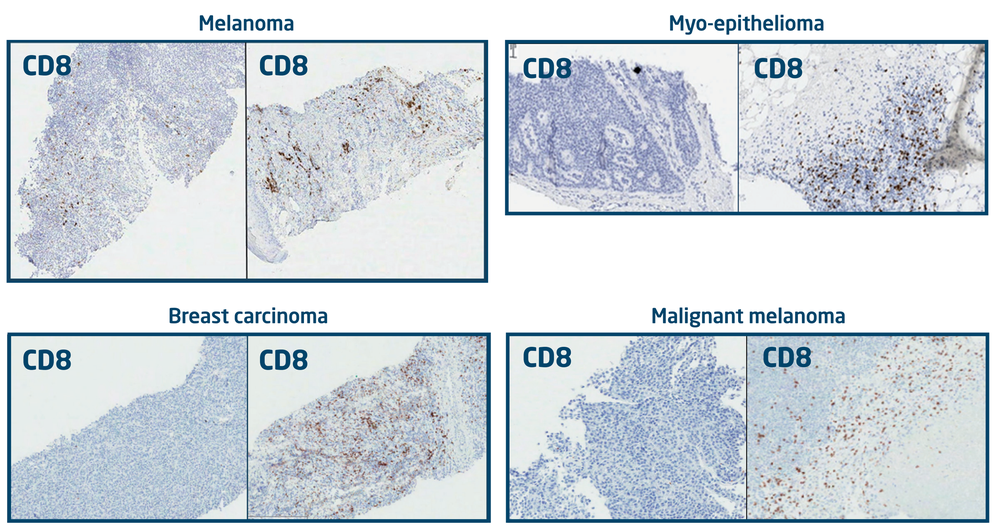
Palpable B16 melanomas were injected with sterile 0.9% NaCl (vehicle controls) **(a)**, with 1 mg LTX-328 **(b)**, or with 1 mg LTX-315 **(c)** once per day on day 12, 13 and 14 after tumor challenge. The survival curves are represented in **(d)** (p = 0.0005) (Camilio et al. 2014).

Fig. 5 LTX-315 increased anticancer immunity mediated by CTLA-4 blockade



a) Experimental setting. **b)** Tumor growth kinetics in the presence (right panel) or absence (left panel) of anti-CTLA4 (100 µg/mouse, 3 injections weekly for two weeks) or isotype control mAb injected **before** LTX-315. Comparison of Kaplan-Meier survival curves were performed using the Log-rank Mantel-Cox test : ****p < 0.0001.

Fig. 6 LTX-315 enhances T-cell infiltration in Phase 1 cancer patients



- Staining of CD8+ cytotoxic T cells in baseline and post-treatment biopsies from tissue samples isolated from 4 representative cancer patients.
- Enhanced infiltration of CD8+ T-cells in injected lesions have so far been documented in 8 out of 13 patients (60%).
- Stable disease (SD), as best response, has been observed to date in 7 of 11 patients (median duration 14 weeks) as assessed by irRC response criteria (4 of 4 melanoma patients achieved SD).

Conclusions

- LTX-315`s ability to increase T-cell infiltration and T-cell clonality makes it ideal as a combination partner for other immunotherapies
- Combination of LTX-315 and immune checkpoint inhibitors (anti-CTLA4) demonstrate significant synergy
- LTX-315 combined with either anti-PD1 or anti-CTLA-4 in a clinical setting is under planning

REFERENCES

- Camilio et al. Cancer Immunol Immunother 2014
- Camilio et al Oncoimmunolgy 2014
- Eike et al. Oncotarget 2015
- Zhou et al. Oncotarget 2015
- Zhou et al. Cell Death and Disease 2016
- Yamasaki et al. Cell Death and Differentiation 2016

Lytix Biopharma AS | P.O. Box 6447 | NO-9294 Tromsø, Norway | E-mail: post@lytixbiopharma.com | Phone: +47 77 67 55 00 | Fax: +47 77 67 55 01