Complete and specific regression of disseminated tumors in a novel rat mesenchymal three-tumor model after intralesional treatment with the nonapeptide LTX-315 (Oncopore™)

Ø. REKDAL[1], J. NESTVOLD[3], M. WANG[2], K. CAMILIO[4], A. AREFFARD[1], B. SVEINBJØRNSSON[4], G. KVALHEIM[2].
[2]The Norwegian Radium Hospital, [3]University of Oslo, [4]University of Tromsø, [1]Lytix Biopharma AS, NO-9294 Tromsø, Norway.

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Background

Intralesional administration of anticancer drugs derived from host defense peptides represents a novel innovative immunotherapeutic strategy.

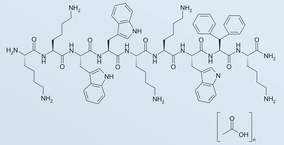
Host defense peptides are present in most living species and have a diverse range of functions including direct killing of pathogens and immune-modulating properties (1). Some host defense peptides are more potent against cancer cells than normal eukaryotic cells due to increased number of anionic membrane components at the surface of the plasma membrane (2).
Our structure-activity relationship studies on host defense peptides has culminated in the engineering of small peptides with enhanced anticancer activity (3). Ultimately, these efforts have led to the development of a chemically modified 9-mer peptide, LTX-315 (Fig 1).

LTX-315 has shown to induce complete tumor regression and prevent relapse and metastasis in several animal models (4).
A phase 1 study has been completed with LTX-315 and a new clinical study is ongoing.

Aim

To investigate whether LTX-315 induces abscopal effects in a novel rat mesenchymal three-tumor model (rTMS).

LTX-315

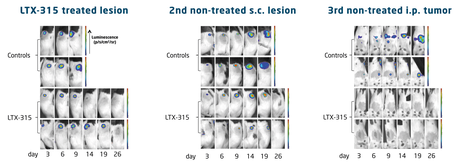


LTX-315 (Oncopore™) consist of 5 lysine residues, three Trp residues and one diphenylalanine residue and is amidated at the C-terminus.

Results

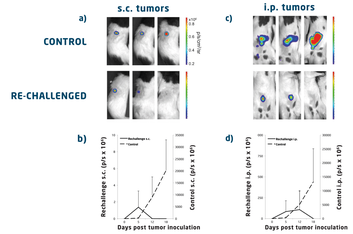
- Intralesional treatment of one single lesion with LTX-315 cured animals with several disseminated tumors (Fig 1).
- A long term memory was obtained in the LTX-315 cured animals (Fig 2).
- Transfer of splenocytes from cured animals prevented tumor growth when adoptively transferred into lymphopenic naïve rats (Fig 3).
- In vitro and in vivo experiments, including a different syngeneic tumor cell line, Roser Leukemia (RL), confirmed that T cells are responsible for the persistent tumor-specific immune responses in animals treated with LTX-315 (Fig 4).
- Complete tumor regression has been obtained in several animal models and protective immune-responses were obtained in all syngeneic models (Table 1).

Fig. 1 LTX-315 eradicates treated and non-treated lesions in the three-tumor rTMS model



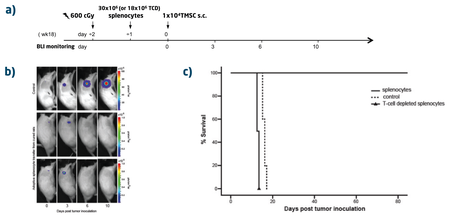
- Tumor cells were inoculated s.c. at day ÷2 on right flank (treated lesion) and at day 0 on contralateral flank (second non-treated lesion) and i.p. (third non-treated lesion) in syngeneic PVG rats.
- The first lesion was treated intra-lesionally with 1mg LTX-315 at day 3 - 9. Representative whole-body bioluminescence images of LTX-315 treated and non treated lesions are presented.

Fig. 2 LTX-315 induces long term protective immune responses



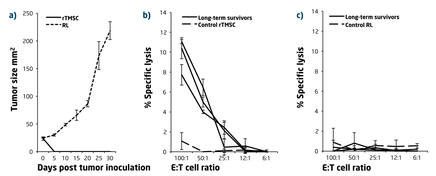
- Each animal previously cured by LTX-315 was re-challenged both s.c. and i.p. with rTMSCs 8 months later.
- **a)** Representative luminescence images of tumor growth after re-challenging with $1{\cdot}10^6$ rTMSCs s.c. (n=6).
- **b)** Tumor growth s.c. in re-challenged and control animals (n=6).
- **c)** Representative luminescence images of tumor growth after re-challenging with $1{\cdot}10^6$ rTMSCs i.p.
- **d)** Tumor growth i.p. in re-challenged and control rats. The data are the average ± SD (n=6).

Fig. 3 Transfer of adaptive immunity by splenocytes from animals cured by LTX-315



a) Rats received either isolated spleen cells from cured rats (n=15), or T-cell-depleted (TCD) spleen cells (n=3). Control animals received splenocytes from naïve rats (n=5). One day after injection of spleen cells, animals were inoculated with rTMSCs s.c. **b)** Representative images of tumor growth. **c)** Survival curves

Fig. 4 LTX-315 induces tumor specific protective immune responses



- Following adoptive transfer of splenocytes from animals cured by LTX-315, recipient rats were challenged with rTMSCs and with a different syngeneic tumor cell line, Roser Leukemia in opposite flanks (a).
- Tumor regression was rapid with complete distinction of the right flank rTMS tumor within 10 days, while the left flank RL tumor continued to develop until day 30, when the tumors were resected due to large size.
- T cells enriched from spleens of cured animals were tested in cytotoxicity assays at varying effector: target (E:T) ratios against rTMSCs and RL cells. The T cells were able to recognize and lyse rTMS targets (b) without any in vitro stimulation whereas no cytotoxic activity against RL targets was obtained (c)

Table 1 Effect of LTX-315 in rodent tumor models

Species	Tumor type	Number Inject.	Dose	Necrosis	Complete Regression	Immuno-protection
Syngenic Mice	A20 B-lymphoma	3	0,5-2 mg	Yes	Yes	Yes
	B16 melanoma	3	1 mg	Yes	Yes	Yes
	CT 26 Colon Carcinoma	3	0,5 - 2mg	Yes	Yes	Yes
Human Xenograft Nude mice	MCF-7 Breast Carcinoma	3	0,75 - 1mg	Yes	Yes	-
	SK MEL-5 Melanoma	3	0,75 - 1mg	Yes	No	-
Syngenic rats	trMSC, Transformed mesenchymal sarcoma	4-6	1-2 mg	Yes	Yes	Yes
	Hepatocellular Carcinoma	6-8	1 mg	Yes	Yes	Yes

Conclusions

- Here we show for the first time that intralesional treatment of one single lesion with LTX-315 (OncoporeTM) is sufficient to cure animals with disseminated tumors.
- Systemic and long lasting protective immune responses were obtained in LTX-315 cured animals.
- T cells are instrumental for the tumor specific protective immune responses obtained in both cured animals and in naïve animals receiving splenocytes from LTX-315 cured animals.
- LTX-315 represents a novel intralesional therapeutic strategy with potential to induce clinical responses in metastatic diseases.
- A phase 1/2a study is ongoing with LTX-315.

References

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Oslo University Hospital

UiO : University of Oslo

UNIVERSITY OF TROMSØ

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