Abstract #5249

Complete Regression and Systemic Protective Immune Responses obtained in B16 Melanomas after Treatment with LTX-315 (Oncopore™)

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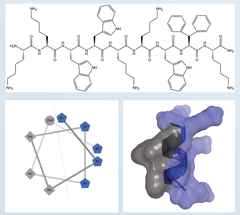
Background

Malignant melanoma, which develops from a neoplastic transformation of melanocytes, is the most aggressive form of skin cancer. With a worldwide increase in the number of patients the need for new and improved therapies is urgent.
LTX-315 (Oncopore™) is a novel cationic anticancer nonapeptide derived from the naturally occurring antimicrobial peptide, bovine lactoferricin. By adopting an amphipathic helical structure, LTX-315 interacts electrostatically with the anionic components of negatively charged cancer cell membranes. LTX-315 induces a destabilization and disruption of the cancer cell membrane, causing cellular lysis and a subsequent release of endogenous cellular content. A phase 1/2a study is ongoing with LTX-315.

Aim

Investigate the anticancer effects of LTX-315 following intralesional administration using the B16 melanoma mouse model, and whether intralesional treatment with LTX-315 resulted in tumor-specific immune responses.

LTX-315



Chemical structure of LTX-315 (top) including a helical wheel projection and its ability to adopt an amphipathic structure (bottom). Cationic residues are in blue and aromatic residues in grey.

Results

Fig. 1 - Malignant melanoma cells are more sensitive to LTX-315 compared to non-malignant cells and display rapid kill kinetics compared to conventional chemotherapeutics

Cell line	LTX-315 IC_{50} ± SD (μM)	LTX-328 IC_{50} ± SD (μM)
B16F1	13.3 ± 0.4	>350
A375	12.7 ± 2.7	
Fem-X	15.3 ± 2.1	
PCS-200-013	19.0 ± 5.7	
MRC-5	25.8 ± 2.0	
HUV-EC-C 2	7.6 ± 3.3	
RBC	>695	

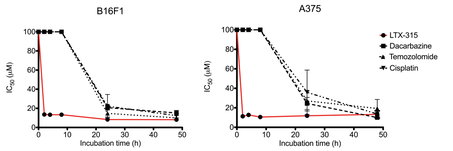
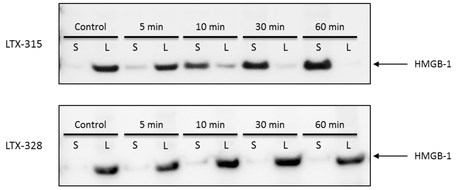
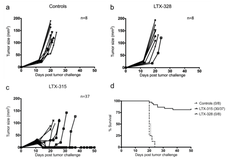


Fig. 2 - B16F1 melanoma cells treated with LTX-315 release High Mobility Group Box-1 (HMGB1) in vitro



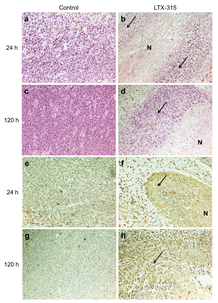
B16F1 melanoma cells treated with 35 μM of either LTX-315 (top) or the non-active control nonapeptide LTX-328 (bottom) for selected time points (5-60 min). The HMGB1 protein is extracellularly released and translocates from the lysate (L) to the supernatant (S) following treatment with LTX-315.

Fig. 3 - LTX-315 induces complete regression of palpable B16F1 tumors following intralesional administration



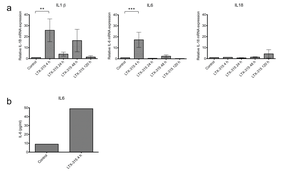
Palpable B16 melanomas were injected with sterile 0.9% NaCl (vehicle controls) (a), with 1 mg of the control peptide LTX-328 (b), or with 1 mg LTX-315 (c) once per day on day 12, 13 and 14 after tumor challenge. The survival curves are represented in (d) ($p = 0.0005$).

Fig. 4 - LTX-315 treatment stimulates T cell infiltration



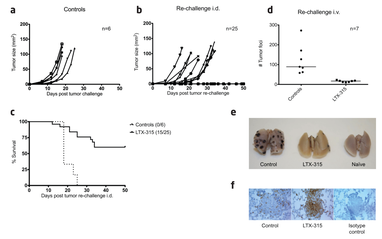
B16 tumors were surgically excised 24h and 120h post-injection with vehicle (a, c, e and g) or LTX-315 (b, d, f and h). Tumors injected with LTX-315 exhibited tumor tissue necrosis (N). Immunolabeling with anti-CD3 showed many of the infiltrating immune cells to be CD3+ T cells (f and h), compared to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

Fig. 5 - LTX-315 treatment mounts an inflammatory response in B16 tumors



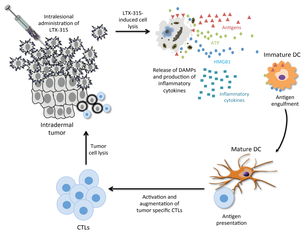
Following a single intralesional injection of sterile 0.9% NaCl (control), or with 1 mg LTX-315, tumor tissue (a) and plasma (b) were harvested and analyzed for cytokine mRNA expression in tumor tissue or for cytokine content in plasma. LTX-315 treatment induced an upregulation of pro-inflammatory cytokines IL1 and IL6 in the tumor tissue and IL6 in plasma samples.

Fig. 6 - LTX-315-treatment of B16 melanomas induces systemic protective immune responses and inhibits lung tumor foci formation



Tumor growth in non-treated control animals (a) was compared to animals previously cured by LTX-315 treatment (b and d). Animals were re-challenged intradermally with 5 x 10^4 viable B16F1 cells contra-lateral to the first tumor site (b) or intravenously with 2 x 10^5 viable B16F1 cells (d). The survival curves of animals re-challenged intradermally is represented in (c) ($p < 0.0001$). A digital image illustrates representative lungs from the different groups re-challenged intravenously (e). The tumor foci of animals previously cured by LTX-315 were highly infiltrated by CD3+ T cells compared to control animals (f).

Fig. 7 - A proposed mechanism of action for intralesional treatment with LTX-315



Conclusions

- LTX-315 induced complete regression of syngeneic B16 melanomas
- Intralesional treatment with LTX-315 induced an inflammatory response and a subsequent infiltration of T cells into the tumor parenchyma
- Intralesional treatment with LTX-315 provided local tumor control followed by systemic protective immune responses and inhibition of metastasis, and thus has potential as a novel immunotherapeutic agent

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