LTX-315 (Oncopore™) as an oncolytic peptide immunotherapy for the treatment of melanoma

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Background

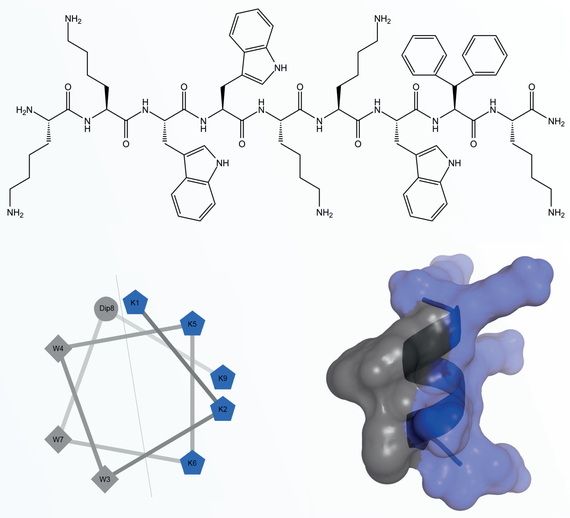
Malignant melanoma, which develops from a neoplastic transformation of melanocytes, is the most aggressive form of skin cancer. With a worldwide increasing incidence of malignant melanoma, there is a continued need for new and improved treatment.

LTX-315 (Oncopore™) is a novel cationic oncolytic peptide designed for the treatment of solid tumors. 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.

Aim

Investigate the anticancer effects of LTX-315 against malignant melanoma following intratumoral administration. We also wanted to elucidate whether intratumoral treatment with LTX-315 resulted in an activation of the immune system and tumor-specific immune responses.

LTX-315



Structural representations of LTX-315

Chemical structure of LTX-315 (top) and helical wheel representations as well as a secondary structure (bottom). Cationic residues are in blue and aromatic residues in grey.

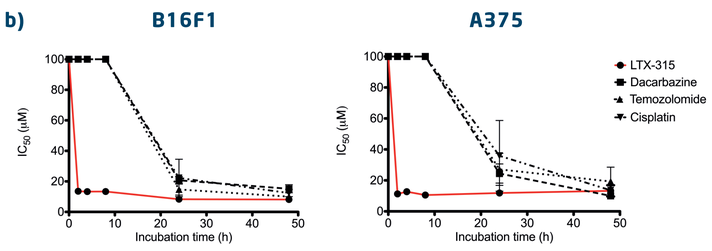
Results

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

a)

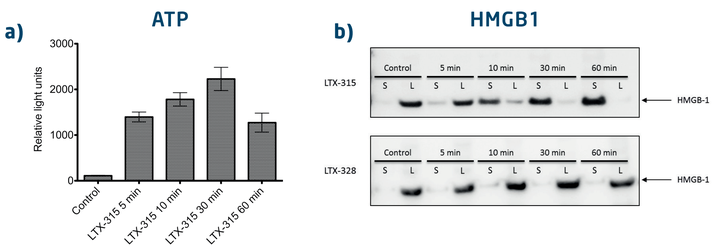
Cell line	LTX-315 IC_{50} ± SD (μM)	LTX-328 IC_{50} ± SD (μM)
B16F1	13.3 ± 0.4	>350
A375	12.7 ± 2.7	
Fem-X	15.3 ± 2.1	
PCS-200-013	19.0 ± 5.7	
MRC-5	25.8 ± 2.0	
HUV-EC-C	27.6 ± 3.3	
RBC	>695	

b)



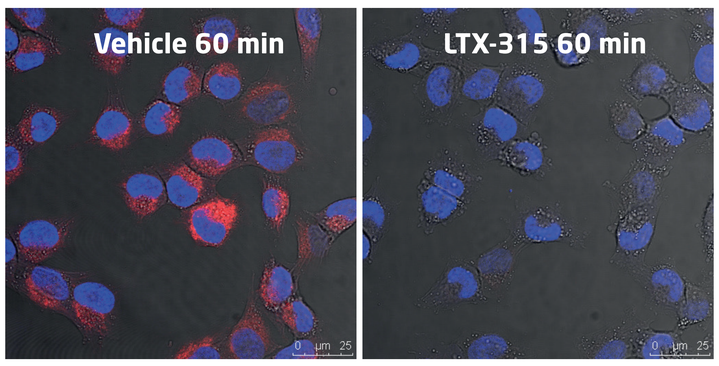
In vitro cytotoxicity of LTX-315 and LTX-328 against cancer cell lines and normal cell lines **(a)**. *In vitro* cytotoxicity data demonstrating IC_{50} values of LTX-315 (*red line*) and three different chemotherapeutic drugs against B16F1 (murine) and A375 (human) melanoma cells **(b)**.

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



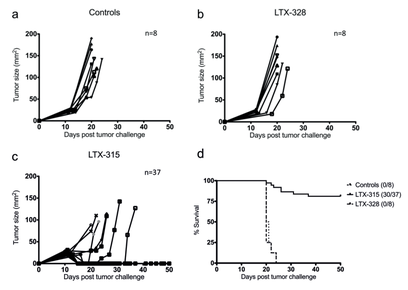
(a) B16F1 melanoma cells treated with 35 μM and analyzed for the release of ATP. **(b)** B16F1 melanoma cells treated with 35 μM of either LTX-315 (*top*) or LTX-328 (*bottom*) for selected time points (5-60 min). The HMGB1 protein is extracellularly released and translocate from the lysate (L) to the supernatant (S) following treatment with LTX-315.

Fig. 3 LTX-315 induces disintegration of mitochondria



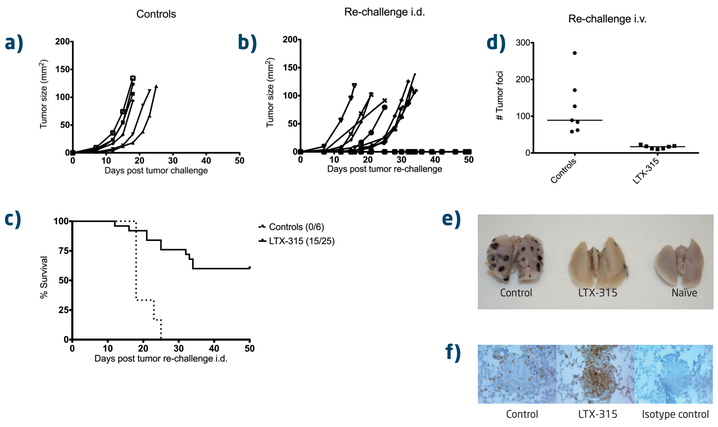
A375 cells were labeled with Mitotracker (Red) and nucleus stained with (DAPI) (left). Treatment with LTX-315 (6 μM) caused disintegration of mitochondria (right).

Fig. 4 LTX-315 induces complete regression of palpable B16F1 tumors following intratumoral administration



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)** ($p = 0.0005$).

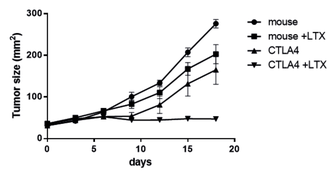
Fig. 5 LTX-315-treatment of B16 melanomas induces systemic protective immune responses and inhibits lung tumor foci formation in a B16 metastasis model



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)**.

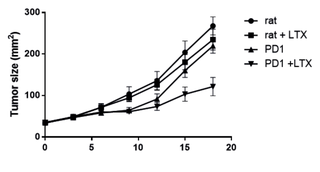
Fig. 6 LTX-315 is a synergistic combination therapy to immune checkpoint inhibitors

Anti-CTLA4 and LTX-315



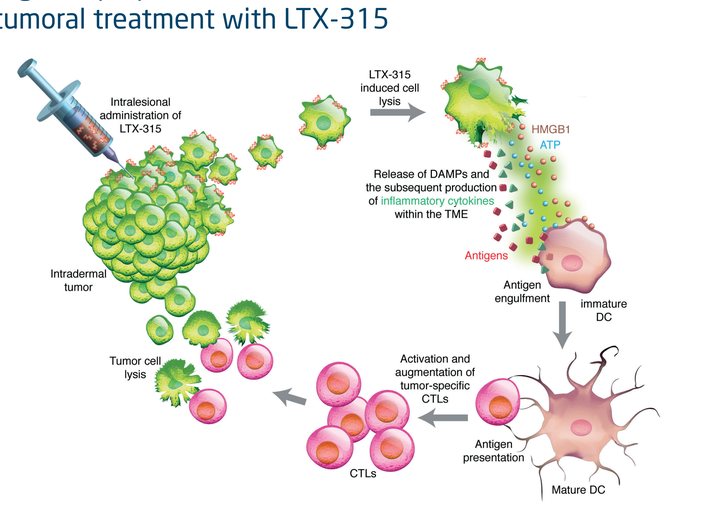
LTX-315 show strong pre-clinical synergistic effect with anti-CTLA-4

Anti-PD1 and LTX-315



LTX-315 show synergistic pre-clinical effect with anti-PD-1 in animals

Source: Laurence Zitvogel, Institut de cancerologie Gustave Roussy



Conclusions

- LTX-315 induced complete regression of syngeneic B16 melanomas
- Intratumoral treatment with LTX-315 induced an inflammatory response and a subsequent infiltration of T cells into the tumor parenchyma
- Intratumoral treatment with LTX-315 provided local tumor control followed by systemic protective immune responses and inhibition of metastasis, and has thus potential as a novel immunotherapeutic agent
- LTX-315 shows synergistic anticancer effects when combined with conventional anticancer therapies such as immune checkpoint inhibitors
- A clinical phase I study investigating the safety and efficacy of LTX-315 has been done and a Phase I/IIa study is ongoing in Europe (ClinicalTrials.gov NCT01986426)
- In the first study, tumour infiltrating lymphocytes and tumour regression were observed in some patients and main safety issues were primarily dose-related flushing and transient hypotension

REFERENCES

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