LTX-401 AS A NOVEL ANTITUMOR AND IMMUNOTHERAPEUTIC AGENT IN AN EXPERIMENTAL LIVER CANCER MODEL

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Background

Hepatocellular carcinoma (HCC) is a malignant disease characterized by few treatment options. Only in early stages of HCC are patients eligible for potentially curable surgical procedures such as resection, transplantation and minimally invasive ablation techniques. HCC is a highly heterogenous cancer and therapies designed to maximize the liberation of tumor antigens in an immunogenic fashion will potentially induce durable clinical responses.

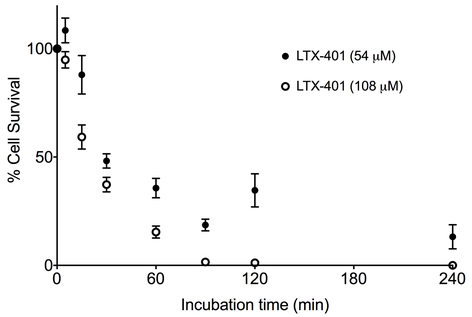
Structure-activity relationship studies on host defense peptides have allowed us to design smaller derivatives with bulky and lipophilic moieties for improved anticancer activity and have culminated in the engineering of LTX-401, a cytolytic immunotherapeutic agent designed for intratumoral injection.

Owing to its amphiphatic nature, LTX-401 may affect the integrity of cancer cell membranes and induce rapid cell death resulting in necrosis, followed by the release of immune-stimulating 'danger signals' or DAMPs that may potentiate antitumor immunity.

Aim

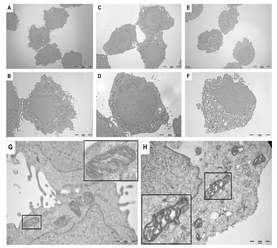
This study was undertaken to investigate the applicability and efficacy of LTX-401 against both s.c. and orthotopic tumors in an immunocompetent rat model of HCC.

FIG. 1: LTX-401 displays rapid killing kinetics towards rat JM1 hepatoma cells



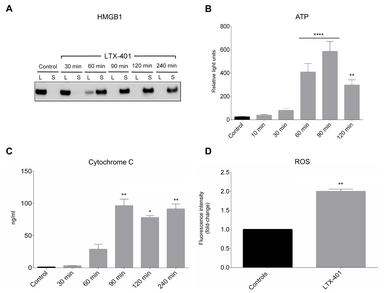
Cells were treated with 54 and 108 μM of LTX-401 for designated time points (5, 15, 30, 60, 90, 120 and 240 min) and assessed for viability using the MTT assay.

FIG. 2: Ultrastructural characteristics of LTX-401-induced cell death



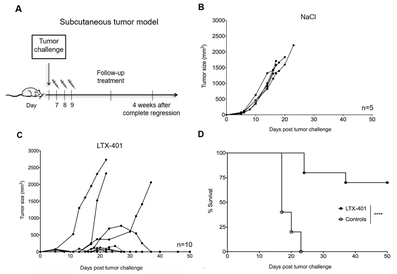
Representative TEM micrographs of JM1 cells treated with 108 μM (4 x $IC50^{4h}$ value) of LTX-401 for various time points. Untreated cells (A, B) were kept in serum-free RPMI 1640 and compared with cells treated with LTX-401 for 5 min (C, D) and 60 min (E, F). Bottom panels; mitochondrial morphology in untreated (G) compared to LTX-401-treated cells (H).

FIG. 3: JM1 cells release DAMPs and ROS when treated with LTX-401



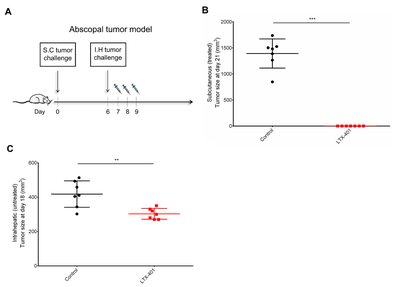
(A) JM1 cells release HMGB1 from the lysate (L) to supernatant (S) after being stimulated with 108 μM LTX-401. (B) ATP is released from JM1 cells into supernatant following treatment with the 54 μM LTX-401. (C) Release of cytochrome *c* into supernatant of LTX-401 treated JM1 cells (108 μM). (D) JM1 cells release ROS after being treated with 271 μM LTX-401 for 45 min. Data are expressed as fold-change in ROS release relative to control.

FIG. 4: Therapeutic efficacy of LTX-401 against subcutaneous JM1 tumors



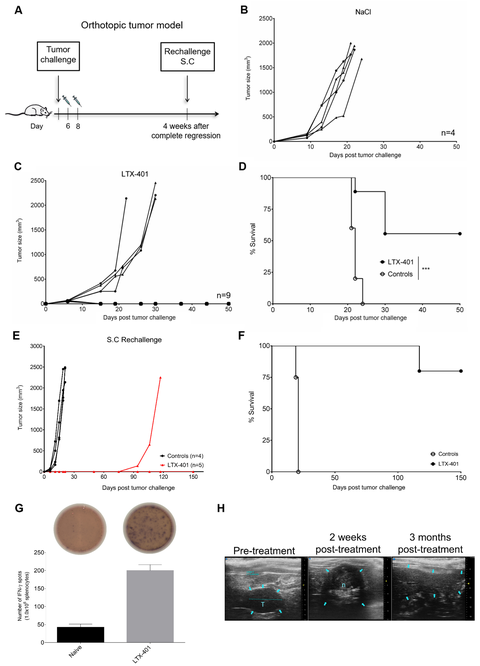
(A) Schematic depiction of the treatment schedule. Subcutaneous JM1 tumors were injected intratumorally with either (B) sterile 0.9 % NaCl (control) or (C) with 0.4 mg LTX-401 once a day for three consecutive days (7, 8, 9). (D) Survival curves of animals treated with LTX-401.

FIG. 5: Partial abscopal response after local therapy with LTX-401



(A) Schematic depiction of the abscopal tumor model. Animals were inoculated subcutaneously with 1 x 10^5 JM1 cells on day 0 followed by intrahepatic inoculation with 1 x 10^5 JM1 cells on day 6. The subcutaneous tumor was treated with 0.4 mg LTX-401 pr. injection or sterile 0.9 % NaCl (control). (B) Subcutaneous (treated) tumor size at day 21 (tumor end-point). Intrahepatic (untreated) tumor size at (C) day 18. One dot represents one animal.

FIG. 6: Therapeutic efficacy of LTX-401 against orthotopic JM1 liver tumors



(A) Schematic depiction of the treatment schedule. Intrahepatic JM1 tumors were injected intratumorally with either (B) sterile 0.9 % NaCl (control) or (C) 1.5 mg LTX-401 on two occasions (day 6 and 8) after tumor cell inoculation. (D) Survival curves of animals treated with LTX-401. (E) Animals cured by LTX-401 treatment were 4 weeks later rechallenged subcutaneously with 1 x 10^5 viable JM1 cells and monitored for tumor growth while (F) indicates survival outcome after rechallenge. (G) Measurement of IFN-γ secretion following co-incubation of splenocytes harvested from long-term survivors with JM1 tumor cells. (H) Ultrasound images depicting tumor and liver pre-treatment, 2 weeks post-treatment and 3 months post-treatment. T = tumor, n = necrosis.

Conclusions

LTX-401:

- Intratumoral injection of LTX-401 was well tolerated, and no treatment-related deaths or systemic toxicity occurred
- When injected into subcutaneous and orthotopic JM1 tumors, LTX-401 treatment resulted in a strong antitumoral effect followed by complete tumor regression in the majority of animals
- LTX-401 provides local tumor control followed by protective immune responses and may be exploited as a novel immunotherapeutic agent in hepatocellular carcinoma.

References

Eike LM, Mauseth B, Camilio KA, Rekdal O, Sveinbjornsson B.
The Cytolytic Amphipathic beta(2,2)-Amino Acid LTX-401 Induces DAMP Release in Melanoma Cells and Causes Complete Regression of B16 Melanoma. Plos One. *2016;11(2):e0148980.*

Zhou H, Sauvat A, Gomes-da-Silva LC, Durand S, Forveille S, Iribarren K, et al.
The oncolytic compound LTX-401 targets the Golgi apparatus. Cell Death Differ. *2016;23(12):2031-41.*

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