Anticancer effects obtained against A20 lymphomas following treatment with LTX-315 (Oncopore™) in combination with low-dose cyclophosphamide

KETIL ANDRÉ CAMILIO[1,2], LIV-MARIE EIKE[2], ØYSTEIN REKDAL[1,2] AND BALDUR SVEINBJØRNSSON[2]
[1]Lytix Biopharma AS, P.O. Box 6447, NO-9294 Tromsø, Norway
[2]Department of Medical Biology, Faculty of Health Sciences, University of Tromsø, Tromsø, Norway

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Background

The need for new and improved anticancer therapies is imperative, with an increased focus on immunotherapy and the combination of different treatments to achieve an additive anticancer effect and maximize the following immune engagement and activation.

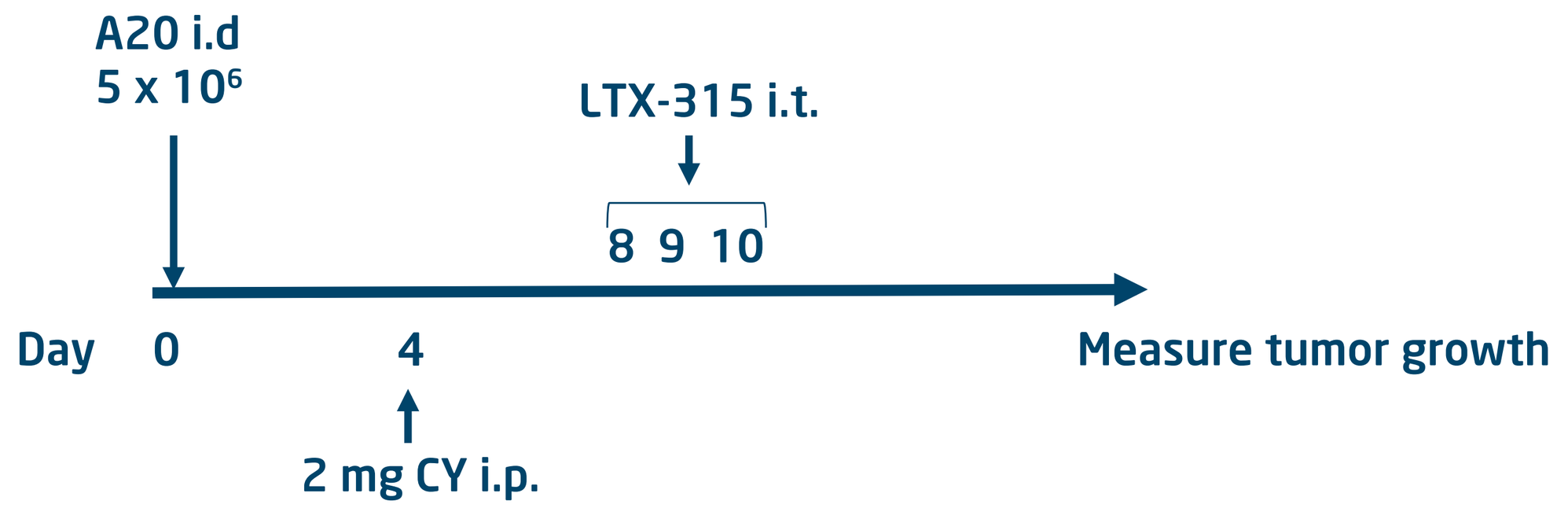
LTX-315 (Oncopore™) is a novel oncolytic peptide derived from the naturally occurring host defense peptide, bovine lactoferricin. By adopting an amphipathic helical structure, LTX-315 interacts electrostatically with the anionic components of negatively charged cancer cell membranes. LTX-315 induces a destabilization and disruption of the cancercell membrane, causing cellular lysis and a subsequent release of endogenous cellular content and danger signals.

Low-dose cyclophosphamide has been shown to enhance immune responses, mostly through the inhibition of immune-suppressive cells such as MDSCs and Tregs, while simultaneously increasing the numbers of $CD4^+$ and $CD8^+$ T cells in the spleen of the host.

Aim

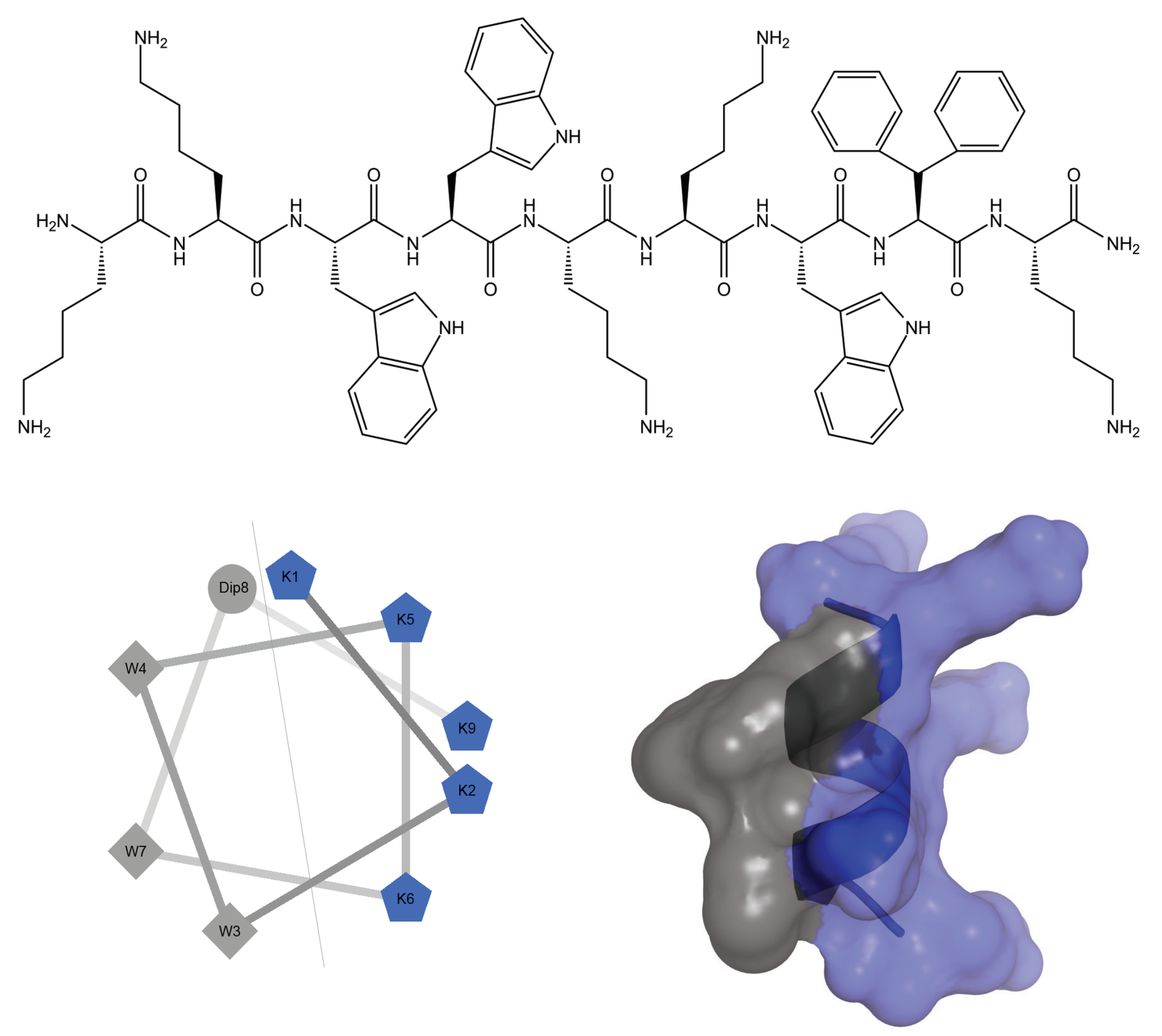
To investigate the anticancer effects of LTX-315 following intralesional administration in combination with low-dose cyclophosphamide against the A20 mouse lymphoma model.

Experimental setup



LTX-315

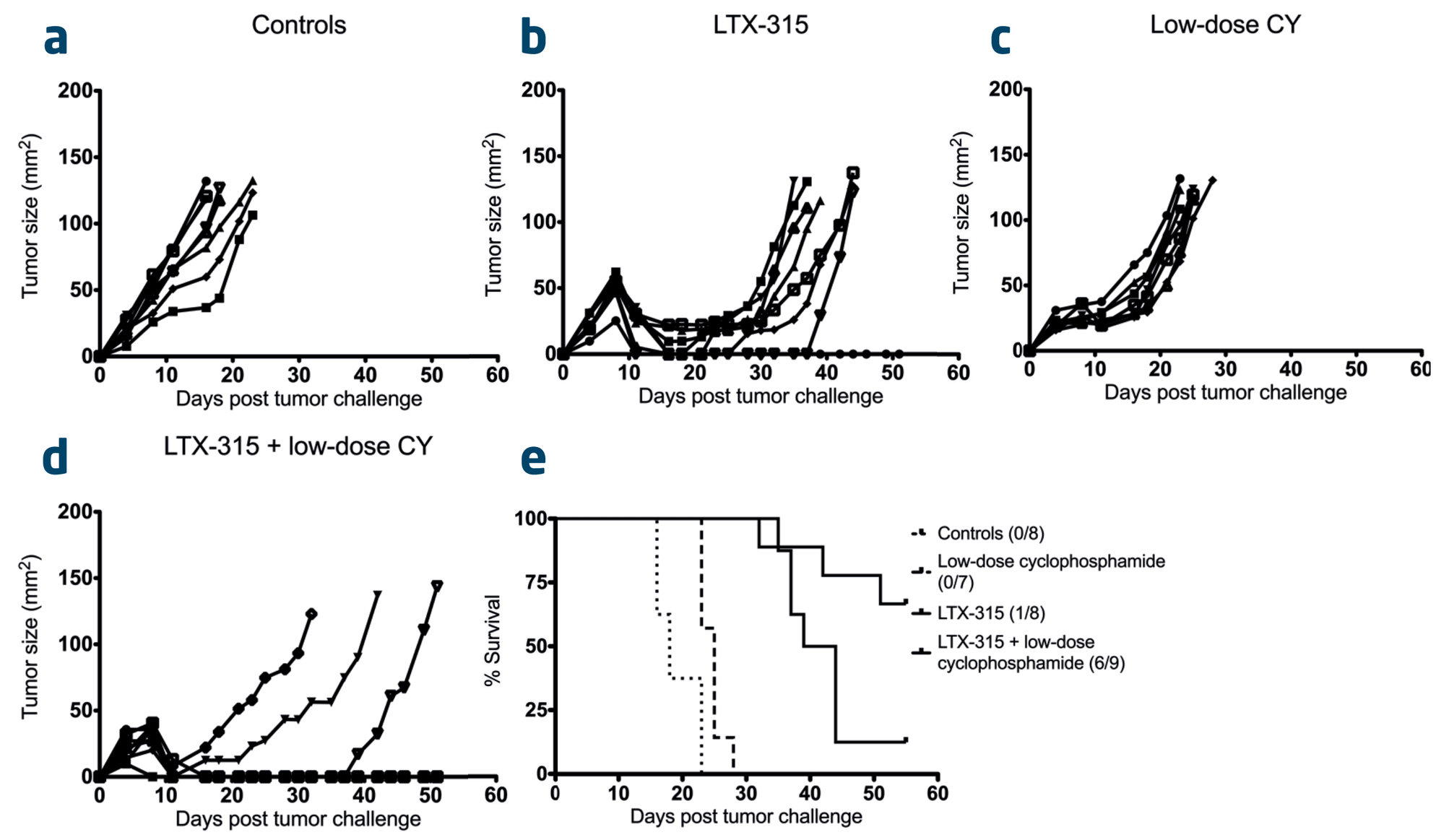
Fig. 1 - Structural representations of LTX-315



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.

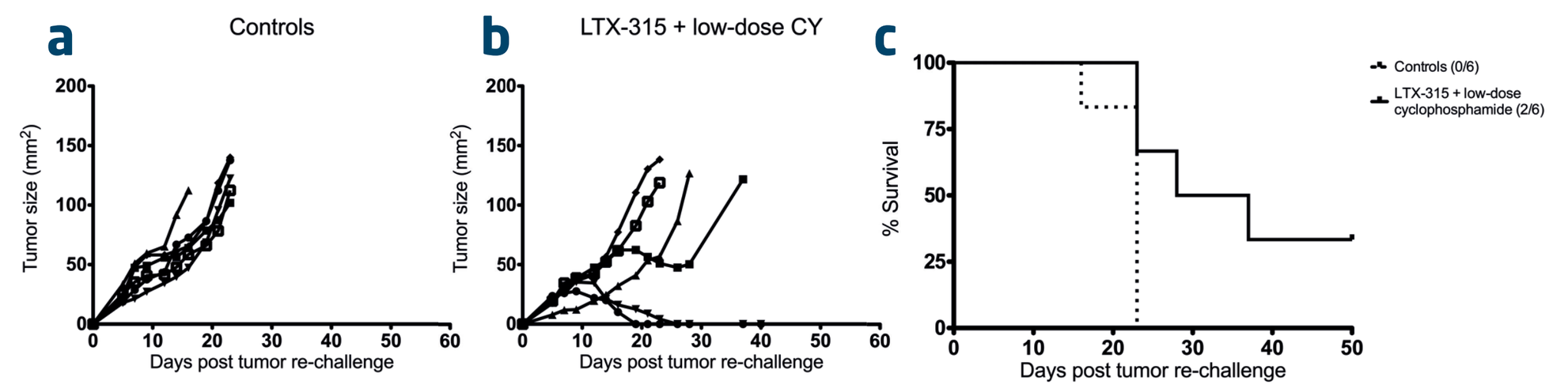
Results

Fig. 2 - LTX-315 induces complete regression of palpable A20 tumors following intralesional administration in combination with low-dose cyclophosphamide



Palpable A20 lymphomas were injected intralesionally with sterile 0.9% NaCl (vehicle controls) **(a)** or with 1 mg LTX-315 **(b)**. Animals were injected intraperitonially with 2 mg CY **(c)** or with 2 mg CY i.p. in combination with 1 mg LTX-315 i.t. **(d)**. The survival curves are represented in **(e)** ($p < 0.0001$).

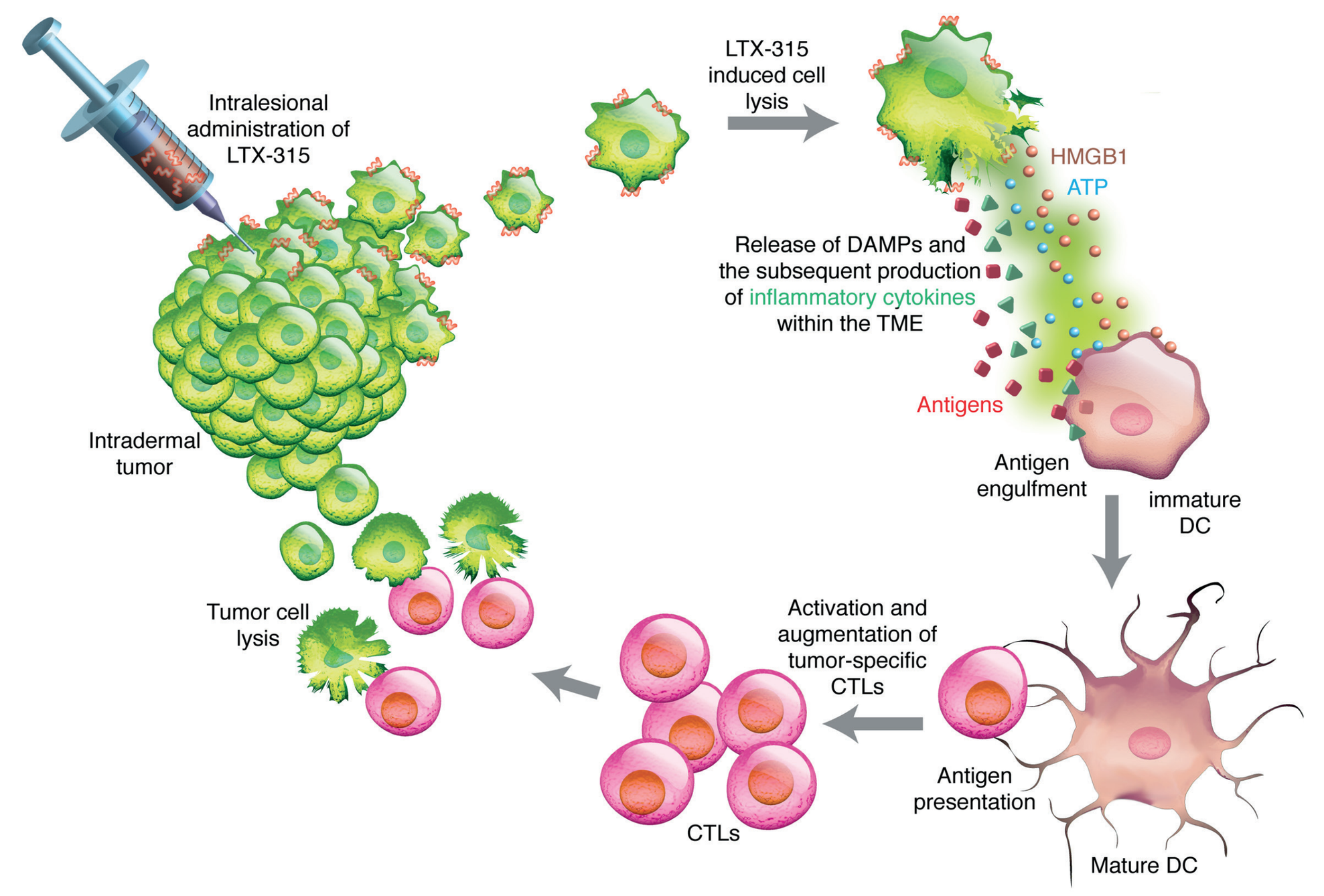
Fig. 3 - LTX-315 treatment combined with low-dose cyclophosphamide resulted in protective systemic immune responses against A20 lymphomas



Naïve control animals **(a)** were inoculated with 5×10^6 A20 lymphoma cells and the tumor growth was compared to the tumor growth in animals previously cured by LTX-315 in combination with low-dose cyclophosphamide **(b)**, also re-challenged with 5×10^6 A20 lymphoma cells. The survival curves are represented in **(c)**.

Mechanism of action

Fig. 4 - A proposed mechanism of action model for intralesional treatment with LTX-315


Intralesional administration of LTX-315
LTX-315 induced cell lysis
HMGB1
ATP
Release of DAMPs and the subsequent production of inflammatory cytokines within the TME
Antigens
Intradermal tumor
Antigen engulfment
immature DC
Tumor cell lysis
Activation and augmentation of tumor-specific CTLs
Antigen presentation
CTLs
Mature DC

LTX-315-induced cellular stress may lead to a cascade of events stimulating the immune system. LTX-315 binds to the cancer cell membrane and kill the cells by membrane lysis. Consequently, intracellular content consisting of DAMPs such as ATP and HMGB1, together with tumor antigens, are released to the micro-environment. This can induce an inflammatory response and subsequently initiate the maturation and recruitment of DCs into the tumor bed. Activated DCs are then primed for antigen engulfment and antigen presentation to T cells, creating tumor-specific CTLs capable of eradicating residual cancer cells.

Conclusions

- LTX-315 induced complete regression of syngeneic A20 lymphomas when combined with low-dose cyclophosphamide.
- Intralesional treatment with LTX-315 in combination with low-dose cyclophosphamide provided local tumor control followed by systemic protective immune responses.
- LTX-315 has the potential to be used as a novel immunotherapeutic agent in combination with already existing anticancer therapies.
- A phase 1/2a study is in progress with LTX-315.

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