Cancer treatment by conventional chemotherapy is limited by factors such as toxic side effects and the development of multi-drug resistance by cancer cells. There is an increasing need for new anticancer therapies with a higher selectivity for neoplastic cells compared with chemotherapy, thereby leading to less cytotoxic side effects during treatment, as well as avoiding the problem of chemoresistance. The field of targeted therapy and immunotherapy has received renewed interest in recent years, and is currently growing rapidly. Successful stories include that of sipuleucel-T (Provenge®) and Ipilimumab (Yervoy®), which are now approved by the US Food and Drug Administration for the treatment of prostate cancer and metastatic melanoma, respectively.

Several cationic antimicrobial peptides demonstrate promising anticancer effects. We have recently described the anticancer properties of LTX-315, a novel synthetic anticancer peptide, against syngeneic B16 melanomas. LTX-315 induced a complete regression of B16 melanomas and systemic protective immune responses following intralesional administration of the peptide.

A short synthetic anticancer peptide and novel immunotherapeutic agent

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**Abbreviations:** CAP, cationic antimicrobial peptide; LfcinB, bovine lactoferricin; DAMP, danger-associated molecular pattern molecule; HMGB1, high mobility group box protein 1; ICD, immunogenic cell death; IL, interleukin; TIL, tumor-infiltrating lymphocyte.

Cationic antimicrobial peptides (CAPs) are small molecules found in a large diversity of species including plants and animals.¹ CAPs vary extensively in the amino acid sequence and encompass a wide variety of structural motifs. However, recurrent structural and functional aspects are observed among peptides from different species, particularly in relation to their cationicity and amphipathicity. These qualities enable them to both interact with- and disrupt lipid membranes. Due to their diverse activities and direct cytotoxic effect, they are often an integral part of the eukaryotic immune system mounting a first-line defense against pathogens. Some CAPs have also been found to have immune modulatory effects.² In addition to their recognized antibacterial activities, several CAPs have promising anticancer properties.³

CAPs are able to kill cancer cells at concentrations that are harmless to untransformed cells⁴ due to the typical anionic nature of cancer cell membranes compared with nonmalignant cells, thereby displaying a selectivity not achievable with chemotherapeutic drugs. CAPs are also predicted to be able to kill dormant or slowly growing malignancies because of their membranolytic effect, thus leading to cell membrane lysis independent of target cell proliferative status, in contrast to chemotherapy that preferentially kills rapidly dividing cells and is ineffective against dormant or slowly proliferating cells.³ In recent years, several groups have attempted to create novel and more efficient synthetic CAPs based on structural parameters important for anticancer activity. It has previously been demonstrated that bovine lactoferricin (LfcinB), a naturally occurring bovine milk protein-derived CAP, has potent anticancer properties, both in vitro and in vivo.⁵,⁶ By chemically modifying LfcinB, shorter and more effective LfcinB derivatives were made using an array of structure-activity relationship studies.

LTX-315 (K-K-W-W-K-K-W-Dip-K-NH₂), a novel synthetic anticancer peptide, has the potential to adopt an amphipathic helical coil structure and was designed for intralesional treatment of tumors. In our study,⁸ we demonstrated that LTX-315 was highly active against both murine and human melanoma cell lines in vitro, while displaying low EC₅₀ cytotoxic activity against human red blood cells. Furthermore, when syngeneic B16 melanomas were treated intralesionally, a majority of the animals (~80%) treated with LTX-315 experienced a complete and long-lasting tumor regression. Histological examinations revealed that

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Intralhesional administration of LTX-315 induced cell lysis through membrane destabilization, leading to a cascade of events that stimulate the immune system. Intracellular content consisting of DAMPs such as ATP and HMGB1, together with tumor antigens, are released into the tumor microenvironment. This induces an inflammatory response and the subsequent production of local inflammatory cytokines, which will 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 cytotoxic CD8+ T lymphocytes capable of eradicating residual cancer cells. ATP, adenosine triphosphate; DAMPs, danger-associated molecular pattern molecules; DC, dendritic cell; CTLs, cytotoxic CD8+ T lymphocytes; HMGB1, high mobility group box protein 1; TME, tumor microenvironment.

LTX-315 has been shown to induce the release of ATP and HMGB1 in vitro, both being DAMPs involved in ICD. When B16 melanomas were analyzed for pro-inflammatory cytokines, the presence of an inflammatory response was observed in vivo. An upregulation of the mRNA levels of inflammatory cytokines such as IL1B, IL6, and IL18 was demonstrated in the tumor tissue, and an elevation of IL6 was observed in plasma samples. Animals that had a complete regression of B16 melanomas were later protected against a re-challenge with viable B16F1 tumor cells, both intradermally and intravenously in an experimental B16 lung metastasis model. In addition, an increase in the amount of infiltrating CD3+ T cells into the re-challenged lung tumor foci was observed. We have previously shown that the immune protection is transferable between animals using adoptive transfer techniques, after the intralhesional treatment of syngeneic A20 lymphomas with a peptide analog to LTX-315.
named LTX-302 (W-K-K-W-Dip-K-K-W-K-NH₂), thereby demonstrating that the immune protection is T cell-dependent. Furthermore, LTX-302 did not induce the complete regression of A20 lymphomas in immunodeficient nude mice, further validating that the mechanism of action requires an intact immune system. The LTX-302-induced antitumor immune responses were also tumor-specific, as animals were not protected against another syngeneic tumor type (Meth A), only A20 lymphomas. The mechanism of LTX-315 is thought to be similar to that of LTX-302. We postulate that LTX-315 induces long-term, specific cellular immunity against B16 melanomas through membrane-induced cellular lysis and the extracellular release of DAMPs such as ATP and HMGB1. This LTX-315-induced immunogenic cell death should result in the maturation of antigen presenting cells and a subsequent presentation of tumor antigens to T cells, hence creating specific cytotoxic T cells capable of eradicating residual cancer cells (Fig. 1).

In conclusion, our observations in vitro and in vivo indicate that the intralesional administration of LTX-315 leads to immunogenic cancer cell death through tumor necrosis initiated by a direct disruptive effect of the peptide on the plasma membrane of tumor cells. Moreover, the lytic effect of LTX-315 leads to the release of DAMPs that stimulate immune responses and the infiltration of TILs into the tumor parenchyma, both of which may be crucial to the eradication of solid B16 melanomas due to their putative role in inducing a long-lasting tumor immunity cascade. Thus, LTX-315 may represent a new approach to cancer immunotherapy, and has potential as a novel immunotherapeutic agent. A clinical Phase I/IIa is currently ongoing with LTX-315.

Disclosure of Potential Conflicts Of Interest
K.A.C. and B.S. receive financial support by and are shareholders in Lytix Biopharma AS. Ø.R. is an employee and shareholder in Lytix Biopharma AS.

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