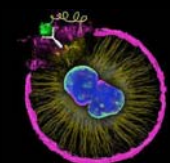


Antibody-Lytic Peptide Conjugates for Cancer Therapy



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Abstract D10201

Esperance is developing its CLYP™ platform technology for targeted delivery of membrane disrupting peptides (MDPs) as payloads to seek and destroy cancer cells. One of the MDPs is CLIP-71, which is a novel amphipathic α -helical lytic peptide that has been optimized for its potent cytolytic effects on cancer cells. CLIP-71 was conjugated chemically or recombinantly to anti-Her2 antibodies. *In vitro* and *in vivo* anti-cancer effects of the antibody conjugates were tested. Her2/neu receptor positive cells (SKOV-3, SKBR-3) and receptor negative (MDA-MB-231) were incubated for 48 hours with the antibody-MDP conjugates or naked antibodies. The IC_{50} values were 27-55 nM. Recombinant antibody conjugated to CLIP-71 was superior to a conjugate containing (KLAKLAK)₂KLAK (IC_{50} 247-338 nM). Her2/neu receptor negative (MDA-MB-231) cells were not killed, indicating that the antibody conjugates specifically targeted cells that expressed Her2/neu receptors. Unconjugated (naked) antibodies did not kill the cells. Intravenous injections of the antibody-MDP conjugates reduced tumor volumes in established SKOV-3 xenografts in nude mice. The naked antibodies were not effective. These results indicate that Esperance's CLYP™ technology is a novel approach to enhance the activity of anti-cancer antibodies. They also show that the antibody-MDP conjugates can be produced by chemical or recombinant methods. Esperance's CLYP™ platform technology is a novel approach to empower antibodies. The potent MDP-antibody conjugates exert their cytotoxic activities via a unique mechanism of action. They can be synthesized recombinantly to produce homogenous products.

Background

Esperance Pharmaceuticals is developing targeted Cationic Lytic Peptide (CLYP™) technology for killing cancer cells. The small targeted oncolytic peptides seek and destroy cancer cells without harming normal cells. The peptides are linear, alpha helical, cationic and they directly interact with negatively charged membranes resulting in their disruption and cell death. As a result of this technology, Esperance Pharmaceuticals™ is developing a new generation of oncolytic peptides. Currently, EP-100, a LHRH targeting CLYP conjugate, is in Phase I clinical trial for the treatment of solid tumors. Immunotoxins are designed to efficiently deliver a payload. They are dependent on internalization and release of the toxin from the antibody to elicit their activity. Often immunotoxins are large molecules that are highly antigenic. Chemically linked antibody-drug conjugates lack a defined stoichiometry. In contrast, protein antibody drug conjugates can be produced recombinantly, generating stoichiometrically defined products. Membrane disrupting peptides (MDP), like (KLAKLAK)₂, have been reported to be active when chemically linked to anti-PSMA or antiCD33 antibodies (Rege et al 2007, Marks et al 2005). To test the concept that CLYP-antibody conjugates kill cancer cells, we have recombinantly produced Her2/neu receptor targeting antibody-CLYP and antibody-(KLAKLAK)₂KLAK conjugates in *E. coli*. The efficacy of the recombinantly produced MDP-antibody conjugates were compared to chemically linked anti-Her2/neu CLYP antibody conjugates *in vitro* and *in vivo*.

Objectives

- In vitro* testing
 - Cytotoxicity of antibody-MDP conjugates recombinantly and chemically produced *in vitro*
 - Kinetics of cytotoxicity *in vitro* IC_{50}
- In vivo* testing:
 - In vivo* efficacy study in SKOV-3 xenografts comparing recombinantly and chemically produced antibody-MDP conjugates and naked antibodies.

Materials & Methods

- In vitro* efficacy** studies were conducted in 96 well plate format (10,000 cells/well) with chemically linked or recombinantly produced MDP-antibody conjugates with CLIP-71 or (KLAKLAK)₂KLAK for 48 h; Cell viability was determined using formazan conversion assays (CellTiter Aqueous ONE, Promega).
- In vitro* Kinetics** were determined in SKOV-3 cell lines in a 96 well plate format (5,000 cells/well) for 4h, 24 and 48 h incubations with recombinantly produced MDP-antibody conjugates. Cell viability was determined using luminometric assays for cell membrane integrity (Cytotox Glo, Promega) for the 4 h time point and CellTiter Glo for 24 and 48 h timepoints.
- In vivo* efficacy studies:** SKOV-3 (N=8-9) xenografts were propagated in female athymic nude mice by subcutaneously injecting a Matrigel/cell suspension (4×10^6 cells/mouse). 42 days after xenograft propagation mice were randomized into treatment groups consisting of 8-9 mice. 9 mice were killed on day 42 and served as baseline controls. EP-420 and EP-422 are naked whole antibody and recombinant antibody. EP-421 is chemically linked CLIP-71 to whole antibody. EP-423 is the recombinantly produced MDP-antibody conjugate. Treatment groups consisted of : Saline Controls (N=9), 3 mg/kg EP-420 (N=9), 0.3 mg/kg EP-421, (N=8), 3.0 mg/kg EP-421, (N=8), 3.0 mg/kg EP-422 (N=9), 0.3 mg/kg EP-423 (N=8) and 3.0 mg/kg EP-423 (N=9). Treatment was conducted through lateral tail vein injection on days 43, 47, 50, 54, 57 and 60. Final necropsy was conducted on day 64 after tumor propagation. Tumor volumes and bodyweights were determined twice weekly, tumor weights and tumor volumes, bodyweights were determined at necropsy. Histology of tumors was evaluated from H&E stained tumor sections.

Recombinantly Produced MDP-antibody Conjugates Targeted to Her2/neu Kill SKBR-3 and SKOV-3 Cells

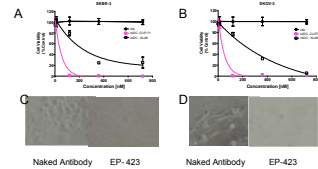


Figure 1: EP-423 recombinantly produced MDP-antibody conjugates, bearing CLIP-71 or KLAK as MDP moiety, killed Her2/neu expressing breast cancer (SKBR-3, A, C) and ovarian cancer cell lines (SKOV-3, B, D) in 48 h incubations. CLIP-71 conjugates were more potent than KLAK conjugated MDP-antibody conjugates. The naked antibodies were not toxic. Naked antibodies did not reach 100% cell death. Photomicrographs C and D show cells incubated with naked antibody and MDP-antibody CLIP-71 conjugates for SKBR-3 (C) and SKOV-3 (D) cells after 48 h incubations.

Recombinantly MDP-antibody Conjugates Kill through Membrane Disruption

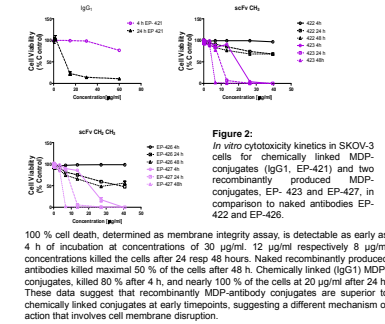


Figure 2: *In vitro* cytotoxicity kinetics in SKOV-3 cells for chemically linked MDP-conjugates (IgG1, EP-421) and two recombinantly produced MDP-conjugates, EP-423 and EP-427, in comparison to naked antibodies EP-422 and EP-426. 100% cell death, determined as membrane integrity assay, is detectable as early as 4 h of incubation at concentrations of 30 μ g/ml. 12 μ g/ml respectively 8 μ g/ml concentrations killed the cells after 24 resp 48 h. Naked recombinantly produced antibodies killed maximal 50% of the cells after 48 h. Chemically linked (IgG1) MDP-conjugates, killed 80% after 4 h, and nearly 100% of the cells at 20 μ g/ml after 24 h. These data suggest that recombinantly MDP-antibody conjugates are superior to chemically linked conjugates at early timepoints, suggesting a different mechanism of action that involves cell membrane disruption.

	CLIP71 [nM]	EP-421 [nM]	EP-422 (naked) [nM]	EP-423 [nM]	EP-426 (naked) [nM]	EP-427 [nM]
4 h	ND	ND	Not toxic*	207.5*	Not toxic*	238.7*
24 h	ND	ND	1292	116	518	112.5
48 h	18180	55	545.7	56.3	327.1	53.7

Table 1: EP-423 and EP-427, recombinantly produced MDP-conjugates, are cytotoxic in the nanomolar range as early as 4 h to SKOV-3 cells reaching their maximal toxicity at 53 nM after 48 h. EP-421, chemically linked MDP antibody shows similar toxicity after 48 h. Naked antibodies (EP-422 and EP-426) are 5-10 fold less toxic than the MDP-antibody conjugates. Unconjugated CLIP-71 is toxic at 18 μ M. Naked antibodies do not cause 100% cell death. ND = Not determined.

Results in vivo studies

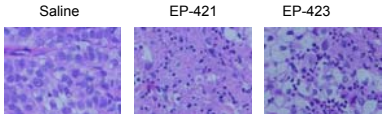


Figure 3: SKOV-3 tumor sections from mice injected with saline, chemically linked antibody conjugates (EP-421 at 3 mg/kg), and recombinantly produced MDP-antibody conjugates (EP-423 at 3 mg/kg). Xenografts from saline treated controls show visible cells with mitotic figures, whereas tumor obtained from MDP antibody conjugate treated animals show cell death characterized by necrosis (Pyknotic) and apoptosis (condensed nuclei) and inflammatory cellular infiltration. Magnification 400x.

Results in vivo Studies

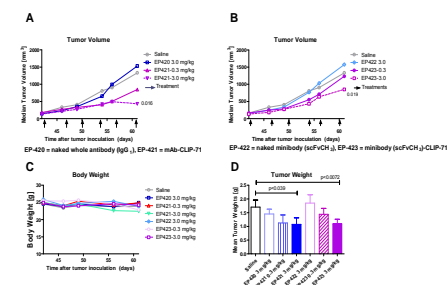


Figure 4: Effect of chemically linked MDP-conjugates (IgG1, EP-421, A) and recombinantly produced MDP-conjugate (B). EP-423 in comparison to naked whole antibodies EP-422 and EP-422. Effect of EP-420, EP-421, EP-422 and EP-423 on tumor growth volumes, A, B) bodyweight (C) and tumor weights (D) of SKOV-3 xenografts. Treatments were administered through lateral tail vein injection (Days 43, 47, 50, 54, 57, 60) as single bolus injections. Necropsies were conducted on day 64. Data are presented as mean \pm SEM (N=8). Treatment response measured as tumor volumes was shown in mice treated with EP-421 and EP-423 at 3.0 mg/kg ($p=0.016$ and 0.019 vs saline controls). Tumor weights was shown in mice treated with EP-421 and EP-423 at 3.0 mg/kg ($p=0.04$ and 0.007 vs saline controls).

Summary

- ✓MDP-Antibody conjugates can be produced recombinantly
- ✓CLIP conjugates are superior to KLAK
- ✓EP-423 and EP-427 destroy cancer cells *in vitro* within 4 h at 207 and 238 nM
- ✓The maximal toxicity *in vitro* is reached at 48 h at 56-57 nM for the recombinant product
- ✓Mechanism action of the MDP-antibody conjugates suggest membrane disruption
- ✓The anti-tumor effects of MDA-antibody conjugates were mediated by necrosis and apoptosis

Conclusion

These results indicate that Esperance's CLYP™ technology is a novel approach to enhance the activity of anti-cancer antibodies. They also show that the antibody-MDP conjugates can be produced by chemical or recombinant methods.

References

1. Rege K et al; Cancer Research, 2007, 67, 6368-6375
2. Marks A et al; Cancer Research 2005, 65, 2373-2377

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