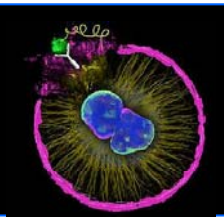
TARGETED ONCOLYTIC PEPTIDE FOR TREATMENT OF OVARIAN CANCERS

Carola Leuschner, Janice Keener, Rebecca McCabe, Nipur Patel, Hector Alila

Esperance Pharmaceuticals, Inc., Baton Rouge, LA



Abstract 4666

Ovarian cancer is the fifth leading cause of death from cancer in women. New therapies are needed to improve the survival of patient.

EP-100 is a novel oncolytic peptide that is being developed to selectively target surface luteinizing hormone releasing hormone (LHRH) receptors. It has been reported that approximately 80 % human ovarian cancer cells and their recurrences over-express LHRH receptors. EP-100 consists of an LHRH receptor-targeting ligand conjugated to a novel lytic peptide (CLIP71). EP-100 was tested *in vitro* and *in vivo* for activity in LHRH receptor over-expressing OVCAR-3 cells. LHRH receptor-negative SKOV-3 ovarian cancer cells were used as negative controls. Cells were cultured in the presence of various concentrations (0.001-100 μM) of EP-100 or unconjugated CLIP 71 for 1 to 24h. Viability was measured by formazan conversion assays. In addition, LHRH receptor expression in cancer cells was determined by immunohistochemistry and quantified by a computerized image scoring system: 0 for no receptor expression and 3 for maximum receptor expression. *In vivo* efficacy of EP-100 was conducted in nude mice implanted with OVCAR-3 cells. Nu/Nu female mice were injected subcutaneously with OVCAR-3 cells in Matrigel suspension. Treatment was started on day 33 after tumor cell injection when the tumors were established and continued on days 41 and 47 with a final necropsy on day 52. The doses for the 3 weekly injections were 0.02, 0.2 and 2 mg/kg body weight, given as a bolus single intravenous injection via lateral tail vein. Treatment groups included controls (10 mice each) injected with saline or CLIP71 (2 mg/kg); and EP-100 (0.02 (N=10), 0.2 (N=10), and 2 mg/kg (N=9),), and Cisplatinum (10 mg/kg, 3qd (N=10),). Tumors were obtained on the day of the first treatment to determine baseline measurements (N=9). Serum CA125 was used as a measure of drug activity. PET Scans were conducted in treated mice and saline controls after administration with [^{18}F]—2fluoro-2-deoxy-D-glucose. EP-100 caused a dose-dependent cell killing of OVCAR-3 cells with an IC_{50} of 4.9 μM at 1 h and 3 μM at 24h. whereas IC_{50} for CLIP71 was significantly higher 33 μM at 1 h and 11.5 μM at 24 h ($p<0.005$). The IC_{50} in LHRH-receptor negative SKOV-3 cells for EP-100 was also higher 11.5 μM after 24 h of incubation compared with CLIP71 incubations resulted in IC_{50} values of 50 μM ($p<0.005$). EP-100 regressed tumors significantly ($p < 0.001$) at doses as low as 0.2mg/kg with several mice remaining tumor free. CLIP71, cisplatinum or saline had no effect on tumor growth. Tumor regression was greatest in mice treated with EP-100 at 0.2 mg/kg ($p<0.03$ vs baseline). Tumor free mice were found in groups 0.2 and 2 mg/kg of EP-100. Cisplatinum and CLIP71 were not effective in reducing tumor weights. Serum CA125 secretion was reduced in EP-100 treated mice at 0.2 and 2 mg/kg ($p<0.0002$) compared to saline controls. These results indicate that EP-100 selectively targets and kills only cancer cells that over-express LHRH receptors. They demonstrate that EP-100 is a potential therapy for multi-drug resistant ovarian cancer in humans.

Background

Traditional non-targeted treatments are often associated with serious side effects, are systemically active and do not discriminate between cancer and normal cells in vital organs. Further, patient's tumors often develop multi-drug resistance and the recurrence of their cancers often presents a more aggressive form of the disease resulting in death.

An alternative approach under development by Esperance Pharmaceuticals for killing cancer cells involves oncolytic peptides that are liner, alpha helical, cationic and short that directly interact with negatively charged membranes resulting in cell death. Major advantages of membrane disrupting peptides over traditional non-targeted treatments include the following: 1. Preferentially destroy negatively charged cells, such as cancer cells, (cancer cells are up to 50 times more sensitive to oncolytic peptides compared to normal cells [1], 2.Fast acting - causing necrosis within minutes through direct membrane interaction [2], 3. Activity is independent of intracellular uptake, 4.Activity is independent of multi-drug resistance, 5.Short half life in vivo, destroying cancer cells independent of proliferation, 6. No interaction with physiological pathways required [3]. A number of cancers over-express receptors for luteinizing hormone releasing hormone (LHRH); these cancers belong to the 10 most frequently occurring cancer incidences such as breast (52 %), ovarian (80 %), endometrial (80%), prostate (86 %), and pancreatic (68 %) cancer [4]. This high incidence of over-expression of LHRH receptors in a wide range of cancers provides the rationale for targeting these receptors.

Background continued

First generation targeted oncolytic peptides consisted of synthetic lytic peptides conjugated to human chorionic gonadotropin or LHRH [5-7]. They selectively kill cells that over-express hCG or LHRH receptors within hours of contact with the cells. The direct contact and interaction with the cancer cell membrane causes their disruption and cell death by necrosis [5-7].

Esperance Pharmaceuticals™ has developed a new generation of oncolytic peptides conjugated to LHRH, that regress xenografts of breast, ovarian and prostate cancers in nude mice. EP-100 is a novel oncolytic peptide that selectively targets surface LHRH receptors. EP-100, has been tested extensively in vitro and in vivo and shows no adverse effects in various species when administered in repeated injections. EP-100 is a fast acting compound, that lacks hemolytic activity and is not immunogenic.

Objectives

- To test *in vitro* efficacy of EP-100 in ovarian cancer cell lines
- To test *in vivo* efficacy of EP-100 in an OVCAR-3 xenograft model

Results – *in vitro* Studies

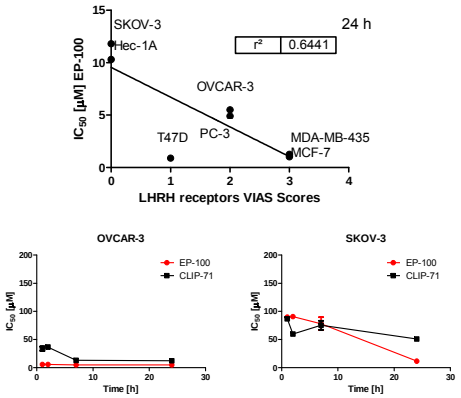


Figure 1:
EP-100 activity correlates with LHRH receptor levels.
EP-100 reached its maximal activity in OVCAR-3 (LHRH receptor positive) cells within one hour (4.9 μM), whereas unconjugated CLIP71 resulted in IC_{50} values of 33, 36, 12.5 and 11.9 [μM] ($p<0.005$) with increasing incubation time. EP-100 is a fast acting agent (4.9 μM) compared to the unconjugated drug.
The SKOV-3 cell line (LHRH receptor negative) showed similar sensitivity regardless of conjugation to LHRH. EP-100 exhibited its maximal efficacy (11.5 μM) after 24 h of incubation whereas CLIP71 incubations resulted in IC_{50} values of 86, 96, 53 and 50 [μM] ($p<0.005$) with increasing incubation time. Data are presented as mean ± SEM, N = 8.
EP-100 is not an appropriate target for SKOV-3 cells that do not express LHRH receptors

Results *in vivo* studies

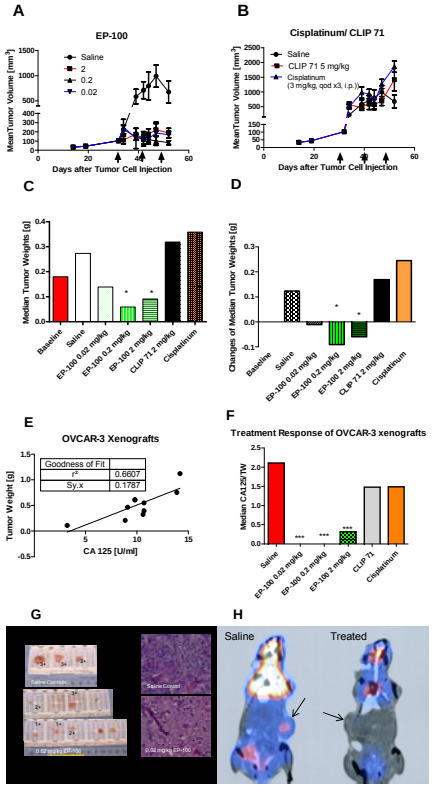


Figure 2:
A, B) Effect of EP-100 and cisplatinum on growth (volume) of OVCAR-3 xenografts. Treatments were administered through lateral tail vein injection (D 33, 41 and 47) as single bolus injection. Necropsies were conducted on day 52. Data are presented as mean, SEM. Arrows show dosing. **C**) shows the median tumor weights, **D**) median changes in tumor weights compared to baseline values. Treatment response measured as tumor regression was greatest in mice treated with EP-100 at 0.2 mg/kg ($p<0.03$ vs baseline). Reduced tumor weights compared to saline controls and CLIP71 ($p<0.05$) were obtained in the groups for 0.2 and 2mg/kg dosages of EP-100. Cisplatinum and CLIP 71 were not effective in reducing tumor weights. **E**) Tumor marker ovarian cancer antigen CA125 relative to tumor weights at necropsy from OVCAR-3-xenograft study, Treatment response as tumor viability from median CA125 secretion compared to saline controls was greatest in mice treated with EP-100 at 0.2 and 2 mg/kg, ($p<0.0002$). **F**) Treatment response as median CA125 secretion/Tumor weight. **G**) Tumors and LHRH receptor levels in control and treated tumors, Histological section of treated and control tumors (H&E, 40 x). **H**) PET image of control and treated mice show lack of viable tumor cells after treatment.

Materials & Methods

In vitro efficacy studies were conducted in 96 well plate format (10,000 cells/well)
Human ovarian cancer cell lines SKOV-3 (negative for LHRH receptors) and OVCAR-3 (positive for LHRH receptors) were incubated for 1, 2, 6 and 24 hours with EP-100 or CLIP-71
Cell viability was determined using formazan conversion assays

OVCAR-3 xenograft model: Nu/Nu female nude mice bearing OVCAR-3 xenografts. Treatment was initiated on day 33 after tumor cell injection and continued on days 41 and 47 with a final necropsy on day 52. The doses for the 3 weekly injections were 0.02, 0.2 and 2 mg/kg body weight, given as a bolus single intravenous injection via lateral tail vein. Treatment groups included controls (10 mice each) injected with saline or CLIP71 (2 mg/kg); and EP-100 (0.02 (N=10), 0.2 (N=10), and 2 mg/kg (N=9),), and Cisplatinum (10 mg/kg, 3qd (N=10),). Tumors were obtained on the day of the first treatment to determine baseline measurements (N=9). Serum CA125 was used as a measure of drug activity.

PET imaging was conducted in treated and untreated mice bearing OVCAR-3 xenografts.

LHRH receptor expression was determined through quantitative immunoperoxidase analysis on cell cultures and tumor sections.

Summary

1. Efficacy of EP-100 correlated with LHRH receptor expression.
2. EP-100 destroyed LHRH receptor expressing OVCAR-3 cells within an hour (4.9 μM)
3. Unconjugated drug (CLIP 71) was slow acting
4. LHRH receptor negative cells (SKOV-3) showed similar sensitivity to EP-100 and CLIP71 regardless of conjugation to LHRH
5. EP-100 is specific for cells that express LHRH receptors
6. In vivo EP-100 reduced OVCAR-3 xenografts in weekly injections at doses as low as 0.2 mg/kg bodyweight
7. Controls (saline) and cisplatinum or CLIP71 treated mice showed tumor growth
8. Tumor weights were reduced in EP-100 treated groups
9. As measure of tumor viability serum CA125 was reduced in treatment groups
10. LHRH receptor levels were reduced in EP-100 treated tumors
11. EP-100 treated tumors were necrotic
12. Treated tumors lacked [^{18}F]-FDG uptake
13. All treatment groups tolerated the injections well
14. No changes were observed in serum chemistry or complete blood count parameters or gross pathology

Conclusion

EP-100 selectively targets and kills cancer cells that over-express LHRH receptors. These data indicate that EP-100 is a potential therapy for multi-drug resistant ovarian cancers in humans.

References

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