

EP-100 IS A TARGETED PEPTIDE FOR TREATMENT OF CANCERS THAT OVER-EXPRESS LHRH-RECEPTORS

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Abstract

Ovarian cancer is the fifth leading cause of cancer deaths in women with estimated 21,550 new cases and 14,600 deaths in 2009. Current treatments are not effective. EP-100 is a novel targeted cancer drug currently in Phase 1 clinical trials; being developed by Esperance Pharmaceuticals Inc. (Baton Rouge, Louisiana) to treat cancers that over-express luteinizing hormone-releasing hormone (LHRH) receptors. Ovarian cancer is one of the cancers that over-express the target LHRH receptors and is the proposed first indication for EP-100. Approximately 80% of ovarian cancer biopsies over-express LHRH receptors. EP-100 was tested *in vitro* and *in vivo* using LHRH receptor-positive and multi-drug resistant OVCAR-3 ovarian cancer cells and LHRH receptor-negative SKOV-3 cells. EP-100 caused rapid killing of cells in a dose-dependent manner ($IC_{50} = 4.9 \mu M$) within 1 hour. The non-targeted lytic peptide, CLIP71, was inactive ($IC_{50} = 33 \mu M$). EP-100 was also inactive in SKOV-3 cells ($IC_{50} = 86 \mu M$). Nude mice bearing OVCAR-3 xenografts received weekly intravenous EP-100 for 3 weeks and tumors were obtained a week after the last injection. The tumors were significantly ($p < 0.001$) regressed at doses as low as 0.2mg/kg. Cisplatin and CLIP71 were inactive. Serum CA125 was significantly ($p < 0.0002$) reduced in EP-100-treated mice compared to saline-treated 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 treatment for ovarian cancer and other LHRH-receptor over-expressing cancers including breast, prostate, endometrial, pancreatic, colon, skin and testicular cancers.

Background

Ovarian cancer is the fifth leading cause of cancer deaths in women with estimated 21,550 new cases and 14,600 deaths in 2009. Current treatments are not effective. Traditional treatments such as chemotherapy or radiation are often associated with serious side effects partly because they are systemically active and do not discriminate between cancer and normal cells in vital organs. Furthermore, 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 targeting drugs that kill only cancer cells without harming normal cells. The drugs bind to cell surface receptors and they kill cells directly via interaction with cell membranes. The company has developed a novel generation of targeted anticancer peptides conjugated to LHRH, that seeks and destroys breast, ovarian and prostate cancer xenografts in nude mice. EP-100 is currently in Phase 1 clinical trials in patients with solid tumors.

A wide variety of major human cancers over-express LHRH receptors (Table below).

Cancer Type	Percent Over-expressing LHRH-Receptors
Breast	52 %
Prostate	86 %
Ovarian	80 %
Endometrial	80 %
Pancreatic	68 %
Melanoma	Yes
Colon	Yes
Oral	Yes

Studies were conducted in vitro and in vivo to demonstrate the selective targeting of EP-100 in ovarian and prostate cancer. This information is applicable to other cancers that over-express LHRH receptors.

Materials and Methods

Cytotoxicity of EP-100 was tested in various human cell lines including ovarian cancer cell lines SKOV-3 (negative for LHRH receptors) and OVCAR-3 (positive for LHRH receptors). The cells were incubated for 1, 2, 6 and 24 hours with EP-100 or CLIP71. Cell viability was determined using formazan conversion assays.

In vivo efficacy studies were conducted using OVCAR-3 and PC-3 cells implanted in female or male nude mice. Treatment was initiated for the OVCAR-3 xenograft (N=10) on day 33, 40 and 47 and day 15, 22 and 29 for PC-3 xenografts (N=11). The doses for OVCAR-3 xenografts were 0.02, 0.2 and 2 mg/kg and 0.002, 0.02, 0.2 and 2 mg/kg body weight in PC-3 xenografts. The 3 weekly injections were given as a bolus single injection via the lateral tail vein. Treatment groups included controls (10 mice each) injected with saline or untargeted peptide (CLIP71) dosed at 2 mg/kg; and Cisplatin (10 mg/kg, 3qd). Tumors were obtained on the day of the first treatment to determine baseline measurements (N=9). Serum CA125 in OVCAR-3 xenografts was used as a measure of drug activity. PET imaging was conducted in mice bearing OVCAR-3 xenografts.

Results – in vitro Studies

EP-100 Kills Several Selected Human Cancer Cells Including Multi-drug Resistant Cell Lines

Cell Line	Cisplatin	EP-100 (IC_{50} - μM)
		24 h
MDA-MB-435S (Breast)	ND	0.9
MDA-MB-231 (Breast)	Resistant	1.9
MCF-7 (Breast)	ND	1.0
AN3-CA (Endometrial)	11.9	3.8
Hec-1A (Endometrial)	ND	10.3
OVCAR-3 (Ovarian)	184	3.0
SKOV-3 (Ovarian)	321	11.8
A2780 (Ovarian)	ND	2.0
LNCAp (Prostate)	19.9	1.6
PC-3 (Prostate)	ND	5.5
MiaPaCa (Pancreatic)	ND	6.5
LoVo (Colon)	ND	5.5

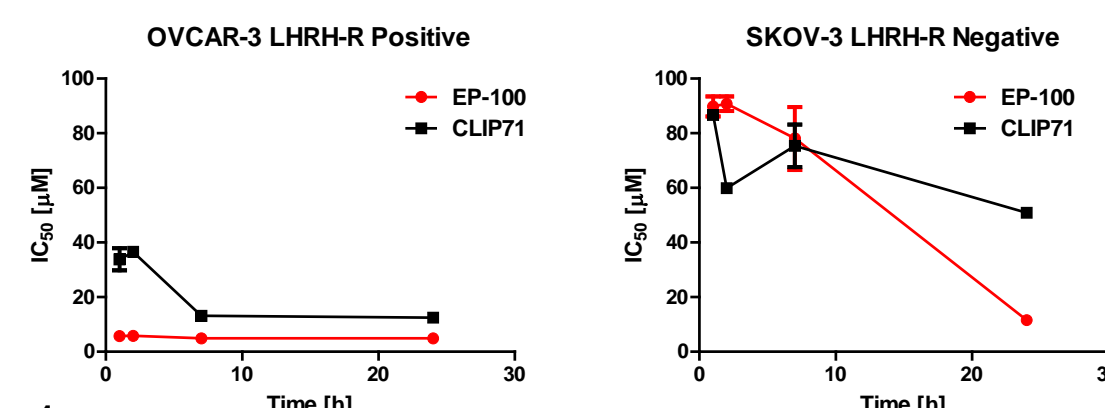


Figure 1 : EP-100 rapidly killed OVCAR-3 (LHRH receptor positive) cells within one hour (4.9 μM). Unconjugated CLIP71 was not active with IC_{50} values of 33, 36, 12.5 and 11.9 μM ($p < 0.005$) with increasing incubation time. The SKOV-3 cell line (LHRH receptor negative) was not sensitive to EP-100 and 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 \pm SEM, N = 8.

Results in vivo studies

EP-100 Destroys Ovarian Cancer in Mice

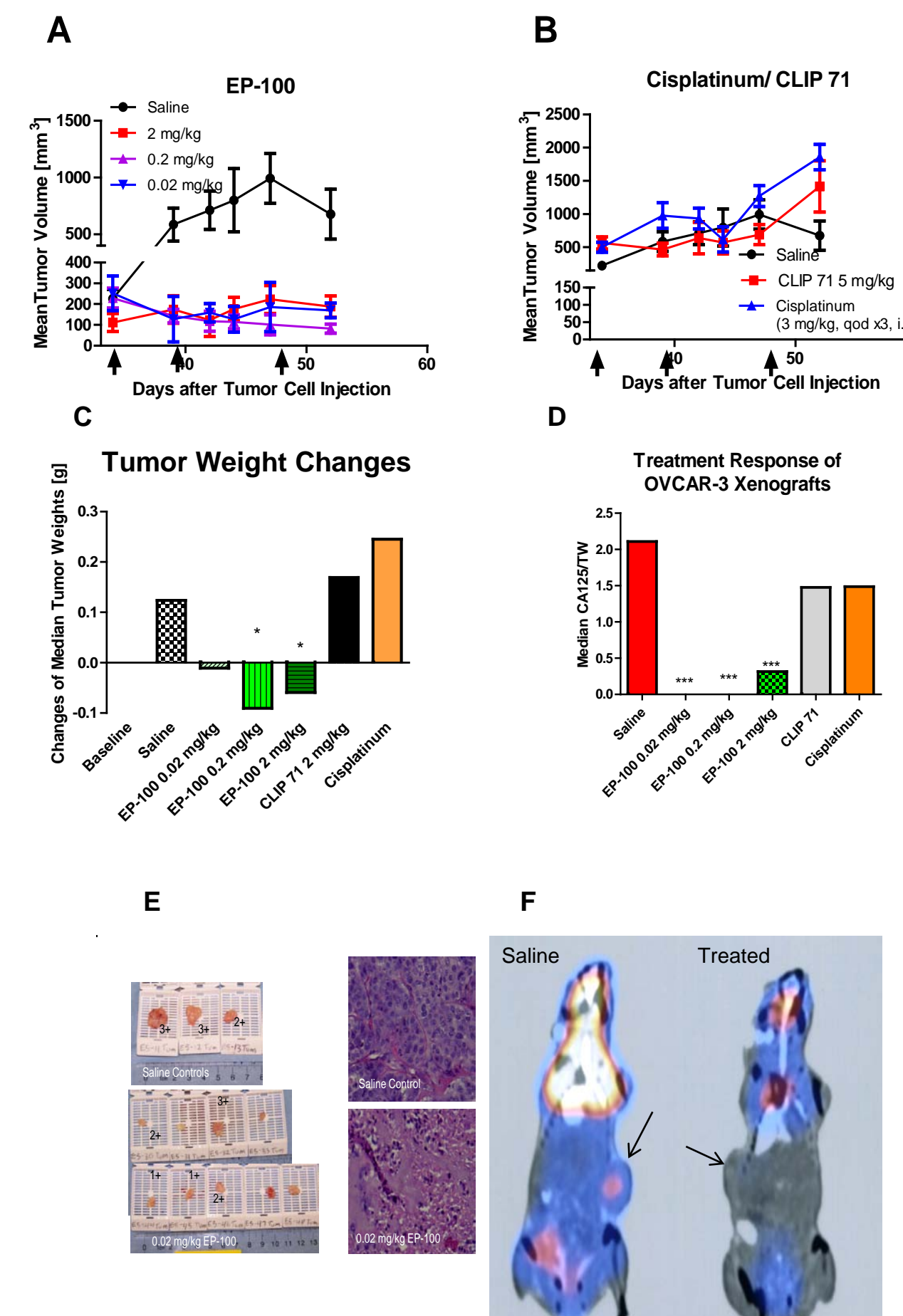


Figure 2: A, B) Effect of EP-100 and cisplatin on growth (volume) of ovarian (OVCAR-3) cancer xenografts. Treatments were administered intravenously on days 33, 40 and 47 post-implantation as single bolus injection. Necropsies were conducted a week after the last injection. Data are presented as mean \pm SEM. Arrows show dosing. C) Median tumor weight changes compared to baseline. Treatment response 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. Cisplatin and CLIP71 were not effective in reducing tumor weights. D) Tumor marker ovarian cancer antigen (CA125) highly correlated to tumor weights at necropsy in saline controls from OVCAR-3-xenograft. 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, ($p < 0.0002$). E) 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.

Results in vivo studies

EP-100 Destroys Prostate Cancer in Mice

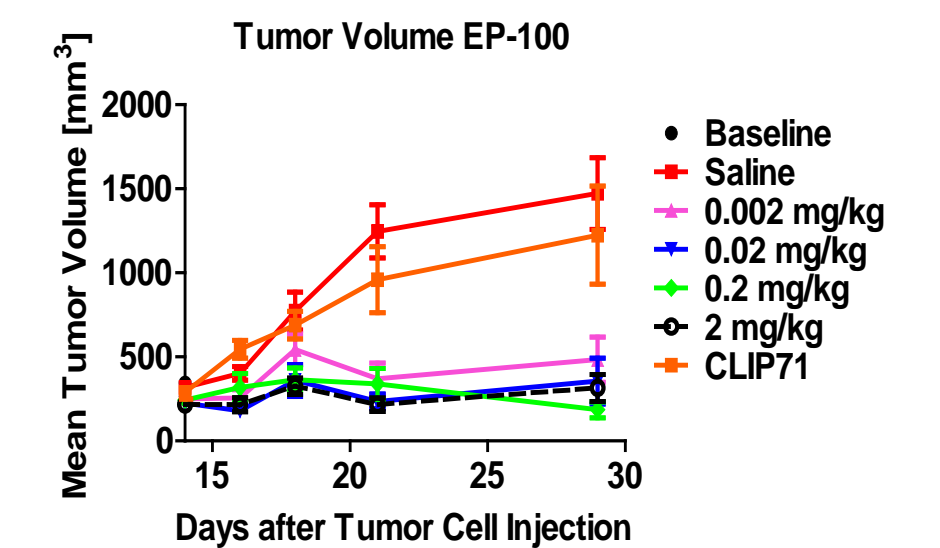


Figure 3: Efficacy of EP-100 in Prostate Cancer Xenografts: Effect of EP-100 on growth (volume) of PC-3 xenografts. Three weekly IV injections on Days 15, 22 and 29, N=11, $p < 0.002$ vs saline control and CLIP71. EP-100 is highly effective at doses as low as 0.002 mg/kg, human equivalent dose 0.006 mg/m².

Summary

1. EP-100 kills a wide variety of human cancer cells.
2. EP-100 kills cancer cells within minutes.
3. EP-100 specifically destroyed LHRH-receptor expressing and multi-drug resistant ovarian (OVCAR-3) and prostate (PC-3) cancer cells in vitro and in vivo.
4. Serum CA125 was reduced in treated animals implanted with ovarian cancer cells.
5. LHRH receptor levels were reduced in EP-100 treated tumors
6. EP-100 treated tumors were killed by necrotic mechanism
7. Treated tumors did not incorporate [¹⁸F]-FDG indicating that the cells were not viable.
8. EP-100 did not cause any adverse effects.
9. Bodyweights of treated mice were not affected.

Conclusion

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