# SYNERGISTIC ACTIVITY OF EP-100 AND CHEMOTHERAPIES IN CANCER CELL LINES


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## Abstract 978


EP-100 is an anti-cancer drug comprised of a membrane-disrupting peptide (MDP) fused to Luteinizing Hormone Releasing Hormone (LHRH) as a targeting moiety. It is currently in Phase 2 clinical trial in tumors that over-express LHRH receptors in combination EP-100 with paclitaxel. EP-100 kills cancer cells via direct membrane disruption. Combinations of EP-100 and paclitaxel, vinorelbine, doxorubicin, cisplatinum, vincristine and 5 fluorouracil (5FU) in two different cancer cell lines were tested to generate a list of potential combinations with EP-100. Half-lives of chemotherapeutics are typically more than 18 hours, whereas EP-100 has a half-life of 15 minutes. Sequential exposure studies were conducted *in vitro* to determine conditions that generate highest potency.

Cells were cultured in the presence of each single agent and in combination with EP-100 for 48 and 72h in non-constant and constant ratio formats (N=6). Viability was measured in luminometric assays. Data were analyzed as $IC_{50}$ values for each single drug and the combination with EP-100 (GraphPad Prism GraphPad Software). Combination indices (CI) on interaction of EP-100 and each chemotherapeutic compound were determined (CompuSyn Software).

The LHRH receptor positive, multi-drug resistant human uterine sarcoma cell line MES-SA-Dx5 and the human breast cancer cell line MDA-MB-231 were killed by EP-100 at low micromolar concentrations after 72 h . Both cell lines showed resistance and sensitization in combination with EP-100 for paclitaxel, vinorelbine, vincristine and cisplatinum. MES-SA-Dx5 cells were resistant to doxorubicin and the combination increased sensitivity. Both cell lines were resistant to 5FU ($IC_{50}$ values 72.4 5.2 µM and 24.3 3.3 µM) with no potentiation observed in combination. Combinations of EP-100 and paclitaxel, vincristine, vinorelbine, doxorubicin and cisplatinum resulted in potentiation of activity in a synergistic manner for both uterine sarcoma and breast cancer cell lines. The combination effects were synergistic with CI of less than 0.2. The sequence of exposure was most potent when EP-100 was given first followed by incubation with paclitaxel, doxorubicin or vinorelbine.

EP-100 and paclitaxel, doxorubicin, vinorelbine, vincristine and cisplatinum may be used in combination for treatment of LHRH receptor positive drug resistant cancers.


## Background

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. Esperance Pharmaceuticals is developing targeted Cationic Lytic Peptide (CLYP™) technology for killing cancer cells. It involves small targeted lytic peptides that seek and destroy cancer cells without harming normal cells. These peptides are linear, alpha helical, cationic and they directly interact with negatively charged membranes resulting in their disruption and 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 lytic peptides compared to normal cells [1], 2.Fast acting - causing necrosis within minutes through direct membrane interaction [2], a mechanism not likely to cause resistance, 3. is independent of intracellular uptake, 4. is independent of multi-drug resistance, 5. has a short half life *in vivo*, destroying cancer cells independent of cell division, and 6. no interaction with physiological pathways is required for its activity[3]. As a result of this technology, Esperance Pharmaceuticals is developing a new generation of ligand-lytic peptide conjugates that bind to target molecules that are over-expressed on cancer cells. Esperance's lead candidate, EP-100, consists of LHRH (10 amino acids) joined to a novel 18 amino acid lytic peptide payload designated CLIP-71 to target cancers that over-express LHRH receptors. EP-100 has completed a Phase 1 clinical trial, is now in Phase 2 clinical trial in combination with paclitaxel for treatment of advanced LHRH receptor positive ovarian cancer(NCT014858). LHRH receptors are over-expressed in a wide range of human cancers including breast, prostate, ovarian, endometrial, pancreatic, colon, renal, testicular, liver, adrenal cancers and hematological malignancies. EP-100 is manufactured by standard solid phase peptide chemistry to produce highly homogeneous and pure product that is highly water soluble. EP-100 is not immunogenic and has been well tolerated in humans.

Treatment of cancer with standard chemotherapeutics often leads to multi-drug resistance. One of the mechanisms of multi-drug resistance is due to overexpression of the MDR1 gene that leads to increased levels of Pgp in cancer cell membranes. P-gp, also known as MDR1 and ABCB1, is a 170 kDa integral plasma membrane protein that functions as an ATP-dependent drug efflux pump. Compounds that interact with P-gp can be identified as stimulators (substrates for transport) or inhibitors of its ATPase activity. Lytic peptides have been shown to increase potency of doxorubicin and paclitaxel in a synergistic manner probably due to inhibition of P-gp [6]. Compounds that bind to the Pgp-efflux pump include classical chemotherapeutic drugs (such as anthracyclines (doxil, doxorubicin), *Vinca* alkaloids (Vincristine), and taxols (Paclitaxel, Docetaxel). Other substrates are anticancer agents such as tyrosine kinase inhibitors, human immunodeficiency virus (HIV) protease inhibitors, immunosuppressants, ionophores, peptides, fluorescent dyes, steroids, cardiac glycosides, and many others including organic molecules, amphipathic and lipid soluble peptides [7]. Many substrates that bind to Pgp are positively charged at physiological pH. These include lytic peptides that are amphipathic and highly positively charged at physiological pH. The binding of lytic peptides to Pgp has been demonstrated (AACR 2012, Abstracts 3715 and 2829). In this study we tested the synergy of EP-100 with chemotherapeutic P-gp substrates: doxorubicin, vincristine, vinorelbine, paclitaxel andtwo non-Pg-p substrate chemotherapeutics 5-fluorouracil and cisplatinum in combination with EP-100 in a multi-drug resistance human uterine sarcoma model and a triple negative breast cancer cell line that overexpress LHRH receptors. The results will serve as a rationale for combinations with EP-100 for reverting drug resistance.

## Objectives

1. To determine single agent responses *in vitro* in multi-drug resistant cancer cell lines
2. To determine responses of cells to combination of EP-100 with standard chemotherapeutics
3. To determine responses to sequential or continuous exposure to combination of EP-100 and chemotherapeutics

## Materials & Methods

- **Cell lines** tested were human uterine sarcoma multi-drug resistant (MES-SA-Dx5) and the triple negative breast cancer (MDA-MB-231).
- **In vitro efficacy** studies were conducted in 96 well plate format (2,000 cells/well) with single agents ( EP-100 or Paclitaxel , 5-fluorouracil, doxorubicin, vinorelbine, vincristine, cisplatinum) and in combination with EP-100. Incubations were conducted for 72 h. Cell viability was determined using luminometric assays. Saline/Vehicle and 0.1 % Triton served as controls for 100 % viability and complete cell death.
- **Data were analyzed** as $IC_{50}$ values using the GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com for the Hill Equation and GraphPad InStat version 3.00 for Windows 95, GraphPad Software, San Diego California USA, www.graphpad.com for statistical analyses.
- The combination effects of EP-100 and chemotherapeutics were determined as Combination Index (CI) using the CompuSyn Software analysis program(Chou and Martin, CompuSyn software for drug combinations for general dose-effect analysis, ComboSyn, Inc Paramus NJ, 2007
- Combination Indices (CI of < 0.9 represent synergism, of 0.9-1.0 additive effects and > 1.0 antagonism

## Results – *in vitro* Studies

***Combination of EP-100 and cisplatinum is potentiated in two CDDP resistant cell lines but not for EP-100 and 5-fluorouracil – possible association with efflux pump***

| | MES-SA-Dx5 ($IC_{50}$ – µM) | Combination Index | Potentiation | MDA-MB-231 ($IC_{50}$ – µM) | Combination Index | Potentiation |
|---|---|---|---|---|---|---|
| EP-100 Alone | 3.04±0.5 | | | 1.4 ± 0.3 | | |
| CDDP | 54.5±5.8 | | | 19.9±6.2 | | |
| EP-100 (5 nM) +CDDP | 25.8±4.5 | 0.4 | 2.1 | 15.4±3.1 | 0.9 | 4.1 |
| EP-100 (50 nM) +CDDP | 23.5±4.0 | 0.6 | 2.3 | 15.1±2.4 | 0.1 | 9.1 |
| EP-100 (500 nM) +CDDP | 8.0±2.6 | 0.2 | 6.8 | 8.1±1.9 | 0.1 | 2.5 |
| 5-Fluorouracil (5-FU) | 72.4±5.2 | | | 24.3±3.3 | | |
| EP-100 (5 nM) +5-FU | 67.3±5.3 | NA | 1.1 | 22.1±7.5 | NA | 1.1 |
| EP-100 (50 nM) +5-FU | 65.1±3.2 | NA | 1.1 | 19.4±5.2 | NA | 1.2 |
| EP-100 (500 nM) +5-FU | 65.6±5.1 | NA | 1.1 | 19.5±4.3 | NA | 1.2 |

**Table 1:** EP-100 sensitizes multi-drug resistant cancer cells expressing LHRH receptors resulting in potentiation up to 7 fold in a synergistic manner in combination with CDDP. The sensitization is dose dependent for CDDP but not for 5-FU. Combinations of EP-100 and CDDP were highly synergistic with CI < 1. The tested anti-cancer drugs are not Pgp-inhibitors.

## Results ctd.

***EP-100 synergizes in combination with Paclitaxel, Vincristine, Vinorelbine and doxorubicin***

| | MES-SA-Dx5 ($IC_{50}$ – nM) | Combination Index | Potentiation | MDA-MB-231 ($IC_{50}$ – nM) | Combination Index | Potentiation |
|---|---|---|---|---|---|---|
| EP-100 Alone | 3044±558 | | | 1400 ± 300 | | |
| Paclitaxel (PTX) | 94.1±0.3 | | | 86.2±5.9 | | |
| EP100 (5 nM) +PTX | 33.7±2.2 | 0.7 | 2.8 | 22.3±0.5 | 0.43 | 4 |
| EP100 (50 nM) +PTX | 0.026±0.001 | 0.18 | 3620 | 17.9±0.6 | 0.25 | 4.8 |
| EP100 (500 nM) +PTX | 0.014±0.002 | 0.001 | 6720 | 15.9±0.8 | 0.22 | 5.4 |
| Vincristine (VCR) | 124.7±16.3 | | | 26.3±4.1 | | |
| EP-100 (5nM) +VCR | 53.5±4.7 | 0.23 | 2.3 | 6.4±1.5 | 0.9 | 4.1 |
| EP-100 (50 nM) +VCR | 40.1±8.1 | 0.16 | 3.1 | 2.95±0.3 | 0.1 | 9.1 |
| EP-100 (500 nM) +VCR | 5.7±0.9 | 0.02 | 21.7 | 1.8±0.1 | 0.09 | 15 |
| Vinorelbine (Vin) | 63.22±15.9 | | | 23.7±3.4 | | |
| EP-100 (5nM) +(Vin) | 13.5±3.3 | 0.14 | 4.8 | 8.5±1.4 | 0.38 | 2.8 |
| EP-100 (50 nM) +(Vin) | 13.8±2.3 | 0.1 | 4.8 | 3.1±0.7 | 0.14 | 7.6 |
| EP-100 (500 nM) +(Vin) | 6.0±1.6 | 0.06 | 10.5 | 3.1±0.9 | 0.1 | 7.6 |
| Doxorubicin (Dox) | 32.6±4.3 | | | 1.26±0.4 | | |
| EP-100 (5 nM) +(Dox) | 12.2±0.8 | 0.7 | 2.6 | 1.14±0.07 | NA | 1 |
| EP-100 (50 nM) +(Dox) | 5.5±0.2 | 0.2 | 6.4 | 1.2±0.2 | NA | 1 |
| EP-100 (500 nM) +(Dox) | 3.3±0.6 | 0.09 | 9.6 | 1.1±0.04 | NA | 1 |

**Table 2:** EP-100 sensitizes multi-drug resistant cancer cells expressing LHRH receptors resulting in potentiation of cell killing in a synergistic manner. The sensitization is dose dependent for paclitaxel (PTX), vincristine (VCR), vinorelbine (Vin) and doxorubicin (Dox). Cell viability was determined after 72 hours using a luminometric viability assay. Saline and 0.1% Triton served as controls. Combinations of EP-100 and PTX, VCR, Vin and Dox were highly synergistic with CI < 1. The tested anti-cancer drugs are known Pgp-inhibitors.

## Results ctd.

***The potency of the combination of EP-100 and Paclitaxel or EP-100 and Vinorelbine is dependent on the order of administration and is highest for long term exposure or when EP-100 is given first***

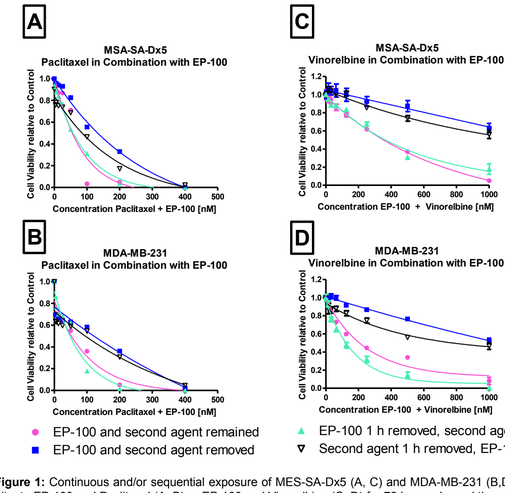


**Figure 1:** Continuous and/or sequential exposure of MES-SA-Dx5 (A, C) and MDA-MB-231 (B,D) cell lines *in vitro* to EP-100 and Paclitaxel (A, B) or EP-100 and Vinorelbine (C, D) for 72 hours showed the maximal effect. The smallest effect was seen when the supernatant was replaced by culture media after 1 hour and the cell viability determined after 72 h. Sequential incubations with EP-100 exposure first were comparable to the constant exposure with both reagents whereas removal of either Paclitaxel or vinorelbine followed by EP-100 resulted in low efficacy profiles similar to the short term exposure of the combinations. compared to 1 h incubations with either EP-100 and Paclitaxel alone followed by 72 h incubation with the second agent. Constant ratio was given 1:20 for Paclitaxel and EP-100 and 1:11 resp 1:4 for Vinorelbine and EP-100. (N=6). EP-100 sensitizes multi-drug resistant cell lines.

## Summary

- ✓EP-100 increased the potency of Paclitaxel, doxorubicin, vincristine, vinorelbine and cisplatinum in drug resistant cancers
- ✓The effect was synergistic and dependent on LHRH receptor expression
- ✓Potency was increased up to 6000-fold compared to the single agent Paclitaxel
- ✓The potency of the combination of EP-100 and Paclitaxel or EP-100 and Vinorelbine was dependent on the sequence of administration and was highest for long term exposure and when EP-100 was added first
- ✓Among chemotherapeutics tested, Pgp substrates and cisplatinum synergized with EP-100
- ✓ 5-fluorouracil did not synergize with EP-100

## Conclusion

EP-100 sensitizes multi-drug resistant cancer cells expressing LHRH receptors to chemotherapeutics. The combination effect is highly synergistic and requires nanomolar concentrations of EP-100. These results indicate that EP-100 can be a potent anti-cancer agent when used in combination with doxorubicin, paclitaxel, vincristine, vinorelbine or cisplatinum.

## References


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