A Peptide-Paclitaxel Conjugate outperforms Paclitaxel in breast and ovarian cancer cell models where TRPV6 is over-expressed.

N. Bartlett[1], S. Gormley[2], V. Lloyd[1], T. Lutes[2], S. Ramsaywack[3], C. Rice[2], J. Stewart[2], S. Westcott[3].
1.Department of Biology, Mount Allison University, Sackville NB, Canada. 2. Soricimed Biopharma Inc., Sackville, NB, Inc.
3. Department of Chemistry and Biochemistry, Mount Allison University, Sackville, NB, Canada

ABSTRACT: The concept of Peptide-Drug Conjugates is similar to that behind Antibody Drug Conjugates. A peptide that strongly binds to a cancer-specific surface target is used to deliver a covalently linked drug directly to the tumour. Synthetic peptides may overcome some difficulties of antibodies for drug delivery (characterization, stability, regulatory, PK parameters). A series of synthetic peptides derived from the C-terminus of soricidin, the paralytic peptide in the saliva of the Northern Short-tailed shrew (*Blarina brevicauda*), bind strongly (EC_{50} ~ 14 - 60 nM) to transient receptor potential vanilloid family, six (TRPV6). This calcium channel is highly up regulated in many epithelial cancers (breast, ovarian, prostate, etc.) with little TRPV6, if any, in most healthy tissues. We conjugated Paclitaxel to one of our peptides and compared the effect of this PDC on cell cultures of breast (MCF-7, T 47D, MB-468) and ovarian (OVCAR-3, SKOV-3 and CaOV-3) cancers at 10 – 15 nM. The PDC outperforms Paclitaxel at decreasing cell viability by 30-50% in four of the six cell lines; CaOv3 and OVCAR-3 responded to the same degree as the drug alone. Xenograft models with TRPV6-rich tumours in mice are presently underway. These observations support the idea that our proprietary C-peptides provide a drug delivery platform for TRPV6-rich tumours that is strictly chemically characterized, easily synthesized and highly adaptable. We expect this platform will accommodate a number of different drugs of interest.

Introduction

Many epithelial cancer types over-express TRPV6, a non-voltage gated calcium channel (Zhuang et al., 2002). Inhibition of the TRPV6 channel activity is anti-neoplastic and Soricimed has a Phase I clinical trial underway in Canada and the USA for such an inhibitor. The subject of the clinical trial is a fragment of a parent paralytic peptide isolated from the saliva of the Northern Short-tailed shrew (*Blarina brevicauda*). A peptide targeted to TRPV6 (in this case SOR-C27) can deliver MRI enhancing reagents and fluorescent imaging reagents to TRPV6-rich tumours.

Figure 1 (right) shows tumour fluorescence after administration of SOR-C27-cy5.5 to ovarian (left, SKOV-3) and prostate (right, DU145) xenografts in mice. The image is a digital plane through the centre of the tumour masses and shows accumulation of the tagged peptide (Bowen et al., 2013). Similar studies with Peptide-SPIO nanoparticles showed retention in the tumour and negative MRI enhancement of the tumour mass.

The logical next step in this process was to pursue delivery of a drug to the tumour site by way of a peptide drug conjugate (PDC) and observe the effects on growth behaviour.

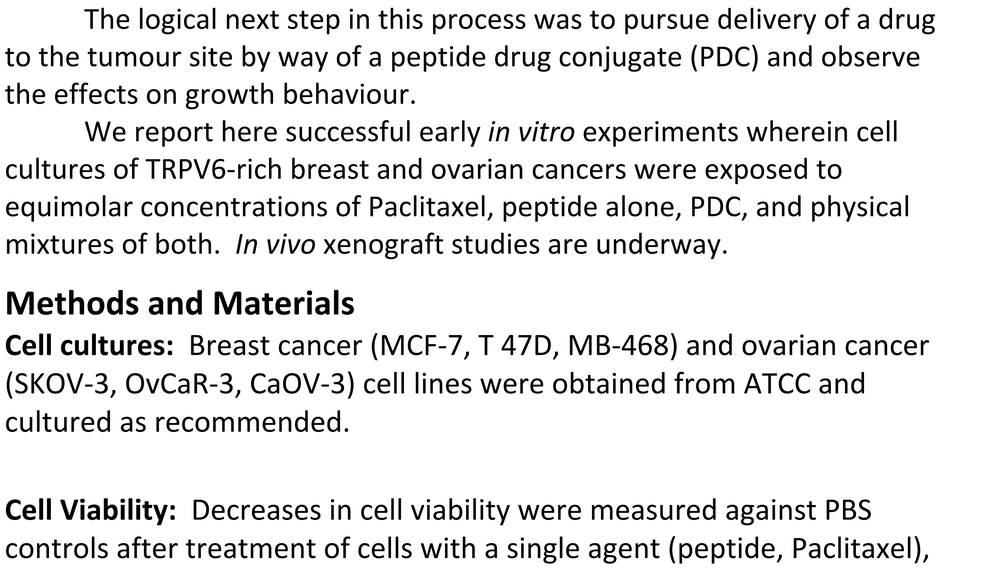
We report here successful early *in vitro* experiments wherein cell cultures of TRPV6-rich breast and ovarian cancers were exposed to equimolar concentrations of Paclitaxel, peptide alone, PDC, and physical mixtures of both. *In vivo* xenograft studies are underway.

Methods and Materials

Cell cultures: Breast cancer (MCF-7, T 47D, MB-468) and ovarian cancer (SKOV-3, OvCaR-3, CaOV-3) cell lines were obtained from ATCC and cultured as recommended.

Cell Viability: Decreases in cell viability were measured against PBS controls after treatment of cells with a single agent (peptide, Paclitaxel), mixture of agents (peptide plus Paclitaxel) and a peptide-Paclitaxel conjugate. Cell viability was measured with CellTitre Blue. Data were expressed as percentage of controls. PDC and Paclitaxel containing treatments were compared with a simple Students' t-test.

Peptide-Paclitaxel Conjugate: (Kirschberg et al., 2003)



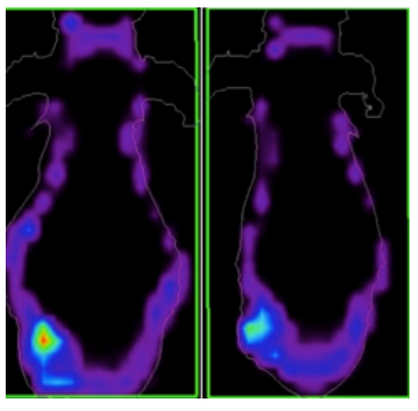


Figure 1: Digital plane through centre of ovarian (Left, SKOV-3) and prostate (Right, DU 145) xenografts imaged with Peptide-Cy5.5 conjugate.

Results

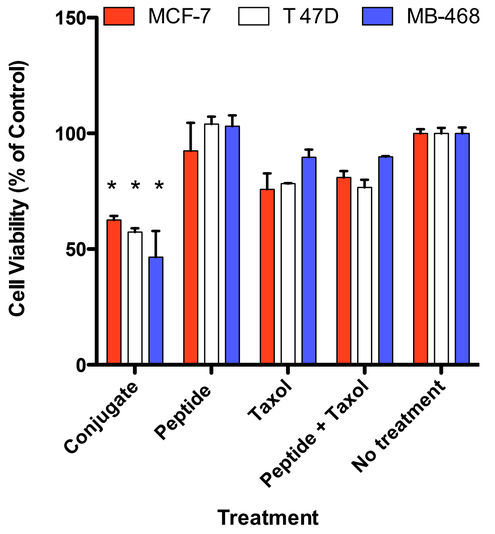


Figure 2: Decreased proliferation of breast cancer cell lines compared to PBS controls. The concentrations of the treatments were equimolar (10 nM). The data are mean ± SEM, n = 3. '*' indicates p<0.05 compared to Paclitaxel alone

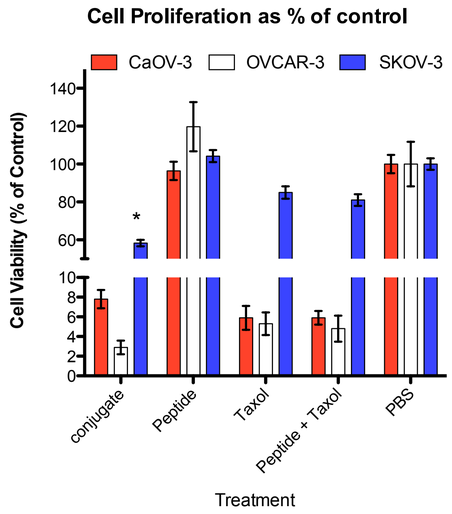


Figure 3: Decreased proliferation of ovarian cancer cells compared to PBS controls. Concentrations were 10 nM but 15 nM for SKOV-3. The data are mean ± SEM, n = 3. '*' indicates p<0.05 compared to Paclitaxel alone

Conclusions

- **SOR-C27 can deliver and accumulate conjugated fluorescent tag (cy5.5) to TRPV6-rich tumours (Figure 1: Bowen et al., 2013).**
- **The C-peptides, represented by SOR-C27, provide a targeting mechanism by which conjugated drugs (Paclitaxel) can be delivered to TRPV6-rich cancer cells. The PDC is more effective at reducing cell viability in breast and ovarian cancer cell cultures by about 2-fold over equimolar Paclitaxel alone (Figures 2 and 3).**
- **This peptide could carry any drug of choice, could be engineered to have a defined drug load or could accommodate multiple drugs.**

References:
Bowen, C.V., DeBay, D., Ewart, H.S., Gallant, P., Gormley, S., Ilenchuk, T.T., Iqbal, U., Lutes, T., Martina, M., Mealing, G., Merkley, N., Perker, S., Mereno, M.J., Rice, C., Syvitski, R.T., Stewart, J.M. 2013. *In vivo* Detection of Human TRPV6-Rich Tumors with Anti-Cancer Peptides Derived from Soricidin. PLOS ONE 10.1371/journal.pone.0058866
Kirschberg, T.A., VanDeusen, C. L., Rothbard, J. B., Yang, M., Wender, P. A.2003. Arginine-based molecular transporters: the synthesis and chemical evaluation of releasable taxol-transporter conjugates. *Org. Lett.* *5*: 3459–3462.
Zhuang L, Peng JB, Tou L, et al. 2002. Calcium-selective ion channel, CaT1, is apically localized in gastrointestinal tract epithelia and is aberrantly expressed in human malignancies. Lab Invest 82:1755 – 1764.

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