#514: Targeting urothelial neoplasia using an investigational virus-like drug conjugate

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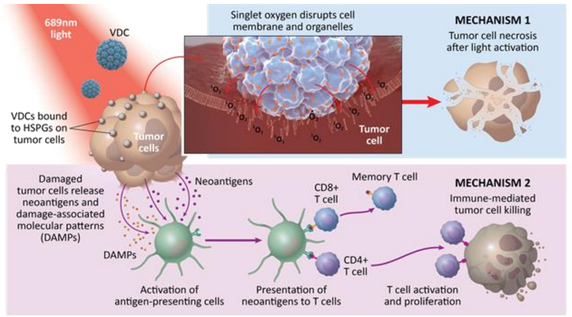
Background

- Human papillomavirus virus-like particles (HPV VLP) preferentially target tumor cells via cell surface modified heparan-sulfate proteoglycans (HSPG).[1]
- AU-011 is an investigational virus-like drug conjugate composed of an HPV modified VLP and a light activatable small molecule.[2]
- Upon activation with near infrared light (nIR), AU-011 causes acute *in vivo* tumor cytotoxicity in a murine flank model using bladder cancer cells (MB49luc). AU-011 treatment results in the activation of cell-mediated anti-tumor immunity capable of preventing tumor recurrence.[3]

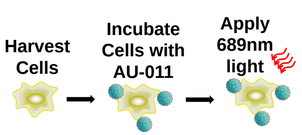


Study Goal

To further explore the use of AU-011 using human bladder cancer cell lines, human bladder biopsy samples and an in-situ murine model of bladder cancer using intravesical delivery.

Methods

- *In vitro* binding and cytotoxicity of AU-011 was assessed using a panel of six human bladder cancer cell lines *in vitro.*



- Binding and distribution of VLPs using human bladder tumor biopsy samples *ex vivo* +/- pre-treatment with hyaluronidase I or Hylenex® to remove the glycocalyx layer. Tissues were stained with an antibody against the VLP.
- Tumor distribution of AU-011 *in vivo* 12 hours after intravesical instillation in the orthotopic MB49luc murine model. Pre-treatment with hyaluronidase (Hylenex®) or formulation of AU-011 with the polyamide Syn3 were tested. Tissues were stained with an antibody against AU-011.

- **AU-011 is a virus-like drug conjugate composed of a modified HPV VLP conjugated with a light activatable small molecule.**
- **AU-011 is capable of binding human bladder cancer cell lines of varying disease stages and is cytotoxic upon nIR activation.**
- **The VLP can bind to human bladder cancer biopsy samples and its distribution is enhanced with the use of hyaluronidase.**
- **AU-011 was detected in murine bladder tumors after intravesical instillation and the accumulation was enhanced with hyaluronidase pre-treatment or when co-delivered with Syn3.**

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References

1. Kines and Cerio, et al. Int. J Cancer, 138(4):901-11, 2016.
2. Kines, et al. Mol Cancer Ther, 17(2):565-574, 2018.
3. Kines et al. Can Immunol Res 9(6):693-706, 2021.

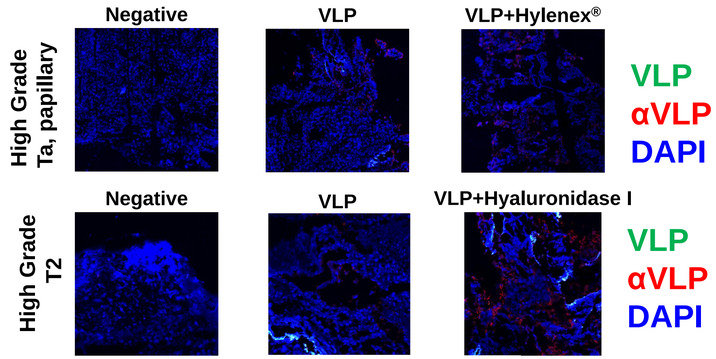
Results

AU-011 binding and potency using human bladder cancer cell lines:

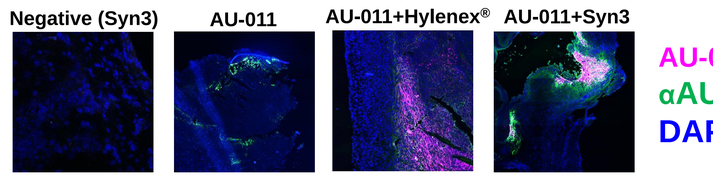
Cell Name	Cell Type	EC_{50} Binding (pM)	EC_{50} Potency (pM)
5637	Grade II carcinoma	24.04	49.9
RT4	Transitional cell papilloma (pT1, grade I-II)	52.79	41.31
SW780	Transitional cell carcinoma (grade I)	15.02	29.89
UM-UC-3	Transitional cell carcinoma (grade III)	21.01	16.66
HT-1179	Transitional cell carcinoma (grade IV)	52.52	62.75
TCCSUP	Transitional cell carcinoma (grade IV)	13.24	36.00

VLP (AF488 dye) distribution in human biopsy samples *ex vivo*:

*VLP AF488 is a surrogate for AU-011 with similar physiochemical properties since AU-011 does not fluoresce strongly



AU-011 distribution after intravesical administration *in vivo* using an orthotopic murine model for bladder cancer (MB49luc):



Future Directions

- Explore AU-011 distribution and efficacy in a rat bladder tumor model that mimics natural disease progression
- Develop *in vitro* methods to understand the role of the glycocalyx and AU-011 tumor targeting.
- Further characterize AU-011's tumor targeting ability using human tumor biopsy samples and potential use in NMIBC.