Poster #1730

A novel chimeric virus-like drug conjugate for the potential treatment of HPV+ tumors

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ABSTRACT

Introduction: Human papillomavirus (HPV) virus-like particles (VLPs) bind to a wide variety of tumor types via modified glycosaminooglycans (GAGs) found on the tumor cell surface. This finding led to the development of the investigational virus-like drug conjugate (VDC) belzupacap sarotalocan (bel-sar, formerly AU-011), an HPV-derived VLP conjugated to a light-activated cytotoxic payload. When activated by near-infrared light, bel-sar induced rapid tumor necrosis resulting in pro-immunogenic cell death, release of tumor neoantigens and long-term anti-tumor immunity. When HPV16 E6 and E7 expressing TC-1 mouse tumors were treated with the VDC, E7-specific T-cells were detected in the absence of provided tumor antigens. A novel chimeric VDC (cVDC) is now in development, in which E6 and E7 are fused to the L2 capsid protein as a means to potentially further enhance the observed anti-tumor response. This cVDC could allow for the targeted cytotoxicity of HPV-positive tumors in addition to the release of supplemental tumor antigens E6 and E7 within the now pro-immunogenic tumor milieu, potentially leading to increased long term anti-tumor response.

Methods: The detoxified sequences of E6 and E7 were engineered as one fusion polypeptide on the C-terminus of the L2 minor capsid protein. Both L2/E6/E7 and L2/E7/E6 protein expression vectors were generated to determine if the order of the proteins impacted L2's ability to co-assemble with L1, the major capsid protein. The plasmids were co-expressed alongside L1 using the mammalian 293TT expression system. cVLPs were conjugated with the light-activated payload and their binding and potency were measured using HPV16+ human tumor cell lines.

Results: Both the L2/E6/E7 and L2/E7/E7 fusion proteins were expressed and co-assembled with L1 into chimeric VLPs. Fusion protein expression was validated by western blots for L2, E6 and E7, and VLPs were confirmed by electron microscopy. Binding and potency were comparable to wild-type L2 containing VDCs and they retained their glycosaminoglycan targeting specificity.

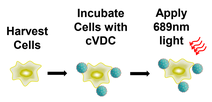
Conclusions: Preliminary data indicate that chimeric VDCs containing E6 and E7 can successfully be generated using the 293TT mammalian expression system. Studies evaluating the cytotoxicity and E6 and E7 immunogenicity of the cVDC as well as the impact on tumor targeting are underway.

STUDY GOAL

Generate near infrared (nIR) dye conjugated chimeric VLPs (cVDC) containing capsid protein L1 and capsid protein L2 expressed as a fusion polypeptide with the HPV16 "detoxified" tumor antigens E6 and E7 (rendered no longer oncogenic). The goal is to use the cVDC as a means of delivering the tumor antigens, E6 and E7, to HPV16+ tumors to further enhance the anti-tumor immunity generated by cVDC binding and light activation.

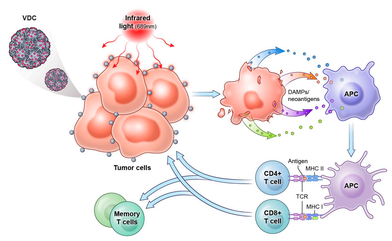
METHODS

- Generate detoxified polypeptide sequences of L2-E6-E7 and L2-E7-E6 and confirm that, when co-expressed with L1, VLPs can form and the full fusion polypeptide is incorporated within the chimeric VLP (cVLP).
- Conjugate the cVLP with a nIR photoactivatable dye and ensure tumor binding specificity and cytotoxicity is retained.
- *In vitro* binding and cytotoxicity of AU-011 was assessed using a panel of HPV16+ human cancer cell lines and TC-1, a murine tumor line expressing HPV16 E6 and E7. Binding and potency EC_{50} values were generated and glycosaminoglycan (GAG) targeting was assessed by inclusion of heparin in the binding assay.



BACKGROUND

- Human papillomavirus virus-like particles (HPV VLP) preferentially target tumor cells via specifically modified glycosaminoglycans (GAG) on the cell surface.[1]
- Belzupacan saratocalan (Bel-sar) is an investigational virus-like drug conjugate (VDC) composed of a modified HPV VLP and a nIR activatable small molecule.[2]
- Upon activation with nIR light, AU-011 causes acute tumor cytotoxicity *in vitro* and *in vivo*.
- Bel-sar mediated tumor cell death is highly inflammatory and leads to an upregulation of markers of immunogenic cell death. Strong anti-tumor immunity against tumor neoantigens (e.g. E6, E7) is induced capable of generating long-term protection from tumor rechallenge.[2,3]



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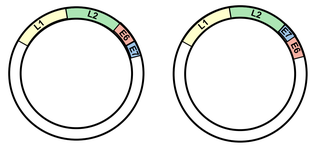
2. Kines, R., et al. *Mol Cancer Ther*, 17(2):565-574, 2018.
3. Kines, R., et al. *Can Immunol Res* 9(6):693-706, 2021.
4. Pastrana, D., et al. *Virology* 321(2):205-16, 2004.
5. https://github.com/BUCK-LCO-NCI/Codmod_as_different_as_possible
6. Peng, S., et al. *Mbio* 12(1): e03224-20, 2021.

RESULTS

1. Cloning strategy:

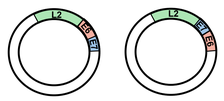
- Use full length HPV16L2 (L2wt) and remove stop codon.
- Add detoxified E6E7 or E7E6 to the C-terminus of L2.
- The HPV16 E6 and E7 genes were codon optimized[4,5] and the following mutations/deletions were made for the purposes of rendering the oncoproteins inert (detoxified, dx)[6]:
  - 16dxE6 **C63G** and **C106G** (two zinc finger domains can't degrade p53, prevents immortalization; prevents telomerase activation); C-terminal 5 residues deleted (Δ147-151; PDZ domain).
  - 16dxE7 **C24G** and **E26G** (Rb binding) and **C91G** (Zinc finger/immortalization/HDAC, c-jun, BRA1).
- Bicistronic (L1/L2) and L2 only constructs were generated

Bicistronic (L1/L2)



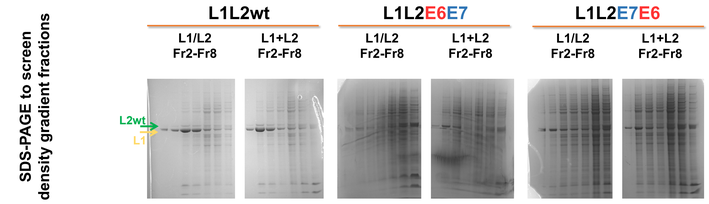
L2 only

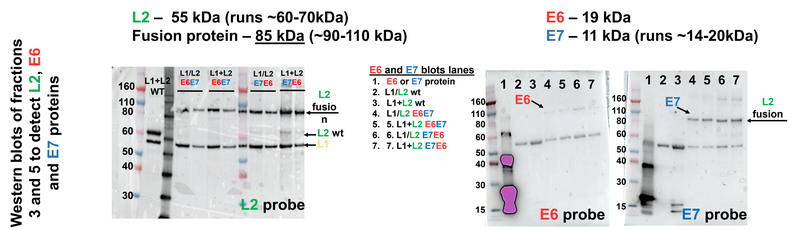
allows for adjusting L1:L2 ratio with L1 plasmid co-transfection

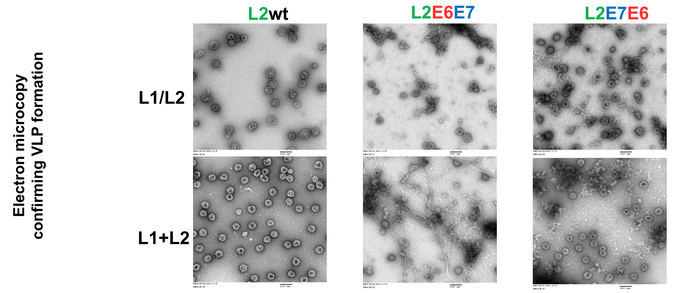


2. Protein expression and characterization:

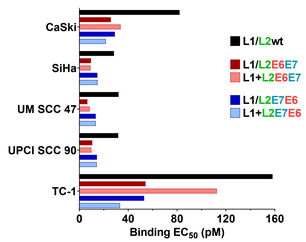
- 293TT cells were transfected with bicistronic plasmids (L1/L2) or L1 and L2 plasmids (L1+L2).
- Cell lysates were purified over density gradient and the collected fractions were screened by SDS-PAGE
- Samples were tested by western blot to determine if E6 and E7 were expressed and incorporated in the cVLP.
- Electron microscopy to verify VLP formation

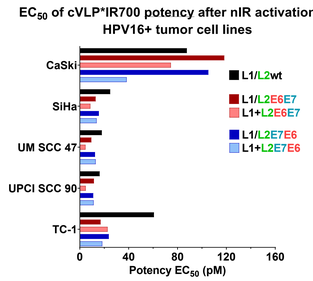




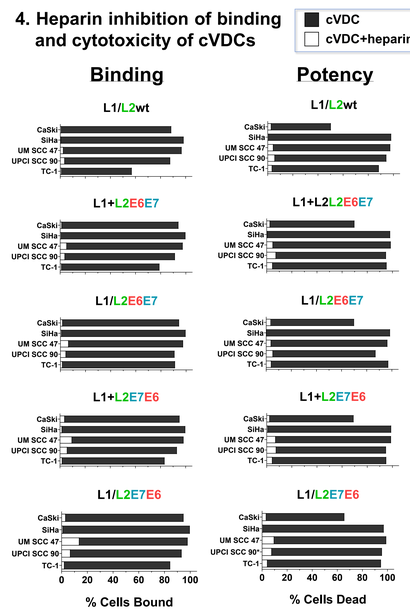


3. Binding and cytotoxicity of cVDCs





4. Heparin inhibition of binding and cytotoxicity of cVDCs



CONCLUSIONS

- Virus-like particles containing the fusion polypeptides of L2-E6-E7 and L2-E7-E6 could successfully be generated using the 293TT mammalian cell expression system.
- The presence of the fusion polypeptides was confirmed by western blot and the VLPs were visualized using electron microscopy.
- The chimeric VLPs were successfully labeled with the nIR photoactivatable molecule and were comparable to L2wt VLPs.
- The chimeric virus-like drug conjugates (cVDC) bound a panel of HPV16+ human tumor cell lines, as well as the murine E6/E7+ tumor line, TC-1. Upon activation with nIR light, the cVDCs demonstrated comparable potency to the L2wt VDC.
- Binding and potency of the cVDCs was heparan sulfate dependent, as both were inhibited by heparin indicating that, 1) incorporation of the polypeptide L2-E6-E7 or L2-E7-E6 into the VLP and 2) labeling with the photosensitizer did not affect tumor GAG tropism.

FUTURE DIRECTIONS

- Estimate L2 occupancy in the cVLP preparations and alter the ratio of L1 and L2 plasmid in order to achieve full occupancy of the cVLP.
- Examine immunogenicity of the cVLPs and cVDCs *in vivo* to determine if they are able to generate E6 and E7 immune responses
- Use the cVDCs to treat TC-1 tumors *in vivo* and compare their efficacy to the L2wt VDCs to determine if the delivery of tumor antigens, E6 and E7, improves overall survival, enhanced E6 and E7 T-cell responses, and long-term tumor protection.