NORTHERN ARIZONA UNIVERSITY

VACCINES OF THE FUTURE:
Why do we need better HPV vaccines?

Using Self-Assembling Peptides (Q11 and KFE8) as a Platform to Create New HPV Vaccine Candidates

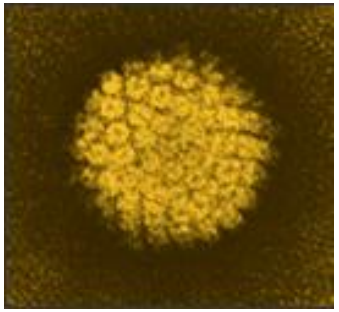
Crystal Morales, BS
Graduate Assistant, Department of Chemistry and Biochemistry
PI: Naomi Lee, PhD

National Institute of General Medical Sciences

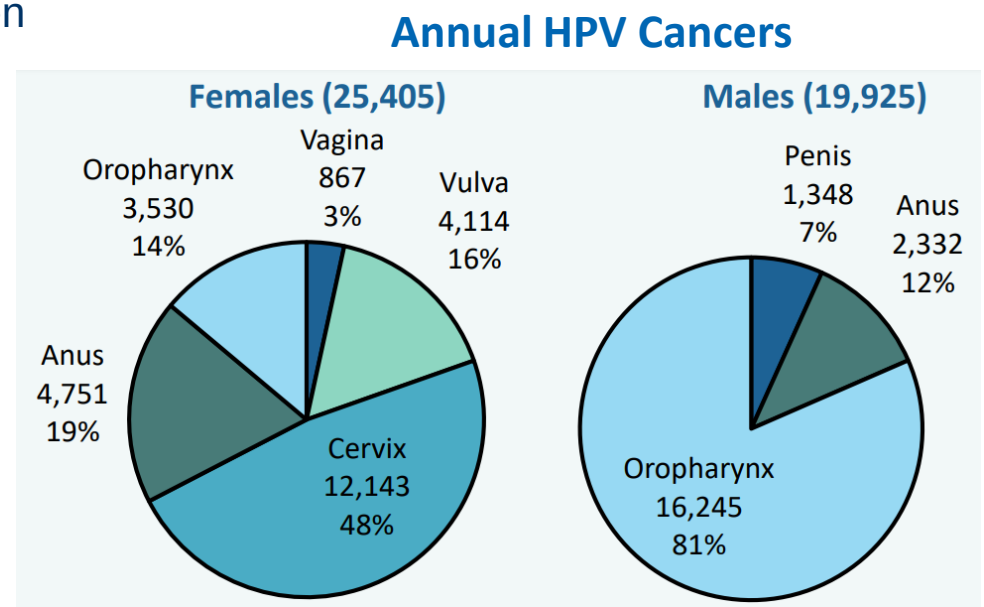
NIH Funded by NIH/NIMHD RCMI U54MD012388 and RISE 1R25GM127199-01 and NACP NCI grant U54CA143925.

HUMAN PAPILLOMAVIRUS IS THE MOST COMMON STI

- Over 150 distinct types have been identified.
  - Most are non-oncogenic (about 30 can cause warts)
  - 14 oncogenic/high risk types associated with cancer
  - You can only prevent HPV infections; there are no antivirals
- Two HPV types (16 and 18) account for most (~70%) cervical cancers
- No FDA approved HPV screening for men



Electron micrograph of human papillomavirus (HPV). Courtesy of NCI. 1986.



Meites, Elissa, et al. *Human Papillomavirus*. 2019, www.cdc.gov/vaccines/pubs/pinkbook/hpv.html. Accessed 12 Nov. 2020.

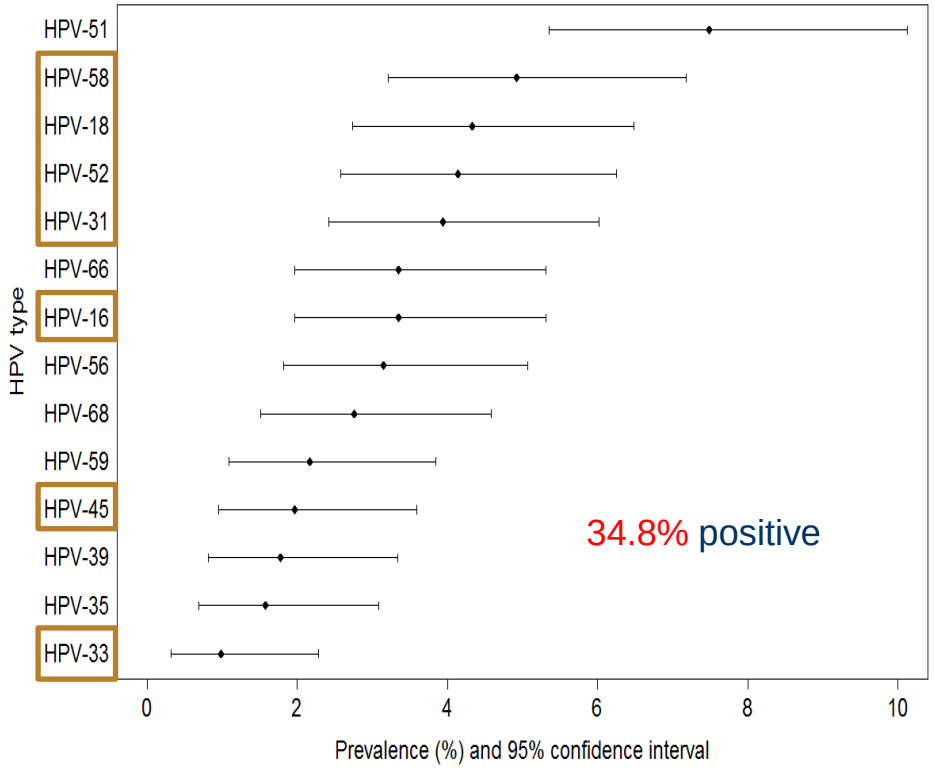
CDC, 2021. https://www.cdc.gov/cancer/uscs/about/data-briefs/no18-hpv-assoc-cancers-UnitedStates-2013-2017.htm

Lee, N. R., et al (2019). Human Papillomavirus Prevalence Among American Indian Women of the Great Plains. *Journal of Infectious Diseases, 219* (6), 908-915

NATIVE WOMEN FROM THE GREAT PLAINS HAVE A HIGH PREVALENCE OF HPV

- ~25% of women in the US are positive for HPV that causes cancer
- Native women are not usually included in national studies
  - Only a few studies
  - 22.2% of Hopi women tested positive
- 34.8% of Native women from Great Plains were positive (2014-2015)
  - Largest study to assess HPV in Native women (n=700)

Great Plains Tribal Community

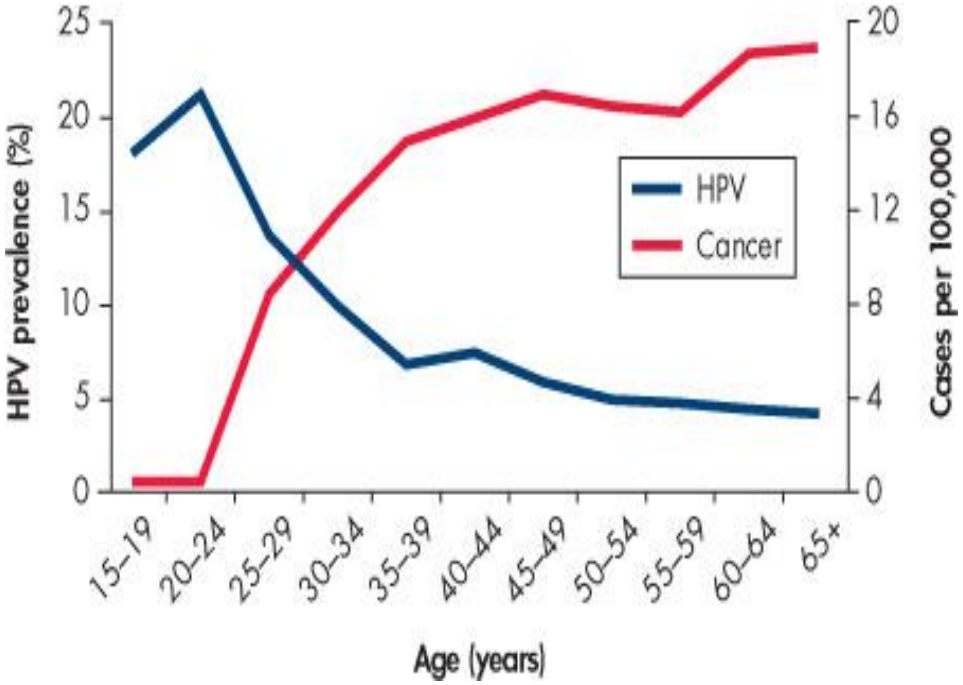


HPV types covered by the current vaccine

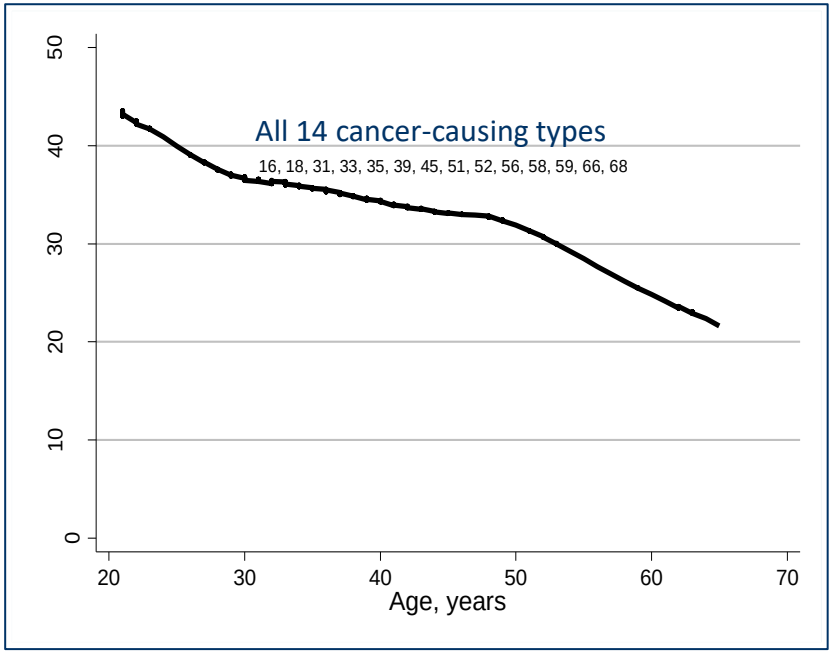
Lee, N. R., et al (2019). Human Papillomavirus Prevalence Among American Indian Women of the Great Plains. *Journal of Infectious Diseases, 219* (6), 908-915.

OLDER NATIVE WOMEN (30-65 YEARS) ARE NOT CLEARING HPV INFECTIONS EFFICIENTLY

AGE IS A RISK FACTOR FOR CANCER



GREAT PLAINS TRIBAL COMMUNITY

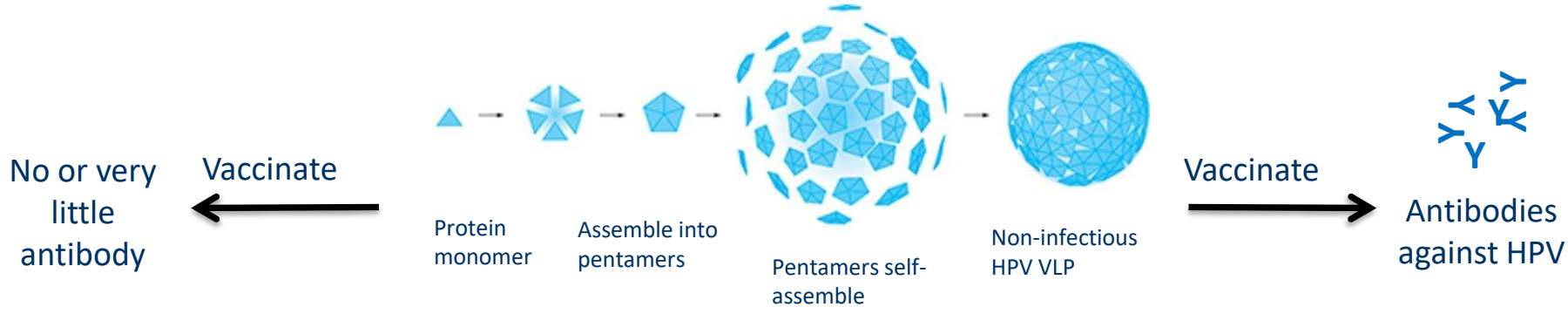


- Older women weren't eligible to receive the vaccine
- Older women are more at risk for developing cancers due to prolonged infections

Lee, N. R., et al (2019). Human Papillomavirus Prevalence Among American Indian Women of the Great Plains. *Journal of Infectious Diseases, 219* (6), 908-915.
Wheeler CM et al. A Population-based Study of HPV Genotype Prevalence in the United States: Baseline Measures Prior to Mass HPV Vaccination. International Journal of Cancer. 2013;132(1):1-19.

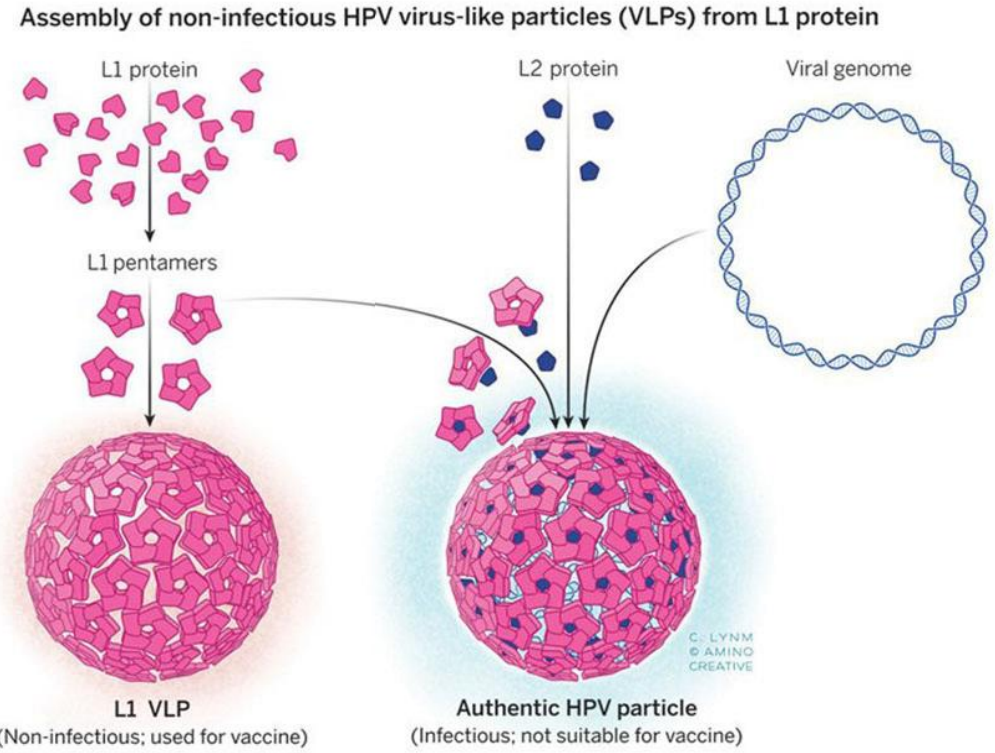
VACCINE - AM I BEING INJECTED WITH THE VIRUS?

- **HPV vaccines are made of virus-like particles (VLPs)**
- Your body thinks, “If it looks like a virus, it must be a virus!!”
  - (but it's harmless)
- Protects against 9 types of HPV -> 7 high risk + 2 low risk (genital warts)
- Most common platform because it creates durable and long-lasting antibodies
- **Downside:** VLPs require constant refrigeration, any fail in keeping VLP’s at 2-8° C can compromise the integrity and potency of the vaccines (proteins unfold)



TARGET: HPV CONSENSUS SEQUENCE

- HPV VLP's are made from 2 different capsid proteins – the Major protein that can form a VLP all by itself, and the minor protein.
- Major structural protein (L1) is specific to each type of HPV, the current vaccine 9 different types of L1 VLPs
- New research shows minor structural protein (L2) is conserved across HPV types
- **Consensus sequence** = peptide sequence that provides potential coverage against a range of HPV types

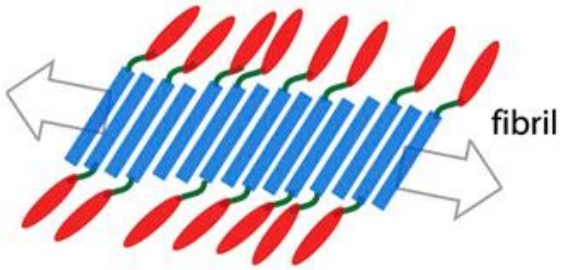


Factors that govern the induction of long-lived antibody response. Bryce Chackerian and David S. Peabody. Review 2020
Gambhira, Ratish, et al. "A Protective and Broadly Cross-Neutralizing Epitope of Human Papillomavirus L2." *Journal of Virology*, vol. 81, no. 24, 10 Oct. 2007, pp. 13927–13931, 10.1128/jvi.00936-07. Accessed 23 Nov. 2020.

THE FUTURE HPV VACCINE

HYPOTHESIS: SELF-ASSEMBLING β-SHEET PEPTIDES ALONGSIDE WITH HPV CONSENSUS STRAND WILL CREATE A MORE STABLE, BROADLY NEUTRALIZING VACCINE

- Mice have also been immunized with the HPV minor protein consensus strand, resulting in antibodies against a **broad range of HPV types**
- Studies with mice being vaccinated with peptides showed that antibodies form against peptides + antigen
  - **T-cell stimulation caused long lasting (6 months) antibodies**



Blue = peptide β-strand
Red = HPV consensus strand

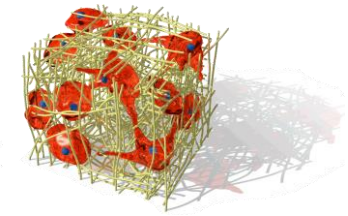
Fuaad, "Polymer-peptide hybrids as a highly immunogenic single-dose nanovaccine."
Eskandari, "Recent advances in self-assembled peptides: Implications for targeted drug delivery and vaccine engineering."
Stephen " Protective Antibody and CD8+ T-Cell Responses to the *Plasmodium falciparum* Circumsporozoite Protein Induced by a Nanoparticle Vaccine"
Roden,"Minor Capsid Protein of Human Genital Papillomaviruses Contains Subdominant, Cross-Neutralizing Epitopes." *Virology*,

PEPTIDE VACCINE – BETTER OPTION?

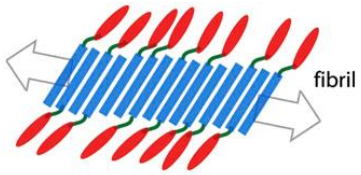
- Need for stable vaccines in range of temperatures to create easier access
- Peptides are able to withstand range of temperatures in or aqueous solutions or as powder form and their immunogenicity is not affected
  - **Q11 self assembles and is stable after 1 week of heating at 45° C – no changes in CD or TEM, mice still have antibodies against Q11**
- Self assembling peptides are easier and faster to make compared to VLPS, used in a range of experiments

Hydrogel

Cell Scaffolding



Vaccine Platform

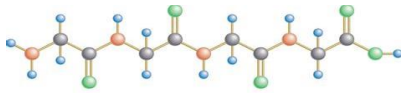


Blue = β-strand
Red = antigen

Thermal stability of self-assembled peptide vaccine materials. Tao sun, huifang han, et al. 2016

SELF-ASSEMBLING (AMPHIPATHIC) PEPTIDES

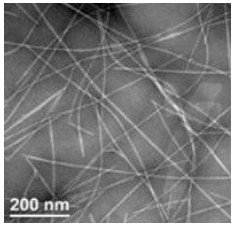
- A peptide is a chain of amino acids - secondary structure when folded



- Occur naturally
  - **Spider silk**

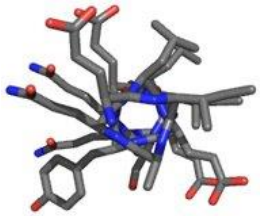
Collagen

Amyloid fibrils

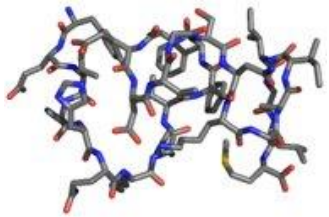


- Many factors that influence secondary structure
  - Amino acid composition (order and length)
  - Media (solvent, pH, ionic strength)
  - External Stimuli (heat, light, reducing agents)

β-Strand



α-Helix



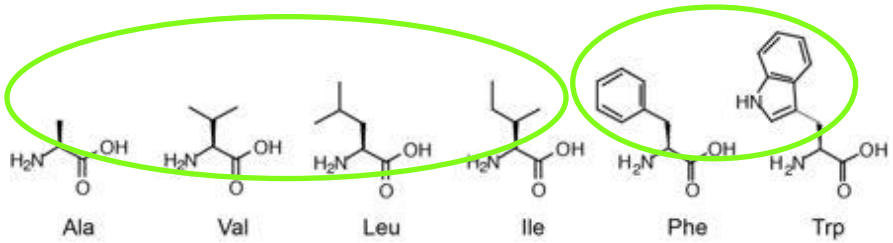
Disordered

Makin, O. S.; Atkins, E.; Sikorski, P.; Johansson, J.; Serpell, L. C. Molecular Basis for Amyloid Fibril Formation and Stability. *Proc. Natl. Acad. Sci.* **2005**, *102* (2), 315–320.

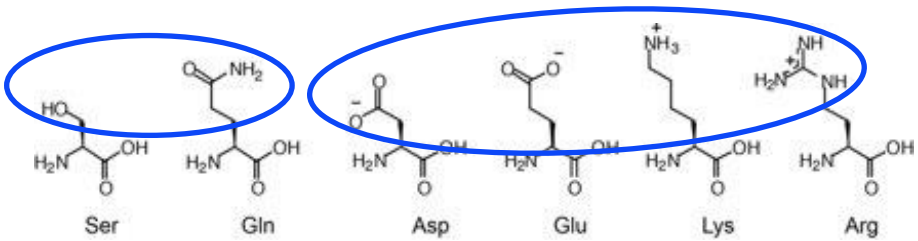
AMPHIPATHIC EXPLAINED

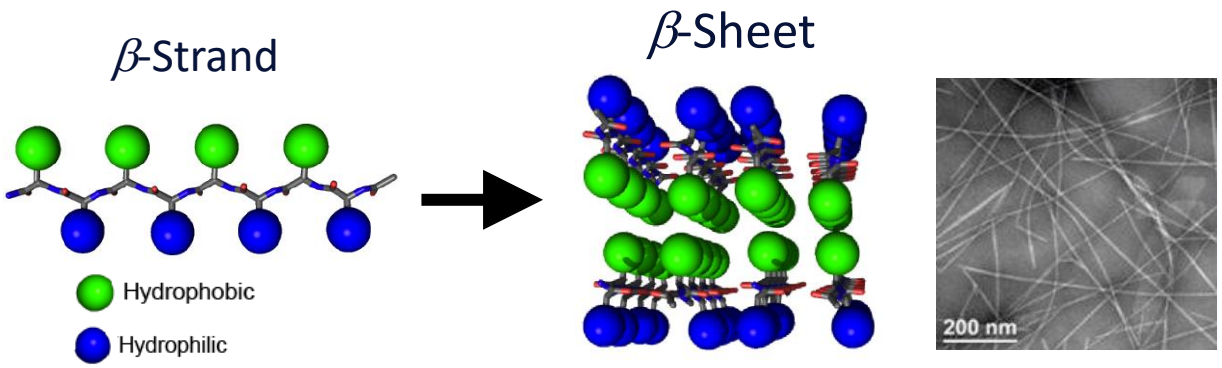
- Peptide containing hydrophobic and hydrophilic amino acids

Hydrophobic
- Aliphatic
- Aromatic



Hydrophilic
- Polar
- Charged





PROGRESS OF VACCINE SYNTHESIS

Synthesize peptides → **Characterize & analyze fibrils** → **Sample prep for animal models** → **Pre-clinical evaluation**

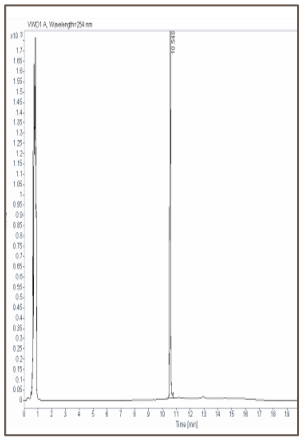
confirm with HPLC & MALDI

TEM imaging & CD

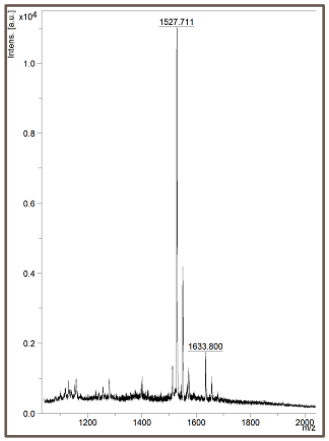
Correct concentration & control

ELISA to measure antibody titer

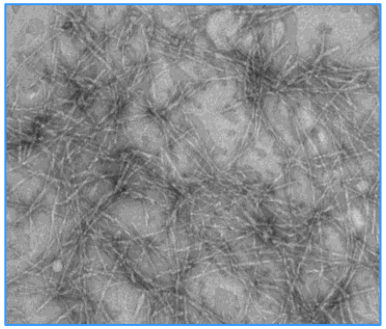
HPLC - Purity



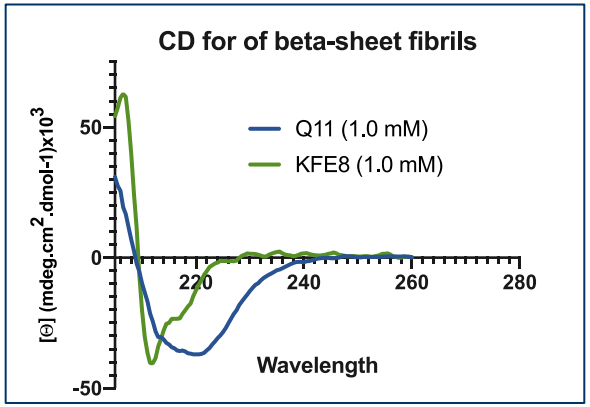
MALDI - Mass



TEM - Fibrils

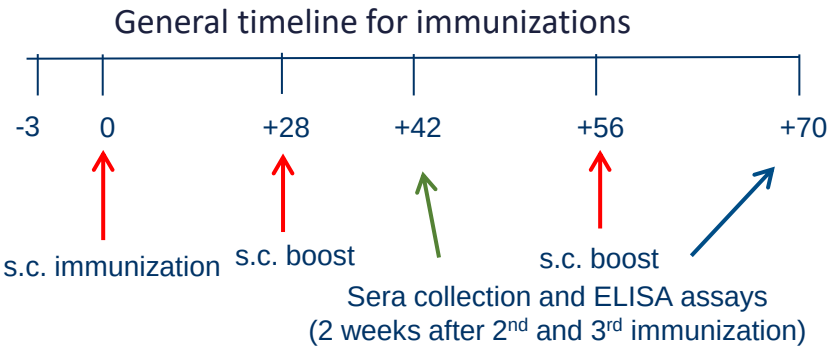


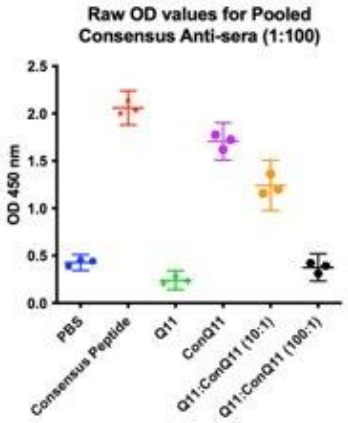
Circular Dichroism – β-sheet fibrils



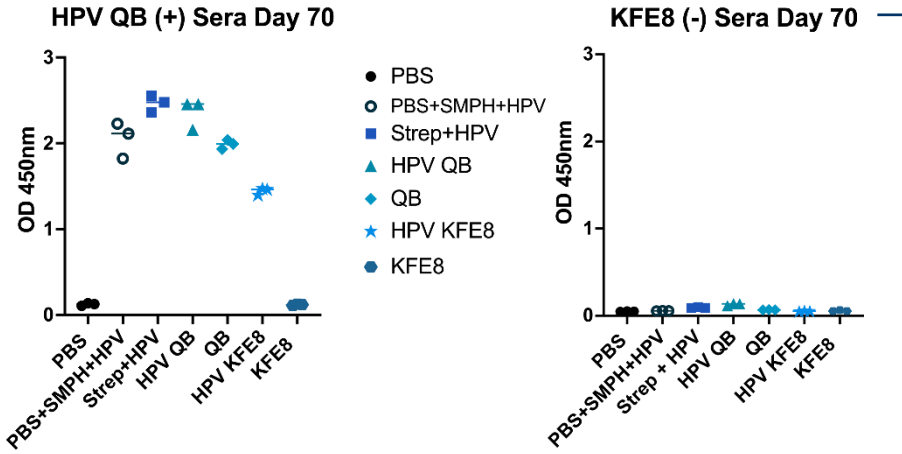
Name	Sequence	Status	Characterized
Q11	Ac-QQKFQFQFEQQ-NH2	Synthesized and fibrillized	HPLC, MALDI, TEM fibrils, CD
KFE8	Ac-FKFEFKFE-NH2	Synthesized and fibrillized	HPLC, MALDI, TEM fibrils, CD
HPV-linker	GTGGRTGYVPLGTRPPTVVDVGGC-NH2	Synthesized	HPLC and MALDI
HPV-KFE8	GTGGRTGYVPLGTRPPTVVDVSGSGFKFEFKFE-NH2	Synthesized and fibrillized	HPLC, MALDI, TEM fibrils,
HPV-Q11	GTGGRTGYVPLGTRPPTVVDVSGSGQQKFQFQFEQQ-NH2	Synthesized and fibrillized	HPLC, MALDI, TEM fibrils,

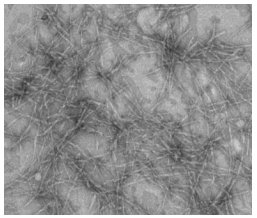
ELISA DATA



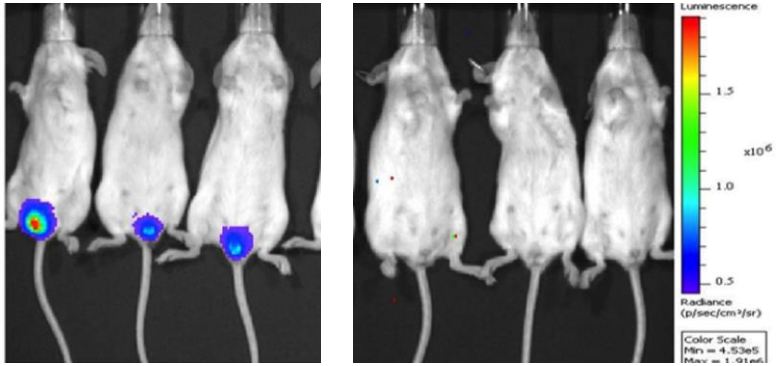


Preliminary Data





FUTURE STEPS & CONCLUSION



Control HPV (+) Immunized HPV (-)

Pseudovirus expresses Luciferin - bioluminescence

- VLPs less stable
- Peptides have high thermostability and accessibility
- HPV vaccines need to be able to protect everyone
- Peptide vaccine with consensus strand could be the solution

1. ELISAs to test for serum Abs responses
2. End point dilutions
3. Challenge studies via Luciferin imaging
4. T cell response vs other adjuvants

Tumban E, Peabody J, Peabody DS, Chackerian B. PLoS One. 2011;6(8):e23310.
Tumban E, Peabody J, Tyler M, Peabody DS, Chackerian B. PLoS One. 2012;7(11):e49751.

THANK YOU

Northern Arizona University

Naomi Lee, PhD
Cathy Propper, PhD
Gabriel Montaño, PhD
Jason Ladner, PhD

Partnership for Native American Cancer Prevention (NACP)

NACP NCI Grant U54CA143925.

University of New Mexico

Bryce Chackerian, PhD
Katheryn Frietze, PhD
Susan Core

Research Initiative for Scientific Enhancement (RISE)

National Institute of General Medical Sciences

Grant: 1R25GM127199-01

Louis Stokes Alliance for Minority Participation (LSAMP)

Southwest Health Equity Research Collaborative

NIH/NIMHD RCMI Grant: U54MD012388