

# Lack of molecular mimicry between HPV vaccine L1 antigen and human proteins by a computational analysis

Kazuhiro Nishioka<sup>1</sup>, Noriomi Matsumura<sup>1</sup>, Reona Shiro<sup>2,3</sup>, Ikuo Tsunoda<sup>2</sup>

<sup>1</sup>Department of Obstetrics and Gynecology, <sup>2</sup>Department of Microbiology  
Kindai University Faculty of Medicine, Sakai, Osaka, Japan

<sup>3</sup>Molecular Genetics & Microbiology, University of New Mexico  
School of Medicine, Albuquerque, NM, USA

PDF poster

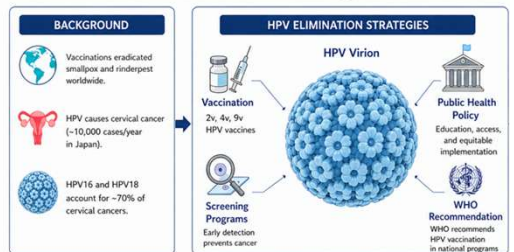


## Abstract

**Introduction** Although human papillomavirus (HPV) vaccines can prevent cervical cancer effectively, the HPV vaccination rate in Japan has remained low because of concerns about alleged adverse events. These concerns were partly based on a flawed hypothesis proposed by Darja Kanduc that molecular mimicry between HPV and human proteins could induce cross-reactive antibodies, causing autoimmune pathology, even when only portions of the amino acid (AA) sequences of the epitopes were identical between HPV and human proteins. **Methods** Using the same computational analysis as Kanduc, we identified 22 linear epitopes (9- to 23-AA length) of the HPV type 16 L1 protein, registered in the Immune Epitope Database (IEDB), as well as epitopes of hepatitis B virus (HBV) and respiratory syncytial virus (RSV). We generated four sets of random AA-sequences. We compared the number of human proteins sharing the same AA-sequences with virus-derived and random sequences using Welch's t-test. **Results** We found that no human epitopes had AA-sequences identical to any entire HPV epitopes. On the other hand, we identified varying numbers of human epitopes whose AA sequences were partially identical to those of HPV, HBV, RSV, and random sequences. HPV had a smaller number of such proteins having "partial" molecular mimicry than HBV and RSV. **Conclusions** No identical AA-sequences to full-length HPV epitopes were found among human epitopes. In addition, HPV epitopes had fewer partial AA-sequence similarities than HBV and RSV epitopes; HPV epitope concordance with human proteins was equivalent to that of random sequences for AA-sequences of eight or more. Therefore, our *in silico* analysis provided no evidence that HPV vaccinations could induce cross-reactive autoantibodies. The flawed molecular mimicry hypothesis should not be used as a scientific basis for alleged HPV vaccine-induced adverse events.

## Background

Vaccinations have helped eradicate two infectious diseases: smallpox and rinderpest. HPV is a major cause of cervical cancer, with 660,000 cases and 348,000 deaths annually worldwide. HPV types 16 and 18 are responsible for approximately 70% of cervical cancers. Among key strategies for HPV elimination (see Figure below), HPV vaccination using bivalent, quadrivalent, or nonavalent vaccines plays a central role; since HPV infects only humans, vaccinations can eliminate HPV from the Earth. The WHO recommends HPV vaccination as an essential component of national cervical cancer prevention programs.



**Three major findings / theories** used by anti-vaccine activists to explain "adverse effects" following HPV vaccinations

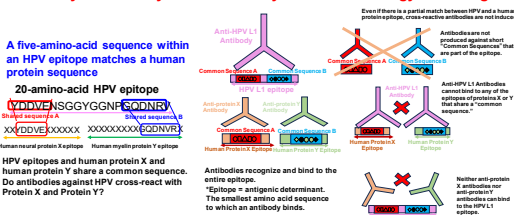
1. Molecular mimicry hypothesis: Similarities between HPV vaccine antigens and human proteins induce cross-reactive autoantibodies.
2. Adjuvant hypothesis: Aluminum adjuvants contained in HPV vaccines cause a novel disease, "macrophagic myofasciitis."
3. Animal experiments: A few animal studies reproduced "HANS (HPV vaccination associated neuro-immunopathic syndrome)."

Findings / theories 2 and 3 have already been disproved<sup>2,3,4,5</sup>.

### Molecular mimicry theory by Darja Kanduc<sup>6</sup>

- Proposed only by the group of Darja Kanduc
- Kanduc proposed, "If HPV shares a portion of amino-acid (AA) sequences with human proteins, the homology inevitably causes cross-reactive autoantibodies, leading to tissue damage."

The theory has already been refuted by basic immunology knowledge



- Epitope: the smallest AA sequence recognized by an antibody
- The HPV antibody recognizes the full-length HPV epitope
- Kanduc identified short sequences shared between HPV epitopes and epitopes of human proteins X or Y.
- Partial sequence matches cannot induce a cross-reactive antibody
- Immunologically, Kanduc's molecular mimicry theory was denounced
- In the current study, we aim to denounce Kanduc's theory experimentally, using the same computational method as used by Kanduc.

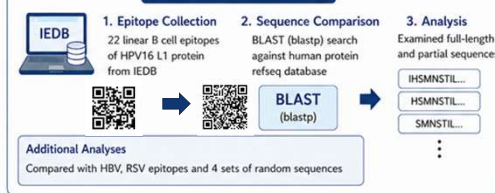
## Aim

To denounce the claim "HPV vaccines induce autoantibodies through molecular mimicry," using the same method as Kanduc

## Materials and methods

- Extract the amino acid (AA) sequences for HPV, hepatitis B virus (HBV), and respiratory syncytial virus (RSV) from the Epitope Database (IEDB), using the same method as Kanduc
- Compare these sequences with human protein epitopes and determine the number of proteins sharing common sequences, using the Basic Local Alignment Search Tool (BLAST)

### METHODS (SUMMARY)



## Results

Examples of HPV epitope AA sequences in the IEDB. The shortest and longest AA sequences consist of 9 and 23.

| HPV epitopes          |                   | Length of the extracted amino acid sequence |    |    |    |    |    |    |    |    |    |    |    |
|-----------------------|-------------------|---------------------------------------------|----|----|----|----|----|----|----|----|----|----|----|
| Amino acid sequence   | Amino acid length | 5                                           | 6  | 7  | 8  | 9  | 10 | 11 | 12 | 13 | 14 | 15 | 16 |
| IHSMNSTIL             | 9                 | 5                                           | 4  | 3  | 2  | 1  | -  | -  | -  | -  | -  | -  | -  |
| GLKAPKPTLGR-KATPTT    | 19                | 15                                          | 14 | 13 | 12 | 11 | 10 | 9  | 8  | 7  | 6  | 5  | 4  |
| LCCLICCPGHEW-GRGSP    | 19                | 15                                          | 14 | 13 | 12 | 11 | 10 | 9  | 8  | 7  | 6  | 5  | 4  |
| LCCLICCPGHEW-GRGSP    | 20                | 16                                          | 15 | 14 | 13 | 12 | 11 | 10 | 9  | 8  | 7  | 6  | 5  |
| VOENVPDLYIK-GRGSP     | 15                | 11                                          | 10 | 9  | 8  | 7  | 6  | 5  | 4  | 3  | 2  | 1  | -  |
| EDTYRFVTSQAI-ACQKHTPA | 21                | 17                                          | 16 | 15 | 14 | 13 | 12 | 11 | 10 | 9  | 8  | 7  | 6  |
| GLKAPKPTLGR-KATPTT    | 20                | 16                                          | 15 | 14 | 13 | 12 | 11 | 10 | 9  | 8  | 7  | 6  | 5  |

Extract sequences with amino acid lengths of 5 and 6 from HPV epitopes

| Amino acid sequence | Amino Acid Length | Length of the extracted amino acid sequence | 5 | 6 |
|---------------------|-------------------|---------------------------------------------|---|---|
| IHSMNSTIL           | 9                 | Number of amino acids extracted             | 5 | 4 |

The amino acid length of "IHSMNSTIL" is 9.

If the length of the amino acid sequence is 5, there are 5 possible sequences.

①IHSMN ②HSMNS ③SMNST ④MNSTI ⑤NSTIL

If the length of the amino acid sequence is 6, there are 4 possible sequences.

①IHSMNS ②HSMNST ③SMNSTI ④MNSTIL

Use BLAST to identify human proteins that share common sequences with each amino acid sequence.

IHSMNSTIL

①IHSMN ②HSMNS ③SMNST ④MNSTI ⑤NSTIL

- ①IHSMN lipopolysaccharide-responsive and beige-like anchor protein isoform 3
  - ②HSMNS heterogeneous nuclear ribonucleoprotein C-like 3
  - heterogeneous nuclear ribonucleoprotein C-like 4
  - heterogeneous nuclear ribonucleoprotein C-like 1
  - B-cell CLL/lymphoma 7 protein family member A isoform b
- human proteins
- The above analysis was performed for longer AA sequences.
  - From all HPV epitopes, we extracted sequences ranging in length from 5 to 13 AA.
  - Extracted HPV AA sequence numbers: 5-AA sequences, 248; 6-AA sequences, 237; 7-AA sequences, 225; 8-AA sequences, 211; 9-AA sequences, 196; 10-AA sequences, 181; 11-AA sequences, 167; 12-AA sequences, 152; and 13-AA sequences, 137

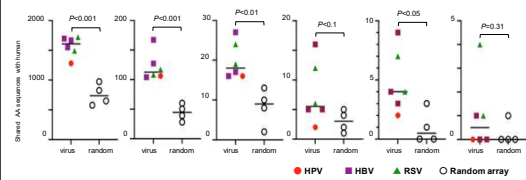
| Number of human proteins with amino acid sequences identical to viral epitope amino acid sequences |                                                | 5    | 6   | 7  | 8  | 9 | 10 | 11 | 12 | 13 |
|----------------------------------------------------------------------------------------------------|------------------------------------------------|------|-----|----|----|---|----|----|----|----|
| HPV16 L1                                                                                           | Large hepatitis B surface antigen core protein | 1235 | 106 | 16 | 2  | 2 | 0  | 0  | 0  | 0  |
|                                                                                                    | HBV                                            | 1605 | 127 | 16 | 5  | 3 | 0  | 0  | 0  | 0  |
|                                                                                                    | HBV                                            | 1549 | 104 | 17 | 5  | 4 | 1  | 0  | 0  | 0  |
|                                                                                                    | HBV                                            | 1607 | 167 | 27 | 16 | 9 | 0  | 0  | 0  | 0  |
| Respiratory Syncytial Virus - RSV                                                                  | DNA polymerase                                 | 1491 | 108 | 19 | 6  | 4 | 1  | 0  | 0  | 0  |
|                                                                                                    | G protein                                      | 1720 | 117 | 24 | 12 | 7 | 4  | 1  | 1  | 0  |
|                                                                                                    | G protein                                      | 1720 | 117 | 24 | 12 | 7 | 4  | 1  | 1  | 0  |
|                                                                                                    | P protein                                      | 825  | 50  | 8  | 5  | 1 | 0  | 0  | 0  | 0  |
| Random sequences                                                                                   | Not applicable                                 | 972  | 60  | 13 | 4  | 3 | 1  | 0  | 0  | 0  |
|                                                                                                    | Not applicable                                 | 378  | 26  | 2  | 1  | 0 | 0  | 0  | 0  | 0  |
|                                                                                                    | Not applicable                                 | 449  | 26  | 10 | 2  | 0 | 0  | 0  | 0  | 0  |
|                                                                                                    | Not applicable                                 | 449  | 26  | 10 | 2  | 0 | 0  | 0  | 0  | 0  |

- We found human proteins containing sequences identical to 5–13-length AA sequences derived not only from HPV but also from hepatitis B virus (HBV), respiratory syncytial virus (RSV), and random sequences.

- The number of matching human proteins decreased as the AA sequence length increased:

- 5-AA, ~1,000 matches; 6-AA, ~100 matches; 7-AA, <30; 8-AA, <20; 9-AA, <10- or more AAs: nearly zero
- HPV epitopes are typically 10 or more amino acids long.

No human AA sequence matched the full length of any HPV epitopes



- Compared with HBV and RSV, HPV has a smaller number of human proteins with matching AA sequences up to a length of 9 amino acids.
- For sequences 10 or more amino acids long, no difference in the number of human proteins whose AA sequences match those of viral epitopes when compared with random sequences.

## Discussion

- Kanduc et al.'s *in silico* analysis did not consider:
  - 1) Three-dimensional protein structures
  - 2) The full length of amino acid sequences
  - 3) Experimental antibody binding
  - 4) Human antigen localization: only cell surface antigens can be the target of antibody-mediated cytotoxicity
- Analysis of 22 linear HPV16 L1 epitopes found no full-length matches with human proteins.
- Short sequence matches were also observed in randomly generated sequences.
- Sequence similarity alone is inappropriate for claiming HPV vaccine risks
- Cross-reactivity and functional tissue damage must be verified experimentally and clinically

## Conclusions

- No human protein epitopes had amino acid (AA)-sequences identical to any entire HPV epitopes
- HPV epitopes had fewer partial AA-sequence similarities than hepatitis B virus (HBV) and respiratory syncytial virus (RSV) epitopes
- HPV epitope concordance with human proteins was equivalent to that of random sequences for AA-sequences of eight or more
- Our *in silico* analysis provided no evidence that HPV vaccinations could induce cross-reactive autoantibodies

The flawed molecular mimicry hypothesis should not be used as a scientific basis for alleged HPV vaccine-induced adverse events.

## References, grant, and COI

1. Nishioka K, Sekiyama K, Shiro R, Tsunoda I, Matsumura N. Lack of molecular mimicry between HPV vaccine L1 antigen and human proteins by a computational analysis. *Int J Clin Oncol*. 31(3): 494-503
2. Matsumura N, Tsunoda I. Scientific evaluation of alleged findings in HPV vaccines: Molecular mimicry and mouse models of vaccine-induced disease. *Cancer Sci*. 2022;113(12):3313-3320.
3. Matsumura N, Shiro R, Tsunoda I. Critical evaluation on roles of macrophagic myofasciitis and aluminum adjuvants in HPV vaccine-induced adverse events. *Cancer Sci*. 2023;114(4):1218-1228.
4. Shiro R, Matsumura N, Tsunoda I. Comparative cytokine & histology studies on HPV and other viral vaccinations: no pathogenicity of HPV vaccine adjuvants. *Cancer Sci*. 2025; 116(12): 3309-3325.
5. Shiro R, Tsunoda I. Towards global HPV eradication: single-dose HPV vaccination vs. pseudoscience. *Pathophysiology* 33(2), 25
6. Kanduc D, Shoenfeld Y. Human papillomavirus epitope mimicry and autoimmunity: the molecular truth of peptide sharing. *Pathobiology*. 2019;86(5-6):285-295.
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