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REVIEW ARTICLE

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Scientific evaluation of alleged findings in HPV vaccines: Molecular mimicry and mouse models of vaccine-induced disease

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Abstract

Cervical cancer is caused by infections of the human papillomavirus (HPV), which can be prevented by vaccinations. In Japan, although about 3000 people die of cervical cancer annually, the HPV vaccination rate has remained extremely low in the eligible population since many Japanese have been concerned that "diverse symptoms," such as chronic pain, movement disorders, and cognitive impairment, may occur as adverse reactions after HPV vaccination. The concern has been raised by media coverage of the ongoing HPV vaccine lawsuits, in which the plaintiffs complained of their symptoms caused by HPV vaccination. The claims have been based on the alleged pathogenic findings in research articles on HPV vaccines, summarized in the document prepared by the plaintiffs' attorneys. We critically evaluated these articles, in which the authors proposed the following findings/hypothesis: (i) molecular mimicry between HPV L1 and human proteins leads to the production of cross-reactive antibodies; and (ii) HPV vaccine injection in mice causes damage in the brain, a mouse model for HPV vaccine associated neuro-immunopathic syndrome (HANS). We found that these hypotheses were based mainly on the findings from a few research groups and that all the articles had flaws in the method, result, or discussion sections. Our current evaluation should help better understand the validity of the findings, which have been often misunderstood as the truth by the general public. We propose to accumulate high-quality data on potential adverse events following HPV vaccination and to continue critically evaluating them.

KEYWORDS

HPV vaccine, molecular mimicry, neuroimmunology, side effect, uterine cervix

Abbreviations: 2vHPV, bivalent HPV vaccine; 4vHPV, quadrivalent HPV vaccine; 9vHPV, nanovalent HPV vaccine; Al, aluminum; BBB, blood-brain barrier; CNS, central nervous system; EAE, experimental autoimmune encephalomyelitis; ELISA, enzyme-linked immunosorbent assay; HANS, HPV vaccine associated neuro-immunopathic syndrome; HBV, hepatitis B virus; HPV, human papillomavirus; MHLW, Ministry of Health, Labor, and Welfare; MMF, macrophagic myofasciitis; MOG, myelin oligodendrocyte glycoprotein; MS, multiple sclerosis; PBS, phosphate-buffered saline; PT, pertussis toxin; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; VLP, virus-like particle.

Noriomi Matsumura and Ikuo Tsunoda contributed equally to this work.

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1 | INTRODUCTION

Cervical cancer is one of the most common cancers and the fourth leading cause of cancer-related deaths. In Japan, the total number of deaths due to cervical cancer has been around 3000 annually. Since cervical cancer is caused by sexually transmitted infections of the human papillomavirus (HPV), it can be preventable by vaccinations against oncogenic HPV. Together with the hepatitis B virus (HBV) vaccine, the HPV vaccine has been demonstrated to reduce the risk of cancer as anticancer vaccines.¹ Currently, three HPV vaccines are available: bivalent HPV vaccine (2vHPV, Cervarix®), quadrivalent HPV vaccine (4vHPV, Gardasil®), and nonavalent vaccine (9vHPV, Gardasil®9/Silgard®9) (Table 1). 2vHPV can prevent infection with two high-risk HPV types: HPV6, 11, 16, and 18. 9vHPV is a vaccine against nine HPV types, which can prevent about 90% of cervical cancer cases.

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In Japan, the HPV vaccination program, using 2vHPV and 4vHPV, was started for girls in the sixth grade of elementary school through the first grade of high school (12–16 years of age) in April 2013.^{2,3} Soon after, news media started reporting the alleged cases of HPV-vaccinated girls developing neuropsychological symptoms or "diverse symptoms," such as chronic pain, movement disorders, and cognitive impairment. This made the general public worry that these symptoms could be adverse reactions to HPV vaccinations. In June 2013, the Ministry of Health, Labor, and Welfare (MHLW) suspended proactive recommendations for HPV vaccinations.⁴ Since then, the HPV vaccination rate has remained below 1% of the eligible population.⁵ Nine years later, in April 2022, the proactive recommendation of HPV vaccination has resumed, although 9vHPV has not been included in the vaccination program as of April 2022.

It is unclear whether the resumed recommendation would increase the rate of HPV vaccination in Japan, since little information is available for general practitioners and the public to determine whether HPV vaccinations really cause the alleged adverse reactions. Despite the international evidence of the safety and effectiveness of HPV vaccinations, many healthcare professionals and the general population in Japan still have concerns about the "diverse symptoms." These concerns are based mainly on media information during the past and ongoing HPV vaccine lawsuits. The number of plaintiffs in the lawsuits has grown to 120.⁴ A ruling in the lawsuit

would significantly impact public understanding/awareness, future media coverage, policymaking, and the HPV vaccination rate in Japan.

The plaintiffs of the lawsuits claimed that they had neuropsychological symptoms caused by HPV vaccination. Their claims are based on several publications on the alleged pathogenic roles of the HPV vaccine, which were summarized in the document posted on their website by the plaintiffs' attorneys.⁶ Among the articles cited in the document, the original manuscripts in the basic research field⁷⁻¹⁵ are listed in Table 2. The attorneys' group has used the findings from these articles as "evidence" in the document, which can be categorized into three findings/hypotheses: (1) molecular mimicry between HPV L1 and human proteins results in the production of cross-reactive antibodies that attack host organs; (2) aluminum (Al) adjuvant contained in HPV vaccine causes neurological and autoimmune diseases such as macrophagic myofasciitis (MMF); and (3) HPV vaccine injection in experimental mice resulted in damage to the central nervous system (CNS). Among the three hypotheses, hypothesis (2) is based on findings related to Al hydroxide, which is a component of 2vHPV, but not 4vHPV or 9vHPV (Table 1); hypothesis (2) cannot explain the "diverse symptoms" commonly claimed in two HPV vaccines. Thus, in the current manuscript, we scientifically evaluated the findings/hypotheses (1) and (3).

The alleged findings have been used repeatedly in the lawsuits or by antivaccine activists, not only in Japan but also in other countries. Here, in this manuscript, as experts in the fields of HPV pathogenesis and neuroimmunology, we evaluated the findings critically. We believe that our evaluation will help readers understand the validity of the findings, which have often been misunderstood as the truth by the general public.

2 | MOLECULAR MIMICRY OF HPV VACCINES

2.1 | No cross-reactive antibodies in HPVvaccinated sera

Molecular mimicry between host proteins and microbes, including viruses and bacteria, has been proposed to cause immune-mediated tissue damage.¹⁶ When humans and microbes share common protein

Vaccine	Bivalent HPV vaccine (2vHPV)	Quadrivalent HPV vaccine (4vHPV)	Nonavalent HPV vaccine (9vHPV)	
Trade name	Cervarix®	Gardasil®	Gardasil® 9/Silgard® 9	
Antigen	HPV L1 protein	HPV L1 protein	HPV L1 protein	
HPV type	16, 18	6, 11, 16, 18	6, 11, 16, 18, 31, 33, 45, 52, 58	
Adjuvant	ASO4: aluminum hydroxide and monophosphoryl lipid A	Aluminum hydroxyphosphate sulfate		
Manufacturer	GlavoSmithKline	Merck		

TABLE 1 Three HPV vaccines and their characteristics

	ely yields hosts and	² 2vHPV and IPV		o statistical	ciate with	alomyelitis;
Scientific evaluation	Flawed methodology, which lik molecular mimicry betweeı any other viral proteins	Al hydroxide is a component ol other vaccines, but not 4vF		Control EAE misled readers. N analysis.	Antibody kinetics did not asso depression	xperimental autoimmune enceph
Alleged findings	Molecular mimicry of 5, 6, or 7 amino-acid length between HPV and host proteins by database analyses	Al hydroxide induces MMF pathology, resulting in clinical symptoms, CNS inflammation, cognitive dysfunction	Al hydroxide induces CNS damage in mice	4vHPV induces CNS disease in experimental mice	4vHPV induces depression and anti-CNS antibody responses	aluminum; CNS, central nervous system; EAE, e)
Lawsuit ref no. ^a	46 47	54-56	58, 59	60	61	ardasil®); Al,
References	Kanduc, J Exp Ther Oncol. 2009 ⁷ Kanduc, Int J Med Med Sci. 2009 ⁸	Gherardi, <i>Brain</i> . 2001, ⁹ Authier, <i>Brain</i> . 2001, ¹⁰ Couette, <i>J Inorg Biochem</i> . 2009 ¹¹	Shaw, <i>J Inorg Biochem</i> . 2009, ¹² Agmon- Levein, <i>J Autoimmun</i> . 2014 ¹³	Aratani, Nakajima et al., <i>Sci Rep.</i> 2016 ¹⁴	Inbar, Shoenfeld et al., <i>Immunol Res.</i> 2017^{15}	Cervarix®); 4vHPV, quadrivalent HPV vaccine (Gé ic myofasciitis.
Hypothesis	I. Molecular mimicry between HPV L1 and human proteins	II. Aluminum adjuvant-induced CNS and autoimmune diseases		III. 4vHPV-induced CNS disease in mice		Abbreviations: 2vHPV, bivalent HPV vaccine (C HPV, human papillomavirus; MMF, macrophagi

¹Reference number in the document⁶ of HPV vaccine lawsuits

Scientific evaluation of hypotheses/alleged pathogenic findings in HPV vaccines

2

TABLE

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sequences/3D structure, the immune response generated against a microbe not only eradicates the infectious agents but also crossreacts host components, leading to tissue damage. For example, due to molecular mimicry between bacterial and neuronal antigens, Campylobacter jejuni infection has been demonstrated to induce cross-reactive antiglycolipid antibodies; these antibodies recognize both the bacterial cell wall and the cell surface molecules on human peripheral nerves, causing Guillain-Barré syndrome, a peripheral immune-mediated neuropathy. On the other hand, HPV encodes two structural proteins, L1 and L2 capsid proteins, and nonstructural E proteins, E1 to E7 proteins (Figure 1). HPV vaccines are viruslike particles (VLPs) containing only L1; antibodies against L1 can prevent virus attachment to host cells. So far, there is no scientific report to demonstrate the presence of a cross-reactive antibody that recognizes both HPV L1 and human proteins in sera from HPVvaccinated people.

2.2 | Molecular mimicry between HPV and human proteins by Kanduc's group

Using the database, a research group led by Darja Kanduc has suggested theoretical risks of HPV vaccinations based on a comparison of amino acid sequences between HPV16 and human proteins. Kanduc found 82 "heptapeptides (7-mer motif, sequence composed of seven amino acids)" sharing the identical 7-mer motif between HPV16 and human proteins. Kanduc concluded that HPV vaccinations would inevitably cause autoimmune diseases by generating cross-reactive antibodies based on molecular mimicry between HPV16 and human proteins.⁷

In this article, however, there was a flaw (or lack of basic knowledge of HPV VLP's components) in the experimental design; Kanduc compared the entire HPV sequences, including L1–2 and E1–7 proteins (Figure 1). Thus, the 82 "heptapeptides" included not only 16 sequences in the L1 protein, but also 66 sequences in the other HPV proteins. This study showed that (1) a simple sequence comparison between host proteins versus HPV-VLP-relevant (L1) or irrelevant viral proteins (L2, E1–E7) similarly yielded many common 7-mer motifs and (2) the L1 protein was not more homologous to the human proteins compared with the other HPV proteins (and likely with proteins of other viruses).

Similarly, using the same approach, Kanduc reported that, among 36,103 human proteins, 1703 (4.7%) shared the identical 6-mer motif "hexapeptides" and 15,361 (42.5%) shared the 5-mer motif "pentapeptides" in the entire polyprotein sequence of HPV16.⁸ Here again, Kanduc did not show whether the HPV L1 protein has more "hexapeptides" or "pentapeptides" having molecular mimicry with host proteins compared with the other HPV proteins or proteins of other viruses (e.g., other viral proteins used in vaccinations, such as the HBs antigen in HBV vaccine and S protein in vaccines against severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]). Although Kanduc concluded that the presence of a common "penta/hexa/heptapeptide" sequence between HPV and host proteins is a risk for induction



 \cdot L1 peptides having molecular mimicry with host proteins need to be antigenic epitopes for antibody induction

 ${\scriptstyle \bullet}$ No evidence that HPV L1 is more homologous to host proteins, compared with other viral proteins

FIGURE 1 Human papillomavirus (HPV) encodes proteins L1–2 and E1–7. HPV vaccine contains only L1 protein; anti-L1 antibodies block virus attachment to host cells. Kanduc reported sequence similarities (molecular mimicry) between L1 and host proteins. However, L2 and E7 also had molecular mimicry; L1 was no more homologous to host proteins than other viral proteins. It is unknown whether the L1 peptides having molecular mimicry are antibody-epitopes or not.



FIGURE 2 "Partial" molecular mimicry between the HPV L1 antibody epitope and human "Proteins X and Y" from the human protein database. HPV L1 and Protein X or Protein Y share only the portion of (not entire) common sequences (common peptide A or B) in their epitopes. Anti-L1, anti-Protein X, and anti-Protein Y antibodies recognize the entire epitopes of L1, Protein X, and Protein Y, respectively, but likely do not bind the portion of the epitope. Thus, the presence of the "partial" molecular mimicry would not confer the production of cross-reactive antibodies recognizing both L1 and Protein X or Protein Y.

of autoimmune cross-reactive antibodies, this is not usually the case. In general, most antibodies and B cells have been shown to recognize the conformational structure of the antigens (conformational epitope), but not sequential amino acids of the antigens (linear epitope), although some antibodies are known to bind linear epitopes of antigens. Thus, in the above molecular mimicry manuscripts, Kanduc should have demonstrated whether the identified "penta/hexa/heptapeptides" really contain the entire sequence of one of the linear epitopes of HPV antibodies or not to propose their potential risk for induction of the autoimmune cross-reactive antibodies.

2.3 | Flawed "partial" molecular mimicry approach

Ten years later, in 2019, by collaborating with Yehuda Shoenfeld, Kanduc conducted a similar analysis, retrieved 186 HPV L1 epitope sequences derived from 15 HPV types (1a, 2, 3, 4, 6b, 6, 11, 16, 18, 29, 31, 33, 44, 52, and 58), and dissected each epitope into "pentapeptides." Kanduc found many "pentapepides" sharing between the HPV L1 epitopes and human proteins.¹⁷

This approach, however, was again flawed; the epitope sequences have been determined or defined as a minimum aminoacid length recognized by B cells (antibodies) or T cells. The "pentapeptides" dissected from the entire epitope most likely do not work as an epitope (antigenic determinant) that elicits B-cell or T-cell responses in most cases (Figure 2). Here, the term "molecular mimicry" is inappropriate since only the portion of the entire epitope sequence was used for comparison; it is appropriate to call it "partial" molecular mimicry, which will not confer the production of cross-reactive antibodies. It was also inappropriate to use the HPV L1 epitope sequences from HPV types irrelevant to vaccines (i.e., HPV1a, 2, 3, 4, 29, and 44).

Furthermore, although Kanduc did not describe whether they used the L1 epitopes from humans or mice, the comparison between

3 | HPV VACCINE-INDUCED CNS DAMAGE IN EXPERIMENTAL MICE

3.1 | Experimental HPV vaccine-induced brain damage

The next category of the alleged findings is that injection of HPV vaccines into experimental mice resulted in CNS damage. Although the article in this category was published only by Shoenfeld's group, there is one retracted manuscript by the group led by Toshiro Nakajima¹⁴ and one unpublished presentation by the group of Shuichi Ikeda¹⁸; these two Japanese groups claimed that they had reproduced CNS pathology in mice, which can be used as a mouse model for "HPV vaccination associated neuro-immunopathetic syndrome (HANS)." The term "HANS" was coined by these Japanese research groups to refer to "diverse symptoms" following HPV vaccination in humans; "HANS" was proposed as an immune-mediated CNS disease caused by HPV vaccination.

Although the publisher retracted Nakajima's article, the PDF file of the article is still available online and has been used as "evidence" in lawsuits. Thus, we decided to evaluate Nakajima's article together with the one from Shoenfeld's group. We also discuss the presentation by Ikeda's group, since it has continuously influenced the decision-making of HPV vaccinations among the general public.

The criteria of "HANS" was first proposed by Nishioka et al.¹⁹ as "Proposed preliminary diagnostic criteria for the diagnosis of HANS" in the rheumatology conference abstract in 2014. Ikeda's group revised and published the criteria with minor changes in 2017 as "Diagnostic criteria for suspected adverse effects after HPV vaccination" in the journal Drug Safety.²⁰ Although the new criteria neither contained the term "HANS" nor "immunopathetic," the authors described it as "immune-related pathological conditions." Currently, the disease entity "HANS" has not been accepted in any major scientific communities, since the criteria and alleged findings in "HANS" have several flaws as follows. (1) Since the criteria did not define the time to disease onset from the HPV vaccination, the average onset time from the first vaccine was 319.7 ± 349.3 days, ranging from 1 to 1532 days. Generally, innate and acquired immune-mediated responses/pathology occur within 1 month following the exposure/inoculations of immunostimulants (e.g., antigens and adjuvants). (2) The criteria excluded the case having "HPV vaccination after 30 years of age," although

people after 30 years are known to be more susceptible to a variety of immune-related diseases. (3) Except for "widespread pain" as one of the major symptoms, the criteria did not contain any immunological symptoms/examinations, including anti-HPV L1 antibody titer. Although the proponents of "HANS" often hypothesized that abnormal anti-HPV antibody responses could lead to immune-mediated damage, no reports have shown the levels of anti-HPV L1 antibodies among "HANS" cases. (4) "HANS" has been proposed to be mediated by anti-HPV-specific immune responses. However, no articles have reported "HANS"-like signs/symptoms among HPV-infected people (natural infection), some of which are expected to develop strong HPV-specific immune responses; more than 80% of women and men have been estimated to acquire HPV by age 45 years.²¹

3.2 | Mouse model of "HANS" by Nakajima's group

Nakajima's group¹⁴ injected 4vHPV (Gardasil®) into mice with or without pertussis toxin (PT), compared with control mice receiving PT alone or PBS. They also induced experimental autoimmune encephalomyelitis (EAE) with myelin oligodendrocyte glycoprotein (MOG)₃₅₋₅₅ peptide, an autoimmune model of multiple sclerosis (MS), and used EAE mice as positive controls that have CNS immunopathology. The severity of neurological signs was assessed by the degree of paralysis of the tail and extremities; this scoring system has been used in EAE studies.

Although tail paralysis was observed in the 4vHPV + PT group, the experimental setting was inappropriate and misleading based on the following concerns. (1) Nakajima's group did not set up the group receiving only the adjuvant contained in 4vHPV or the group receiving a vaccine other than 4vHPV (e.g., HBV or 2vHPV). This made it unclear whether the tail paralysis was HPV vaccinespecific or not. (2) Neither 4vHPV alone nor the 4vHPV+PT group developed limb paralysis, although 12 of 21 mice receiving 4vHPV+PT developed tail paralysis and two of 12 mice receiving 4vHPV developed mild tail paralysis. On the other hand, all EAE mice developed hind or forelimb paralysis and eight of 10 mice died of EAE. Neurological deficits, including tail and limb paralysis, in MOG-induced EAE are known to be caused by inflammatory demyelination with substantial infiltration of T cells and macrophages in the CNS. In contrast, none of the mice receiving 4vHPV had inflammation in the CNS; tail paralysis in the 4vHPV group was not mediated by CNS immune cell infiltration. Thus, using the EAE scoring system (or using EAE as a positive control) was misleading, since it would give the wrong impression that any clinical signs observed in this article were caused by CNS inflammation. (3) The method used in this manuscript was inappropriate for the standard of EAE studies; there was no time-course analysis in EAE scores or body weight changes, and evaluation of EAE scores was not performed in a blind fashion. (4) PT, which is not used for HPV vaccination in humans, was used in the experiment; PT is irrelevant to human HPV vaccination.

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Histologically, the 4vHPV+PT group had "narrowing" of the third ventricle, TUNEL⁺ apoptotic vascular endothelial cells in the thalamus and hypothalamus, and increased tyrosine hydroxylase and decreased glutamic acid decarboxylase and γ -aminobutyric acid in the hypothalamic paranuclei. These neuropathological analyses had the following seven concerns: (1) All histological findings were shown by one microscopic picture/staining/group, but not quantified. The numbers of mice were not shown; no statistical analysis was conducted. (2) It has been known that paraffin sections of normal mouse brains often had no gap in the third ventricle; an atlas of normal mouse brains shows what is equivalent to the figure described as "narrowing" in this article.²² (3) It should be noted that the ventricles become widen when the paraffin sections are incubated in water for a long time before being placed on glass slides; such a sectioning procedure could influence the size of the ventricles. (4) If pathological narrowing or obstruction of the third ventricle occurred, this could lead to damage in other CNS regions, particularly dilatation of other ventricles: the authors did not find the presence of hydrocephalus²³ or other related neuropathology. (5) Micrographs of TUNEL⁺ cells did not show nuclear fragmentation characteristics of apoptosis. Although a marker for vascular endothelium, CD31, was used to identify the apoptotic cells, the authors conducted single immunostaining using serial sections, in which some TUNEL⁺ cells were clearly seen at a distance from the CD31⁺ cells; they did not conduct double-staining of TUNEL and CD31. (6) No statistical correlation was examined between the clinical scores (tail paralysis), third ventricle narrowing, endothelium apoptosis, and changes in neuronal cell subtypes. The authors did not provide their working hypothesis of how these neuropathological changes can be associated with or induced by 4vHPV injection. (7) No experiments were conducted to examine a possible immunopathogenic role of 4vHPV, such as anti-HPV L1 antibody deposition and Al-containing macrophages in the CNS.

Although using EAE as a positive control was misleading in the above study, Hiroyuki Oshiumi's group²⁴ used EAE appropriately to determine whether HPV vaccines could exacerbate EAE or not. In this study, mice were injected with 2vHPV, 4vHPV, or PBS, which was followed by EAE induction with MOG. The authors found no differences in clinical EAE severities among the mice injected with HPV vaccines or PBS during the 1-month observation period, providing evidence for the safety of HPV vaccines in the CNS autoimmune disease.

3.3 | 4vHPV-induced CNS abnormalities in mice by Shoenfeld's group

Shoenfeld's group reported that 4vHPV (Gardasil®) injection in experimental mice led to CNS abnormalities.¹⁵ The article was accepted by the journal *Vaccine* in 2016, but retracted by the journal at the request of the Editor-in-Chief due to serious concerns regarding its scientific soundness. A review by the Editor-in-Chief and evaluation by outside experts confirmed that the methodology was

seriously flawed and that the conclusions in the article were unjustified.²⁵ Subsequently, this paper was published in the special issue of the journal *Immunologic Research* (Shoenfeld was one of the issue editors) with the same contents and authors.¹⁵

The authors injected mice with AI hydroxide, 4vHPV, or 4vHPV+PT; PT was used to damage the blood-brain barrier (BBB). Three behavioral tests were conducted: the forced swimming test (FST) for depression; the staircase test for locomotor, explorative activity, and anxiety and the novel object recognition test for memory. Among the three tests, only the FST showed abnormalities in the experimental groups compared with the control group injected with the vehicle. The FST indicated depression in the 4vHPV and 4vHPV+PT groups 3 months post-injection (p.i.), although the AI hydroxide and 4vHPV+PT groups, but not the 4vHPV group, were more depressed compared with the control group 6 months p.i. Since the FST findings between 3 and 6 months p.i. were inconsistent, one cannot determine whether Al hydroxide, HPV L1, or PT was responsible for the findings. In addition, 4vHPV contains AI hydroxyphosphate sulfate, but not AI hydroxide; the findings of the AI hydroxide group were not comparable with those of the 4vHPV group.

The authors also conducted enzyme-linked immunosorbent assays (ELISAs) and detected antibodies against 4vHPV, brain protein extract, and brain phospholipid extract in the 4vHPV and 4vHPV+PT groups from the sera 1-month p.i., but not 2-months p.i. Overnight adsorption of the sera with brain protein extract reduced the titers of the anti-4vHPV antibody (ELISA plates were coated with 4vHPV; the anti-4vHPV antibody was presumably anti-HPV L1 antibody). There were several concerns over the findings. (1) The adsorption experiment was conducted only with brain protein extract, but not with brain phospholipids or proteins from any other organs; the authors did not test whether adsorption of sera with 4vHPV could reduce the antibody titers against brain protein extract or brain phospholipid extract. Thus, it is unclear whether or not the finding was due to cross-reaction between the brain-specific molecules and HPV. (2) The authors did not determine whether the adsorption could occur only with anti-HPV antibodies or also with other antibodies. Since the authors did not find a reduction of anti-4vHPV antibody titers by brain protein extract in the AI hydroxide group, they suggested the presence of anti-4vHPV antibody that cross-reacts brain protein extract was specific in the 4vHPV group. However, this interpretation was incorrect since the antibody against 4vHPV was not detected in sera from the Al hydroxide group in the first place. (3) Although all the antibody titers were reduced 2-months p.i., the abnormalities in the FST occurred in the 4vHPV groups after 3 or 6 months p.i, and also after 6 months p.i. in the Al hydroxide group. Thus, the kinetics of any antibody levels were not associated with the abnormalities in the FST.

Furthermore, the authors showed that the density of Iba-1 immunostaining in the hippocampus was higher in the 4vHPV group than in the Al hydroxide group, suggesting 4vHPV injection increased microglia activation. This experiment had the following flaws: (1) there was no statistical difference in microglia activation between the control and 4vHPV groups, and activated microglia did not increase in the 4vHPV + PT group; (2) hippocampas is associated with memory, but not depression; and (3) micrographs of Iba-1 staining were not shown.

3.4 | Anti-CNS antibody induction by HPV vaccine in mice by Ikeda's group

Neither Nakajima's group nor Shoenfeld's groups investigated whether HPV vaccines more efficiently induce CNS-specific antibodies or cause neurological damage compared with other vaccines. Ikeda's group presented their experimental results addressing the issue at the March 2016 meeting of the Health and Labor Science Research Project.¹⁸ Ikeda's group administered 2vHPV (Cervarix®), influenza virus vaccine, or HBV vaccine to NF-κBp50 knockout mice, which are known to have potentially abnormal antibody production. They collected sera from the mice and applied the sera to hippocampal sections of naive mice. Since only the 2vHPV group had serum antibody binding on the section, Ikeda's group suggested that 2vHPV vaccination would likely cause brain damage.

This interpretation was inaccurate since (1) anti-CNS antibody does not necessarily cause brain damage and some anti-neuroantigen antibodies have been shown to promote CNS regeneration (remye-lination),¹⁶ and (2) serum anti-CNS antibody does not enter the CNS because of the presence of the BBB. It has been shown that intrave-nous or intraperitoneal injection of autoantibodies to CNS antigens did not cause CNS damage in experimental mice when the BBB was intact.²⁶

Later on, it turned out that Ikeda's group used only one mouse per vaccine. When the number of mice was subsequently increased to three, no sera from the 2vHPV group showed binding to the brain sections. No other research groups have demonstrated that HPV vaccines induced anti-CNS antibody responses more efficiently than other vaccines.

4 | CONCLUSION

It is unfortunate that HPV vaccination status in Japan has been influenced by multiple HPV vaccine lawsuits. In this manuscript, we demonstrated that all experimental findings of the evaluated articles^{7,8,14,15} cited in the lawsuits⁶ had flaws. Although we did not evaluate the other articles cited in the lawsuits,^{9–13} the alleged "adverse reactions" in these articles were from Al hydroxide, which is included in 2vHPV, but not in 4vHPV; "diverse symptoms" specific to two HPV vaccines cannot be explained with the Al hydroxide hypothesis. Thus, the articles were inappropriate to associate HPV vaccination with "diverse symptoms" following HPV vaccination. Without critical scientific reviews/evaluations, the HPV-vaccinated persons with "diverse symptoms" might believe the conclusions drawn from such low-quality articles, which hinders the search for the right solution for the issue.

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We propose to accumulate high-quality data on potential adverse events following HPV vaccination and continue critically evaluating them. A systematic literature review and meta-analysis of published data is one way to draw unbiased conclusions. A recent meta-analysis by Boender et al.²⁷ concluded that the risk of Guillain-Barré syndrome after HPV vaccination lacks statistical significance, which had been inconclusive due to the different results among published articles. Scientific evaluations/conclusions based on these approaches would provide appropriate information for healthcare providers, policymakers, and the general public.

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CONFLICT OF INTEREST

Noriomi Matsumura is an outside director of Takara Bio. Noriomi Matsumura also received a lecture fee and a research grant from AstraZeneca, and a lecture fee from Takeda Pharmaceutical. Those companies have nothing to do with the HPV vaccines. Ikuo Tsunoda has no conflict of interest with any commercial companies.

ETHICS STATEMENT

Approval of the research protocol by an Institutional Reviewer Board: *not applicable* (*N*/*A*). Informed Consent: *N*/*A*. Registry and the Registration No. of the study/trial: *N*/*A*. Animal Studies: *N*/*A*.

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