Stable and Consistent Genetic Profile of Oka Varicella Vaccine Virus Is Not Linked with Appearance of Infrequent Breakthrough Cases Postvaccination

Recently, Dr. Sauerbrei and his colleagues reported (8) on the genetic profile of an Oka varicella vaccine virus variant (V-Oka-zoster) isolated from an infant with herpes zoster. Those authors hypothesized that the isolated variant had an increased ability to cause zoster and was present as a subspecies within the vaccine that was used to immunize the infant. The rationale for linking this variant with the hypothetical altered capacity to cause zoster was based on a limited comparison of its nucleotide sequence with the respective sequence of Oka parental (P-Oka, or "wild-type," parental Oka strain), Oka vaccinal (V-Oka), and Varilrix (GlaxoSmithKline Biologicals) strains. Sauerbrei et al. claimed that several positions, including one within open reading frame 62 (ORF 62) of the V-Oka-zoster variant, had "reverted" to those found in P-Oka. They also claimed that the V-Oka-zoster variant contained six P-Oka bases in single-nucleotide polymorphisms (SNPs) at targeted regions (Table 1), located in ORFs 9A, 10, 21, 52, 55, and 62 (position 108838), that could be associated with an increased rate of zoster reactivation.

Sequencing of the viral regions analyzed by Sauerbrei et al. has been published in a comprehensive analysis of Varivax (Merck) and Varilrix vaccine strains and related viruses (6) and is in agreement with our sequencing data for the same viral regions. A comparison among P-Oka, V-Oka, Varilrix, and Varivax viral sequences reveals that the Varivax strain matches more closely P-Oka in its SNP pattern for the positions, which those authors suggested to be linked with the ability of the vaccine to reactivate and cause zoster. However, this by no means implies that the Varivax strain has an increased ability to reactivate. Postmarketing reports on the safety profile of the Varivax vaccine indicated a rate of reactivation of only 1.3 cases per 100,000 vaccine doses (10), which is in line with postmarketing surveillance of the incidence of zoster after vaccination with Varilrix (data not published). Based on postmarketing data, the U.S. Centers for Disease Control and Prevention have reported a rate of herpes zoster after vaccination of 2.6/100,000 vaccine doses distributed (2).

Previously published reports and our sequencing of the full-length genomes of Varilrix and Varivax strains (GenBank accession numbers DQ008354 and DQ008355) revealed multiple discrepancies with the work done by Sauerbrei et al. (Table 1). There are a number of possible explanations for these discrepancies. As mentioned by those authors in Discussion, "amplification of some regions (e.g., ORF 62 105331, 107252, and 107797) presented technical difficulties that conceivably reduced the quality of correspondent sequence data." Furthermore, and this is a critical issue given the mixed genomic composition of all Oka vaccines, the authors do not comment whether their sequence information was partially obtained by sequencing of plasmid clones or by direct consensus sequencing of PCR amplicons. In addition, the experimental design employed by Sauerbrei et al. included two intermediate cell culture passages of all strains on primary human thyroid cells, followed by two passages on human embryonic lung fibroblasts.

TABLE 1. Comparison of genome sequences of Oka vaccines and "zoster variant"

Feature (in Dumas)	Position[b]	Nucleotide in[a]:								
		Oka-P	AB097933	Oka-V	AB097932	VAX	RIX	A2	A3	Zoster
A→G, ORF 6, S→P	5745	**A**	**A**	G	G	A/G	G	**A**	G	G
T→C/T	10900	**T**	**T**	T/C	T/C	**T**	C/T	**T**	**T**	**T**
C→C/T, ORF 10, A→V	12779	**C**	**C**	C/T	C/T	**C**	C/T	C/T	C/T	**C**
C→T/C, ORF 21, T→I	31732	**C**	**C**	C/T	C/T	**C**	**C**	**C**	**C**	**C**
A→G, ORF 31, I→V	58595	**A**	**A**	A/G	A/G	G	A/G	G	G	G
T→C, ORF 39, M→T	71252	**T**	**T**	T/C*	C	C/T	C	**T**	C	C
T→C/T, ORF 50, S→G	87306	**T**	**T**	C/T	C/T	**T**	**T**	C	**T**	C
A→G, ORF 51, T	89734	**A**	**A**	G	G	**A**	G	**A**	A/G	G
A→A/G, ORF 52, I→V	90535	**A**	**A**	A*	A/G	**A**	A/G	**A**	A/G	**A**
T→C, ORF 54, L	94167	**T**	**T**	C	C	T/C	C	**T**	C	C
G→A/G, ORF 55, A→T	97748	**G**	**G**	A*	A/G	A/G	A/G	A	A	**G**
A→A/G, ORF 59, L→P	101089	**A***	**A/G**	G*	A/G	A/G	A/G	A	G	G
A→A/G, ORF 62, L→S	105310	**A**	**A**	A/G	A/G	A/G	A/G	**A**	G	G
A→G, ORF 62, V→A	105544	**A**	**A**	G	G	G	G	G	G	G
T→C, ORF 62, A	105705	**T**	**T**	C	C	C	C	**T**	C	C
T→C, ORF 62, R→G	106262	**T**	**T**	C	C	C	C	C/T	C	C
T→C, ORF 62, A	107136	**T**	**T**	C	C	C	C	**T**	C	C
T→C, ORF 62, S→G	107252	**T**	**T**	C	C	C	C	C/T	C	C
T→C, ORF 62, P	108111	**T**	**T**	C	C	C	C	C	C	C
A→A/G, ORF 62, M→T	108838	**A**	**A**	A/G	A/G	A/G	A/G	**A**	**A**	**A**

[a] AB097932, GenBank entry that describes the full-length sequence of V-Oka; AB097933, GenBank entry that describes the full-length sequence of P-Oka; Oka-P and Oka-V, sequencing information on respective virus strains obtained by Sauerbrei et al.; zoster, sequencing information obtained by Sauerbrei et al. on the virus strain isolated from a child with herpes zoster; *, indicates positions that do not correspond to the reference AB097932 and AB097933 sequences; VAX and RIX, sequencing information on Varivax and Varilrix, respectively, derived at GSK Bio; A2, sequencing information on the Varilrix lot produced in 1999 and used to vaccinate the child that later developed herpes zoster; A3, sequencing information on the Varilrix lot produced in 1998. Boldface indicates that the position of a nucleotide base is identical in the analyzed vaccine strains and in OKA-P and reference AB097933.

[b] Underlined numbers indicate positions that could contribute to enhance virulence for developing zoster.

This procedure hampers the interpretation of the results about the variant composition of vaccine strains (the observed shift from mixed to single nucleotides present in certain positions). The passage history of the Varivax and Varilrix Oka vaccine viruses after their receipt from Osaka University (1) includes only passages and manufacturing on MRC-5 cells.

Published reports and our own data indicate that mixed nucleotide positions are associated with the presence of a limited number of virus variants in the vaccine, consistent with the original composition of V-Oka (5). Our analysis of multiple, mixed nucleotide positions (SNPs) by the isolation and characterization of individual clones revealed that each clone is associated with a specific pattern of SNPs. This limited number of variants is present in comparable and significant proportions within the vaccine, making it very unlikely to be associated with highly uncommon events such as zoster reactivation.

Sauerbrei et al. stated that the vaccine lot given to the child who developed zoster was "V-Oka-GSK-A2." Strikingly, the SNP pattern of nucleotide positions in the isolated "zoster variant" matches the V-Oka-GSK-A3 vaccine lot much more closely (Table 1). Yet, no Oka vaccine or variant thereof has ever been associated with zoster.

There are no reports that vaccination with GSK Oka vaccine lots produced in different years would have resulted in any fluctuations of immunogenicity or in any increased incidence of zoster or varicella breakthrough cases. The experience with Varivax is the same as that with Varilrix. Therefore, the suggestion that there is an issue with the consistency of vaccine preparations is not supported by extensive clinical evidence.

There are several differences between the V-Oka and P-Oka sequences derived and used for alignment by Sauerbrei et al. and the original sequence of V-Oka from AB097932 and P-Oka from AB097933. For example, 71252 Y is C in AB097932, 90535 A is R in AB097932, 97748 A is R in AB097932, and 101089 G is R in AB097932, and 101089 G is R in AB097933. Our own results (Table 1) from the full-length sequencing of the Varilrix strain revealed that all four positions referred to above are identical to the ones given in the GenBank (AB097932). Sauerbrei et al. should explain these differences. Remarkably, five out of the six positions with suggested P-Oka "wild-type" bases (except the one in ORF 55) reflected a difference of a single versus a mixed (double) position (while the initial nucleotide was still preserved).

In previous studies (7, 9), Sauerbrei et al. analyzed the R5 repeat region of different GSK vaccine lots and found a polymorphism in the R5 repeat region in Varilrix distributed in 1991 and 1999. Our sequencing data on this region indeed confirm its length polymorphism. As has been reported by other investigators, several variants with varied but defined lengths coexist in all vaccine lots, which is consistent with the fact that all vaccine lots have been produced starting from the same working seed. Specific amino acid substitutions in ORF 62 have been linked with enhanced virus growth and spread in cell culture, and substrains purified from the vaccine mixture were shown to display different phenotypes in cell culture (5). Indeed, a comparison between V-Oka and P-Oka sequences revealed 11 bases (560, 5745, 26125, 94167, 105356, 105544, 105705, 106262, 107136, 107252, and 108111, the last 7 of which were in ORF 62) that were completely replaced by other bases (5). None of these 11 bases were demonstrated by Sauerbrei et al. to revert to the wild type in the alleged "zoster" strain.

Commercial V-Oka-strain-derived vaccines (11) have never been cloned, and complete genomic sequencing of the V-Oka

vaccine (AB097932) revealed that it contained several strains that could be separated in tissue culture (3, 4).

Sauerbrei et al. stated that GlaxoSmithKline uses a selected, plaque-purified variant of the V-Oka vaccine. This statement is not correct since the five terminal dilution passages (1) of the sonicated MRC-5 cell V-Oka-infected material aimed to increase the homogeneity of the vaccine population and not to clone out a variant. The latter is consistent with our observation that the vaccine contains a limited number of virus variants.

In summary, we believe that Sauerbrei et al. have made statements and conclusions that are not supported by their own or other published data. This is of concern because people who are not familiar with the published literature on the sequencing of Oka varicella vaccine strains may be misled concerning the safety of these vaccines.

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Authors' Reply

We concur that the Oka varicella vaccine is safe and effective, as we specifically asserted in our introduction; more than 50 million doses have now been administered in the United States, with very low numbers of adverse events (4). Our purpose in pursuing this and other work is not intended to exaggerate the importance of adverse events associated with the varicella vaccine or to cast doubt on the vaccine's efficacy and safety but to learn more about the factors that may contribute

to rare adverse events. Quotation marks aside, we were not suggesting that the strain associated with severe vaccine zoster represents a reversion to the wild type. Rather, as we stated in Discussion, we believe "that an in vivo-selected virus, V-Oka-zoster, isolated from a vaccinated zoster patient, has wild-type ORF 10 and that it emerged from a vaccine preparation containing both vaccine and wild-type gene 10 variants." We did use the word "revertant" in the abstract in a reference to vaccine loci that tend to occur as mixed genotypes in vaccines and, as such, are not useful for discriminating the varicella vaccine strains from wild-type strains. In retrospect, this was a poor choice of words and, to the extent that it led readers to conclude that the vaccine strains are necessarily undergoing further mutation, this was categorically never our intention. We did conclude, however, and continue to maintain that our targeted sequence data reveal some lot-to-lot variations in the viable-strain contents of the vaccine. We and others have demonstrated the presence of strains in the vaccine (probably at low levels) that are maintained during the vaccine production process and that emerge infrequently to cause clinical disease in vaccinated persons.

Regarding our conclusion that strains in the vaccine include a rich assortment of wild-type SNPs associated with vaccine adverse events, this association has been largely confirmed by a more extensive study (2; unpublished data). This finding is no surprise, given that the relatively small number of well-defined genomic SNPs must account, in some fashion, for the attenuation of the Oka vaccine. The occurrence of mixed SNPs at a number of vaccine loci also confirms that all of the Oka vaccine preparations represent mixtures of multiple vaccine strains (1–4). Clearly, the presence of individual clones within the vaccine mixture with "enhanced" reactogenicity, or pathogenic potential, is not sufficient in and of itself to result in clinical disease. If that were the case, the incidence of serious adverse events associated with the Oka vaccine would be unacceptably high. Accordingly, other influences, such as host factors and environmental conditions, must represent essential cofactors for the development of adverse events associated with this vaccine.

In support of a fairly diverse mixture of vaccine strains, consider our findings and those of other laboratories (1–4). Thirteen different viruses obtained by plaque purification of the Biken vaccine preparation were all determined by Gomi et al. (1) to display different arrays of wild-type and vaccine-associated SNPs at ORF 62. Quinlivan and coworkers (3) analyzed SNPs at most of the vaccine loci for 13 clinical isolates obtained from patients with documented Oka vaccine rash or zoster and found no two isolates identical. Moreover, our laboratory has observed the same in clinical isolates from 60 cases of vaccine rash and zoster (2). No two isolates from all 73 cases included in these two studies had identical SNP profiles. Both studies confirmed the consistent expression of several wild-type SNPs in all of the clinical isolates, and both studies revealed that all of the isolates from vaccine adverse events expressed more SNPs as wild-type than vaccine. Cloning viruses from tissue culture would likely underestimate the amount of actual variation, particularly if a number of variants

are present in the stock material in very low numbers. A living human body, in contrast, is likely to provide an environment much more conducive to the selective propagation of reactogenic strains. None of these data, it bears repeating, in any way call into question the performance record of Oka vaccine; in that arena, clinical experience trumps all other findings, and the vaccine in all of its marketed varieties is unmistakably safe and effective. Nonetheless, data that support an association between wild-type-skewed vaccine variants and vaccine adverse events reveal potentially important information about the pathogenesis/attenuation of the virus. Similarly, the observation of lot-to-lot variation in a few of the vaccine SNPs was not intended as an indictment of vaccine efficacy or immunogenicity. In fact, given the very limited varicella-zoster virus (VZV) strain variation observed not only between vaccine Oka and parental Oka but also among globally distributed wild-type strains, it would be fairly remarkable if appreciable differences in immunogenicity between any two strains of VZV could be demonstrated.

In reference to the differences observed in the published Oka Biken vaccine sequence and the sequence for the lot that was available to us for testing, we addressed that issue in our concluding paragraph. We suggested that the vaccines were either changing (in relative strain content, not necessarily reflecting any actual mutation of strains) over time or that possibly different seed lots were used in production. No doubt there are other possible explanations that have not occurred to us.

We are strong proponents of vaccination; few, if any, single public health strategies have had a more dramatic impact on the quality and duration of human life.

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