Developing New Smallpox Vaccines

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New stockpiles of smallpox vaccine are required as a contingency for protecting civilian and military personnel against deliberate dissemination of smallpox virus by terrorists or unfriendly governments. The smallpox vaccine in the current stockpile consists of a live animal poxvirus (*Vaccinia virus* [VACV]) that was grown on the skin of calves. Because of potential issues with controlling this earlier manufacturing process, which included scraping VACV lesions from calfskin, new vaccines are being developed and manufactured by using viral propagation on well-characterized cell substrates. We describe, from a regulatory perspective, the various strains of VACV, the adverse events associated with calf lymph-propagated smallpox vaccine, the issues regarding selection and use of cell substrates for vaccine production, and the issues involved in demonstrating evidence of safety and efficacy.

An attack on the United States with a biological or chemical weapon is an eventuality that must be actively addressed (1,2). Civilian and military public health agencies are preparing to cope with biological terrorism and warfare through efforts to stockpile vaccines and antibiotics needed to protect against diseases caused by potential biological weapons. New vaccines, such as a new smallpox vaccine, are being actively developed, and other biologics are needed to counteract the effects of these weapons on the domestic population and military forces.

Smallpox (*Variola major*) is a particularly dangerous biological weapon threat because of its clinical and epidemiologic properties (2,3). This virus can be manufactured in large quantities, stored for an extended period of time, and delivered as an infectious aerosol. Evidence indicating that potential enemies of the United States possess smallpox virus has led to concerns about the susceptibility of U.S. troops and civilians to the virus and the need to develop defense strategies (4). With the success of the World Health Organization (WHO) campaign in the 1970s to eradicate naturally occurring smallpox and the subsequent discontinuation of vaccination, a large proportion of the population has no immunity. Case-fatality rates could be higher than 25% if smallpox were released as a bioterrorist weapon (5).

The U.S. stockpile of smallpox vaccine is maintained by the Centers for Disease Control and Prevention (CDC). These decades-old doses may be inadequate to meet the vaccination needs projected after a bioweapon incident. The number of doses needed in various scenarios has recently been discussed (6). In 1972, for example, a single case of smallpox in Yugoslavia required 18 million doses of vaccine to stop the spread of disease (7). At least 40 million doses of vaccine are projected to be needed to respond effectively to a terrorist attack in the United States (6).

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Smallpox Vaccine

The only commercially approved smallpox vaccine available for limited use in the United States is Wyeth Dryvax. This vaccine is a lyophilized preparation of live *Vaccinia virus* (VACV), made by using strain New York City calf lymph (NYC_CL), derived from a seed virus of the New York City Board of Health (NYCBH) strain of VACV that underwent 22 to 28 heifer passages. The vaccine consists of lyophilized calf lymph containing VACV prepared from live calves. The animals were infected by scarification, and the skin containing viral lesions was physically removed by scraping. The lyophilized calf lymph type vaccine is reconstituted with a diluent containing 50% glycerin, 0.25% phenol, and 0.005% brilliant green. Vaccine prepared by this traditional manufacturing technique of harvesting VACV from the skin of cows (and sheep) was used in most regions of the world during the smallpox eradication campaign. The facilities, expertise, and infrastructure required for producing the virus in this way are no longer available. Wyeth Laboratories discontinued distribution of smallpox vaccine to civilians in 1983 (8).

This live-virus vaccine also caused rare but serious adverse reactions and common local reactions. Effective vaccination, classified as a "take," was indicated by the observation of a pustular lesion 6 to 10 days after vaccination at the injection site. This lesion represented a localized infection and was associated with the generation of an immune response. The vaccine take rate has been generally accepted as a correlate of vaccine efficacy. Specifically, there is a direct relationship between the intensity and extent of virus multiplication and the magnitude and duration of antibody response (9-13). The vaccine take rate of lesion formation for the currently stockpiled vaccine is >90%. Intradermal and intramuscular administration of VACV vaccine produces less severe local reactions, thus decreasing the risk of inadvertent autoinoculation or transmission; however, this inoculation route produces substantially lower responses as measured by enzyme-linked immunosorbent assay (ELISA) and neutralizing antibody testing (9,13).

Perspectives

Adverse Events Associated with VACV Vaccine

The complications arising from smallpox vaccination were well documented during the vaccination program (14-20). Dermatologic and central nervous system disorders were the most frequently recognized adverse events. Dermal complications included vaccinia necrosum, a complication with case-fatality rates of 75% to 100% that occurred almost exclusively in persons with cellular immunodeficiency (21). Eczema vaccinatum was associated with case-fatality rates of up to 10% overall and 30% to 40% in children <2 years of age (22). Generalized vaccinia was reported and probably resulted from rare bloodborne dissemination of virus in normal persons. Erythematous urticarial eruptions occurred in 1% of all primary vaccinees, and rarely, Stevens-Johnson syndrome occurred. Rarer diseases such as pericarditis (23), arthritis (24), and malignant tumors at vaccination scars (25) have been described in case reports.

During the U.S. smallpox vaccination program, approximately seven to nine deaths per year were attributed to vaccination, with the highest risk for death in infants. Most of these infant deaths were attributed to postvaccinal encephalitis (26). Most primary vaccinations in the United States were administered to children, so less is known about adverse events in adults. Rates tended to be higher in primary vaccinees and also in certain countries such as Austria and Denmark, where strains were used that may have been more virulent.

Of important concern is inadvertent administration of vaccine to persons who are immunodeficient or have other underlying contraindications to VACV vaccination, such as pregnancy. Administration of vaccine in the context of mass vaccination for outbreak control increases the risk for serious adverse events, since careful screening for vaccine contraindications would be problematic. Cultures of the vaccination sites of primary vaccinees have yielded positive cultures from days 3 through 14 after vaccination. Thus, transmission of VACV to close contacts of vaccine recipients does occur (27-30) and, in light of the global HIV epidemic and the large prevalence of patients on immunosuppressive therapy, constitutes a serious concern (31).

Development of New VACV Vaccines

During the early eradication campaign, a number of studies were undertaken to determine the factors that rendered the smallpox vaccine potent and stable. WHO and its Expert Committee provided the initial recommendations for smallpox vaccines in 1959 and updated them in 1965, defining testing procedures and standards, including a required pock count of 1 x 10^8 PFU/mL of undiluted vaccine (32). In 1967, the National Institutes of Health published more stringent requirements, including the use of a national reference vaccine preparation.

Selection of Strain

The smallpox eradication campaign used vaccines derived from many VACV strains. In the United States, these included the New York City calf lymph (NYC_CL) and New York City chorioallantoic membrane (NYC_CAM) strains, both of which were derived from a seed virus of the NYCBH strain. Strains derived from the NYCBH strain caused lower rates of adverse events, especially encephalitis. Other strains used frequently in the global eradication

program were EM-63 (USSR) and Temple of Heaven (China). The Lister or Elestree (United Kingdom) strain, prepared on the skin of sheep, was used extensively in Europe and other parts of the world. The Lister strain, which appeared to cause less illness than some of the other vaccine strains, was distributed by the WHO International Reference Centre to production laboratories for use as seed lots. By 1968, 71 producers used 15 principal strains of VACV, in addition to some unknown strains. From 1968 to 1971, the Lister strain became the most widely used throughout the world (33).

The exact origin and lineage of many of these strains and their relation to each other are not clear; however, all these strains (that were used in settings where smallpox actually occurred) were effective in eliminating the disease. With regard to efficacy, this observation suggests a degree of latitude in the selection of a VACV strain to provide protective immunity. From a scientific and regulatory perspective, many strains of VACV should be appropriate for a new vaccine as long as the manufacturer can demonstrate that the new vaccine is safe and elicits a "take" and an immune response analogous to that observed for the present licensed vaccine.

Selection of Cell Substrate

Historically, most manufacturers produced smallpox vaccine in live animals. However, this harvesting method has important limitations: it is prone to contamination with bacteria and other adventitious agents, and the antigenic and allergenic character of the accompanying animal protein can potentially result in sensitization and allergic reactions. Thus, the use of a well-characterized cell substrate for vaccine production has some potential advantages. The *Orthopoxviridae*, including *Vaccinia*, generally replicate on a wide range of candidate vertebrate fibroblast cell lines. The choice of a well-characterized or easily tested cell substrate for vaccine production can help expedite the review process (34). Issues associated with cell substrates that have been used for the manufacture of licensed live-virus vaccines may be the easiest to anticipate and address.

Use of primary cell substrates, particularly embryonated chicken egg-produced smallpox vaccine, would help to address major issues with regard to preparation of vaccine in cell culture. Cells derived from embryonated chicken eggs (especially chicken embryo fibroblasts) have been used in preparing many safe biological products, including vaccines. A variety of methods to ensure product safety can be evaluated rapidly with a high degree of confidence.

Candidate cell substrates for a new smallpox vaccine also include continuous cell lines or diploid cell strains of human or animal origin. For human cell substrates, the source of cells should be clearly described, including the tissue or organ of origin, ethnic and geographic origin, age, gender, and general physiologic condition, as well as the health or medical condition of the donor, if known. For animal cell substrates, description of the source should include species, strains, breeding conditions, tissue or organ of origin, geographic origin, age, gender, and general condition of the original donor.

The Food and Drug Administration (FDA) has licensed live-virus vaccines, such as varicella and rubella, prepared in diploid cell substrates (e.g., MRC-5, WI-38). Recently,

MRC-5 was used as a cell substrate for the preparation of an experimental smallpox vaccine under a Phase 1 trial (9). Another diploid cell strain, FrhL-2, has been used as a cell substrate for rotavirus vaccine and other live-virus vaccines tested in human clinical trials. The FDA experience in evaluating live-virus vaccines prepared in these diploid cell substrates makes the selection and use of such cell substrates potentially suitable for manufacture of a smallpox vaccine.

The continuous cell line Vero has been used to prepare a U.S.-licensed inactivated virus vaccine, the inactivated polio vaccine. Although the FDA has not yet licensed a live-virus vaccine manufactured in a continuous cell line, international experience with Vero cells suggests that they may be a suitable substrate for a smallpox vaccine. Issues pertaining to Vero cells as a substrate for live vaccines, including tumorigenic potential, were discussed at the May 2000 Vaccines and Related Biological Products Advisory Committee meeting (35). Further information regarding cell substrates can be obtained in the FDA document "Draft Points to Consider in the Characterization of Cell Lines Used to Produce Biologicals" (1993). An FDA letter to vaccine manufacturers concerning the use of Vero cells was recently issued (http://www.fda.gov/cber/letters.htm).

Any new smallpox vaccine ideally should not be less immunogenic than animal-derived vaccines. The properties of *Poxviridae* host range, virulence, and genome composition have been shown to change upon many passages in tissue culture cells. To retain the properties that make VACV a good vaccine against smallpox, the number of passages in the cell culture substrate should be kept to a minimum.

LC16m8, an attenuated VACV strain developed by Japan in 1975 for primary vaccination, was derived by passing the Lister strain 36 times through primary rabbit kidney cells at low temperature (30°C) (36). The LC16m8 strain had a take rate of 95% (compared with 93.7% for Lister), fever rate of 7.7% (compared with 26.6% for Lister), and lower neurovirulence in a monkey assay. The lower fever rate and reduced neurovirulence were considered indications that this was a safer vaccine (37). Antibody titers and induration size were lower than those of the Lister strain; however, the effect of its decreased immunogenicity on the ability of this vaccine to protect against smallpox infection is unknown since the vaccine was never used in a smallpox-endemic region.

Similarly, the Modified *Vaccinia* Ankara was derived from the Ankara *Vaccinia* strain and is one of the most highly attenuated strains. With >570 passages in chicken embryo fibroblasts, it is host restricted and unable to replicate in human and other mammalian cells. Pock lesions did not form at the site of inoculation, and no adverse reactions were observed in clinical trials in persons at high risk with skin lesions (38). Modified *Vaccinia* Ankara was intended to serve as an attenuated smallpox vaccine for primary vaccinations in regions where smallpox was not endemic, since most of the adverse reactions associated with VACV occur after primary vaccination. The vaccine was safely used to vaccinate >120,000 persons in Turkey and Germany; however, its effectiveness against smallpox is unknown.

The cell substrate must be screened extensively for both endogenous and exogenous viral contamination. The measures taken to remove, inactivate, or prevent contamination of the product from any adventitious agent present in the cell substrate should be described. When applicable, carefully designed viral clearance studies should be conducted with different methods of virus inactivation or removal in the same production process to achieve maximum viral clearance. In addition, studies should be performed to assess virus inactivation and removal. The FDA has drafted several documents that provide guidance concerning testing biological products for adventitious agents (Appendix).

Clinical Trials

Phase 1

Phase 1 vaccine trials are primarily designed to evaluate safety and immunogenicity in small groups (e.g., 10 to 20 persons) of closely monitored healthy adult volunteers. Clinical evaluations of safety in Phase 1 studies should include both local injection site (lesion measurement) and systemic reactions, as well as hematologic testing, serum chemistries, and other laboratory studies. At predefined intervals, periodic assessments of the local injection site and systemic signs and symptoms would normally be recorded. For live attenuated VACV vaccines, active monitoring of the immunization site would be required for at least 21 days or until formation and separation of the scab, whichever is longer.

One difficulty in evaluating a new smallpox vaccine is demonstrating that the vaccine generates a protective immune response in the recipient. The appearance of a vaccine take or lesion is thought to be an important correlate of immunity. In Phase 1 trials, the frequency and size of the lesion generated by a new vaccine should be compared with the values observed historically for the current licensed calf lymph-type vaccine. It is also important to compare the breadth and scale of the hosts' immune response with responses generated by vaccines previously used against smallpox infection. One of the challenges will be developing validated assays to evaluate vaccine efficacy. In a Phase 1 trial, VACV-binding antibodies should be determined by ELISA and plaque neutralization assays. Extensive characterization of the immune response, including investigation of the cellular as well as the humoral response, can be pursued in Phase 2 studies.

Situations in prelicensure trials that may lead to safety problems for both vaccinees and their close contacts need to be anticipated. Contraindications for vaccinees include immune disorders, HIV infection, eczema, history of eczema, other skin conditions including burns, immunosuppressive therapy, malignancies, lymphomas and leukemias, and pregnancy. Vaccinees who have close contacts with these contraindications should also be excluded. Mechanisms to address rare serious VACV vaccine complications (e.g., availability of VACV immunoglobulin treatment) need to be addressed in the protocol.

For live attenuated VACV products, sponsors should describe their proposed procedures for containing the live biological material during their clinical studies and should provide data on the expected survival of the organism in the environment. Shedding of live vaccine organisms would need to be evaluated; isolation of volunteers early in clinical development may be necessary to assess the shedding of VACV and the potential for spread to contacts. The vaccination site needs to be covered at all times with a porous bandage until the scab has separated and the underlying skin has healed.

A dry, porous bandage is preferred to prevent the accumulation of perspiration around the inoculation site, which can increase the risk for secondary inoculation (9). Subjects should receive a dressing kit, including a medical waste bag in case the dressing should come off. Subjects and health-care professionals who may handle these dressings should be instructed on the importance of handwashing after contact with the site to prevent both self-inoculation of the virus and contact with the site by unvaccinated persons.

Phase 2

In Phase 2 studies, generally more subjects are enrolled than in Phase 1 studies, and further data are provided on safety and immunogenicity. Phase 2 vaccine studies are often randomized and well controlled in design. One purpose of Phase 2 studies is to identify a preferred vaccine formulation, dose, and schedule for further clinical development in definitive safety and efficacy trials.

Clinical studies to compare the new vaccine with the licensed Dryvax vaccine would be done at this phase. Study size for a pivotal immunogenicity study would be based on statistical design to provide enough power to detect differences in "vaccine take," immune response, and safety (common local reactions) after inoculation by scarification, compared with the licensed Wyeth Dryvax. Both the humoral and cellular arms of the host response should be measured.

Studies to validate immune response assays should begin early in the drug development process. The goal is to have validated assays in place to assess critical immune responses before pivotal immunogenicity studies are initiated (39). At a minimum, seroconversion would have to be determined by plaque neutralization assays, VACV binding antibodies by ELISA, and cellular responses by cytotoxic T-lymphocyte response and lymphoproliferation assays.

Phase 3 and Beyond

Conducting large-scale clinical endpoint efficacy field trials for a new smallpox vaccine cannot be planned at this time, in part because there are no longer any populations at risk for naturally occurring smallpox infection. For new vaccines based on vaccine take rate and the development of neutralizing antibody responses, pivotal comparative immunogenicity studies of the new compared with the licensed vaccines will likely form the basis of efficacy assessment. Studies in humans to evaluate the ability of a vaccine to protect against subsequent challenge with a live attenuated VACV vaccine might also be informative.

Large-scale trials are needed to provide safety data to support the license application, especially to evaluate less common serious adverse events. Randomized, well-controlled trials would provide the most informative safety data.

Plans should be defined for obtaining adequate safety data. Pediatric use could be critical during a bioterrorist event; however, identifying an appropriate pediatric study population for safety and immunogenicity evaluation presents challenges. Plans for pediatric clinical development should be discussed with the FDA (40,41). Finally, given the historical information on adverse events associated with VACV vaccination, a rigorous Phase 4 study commitment is expected (42).

VACV Immunoglobulin Development

VACV immunoglobulin (VIG) is the only approved product currently available for treating complications of VACV vaccination. It is derived from the immunoglobulin fraction of plasma from persons who were immunized with VACV. The Red Cross initially obtained the product from the sera of hyperimmunized army recruits. The current supply of VIG is owned by the Department of Defense, which has provided some of this material to CDC for release in response to emergencies. VIG can be obtained from CDC to treat adverse reactions of VACV vaccine recipients, such as laboratory workers exposed to VACV or related *Orthopoxviridae*.

VIG is believed to be effective against certain complications of VACV vaccinations; it is recommended by the CDC's Advisory Committee on Immunization Practices for use in treating eczema vaccinatum, vaccinia necrosum, severe generalized VACV infections, VACV infections of the eyes (but not keratitis) or mouth, and VACV infections in the presence of other skin lesions such as burns, impetigo, varicella zoster, or poison ivy (43). No randomized controlled clinical trials have been performed to evaluate therapeutic efficacy in patients with VACV complications. However, a standard of care has developed based on data consisting of case series and anecdotal reports, as well as controlled data suggesting that VIG may modify VACV infection if administered concomitantly with vaccine.

Limited data from unblinded controlled studies support the efficacy of VIG in certain situations. In a trial conducted in Madras, India, 705 family contacts of 208 smallpox patients were randomized to receive smallpox vaccine or smallpox vaccine plus VIG as soon as possible after the index patient was admitted to the hospital. Smallpox developed in 5 of 326 contacts who received VIG compared with 21 of 379 controls, for a relative efficacy of 70% in preventing natural smallpox (44) (p<0.05, calculated by the first author).

The potential for VIG to prevent postvaccine encephalitis when administered prophylactically with vaccine was studied among Dutch military recruits in a double-blinded, randomized, placebo-controlled trial (45). More than 106,000 recruits were randomized to receive VIG plus smallpox vaccine or placebo plus smallpox vaccine. Three cases of VACV-associated encephalitis occurred in the VIG group compared with 13 cases of encephalitis in the placebo group (p<0.05, calculated by author).

Published case series of patients with severe VACV vaccination complications treated with VIG suggest that VIG lowered case-fatality rates and shortened the course of disease (20,46-52). Other trials have used antiviral agents in an attempt to treat complications (52-54), and these agents did not appear to have greater benefit than VIG. VIG is not considered to be effective in treating postvaccine encephalitis and is contraindicated for the treatment of vaccinal keratitis (55).

The recommended therapeutic dosage of VIG is 0.6 mL/kg intramuscularly, or 42 mL for a 70-kg adult; this dosage may be repeated as often as weekly. Such high intramuscular volume can be associated with trauma and possible nerve damage. Future development of VIG may include intravenous formulations to obviate these dose-related problems.

A more basic problem for the use of VIG is the availability of licensed product. The amount of VIG needed to respond

to the adverse events associated with a large-scale vaccination program cannot be manufactured from the currently available human sera.

Future Considerations

An important benefit of the eradication of naturally occurring smallpox was the cessation of smallpox vaccination and the elimination of iatrogenic VACV vaccine adverse events. Growing concern about the U.S. population's vulnerability to a potential terrorist attack with biological weapons has led to strong political commitment to develop and stockpile new vaccines and other agents to respond to such an event (56). This response requires the development and manufacture of an effective vaccine, as well as products to treat the potential complications arising from a widespread vaccination program.

The evaluation of these products, especially ones that do not induce a vaccine take and induce an immune response that substantially differs from that induced by the currently licensed vaccine, may pose problems. Specifically, the usual measures of efficacy that require exposure to natural disease currently are not possible because the disease has been globally eradicated. In addition, definitive human challenge and protection studies with *Variola* would not be possible for ethical reasons.

In general, the issue of providing substantial evidence of efficacy when the traditional efficacy studies in humans cannot be done is of concern to the FDA. To address this issue, FDA has published and requested comments on a proposed rule intended to address certain efficacy issues for new agents to be used against lethal or permanently disabling toxic substances (57). The proposed rule attempts to define standards so that new drug and biological products developed to prevent serious or life-threatening conditions could be approved for marketing on the basis of evidence of effectiveness derived from appropriate studies in animals, without adequate and well-controlled efficacy studies in humans (21 CFR 314.126). For example, the wide host range of poxvirus viruses could potentially lead to the exploration of a monkeypox model to obtain supporting data. For vaccines, human safety and immunogenicity data would also be needed to support such approvals. The final rule, when published, would be expected to facilitate the development and licensing of certain new products to protect against biological warfare. This proposal would not apply if approval can be based on other standards in FDA regulations.

The future response to complications inherent in a new smallpox vaccine during a wide-scale vaccination program could entail a combination approach including VIG, antiviral drugs, and immune-based therapy involving humanized antibodies or fragments of antibodies produced in animals. Antiviral medications could be mass-produced, stored for long periods of time, and distributed quickly, if needed. Cidofovir, a DNA polymerase inhibitor developed for *Cytomegalovirus* retinitis, has been found to be active in preventing *Variola* infection in cultured cells and protects mice against lethal VACV challenge (58,59). In the long term, more products should be developed to protect the general population against adverse events due to VACV infection. Each product will pose unique scientific issues for evaluation and licensure.

Acknowledgments

Dr. Rosenthal is a medical officer in the Office of Vaccines Research and Review, U.S. Food and Drug Administration. His work focuses on the review of new vaccine applications, including vaccines against bioterrorism and biowarfare agents.

Appendix

A. Examples of U.S. Food and Drug Administration and International Conference on Harmonization (ICH) Documents Relevant to the Manufacture and Product Quality of New Smallpox Vaccines

Title	Date
FDA points to consider in the characterization of cell lines used to produce biologicals	1993
ICH guidance on quality of biotechnological/biological products: derivation and characterization of cell substrates used for production of biotechnological/ biological products	1998
ICH guidance on viral safety evaluation of biotechnology products derived from cell lines of human or animal origin	1998
FDA guidance for industry: content and format of chemistry, manufacturing and controls information and establishment description information for a vaccine or related product	1999
FDA draft guidance for industry: revised preventive measures to reduce the possible risk of transmission of Creutzfeldt-Jakob Disease (CJD) and Variant Creutzfeldt-Jakob Disease (vCJD) by blood and blood products	2001

To obtain these documents, connect to http://www.fda.gov/cber/ guidelines.htm or call the FDA Office of Communication, Training and Manufacturers Assistance at 1-800-835-4709.)

B. Examples of ICH Documents Relevant to Clinical Testing of Vaccines

Title	Date
Good clinical practice: Consolidated guideline	1997
General considerations for clinical trials	1997
Structure and content of clinical study reports	1996
Statistical principles for clinical trials (draft)	1997

To obtain these documents, connect to http://www.fda.gov/cber/ guidelines.htm or call the FDA Office of Communication, Training and Manufacturers Assistance at 1-800-835-4709.

C. U.S. Code of Federal Regulations (CFR) and U.S. Pharmacopeia (USP) Standards Applicable to Vaccines

Issuance	Subject
21 CFR 50	Protection of Human Subjects
21 CFR 56	Institutional Review Boards
21 CFR 58	Good Laboratory Practices
21 CFR 210, 211	Good Manufacturing Practices
21 CFR 312	Investigational New Drug Applications (INDs)
21 CFR 314.126	Adequate and Well-Controlled Studies
21 CFR 610	General Biological Product Standards
21 CFR 610.12	Sterility Testing
21 CFR 610.13	Purity Testing
USP 24-(85)	Bacterial Endotoxin Test
USP 24-(1510)	Pyrogen Test
USP 24-(1171)	Sterility

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