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Evaluation of a 9-valent HPV vaccine in Sprague-Dawley rats: Nonclinical studies assessing general, reproductive, and developmental toxicity

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ABSTRACT

GARDASIL®9, a 9-valent vaccine against human papillomavirus (9vHPV), was developed to prevent diseases mediated by HPV types 6/11/16/18/31/33/45/52/58. During the development of the vaccine, three nonclinical safety studies were conducted to evaluate repeat-dose toxicity and prenatal and postnatal developmental toxicity in Sprague-Dawley rats. In all studies, the vaccine was administered via intramuscular injections of 0.5 mL (the human dose) divided equally into each quadriceps muscle. In the repeat-dose toxicity study, potential local and systemic toxic effects of the 9vHPV vaccine were evaluated after 4 doses given 21 days apart and after a 21-day recovery period. In the prenatal study, virgin females were dosed at 5 and 2 weeks prior to mating and on Gestation Day [GD] 6 (3 total doses). Potential postnatal developmental toxicity of the vaccine formulation was evaluated after 4 total doses (premating to lactation). There were no treatment-related unscheduled deaths in any studies. In the 3-month repeat-dose toxicity study, no adverse effects in male or female rats were observed. Anticipated systemic effects representing immunological responses and local inflammatory reactions at the injection sites were noted in the vaccine-treated groups, with a trend toward recovery by the end of the 21-day recovery period. In the prenatal developmental toxicity study, there was no evidence of toxicity in females given the vaccine. There were no effects on fertility or reproductive performance of the parental females and no evidence of developmental toxicity. In the postnatal study, there was no evidence of toxicity in vaccine-treated females and no evidence of developmental toxicity based on standard postnatal parameters, including behavioral testing and reproductive performance. The vaccine induced antibody responses in all studies and vaccine-specific antibodies were detected in offspring in the developmental toxicity studies. These results support the favorable safety profile of GARDASIL®9.

1. Introduction

Human papillomavirus (HPV) is a group of more than 170 related viruses with each virus given a number called its HPV type [1]. These viruses contain a circular double-stranded DNA and a viral shell that is composed of 72 capsomeres. Every subunit of the virus is composed of 2 structural proteins, L1 and L2. The first vaccines against HPV targeted the 2 high-risk HPV types 16 and 18, which cause most cervical cancers. CERVARIX® contains L1 proteins for only these 2 HPV types, while GARDASIL® also includes HPV types 6 and 11. Both vaccines use recombinant proteins that self-assemble into hollow, virus-like particles (VLPs) and elicit virus-neutralizing antibody responses that prevent infection with the HPV types represented in the vaccine.

HPV infection causes benign, precancerous, and malignant disease in the anogenital area and upper airway, including cancers and precancers of the cervix, vulva, vagina, anus, and penis, oropharyngeal cancers, anogenital warts, and recurrent respiratory papillomatosis [20]. It has been estimated that worldwide in 2012 as many as 4.5% of new cancer cases, including cancers of the cervix, anogenital tract and head and neck, were associated with HPV infection [2]. HPV types 16 and 18 are responsible globally for 71% of cases of cervical cancer [3,2], and HPV types 6 and 11 play a major role in the incidence of genital warts [5]. An additional 5 HPV types (31, 33, 45, 52, and 58) account for another 20% of cervical cancers. Together these 9 HPV types also cause a total of

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approximately 90% of HPV-related vulvar, vaginal, and anal cancers, 80% of high-grade cervical precancers, and 50% of low-grade cervical lesions [14]. Thus, a 9-valent HPV vaccine was developed to cover these 9 HPV types; it was shown to be efficacious and generally well tolerated in clinical trials, and first licensed in 2014 in the United States under the name GARDASIL®9 [10].

The safety profiles of the bivalent and quadrivalent HPV vaccines have been reviewed extensively by several health organizations. Given the intended population for this vaccine, a primary concern is the potential effects on reproduction and embryonic/fetal development [20]. Vaccination with HPV vaccines is not recommended in pregnancy due to the absence of well-controlled studies in pregnant women. However, as pointed out in the 2017 WHO position paper, "No specific safety concerns have been identified for the outcome of pregnancy or for fetal development in the women given any of the 3 HPV vaccines." That statement was based on data available from inadvertent vaccine exposures during pregnancy, and through established pregnancy registers. Additional studies support this statement [6,19,13,18,8,9]. There is also no evidence of a significant elevated risk of primary ovarian insufficiency after adolescent vaccination with HPV vaccines [12].

Nonclinical studies with the bivalent and quadrivalent vaccines have predicted the lack of effects on fertility and embryonic/fetal development. In a rat study with the quadrivalent vaccine, there were no effects on female fertility, prenatal development, or postnatal development and reproductive performance [22]. Another study from the same laboratory found no evidence of adverse effects on male rat reproductive function, including histomorphology of testes and epididymis, sperm count, and sperm motility [23]. Segal and colleagues [17] reported no effects of the bivalent vaccine in rats on female fertility, prenatal development, or postnatal development. In a subsequent report, single and repeated doses of the bivalent vaccine were determined to be safe and well tolerated in rats and rabbits [16]. These animal studies also demonstrated immunogenicity of the vaccines in the test species, including exposure to the offspring. In addition, no unanticipated effects of the adjuvants alone were noted.

The design and results of three rat studies with the 9vHPV vaccine that assessed local and systemic toxicity, as well as prenatal and postnatal developmental toxicity are described herein. As with the other HPV vaccines, these studies demonstrate no unanticipated toxicological effects of the 9vHPV vaccine or adjuvant in male or female rats or in the offspring of mothers administered the 9vHPV vaccine or adjuvant.

2. Materials and methods

The 3 studies detailed below and in Supplementary M&M were conducted at Merck & Co., Inc. (West Point, PA, USA) under current Good Laboratory Practice regulations for nonclinical laboratory studies. All studies utilized Sprague-Dawley [Crl:CD(SD)] rats obtained from Charles River Laboratories (Raleigh, NC). Rats were approximately 8 weeks of age at study start. All postweaning and adult animals were identified by microchip implant. Animal housing and care procedures were compliant with The Guide for the Care and Use of Laboratory Animals (8th edition). All procedures performed on the animals were reviewed and approved by the Institutional Animal Care and Use Committee. The animal facility was fully accredited with AAALAC International. Details of animal husbandry and physical sign observations are described in Supplementary M&M.

2.1. Test agents

The components of the adjuvant (Merck aluminum adjuvant [MAA], also called amorphous aluminum hydroxyphosphate sulfate) and vaccine formulations used in the 3 studies are shown in Table 1.

The 3 dose levels in Study 1 were intended to cover the ranges of VLP and MAA concentrations planned to be tested in clinical studies. Except for the low-dose formulation, all other doses of antigen and adjuvant are similar or higher than in the marketed product (GARDASIL®9, Study 3 formulation was identical to the marketed product). For all studies, sterile formulations of the 9vHPV vaccine, MAA, and phosphate buffered saline (PBS) were supplied by various units within Merck & Co., Inc., West Point, Pennsylvania, U.S.A. For Study 2, sterile PBS was supplied by HyClone Laboratories, Inc., Logan, Utah, U.S.A. For all 3 studies, the vaccine formulations were determined to be stable over the period and conditions of use in each study and shown to be of acceptable purity and potency by standard release testing.

2.2. Experimental/study designs

Study designs were developed in accordance with WHO and FDA guidelines for assessing the potential toxicity of vaccines for infectious diseases [21,4]. In addition, the developmental toxicity studies conformed to ICH guidelines [7].

In Study 1, five groups of 20 animals/sex were dosed by intramuscular (IM) injection on Study Days (SD) 1, 22, 43, and 64,

Table 1
Constituents of Merck Aluminum Adjuvant (MAA) and 9-valent HPV vaccine formulations.

Ingredient[a]	MAA	Study 1 Low dose	Study 1 Mid dose	Study 1 High dose	Study 2 Vaccine	Study 3 Vaccine*
HPV type 6 VLP	–	40	60	80	60	60
HPV type 11 VLP	–	80	80	80	80	80
HPV type 16 VLP	–	80	160	160	160	120
HPV type 18 VLP	–	40	80	160	110	80
HPV type 31 VLP	–	40	40	60	60	40
HPV type 33 VLP	–	40	40	60	60	40
HPV type 45 VLP	–	40	40	60	60	40
HPV type 52 VLP	–	40	40	60	60	40
HPV type 58 VLP	–	40	40	60	60	40
Aluminum[b] (mg/mL)	1.097	0.788	1	1.097	1	1
Sodium borate (μg/mL)	70	70	70	70	70	70
Sodium chloride (M)	0.32	0.32	0.32	0.32	0.33	0.33
Histidine (mM)	10	10	10	10	10	10
Polysorbate 80 (%)	0.01	0.01	0.01	0.01	0.01	0.01

HPV = Human papilloma virus.
VLP = Virus-like particle.
[a] Prepared in water for injection, pH 6.2. Concentrations of HPV types in units of μg/mL.
[b] As aluminum hydroxyphosphate sulfate.
* This formulation is identical to GARDASIL®9.

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