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The efficacy and safety of Tipapkinogen Sovacivec therapeutic HPV vaccine in cervical intraepithelial neoplasia grades 2 and 3: Randomized controlled phase II trial with 2.5 years of follow-up

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HIGHLIGHTS

- Tipapkinogen sovacivec completely resolves CIN 3 lesions significantly more frequently than placebo.
- Tipapkinogen sovacivec completely clears HPV16 viral DNA associated with CIN 2/3 significantly more often than placebo.
- Tipapkinogen sovacivec has significantly greater complete resolution rates of CIN 2/3 regardless of HR HPV type.
- Tipapkinogen sovacivec offers 36% complete resolution or partial response of CIN2/3 associated with all HR HPV types.

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ABSTRACT

Background. While prophylactic human papillomavirus (HPV) vaccination exists, women are still developing cervical intraepithelial neoplasia (CIN) grade 2 or 3 for which an immunotherapeutic, non-surgical, approach may be effective. The primary aim was to assess the efficacy of tipapkinogen sovacivec (TS) vaccine in achieving histologic resolution of CIN2/3 associated with high risk (HR) HPV types.

Methods. Women 18 years and older who had confirmed CIN2/3 were enrolled in a randomized, double blind, placebo-controlled phase II trial and assigned to drug in a 2:1 ratio (vaccine:placebo). The primary endpoint occurred at month 6 when the excisional therapy was performed; cytology and HR HPV typing were performed at months 3, 6 and every six months through month 30. The safety population included all patients who received at least one dose of study drug.

Results. Of the 129 women randomized to vaccine and 63 to placebo, complete resolution was significantly higher in the vaccine group than placebo for CIN 2/3 regardless of the 13 HR HPV types assayed (24% vs. 10%, $p < 0.05$); as well as for only CIN 3 also regardless of HR HPV type (21% vs. 0%, $p < 0.01$). Irrespective of baseline HPV infection, viral DNA clearance was higher in the vaccine group compared to placebo ($p < 0.01$). The vaccine

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was well tolerated with the most common adverse events being injection site reactions.

Conclusions. The TS vaccine provides histologic clearance of CIN 2/3 irrespective of HR HPV type in one third of subjects and is generally safe through 30 months.

1. Introduction

Immunotherapeutic advances against human papillomavirus (HPV) are the next frontier in treating at least six different cancers or cancer precursors of the cervix, anus, vulva, vagina, penis, and oropharynx [6]. Of these, cervical cancer comprises over 80% of the HPV associated cancer global burden [3,5], and, despite the high surgical cure rate, is the focus of therapeutic vaccine development. While prophylactic HPV vaccines prevent infections from half of the HR HPV types associated with cancer, there is a clinical need for non-surgical, non-ablative therapeutics to control HPV diseases. In addition, surgical and ablative therapies, while effective, are associated with reproductive morbidities [17–19]; and recurrence can occur, especially with positive surgical margins, likely because of persistent viral infection [2,22]. Furthermore, current screening strategies have no therapeutic option for women with persistent HPV infections whose likelihood of cancer progression is unknown [24].

The modified vaccinia virus Ankara (MVA) is a highly attenuated replication-deficient strain of vaccinia virus used widely as a gene-delivery system of vaccines. TS has inserted genes that code for three proteins: human cytokine IL-2, and modified forms of HPV 16 E6 and E7 proteins that have been rendered non oncogenic. MVA by itself contributes to the immune reaction by the induction of an Interferon-alpha response [11]. Upon sub-cutaneous injection, TS infects the surrounding cells. The expressed HPV16 E6 and E7 proteins are then processed and presented by dendritic cells which are co-activated by the viral infection. These dendritic cells migrate to the draining lymph-node and present E6 and E7 peptides to the naive T-cells present in the lymph-node, which should allow development of a targeted cell mediated immune response.

Preclinical and early clinical studies suggest that TS can cause CIN2/3 resolution by induction of a cell mediated immune response [4]. The primary aim of this study was to assess the efficacy of TS compared to placebo to achieve complete histologic resolution (no CIN) at month 6 in subjects with CIN2/3 associated with HPV 16 monoinfection. This analysis is extended to include complete resolution and partial lesion response associated with other HR HPV infections. The secondary aims are to assess viral DNA clearance over 2.5 years after definitive excision at month 6 and safety assessment.

2. Methods

2.1. Study design and participants

This study was a multicenter, prospective, randomized, double-blind, 2-arm parallel group, placebo-controlled, phase IIb trial conducted in the United States, Spain, Belgium, France, and Finland, among 66 study locations. This trial was registered at Clinicaltrials.gov identifier NCT01022346; EudraCT 2008–006946-24. The protocol was approved by the institutional review board or ethics committee at each participating institution, and all patients gave written informed consent. Women were recruited based on known colposcopic biopsy results at their home institutions.

Women aged 18 years and older were invited to enroll if they had a histologically confirmed central pathology review (CPR) panel reconfirmation of a first diagnosis of CIN2/3 associated with single or multiple HR HPV infections. To be included in the study, women had to have residual disease present after biopsy, involving at least one, but no more than two quadrants, and did not have colposcopic characteristics that would be concerning for invasive disease. These restrictions were enforced to rule out potential occult microinvasive cancers [25].

Women who had received a prophylactic HPV vaccine were excluded. Women who had prior excisional or ablative therapy for any CIN, vulvar intraepithelial neoplasia (VIN) or vaginal intraepithelial neoplasia (VaIN), or any atypical endometrial or glandular cells or carcinoma were excluded. In addition, women were excluded if they were pregnant or breast-feeding, co-infected with hepatitis B, hepatitis C, cytomegalovirus, Epstein Barr virus or HIV, or had other immune deficient states.

2.2. Study randomization, dosing and blinding

Study subjects were randomized in a 2:1 ratio (TS vaccine:placebo) and stratified based on the HR HPV type: HPV 16 monoinfection (stratum 1) vs. other HR HPV single or multiple infections (stratum 2), which are defined as the alpha 5, 6, 7 and 9 clades (HPV 51; and HPV 56; and HPV 18, 39, 45, 59, 68; and HPV 16, 31,33, 35, 52, 58, respectively as detailed in Table 1). Placebo doses were prepared and labelled in identical syringes by a study pharmacist at each institution.

The study CPR was blinded to the randomization process, as were the laboratory personnel performing HPV typing and clinical personnel delivering the injections.

2.3. Procedures

Study subjects with CIN2/3 were recruited from August 2009–September 2011. Those meeting study eligibility were assigned to drug or placebo which was injected subcutaneously in the thigh, on days 1, 8, and 15.

Subjects returned at month 3 and month 6 for cytology and HR HPV testing. Colposcopy was performed at month 3 where biopsies were taken only at the investigator's discretion. At month 6, subjects underwent definitive treatment (loop electrosurgical excision procedure (LEEP/LLETZ) or conization) as well as HR HPV typing. Subjects continued follow-up every six months until month 30 with repeat cytology, HR HPV testing, adverse event assessments and colposcopy and biopsy, if clinically needed.

Subjects recorded injection site reactions in their diary every day for seven days following each injection, to assess tenderness, pruritus, erythema, induration and pain. Adverse events were recorded using the Medical Dictionary of Regulatory Activities (MedDRA) version 16.1 throughout the trial. Serious adverse events were recorded and assessed by both the site investigators and the blinded medical monitor. These data, including hematology and blood chemistries, were reviewed for safety in real time.

2.4. Outcomes

The primary efficacy endpoint was the individual histological resolution at six months after the first injection in the HPV16 monoinfected modified intent to treat (mITT) population defined as subjects receiving at least one dose of study drug and who were confirmed to have CIN2 or 3 at baseline by the CPR panel. Other exploratory efficacy endpoints included histological complete resolution or partial response (CIN1) six months after the first injection. These exploratory analyses included age, CIN category, HPV type infection, and number of quadrants of lesion

Table 1
Demographics of study patients at baseline by randomization group: Intent to Treat (ITT) population for safety analysis.

	Tipapkinogen sovacivec vaccine	Placebo
	$N = 136$	$N = 70$
Age, yrs. mean (SD)	30.1 (7.8)	29.8 (7.6)
Range (Min–max), yrs	18–60	19–50
≤30 years, n (%)	84 (62)	44 (63)
>30 years, n (%)	52 (38)	26 (37)
Ethnicity/Race, n (%)		
Hispanic	32 (24)	9 (13)
Not Hispanic, Black	10 (7)	8 (11)
Not Hispanic, White	89 (65)	51 (73)
Not Hispanic, Other	5 (4)	2 (3)
Global, n (%)		
US	84 (62)	40 (57)
Western Europe	52 (38)	30 (43)
Central panel review histologic diagnoses, n (%)		
<CIN 2[a]	5 (4)	6 (9)
CIN 2	53 (39)	30 (43)
CIN 3	76 (56)	33 (47)
Adenocarcinoma-in-situ[a]	1 (1)	1 (1)
Body mass index (BMI) (kg/m^2) mean (SD)	25.2 (5.9)	25.2 (6.8)
History of small pox vaccine, n (%)		
Yes	9 (7)	6 (9)
No	92 (68)	41 (59)
Unknown	35 (26)	23 (33)
Stratum 1 – HPV 16 mono-infections	56 (41)	29 (41)
Stratum 2[b]	80 (59)	41 (57)
Single genotype	27 (34)	12 (29)
Multiple genotypes	53 (66)	29 (71)

[a] Omitted from ITT population to create modified ITT (mITT) population for analysis.
[b] Stratum 2 could be one of 7 different combinations:
1. HPV 16 AND one or more of 31, 33, 35, 52, 58.
2. One or more of 31, 33, 35, 52, 58.
3. One or more of 18, 39, 45, 59, 68.
4. HPV 16 AND 18, 39, 45, 59, 51, 56, 68 AND one or more of 31, 33, 35, 52, 58.
5. One or more of HPV 18, 39, 45, 59, 51, 56, 68 AND one or more of 31, 33, 35, 52, 58.
6. HPV 16 AND one or more of 31, 33, 35, 51, 52, 56, 58, 66, 73, 82.
7. Other combinations of single or multiple genotypes not listed above.

at baseline, as well as race/ethnicity, and number of biopsies. Secondary endpoints included viral DNA clearance of baseline HPV over 2.5 years, as well as long term safety.

2.5. Statistical analyses

The population for safety analysis comprised all women who received either vaccine or placebo injections regardless of study completion. The analysis of efficacy for the study did not have a predefined per-protocol population. The main analysis population was a modified intent to treat (mITT) population defined as only patients who are confirmed to have CIN 2/3 at baseline by the CPR panel and who completed at least the six -onth follow-up surgical excision.

The sample size was not determined by a formal power calculation but is based on a feasibility assessment of possible patient recruitment and the likely distribution of HPV types. An interim administrative look (IAL) was performed on the month 6 consensus histology diagnoses and was based on a Bayesian assessment of the predictive probability of success in a phase III trial given the interim results. The predefined analysis plan was developed for completion regardless of the IAL decision.

We explored the following null and alternative hypotheses: the treatment resolution rate is <60% or the treatment resolution rate is less than double the control resolution rate, compared to whether the treatment resolution rate is at least 60% and the treatment resolution rate is at least double the control resolution rate. The 60% resolution rate was considered the lowest clinically acceptable rate given that surgical cures range from 75 to 100% [2].

Descriptive analyses corresponded to a priori and post hoc stratification variables: by age (>30 years old vs ≤ 30 years old), and by baseline diagnosis (CIN2 vs CIN3) and by HPV infection types (HPV 16 monoinfection, HPV16 infection with any of the other 12 HR HPV types, non-HPV16 HR infections and all 13 HR HPV types). Statistical significance was reported at the $p < 0.05$ and $p < 0.01$ levels for chi-square and Fisher's exact test.

Univariate and multivariate logistic regression were used to evaluate a priori and post hoc impact of the treatment with and without adjustment on age, HPV16 status, HPV type, CIN grade, ethnicity/race, number of biopsies and number of quadrants of disease at baseline. Wald 95% confidence intervals are reported for individual rates and odds ratios with significance at $p < 0.05$.

Viral DNA clearance time was presented using Kaplan-Meier curves where unstratified log-rank tests were used to compare treatment groups by a priori and post hoc stratification. All calculations were performed with SAS 9.4 [23] with significance at $p < 0.05$.

3. Results

700 patients were screened with 206 meeting study eligibility (Fig. 1) between August 2009 and September 2013 (last patient last visit). The intent to treat (ITT) safety population included 136 study subjects who received TS vaccine and 70 who received placebo; 56 subjects were in Stratum 1 (HPV 16 monoinfection) and received vaccine and 29 received placebo. Stratum 2 enrolled 80 to vaccine and 41 to placebo. Table 1 shows equitable distributions between treatment groups for age, race, body mass index (BMI), CIN2/3 distribution and strata allocation in this population.

The modified intent to treat (mITT) population received at least one dose of vaccine and had a confirmed entry biopsy of CIN 2 or 3 by CPR panel, resulting in 129 subjects receiving vaccine vs. 63 receiving placebo. In the HPV16 monoinfection stratum, 55 were randomized to the vaccine and 27 to placebo; in stratum 2, 74 were randomized to vaccine and 36 to placebo.

3.1. Primary aim (Table 2)

Histologic complete resolution from CIN 2/3 at month 6, among the HPV 16 monoinfected women occurred in 18% (95% CI: 8–28%) of the vaccine group compared to 4% (95% CI: 0–11%) of the placebo group, resulting in a vaccine efficacy of 80% (95% CI: 67–88%).

Other exploratory histologic endpoints at month 6 for women infected with at least HPV 16 and any other HR HPV type showed complete resolution from CIN 2/3 in 18% (95% CI: 4–32%) of the vaccine group vs. 8% (95% CI: −7–24%) of the placebo group, with a vaccine efficacy of 53% (95% CI: 47–61%). Complete resolution of women with CIN 2/3 who were infected with any number of HPV infections that are any HR HPV type except HPV 16 occurred in 35% (95% CI: 21–49%) of the vaccine group and 17% (95% CI: 2–32%) of the placebo group, with a vaccine efficacy of 52% (95% CI: 38–66%). Within this HPV infected group complete resolution of CIN 3 alone occurred significantly more often ($p < 0.05$) in the vaccine group (36% (95% CI: 46–57%)) than the placebo group (0% (95% CI: -2–2%)), with a vaccine efficacy of 100% (95% CI: 85–100%). When all HR HPV types were considered, complete resolution from CIN 2/3 occurred significantly more often in the vaccine group (24% (95% CI: 17–31%)) than the placebo group (10% (95% CI: 2–17%)) ($p < 0.05$), with a vaccine efficacy of 60% (95% CI: 54–67%). For the subset of women with any HR HPV type and a CIN 3 baseline lesion, complete resolution occurred significantly more often in the vaccine group (21% (95% CI: 12–30%)) than the placebo group (0% (95% CI: −2–2%)) ($p < 0.01$), with a vaccine efficacy of 100% (95% CI: 95–100%). The complete resolution rate never exceeded the a priori 60% threshold, but the vaccine induced resolution rates exceeded

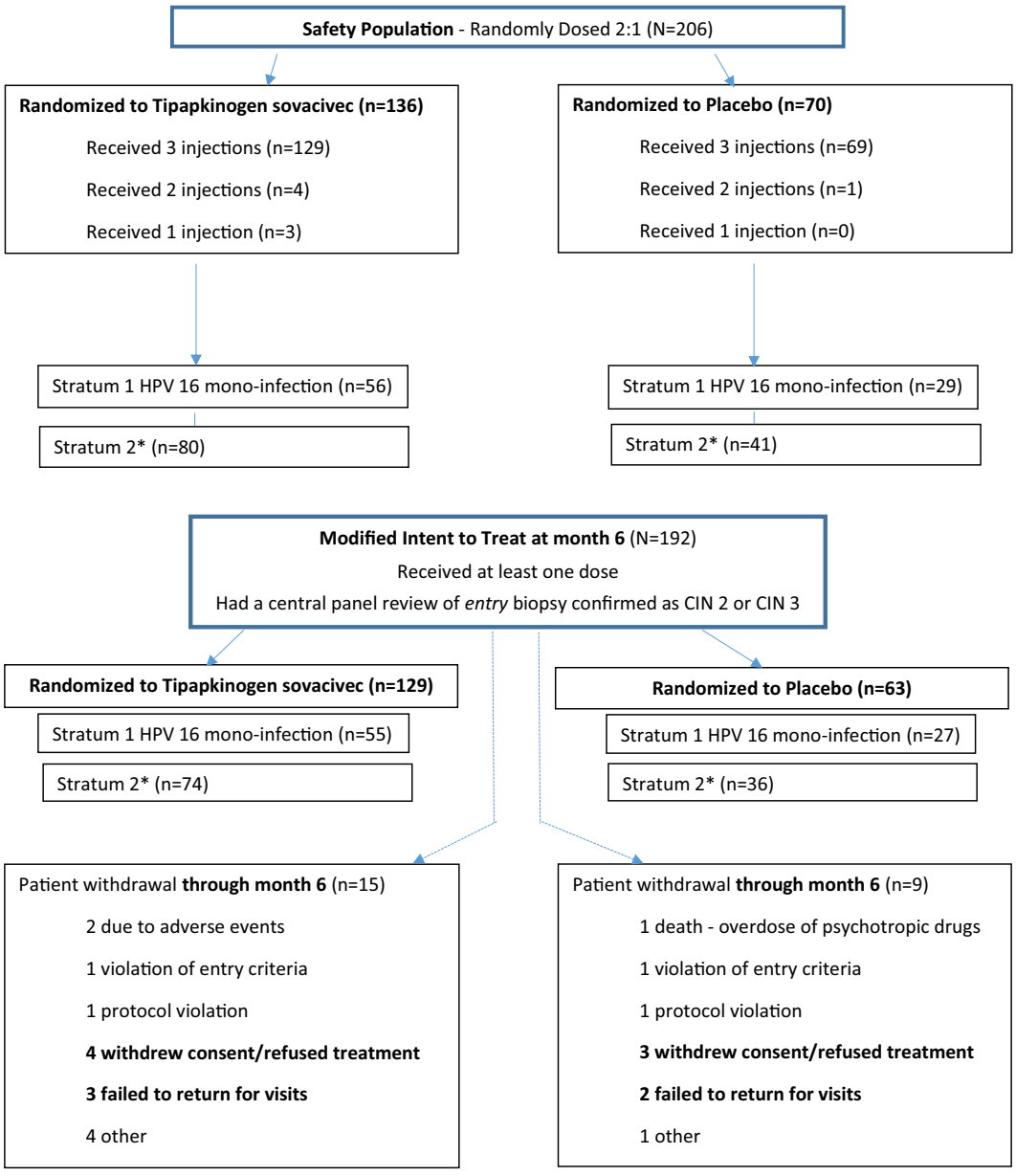

Fig. 1. Trial schematic—700 Patients Screened and subsequent enrollment in the safety population and the modified intent to treat (mITT) population.

twice the placebo rates, meeting part of the a priori hypothesis, in four subgroups: the stratum 1 HPV16 monoinfection for both the CIN 2/3 and CIN 3 populations as well as the non-HPV 16 infections and all HR HPV infections for the CIN 3 population (Supplemental Figure S1).

Histologic partial response at month six was never significantly different between vaccine and placebo groups for any HR HPV stratum. When combining the histologic complete resolution and partial response rates together, the women with CIN 3 associated with any HR HPV type had a significantly higher resolution/response rate of 32% in the vaccinated group vs. 12% in the placebo group ($p < 0.01$); in addition, women with CIN 2/3 associated with any HR HPV type had a 36% resolution/response rate vs. 21% in the placebo group ($p < 0.05$).

3.2. Logistic regression predictions

3.2.1. Univariate

Three univariate models were explored (Table 3). The first two models explored complete resolution vs. no response in two populations: a) among both CIN 2 and 3 baseline lesions, b) among only CIN 3 baseline lesions. In the first model, regardless of treatment, HPV 16 infections were significantly less likely to resolve than any other HR HPV type (OR = 0.42 (95% CI: 0.20–0.88)); and those with three biopsies at baseline were significantly more likely to resolve compared to those with a single diagnostic biopsy (OR = 3.17 (95% CI: 1.03–9.75)). Among only CIN 3 women, those of Hispanic ethnicity/race were significantly more likely to completely resolve their lesion vs. non-Hispanic white women (OR = 10.64 (95% CI: 3.00–37.73)); and women with three biopsies at baseline were also significantly more likely to have lesion resolution compared to a single biopsy (OR = 5.36 (95% CI: 1.14–25.26)).

The third univariate model explored the combination of complete resolution and response vs. no response for the CIN 2/3 baseline. In this model, only ethnicity/race showed significance in resolution/response at month six.

3.2.2. Multivariate

There were three corresponding multivariate models adjusting for all single univariate factors. The first multivariate model showed that vaccine treatment significantly increased the odds of complete

Table 2
Six-month primary endpoints of complete resolution and partial response, mITT population.

	Tipapkinogen Sovacivec			Placebo			
	Complete resolution	Partial response	No response	Complete resolution	Partial response	No response	Vaccine efficacy[a] for complete resolution % (95% CI)
HPV 16 mono infection	10/55 (18.2%)	7/55 (12.7%)	38/55 (69.1%)	1/27 (3.7%)	5/27 (18.5%)	21/27 (77.8%)	80% (67–88%)
Age: ≤30 yrs	6/32 (18.8%)	4/32 (12.5%)	22/32 (68.8%)	0/14 (0.0%)	2/14 (14.3%)	12/14 (85.7%)	
Age: >30 yrs	4/23 (17.4%)	3/23 (13.0%)	16/23 (69.6%)	1/13 (7.7%)	3/13 (23.1%)	9/13 (69.2%)	
CIN2	4/16 (25.0%)	1/16 (6.3%)	11/16 (68.8%)	1/9 (11.1%)	2/9 (22.2%)	6/9 (66.7%)	
CIN3	6/39 (15.4%)	6/39 (15.4%)	27/39 (69.2%)	0/18 (0.0%)	3/18 (16.7%)	15/18 (83.3%)	100% (91–100%)
HPV 16 infection with any other HR HPV type	5/28 (17.9%)	4/28 (14.3%)	19/28 (67.9%)	1/12 (8.3%)	0/12 (0.0%)	11/12 (91.7%)	53% (47–61%)
Age: ≤30 yrs	3/20 (15.0%)	4/20 (20.0%)	13/20 (65.0%)	1/8 (12.5%)	0/8 (0.0%)	7/8 (87.5%)	
Age: >30 yrs	2/8 (25.0%)	0/8 (0.0%)	6/8 (75.0%)	0/4 (0.0%)	0/4 (0.0%)	4/4 (100.0%)	
CIN2	3/13 (23.1%)	3/13 (23.1%)	7/13 (53.8%)	1/8 (12.5%)	0/8 (0.0%)	7/8 (87.5%)	
CIN3	2/15 (13.3%)	1/15 (6.7%)	12/15 (80.0%)	0/4 (0.0%)	0/4 (0.0%)	4/4 (100.0%)	100% (80–100%)
Non-HPV 16 infections of any HR HPV type and number of types	16/46 (34.8%)	4/46 (8.7%)	26/46 (56.5%)	4/24 (16.7%)	2/24 (8.3%)	18/24 (75.0%)	52% (38–66%)
Age: ≤30 yrs	8/27 (29.6%)	3/27 (11.1%)	16/27 (59.3%)	3/16 (18.8%)	1/16 (6.3%)	12/16 (75.0%)	
Age: >30 yrs	8/19 (42.1%)	1/19 (5.3%)	10/19 (52.6%)	1/8 (12.5%)	1/8 (12.5%)	6/8 (75.0%)	
CIN2	8/24 (33.3%)	3/24 (12.5%)	13/24 (54.2%)	4/13 (30.8%)	1/13 (7.7%)	8/13 (61.5%)	
CIN3*	**8/22 (36.4%)**	**1/22 (4.5%)**	**13/22 (59.1%)**	**0/11 (0.0%)**	**1/11 (9.1%)**	**10/11 (90.9%)**	**100% (85–100%)**
All HR HPV[b] types*	**31/129 (24.0%)**	**15/129 (11.6%)**	**83/129 (64.3%)**	**6/63 (9.5%)**	**7/63 (11.1%)**	**50/63 (79.4%)**	**60% (54–67%)**
Age: ≤30 yrs	17/79 (21.5%)	11/79 (13.9%)	51/79 (64.6%)	4/38 (10.5%)	3/38 (7.9%)	31/38 (81.6%)	
Age: >30 yrs	14/50 (28.0%)	4/50 (8.0%)	32/50 (64.0%)	2/25 (8.0%)	4/25 (16.0%)	19/25 (76.0%)	
CIN2	15/53 (28.3%)	7/53 (13.2%)	31/53 (58.5%)	6/30 (20.0%)	3/30 (10.0%)	21/30 (70.0%)	
CIN3**	**16/76 (21.1%)**	**8/76 (10.5%)**	**52/76 (68.4%)**	**0/33 (0.0%)**	**4/33 (12.1%)**	**29/33 (87.9%)**	**100% (95–100%)**

[a] Vaccine efficacy is calculated as the ratio of the difference between the rates of complete resolution of the lesion at month 6 with placebo from the rate from treatment with tipapkinogen sovacivec divided by the rate with complete resolution at month 6 treated with tipapkinogen sovacivec. [(Rate complete resolution$_{(tip\ sov)}$ − Rate complete resolution $_{(placebo)}$) / (Rate complete resolution $_{(tip\ sov)}$)].
[b] All HRHPV means regardless of high risk human papillomavirus type of the 13 types for which we tested.
* $p < 0.05$ in comparison to respective placebo.
** $p < 0.01$.

resolution among the CIN 2/3 baseline population (aOR = 4.68 (95% CI: 1.57–13.98)), and Hispanic women were significantly more likely to completely resolve their CIN 2/3 lesion at month six than non-Hispanic white women (aOR = 3.11 (95% CI: 1.21–8.02)).

The second multivariate model considered all factors in prediction of CIN 3 resolution. Because there were zero complete resolutions in the placebo group, an adjusted model for vaccine effects was not possible. Significant racial differences were seen in complete resolution.

The third multivariate model was similar to the first in that the vaccine treatment significantly increased odds of complete resolution or partial response at month six among the CIN 2/3 baseline population (aOR = 2.82 (95% CI: 1.26–6.30)), but only non-Hispanic Black women remained significantly more likely that non-Hispanic White women to resolve/respond (aOR = 5.91 (95% CI: 1.78–19.55)). Of note, in all multivariate models the type of HPV infection did not significantly predict month six resolution or response.

3.2.3. 2.5 year follow-up

While all women were to be followed to study end, few subjects needed to be histologically evaluated during this time frame (Table 4). Of those who had complete resolution at month 6, 24% (4/17) in the TS arm had worse histology detected at 2.5 years. Likewise, one of two (50%) placebo subjects had worse histology at longer term follow-up.

Of those who had partial response at month 6, 12% (3/25) in the TS arm had worse histology detected at follow up; while one of three (33%) placebo subjects had worse histology at follow-up.

This long-term follow-up study resulted in a mean follow-up time of 822 days for the treatment group and 756 days for the placebo group with 76% of the treatment group and 66% of the placebo group staying in the study for >811 days.

3.3. Secondary aim

Viral DNA clearance of all CIN 2/3 regardless of HR HPV type (Fig. 2A) was significantly greater in the vaccine treated cohort than in placebo cohort ($p < 0.01$); in addition, Fig. 2B shows that viral DNA clearance among the women with baseline CIN 3, regardless of HR HPV type, was significantly superior with vaccine than placebo ($p \leq 0.01$). For specific HR HPV types (Fig. 2C), the vaccine was superior to placebo for the following subgroups: 1) the stratum 1 cohort of HPV 16 monoinfected

Table 3
Predictors of histologic resolution/response.

	Among CIN 2/3 Predicting complete resolution vs. no response		Among CIN 3 Predicting complete resolution vs. no response		Among CIN 2/3 Predicting complete resolution and partial response vs. no response	
	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)	OR (95% CI)	aOR (95% CI)
All HR HPV types						
Vaccine	3.11	4.68	NE	NE	2.13	2.82
	1.21–7.98	1.57–13.98			1.05–4.33	1.26–6.30
Placebo	Referent	Referent	Referent	Referent	Referent	Referent
HPV 16						
Vaccine	4.21				2.09	
	0.91–19.59				0.81–5.34	
Placebo	Referent				Referent	Referent
HPV infection type						
HPV 16	0.42	0.42	0.40	0.37	0.63	0.68
	0.20–0.88	0.18–1.01	0.13–1.18	0.09–1.57	0.33–1.18	0.33–1.39
Non HPV 16	Referent	Referent	Referent	Referent	Referent	Referent
Age	1.02	1.05	1.03	1.02	1.02	1.04
	0.97–1.07	0.99–1.11	0.96–1.10	0.93–1.12	0.98–1.06	1.00–1.09
CIN grade						
CIN 2	Referent	Referent			Referent	Referent
CIN 3	0.49	0.33			0.58	0.46
	0.23–1.02	0.14–0.80			0.31–1.08	0.23–0.93
Ethnicity/race						
White	Referent	Referent	Referent	Referent	Referent	Referent
Black	3.07	4.42	6.50	5.10	4.10	5.91
	0.82–11.51	0.99–19.79	0.95–44.59	0.61–42.77	1.41–11.88	1.78–19.55
Hispanic	3.49	3.11	10.64	6.22	2.55	2.07
	1.49–8.19	1.21–8.02	3.00–37.73	1.47–26.23	1.18–5.50	0.91–4.71
Other	1.34	1.18			1.59	1.40
	0.14–12.67	0.11–12.50			0.28–9.11	0.22–8.82
Number of biopsies						
One biopsy	Referent	Referent	Referent	Referent	Referent	Referent
Two biopsies	1.60	2.82	1.25	1.43	1.34	2.00
	0.67–3.83	0.94–8.49	0.34–4.66	0.26–7.91	0.66–2.69	0.87–4.60
Three biopsies	3.17	3.75	5.36	3.41	2.10	2.51
	1.03–9.75	0.93–15.10	1.14–25.26	0.44–26.57	0.79–5.60	0.79–7.90
Four biopsies	1.85	1.56			1.96	3.36
	0.17–19.85	0.09–25.67			0.30–12.75	0.39–28.95
Number of quadrants of disease						
One quadrant	Referent	Referent	Referent	Referent	Referent	Referent
Two quadrants	0.55	0.31	0.86	1.07	0.53	0.38
	0.24–1.25	0.11–0.91	0.25–2.98	0.16–6.99	0.26–1.07	0.16–0.87

NE means not evaluable.

women, 2) the cohort with any HR HPV infection except HPV 16, and 3) the cohort of HPV16 co-infected with any other HR HPV infection.

3.4. Safety

In general, the TS vaccine was well tolerated but with nearly all recipients having an injection site reaction, 29% being graded as severe according to the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials and Common Terminology Criteria for Adverse Events v3.0, for which two patients discontinued the study (Supplemental Table S1). Of the patients who received placebo, 37% experienced injection site reactions graded as mild, moderate, or severe. All other adverse events or pregnancy outcomes were evenly distributed between the treatment groups. One death occurred in the placebo arm of the study (due to medication overdose) approximately six months after the last study injection; the death was considered unrelated to the study drug. One subject reported severe lymphadenopathy after the first two injections, delaying the third TS injection. One subject in the placebo arm reported invasive lobular breast carcinoma detected prior to month 3.

There were six cancers and three adenocarcinoma-in-situ diagnoses detected between the time of the trial injections (vaccine/placebo) and the six-month excision. All three adenocarcinoma-in-situ were in the placebo arm. Three squamous cell cancers were detected in the vaccine arm, and three cancers (one each: adenosquamous, adeno, and squamous) were detected in the placebo arm. Risk factor analyses of these cancers are discussed in the supplement.

4. Discussion

Our study is the largest study to date following women for the longest time frame trialing a therapeutic vaccine for HPV associated CIN 2/3. The TS vaccine was significantly associated with greater complete resolution rates of histologic CIN 3 disease as well as with significantly greater viral DNA resolution regardless of HR HPV type. The vaccine was expected to act in the HPV 16 alpha 9 clade as determined in phase 1 studies by T cell proliferation, anti-MVA antibodies and anti-E6/E7 neutralizing antibodies [4], but this work shows that all HR HPV types responded to some degree to the vaccine. This presents the hypothesis that CIN 3, the most severe dysplasia, with greater expression of E6/E7 proteins are susceptible to the neutralizing antibodies significantly more than the CIN 2 lesions, and that this immunotherapy might be combined with other checkpoint inhibitors for more advanced stages of HPV associated cancers that are expressing E6/E7 proteins.

We showed significant complete resolution of CIN 2/3 from all HR HPV types at 24% in the vaccine group, similar to a pilot report by Alvarez showing a 26% complete resolution of HPV 16 CIN 2/3 lesions after particle mediated epidermal delivery, intramuscular delivery or intralesional delivery of a DNA based therapeutic HPV 16 E7-calreticulin based compound [1]; and similar to a phase 1 trial of HPV16 E6 adjuvanted with candida (Pepcan) that resulted in 17%–20% complete resolution at higher

Table 4
Histologic follow up through month 30 in mITT population.

	Tipapkinogen Sovacivec	Placebo
	$N = 129$	$N = 63$
Histological assessments available post month 6	57/129 (44%)	24/63 (38%)
Histological assessments available post month 6 for those subjects who had complete **resolution** at month 6	17/31 (22%)	2/6 (33%)
Subjects with complete **resolution** at month 6 who had worse histology at 2.5 years	4/17 (24%)	1/2 (50%)
Histology at 2.5 years	1 subject: CIN 1 1 subject: CIN 2 2 subjects: CIN 3	1 subject: adenocarcinoma in situ
Histological assessment available post month 6 for those subjects who had partial **response** at month 6	25/46 (54%)	3/13 (23%)
Subjects with a partial **response** at month 6 who had worse histology at 2.5 years	3/25 (12%)	1/3 (33%)
Histology at 2.5 years	1 subject: CIN 2 2 subjects: CIN 3	1 subject: intracervical carcinoma with stromal invasion, squamous cell carcinoma

doses [15]. Two small phase 2 trials with a heat shock fusion protein (SGN-00101) [12,21] and a compound similar to TS (TG4001) did not reach complete resolution [28]. In addition, the placebo complete resolution rate (10%) and partial response (11%) at month 6 for all HR HPV types were similar to other immunotherapeutic studies for CIN2/3 [20].

The absolute complete resolution rate did not meet the a priori primary objective, in our study, of 60%, which was identified as a clinically acceptable resolution threshold, but the therapeutic vaccine did meet the resolution rate above twice the placebo resolution rate for HPV 16 monoinfected CIN 2/3 and for all HR HPV CIN 3 lesions. Given the therapeutic harms of surgical excision [17–19] with reproductive morbidity, an argument can be made that a lower therapeutic resolution/response could be both clinically and cost effective from a broader perspective than just cancer prevention.

The combination complete resolution/partial response rates in our study (36%) are similar to the 40% regression rate documented for VGX-3100 against CIN2/3 associated with HPV 16/18 in a phase 2b trial [28]; to that seen by Pepcan in a phase 1 trial [15]; and to the 48% partial resolution seen in the phase 2 TG4001 trial [12].

We cannot know from this study if a higher resolution rate could have been seen if the endpoint had been evaluated later than month 6. For instance, another immunotherapeutic agent, VGX-3100, showed a marginally higher efficacy for CIN2/3 associated with HPV 16/18 regression to normal at month 9, but likewise did not meet the 60% resolution threshold [28]. In addition, we did not test whether a booster vaccination at month 3 could have increased month 6 resolution rates; nor did we test whether fewer than three doses could accomplish the same current resolution or response.

Of those with resolution or response post-surgical treatment only a small proportion of subjects had worsening pathology at 2.5 years follow-up most likely due to incomplete excision of the original lesion. Of the 2.9% of enrolled subjects diagnosed with a malignancy, it is likely that these were prevalent cancers missed at diagnosis, supported by the literature which shows an incident rate of 2.0–4.1% due to the limitations of colposcopy-guided biopsies [7,14].

Viral DNA clearance is a secondary indicator of efficacy. Prior work indicates that women who have viral DNA clearance of their type specific persistent HPV infection are more likely to maintain resolution of their CIN2/3 [26]. In addition, women with surgical excisions resulting in positive endocervical margins have more than two-fold the recurrence rates of CIN 2/3 because of residual viral infection [8]. Unlike the VGX-3100 study [28], viral DNA clearance in our study was measured and analyzed separately from histologic resolution/response. Our vaccine was significantly superior to placebo in complete viral DNA clearance for both CIN 2/3 and CIN 3 for all HR HPV types considered, although it did not provide complete clearance in all subjects. Complete viral DNA clearance was sustained over the 2.5 year follow-up in both vaccine and placebo groups.

This study has several strengths. A simple subcutaneous injection is an easy implementation strategy. Even with less resolution/response than anticipated, this vaccine could offer a preliminary reduction in surgical excisions avoiding their resulting reproductive morbidity harms. In addition, this study had a rigorous adjudicating CPR to review all histologic specimens reducing the misdiagnosis at study entry. The precise HPV detection methodology allowed both HPV 16 monoinfection and other HR HPV types to be detected from all sample aliquots. The rigorous study design protected subjects by detecting the prevalent cancers without impacting trial results. Finally, most of the study participants were followed for a 30-month time frame, the longest duration of follow-up in a therapeutic vaccine trial to date.

The study also has limitations. The study was not powered for a specific efficacy; and the baseline viral load could not differentiate incident from persistent infections. Another limitation was the six-month time frame for immunotherapeutic response which, in hindsight, may have been too short. Finally, balancing the potential harm of CIN 3 progressing to cancer with the need to understand the efficacy of an immunotherapeutic agent is challenging even over a short time frame, as the colposcopic impression is maximally sensitive for CIN3+ disease at only 70% [13].

Immunologic therapeutic HPV vaccines offer a potential non-surgical option for women with cervical pre-cancers which might be extended to other HPV associated precursors or cancers in the future. The spectrum of decreasing incidence and mortality from HPV associated cancers includes prophylactic vaccination [16], skin barriers such as condoms [10], continued education about sexuality [27], creating potentially hormonally hostile environments [9], with the largest success to date being the opportunistic and organized screening programs simplified most recently by the approval of primary HR HPV screening tests at five-year intervals [29]. The treatment of cervical pre-cancerous lesions may eventually include first round therapeutic vaccination which we have also shown to be safe through 30 months prior to going to excisional surgery if necessary.

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This study was conceived jointly by F Hoffmann-La Roche LTD and consultants, some of whom also served as investigators. Roche funded and coordinated this study. Roche sold the compound to Transgene who facilitated the data analysis with the corresponding author. The corresponding author had full access to the clinical study report and had final responsibility for submission for the publication.

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Conflict of interest statement

Dr. Harper's institution reports grants from Roche during the conduct of the study.

Dr. Nieminen reports grants and non-financial support from Roche during the conduct of the study.

Dr. Donders reports nothing to disclose.

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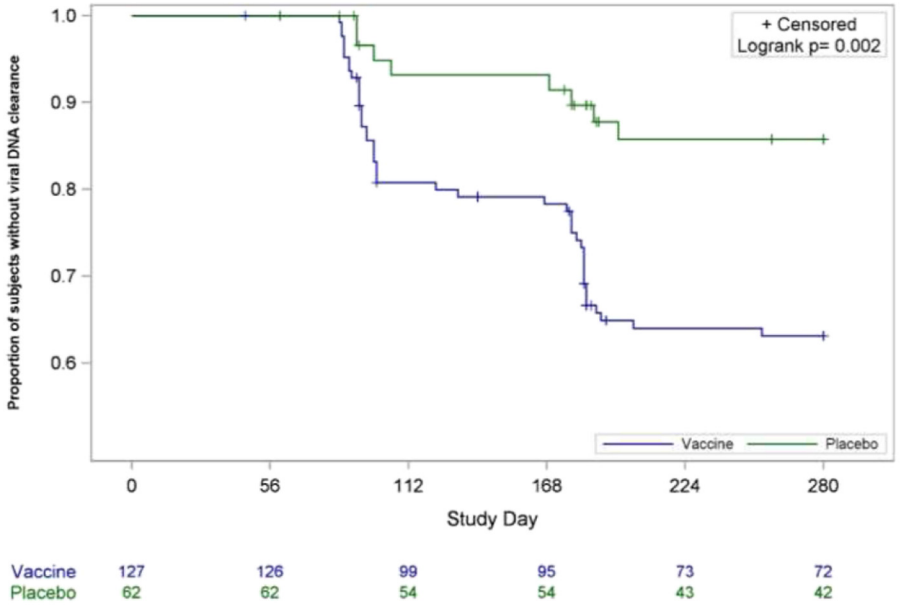
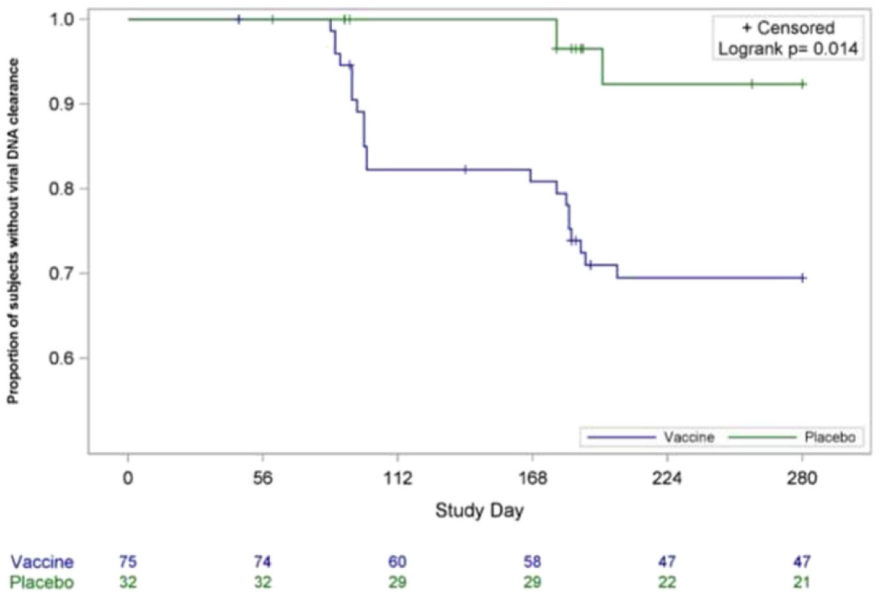
A. Among baseline CIN 2/3 regardless of HR HPV types through month 30, mITT



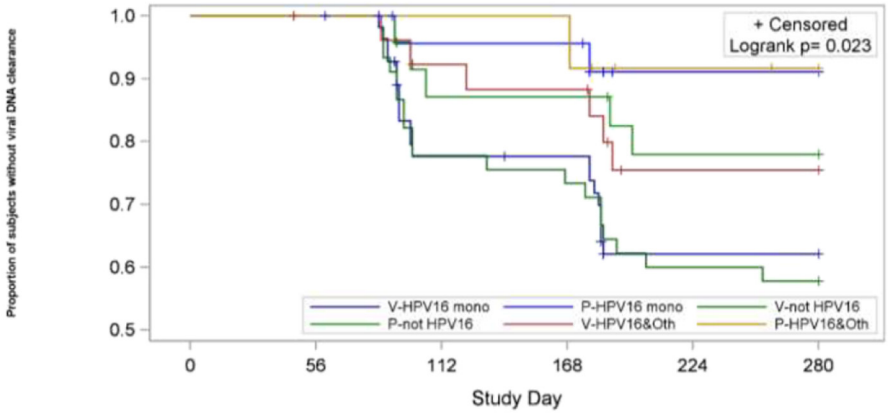
Vaccine	127	126	99	95	73	72
Placebo	62	62	54	54	43	42

B. Among baseline CIN 3 regardless of HR HPV types through month 30, mITT



Vaccine	75	74	60	58	47	47
Placebo	32	32	29	29	22	21

C. Among baseline CIN 2/3 by HR HPV type through month 30, mITT



V-HPV16 mono	55	55	41	40	30	30
P-HPV16 mono	26	26	22	22	17	17
V-not HPV16	45	45	35	33	27	26
P-not HPV16	24	24	20	20	17	17
V-HPV16&Oth	27	26	23	22	16	16
P-HPV16&Oth	12	12	12	12	9	8

Fig. 2. Viral DNA clearance by Roche Linear Array Assay Fig. 2A. Among baseline CIN 2/3 regardless of HR HPV types through month 30, mITT. Fig. 2B. Among baseline CIN 3 regardless of HR HPV types through month 30, mITT. Fig. C. Among baseline CIN 2/3 by HR HPV type through month 30, mITT.

work; he has advised or participated in educational speaking activities but does not receive an honorarium from any companies. In specific cases, his employers have received payment for his time spent for these activities from Papivax, Cynvec, Altum Pharma, Photocure, Becton Dickenson, and PDS Biotechnologies. If travel required for meetings with industry, the company pays for Dr. Einstein's travel expenses. Rutgers has received grant funding for research-related costs of clinical trials that Dr. Einstein has been the overall or local PI within the past 12 months from J&J, Pfizer, AstraZeneca, Advaxis, and Inovio.

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Dr. Glavini reports she is an employee of Roche.

Dr. Attley reports she is an employee of Roche.

Dr. Limacher reports being an employee and shareholder from 2004 to 2016 at Transgene SA, during the conduct of the study.

Mrs. Bastien reports nothing to disclose.

Dr. Calleja reports she was an employee of Roche during the study period.

Author contributions

DMH, PN, GD, MHE, WKH contributed patients to the trial.

DMH, KG, GA, JML, contributed to the literature search.

DMH, BB contributed to the figures.

DMH, PN, MHE, FG, WKH, MHS, KG, GA, JML, EC contributed to the study design.

DMH, PN, GD, WKH, MHE, MHS, contributed to data collection.

DMH, PN, GD, MHE, FG, WKH, MHS, KG, GA, JML, BB, EC contributed to the data analysis, data interpretation, writing and reviewing of final manuscript.

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