はわが国では1988年に承認された．一方，小児用７価肺炎球菌コンジュゲートワクチン（PCV7）は2009年10月にわが国で承認された．本稿では，PPV23とPCV7を中心とする肺炎球菌ワクチンの免疫原性，特異抗体検査，臨床効果および医療経済効果，今後の臨床応用の展開について概説する．

2.　肺炎球菌の保菌と肺炎球菌感染症の疫学

肺炎球菌は気道親和性のグラム陽性双球菌であり，乳幼児の約20～40％が鼻咽頭に肺炎球菌を保菌している[1]．一方，高齢者では保菌の頻度は３～５％とされている[2]．小児，成人において本菌は鼻咽頭から直接進展により中耳炎を発症し，また下気道への誤嚥により気管支炎，肺炎を発症する（図１）．一方，血液中に侵入し敗血症，髄膜炎などの侵襲性感染症を惹起する[3]．一般に，小児，成人いずれの年齢層においても，菌血症が髄膜炎より頻度が高い．わが国における５歳未満の小児における侵襲性感染症の罹患率（10万人あたり・年）は21.7～23.6とされている．一方，成人における市中肺炎の罹患率（1,000人あたり・年）は65歳以上の高齢者で高くなり，わが国における高齢者入所施設においては90(1,000人あたり・年）と高頻度であることが報告されている[4]．また，わが国では成人の市中肺炎の大半は菌血症を伴わない肺炎であり，菌血症を伴う肺炎は10％未満である．また，市中肺炎は定型細菌，非定型細菌，ウイルスなど多様な微生物によって惹起される．成人における肺炎球菌の病因の20～40％が肺炎球菌に起因するとされている．

3.　わが国における肺炎球菌の血清型分布

肺炎球菌の血清型はその菌表層の莢膜ポリサッカライド（CPS）により決定され，現在までに少なくとも93血清型が存在する．表１には血清型と亜型，PPV23，PCV7，PCV13に含まれる血清型を示した．また，地域，成人・小児の別，疾患によってその血清型分布は異なっている．図２にはわが国の小児における侵襲性感染症の血清型分布，図３にはわが国における成人の侵襲性感染症と肺炎由来の血清型分布を示した[5]．わが国の小児の侵襲性感染症においては，その主要血清型は6B，19F，14，23Fであり，PCV7含有血清型は75.4％，PCV13含有血清型は93.7％であった．一方，成人の侵襲性感染症の主要血清型分布は12F，3，6B，14，4，23Fなどであり，とりわけ，侵襲性感染症由来の12Fについては国内における単一のクローン株の伝播によることが示されている[6]．一方，市中肺炎の主要血清型は19F，23F，6B，3，14などであり，成人における侵襲性感染症における分布とはかなり異なることが判る．また，市中肺炎由来株のPPV23の含有血清型は，6Bの交差免疫抗原である6Aも含めると，82.5％であった[7]．

4.　肺炎球菌ワクチンの免疫原性とその評価法

1）　特異抗体産生誘導

PPV23を構成するCPS抗原はＴ細胞非依存性抗原であり初回接種で抗体応答が認められる

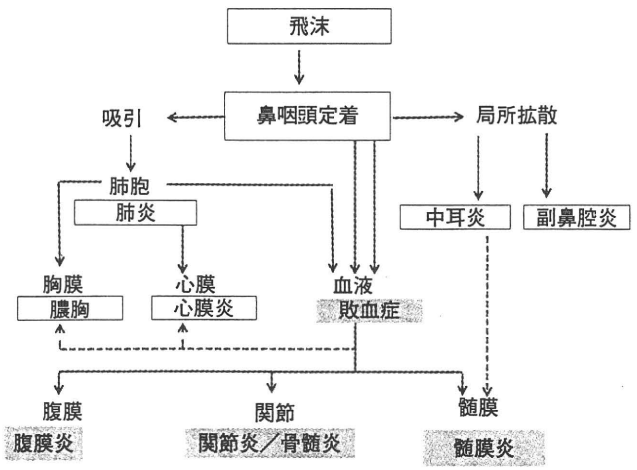

図１　肺炎球菌感染症の臨床病像（文献３より改変）

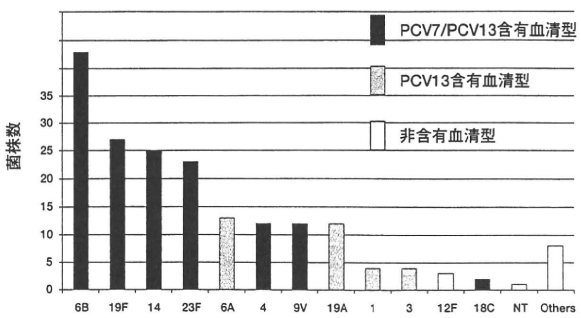

図２　わが国における小児の侵襲性感染症197症例の原因菌血清型分布
PCV7含有血清型は75.4％，PCV13含有血清型は93.7％とされている．（文献５より改変）

表1　肺炎球菌の血清型と各肺炎球菌ワクチンの含有血清型

血清型	亜型	PPV23	PCV7	PCV13
1				1
2				
3		3		3
4		5	4	4
5				5
6	6A, 6B, 6C, 6D	6B	6B	6A, 6B
7	7F, 7A, 7B, 7C	7F		7F
8				
9	9A, 9L, 9N, 9V	9N	9V	9V
10	10F, 10A, 10B, 10C	10A		
11	11F, 11A, 11B, 11C, 11D, 11E	11A		
12	12F, 12A, 12B	12F		
13				
14			14	14
15	15F, 15A, 15B, 15C	15B		
16	16F, 16A			
17	17F, 17A	17F		
18	18F, 18A, 18B, 18C	18C	18C	18C
19	19F, 19A, 19B, 19C	19F	19F	19A, 19F
20				
21				
22	22F, 22A	22F		
23	23F, 23A, 23B	23F	23F	23F
24	24F, 24A, 24B			
25	25F, 25A			
26				
27				
28	28F, 28A			
29				
30				
31				
32	32F, 32A			
33	33F, 33A, 33B, 33C, 33D	33F		

本表の血清型以外に血清型35(F, A, B, C), 36, 37, 38, 39, 40, 41(F, A), 42, 43, 44, 45, 46, 47(F, A), 48がある.

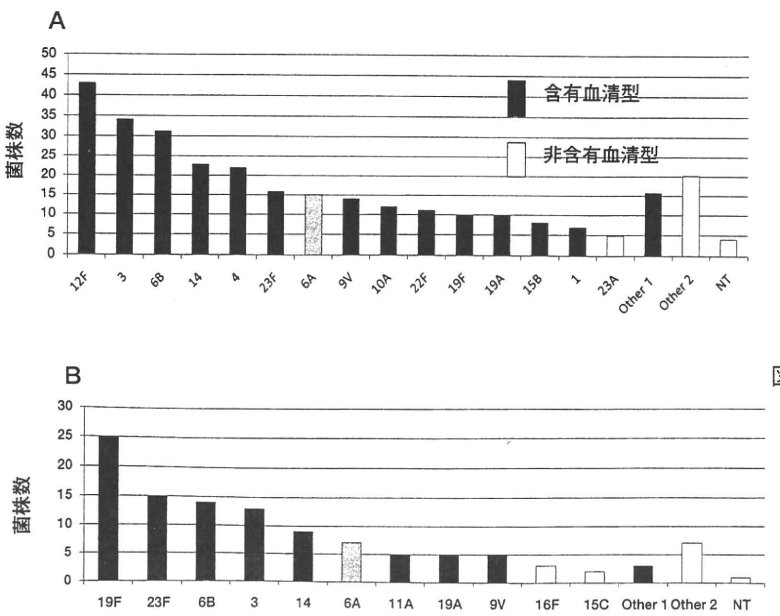

図3　わが国における成人の侵襲性感染症303症例（A）と市中肺炎114症例（B）の原因菌血清型分布

侵襲性感染症の原因菌のPPV23含有血清型は85.4%で，市中肺炎の原因菌のPPV23含有血清型は82.5%であった．血清型6A(グレー）は6Aの交差免疫抗原（文献5，6より改変）

が，メモリーB細胞が誘導できないために2回目以降の接種によるブースター効果はない．血清型特異抗体のうちその主要な役割を果たすのはIgG2サブクラスであり，その感染防御能は補体依存性オプソニン活性として示される．一方，PCVはCPS抗原に無毒化ジフテリアトキソイドCRM_{197}などの蛋白質を結合させT細胞性依存性にし，乳幼児における血中血清型特異IgG抗体産生を誘導し，そのサブタイプはIgG1が主体である．

2)　ELISA IgG

現行の血清型特異IgG抗体測定は，Frash CEらが開発したサンドイッチタイプの第三世代ELISAである．血清中の肺炎球菌の共通抗原（cell wall polysaccharideと22F CPS）に対する抗体を吸収後に，ELISAプレートに固層化した個々の血清型のCPS抗原と結合するIgG抗体を定量的に測定する[8]．図4Aには乳幼児におけるPCV接種前，1回目接種後，2回目接種後，3回目接種後の血清型23Fに対する特異IgG抗体濃度の推移を示している[9]．このように，初回接種では明らかな特異IgG抗体の増加は明らかでないが，2回目，3回目の接種によりブースター効果が認められる．一方，図4Bには慢性肺疾患患者におけるPPV23接種前，接種後2年間の血清型23Fに対する特異IgG抗体濃度の推移を示している[10]．PPV23接種後，応答者においては接種後1月後に血中濃度のピークを示し，少なくとも2年間は接種前にベースラインより高い血中濃度を維持していることが判る．50歳以上の成人に対するPPV23の初回接種および再接種により，4，6B，8，9V，12F，14，23Fに対する特異IgG抗体は，2～5年間未接種者のそれより高いレベルを保つことが示されているが，血清型3に対する特異IgG抗体は2年までにベースラインレベルに低下するとされている[11]．

3)　OPA

アラバマ大学のNahm Mは，肺炎球菌ワクチン接種後の血清型特異抗体の機能評価のために，複数の血清型に対するオプソニン活性を測定を可能にするOPA(MOPA) を開発した(http://www.vaccine.uab.edu/)[8]．図5Aには血清型23Fの肺炎球菌に対する特異IgG抗体の補体依存性のオプソニン（OPK）活性を図示した．図5Bには，PPV23接種前（1041-1）および接種1ヶ月後（1041-2）の血清のOPK活性の結果を示している．このようにPPV23接種前（125）から接種後（1611）に血清中OPK活性が増加していることが判る．

また，Nahmらは肺炎球菌ワクチン接種後の若年者と高齢者の血清中ELISA IgGレベルとOPKレベルの蓄積分布曲線を示し，両グループのELISA IgGレベルはほぼ同等であるのに対し，OPKレベルは高齢者に比較して若年者が高いことを報告している[12]．これらの結果から，ワクチン接種後の高齢者の血清オプソニン活性は若年者に比較して減弱していることが明らかになった．一方，著者らもワクチン接種前の東アフリカ，ウガンダの成人において，血清

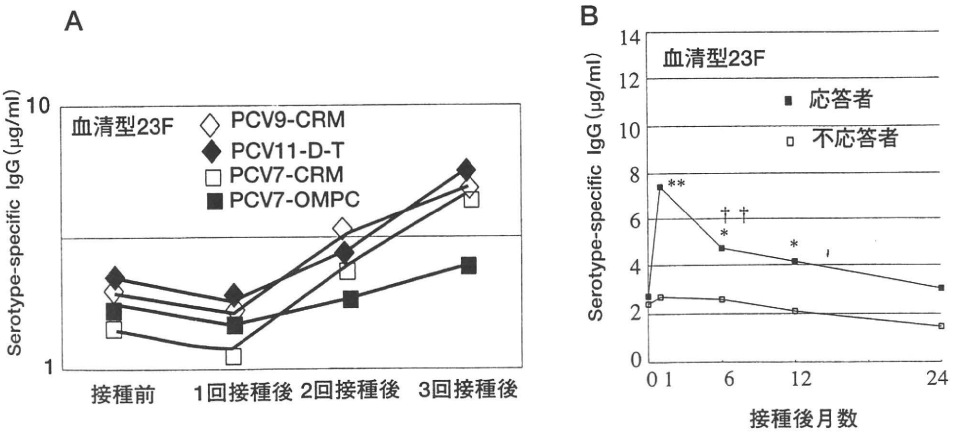

図4　肺炎球菌コンジュゲートワクチン（A）と肺炎球菌ポリサッカライドワクチン（B）接種後の血清中抗23F特異IgG抗体の推移．（Aは文献9，Bは文献10より改変）

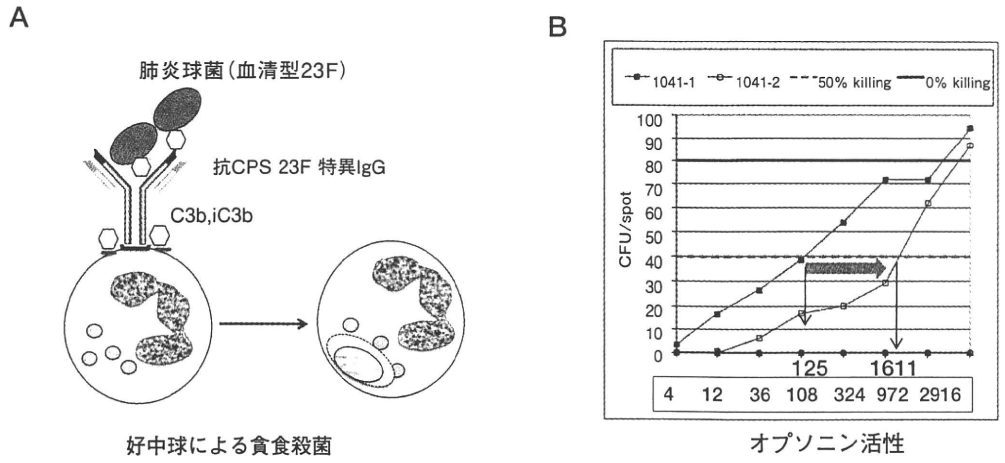

図5　血清型特異IgG抗体の機能の模式図（A）と23価肺炎球菌ポリサッカライドワクチン接種前，接種1ヶ月後のOPAによる血清オプソニン活性の推移
OPKタイター：補体存在下で接種菌数（ここでは80cfu/spot）を50%殺菌できる血清希釈倍数の逆数で表現.

特異IgG抗体のオプソニン活性が減弱していることを報告している[13]．これらの結果から，少なくとも成人においてはELISA IgGのみの評価は不十分で，OPK活性の評価が必要と考えられる.

5. 小児におけるPCVの効果

米国カリフォルニア州で行われた37,868人の乳幼児を対象とした二重盲検試験において，PCV7の3回以上接種または3回接種と追加接種1回（PP解析）により侵襲性感染症が97.4%減少した（表2）[14]．また，1回以上の接種（ITT解析）では侵襲性感染症を93.9%減少した．その後，米国でPCV7は2000年に小児に対する定期接種として導入された．図6にはPCV7の定期接種導入後，米国8州における2005年までの1歳未満，1歳の乳幼児における侵襲性感染症罹患率の劇的な減少を示した[15]．さらに，最近の報告では5歳未満の小児における侵襲性感染症の罹患率は導入前の81.9人/10万人・年から2006～2007年には0.4人/10万人・年まで減少したとされている[16]．また，小児におけるPCV7の定期接種のカバー率が90%まで達した状況下で，65歳以上の侵襲性感染症の罹患率が減少する事実が示され[16]，集団免疫効果と考えられている．しかしながら，PCV7の定期接種導入によってワクチン非含有血清型である19A，15A，35B，23A，6C等の非ワクチン血清型による侵襲性感染症が増加し，さらには19Aのみならず15A，23A，35A,6Cにおけるペニシリン非感受性菌の頻度が増加している問題がクローズアップされている[17]．

一方，前述の米国カリフォルニア州で行われた二重盲検試験において，PCV7接種によりWHO基準による胸部X線所見で診断した肺炎がPP解析で30.3%，ITT解析では25.5%減少した（表2）[18]．さらに，フィンランドで実施された1,662人の乳幼児を対象とした二重盲検試験において，PCV7接種によりワクチン含有血清型による中耳炎は57%減少した（表2）[19]．このように，PCVは乳幼児の侵襲性感染症に対する高い予防効果，肺炎や中耳炎に対しても予防効果を示した.

PCV接種により米国では10万人あたり6人の死亡を減らすのに対しアフリカのガンビアでは10万人あたり700人の死亡を減らすことが期待される[20]．また，総年収US$1,000以下のGAVI支援該当72ヶ国を対象とした乳幼児に対するPCV接種した場合，その生後3～29ケ月の小児の死亡379万人の7%にあたる約26万人の死亡を予防できることも予測されている[21]．これらの予測から，アジア，アフリカの途上国の乳幼児に対するPCVの定期接種化が期待されている．わが国においても，2010年10月に厚生労働省は小児におけるPCV7の定期接種化の方針を決定した.

表2　肺炎球菌コンジュゲートワクチン（PCV7）の乳幼児における臨床効果

	ワクチン効果(%)	95%信頼区間	P
侵襲性感染症			
PP 解析	97.4	82.7～99.9	＜0.001
ITT 解析	93.9	79.6～98.5	＜0.001
肺炎			
PP 解析	30.3	10.7～45.7	0.043
ITT 解析	25.5	6.5～40.7	0.011
中耳炎	57.0	44.0～67.0	—

PP：Perprotocol，ITT：Intention to treat　（文献14，18，19より）

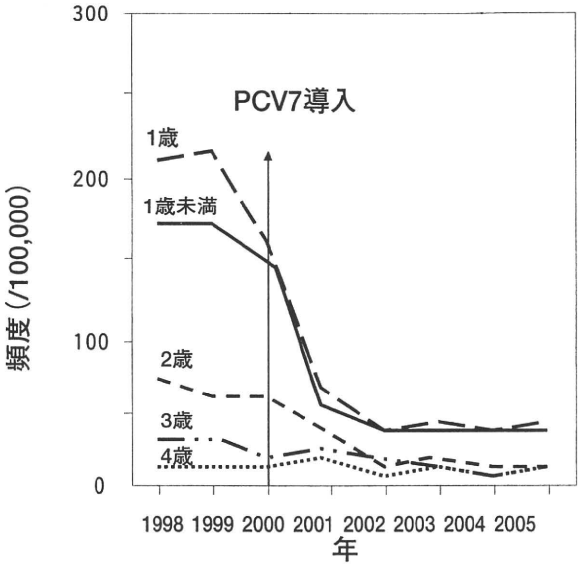

図6　米国8州における肺炎球菌コンジュゲートワクチン（PCV7）の定期接種導入後の小児における侵襲性感染症の劇的な効果．（文献15より改変）

6.　成人における肺炎球菌ワクチンの効果

これまでに蓄積された多くの成人に対するPPV23の臨床試験において，免疫不全のない高齢者における菌血症を伴う肺炎，髄膜炎などの侵襲性肺炎球菌性感染症に対する予防効果が報告されているものの，すべての原因による肺炎の予防効果は明らかになっていない[22]．しかしながら，これまでにPPV23接種による成人肺炎の重症度，死亡リスクの低下が報告されている[23,24]．

最近になって，PPV23接種のわが国における高齢者に対する肺炎予防効果が2グループから報告されている．Maruyamaらは，1,006人の高齢者介護施設入所者（平均年齢85歳）を無作為にPPV23接種群（502人）と非接種群（504人）に割りつけ，3年間の肺炎，肺炎球菌肺炎の発症および死亡について比較検討した二重盲検試験の結果を報告した[4]．この研究では，PPV23接種群では肺炎球菌肺炎のみならず，すべての肺炎に対する予防効果が認められ，さらにPPV23群では肺炎球菌性肺炎による死亡率が有意に減少した．

さらに，著者らはインフルエンザワクチン定期接種を受けた65歳以上の高齢者786人を対象として，PPV23接種群（391人）と非接種群（387人）の2群に割りつけたオープンラベル無作為比較試験の結果を報告した[25]．本研究において，全症例（65歳以上の高齢者）ではPPV23接種群，PPV23非接種群において肺炎罹患率に有意な差は認めなかったものの，75歳以上の高齢者ではPPV23接種群で肺炎罹患率が有意に減少した．また，慢性肺疾患，歩行困難者においてもPPV23接種により肺炎罹患率の有意な減少が認められた．さらに，65歳以上の高齢者全体においてPPV23接種群ですべての肺炎による医療費の削減効果も示された．このように，わが国におけるPPV23の高齢者肺炎に対する予防効果，死亡抑制効果，医療費削減効果が明らかにされたことから，高齢者に対するPPV23の定期接種化の早期実現が望まれる．

サハラ以南のアフリカではHIV感染者における肺炎球菌による侵襲性感染症や肺炎の罹患率が高い．しかしながら，HIV感染成人に対するPPV23の臨床効果は明らかになっていない．

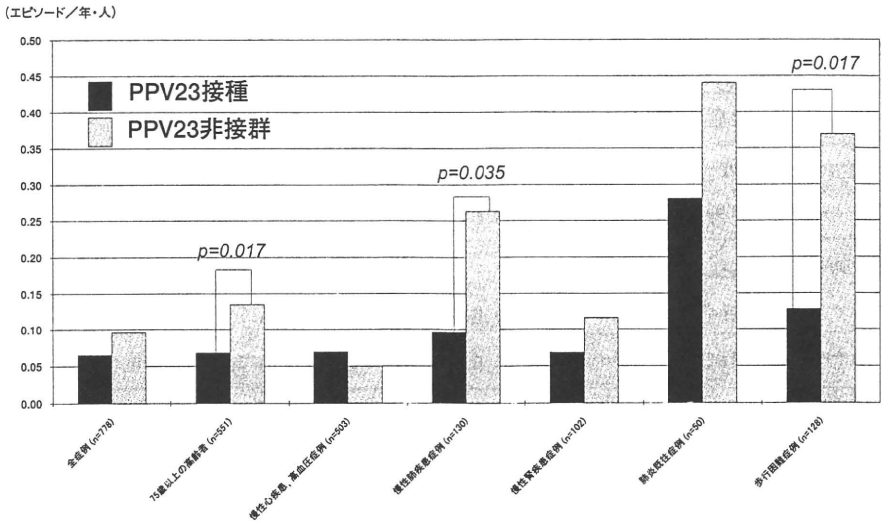

図7　高齢者における23価肺炎球菌ポリサッカライドワクチン接種によるすべての肺炎に対する予防効果．（文献25より改変）

このため，French Nらは496名マラウイの青年と成人（88％がHIV感染者）を対象としてPCV7の二重盲検試験を実施し，PCV7接種がワクチン（ワクチンは4週間の間隔で2度接種）含有血清型と6Aによる繰り返す侵襲性肺炎球菌感染症を74％予防することを報告した[26]．

また，Dansfieldらは COPD 患者においてはPCV7と PPV23の初回接種の免疫原性を ELISA IgG と OPA で比較検討し，PCV7が PPV23に比較して優れていると結論している[27]．さらには，成人における PCV7および PPV23の連続接種による複数の免疫原性試験が実施されており[28-30]，今後の成人におけるこれらのワクチンの交互接種の可能性が示唆されている．このように，成人においても PPV23のみならず，PCV の役割が少しずつ明らかにされつつある．

7. おわりに

小児と成人における肺炎球菌感染症に対する予防効果がポリサッカライドベースワクチンにより可能になった．さらには，世界的には小児における PCV7は PCV13に切り替えが進んでおり，わが国でも小児に対する PCV13は臨床試験中，成人に対する PCV13は臨床試験が終了している．今後，成人においては，PPV23とPCV13の組み合わせ接種法についての検討が必要と考えられる．

参考文献

1) van der Poll T, Opal SM：Pathogenesis, treatment, and prevention of pneumococcal pneumonia. Lancet 374：1534-1556, 2009

2) Flamaing J, Peetermans WE, Vadeven J, Verhaegen TJ：Pneumococcal colonization in older persons in a nonoutbreak setting. J Am Geriatri Soc 58：396-398, 2010

3) Bogaert D, De Groot R, Hermans PW：Streptococcus pneumoniae colonisation:the key to pneumococcal disease. Lancet Infect Dis 4：144-154, 2004

4) Maruyama T, Taguchi O, Niederman MS, Morser J, Kobayashi H, Kobayashi T, D'Alessandro-Gabazza C, Nakayama S, Nishikubo K, Noguchi T, Takei Y, Gabazza EC：Efficacy of 23-valet pneumococcal vaccine in preventing pneumonia and improving survival in nursing home residents：double blind, randomized and placebo controlled trial. BMJ 340：c1004, 2010

5) Chiba N, Morozumi M, Sunaoshi K, Takahashi S, Takano M, Komori T, Sunakawa K, Ubukata K：IPD Surveillance Study Group：Serotype and antibiotic resistance of isolates from patients with invasive pneumococcal disease in Japan. Epidemiol Infect 138：61-68, 2010

6) Available at：http://strep.umin.jp/

7) Oishi K, Yoshimine H, Watanabe H, Watanabe K, Tanimura S, Kawakami K, Iwagaki A, Nagai H,

Goto H, Kudoh S, Kuriyama T, Fukuchi Y, Matsushima T, Shimada K, Matsumoto K, Nagatake T : Drug-resistant genes and serotypes of pneumococcal strains of community-acquired pneumonia among adults in Japan. Respirology 11 : 429-436, 2006

8) Available at : http://www.vaccine.uab.edu/

9) Kayhty H, Lockhart S, Schuerman L : Immunogenicity and reactogenicity of pneumococcal conjugate vaccines in infants and children. *In* Textbook of Pneumococcal vaccines. Eds. Siber GR, Klugman KP, Makela PH. ASM Press, Washington, D.C., 2007, pp.227-243

10) Chen M, Hisatomi Y, Furumoto A, Kawami K, Masaki H, Nagatake T, Sueyoshi Y, Iwanaga T, Aizawa M, Oishi K : Comparative immune response of patients with chronic pulmonary diseases during the 2 year period after pneumococcal vaccination Clin Vaccine Immunol 14 : 139-145, 2007

11) Musher DM, Monoff SB, Liss C, McFetridge RD, Marchese RD, Bushnell B, Alveretz F, Painter C, Bum MD, Siber JL : Safety and antibody response, including antibody persistence for 5 years, after primary vaccination or revaccination with pneumococcal polysaccharide vaccine in middle-ages and older adults. J Infect Dis 201 : 516-524, 2010

12) Schenkein JG, Park S, Nham MH : Pneumococcal vaccination in older adults increases antibodies with low opsonic capacity and reduced antibody potency. Vaccine 26 : 5521-5526, 2008

13) Chen M, Ssali F, Mulungi M, Awio P, Yoshimine H, Kuroki R, Furumoto A, Tanimura S, Kityo C, Nagatake T, Mugyenyi P, Oishi K : Induction of opsonophagocytic killing activity with pneumococcal conjugate vaccine in human immunodeficiency virus-infected Ugandan adults. Vaccine 26 : 4962-4968, 2008

14) Black SB, Shinefield H, Fireman B, Lewis E, Ray P, Hansen JR, Elvin L, Ensor KM, Hackell J, Siber G, Malinoski F, Madore D, Chang I, Kohberger R, Watson W, Austrian R, Edwards K : Efficiency, safety and Immunogenicity of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. Pediatr Infect Dis J 19 : 187-195, 2000

15) Invasive pneumococcal disease in children 5 years after conjugate vaccine introduction — eight states, 1998-2005. MMWR 57 : 114-148,2008

16) Pilishvili T, Lexau C, Farley MM, Hadler J, Harrison LH, Bennett NM, Reingold A, Thomas A, Schaffner W, Craig AS, Smith PJ, Beall BW, Whitney CG, Moore MR : Active Bacterial Core Surveillance/Emerging Infections Program Network : Sustained reductions in invasive pneumococcal disease in the era of conjugate vaccine. J Infect Dis 201(1) : 32-41, 2010

17) Gertz RE Jr, Li Z, Pimenta FC, Jackson D, Juni BA, Lynfield R, Jorgensen JH, Carvalho Mda G, Beall BW : Active Bacterial Core Surveillance Team : Increased penicillin nonsusceptibility of nonvaccine-serotype invasive pneumococci other than serotypes 19A ad 6A in psot-7-valent conjugate vaccine era. J Infect Dis 201 : 770-775, 2010

18) Black SB, Shinefield HR, Ling S, Hansen J, Fireman B, Spring D, Noyes J, Lewis E, Ray P, Lee J, Hackell J : Effectiveness of heptavalent pneumococcal conjugate vaccine in children younger than five years of age for prevention of pneumonia. Pediatr Infect Dis J 21 : 810-815, 2002

19) Eskola J, Kilpi T, Palmu A, Jokinen J, Haapakoski J, Herva E, Takala A, Käyhty H, Karma P, Kohberger R, Siber G, Mäkelä PH : Finnish Otitis Media Study Group : Efficacy of a pneumococcal conjugate vaccine against acute otitis media. N Eng J Med 344 : 403-409, 2003

20) Levine OS, Greenwood B : Opportunities and challenges for pneumococcal conjugate vaccines in low-and middle-income countries. *In* Textbook of Pneumococcal vaccines. Eds. Siber GR, Klugman KP, Makela PH. ASM Press, Washington, D.C, 2007, pp.405-418

21) Sinha A, Levine O, Knoll MD, Muhib F, Lieu TA : Cost-effectiveness of pneumococcal conjugate vaccination in the prevention of child mortality : an international economic analysis. Lancet 369 : 389-396, 2007

22) Jackson LA, Janoff EN : Pneumcoccal vaccination of elderly adults : new paradigms for protection.

Clin Infect Dis 47 : 1328-1338, 2008

23) Fisman DN, Abrutyn E, Spaude KA, Kim A, Kirchner C, Daley J : Prior pneumococcal vaccination is associated with reduced death, complication, and length of stay among hospitalized adults with community-acquired pneumonia. Clin Infect Dis 42 : 1093-1101, 2006

24) Johnstone J, Marrie TJ, Eurich DT, Majumdar SR : Effect of pneumococcal vaccination in hospitalized adults with community-acquired pneumonia. Arch. Intern Med 167 : 1938-1943, 2007

25) Kawakami K, Ohkusa Y, Kuroki R, Tanaka T, Koyama K, Harada Y, Iwanaga K, Yamaryo T, Oishi K : Effectiveness of pneumococcal polysaccharide vaccine against pneumonia and cost analysis for the elderly who receive seasonal influenza vaccine in Japan. Vaccine 28 : 7063-7069, 2010

26) French N, Gordon SB, Mwalukomo T, White SA, Mwafulirwa G, Longwe H, Mwaiponya M, Zijlstra EE, Molyneux ME, Gilks CF. A trial of a 7-valent pncumococcal conjugate vaccine in HIV-infected adults. N Eng J Med 362 : 812-822, 2010

27) Dransfield MT, Nahm MH, Han MK, Harnden S, Criner GJ, Martinez FJ, Scanlon PD, Woodruff PG, Washko GR, Connett JE, Anthonisen NR, Bailey WC : COPD Clinical Research Network. Superior immune response to protein-conjugate versus free pneumococcal polysaccharide vaccine in chronic obstructive pulmonary disease. Am J Crit Care Respir Med 180 : 499-505, 2009

28) de Roux A, Schmoele-Thoma B, Siber GR, Hackell JG, Kuhnke A, Ahlers N, Baker SA, Razmpour A, Emini EA, Fernsten PD, Gruber WC, Lockhart S, Burkhardt O, Welte T, Lode HM : Comparison of pneumococcal conjugate polysaccharide and free polysaccharide vaccines in elderly adults : conjugate vaccine elicits improved antibacterial immune responses and immunological memory. Clin Infect Dis 46 : 1015-1023, 2008

29) Musher DM, Rueda AM, Nahm MH, Graviss EA, Rodriguez-Barradas MC : Initial and subsequent response to pneumococcal polysaccharide and protein-conjugate vaccines administered sequentially to adults who have recovered from pneumococcal pneumonia. J Infect Dis 198 : 1019-27, 2008

30) Goldblatt D, Southern J, Andrews N, Ashton L, Burbidge P, Woodgate S, Pebody R, Miller E : The immunogenicity of 7-valent pneumococcal conjugate vaccine versus 23-valent polysaccharide vaccine in adults aged 50-80 years. Clin Infect Dis 49 : 1318-1325, 2009

Efficacy of Human Papillomavirus 16/18 AS04-Adjuvanted Vaccine in Japanese Women Aged 20 to 25 Years

Interim Analysis of a Phase 2 Double-Blind, Randomized, Controlled Trial

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Background: A phase 2 double-blind, controlled, randomized multicenter study with human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine is ongoing in healthy Japanese women aged 20 to 25 years. We assessed the vaccine efficacy in the event-triggered analysis.

Methods: Japanese women aged 20 to 25 years were randomly assigned to receive either HPV-16/18 AS04-adjuvanted (n = 519) or hepatitis A (n = 521) vaccine at 0, 1, and 6 months. The women were assessed for virological and cytological end points associated with HPV-16/18 in cervical specimens and for the vaccine safety and immunogenicity.

Results: The mean length of follow-up for women in the primary analysis for efficacy at the time of a prespecified event-triggered interim analysis was 13.6 months after the first vaccination. Vaccine efficacy against HPV-16/18 persistent infections (6-month definition) in the according-to-protocol cohort for efficacy was 100% (99% confidence interval, 20.5–100, $P = 0.0037$). At 6 months after the third dose of vaccine, geometric mean titers against HPV-16 and HPV-18 were 2899.3 and 1352.2 enzyme-linked immunosorbent assay units per milliliter, respectively, that is, 97- and 60-fold higher than geometric mean titers observed after natural infection. There were no clinically meaningful differences in safety between the HPV and control group.

Conclusions: The HPV-16/18 AS04-adjuvanted vaccine was as efficacious in Japan as in other countries and was generally safe and highly immunogenic in Japanese women.

Key Words: Human papillomavirus, HPV-16/18 AS04-adjuvanted vaccine, Cervical cancer, Persistent infection, Efficacy

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Cervical cancer is the second most common cancer, and it is estimated that approximately 2000 new cases and 200 deaths occur every year in Japanese women in their 20s and 30s.[1,2] The incidence and the mortality in young Japanese women have recently been increasing.[1,2] It is commonly understood that persistent infection with oncogenic

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human papillomavirus (HPV) is involved in the induction of dysplasia in cervical epithelial cells, which may ultimately evolve into invasive cervical cancers through progression of precancerous lesions.[3] HPV types 16 and 18 are predominantly found in cancerous cervical tissue in Japan and worldwide.[4,5] Epidemiological data from Japan suggest that the detection rate of HPV-18 is 54% and tends to be rather high in Japanese women in their 20s and 30s.[6] In addition, the overall frequency of the detection rate of both HPV-18 and HPV-16 reaches 80% of cervical cancer tissues from Japanese women in their 20s or 30s.[6] Moreover, cervical lesions associated with HPV-16 and HPV-18 may progress rapidly to higher grades of cervical intraepithelial neoplasia (CIN2/3) in comparison with other oncogenic HPV types.[7]

In general, HPV-16 is globally the most common type found in both cervical squamous cell carcinoma (SCC) and adenocarcinoma (ADC), and it accounts for 58.8% of SCC and 52.1% of ADC.[8] On the other hand, HPV-18 is found more frequently in ADC (39.0%) rather than in SCC (18.0%).[8] Prevalence of HPV-16 in cervical cancers may be slightly lower in a Japanese population for both cancer types (45.8% for SCC and 31.3% for ADC), but HPV-18 in return is detected more frequently in ADC (58.2%) in Japanese women in comparison with other ethnic populations; in addition, HPV-18 accounts for 10.8% of SCC in Japan.[4] After HPV-16 and HPV-18, types 52, 58, and 33 are the next most prevalent in all cervical cancers in Japan.[4] HPV type 52 accounts for 7.4% of SCC and 1.5% of ADC, and HPV-58 accounts for 7.1% of SCC and 1.5% of ADC.[4] The proportion of ADC is increasing in all cervical cancers in Japan, particularly in the younger generation.[9] Cervical ADC is rather difficult to detect and often slips through standard cytological screening.[8,10] In addition, ADC is likely to be associated with a higher rate of relapse and poorer outcomes than SCC.[8,11] Therefore, it is very important to prevent the infection with HPV-18 and HPV-16 so that cervical cancer mortality is reduced in Japan.

An HPV-16/18 L1 viruslike particle (VLP) vaccine formulated with the AS04 adjuvant system (GlaxoSmithKline Biologicals, Rixensart, Belgium) has demonstrated its prophylactic efficacy for persistent infections and CIN2+ with HPV-16 and/or HPV-18 in several clinical trials.[12,13] The efficacy has been maintained through 7.3 years,[14] together with sustained levels of antibodies against both HPV-16 and HPV-18 that are at least 10-fold higher than those antibody levels in women who had cleared natural HPV-16 or HPV-18 infections, respectively.[14] Based on various models, the antibody levels are expected to remain higher than the natural infection level for 20 years or possibly for life depending on the statistical model used.[15] Vaccine efficacy against CIN2+ associated with HPV-16/18 has also been demonstrated up to 7.3 years in a long-term follow-up study (NCT00120848).[14] In addition, the data have provided evidence of cross-protection against CIN2+ with HPV-31, HPV-33, and HPV-45 in a pivotal phase 3 study (NCT00122681).[16] Although previous efficacy studies have been conducted in a large number of women from several countries and therefore included various ethnicities, these studies did not include women enrolled from Japan.[12,13,16] This phase 2 study was therefore conducted to specifically evaluate the prophylactic efficacy of HPV-16/18 AS04-adjuvanted vaccine in Japanese women aged 20 to 25 years.[17] Detection of CIN2+ lesions associated with HPV-16/18 infection is a good primary end point in a clinical trial.[18] However, as time to progression from HPV infection to CIN2+ in carriers is variable, a large number of subjects and long-term follow-up are required to examine vaccine efficacy through a clinical trial with histopathological end points.[14,16] Interestingly, persistent oncogenic HPV infection has been reported to be consistently associated with an increased risk of CIN2+ lesions and therefore was considered as a reliable surrogate marker for cervical cancer.[3] Persistent infection of oncogenic HPV can induce cytological abnormality and may induce low- and high-grade histopathological lesions and ultimately cancer.[3,19] Therefore, and because the persistent infection is considered to be a robust virological end point that is consistently and strongly related to CIN2+, we used 6-month persistent infection of HPV-16/18 as a primary end point in our clinical trial in Japan.

Previously, we reported the first interim analysis 1 month after the third vaccine dose and demonstrated that the vaccine was highly immunogenic and well tolerated in Japanese women.[17] Here, we report the event-triggered interim analysis of this vaccine against a 6-month persistent infection associated with HPV-16/18, which was the primary objective of this study.

PATIENTS AND METHODS

Study Population

Healthy women aged 20 to 25 years were recruited for a phase 2 double-blind (observed-blind), controlled, randomized multicenter study (NCT00316693) between April and October 2006 in 13 centers in Japan. Study participants were not screened before enrollment with respect to baseline serological, cytological, or HPV DNA status. Subjects who had a urine pregnancy test with a negative result, who agreed to use adequate contraception over the vaccination period, and who had an intact cervix were eligible for inclusion. Exclusion criteria were previous vaccination with HPV vaccine or hepatitis A vaccine (HAV), previous 3-*O*-desacy 1-4'-monophosphoral lipid A administration, hepatitis A infection and various clinically significant diseases, previous colposcopic examination to evaluate for cervical cytological abnormality, pregnancy, and lactation. The study was conducted following the Declaration of Helsinki, and all participants provided written informed consent. All recruitment materials, informed consent, protocols, and amendments were approved by independent institutional review boards.

Procedures

Women were randomized in a 1:1 fashion to receive either the HPV-16/18 AS04-adjuvanted vaccine (containing 20 μg each of HPV-16 L1 VLP and HPV-18 L1 VLP adjuvanted with 50 μg of 3-O-desacyl-4'-monophosphoryl lipid A and 0.5 mg of aluminum hydroxide) or the Japan-licensed HAV (Aimmugen; The Chem-Sero-Therapeutic Research Institute, Kumamoto, Japan) containing 0.5 μg of inactivated hepatitis A antigen as the control vaccine. Both vaccines were administered intramuscularly according to 0-, 1-, and 6-month

schedules. The collection of blood samples and the evaluation of immunogenicity and HPV DNA were as previously described.[12,17] The investigator obtained cervical specimens with a cervical brush for cytological evaluation and HPV DNA testing at month 0, 6, 12, 18, and 24. Cytological condition was assessed with a liquid-based cytology system (ThinPrep; Cytyc Corporation, Marlborough, MA) using central laboratory testing (Quest Diagnostics, Teterboro, NJ) at month 0, 12 and 24. Results of the cytological assessment were reported using the 2001 Bethesda classification system. We did cytological assessments at a 6-month interval if previous findings showed atypical squamous cell of undetermined significance, and positive by Hybrid Capture II (Digene Corporation, Gaithersburg, MD), or low-grade squamous intraepithelial lesion (LSIL). HPV DNA isolated from the cytological specimens was detected by the polymerase chain reaction (PCR) SPF_{10}-$LiPA_{25}$ system, assessing 14 oncogenic HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 11 low–oncogenic risk HPV genotypes (6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74). If the sample was negative for HPV-16 or HPV-18 DNA by the SPF_{10}-$LiPA_{25}$ system (DDL Diagnostics Laboratory, Voorburg, The Netherlands), type-specific PCR for HPV-16 or HPV-18 was performed.[13,16]

As a safety assessment, women were asked to record on diary cards local symptoms (pain, redness, and swelling) and systemic symptoms (arthralgia, fatigue, fever, gastrointestinal symptoms, headache, myalgia, rash, and urticaria) experienced during the first 7 days after vaccination, with a 3-grade scale of symptom intensity. Additionally, they were to record any other adverse events occurring within 30 days after vaccination. Information on serious adverse events (SAEs) and pregnancies was collected throughout the study.

The primary objective of the study was to demonstrate the vaccine efficacy in preventing 6-month persistent cervical infections associated with HPV-16 and/or HPV-18 in women who are seronegative and DNA negative (by PCR) for the corresponding HPV DNA at the study start (month 0) and at month 6. Secondary and exploratory objectives included evaluation of vaccine efficacy against incident and 12-month persistent cervical infection, cytological abnormality, and cervical lesions that are associated with HPV-16/18 or other oncogenic HPV types and evaluation of immunogenicity and safety.

Statistical Analysis

Interim analysis of the data was triggered with an event-defined analysis plan when at least 8 cases of persistent infection of HPV-16 and/or HPV-18 were detected in the according-to-protocol (ATP) cohort for efficacy. The ATP cohort for efficacy was defined as the subject population who (1) met all eligibility criteria, (2) complied with protocol procedures, (3) received all 3 doses of vaccine according to protocol, (4) were DNA negative (by PCR) for the corresponding HPV types at months 0 and 6, (5) had efficacy end point measures available, (6) had no or low-grade cytological abnormality (atypical squamous cell of undetermined significance or LSIL) at month 0, and (7) were seronegative for the corresponding HPV type at month 0. The annual attack rate of persistent infection with HPV-16 or HPV-18 was assumed to be approximately 2%. A minimum of 8 cases of persistent infection at the time of the interim analysis were therefore assumed to provide 80% power to confirm a 99% confidence interval (CI) lower limit greater than 0%. The type I error α was divided as 0.01 and 0.045 between this interim analysis and the final analysis planned at the end of the study. Events rates were calculated as the number of cases divided by the accrued person-time since enrollment in both treatment groups and are expressed per 100 women-years. Vaccine efficacy was estimated using a conditional exact method. This method computes CI around the rate ratio (ratio of the event rates in the vaccinated versus control group) and takes into account the follow-up time of the subjects within each group. Vaccine efficacy was defined as 1 minus the rate ratio. Statistical significance was defined as a lower limit of the 99% CI greater than 0% (corresponding to an α of 1%). Additionally, P values were calculated using the Fisher exact test to compare the attack rates between both groups. The total vaccinated cohort (TVC) for efficacy was defined as all women who were given at least 1 vaccine dose and had no or low-grade cytological abnormality at month 0. The definition of the ATP cohort for analysis of immunogenicity was previously described.[17] This interim analysis was performed by an independent and external statistician, and the study blinding was maintained for all GlaxoSmithKline personnel, investigators, study collaborators, and subjects.

RESULTS

The study cohorts for efficacy analysis are defined according to the algorithm (Fig. 1). A total of 1040 eligible women were vaccinated and designated as the TVC: 519 in the HPV group and 521 in the control group. Ten subjects were excluded from the TVC because of high-grade cytological abnormality or missing cytological result at baseline; therefore, 1030 women (514 in the HPV group and 516 in the control group) were in the TVC for efficacy. Twenty-eight women (13 in the HPV group and 15 in the control group) were excluded from the TVC for efficacy to establish the ATP cohort for efficacy. One thousand two women (501 in the HPV group and 501 in the control group) were in the ATP cohort for efficacy. The mean (SD) follow-up time of the subjects at the time of this analysis was 13.6 (3.7) months in the ATP cohort for efficacy. The demographic characteristics of women enrolled in this study were previously described.

At interim analysis, 9 cases of primary end points (ie, 6-month persistent infection associated with HPV-16 and/or HPV-18) had occurred, all in the control group (Table 1). Of these 9 cases, 6 cases were associated with HPV-16, and 3 cases were associated with HPV-18. The vaccine efficacy was 100% (99% CI, 20.5–100; $P = 0.0037$). The vaccine efficacy against incident infection associated with HPV-16/18 was 82.2% (99% CI, 46.2–95.8; $P < 0.0001$), with 6 cases in the HPV group and 33 in the control group (Table 2). The efficacy rates against incident infection with HPV-16 alone and HPV-18 alone were 76.5% (99% CI, 5–96.5; $P = 0.0064$) and 88.6% (99% CI, 34.1–99.5; $P = 0.0003$), respectively. In addition to vaccine efficacy against HPV-16/18 infection, the efficacy to prevent against cytological abnormalities associated with HPV-16/18 infections was examined (Table 3). The

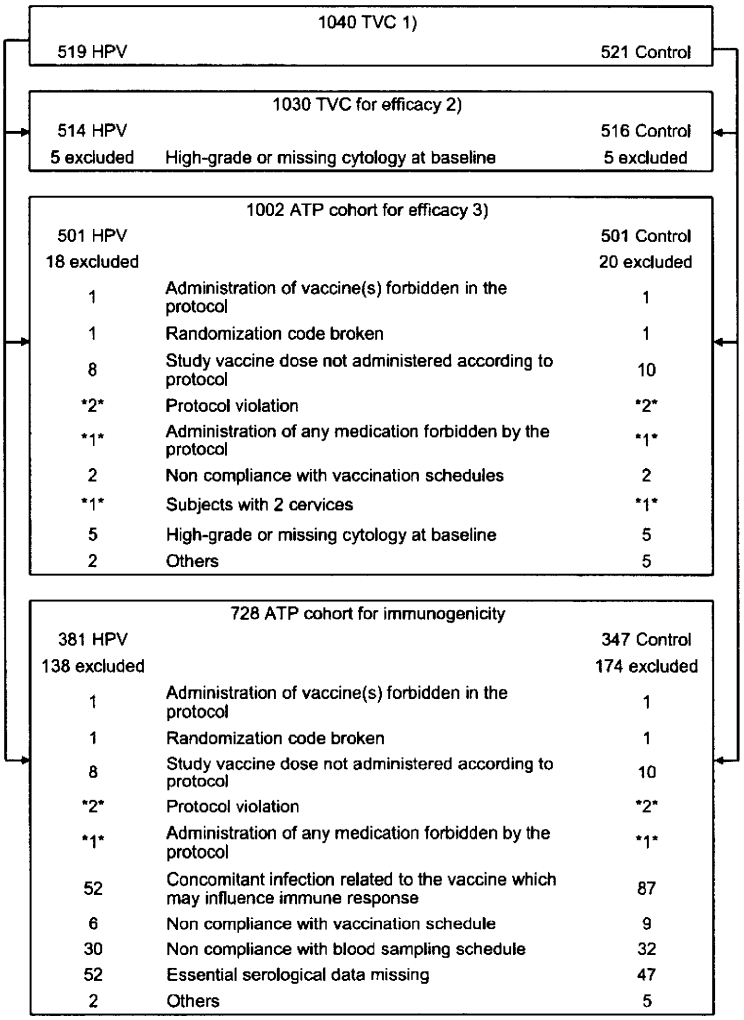

FIGURE 1. Description of study cohorts for analysis of end points. HPV indicates HPV-16/18 AS04-adjuvanted vaccine; control, hepatitis A vaccine; 1, TVC includes all women who received at least 1 vaccine dose. All women in the TVC were assessed for efficacy, safety, and immunogenicity end points. 2, TVC for efficacy includes women with no or low-grade cytological abnormality at month 0, who received at least 1 vaccine dose and had data available concerning the efficacy end point assessed. 3, ATP cohort for efficacy includes women with no or low-grade cytological abnormality at month 0, who met the eligibility criteria, complied with protocol procedures, had received all 3 vaccine doses, and had data available concerning the efficacy end point assessed. *n*, n cases described in 1 group and none in the other group. These cases remain blinded, as this study is ongoing.

vaccine efficacy was 83.4% (99% CI, −116.2–99.9; P = 0.1234), with 1 case in the HPV group and 6 in the HAV group. One case in the HPV group had a diagnosis of an LSIL at month 12, and multiple HPV infections were detected at all visits: HPV-6, HPV-31, and HPV-51 at study entry; HPV-31, HPV-51, HPV-53, and HPV-56 at month 6; and HPV-18 and HPV-31 at month 12. At the time of interim analysis, no histopathological data were available for analysis.

In the ATP cohort for immunogenicity, in the HPV group, anti–HPV-16 and anti–HPV-18 seropositivity at month 12, that is, 6 months after the third dose of vaccine, was still 100% (Table 4). At month 7, anti–HPV-16 GMT of the HPV group was 8033.3 enzyme-linked immunosorbent assay units per milliliter (EL.U/mL); it was 270-fold higher than the natural infection level that was defined as the anti–HPV-16 antibody level in women who had cleared HPV-16 infection. Similarly, anti–HPV-18 GMT of the HPV group was 4075.7 EL.U/mL, 180-fold higher than that after natural infection (GMT, 22.6 EL.U/mL) for HPV-18. At month 12, anti–HPV-16 and anti–HPV-18 GMTs were 2899.3 and 1352.2 EL.U/mL, respectively; they were still 97- and 60-fold higher than each natural infection level, respectively.

At the time of this event-triggered interim efficacy analysis, 16 women in the HPV group reported 20 SAEs,

TABLE 1. Vaccine efficacy to prevent persistent HPV-16/18 infections

	Group	N	n	Vaccine Efficacy, % (99% CI)	P
ATP cohort for efficacy					
HPV-16/18	HPV	358	0	100 (20.5–100)	0.0037
	Control	367	9		
HPV-16	HPV	305	0	100 (−44.5–100)	0.0307
	Control	319	6		
HPV-18	HPV	321	0	100 (−373.6–100)	0.2488
	Control	321	3		
TVC for efficacy					
HPV-16/18	HPV	411	0	100 (54.4–100)	0.0001
	Control	417	14		
HPV-16	HPV	352	0	100 (4.5–100)	0.0076
	Control	364	8		
HPV-18	HPV	365	0	100 (−41.4–100)	0.0306
	Control	370	6		

HPV, HPV-16/18 AS04-adjuvanted vaccine; Control, HAV; N, number of women included in each group; n, number of women reporting at least 1 event in each group.

TABLE 3. Vaccine efficacy to prevent cytological abnormalities associated with HPV-16/18 infections

	Group	N	n	Vaccine Efficacy, % (99% CI)	P
ATP cohort for efficacy					
HPV-16/18	HPV	402	1*	83.4 (−116.2–99.9)	0.1234
	Control	406	6		
HPV-16	HPV	345	0	100 (−173.4–100)	0.124
	Control	353	4		
HPV-18	HPV	356	1*	51.2 (−2161.0–99.8)	0.6239
	Control	355	2		
TVC for efficacy					
HPV-16/18	HPV	416	1*	88.8 (−20.1–100)	0.0208
	Control	426	9		
HPV-16	HPV	357	0	100 (−44.2–100)	0.0308
	Control	371	6		
HPV-18	HPV	369	1*	66.6 (−704.6–99.9)	0.6241
	Control	377	3		

*The case had multiple HPV infections detected: HPV-6, HPV-31, and HPV-51 at study entry; HPV-31, HPV-51, HPV-53, and HPV-56 at month 6; and HPV-18 and HPV-31 at month 12.

and 15 women in the control group reported 17 SAEs. Thirty-six pregnancies were reported in each group. In the HPV group, the outcomes of these pregnancies were 10 healthy infants, 5 spontaneous abortions, and 12 elective terminations, with 9 pregnancies still ongoing at the time of analysis. In the control group, pregnancy outcomes were 13 healthy infants, 3 spontaneous abortions, and 15 elective terminations, with 5 pregnancies still ongoing.

TABLE 2. Vaccine efficacy to prevent incident HPV-16/18 infections

	Group	N	n	Vaccine Efficacy, % (99% CI)	P
ATP cohort for efficacy					
HPV-16/18	HPV	403	6	82.2 (46.2–95.8)	<0.0001
	Control	406	33		
HPV-16	HPV	346	4	76.5 (5.0–96.5)	0.0064
	Control	353	17		
HPV-18	HPV	357	2	88.6 (34.1–99.5)	0.0003
	Control	355	17		
TVC for efficacy					
HPV-16/18	HPV	432	8	82 (53.1–94.6)	<0.0001
	Control	445	44		
HPV-16	HPV	373	6	73.4 (14.9–93.9)	0.002
	Control	389	23		
HPV-18	HPV	380	3	87.5 (46.0–98.7)	<0.0001
	Control	394	24		

DISCUSSION

The HPV-16/18 AS04-adjuvanted vaccine is currently licensed in more than 95 countries on the basis of an extensive database on safety, immunogenicity, and efficacy. National and regional immunization programs aimed at young adolescent girls have been widely implemented and include catch-up programs in some countries up to the age of 18 years or older. Our previous publication reported that the HPV-16/18 AS04-adjuvanted vaccine was highly immunogenic and well tolerated in healthy Japanese women aged 20 to 25 years.[17] Immunogenicity and safety results were in line with results of the large, international phase 3 efficacy study conducted globally (NCT00122681); therefore, vaccine efficacy as demonstrated in this pivotal efficacy trial was also expected to be observed in Japanese women.[16] The event-triggered efficacy analysis was conducted as 9 cases of 6-month persistent infection with HPV-16/18 were confirmed in the ATP cohort for efficacy. All cases were reported in the control group, resulting in 100% efficacy to prevent persistent HPV-16/18 infections at 6-month intervals in Japanese women. The HPV-16/18 AS04-adjuvanted vaccine appeared to be as efficacious in the Japanese study population as it had proven to be in other populations. It is reported that persistent infection is a robust surrogate marker for cervical cancer development and a valid end point in clinical trials with prophylactic HPV vaccine.[3] Persistent infection with HPV is strongly associated with cervical precancerous lesions such as CIN; therefore,

TABLE 4. Seropositivity rate and GMTs for HPV-16 and HPV-18 VLP immunoglobulin G antibodies in subjects who were seronegative before vaccination (ATP cohort for immunogenicity)

Antibody	Group	Timing	N	Seropositivity, %	GMT (95%CI)
Anti–HPV-16	HPV	Enrollment	323	0.0	4.0 (4.0–4.0)
		Month 6	323	100	648.9 (592.7–710.4)
		Month 7	323	100	8033.3 (7381.9–8742.2)
		Month 12	317	100	2899.3 (2616.9–3212.1)
	Control	Enrollment	308	0.0	4.0 (4.0–4.0)
		Month 6	308	2.6	4.2 (4.0–4.3)
		Month 7	304	1.6	4.1 (4.0–4.2)
		Month 12	298	5.0	4.3 (4.1–4.4)
Anti–HPV-18	HPV	Enrollment	323	0.0	3.5 (3.5–3.5)
		Month 6	323	100	486.7 (443.9–533.5)
		Month 7	323	100	4075.7 (3745.0–4435.6)
		Month 12	318	100	1352.2 (1218.4–1500.6)
	Control	Enrollment	302	0.0	3.5 (3.5–3.5)
		Month 6	300	3.3	3.6 (3.5–3.7)
		Month 7	299	3.7	3.6 (3.6–3.7)
		Month 12	290	6.9	3.8 (3.7–4.0)

GMT, geometric mean antibody titer; N, number of women with prevaccination results available.

both qualify as end points for clinical efficacy trails with prophylactic HPV vaccines, which are recognized by regulatory authorities worldwide.[3,18] HPV is usually eliminated by the individual's immune system once the virus has infected cervical epithelial cells.[19] Incident infection therefore is less likely to progress to either precancerous lesions or cervical cancers. Persistent infection with oncogenic HPV raises the risk of cervical lesions because intrinsic oncogenic proteins such as E6 and E7 facilitate cell transformation and ultimately may lead to precancerous lesions or even cancer.[19] The present event-triggered efficacy analysis demonstrates 100% protection against persistent infection with HPV-16/18, whereas efficacy to prevent incident HPV-16/18 infection was approximately 80%. Similar efficacy against persistent and incident infection has been shown in other trials.[12,13,16] One subject in the HPV group had a diagnosis of an LSIL with HPV-18 at month 12; this observation merits some attention. Multiple HPV infections were detected in this subject: HPV-6, HPV-31, and HPV-51 at study entry; HPV-31, HPV-51, HPV-53, and HPV-56 at month 6; and HPV-18 and HPV-31 at month 12. Human papillomavirus type 18 was thus only detected at month 12, but HPV-31 was detected at all time points. The cytological abnormality in this woman may therefore be causally attributed to sustained HPV-31 infection rather than to the incident infection with HPV-18.

The interim analysis took place after a mean subject follow-up time of approximately 13.6 months. Therefore, the number of cases in the secondary end points such as a 12-month persistent infection and cytological and histological abnormalities was limited. At the final analysis, larger numbers will be accrued; this should allow us to evaluate some more objectives against nonvaccine HPV types in the study, and these results will be reported in the next publication.

Previous data demonstrated that 12 months after the first vaccination, the GMTs against both HPV-16 and HPV-18 in the subjects (15- to 25-year-old women) were 42- or 44-fold higher than those after natural infection in women who had cleared correspondence HPV infection and that these antibodies persisted at levels more than 10-fold higher than natural infection levels even 7.3 years after the first vaccination (NCT00120848).[12–14] The same study also demonstrated 100% vaccine efficacy to prevent CIN1+ and persistent HPV-16/18 infection for up to 7.3 years (NCT00120848).[12–14] Although a correlate of protection in antibody levels that are predictive of clinical protection against HPV-related disease is not yet defined, there seems to be a good correlation between sustained antibody response and long-term protection against cervical dysplasia and persistent infection. In our study, anti–HPV-16 and anti–HPV-18 GMTs in the women vaccinated with HPV-16/18 AS04-adjuvanted vaccine were comparable with the ones observed in the larger international phase 3 study (NCT00122681) and were significantly higher than antibody levels after natural infection.[16] Actual data on how long the immunological response is maintained after the 3 dose vaccination schedules are not yet available, but on the basis of assumptions in statistical models, protection could be lifelong when women aged 15 to 25 years are vaccinated with the HPV-16/18 AS04-adjuvanted vaccine.[15] These results suggest that long-term protection against invasive cervical cancer may be achieved in women if they had the opportunity to be vaccinated.

There was no overall difference in the number of SAEs reported and pregnancy outcomes between the HPV and control groups in this study as in other studies.[12,13,16] Recently, a pooled analysis of the safety data of the HPV-16/18 AS04-adjuvanted vaccine in a cohort of almost 30,000 females aged 10 years and older was reported and resulted in no clinically relevant differences between the HPV group and the pooled control groups in rates of SAEs (2.8% vs 3.1%) and pregnancy outcomes (rates of spontaneous abortion, 9.4% vs 8.6%); this is in line with the present study wherein the HPV-16/18 AS04-adjuvanted vaccine is generally safe and well tolerated.[20]

We can conclude from this interim analysis that the HPV-16/18 AS04-adjuvanted vaccine showed excellent vaccine efficacy, potent immunogenicity, and a good safety profile in the study population consisting of Japanese women. Vaccination of Japanese women with HPV-16/18 AS04-adjuvanted vaccine may contribute to a decrease of the burden of cervical cancer in Japan.

CONTRIBUTORS

Fabian Tibaldi (Global Clinical Research and Development Department, GlaxoSmithKline Biologicals, Rixensart, Belgium) contributed to the statistical analyses. Gary Dubin (Global Clinical Research and Development Department, GlaxoSmithKline Biologicals, Marietta, PA), Dominique Descamps (Global Clinical Research and Development Department, GlaxoSmithKline Biologicals, Rixensart, Belgium), and Olivier Godeaux (Global Clinical Research and Development Department, GlaxoSmithKline Biologicals, Rixensart, Belgium) contributed to the study conception and design. Nobuhiro Noro (Vaccine Clinical Development, GlaxoSmithKline K.K., Tokyo, Japan) contributed to data interpretation.

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REFERENCES

1. Vital Statistics of Japan (Ministry of Health, Labour & Walfare). Available at: http://ganjoho.ncc.go.jp/professional/statistics/statistics.html. Accessed August 13, 2009.
2. Marugame T, Matsuda T, Kamo K, et al. Cancer incidence and incidence rates in Japan in 2001 based on the data from 10 population-based cancer registries. *Jpn J Clin Oncol.* 2007;37:884–891.
3. Koshiol J, Lindsay L, Pimenta JM, et al. Persistent human papillomavirus infection and cervical neoplasia: a systematic review and meta-analysis. *Am J Epidemiol.* 2008;168:123–137.
4. Miura S, Matsumoto K, Oki A, et al. Do we need a different strategy for HPV screening and vaccination in East Asia? *Int J Cancer.* 2006;119:2713–2715.
5. Munoz H, Bosch FX, Castellsague X, et al. Against which human papillomavirus types shall we vaccinate and screen? The international perspective. *Int J Cancer.* 2004;111:278–285.
6. Onuki M, Matsumoto K, Satoh T, et al. Human papillomavirus infections among Japanese women: age-related prevalence and type-specific risk for cervical cancer. *Cancer Sci.* 2009;100:1312–1316.
7. Winer RL, Kiviat NB, Hughes JP, et al. Development and duration of human papillomavirus lesions, after initial infection. *J Infect Dis.* 2005;191:731–738.
8. Schwarz TF. AS04-adjuvanted human papillomavirus-16/18 vaccination: recent advances in cervical cancer prevention. *Expert Rev Vaccines.* 2008;7:1465–1473.
9. Takekida S, Maeno Y, Oki N, et al. A retrospective study of young cervical cancer at the Hyogo medical center for adults from 1976 to 2005 [in Japanese]. *Sanpu No Shinpo.* 2007;59:111–114.
10. Castellsague X, Diaz M, de Sanjose S, et al. Worldwide human papillomavirus etiology of cervical adenocarcinoma and its cofactor: implications for screening and prevention. *J Natl Cancer Inst.* 2006;98:303–315.
11. Smith HO, Tiffany MF, Qualls CR, et al. The rising incidence of adenocarcinoma relative to squamous cell carcinoma of the uterine cervix in the United States—a 24 year population-based study. *Gynecol Oncol.* 2000;78:97–105.
12. Harper DM, Franco EL, Wheeler C, et al. Efficacy of a bivalent L1 virus-like particle vaccine in prevention of infection with human papillomavirus types 16 and 18 in young women: a randomised controlled trial. *Lancet.* 2004;364:1757–1765.
13. Harper DM, Franco EL, Wheeler CM, et al. Sustained efficacy up to 4.5 years of a bivalent L1 virus-like particle vaccine against human papillomavirus types 16 and 18: follow-up from a randomised control trial. *Lancet.* 2006;367:1247–1255.
14. Carvalho D, Roteli C, Teixeira J, et al. Immunogenicity and safety of the HPV-16/18 AS04-adjuvanted vaccine up to 7.3 years. 25th International Papillomavirus Conference and Clinical Workshop; 2009; Malmo, Sweden.
15. David MP, Herck KV, Hardt K, et al. Long-term persistence of anti–HPV-16 and -18 antibodies induced by vaccination with the AS04-adjuvanted cervical cancer vaccine: Modeling of sustained antibody responses. *Gynecol Oncol.* 2009;115: S1–S6.
16. Paavonen J, Naud P, Salmeron J, et al. Efficacy of human papillomavirus (HPV)–16/18 AS04-adjuvanted vaccine against cervical infection and precancer caused by oncogenic HPV types (PATRICIA): final analysis of a double-blind, randomised study in young women. *Lancet.* 2009;374:301–314.
17. Konno R, Dobbelaere KO, Godeaux OO, et al. Immunogenicity, reactogenicity and safety of human papillomavirus (HPV)-16/18 AS04-adjuvanted vaccine in Japanese women: interim analysis of a phase II double-blind, randomized controlled trial at month 7. *Int J Gynecol Cancer.* 2009;19:905–911.
18. Pagliusi SR, Teresa AM. Efficacy and other milestones for human papillomavirus vaccine introduction. *Vaccine.* 2004;23:569–578.
19. Stanly MA, Pett MR, Coleman N. HPV: from infection to cancer. *Biochem Soc Trans.* 2007;35:1456–1460.
20. Descamps D, Hardt K, Spiessens B, et al. Safety of human papillomavirus (HPV)–16/18 AS04 adjuvanted vaccine for cervical cancer prevention: a pooled analysis of 11 clinical trials. *Hum Vaccin.* 2009;5:332–340.

Efficacy of Human Papillomavirus Type 16/18 AS04–Adjuvanted Vaccine in Japanese Women Aged 20 to 25 Years

Final Analysis of a Phase 2 Double-Blind, Randomized Controlled Trial

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Background: Human papillomavirus (HPV) type 16/18 AS04–adjuvanted vaccine was shown to be highly immunogenic and generally well tolerated in the interim analysis of a phase 2 double-blind, randomized controlled multicenter study in Japanese healthy women aged 20 to 25 years. Vaccine efficacy, immunogenicity, and safety are assessed in this study through 24 months after the first vaccination.

Methods: Japanese women aged 20 to 25 years were randomly assigned to receive either HPV-16/18 AS04–adjuvanted vaccine (n = 519) or hepatitis A vaccine (n = 521) at 0, 1, and 6 months. Women were assessed for virological, cytological, and histological end points associated with HPV-16/18 and 12 other oncogenic HPV types (types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) in cervical specimens and for the vaccine safety and immunogenicity. Antibody concentrations were measured by an enzyme-linked immunosorbent assay. Primary efficacy analysis was performed in the according-to-protocol cohort for efficacy, primary immunogenicity analysis was performed in the according-to-protocol cohort for immunogenicity, and primary safety analysis was done in the total vaccinated cohort.

Results: Vaccine efficacy against persistent infections (6 month definition) associated with HPV-16/18 was 100% (95.5% confidence interval, 71.3–100; $P < 0.0001$). Vaccine efficacy against cervical intraepithelial neoplasia 1+ associated with 14 oncogenic HPV types was 64.9% (95.5% confidence interval, 4.9–89.0; $P = 0.02$). At 24 months after the first dose of the vaccine, geometric mean antibody titers against HPV-16 and HPV-18 were 1521.5 enzyme-linked immunosorbent assay U/mL and 627.4 enzyme-linked immunosorbent assay U/mL, respectively. The HPV-16/18 AS04–adjuvanted vaccine had a clinically acceptable safety profile.

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Conclusions: The HPV-16/18 AS04–adjuvanted vaccine showed excellent prophylactic efficacy against 6-month persistent infection with HPV-16/18. The HPV-16/18 AS04–adjuvanted vaccine was generally well tolerated and immunogenic in the study population of healthy Japanese women aged 20 to 25 years.

Key Words: Human papillomavirus, HPV-16/18 AS04–adjuvanted vaccine, Cervical cancer, Persistent infection, Efficacy

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Cervical cancer is the second most prevalent cancer affecting women worldwide.[1,2] The burden associated with cervical cancer could be reduced by implementing prophylactic vaccination against human papillomavirus (HPV). Indeed, HPV is commonly found in the genital mucosa of sexually active women and may lead to cervical neoplasia.[3,4] Every year in Japan, approximately 15,000 women are diagnosed with cervical cancer, and approximately 3500 women die of it.[5] Human papillomavirus types 16 and 18 are the most prevalent types and account for more than 70% of all invasive cervical cancers worldwide; HPV-45 and HPV-31 are responsible for an additional 10% of cases.[6] Although HPV-16 and HPV-18 also seem to be the most frequently identified HPV types in invasive cervical cancers in Japan (64.9%), HPV-52 and HPV-58 seem to be next most common types and account for 11.5% of cervical cancer in Japanese.[7]

Human papillomavirus type 16/18 AS04–adjuvanted vaccine (GlaxoSmithKline Biologicals; Wavre, Belgium) has been shown to protect against cervical intraepithelial neoplasia (CIN) 2+ associated with HPV-16/18 in a large phase 3 efficacy trial (PATRICIA).[8,9] Sustained protection and immune response have been documented up to 7.3 years.[10–14] Vaccine-induced antibody titers for HPV-16 and HPV-18 are several-fold higher than those induced by natural infection.[8,9] Based on mathematical models, antibodies are expected to persist for at least 20 years and possibly lifelong.[15]

The efficacy of the HPV-16/18 AS04–adjuvanted vaccine for prevention of cervical dysplasia and persistent infection and its safety profile have primarily been demonstrated in white populations.[8–12] This phase 2 study was specifically designed to assess the immunogenicity, safety, and efficacy of the HPV-16/18 AS04–adjuvanted vaccine in Japanese women.[16]

We previously reported interim results about the efficacy of the vaccine against HPV-16/18–associated end points and about its immunogenicity and safety.[16,17] Here we report the final analysis of this study through 24 months after the first vaccination, evaluating the efficacy against persistent infection associated with HVP-16/18 (primary end point of the study), as well as the immunogenicity through 2 years. In addition, the efficacy against cytological abnormalities and histopathologic lesions associated with HPV-16/18 and oncogenic HPV types has been assessed.

METHODS

Study Population

This phase 2, observed-blind, randomized controlled study (104798, NCT00316693) was conducted at 13 centers in Japan between April 2006 and February 2009 in healthy female volunteers aged 20 to 25 years at the time of first vaccination.

Study participants were not screened before enrollment with respect to baseline serological, cytological, or HPV DNA status. Inclusion criteria and exclusion criteria were previously described.[16] The study was conducted following the Declaration of Helsinki (version 1996), and all participants provided written informed consent. All recruitment materials, informed consents, protocols, and amendments were approved by independent institutional review boards.

Procedures

Subjects were randomized in a 1:1 fashion to receive either the HPV-16/18 AS04–adjuvanted vaccine (containing 20 μg of HPV-16 L1 VLP and 20 μg of HPV-18 L1 VLP adjuvanted with 50 μg 3-*O*-desacyl-4′-monophosphoryl lipid A and 0.5 mg aluminum hydroxide) or Japan-licensed hepatitis A vaccine (Aimmugen; Chem-Sero-Therapeutic Research Institute, Kumamoto, Japan; containing 0.5 μg inactivated hepatitis A antigen) as control vaccine. Both vaccines were administered intramuscularly according to a 0-, 1-, and 6-month schedule. The collection of blood and cervical samples for evaluation of immunogenicity and HPV DNA was performed as previously described.[10,11,16,17] Investigators obtained cervical specimens with a cervical brush for cytology and HPV DNA testing at months 0, 6, 12, 18, and 24. Cytology was assessed with liquid-based cytology (ThinPrep; Cytyc Corporation, Marlborough, Mass) using a central laboratory (Quest Diagnostics, Teterboro, NJ) at months 0, 12, and 24. Cytology results were reported using the 2001 Bethesda System. Cytology assessments were repeated at 6-month interval if previous findings showed atypical squamous cell of undetermined significance (ASC-US) and were positive by Hybrid Capture II (Digene Corp, Gaithersburg, Md) or in case of low-grade squamous intraepithelial lesion (LSIL). Human papillomavirus DNA isolated from the cytology specimens was detected by the polymerase chain reaction (PCR) SPF_{10}-$LiPA_{25}$ system, assessing 14

oncogenic HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) and 11 low-oncogenic-risk HPV genotypes (6, 11, 34, 40, 42, 43, 44, 53, 54, 70, and 74). If the sample was negative for HPV-16 or HPV-18 DNA by the SPF_{10}-$LiPA_{25}$ system, type-specific PCR for HPV-16 or HPV-18 was performed.[10,11,16,17]

Protocol guidelines recommended colposcopy after 2 consecutive reports of oncogenic HPV DNA–positive (with Hybrid Capture II) ASC-US or LSIL (independent of HPV DNA results) or 1 report of high-grade squamous intraepithelial lesion (HSIL), atypical glandular cells, or atypical squamous cells cannot exclude HSIL (ASC-H). Biopsy was required for any suspected lesions on colposcopy. The central laboratory (Quest Diagnostics) processed and interpreted results from histology samples. All CIN end points were confirmed by an expert histopathology review panel that was blinded to vaccine status, HPV DNA status before biopsy, and cytology reports. Human papillomavirus DNA testing was done at DDL Diagnostic Laboratory (Voorburg, the Netherlands).

The primary objective of the study was to demonstrate the vaccine efficacy to protect against 6-month persistent cervical infections associated with HPV-16 and/or HPV-18 in women who were seronegative at study entry (month 0) and DNA negative (by PCR) for the corresponding HPV DNA at months 0 and 6. Secondary and exploratory objectives included evaluation of vaccine efficacy against incident and 12-month persistent cervical infections, and cytological and histopathologic abnormalities associated with HPV-16/18 or any oncogenic HPV types, as well as immunogenicity and safety. Cytological abnormalities included ASC-US, LSIL, ASC-H, HSIL, and atypical glandular cells, whereas histological abnormalities included CIN1 or worse (CIN1+).

Serious adverse events (SAEs), medically significant conditions (ie, adverse events prompting either emergency room visit or physician visits that were not related to common diseases, eg, sinusitis and pharyngitis), new-onset chronic diseases, pregnancy, and pregnancy outcomes were collected throughout the study.

Statistical Analysis

Figure 1 shows the descriptions of the analysis cohorts. The total vaccinated cohort (TVC) included all women who received at least 1 vaccine dose. The TVC for efficacy (TVC-E) included all women who received at least 1 vaccine dose, had normal or low-grade cytology (negative, ASC-US, or LSIL) at month 0, and were evaluable for efficacy. The according-to-protocol cohort for efficacy (ATP-E) included all evaluable women (ie, meeting all eligibility criteria, complying with protocol procedures, without any protocol violations) who completed the 3-dose schedule, had normal or low-grade cytology at month 0, and were evaluable for efficacy. The HPV-16/18 type–specific analyses in the ATP-E and TVC-E were stratified by serostatus at month 0. Human papillomavirus type–specific analyses were performed in women who were HPV DNA negative for the corresponding type at months 0 and 6 for the ATP-E and at month 0 for TVC-E. Primary analysis of efficacy was performed in the ATP-E; the same analyses were also performed on the TVC-E. Primary analysis of immunogenicity was performed in the according-to-protocol cohort for immunogenicity (previously described in Konno et al[16]), and primary safety analysis was performed on the TVC.

The enrollment of an estimated target of 1000 unscreened women was estimated to provide 800 women who were DNA negative for HPV-16 or HPV-18 at months 0 and 6 and who would be evaluable for assessment of the primary end point in the ATP-E. If an annual attack rate of persistent infection of 2% and vaccine efficacy against 6-month persistent infection of 90% were assumed, the final efficacy analysis would provide 80% power to achieve the primary objective.

Vaccine efficacy for all virological, cytological, and histopathologic end points was calculated with a conditional exact method. For the final analysis, we defined significance when the lower limit of the 95.5% confidence interval (CI) for vaccine efficacy was greater than 0 (for all end points). The type I error α was divided as 0.01 and 0.045 between

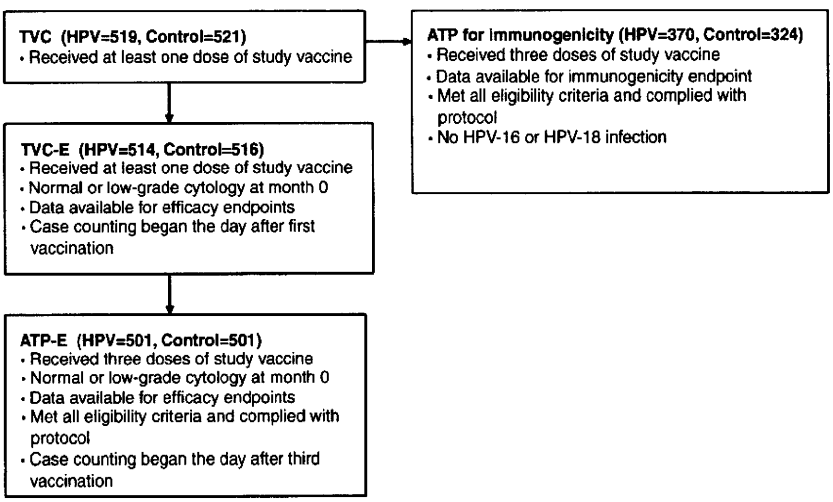

FIGURE 1. Definition of study cohorts.

the second interim analysis and the final analysis. In addition, *P* values were calculated using the Fisher exact test to compare the attack rates between both groups.

RESULTS

The study cohorts for immunogenicity analysis and the demographic characteristics of women enrolled in this study were previously described.[16] A total of 1040 eligible women were vaccinated (TVC): 519 in the HPV vaccine group and 521 in the control vaccine group. Ten subjects were excluded from the TVC-E because of high-grade or missing cytology at baseline. The TVC-E therefore included 1030 women: 514 in the HPV vaccine group and 516 in the control group). Twenty-eight women (13 in the HPV vaccine group, 15 in the control group) were excluded from the ATP-E. The ATP-E included 1002 women: 501 in the HPV vaccine group and 501 in the control vaccine group.

The primary end point analysis was performed in the ATP-E cohort in women who were seronegative at month 0 and HPV DNA negative at both months 0 and 6. At final analysis, 15 cases of 6-month persistent infection associated with HPV-16 and/or HPV-18 were identified in the ATP-E, all in the control group (Table 1). Of these 15 cases, 11 cases were associated with HPV-16, and 5 cases were associated with HPV-18. Dual infection was confirmed in 1 case. The primary objective of the study was met, with a calculated vaccine efficacy of 100% (95.5% CI, 71.3%–100%; $P < 0.0001$). Other analyses were also performed with regard to vaccine efficacy against incident infections, 12-month persistent infections, and cytological abnormalities associated with HPV-16/18 (Table 2). Vaccine efficacy against incident infections associated with HPV-16/18 was 82.5% (95.5% CI, 59.8%-93.6%; $P < 0.0001$), with 7 cases in the HPV vaccine group and 39 in the control group in the ATP-E. Vaccine efficacy against HPV-16/18 12-month persistent infections was 100% (95.5% CI, 11.2%–100%; $P = 0.0306$), with no case in the HPV vaccine group and 6 in the control group in the ATP-E. Vaccine efficacy against cytological abnormalities associated with HPV-16/18 was 91.7% (95.5% CI, 42.1%–99.8%; $P = 0.0017$), with 1 case in the HPV vaccine group and 12 in the control group in the ATP-E. The subject in the HPV vaccine group was diagnosed as an LSIL at month 12, and multiple HPV infections were detected at all visits: HPV-6, HPV-31, and HPV-51 at study entry; HPV-31, HPV-51, HPV-53, and HPV-56 at month 6; HPV-18 and HPV-31 at month 12; and HPV-31, HPV-52, and HPV-74 at month 24. Specimen at month 18 was not taken, and therefore cytological data and HPV DNA status were not available for the subject at that point in time. In addition to vaccine efficacy for virological end points and cytological abnormalities, the efficacy to prevent histopathologic abnormalities associated with HPV-16/18 infections was examined. Three cases of CIN1+ and 1 case of CIN2+ associated with HPV-16 and/or HPV-18 were confirmed in the control group in the ATP-E.

Vaccine efficacy to prevent infections and cytological or histopathologic abnormalities associated with any of 14 oncogenic HPV types (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) was also examined (Table 3). Significant protection against incident infections, persistent

TABLE 1. Vaccine efficacy to prevent persistent infections (6-month definition) associated with HPV-16/18

	Group	N	n	Vaccine Efficacy (95.5% CI), %	*P*
ATP-E					
HPV-16/18	Vaccine	387	0	100 (71.3–100)	<0.0001
	Control	392	15		
HPV-16	Vaccine	332	0	100 (58.4–100)	0.0009
	Control	340	11		
HPV-18	Vaccine	346	0	100 (−12.7 to 100)	0.0301
	Control	343	5		
TVC-E					
HPV-16/18	Vaccine	418	0	100 (79.4–100)	<0.0001
	Control	418	20		
HPV-16	Vaccine	359	0	100 (65.9–100)	0.0002
	Control	365	13		
HPV-18	Vaccine	368	0	100 (39.0–100)	0.0075
	Control	371	8		

ATP-E: For combined types: subjects DNA negative at months 0 and 6 and seronegative at month 0 for at least 1 HPV type. For single type: subjects DNA negative at months 0 and 6 and seronegative at month 0 for the corresponding HPV type. TVC-E: For combined types: subjects DNA negative and seronegative at month 0 for at least 1 HPV type. For single type: subjects DNA negative and seronegative at month 0 for the corresponding HPV type.

control, hepatitis A vaccine; n, number of women reporting at least 1 event in each group; N, number of women included in each group; vaccine, HPV-16/18 AS04–adjuvanted vaccine.

TABLE 2. Vaccine efficacy to prevent incident infections, cytological abnormalities, persistent infection (12-month definition), and CIN1+ and CIN2+ associated with HPV-16/18

	Group	N	n	Vaccine Efficacy (95.5% CI), %	*P*
ATP-E					
Incident infection	Vaccine	408	7	82.5 (59.8–93.6)	<0.0001
	Control	406	39		
Persistent infection (12-mo definition)	Vaccine	365	0	100 (11.2–100)	0.0306
	Control	369	6		
Cytological abnormalities	Vaccine	408	1*	91.7 (42.1–99.8)	0.0017
	Control	406	12		
CIN1+	Vaccine	408	0	100 (−156.8 to 100)	0.1241
	Control	407	3		
CIN2+	Vaccine	408	0	100 (−428.9 to 100)	0.4994
	Control	407	1		
TVC-E					
Incident infection	Vaccine	432	9	82.0 (62.5–92.4)	<0.0001
	Control	445	49		
Persistent infection (12-mo definition)	Vaccine	406	0	100 (47.4–100)	0.0037
	Control	411	9		
Cytological abnormalities	Vaccine	422	1*	93.3 (55.3–99.9)	0.0005
	Control	427	15		
CIN1+	Vaccine	422	0	100 (−15.2 to 100)	0.0618
	Control	427	5		
CIN2+	Vaccine	422	0	100 (−476.0 to 100)	0.4994
	Control	427	2		

*Multiple HPV infections were detected in this subject: HPV-6, HPV-31, and HPV-51 at study entry; HPV-31, HPV-51, HPV-53, and HPV-56 at month 6; HPV-18 and HPV-31 at month 12; and HPV-31, HPV-52, and HPV-74 at month 24. Specimen at month 18 was not taken, and therefore cytological data and HPV DNA status were not available for the subject at that point in time.

ATP-E: For combined types: subjects DNA negative at months 0 and 6 and seronegative at month 0 for at least 1 HPV type. For single type: subjects DNA negative at months 0 and 6 and seronegative at month 0 for the corresponding HPV type. TVC-E: For combined types: subjects DNA negative seronegative at month 0 for at least 1 HPV type. For single type: subjects DNA negative and seronegative at month 0 for the corresponding HPV type.

n, number of women reporting at least 1 event in each group; N, number of women included in each group.

infections (6-month definition), cytological abnormalities, and CIN1+ was demonstrated in the ATP-E, with corresponding vaccine efficacies of 31.2% (95.5% CI, 9.5%–47.8%; P = 0.0036), 50.6% (95.5% CI, 19.3%–70.5%; P = 0.0022), 43.9% (95.5% CI, 4.2%–67.9%; P = 0.0207), and 64.9% (95.5% CI, 4.9%–89.0%; P = 0.02), respectively. Vaccine efficacy against CIN2+ associated with 14 oncogenic HPV types was not statistically significant, that is, 75.1% (95.5% CI, −28.4% to 97.9%; P = 0.0618) and 66.6% (95.5% CI, −12.6% to 92.4%; P = 0.0468) in ATP-E and TVC-E, respectively.

Seropositivity for both HPV-16 and HPV-18 in the according-to-protocol cohort for immunogenicity was 100% in all tested time points. At month 24, antibody titers were 1521.5 and 627.4 enzyme-linked immunosorbent assay U/mL for anti–HPV-16 and anti–HPV-18 antibodies respectively, that is, 51- and 28-fold higher than titers after natural infection as obtained from subjects in study HPV-008 (PATRICIA) who were seropositive and HPV DNA negative for the respective HPV type at baseline. Anti–HPV-16 and anti–HPV-18 antibody levels peaked at 1 month after the third dose (month 7)[16] and reached a plateau between months 18 and 24 after the first dose (Fig. 2).

Serious adverse events were reported by 18 women (3.5%) in the HPV vaccine group and 19 women (3.6%) in the control group. One SAE (0.2%), a spontaneous abortion, was considered to be possibly related to vaccination by the investigator in charge of the subject because the event occurred approximately 15 days after vaccination, and the temporal relationship of the event to vaccination was considered in the HPV vaccine group. Medically significant conditions and new-onset chronic diseases were reported for 91 (17.5%) and 5 women (1%) in the HPV vaccine group, and 107 (20.5%) and 6 women (1.2%) in the control group, respectively. Forty-six and 43 pregnancies were reported in the HPV vaccine group and the control vaccine group, respectively. In the HPV vaccine group, the outcomes of these