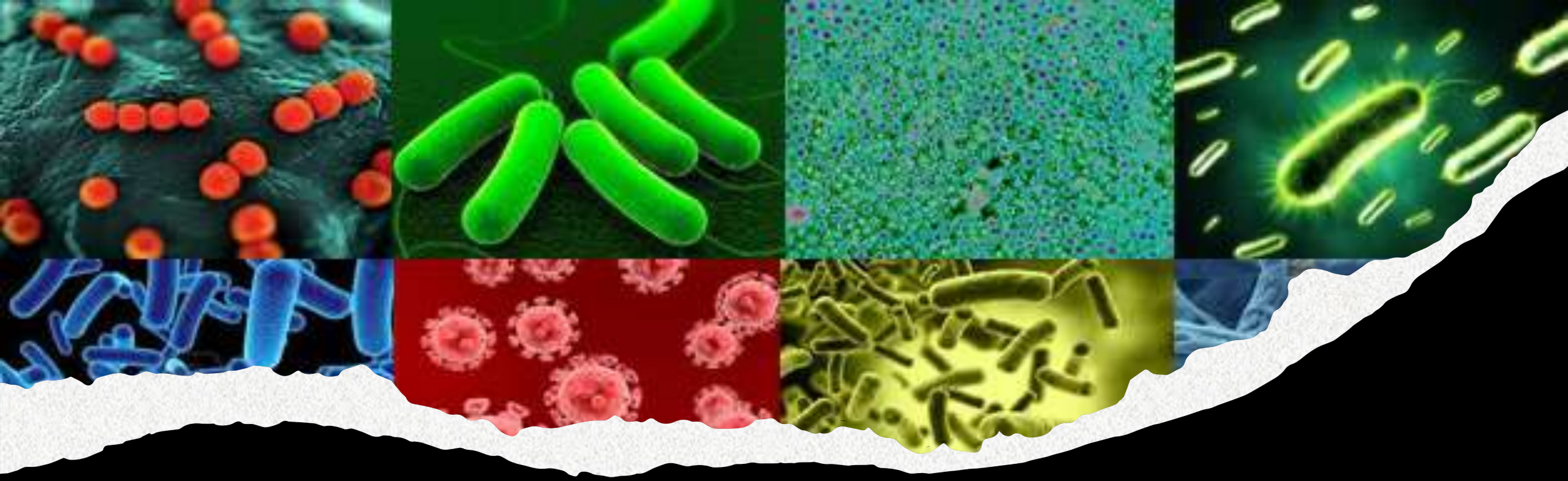


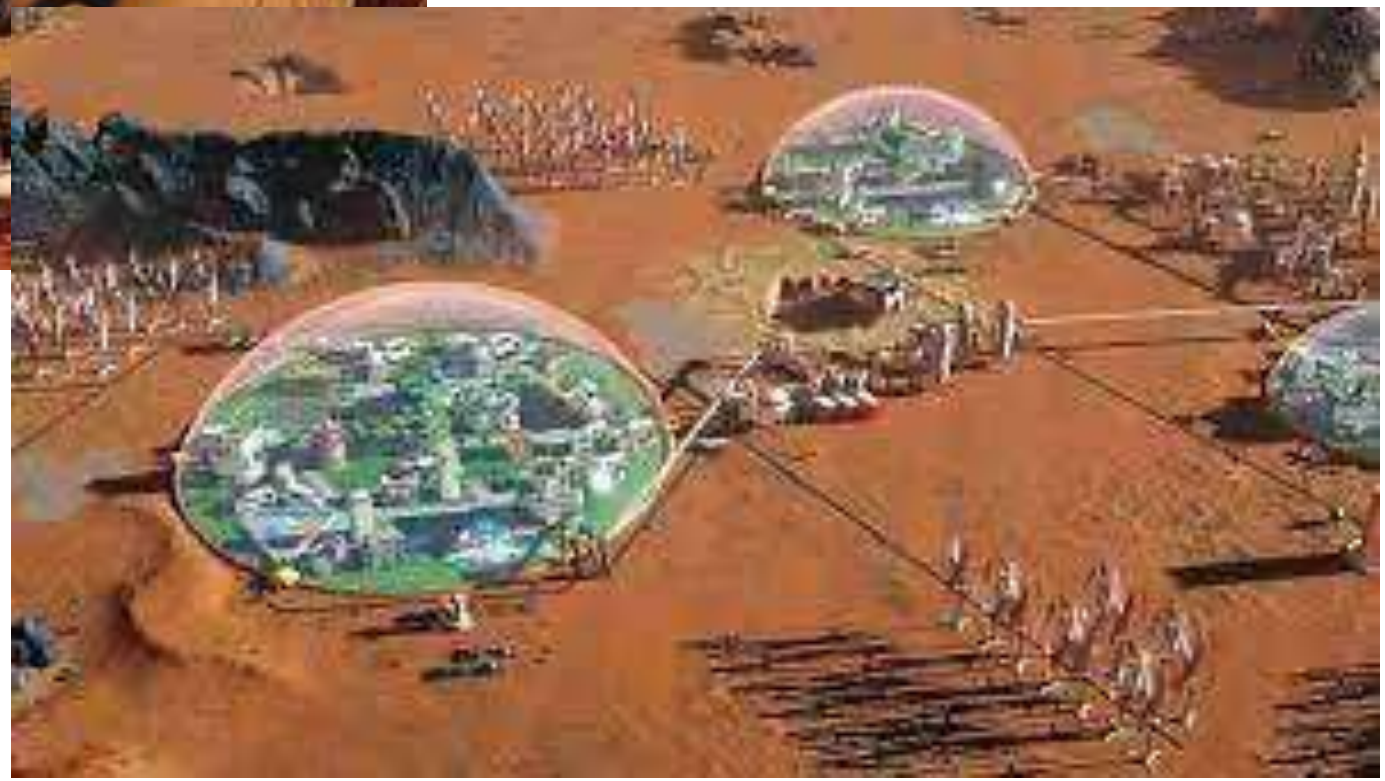
Latent Virüsler ve İmmünizasyon

Doç. Dr. Güle Çınar

Ankara Üniversitesi Tıp Fakültesi

İnfeksiyon Hastalıkları ve Klinik Mikrobiyoloji Anabilim Dalı


Ay Yürüyüşü
Neil Armstrong



frontiers in Microbiology

REVIEW
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Herpes Virus Reactivation in Astronauts During Spaceflight and Its Application on Earth

*Bridgette V. Rooney[1], Brian E. Crucian[2], Duane L. Pierson[2], Mark L. Laudenslager[3] and Satish K. Mehta[4]**

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Latent herpes virus reactivation has been demonstrated in astronauts during shuttle (10–16 days) and International Space Station (≥180 days) flights. Following reactivation, viruses are shed in the body fluids of astronauts. Typically, shedding of viral DNA is asymptomatic in astronauts regardless of mission duration; however, in some cases, live/infectious virus was recovered by tissue culture that was associated with atopic-dermatitis or skin lesions during and after spaceflight. Hypothalamic-pituitary-adrenal (HPA) and sympathetic-adrenal-medullary (SAM) axes activation during spaceflight occurs as indicated by increased levels of stress hormones including cortisol, dehydroepiandrosterone, epinephrine, and norepinephrine. These changes, along with a decreased cell mediated immunity, contribute to the reactivation of latent herpes viruses in astronauts. Currently, 47/89 (53%) astronauts from shuttle-flights and 14/23 (61%) astronauts from ISS missions shed one or more herpes viruses in saliva/urine samples. Astronauts shed Epstein–Barr virus (EBV), varicella-zoster virus (VZV), and herpes-simplex-1 (HSV-1) in saliva and cytomegalovirus (CMV) in urine. Larger quantities and increased frequencies for these viruses were found during spaceflight as compared to before or after flight samples and their matched healthy controls. The shedding did not abate during the longer ISS missions, but rather increased in frequency and amplitude. These findings coincided with the immune system dysregulation observed in astronauts from shuttle and ISS missions. VZV shedding increased from 41% in space shuttle to 65% in ISS missions, EBV increased 82 to 96%, and CMV increased 47 to 61%. In addition, VZV/CMV shed ≤30 days after ISS in contrast to shuttle where VZV/CMV shed up to 5 and 3 days after flight respectively. Continued shedding of infectious-virus post-flight may pose a potential risk for crew who may encounter newborn infants, sero-negative adults or any immunocompromised individuals on Earth. Therefore, developing spaceflight countermeasures to prevent viral reactivation is essential. Our spaceflight-developed technologies for saliva collection/rapid viral detection have been extended to include clinical applications including zoster patients, chicken pox, post-herpetic neuralgia, multiple sclerosis, and various neurological disorders. These protocols are employed in various clinics and hospitals including the CDC and Columbia University in New York, as well as overseas in Switzerland and Israel.

Keywords: herpes, latency, viral reactivation, spaceflight, immunity

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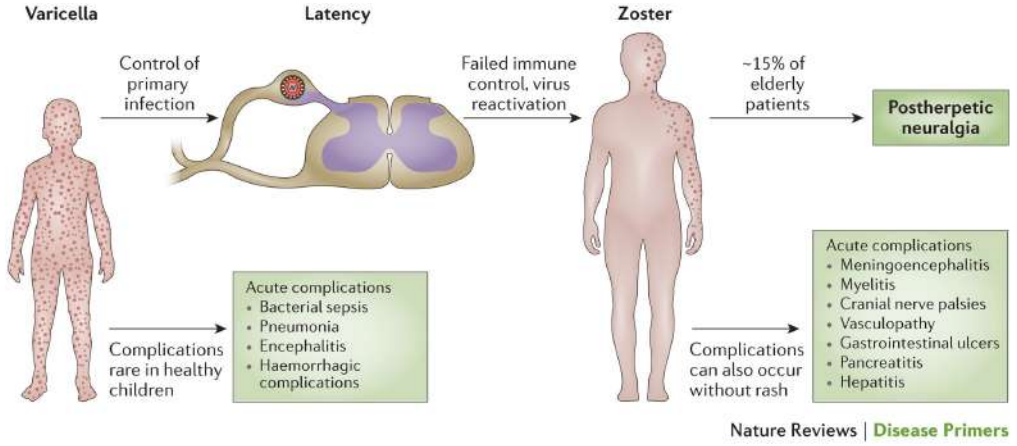
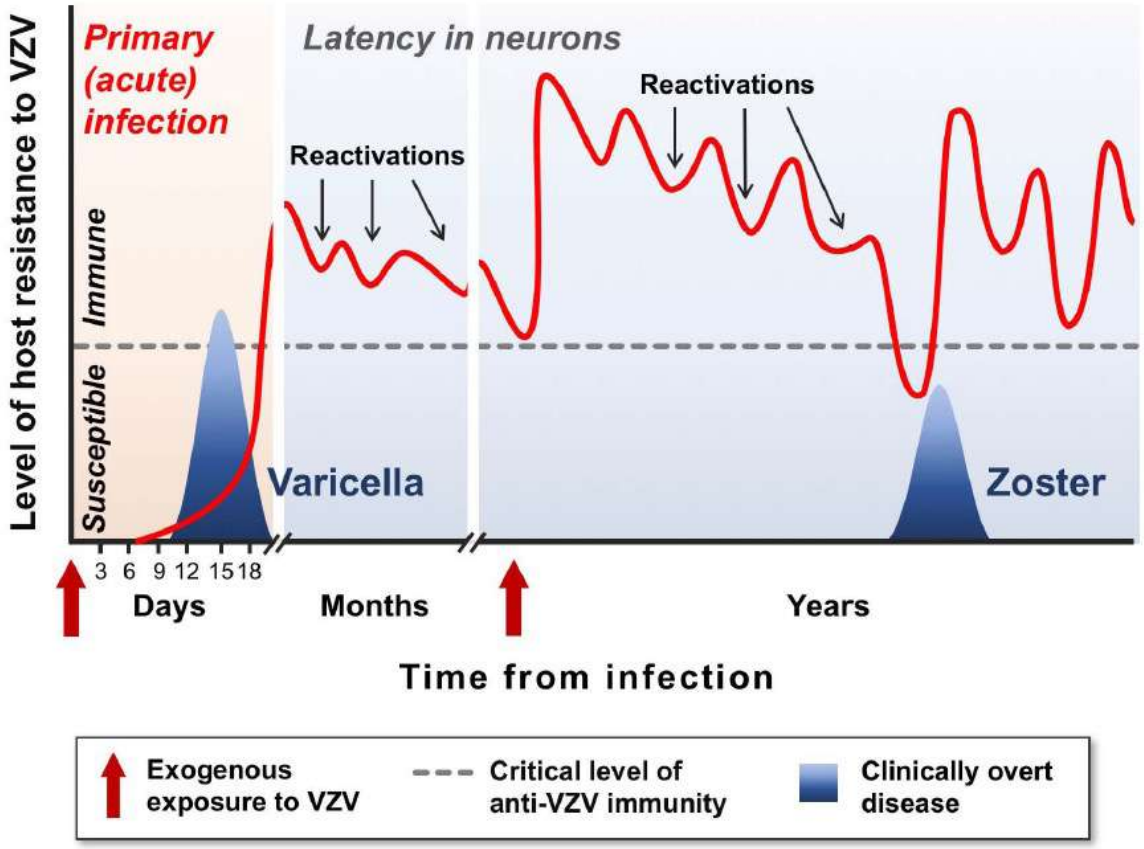
- VZV duyusal ve motor nöronlarda latent kalır.
- HZ insidansı 50-59 yaşlarında 6-8 vaka/1000 kişi-yıl
- >70 yaş 11 vaka/1000 kişi-yıl
- HZ'nin şiddeti ve komplikasyonları yaşla birlikte artar.
- T hücre aracılı bağışıklık reaktivasyonu önlemede önemli
- Yaşa bağlı olarak HB’da düşüş
- Aşılar VZV'ye özgü bellek T hücresi düşüşlerini önlüyor.



Hope-Simpson Hipotezi



The Journal of Infectious Diseases
VACCINE TRIUMPHS SUPPLEMENT

Herpes Zoster Vaccines

Ruth Harbecke,[1,2] Jeffrey I. Cohen,[3] and Michael N. Oxman[1,2,4]
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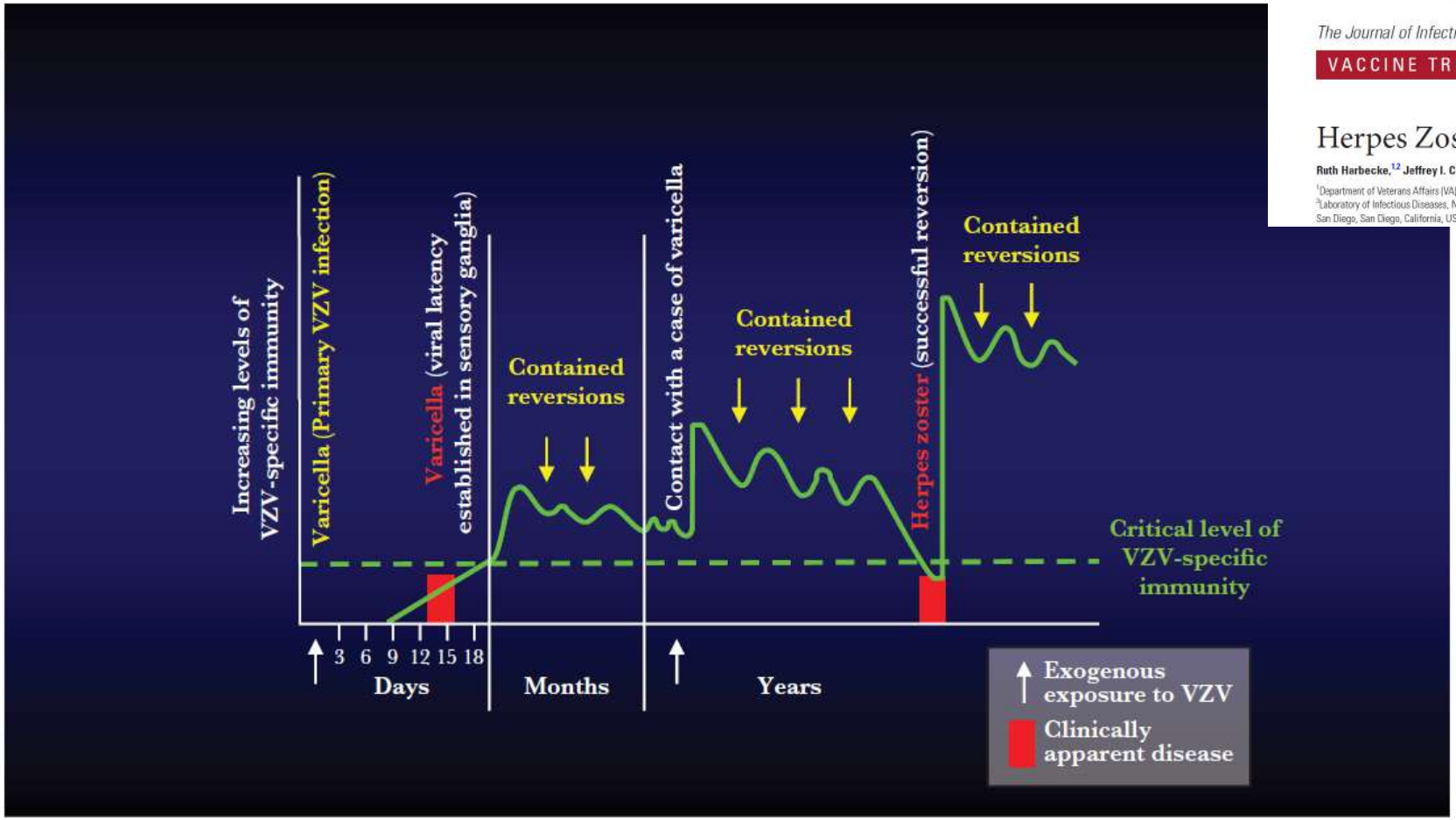
Herpes Zoster'a Karşı Aşı Olmanın Zorluğu

Suçiçeği aşısı da dahil olmak üzere çoğu aşı, duyarlı kişilere patojen tarafından infeksiyon bulaşmadan önce uygulanır.

Bu aşılar, birincil infeksiyonu ve/veya hastalığı önleyen bağışıklığı indükler.

Buna karşılık, HZ'ye karşı aşılama, daha önce VZV ile infekte olmuş ve VZV'e karşı bir bağışıklığa sahip olan, ancak yeniden aktive olabilen ve HZ'ye neden olabilen latent VZV'yi barındıran kişilere yöneliktir.

Zoster aşısının etkili olabilmesi için, "terapötik bir aşı" işlevi görmesi ve önceden VZV'ye karşı bağışıklığı olan ve zaten infekte olmuş bir kişide latent VZV'nin yeniden aktivasyonunu önlemek için daha güçlü bir bağışıklık tepkisi oluşturması gerekir.



The Journal of Infectious Diseases
VACCINE TRIUMPHS SUPPLEMENT

Herpes Zoster Vaccines

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Anamnestik bir konakçı bağışıklık tepkisi genellikle VZV replikasyonunu HZ gelişimini önlemek için zamanında durdurabilir.

Yine de meydana gelen VZV replikasyonu, konağın VZV'ye özgü bağışıklık tepkilerini artırmak için yeterlidir.

Bunlar suçiçeğine karşı bağışıklığı korur ve hücresel immünitedeki yaşa bağlı düşüşü geciktirerek yaşa özel HZ insidansını azaltır.

Su çiçeği veya HZ'li kişilerle temasın bir sonucu olarak VZV'ye yeniden maruz kalma sırasında VZV'ye karşı bağışıklıkta benzer artışlar gözlenir.

HZ VZV'ye karşı hücresel immünite azaldığında ortaya çıkar.

ZVL, Zostavax, Merck, 2006 yılında ≥50 yaş yetişkinler için FDA tarafından ruhsatlandırılmış ve ACIP tarafından 60 yaş ve üstü kişiler için önerilen canlı zayıflatılmış bir aşıdır.
Zostavax, 18 Kasım 2020'den beri Amerika Birleşik Devletleri'nde kullanılmamaktadır.

2018'de ACIP tarafından ≥50 yaş yetişkinler için tercih edilmesini tavsiye etmiştir.

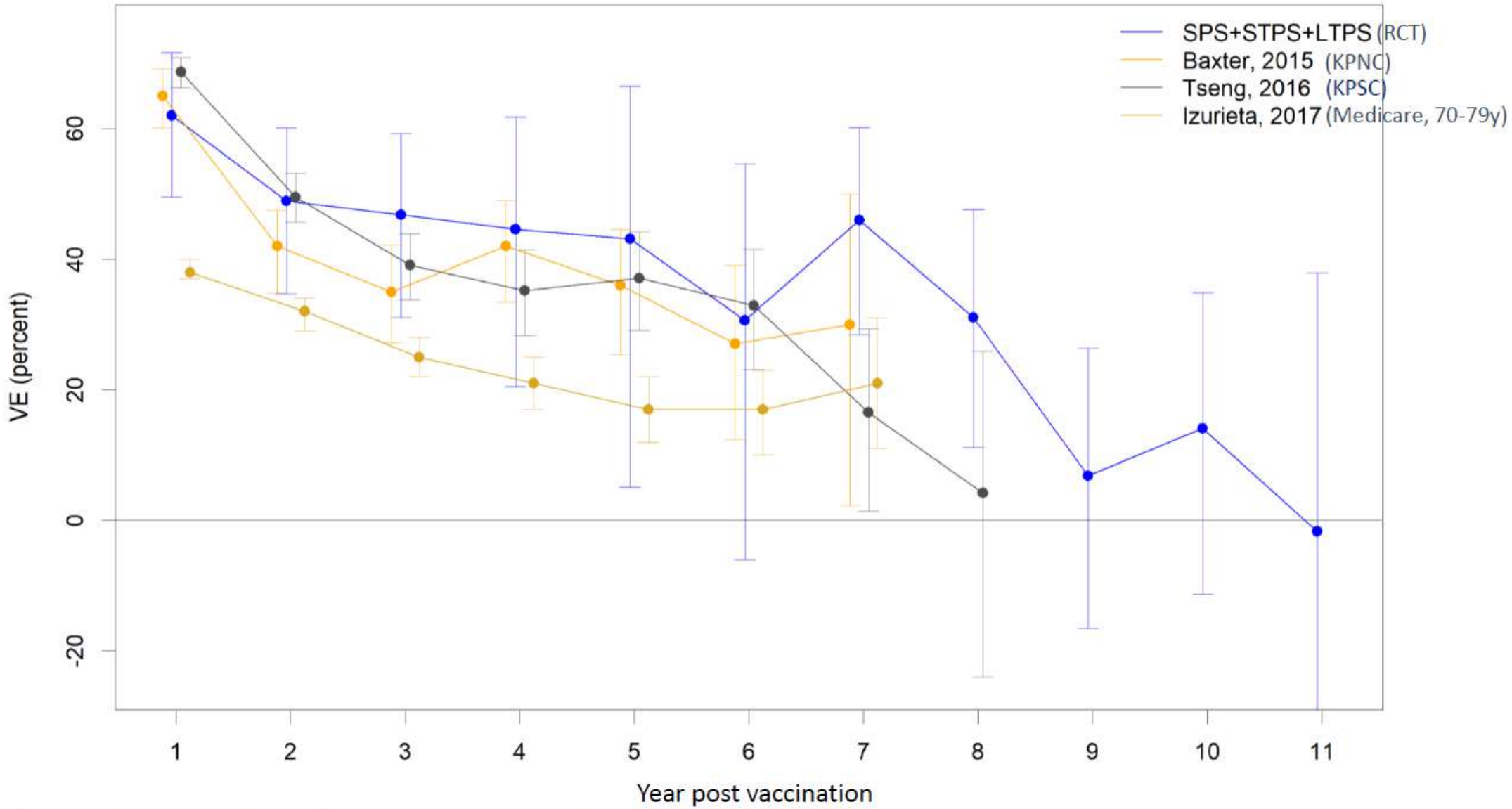
		cine; GlaxoSmithKline)
		cture when stored at +2°C
		upper arm, 2 to 6 months apart, 0.5 mL/dose
Reactogenicity	Low	High
Overall efficacy against incidence of HZ	51.3%	97.2%
Overall efficacy against	66.5%	91.2%
Pe		tudied up to 10 years)
FD		2017 for adults aged ≥50 yoa 1 for adults ≥18 yoa who are or will be at increased due to immunodeficiency or immunosuppression y known disease or therapy
ACIP recommendations	For use in immunocompetent adults aged ≥50 years	(1) For use in immunocompetent adults aged ≥50 yoa; (2) For use in immunocompetent adults aged ≥50 yoa who previously received ZOSTAVAX; (3) Preferred over ZOSTAVAX. Should wait at least 8 weeks if previously administered ZOSTAVAX.

Abbreviations: ACIP, Advisory Committee on Immunization Practices; FDA, US Food and Drug Administration; gE, glycoprotein E; HZ, herpes zoster; IM, intramuscular; PFU, plaque-forming units; PHN, postherpetic neuralgia; SQ, subcutaneous; VZV, varicella-zoster virus; yoa, years of age.

Canlı Zayıflatılmış Zoster Aşısı (ZVL) (Oka/Merck)

- Canlı Zayıflatılmış vOka
- Hemen hemen tüm yetişkinlerin suçiçeğine karşı bağışıklığı olduğundan, zoster aşısındaki vOka miktarı suçiçeği aşısındaki miktarın 14 katından fazlasına çıkarılmıştır.
- SPS, 60 yaş ve üzeri 38 546 yetişkinin dünya çapında 22 çalışma sahasında tek doz subkutan yüksek potensli canlı zayıflatılmış VZV aşısı (ZVL) veya plasebo almak üzere randomize edildiği çift kör, plasebo kontrollü bir çalışma
- Canlı zoster aşısı, "HZ'ye bağlı hastalık yükünü" (birincil çalışma bitiş noktası, zaman içinde zoster ağrısının şiddeti değerlendirilerek belirlenen klinik olarak ilgili bir hastalık şiddeti ölçüsü) %61,1 azaltmış.
- ‘Klinik olarak anlamlı PHN insidansını’ (HZ'ye bağlı ağrı ve rahatsızlık, 0-10 ölçeğinde ≥3 olarak puanlanan, döküntü başlangıcından sonra >90 gün devam eden) %66,5 azaltmış.
- “HZ insidansını” %51,3 oranında azaltmış.
- HZ insidansı için yaşa özel ZVL etkinliği 60-69 yaşlarında %64 iken, ≥70 yaşlarında sadece %37.6 idi.

ZVL'nin HZ'e karşı koruyuculuk süresi



Note: The Shingles Prevention Study, Short-term Persistence Study, and Long-term Persistence Study followed the same study population in a randomized control trial over time. Baxter (2015), Tseng (2016), and Izurieta (2017) are observational studies. Studies were done in different time periods and among different study populations that had different age structures.

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Current Opinion in Immunology

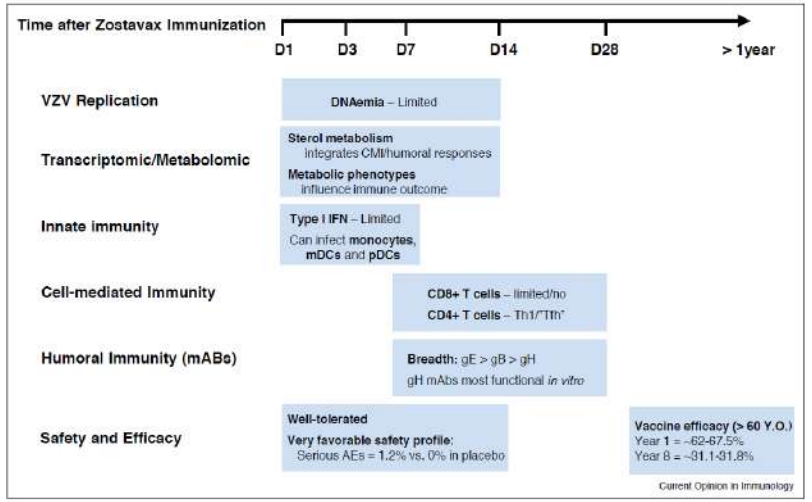
Understanding the immunology of the Zostavax shingles vaccine

Nicole L Sullivan[1], Christiane S Eberhardt[2,3], Andreas Wieland[2], Kalpit A Vora[1], Bali Pulendran[4] and Rafi Ahmed[2]

- 2017'de, Zostavax aşılamasından sonra transkriptomik, metabolomik, hücre frekansları ve immün yanıtın bütünleyici bir analizi yapıldı.
- Zostavax aşılaması, MX1 ve IFI44L gibi interferon kaynaklı antiviral genlerin ve TNFRSF17 ve MZB1 gibi antikor üretimine dahil olan genlerin up-regülasyonu ile sonuçlandı.
- Zostavax'ın doğal immünitenin ve dendritik hücre aktivasyonunun zayıf bir uyarıcısı olduğu gösterildi.
- Bu gözlemlere paralel olarak Zostavax/VZV, CD80 ekspresyonu ve TNF ve IL-6 salgılanması ile ölçüldüğü üzere, bu hücrelerin önemli ölçüde aktivasyonu olmadan in vitro olarak monositleri, monosit türevli DC'leri (mDC'ler) ve plazmasitoid DC'leri (pDC'ler) etkili bir şekilde infekte edebildi.
- pDC'ler, TLR9 agonist uyarımından (CpG-A) sonra IFN-α üretti, ancak Zostavax/VZV ile infeksiyondan sonra üretmedi.
- Bununla birlikte, infekte hücrelerin önemli bir kısmında CXCL8 (IL-8), CCL2 (MCP-1), CXCR9 (MIG) ve CXCL10 (IP-10) gibi kemokinler tespit edildi.
- Zostavax/VZV monositleri ve DC'leri in vitro olarak infekte edebilmesine rağmen, bu hücrelerin önemli doğuştan gelen immün aktivasyonunu indüklemez.

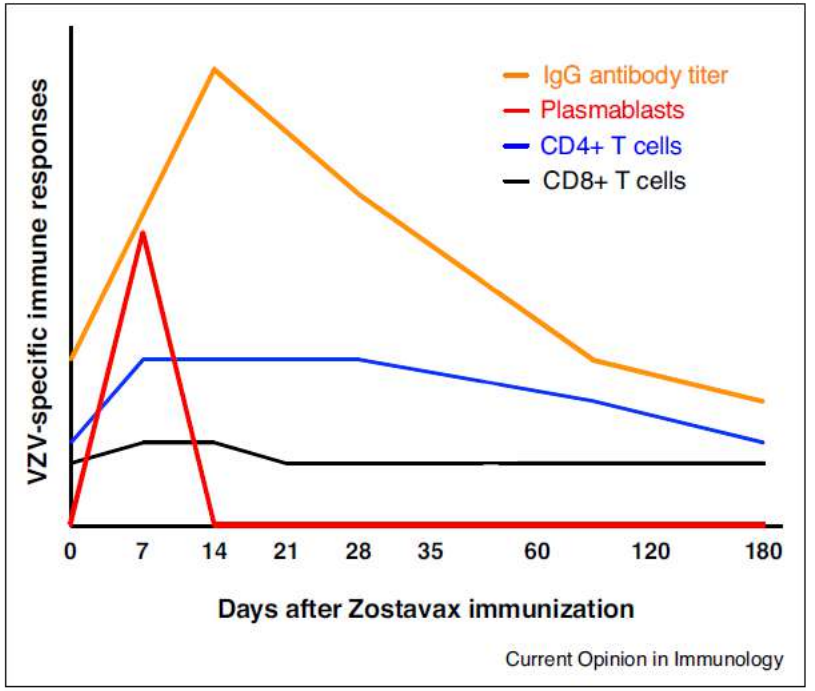
- Zostavax aşılaması, aşılamadan sonraki yedinci günde zirve yapan bir plazmablast yanıtını (IgG ve IgA) indükler.
- VZV total glikoproteine özgü IgG ve IgA titreleri hem genç hem de yaşlı erişkinlerde önemli ölçüde artar, bağışıklamadan 2-4 hafta sonra zirveye ulaşır ve başlangıca yakın seviyeye düşer.
- Bununla birlikte, VZV'ye özgü IgG titrelerindeki artış, genç erişkinlerde önemli ölçüde daha fazlaydı.
- Genel olarak, VZV'ye özgü IgG titrelerindeki kat artışı ile başlangıç seviyeleri arasında negatif bir korelasyon vardı.
- Bu veriler, VZV'ye karşı önceden var olan yüksek antikor titrelerinin, Zostavax aşısının, muhtemelen viral replikasyonu sınırlandırarak, bağışıklama sonrası daha yüksek seviyelerde antikor indükleme yeteneğini sınırlayabileceğini düşündürmektedir.
- Doğal VZV infeksiyonunu takiben, glikoprotein E'ye yönelik antikor titreleri en yüksektir, ardından glikoprotein B ve ardından glikoprotein H gelir.
- Zostavax ile aşılamadan sonra, gE > gB > gH antikor titrelerinin bu hiyerarşisinin, aşılamadan yedi gün sonra plazmablastlardan monoklonal antikorlar üreterek tek hücre seviyesinde de gözlemlendiğini gösterilmiştir.
- Gözlemlenen plazmablast yanıtının, çok sayıda somatik hipermutasyon nedeniyle VZV'ye özgü bellek B hücre yanıtından kaynaklandığını varsayılmaktadır.

Figure 1



Immune response, safety profile and durability after Zostavax immunization.
Schematic showing the time course of the peak responses in DNAemia, metabolites, innate immunity, cell-mediated immunity, humoral immunity (data obtained from monoclonal antibodies isolated from human subjects that received Zostavax) after immunization with Zostavax. Additionally, overall safety and durability after immunization are listed. 'Tfh' = Tfh-like cells measured in the peripheral blood express CXCR5, CXCR3 and ICOS.

Figure 2



Kinetics of adaptive immune responses to Zostavax immunization. Schematic illustrating the kinetics of VZV-specific CD4+ T cells (blue), CD8+ T cells (black), plasmablasts (red) and anti-VZV IgG antibody titers (orange) after immunization with Zostavax.

- Yaşları 50-59 arasında değişen 22 439 kişide yapılan randomize, plasebo kontrollü ZOSTAVAX Etkinlik ve Güvenlik Çalışması (ZEST), HZ insidansı için ZVL etkinliğinin ortalama 1,3 yıllık bir takip süresinde %69,8 olduğunu göstermiştir.

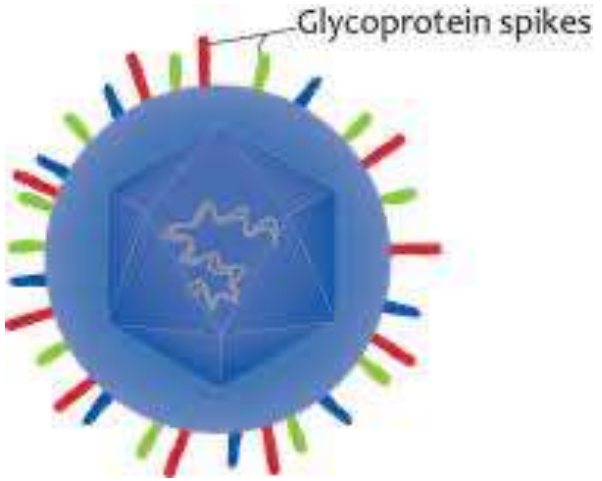
- Aşı etkinliği HZ hastalık yükü ve PHN insidansı için 10. yılda ve HZ insidansı için 8. yılda devam etmiştir.

- Zoster aşısının HZ hastalık yükü için etkinliği %61,1'den %37,3’e

  PHN insidansı için etkinliği %66,5'ten %35,4’e

  HZ insidansı için etkinliği %51,3'ten %21,1'e düştü.

Rekombinant Subunit Herpes Zoster Aşısı (RZV)



- Rekombinant VZV gE ve $AS0_1B$ adjuvan sistemi içeren protein bir subunit aşı (RZV, HZ/su), Glaxo Smith Kline Vaccines (Wavre, Belçika) tarafından geliştirilmiştir.

- gE, VZV viryonlarında ve infekte olmuş hücrelerde en fazla bulunan glikoproteindir; virus replikasyonu ve hücreden hücreye yayılma için esastır ve VZV'ye özgü $CD4^{+2}$ T-hücre yanıtları için ana hedeftir.
- Hem nötralize edici antikor hem de CD4 T-hücre yanıtlarını indükler.

Lipozom bazlı $AS01_B$ adjuvan sistemi 2 immüno-uyarıcı içerir:

- NF-κB transkripsiyonunu ve sitokin üretimini uyaran ve antijen sunan hücreleri aktive eden bir TLR4 agonisti olan monofosforil lipid A

- Antijene özgü antikoru ve $CD4^{+2}$ T-hücre yanıtlarını destekleyen doğal bir saponin olan QS-21

- RZV, 100 µg $AS01_B$ (50 µg MPL ve 50 µg QS-21) içinde karıştırılmış 50 µg gE içerir.

- IM, Deltoid, 2 doz


The Journal of Infectious Diseases

MAJOR ARTICLE

IDSA Infectious Diseases Society of America hivma hiv medicine association

Immune Responses to a Recombinant Glycoprotein E Herpes Zoster Vaccine in Adults Aged 50 Years or Older

Anthony L. Cunningham,[1,a] Thomas C. Heineman,[2,4,5] Himal Lal,[2,c] Olivier Godeaux,[3,d] Roman Chlibek,[6] Shinn-Jang Hwang,[5] Janet E. McElhaney,[8] Timo Vesikari,[7] Charles Andrews,[8] Won Suk Choi,[9] Meral Esen,[10] Hideyuki Ikematsu,[11] Martina Kovac Chorna,[12] Karlis Pauksens,[13] Stéphanie Ravault,[14] Bruno Salaun,[14] Tino F. Schwarz,[15] Jan Smetana,[4] Carline Vanden Abeele,[3] Peter Van den Steen,[3] Ilse Vastiau,[3] Lily Yin Weckx,[16] and Myron J. Levin[17]; for the ZOE-50/70 Study Group

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Current Opinion in Immunology

Understanding the immunology of Shingrix, a recombinant glycoprotein E adjuvanted herpes zoster vaccine

Thomas C Heineman[1], Anthony Cunningham[2] and Myron Levin[3]

- Aşı formülasyonunda gE, Çin hamsterı over hücrelerinde üretilen membran ankoru ve karboksi terminal alanlarından silinmiş bir rekombinant proteindir (Haumont et al, 1996).
- $AS01_B$, antijen sunan dendritik hücrelerin toplanmasına ve aktivasyonuna yol açan doğuştan gelen yanıtın yerel ve geçici bir aktivasyonunu uyarır.
- $AS01_B$ adjuvan sistemi ile birleştirilen gE'nin, gE'ye özgü en güçlü CD4+ T hücre yanıtlarının yanı sıra güçlü gE'ye özgü antikor yanıtlarını verdiğini gösterilmiştir (Hücre aracılı yanıtları indüklemedeki etkinliğini artırmak için).

- QS-21, geçici lokal sitokin tepkilerini ve kastaki dendritik hücrelerin ve makrofajların aktivasyonunu ve hayvan modellerinde drene olan lenf düğümlerini indükleyen bir adjuvandır. Doğuştan gelen bağışıklık hücrelerinde, drene olan lenf düğümlerinin periferik makrofajlarında inflamasyonu uyarır.

- Bu, NK hücrelerini ve CD8+ T hücrelerini IFNγ salması için uyarır, bu da sırayla gE'yi CD4+ T hücrelerine almak ve sunmak için kan monosit türevi ve yerleşik lenf düğümü dendritik hücrelerinin aktivasyonu ve toplanmasını uyarır.

- Ayrıca, NK hücrelerine ve CD8+ T hücrelerine ek olarak $AS01_B$, CD4+ T hücrelerinden IFNγ salınımını uyarır.

- IFNγ genellikle viral replikasyonu inhibe eder ve ayrıca T hücre yanıtlarını ve antikor izotip değişimini geliştirir.

- Toll benzeri reseptör tip 4 agonisti MPL, interferon-gama (IFN-γ) üretimi yoluyla birlikte uygulanan antijene karşı bağışıklık tepkisini arttırmak için QS-21 ile sinerji oluşturur. QS-21, antijenin dendritik hücreler tarafından emilimini ve tutulmasını arttırır (Coffman et al, 2010; Lal et al, 2013).

Clinical Infectious Diseases
MAJOR ARTICLE

The Adjuvanted Recombinant Zoster Vaccine Confers Long-Term Protection Against Herpes Zoster: Interim Results of an Extension Study of the Pivotal Phase 3 Clinical Trials ZOE-50 and ZOE-70

Céline Boutry,[1,a] Andrew Hastie,[2] Javier Diez-Domingo,[3] Juan Carlos Tinoco,[4] Chong-Jen Yu,[5] Charles Andrews,[6] Jean Beytout,[7] Covadonga Caso,[8] Huey-Shinn Cheng,[9] Hee Jin Cheong,[10] Eun Ju Choo,[11] Dan Curiac,[12] Emmanuel Di Paolo,[13] Marc Dionne,[14] Tamara Eckermann,[15] Meral Esen,[16] Murdo Ferguson,[17] Wayne Ghesquiere,[18] Shinn-Jang Hwang,[19,20] Thiago Junqueira Avelino-Silva,[21] Pavel Kosina,[22] Chiu-Shong Liu,[23] Jukka Markkula,[24] Beate Moeckesch,[25] Cláudia Murta de Oliveira,[26] Dae Won Park,[27] Karlis Pauksens,[28] Paola Pirrotta,[29] Georg Plassmann,[30] Carol Pretswell,[31] Lars Rombo,[32] Bruno Salaun,[33] Johan Sanmartin Berglund,[33] Isabelle Schenkenberger,[34] Tino Schwarz,[35] Meng Shi,[2] Benita Ukkonen,[36] Toufik Zahaf,[29] Cristiano Zerbini,[37] Anne Schuind,[2,b] and Anthony L. Cunningham[38,39], on behalf of the Zoster-049 Study Group*

- ≥50 yaş (ZOE-50) ve ≥70 yaş(ZOE-70) yetişkinlerde HZ ve PHN'ye karşı güvenliliğini ve etkililiğini belirlemek için Kuzey Amerika, Avrupa, Asya, Avustralya ve Latin Amerika'daki 18 ülkede eşzamanlı olarak iki randomize, plasebo kontrollü faz III etkinlik çalışması, 6 yıl

- Aşılamadan sonra Y6'dan itibaren HZ'a karşı etkinlik ve Y5'ten sonra anti-gE antikor konsantrasyonları ve gE'ye özgü $CD4^{+2}$ T-hücresi (değerlendirilen 4 aktivasyon belirtecinden IFNy, IL2, TNF α, CD40 ligandı ≥2'sini eksprese eden) frekansları için yıllık değerlendirmeler

- HZ insidansı için rekombinant zoster aşı etkinliği yetişkinlerde ≥50 yaşında %97.2 ve ≥70 yaşında erişkinlerde %89.8

- Hem ZOE-50 hem de ZOE-70'te, HZ insidansı için RZV etkinliği artan yaşla birlikte önemli ölçüde azalmadı ve ortalama 3,7 yıllık takipten sonra yüksek kaldı.

- Anti-gE antikor geometrik ortalama konsantrasyonları ve gE'ye özgü $CD4^{+2}$ T-hücresi medyan frekansları, aşılama öncesi seviyelerin yaklaşık 6 katı üzerinde bir platoya ulaştı.

- Yaşlı erişkinlerde RZV'nin klinik yararı aşılamadan sonra en az 7 yıl devam eder.

Open Forum Infectious Diseases

BRIEF REPORT

- Rekombinant zoster aşısı (RZV) ile aşılamadan yaklaşık 10 yıl sonra, ZOE-50/70 takip çalışmasının ara analizi, herpes zoster'a karşı etkinliğin yüksek kaldığını gösterdi.

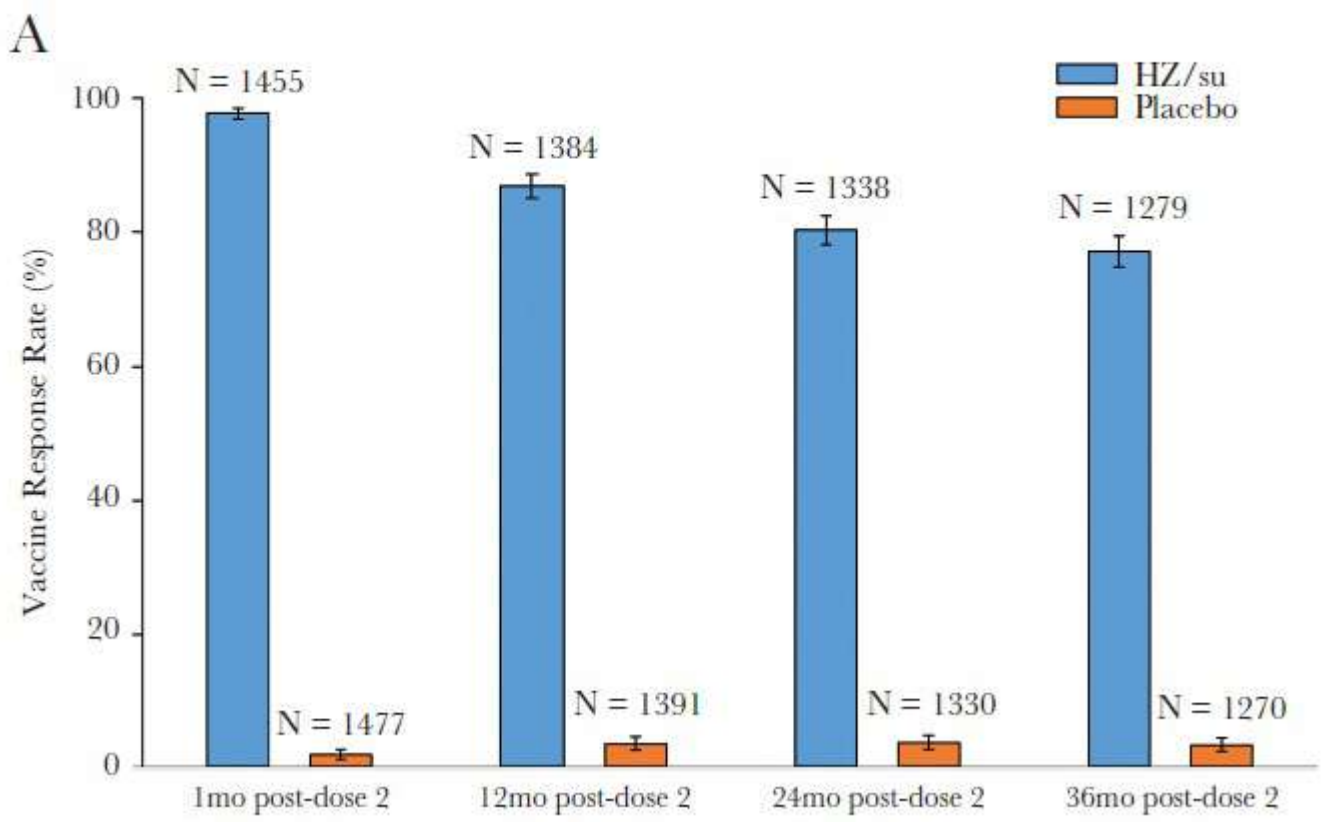
- Ayrıca, güvenlilik profili klinik olarak kabul edilebilir kalmıştır ve bu da RZV'nin ≥50 yaşındaki hastalardaki klinik yararının 10 yıla kadar sürdüğünü düşündürmektedir.

Long-term Protection Against Herpes Zoster by the Adjuvanted Recombinant Zoster Vaccine: Interim Efficacy, Immunogenicity, and Safety Results up to 10 Years After Initial Vaccination

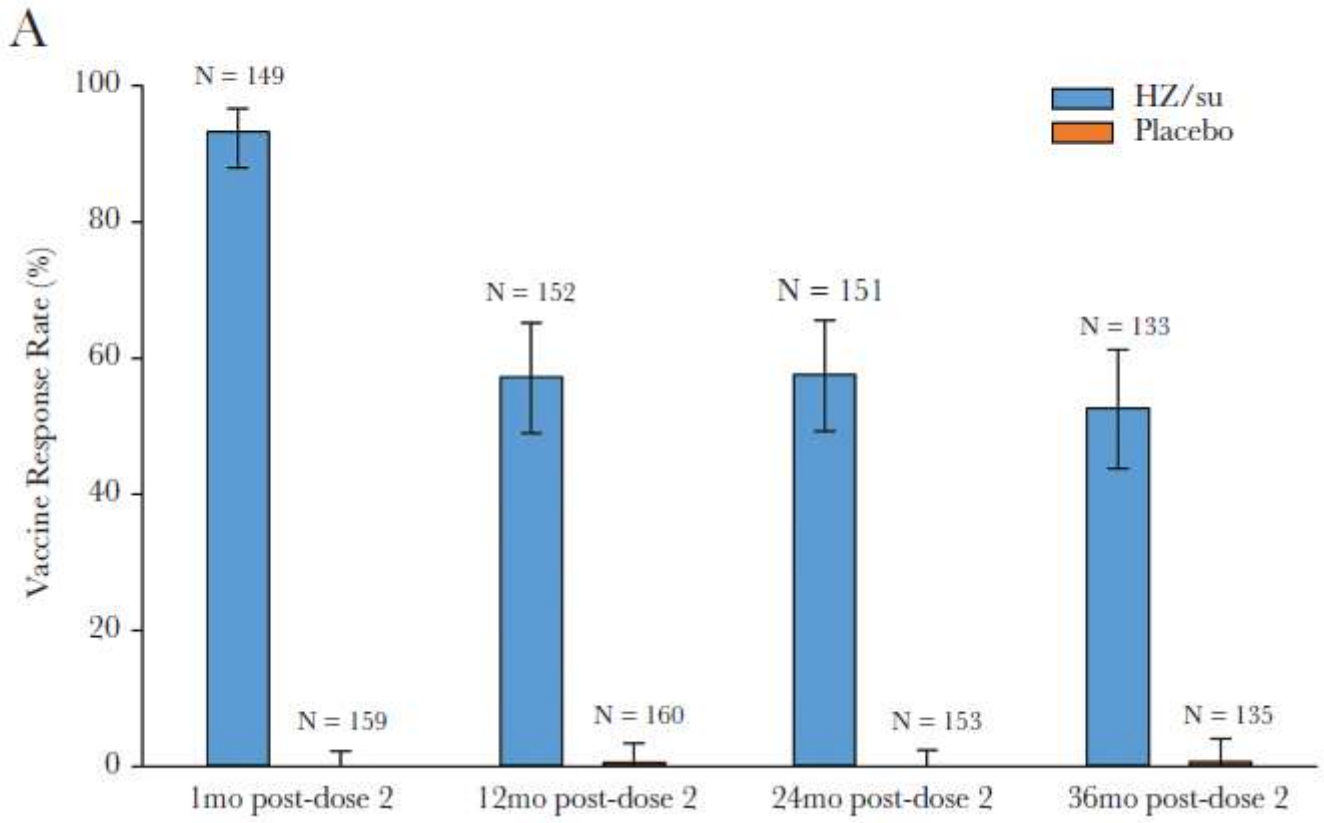
Ana Strezova,[1] Javier Diez-Domingo,[2] Kamal Al Shawafi,[3] Juan Carlos Tinoco,[4] Meng Shi,[5] Paola Pirrotta,[6] and Agnes Mwakingwe-Omari[5] on behalf of the Zoster-049 Study Group[a]

[1]GSK, Rixensart, Belgium, [2]FISABIO Fundación para el Fomento Investigación Sanitaria y Biomédica de la Comunitat Valenciana, Valencia, Spain, [3]Modis, Belgium c/o GSK, Wavre, Belgium, [4]Hospital General de Durango, Durango, Mexico, [5]GSK, Rockville, Maryland, USA, and [6]GSK, Wavre, Belgium

Humoral Bağışıklık Yanıtı / gpE Antikorları



Hücre Aracılı Bağışıklık Yanıtı/ gE'ye özgü $CD4^{+2}$ T-hücresi frekansları



The Journal of Infectious Diseases

SUPPLEMENT ARTICLE

Comparative Immune Responses to Licensed Herpes Zoster Vaccines

Adriana Weinberg,[1,2,3] Miranda E. Kroehl,[4] Michael J. Johnson,[1] Andrew Hammes,[4] Dominik Reinhold,[4] Nancy Lang,[1] and Myron J. Levin[1,2]

Departments of [1]Pediatrics, [2]Medicine, and [3]Pathology, School of Medicine, and [4]Department of Biostatistics and Informatics, School of Public Health, University of Colorado Denver, Anschutz Medical Campus, Aurora

JCI The Journal of Clinical Investigation

Tale of two vaccines: differences in response to herpes zoster vaccines

Anne A. Gershon

J Clin Invest. 2018;128(10):4245-4247. https://doi.org/10.1172/JCI123217.

- Bağışıklık yaşlanması, artan yaşla birlikte bağışıklık sisteminin kapasitesini bozar ve yaşlıları infeksiyona duyarlı hale getirir.

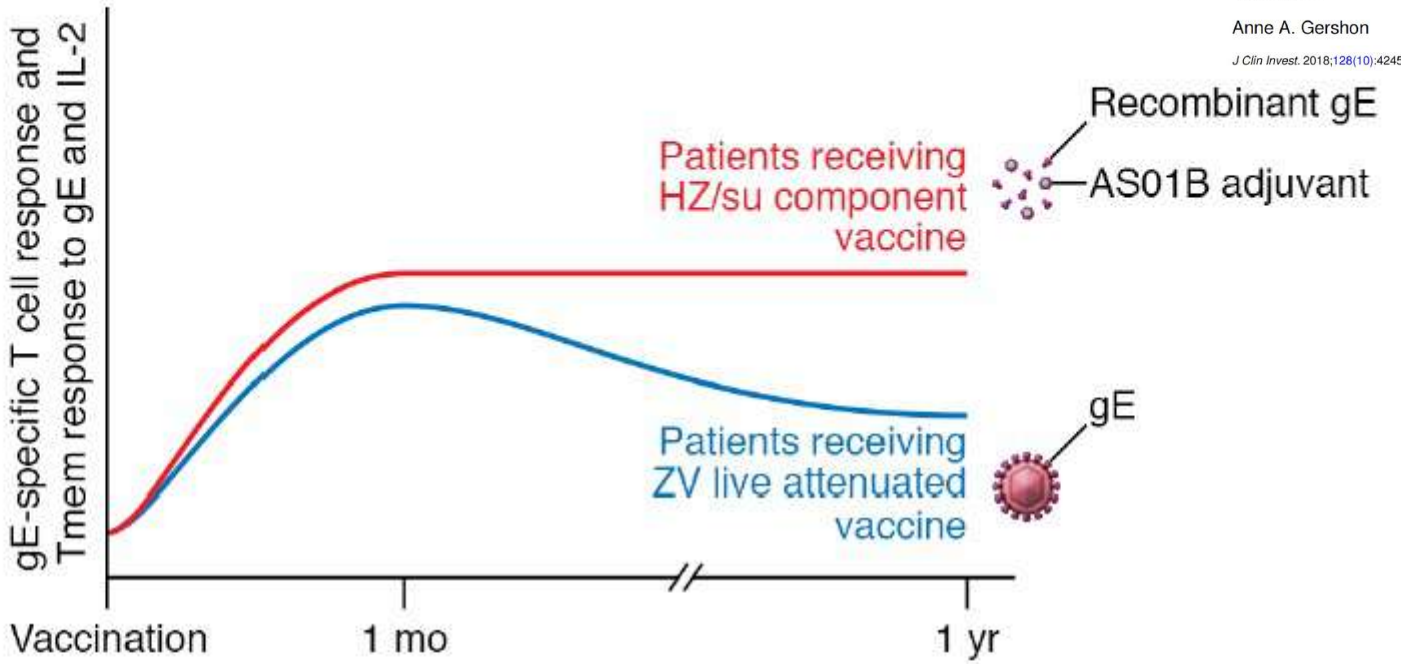
RZV etkililik tahminleri, tüm yaş gruplarında ZVL tahminlerinden önemli ölçüde daha yüksektir:

- 60-69 yaş: 97% - 64%
- 70-79 yaş: 91% - 41%
- >80 yaş: 91% - 18%

- Adjuvan teknolojisindeki gelişmeler, özellikle yaşlı insanlar tarafından kullanılması amaçlanan aşıların immünojenisitesini arttırmada yardımcı olmuştur.

- İçerdiği adjuvan nedeniyle yaşa bakılmaksızın uzun süreli ve kararlı bir yanıtın indüklenmesi, RZV'nin ZVL'ye göre açık bir avantajıdır.

JCI The Journal of Clinical Investigation
Tale of two vaccines: differences in response to herpes zoster vaccines
Anne A. Gershon
J Clin Invest. 2018;128(10):4245-4247. https://doi.org/10.1172/JCI123217.



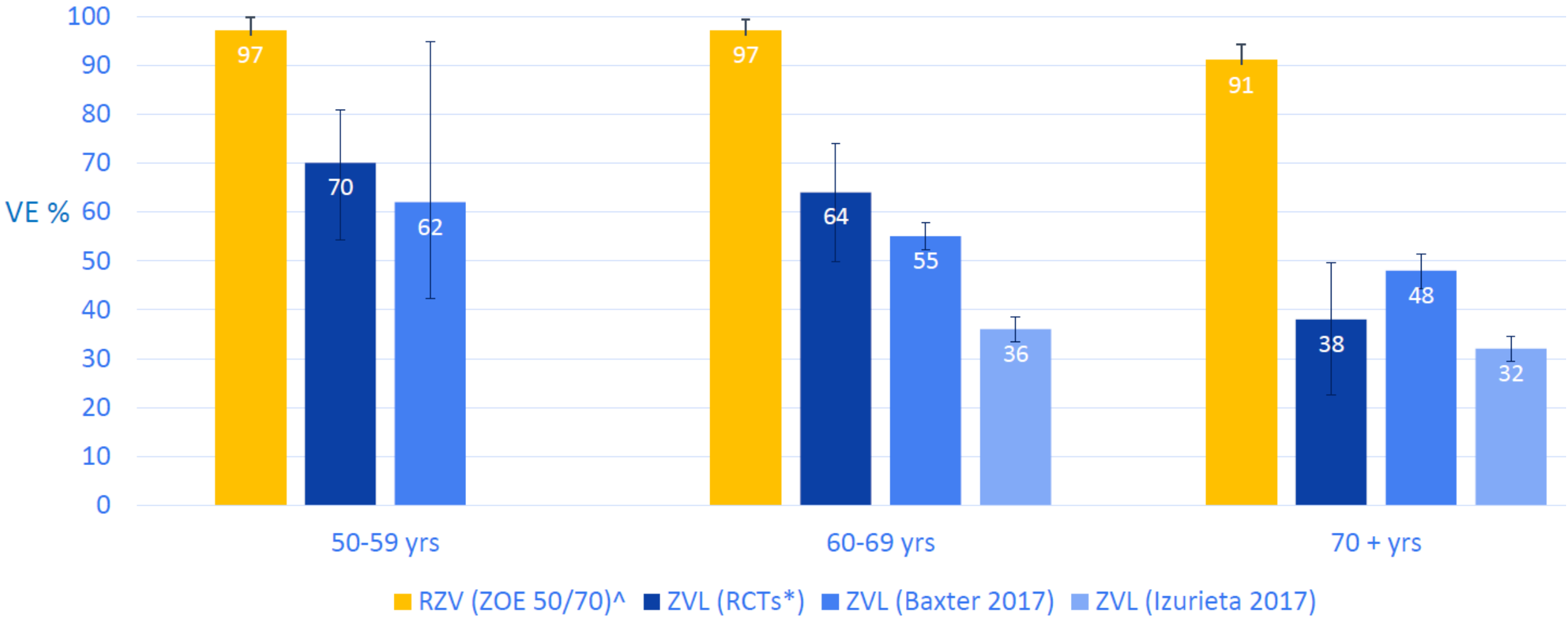
RZV ile aşılanan denekler, ZVL tarafından indüklenenlerden on kat daha yüksek olan ve aşılamadan 12 ay sonra ölçülebilen gE'ye özgü T hücre yanıtları geliştirmekte.

gE ve IL-2'ye bellek T hücresi yanıtları da RZV ile ZVL'den daha yüksek

Aşılamadan 1 ay sonra (ZVL için 1 doz ve RZV için 2 dozdan sonra) IL-2'ye karşı en yüksek bellek yanıtları gözlendi ve T helper yanıtlarının kalıcılığı için gerekli

Adjuvanlı RZV alıcılarında, T memory yanıtları, ZVL'nin etkisinden daha verimli bir şekilde devam etti.

HZ İlk 4 Yıl

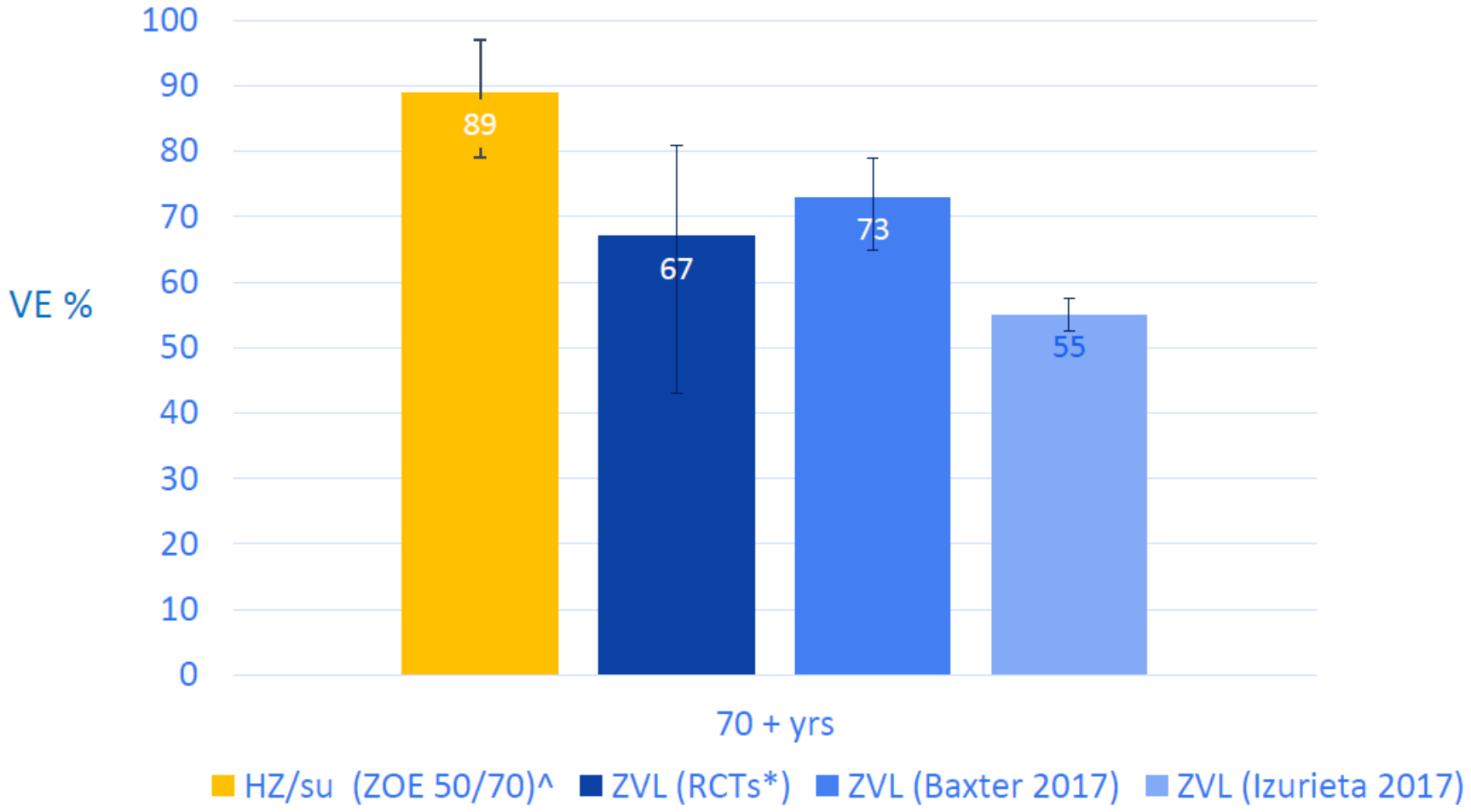


‡ Median follow up may be less than 3 yrs: Schmader 2012= 1.3 yrs

^ ZOE 50/70= 50-59 & 60-69yr: Lal 2015, 70+yrs: Cunningham 2016

* RCTs= 50-59 yrs: Schmader 2012, 60-69 and 70+ yrs: Oxman 2005,

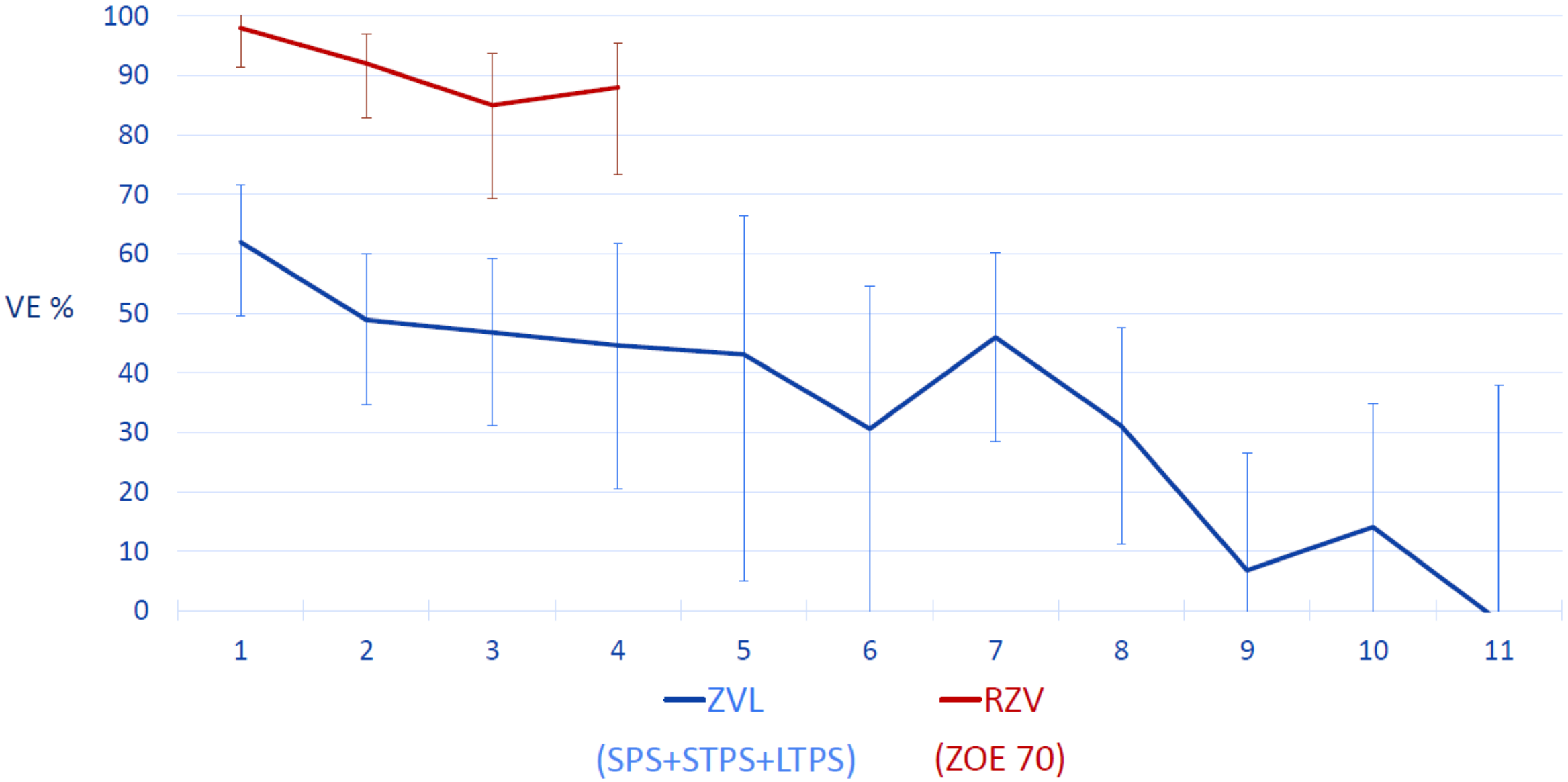
PHN İlk 4 Yıl



^ Pooled ZOE 50/70: Cunningham 2016

* Shingles Prevention Study: Oxman 2005,

Yıl bazında aşı etkinliği



Note: The Shingles Prevention Study, Short-term Persistence Study, and Long-term Persistence Study followed the same study population over time.

- Shingrix, daha önce Zostavax almış olanlar da dahil olmak üzere, 50 yaş ve üzerindeki bağışıklığı yeterli yetişkinlerde herpes zoster ve buna bağlı komplikasyonların önlenmesi için önerilir.

- ACIP, Ocak 2018'de 50 yaş ve üzerindeki bağışıklığı yeterli yetişkinler için zoster aşılama önerilerini yayınladı: www.cdc.gov/mmwr/volumes/67/wr/pdfs/mm6703a5-H.pdf.

- 20 Ekim 2021'de ACIP, hastalık veya tedavi nedeniyle bağışıklığı yetersiz veya bağışıklığı baskılanmış olan veya olacak olan 19 yaş ve üstü yetişkinlerde herpes zoster ve ilgili komplikasyonların önlenmesi için 2 doz RZV önerdi.

- ACIP, Ocak 2022'de bağışıklığı baskılanmış olan veya olacak olan 19 yaşında veya daha büyük yetişkinlerde rekombinant zoster aşısının kullanımına ilişkin tavsiyelerini yayınladı: www.cdc.gov/mmwr/volumes/71/wr/pdfs/mm7103a2-H.pdf.

- 2 ila 6 aylık aralık içinde iki doz Shingrix

- Shingrix'in ilk dozunun üzerinden 6 aydan fazla zaman geçtiyse, mümkün olduğunda ikinci dozu uygulanmalı, aşı serisini yeniden başlatılmamalı

Table 2. Other Zoster Vaccines and Zoster Vaccine Candidates

Company Name	Country	Vaccine Name	Characteristics	Status	Reference
SK Bioscience	South Korea	SKY Zoster (NBP608)	Live-attenuated Oka/SK strain	NCT03120364 Phase 3 (completed); approved in 2017 in Korea for adults ≥50 yoa	Choi WS, Choi JH, Jung DS, et al [255]
Curevo/GC Pharma/IDRI	USA/South Korea	CRV-101	gE subunit vaccine with proprietary adjuvant	NCT03820414 Phase 1 (completed)	https://curevovaccine.com/2020/09/curevo-vaccine-announces-robust-antibody-response-results-of-phase-i-clinical-trial-of-investigational-vaccine-for-shingles-crv-101/
EyeGene/ Novotech	South Korea/ Australia	EG-HZ	Adjuvanted recombinant VZV gE protein	NCT04210752 Phase 1 (completed)	http://eyegene.co.kr/eng/product_pipeline/EG_HZ/?lang = en_US
BCHT Biotechnology	China	Zoster Vaccine, Live	Live-attenuated Oka VZV vaccine	NCT04334577 Phase 3 (not yet recruiting)	https://ichgcp.net/clinical-trials-registry/NCT04334577
Vaccitech/ CanSino Biologics	United Kingdom/ Hong Kong	VTP-400 (CSB016)	Adenoviral vaccine (ChAdOx1) encoding VZV gE	Preclinical	www.vaccitech.co.uk/pipeline/
GeneOne Life Science	South Korea	GLS-5100	plasmid containing VZV-derived gene encoding a VZV protein; administered to the body using electroporation	Preclinical	www.genels.com/en/sub/technology/vaccine.asp
Akshaya Bio	Canada	Chimigen ShingVax	recombinant proteins, antigens fused to the Fc fragment of a murine monoclonal antibody through proprietary peptide linkers.	Preclinical	www.akshayabio.com/technology.html
Merck/Moderna	USA	VZV gE mRNA/LNP	mRNA expressing truncated VZV gE protein in lipid nanoparticles	Nonhuman primate studies	Monslow MA, Elbashir S, Sullivan NL, et al [260]
CPL Biologicals	India	VZV Vaccine	Based on nanoparticle technology from Novavax (will target varicella and HZ)	In development	http://cplbio.com/rd/rd-pipeline/

Abbreviations: gE, glycoprotein E; HZ, herpes zoster; IDRI, Infectious Disease Research Institute; LNP, lipid nanoparticle(s); mRNA, messenger ribonucleic acid; VZV, varicella-zoster virus; yoa, years of age.

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REVIEW
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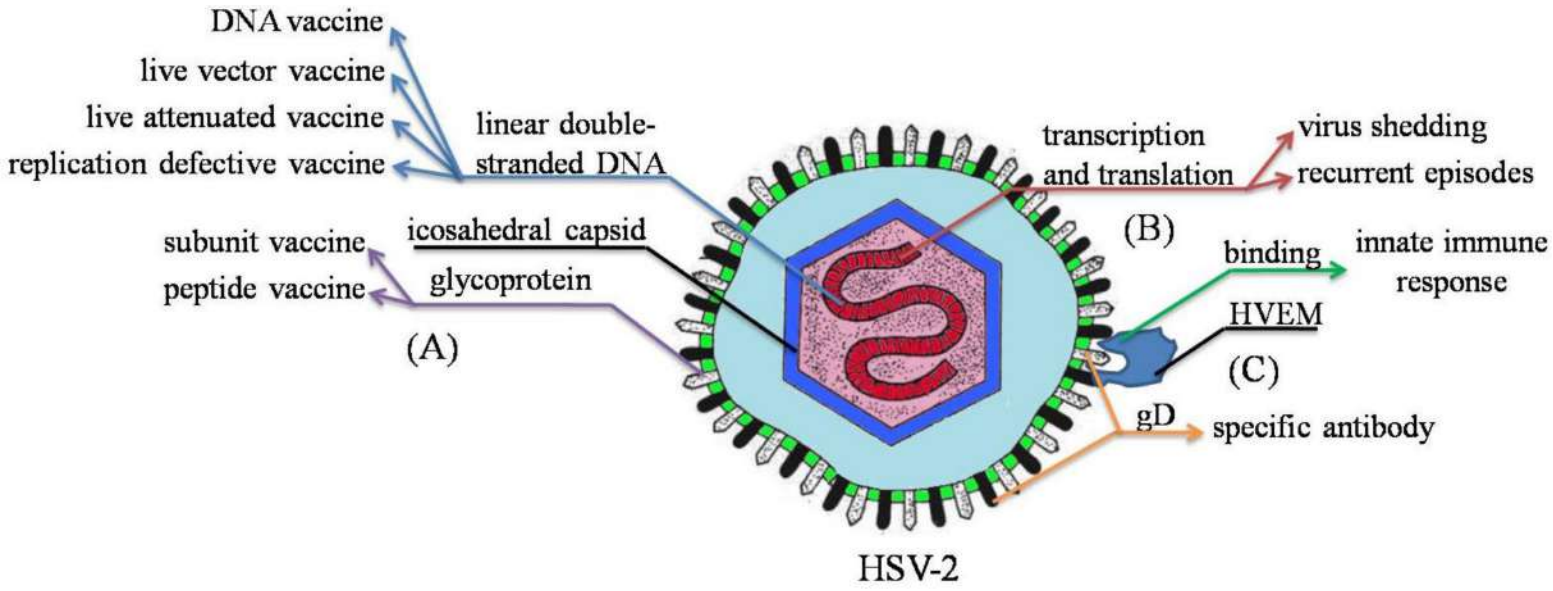
Combinatorial Herpes Simplex Vaccine Strategies: From Bedside to Bench and Back

Aziz A. Chentoufi[1], Nisha R. Dhanushkodi[1], Ruchi Srivastava[1], Swayam Prakash[1], Pierre-Gregoire A. Coulon[1], Latifa Zayou[1], Hawa Vahed[2], Hiba A. Chentoufi[3], Kathy K. Hormi-Carver[1] and Lbachir BenMohamed[1,3,4]*

Herpes simpleks virusu tip 1 (HSV-1) ve tip 2 (HSV-2) infeksiyonları ve hastalıklarıyla savaşmak için 4 ana aşı yaklaşımı tasarlandı ve test edildi.

- İnaktive edilmiş HSV aşıları
- Canlı zayıflatılmış HSV aşıları
- Replikasyon kusurlu HSV aşıları
- Subunit HSV aşıları

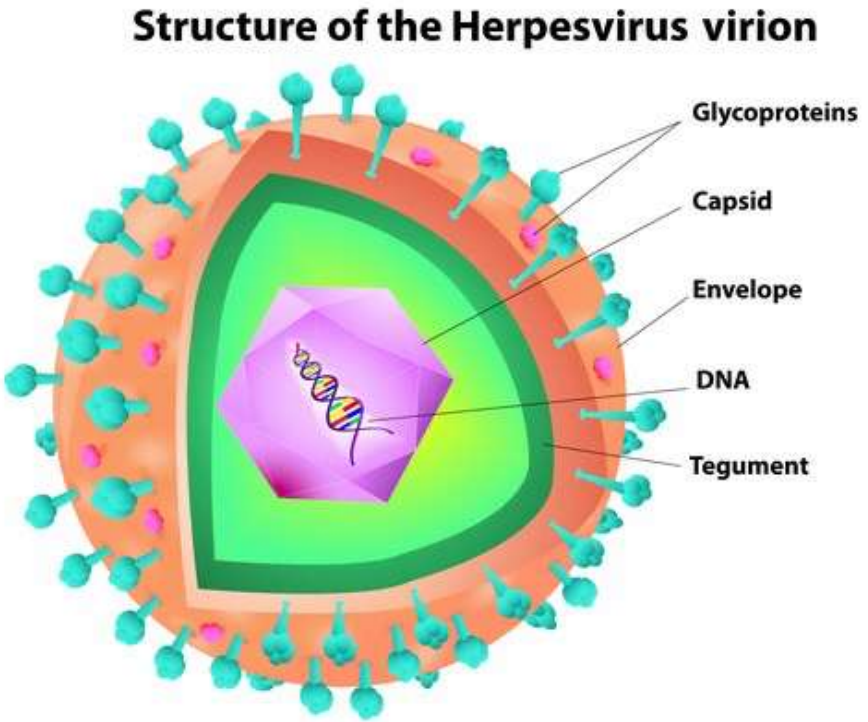


- Güvenlik, immünojenisite ve koruyucu etkinlik söz konusu olduğunda, bu tür aşı yaklaşımlarının her birinin artıları ve eksileri vardır.

İnaktif HSV Aşıları

- 70'ler ve 80'lerde, ilk tam inaktive HSV aşısı yaklaşımı, ısıya, UV ışığına veya kimyasallara maruz kaldıktan sonra tüm virusu "öldürmek" yaklaşımına dayanmakta

- Bu aşıların antikor yanıtını indükledi, ancak T hücrelerini etkilemedi ve bu nedenle, tekrarlayan HSV-1 veya HSV-2 infeksiyonlarına ve hastalıklarına karşı korumada başarılı olmadı.



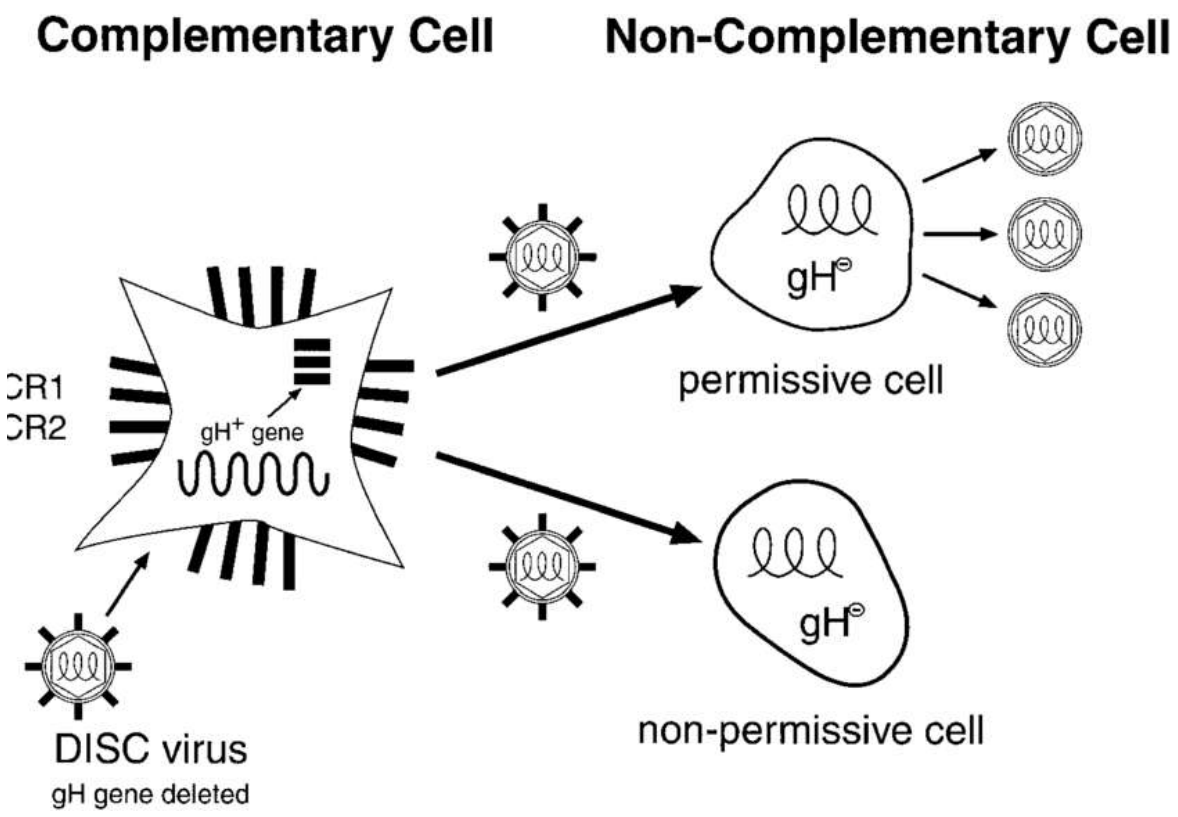
Replikasyon Kusurlu HSV Aşıları

- DISC (Disabled Infectious Single Cycle) virus aşıları olarak da adlandırılan bu aşılar, viral genom replikasyonu veya viral partiküllerin sentezi ve montajı için gerekli olan bir veya daha fazla gen için kusurludur.

- Normal hücrelerde, viral gen ürünlerini ifade ederler, ancak viryonları oluşturmak için çoğalmazlar.

- Çoğaltma kusurlu HSV aşıları, bağışıklık tepkilerini uyarabilir ancak hiçbir viral partikül üretmez.

- Bununla birlikte, konakçıda çoğalmadıkları ve yayılmadıkları için, daha az immünojenik olabilirler, özellikle daha az T hücresi uyarısına yol açabilirler. Çünkü profesyonel antijen sunan hücreleri (yani, B, makrofaj ve dendritik) aktive etmek için nispeten sınırlı bir kapasiteye sahiptirler.



Subunit HSV Aşıları

- Proteinler, DNA ve peptit epitop bazlı aşılar dahil olmak üzere çeşitli subunit HSV aşı yaklaşımları geliştirilmiştir.

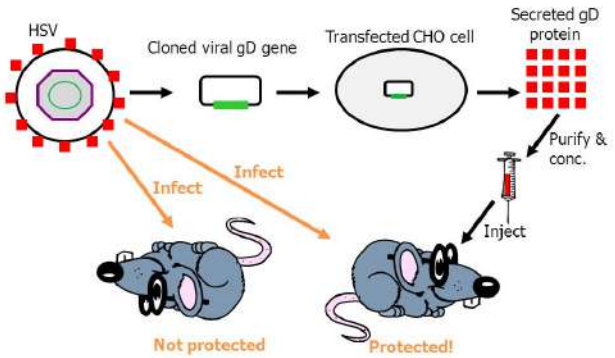
- Geleneksel protein bazlı aşılar, canlı zayıflatılmış ve replikasyon kusurlu HSV aşılarına kıyasla güvenlidir.

- Rekombinant çözünür HSV-2 glikoprotein D (gD), kapsamlı klinik değerlendirmeye giren en umut verici alt birim aşı olmuştur.

- Son 25 yılda, tümü HSV-2 gD kullanan (veya bir denemede gB ile karıştırılmış) bir Faz II terapötik genital herpes aşısı ve profilaktik alt birim aşılarının Faz III çalışmaları yapılmıştır.

- İlk terapötik gD/Alum aşı denemesinden elde edilen hayal kırıklığı yaratan sonuçlar, terapötik koruma için;
  (1) Nötralize edici antikorlara ek olarak CD4+ ve CD8+ T hücre tepkilerini indüklemeli
  (2) gD dışındaki HSV-2 antijenlerini içermeli
  (3) Alum dışında farklı adjuvanları test etmelidir.

Fig. 12.2 A subunit vaccine against HSV



- 1997'de, Chiron aşı deneyi, MF59 ile birlikte verilen gD ve gB'nin bir kombinasyonunu kullandı.

- Bu gB/gD/MF59 aşısı, T hücresi tepkilerini ortaya çıkarmadı, HSV2'ye karşı yüksek seviyelerde nötralize edici antikor üretti, ancak yalnızca %9 etkililiğe sahipti.

- Bu çalışma şunları önerdi:

  (1) nötralize edici antikorların yanı sıra koruyucu bir aşı, antiviral CD4+ ve CD8+ T hücre tepkilerini indüklemelidir

  (2) bir terapötik aşı, gB ve gD dışındaki HSV-2 antijenlerini içermelidir

  (3) Alum ve MF59 dışındaki farklı adjuvanları test etmelidir.

- Daha sonra, iki aşı denemesinde (biri 2004'te, diğeri 2012'de bildirildi), farklı bir adjuvan olan 3-0-deasile edilmiş monofosforil lipid A (MPL), bir TLR4 agonisti (93) ile birlikte verilen gD proteini kullanıldı.
- İlk klinik çalışmada, uyumsuz çiftlerin ayırt edici özelliği, infekte olmayan partnerin potansiyel olarak infekte partner tarafından tekrar tekrar HSV'ye maruz kaldığı oldukça seçilmiş bir grup olmalarıydı. Bu muhtemelen infeksiyon ve hastalık riskini artırdı, dolayısıyla terapötik aşının önemli bir etkisinin görülme eşiğini düşürdü.

- 2016-2018'de, bir aşı denemesinde (Gen-003 olarak belirlenmiş), Matrix M2 (MM-2) adlı yeni bir adjuvan ile ICP4 ve gD2 kesilmiş proteinlerin bir kombinasyonu kullanılmıştır.
- Matrix M, dengeli bir B ve T hücresi immüno-uyarıcı profiline sahip olan saponin bazlı bir adjuvandır.
- Bu deneme, tekrarlayan herpes lezyonlarında ve genital viral saçılımda önemli bir azalma olduğunu bildirdi.
- Bu koruma, kandan türetilen antiviral CD4+ ve CD8+ T hücre yanıtlarıyla korele görünüyordu.
- Etik ve pratik sınırlamalar nedeniyle, aşı klinik deneylerinin hiçbiri dorsal kök ganglionlarında (DRG) ve vajinal mukozal dokularda yerel dokuda yerleşik CD4+ ve CD8+ T hücrelerini araştırmamıştır.

Modifiye RNA (mRNA) Aşıları

- Friedman grubu glikoproteinler gC, gD ve gE'yi kodlayan lipid nanopartikül aşısındaki nükleosid modifiye mRNA'nın farelerde akut ve latent herpes simpleks virusu tip 2 infeksiyonuna karşı güçlü ve koruyucu bağışıklığı indüklediğini göstermiştir.

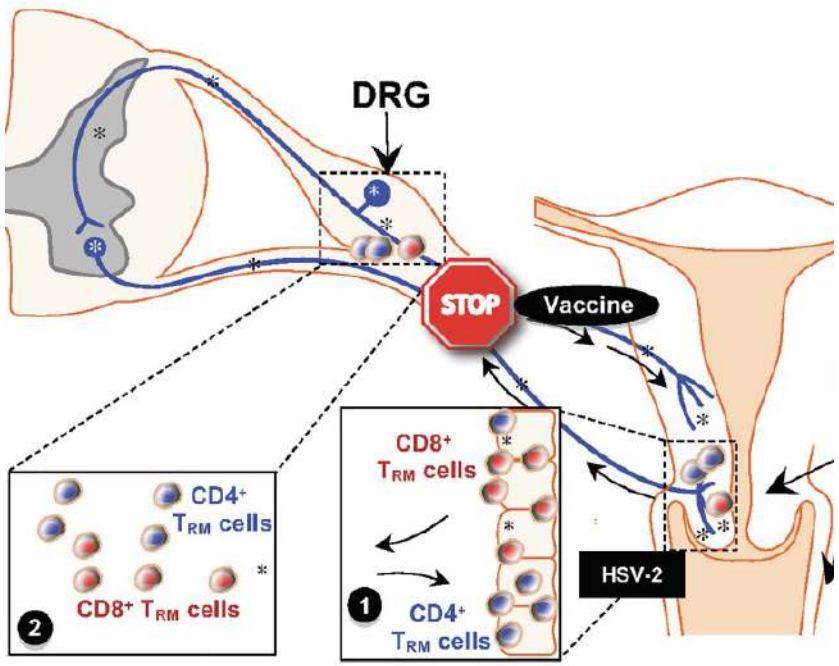
- Bir çalışmada:

  (1) Trivalent gC2/gD2/gE saflaştırılmış glikoproteinler adjuvanlar (CpG ve Alum) 19 ile

  (2) lipitte formüle edilmiş 3 glikoproteini kodlayan modifiye mRNA nanoparçacıklar (LNP)

- RNA, hücresel alımı artırmak ve doğuştan gelen bağışıklık sensörlerinin çeviri mekanizmasını engellemesini önlemek için modifiye edildi.

- mRNA-LPN aşısının, HSV1 ve HSV2 genital infeksiyonunu önlemede ve fareleri ve kobayları intravajinal HSV2 infeksiyonuna karşı korumada glikoprotein bazlı aşıdan daha iyi performans gösteren yüksek titrelere ve dayanıklı antikor yanıtlarına çevrilen etkili foliküler T helper ve germinal merkez B hücre yanıtlarını indüklediği gösterilmiştir.



Schematic of Prime-Pull-Keep Therapeutic Vaccine (PPK Vaccine). The PPK vaccine is designed to boost Neutralizing IgG/I
mber and function of antiviral CD4+ and CD8+ T_{RM} cells within the cervico genital muco-cutaneous [CGMC, **(1)**] and dorsal r
PPK vaccine is expected to help STOP the virus reactivation from latently infected DRG, virus shedding and virus replication
urrent genital herpes disease. *, represent virus.

TABLE 1 | Herpes Vaccine Strategies.

Type of Vaccine	Vaccine Construct	Administration Route	Phase ofTrial	Virus Subtype	Results	Limitations	Ref.
Inactivated vaccine	**HSV-1 gH deletion (SC16ΔgH)**	Subcutaneous in human	Clinical trial	HSV-2	• Unable to show protection against acute or recurrent genital herpes infection • Does not show improvement in recurrences and disease severity. • Does not affect on viral shedding	• Vaccine did not achieve clinical usefulness • Alternative approaches could be proposed	(11) Akhrameyeva NV, Zhang P, Sugiyama N, Behar SM, Yao F. Development of a glycoprotein D- expressing dominant- negative and replication- defective herpes simplex virus 2 (HSV-2) recombinant viral vaccine against HSV-2 infection in mice. *J Virol*, 85(10), 5036-5047 (2011).
		Subcutaneous and intravaginal in guinea pig	Preclinical trial	HSV-2	• Provides complete protection against primary and recurrent HSV infection • Induces high neutralizing antibody titers • Induces long- lasting immune responses i.e., over 6 months • Develops high potency for complete HSV protection	• Missing reproducibility on correlation between antibody titers and recurrent infection pattern • The immune mechanisms involved in the control of recurrent infection need to be elucidated	(12) Reszka NJ, Dudek T, Knipe DM. Construction, and properties of a herpes simplex virus 2 dl5-29 vaccine candidate strain encoding an HSV-1 virion host shutoff protein. *Vaccine*, 28(15), 2754-2762 (2010)
		Intraepithelial and intravaginal in guinea pig	Preclinical trial	HSV-2	• Reduces HSV symptoms • Gives quicker symptomatic episodes • Prevents local HSV-2 replication • offers Improved protection against HSV severity via Intravaginal route	• High risk of genetic recombination • Unable to block the virus reactivation to prevent disease recurrences • This study needs more animal experiment for statistical significance	(13) Belshe PB, Leone PA, Bernstein DI *et al.* Efficacy Results of a Trial of a Herpes Simplex Vaccine. *The New England journal of medicine*, 366, 34-43 (2012).
		Scarification via ear pinna route in mice	Preclinical trial	HSV-1	• Establishes self-limiting HSV infection • Induces DTH response • Provides protection against acute HSV infection	• May reactivate latent HSV • Viral latency and reactivation should be studied in more suitable animal model	(14) Bernard MC, Balban V, Pradezynski F *et al.* Immunogenicity, protective efficacy, and non-replicative status of the HSV-2 vaccine candidate HSV529 in mice and guinea pigs. *PLoS One*, 10(4), e0121518 (2015).
	HSV-2 ICP8 replication defective + B7 co-stimulation	Subcutaneous in mice	Preclinical trial	HSV-2	• Increases IFN-g-producing T- cells • Decreases HSV replication in genital mucosa • Lowers HSV related genital and neurological disease • Reduces mortality	• The protective immunity mediated by antibody and T- cells	(15, 16) Ohashi M, Bertke AS, Patel A, Krause PR. Spread of herpes simplex virus to the spinal cord is independent of spread to dorsal root ganglia. *J Virol*, 85(6), 3030-3032 (2011). Dasgupta G, Chentoufi AA, Kalantari M *et al.* Immunodominant "asymptomatic" herpes simplex virus 1 and 2 protein antigens identified by probing whole-ORFome microarrays with serum antibodies from seropositive asymptomatic versus symptomatic individuals. *J Virol*, 86(8), 4358-4369 (2012).
	Multiple genes Deletion of HSV-2	Subcutaneous in mice	Preclinical trial	HSV-2	• Reduces viral titer and viral shedding • Suppreses viral replication and latency • Theoretically provides protection against double- mutant virus even in immunocompro mised individuals	• The genetic basis underlying the latency defect should be elucidated	(17) Dasgupta G, Nesburn AB, Wechsler SL, BenMohamed L. Developing an asymptomatic mucosal herpes vaccine: the present and the future. *Future Microbiol*, 5(1), 1-4 (2010).
	HSV-2 ICP10ΔPK deletion	Subcutaneous in mice	Preclinical trial	HSV-2	• Induces memory T-cells and establish strong T-helper type 1 (Th1) immune response • Increases IL-12 secretion by DCs	• Does not readily begin latency • Must show the frequency and duration of memory T-cells • Assess the ability to activate p38MAPK in T- cells	(18) Chentoufi AA, BenMohamed L. Future viral vectors for the delivery of asymptomatic herpes epitope-based immunotherapeutic vaccines. *Future virology*, 5(5), 525-528 (2010).
	HSV-2 UL5 & UL29 genes deletion	Intramuscular in humans	Clinical trial	Multiple mutated HSV-1 and HSV-2 combina tions	• Safe and well tolerated	• More reactions than placebo on the injection site	(19) Schiffer JT, Abu-Raddad L, Mark KE *et al.* Mucosal host immune response predicts the severity and duration of herpes simplex

TABLE 1 | Continued

Type of Vaccine	Vaccine Construct	Administration Route	Phase ofTrial	Virus Subtype	Results	Limitations	Ref.
					• Produces neutralizing antibody along with CD4+ and CD8+ T-cell responses in HSV seronegative individuals • Produces only CD4+ T-cell responses in HSV seropositive individuals	• Should modify vaccine by increasing the expression of certain viral proteins • Should inhibits the expression of viral immune evasion genes, or adding an adjuvant	virus-2 genital tract shedding episodes. *Proc Natl Acad Sci U. S. A.*, 107(44), 18973-18978 (2010).
		Subcutaneous, and intramuscular in mice	Preclinical trial	HSV-2	• Decreases genital infection and viral shedding • Produces strong immune response • Gives protection against many HSV-2 viral strains • Shows better protection via intramuscular route	• Should study the role and type of DC involved in priming immunity against the intramuscular vaccine	(20) Chentoufi AA, Binder NR, Berka N *et al.* Asymptomatic human CD4+ cytotoxic T-cell epitopes identified from herpes simplex virus glycoprotein B. *J Virol*, 82(23), 11792-11802 (2008).
	HSV-2 gD (ΔgD-2) deletion	Intramuscular in mice	Preclinical trial	HSV-2 and superin-fection (HSV-1*)	• Induces IgG2 response • Fully protects HSV-2 spreading to the sacral ganglia and mortality • Shows almost no signs of disease	• voir in the • Should use guinea pigs as an animal model to study recurrent diseases • Should incorporate murine superinfection model in predinical evaluation of HSV- vaccine candidates	(21) Dervillez X, Qureshi H, Chentoufi AA *et al.* "Asymptomatic" HLA- A*02:01-Restricted Epitopes from Herpes Simplex Virus Glycoprotein B Preferentially Recall Polyfunctional CD8+ T Cells from Seropositive Asymptomatic Individuals and Protect HLA Transgenic Mice Against Ocular Herpes. *J Immunol*, (2013).
Live attenuated vaccine	**R7017 Deletion of HSV-1 thymidine kinase**	Intracerebral in mice, vaginal, intradermal, and intramuscular in guinea pigs and scarification of cornea in rabbits	Preclinical trial	HSV-1 and HSV-2	• Protects against severe HSV infections • HSV lesions are localized, superficial and heals more rapidly	• It establishes low frequency of latent infections in all hosts (R7020) • It also establishes latent infection in rabbits (R7017)	(22) Dervillez X, Gottimukkala C, Kabbara KW *et al.* Future of an "Asymptomatic" T-cell Epitope-Based Therapeutic Herpes Simplex Vaccine. *Future virology*, 7(4), 371-378 (2012).
	RAV9395 (Deletion of HSV-2 γ134.5 gene, UL55 and UL56 ORF)	Intramuscular	Preclinical trial	HSV-2	• Decreases lesion development and HSV infection severity • Decreases frequency of HSV reactivation from explanted DRG	N/A	(23) Pope C, Kim SK, Marzo A *et al.* Organ-specific regulation of the CD8 T cell response to Listeria monocytogenes infection. *Journal of immunology*, 166(5), 3402-3409 (2001).
	VC2 (mutations in gK and UL20)	Intramuscular	Preclinical trial	HSV-1 and HSV-2	• Fully protects against lethal intravaginal HSV challenge • Presents cross-protective humoral and cellular immunity • Absence of viral DNA in ganglionic tissues	N/A	(24) Gebhardt T, Whitney PG, Zaid A *et al.* Different patterns of peripheral migration by memory CD4+ and CD8+ T cells. *Nature*, 477 (7363), 216-219 (2011).
		Intramuscular	Preclinical trial	HSV-2	• Decreases acute viral replication in vagina, amount of virus in neural tissue, subsequent recurrent disease, and viral shedding • Delivers protection after 6 months	• Applying the criteria used for human trials	(25) Nelson MH, Bird MD, Chu CF *et al.* Rapid clearance of herpes simplex virus type 2 by CD8+ T cells requires high level expression of effector T cell functions. *J Reprod Immunol*, 89(1), 10-17 (2011).
	HSV-2 ICP0-ΔNLS)	Footpad injection	Preclinical trial	HSV-2	• Significantly reduces viral shedding in vagina • No detectable infection	N/A	(26) Bertke AS, Patel A, Imai Y, Apakupakul K, Margolis TP, Krause PR. Latency-associated transcript (LAT) exon 1 controls herpes simplex virus species-specific phenotypes: reactivation in the guinea pig genital model and neuron subtype-specific latent expression of LAT. *J Virol*, 83(19), 10007-10015 (2009).
	HSV-2 gE deletion	Intramuscular, intravaginal, and intravenous	Preclinical trial	HSV-2	• No disease mortality • Absence of infectious virus in DRG and recurrent HSV shedding in vagina • Decreases recurrent genital HSV lesions • Gives better efficacy through intramuscular route than subcutaneous route	• Provides incomplete protection	(27) Schiffer JT, Corey L. Rapid host immune response and viral dynamics in herpes simplex virus-2 infection. *Nat Med*, 19(3), 280-290 (2013).

Type of Vaccine	Vaccine Construct	Administration Route	Phase ofTrial	Virus Subtype	Results	Limitations	Ref.
	VC2 (gKD31-68 deletion of HSV-1)	Intramuscular	Preclinical trial	HSV-2	• Shows poor HSV replication at the immunization site • Rarely infects neural tissue • Lack of any genital disease • Reduces severity of acute and recurrent HSV-2 shedding in vagina and quantity of virus in DRG • Better selection as a prophylactic vaccine	• Not effective as a therapeutic vaccine	(28) Tang VA, Rosenthal KL. Intravaginal infection with herpes simplex virus type-2 (HSV-2) generates a functional effector memory T cell population that persists in the murine genital tract. *J Reprod Immunol*, 87(1-2), 39-44 (2010).
		Intramuscular	Preclinical trial	HSV-1	• Gives protection against HSV-1- induced ocular pathogenesis • Provides complete recovery from initial conjunctivitis • Increases neutralizing antibody titers along with CD3+, CD4+ and CD8+ T-cells • Decreases infiltration of Iba1+ macrophages	N/A	(29) van Lint A, Ayers M, Brooks AG, Coles RM, Heath WR, Carbone FR. Herpes simplex virus specific CD8+ T cells can clear established lytic infections from skin and nerves and can partially limit the early spread of virus after cutaneous inoculation. *J Immunol*, 172(1), 392-397 (2004).
	R2 (HSV-1 mutation in region 2 of pUL37)	Intramuscular, intradermal, and intravaginal	Preclinical trial	HSV-2	• Increases neutralizing antibodies • Decreases acute and recurrent HSV latent virus detection in DRG and recurrent shedding • Rarely infects neural tissue • Shows more effectivity via intradermal route	N/A	(30) Rott LS, Briskin MJ, Andrew DP, Berg EL, Butcher EC. A fundamental subdivision of circulating lymphocytes defined by adhesion to mucosal addressin cell adhesion molecule 1. Comparison with vascular cell adhesion molecule-1 and correlation with beta 7 integrins and memory differentiation. *J Immunol*, 156(10), 3727-3736 (1996).
	HSV-1 ICP0ΔNLS	Subcutaneous and intramuscular	Preclinical trial	HSV-1	• Shows less infectious virus during acute infection in TG and brainstem • Stimulates an immune response by increasing the gB-elicited interferon (IFN)-γ, granzyme B and CD107a; and decreasing LAG-3, PD-1, and TIM-3 • Gives protection against ocular HSV-1 challenge by reducing ocular neovascularization and suppressing peripheral nerve virus replication	• T-cell response is only observed at a single time point	(31) Mebius RE, Streeter PR, Michie S, Butcher EC, Weissman IL. A developmental switch in lymphocyte homing receptor and endothelial vascular addressin expression regulates lymphocyte homing and permits CD4+ CD3- cells to colonize lymph nodes. *Proc Natl Acad Sci U S A*, 93(20), 11019-11024 (1996).
Naked DNA vaccine	**pSVL- HSV-1 gD, pRc/CMV- HSV-1 gD**	Intramuscular	Preclinical trial	HSV-1	• Reduces serum anti-gD antibody, anti-HSV1 neutralizing antibody and anti-gD ELISA responses • Gives non- specific changes in ELISA and neutralization antibody titers	• Provides low protection against HSV-1 • Not a useful alternative of a gD subunit vaccine	(32) Mackay CR, Andrew DP, Briskin M, Ringler DJ, Butcher EC. Phenotype, and migration properties of three major subsets of tissue homing T cells in sheep. *Eur J Immunol*, 26(10), 2433-2439 (1996).
	pDNA encoding HSV-2 gD2	Intramuscular	Clinicaltrial	HSV-1-/HSV-2-, HSV-1+/HSV-2-	• Provides safe and well tolerated with no dose-limiting toxicities • Increases D2-specific cytotoxic T- cell and lymphoproliferati on immune responses	• Produces adverse events that are mostly local site reactions	(33) Abitorabi MA, Mackay CR, Jerome EH, Osorio O, Butcher EC, Erle DJ. Differential expression of homing molecules on recirculating lymphocytes from sheep gut, peripheral, and lung lymph. *J Immunol*, 156(9), 3111-3117 (1996).
	pDNAs encoding HSV-2 gD2	Subcutaneous	Preclinical trial	HSV-2	• Provides fully protection against lethal intravaginal HSV-2 infection • Produces strong HSV-2 virion- specific IgG and neutralizing antibody responses • Reduces all levels of recurrent HSV-2 significantly • Reduces acute and recurrent disease, recurrent lesion days and latent HSV-2 load	• Should be studied in a greater number of guinea pigs	(34) von Andrian UH, Mackay CR. T-cell function and migration. Two sides of the same coin. *N Engl J Med*, 343(14), 1020-1034 (2000).
	pDNA encoding HSV-2 gD2 coupled with Vaxfectin®	Intramuscular	Preclinical trial	HSV-2	• Increases IgG antibody titers	• Limited sensitivity for IgG assay	(35) Mackay LK, Wakim L, van Vliet CJ *et al.* Maintenance of T cell function in the face of chronic antigen stimulation and repeated

Type of Vaccine	Vaccine Construct	Administration Route	Phase ofTrial	Virus Subtype	Results	Limitations	Ref.
					• Provides protection against lethal HSV-2 challenge • Reduces vaginal HSV load and viral latency in DRG		reactivation for a latent virus infection. *J Immunol*, 188(5), 2173-2178 (2012).
	pDNA encoding HSV-2 gD2 and UL46 and UL47 genes coupled with Vaxfectin®	Intramuscular	Preclinical trial	HSV-2	• Reduces viral replication and shedding in genital tract, latent HSV-2 DNA in DRG, and frequency of recurrent disease • Completely protects from both primary and recurrent genital disease	• Includes additional controls including irrelevant plasmids coupled with Vaxfectin®	(35) Mackay LK, Wakim L, van Vliet CJ *et al.* Maintenance of T Cell Function in the Face of Chronic Antigen Stimulation and Repeated Reactivation for a Latent Virus Infection. *J Immunol*, (2012).
	Codon-modified polynucleo-tide vaccine **COR-1: (1) Full-length HSV-2 envelope gD2 and (2) truncated version of gD2 fused to a ubiquitin sequence**	Intradermal in forearm	Clinical trial	HSV-2	• Provides safe and well tolerated protection with no moderate or serious adverse effects • Increases immune cellular activity • Presence of CD45+, CD4+, CD68+ macrophages and polymorphonude ar neutrophils at site of immunization • Decreases mean number of outbreaks and viral shedding	• Minimal antibodies increase with overall no statistical significance • Insufficient number of subjects to determine a significant placebo effect	(36) Mackay LK, Stock AT, Ma JZ *et al.* Long-lived epithelial immunity by tissue-resident memory T (TRM) cells in the absence of persisting local antigen presentation. *Proc Natl Acad Sci U S A*, 109(18), 7037-7042 (2012)
	SLV-20: (1) pGX27 with tissue plasmino- gen activator (tpa), Flt3L and HSV-2 gB and UL39, (2) pGX27 with gD2, ICP0 and ICP4 and (3) pGX27 with IL-12- IL-21 and MIP-1α	Intramuscular	Preclinical trial	HSV-2	• Inhibits pathological progression after viral infection • Increases survival rate • Reduces virus titer and viral shedding • Increases IFN- γ, CD4+, CD8+ and CD44hiCD62Lhi central memory T-cells expression	• Does not show any significant differences in immunoglobulin IgA, IgM, IgG1 and IgG3 levels	(37) Masopust D, Picker LJ. Hidden memories: frontline memory T cells and early pathogen interception. *J Immunol*, 188(12), 5811-5817 (2012).
Protein-based subunit vaccine	**HSV-2 gD2t with 3-O-deacylated mono-phosphoryl**	Intramuscular	Preclinical trial	HSV-1	• Reduces latent viral load significantly • Provides protection against acute and recurrent HSV-2 infection	• Not as effective as replication- defective *dl5-29*	(38) Suni MA, Ghanekar SA, Houck DW *et al.* CD4(+) CD8(dim) T lymphocytes exhibit enhanced cytokine expression, proliferation, and cytotoxic activity in response to HCMV and HIV-1 antigens. *Eur J Immunol*, 31(8), 2512-2520 (2001).
	lipid A (MPL)- aluminum hydroxide (alum)	Subcutaneous	Preclinical trial	HSV-2	• Provides protection against acute and recurrent HSV infection and acute viral shedding • Reduces recurrent lesion days; sufficient to prevent most recurrent lesion episodes significantly	• Does not show significant reduction in the mean number of days with recurrent diseases • Not sufficient to suppress early stages of viral reactivation • Produces low levels of HSV-2 virion-specific antibodies	(34) von Andrian UH, Mackay CR. T-cell function and migration. Two sides of the same coin. *N Engl J Med*, 343(14), 1020-1034 (2000).
	HSV-2 gD with MPL-alum	Intramuscular	Clinical trial	HSV-1-/HSV-2-, HSV-1+/HSV-2+	Presents a protective effect in those women who were HSV-1 and HSV-2 seronegative	• Ineffective in women who are seropositive for HSV-1 but seronegative for HSV-2 • Ineffective in men regardless of serologic status	(39) Jiang X, Chentoufi AA, Hsiang C *et al.* The herpes simplex virus type 1 latency associated transcript (LAT) can protect neuronal derived C1300 and Neuro2A cells from Granzyme B induced apoptosis and CD8 T-cell killing. *J Virol*, (2010).
		Subcutaneous	Preclinical trial	HSV- 1 and HSV- 2	• Gives almost complete protection against primary infection • Presents better protection against latent infection	• Does not prevent mucosal infection	(40)
	HSV-2 gD and gB adjuvanted with a novel T- cell antigen and tegument protein UL40	Intramuscular	Preclinical trial	HSV-2	• Increases HSV-2 antigen-specific CD8+ T- cell responses • Stimulates high titers of neutralizing antibodies • Reduces HSV shedding in vagina, lesion scores and latent infection	N/A	(41) Jameson SC, Masopust D. Diversity in T cell memory: an embarrassment of riches. *Immunity*, 31(6), 859-871 (2009).

Type of Vaccine	Vaccine Construct	Administration Route	Phase ofTrial	Virus Subtype	Results	Limitations	Ref.
	HSV-2 gD2 and gB2 formulated in a nano-emulsion adjuvant (NE01- gD2/gB2)	Intranasal and intramuscular	Preclinical trial	HSV-2	• Increases neutralizing antibodies levels • Reduces acute and recurrent disease scores and shedding of virus • Reduces detection of latent virus in DRG	• Less efficiently induces neutralizing antibodies than intramuscular IgD2 with MPL- alum vaccine	(42) Khan AA, Srivastava R, Spencer D *et al.* Phenotypic and Functional Characteitzation of Herpes Simplex Virus Glycoprotein B Epitope-specific Effector and Memory CD8+ T Cells from Ocular Herpes Symptomatic and Asymptomatic Individuals. *Journal of virology*, (2015).
	Trivalent (gC2, gD2, gE2) subunit vaccine mixed with CpG and alum	Intramuscular	Preclinical trial	HSV-2	• Produces antibodies that binds to gC2 and blocks its ability to bind C3b for immune evasion	• gC2 are not immunogenic Without adjuvant during natural HSV-2 infection in humans or HSV-2 infected guinea pigs	(43) Shin H, Iwasaki A. A vaccine strategy that protects against genital herpes by establishing local memory T cells. *Nature*, 491 (7424), 463-467 (2012).
		Intramuscular	Preclinical trial	HSV-1 and HSV-2	• Increases HSV glycoprotein- specific antibodies which neutralizes HSV-1 and HSV-2 • Provides remarkable durability of vaccine response (continues up to 21 months post- immunization) • Exhibits little to no viral replication • Absence of viral DNA in brains or trigeminal ganglia • Provides protection against nHSV (maternal immunization promotes transfer of neutralizing antibodies and protects offspring from disseminated disease, weight loss, anxiety-like behaviour, and mortality)	N/A	(44) Khan AA, Srivastava R, Chentoufi AA *et al.* Bolstering the Number and Function of HSV-1-Specific CD8(+) Effector Memory T Cells and Tissue-Resident Memory T Cells in Latently Infected Trigeminal Ganglia Reduces Recurrent Ocular Herpes Infection and Disease. *J Immunol*, 199(1), 186-203 (2017).

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Cellular and Molecular Life Sciences

REVIEW

Vaccination against the Epstein–Barr virus

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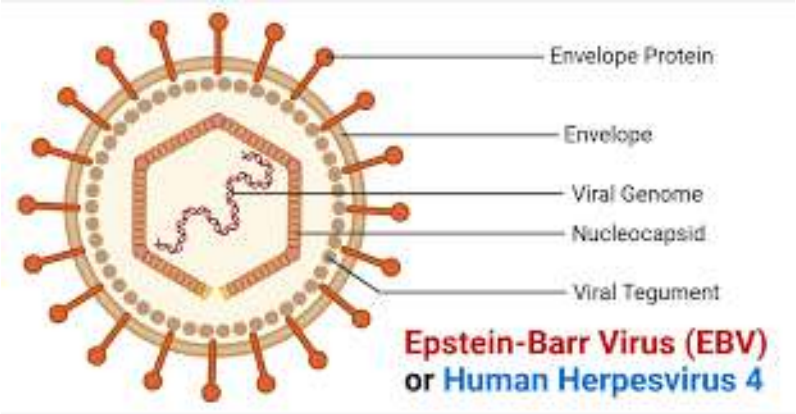
- Epstein-Barr virusu (EBV), keşfedilen ilk insan tümör virusu ve bugüne kadar in vitro olarak hücreleri dönüştürebilen tek insan patojeni olmaya devam ediyor.

- Rekombinant viral vektörler ve bunların heterolog prime-boost aşıları, EBV türevli virus benzeri partiküller ve viral zarf glikoprotein formülasyonları araştırılmıştır.



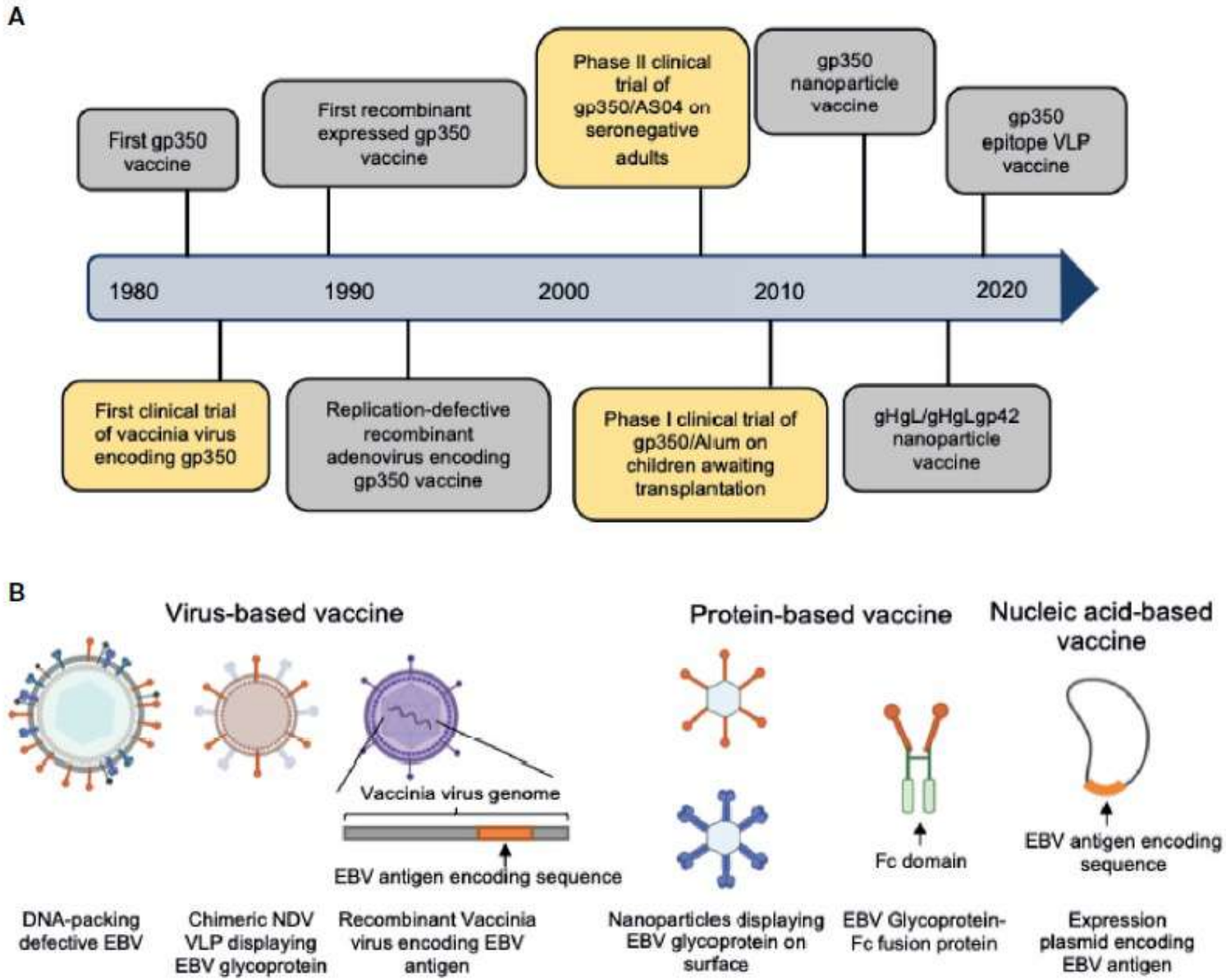
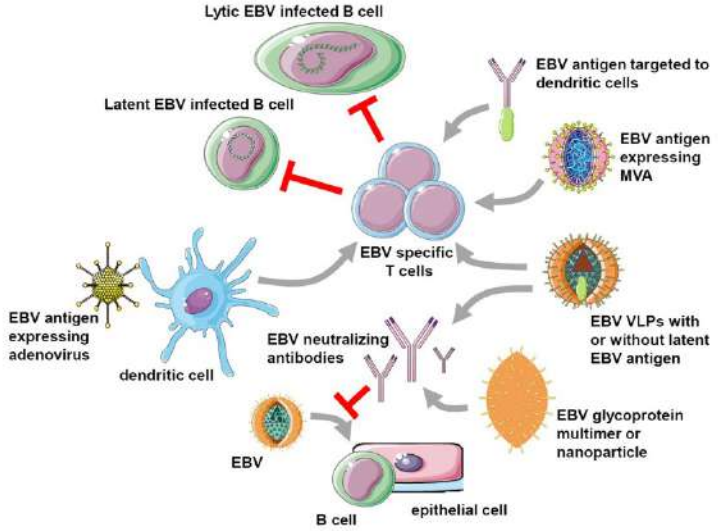


FIGURE 2 | **(A)** Hallmarks of prophylactic EBV vaccine development using EBV glycoproteins as antigens. Clinical trials are marked in yellow box and others are marked in gray box. **(B)** Current candidate platforms for EBV vaccines, including virus-based, protein-based and nucleic-acid-based vaccines.

Rekombinant viral vektör aşıları

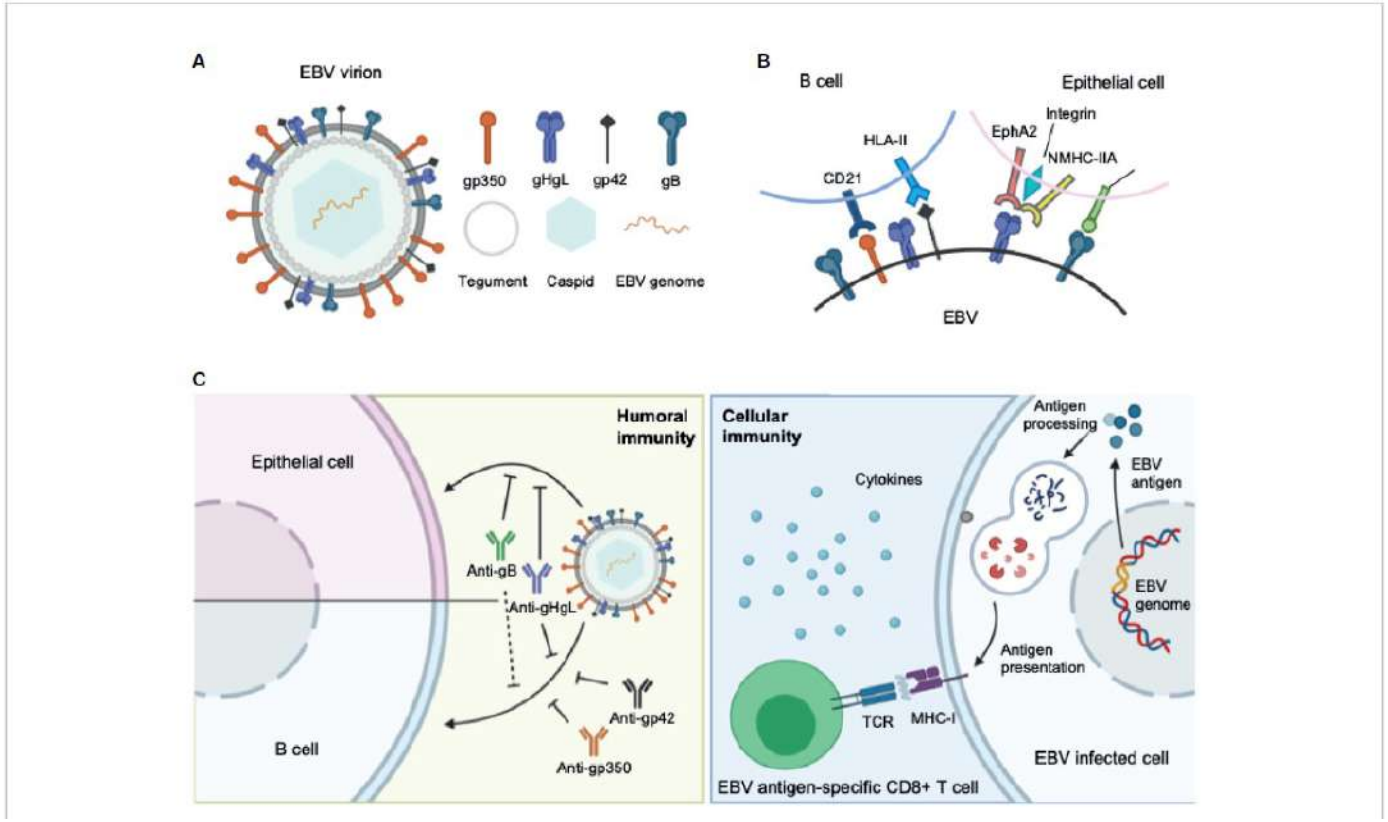
- Rekombinant viral vektör aşıları, bağışıklık tepkilerinin istendiği ek proteinleri ifade etmek üzere tasarlanmış canlı viruslardır.
- Bu aşı platformları nispeten yenidir ve geleneksel aşılara göre birçok avantajı vardır.
- İlk olarak, viral vektör aşıları, özellikle infekte olmuş ve tümör hücrelerinin temizlenmesinde önemli olan CD8+ sitotoksik T lenfosit (CTL) yanıtlarında artış olmak üzere, geniş bir yelpazede bağışıklık tepkilerini indükleyebilir.
- Viral vektör hedef hücreleri infekte eder ve sitozolde antijen ekspresyonuna yol açar, burada klasik MHC sınıf I-işleme yoluna kolay erişim elde edebilir ve ardından elde edilen peptit epitoplarının MHC sınıf I molekülleri üzerinde antijene özgü bir CD8+'yı uyarmak için sunumuna yol açar.
- İkincisi, viruslar doğal olarak immünojeniktir ve bu nedenle, bir inflamatuar yanıtı başlatmak için bir dizi patojenle ilişkili moleküler modeli (PAMP'ler) ifade ettikleri için adjuvanlardır.
- Bu adjuvan etki, koruyuculuğu arttırmak için çok önemlidir.
- Üçüncüsü, viral vektör aşıları, yüksek bir gen transdüksiyon etkinliğine sahiptir ve kullanılan viral vektörlerin tropizmine bağlı olarak antijenleri farklı hücre tiplerine iletebilir.



Virus benzeri parçacıklar

- Virus benzeri parçacıklar (VLP'ler), herhangi bir viral nükleik asit içermeyen virus parçacıkları olarak tanımlanır.

- 2012 yılında Pavlova ve ark. EBNA1 ve EBNA3C gibi latent antijenleri kaynaştırarak DNA içermeyen EBV VLP'leri oluşturmayı başardı.



Heterolog prime-boost aşılama

- Adenovirus aşıları üzerinde yapılan ilk çalışmalarda, insan adenovirus 5 (hAd5) gibi serotipler kullanıldı, ancak viral vektörü nötralize edebilen önceden var olan bağışıklık, insan popülasyonunda yaygındır, bu nedenle gücünü sınırlar ve klinik kullanımını engeller.

- Şempanze adenovirus vektörleri daha sonra bu önceden var olan nötralize edici bağışıklığı önlemek için geliştirildi.

- Ne yazık ki, bu vektörlerin immün sistem üzerine etkisi, ek aşılama sırasında farklı viral vektörlerin kullanılmasını gerektiren ikincil enjeksiyonlar için kapasitesini sınırlayan nötralize edici tepkiler oluşturabilir.

- Aslında, iki antijen formülasyonunu kullanan heterolog primeboost stratejileri, gelişmiş bir bağışıklama yolu olarak kabul edilmiştir.

- EBNA1'e karşı adenovirus prime ve MVA boost aşılamasının, EBV antijeni eksprese eden lenfomalara karşı korumaya dönüşebilen kapsamlı CD4+ ve CD8+ T hücre yanıtlarını ortaya çıkarmada etkili olduğunu gösterilmiştir.

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Protective anti-gB neutralizing antibodies targeting two vulnerable sites for EBV-cell membrane fusion

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- Glikoprotein B (gB), EBV'nin konakçı hücrelere girişi için gerekli olan birincil füzyon proteinidir.

- EBV gB'ye özgü nötralize edici antikorlar, 3A3 ve 3A5; her ikisi de B ve epitel hücrelerinin dual-tropik EBV infeksiyonunu etkili bir şekilde nötralize etti.

- Farelerde, her iki antikor da EBV'nin neden olduğu lenfoproliferatif bozukluklara karşı etkili koruma gösterdi.

- Kriyoelektron mikroskobu analizleri, yapıya dayalı mutajenez, 3A3 ve 3A5'in, gB-hücre etkileşimi ve gB aktivasyonu ile etkileşimi içeren farklı mekanizmalar yoluyla zar füzyonunu inhibe ettiğini ortaya çıkardı.

- Daha da önemlisi, 3A3 ve3A5 epitopları, insanlarda doğal EBV infeksiyonu tarafından ortaya çıkan koruyucu gB'ye özgü nötralize edici antikorların ana hedefleridir ve antiviral terapiler ve aşılar için potansiyel hedefler sağlar.

TABLE 1 | Summary of EBV vaccine animal trials.

Year	Platform/ Adjuvant	Antigen	Animals	Results
1984	Subunit vaccine/ liposome, Freund's adjuvant, lipid A	Full length membrane gp340 (gp350) purified from virus	Mice, rabbit and cottontop tamarins	Antibody responses were induced similarly in mice and cotton-top tamarins, among which groups adjuvanted with liposome and lipid A elicited antibody responses earlier; Antibody responses in rabbits were rather weak (32).
1985	Prototype subunit vaccine	Full length membrane gp340 (gp350) purified from virus	cottontop tamarins	Privided protection against malignant lymphoma (21)
1985	Recombinant vaccinia virus(WR strain)	gp340	rabbits	Neutralizing antibodies against gp340 could be detected (22).
1986	Subunit vaccine	gp340 produced by immunoaffinity chromatography from B95-8.	cottontop tamarins	No protection against malignant lymphoma (23)
1988	Subunit vaccine	gp350/gp220 produced by immunoaffinity chromatography from yeast and mammalian cells	——	All of the mammalian cell-derived versions of the membrane antigen were found capable of inducing EBV-specific neutralizing antibodies as well as B95-8 (33).
1988	Subunit vaccine/ ISCOMS	gp340 incorporated into immune-stimulating complexes (ISCOMS)	cottontop tamarins	Provided protection against malignant lymphoma (34)
1988	Recombinant vaccinia virus (Wyeth or WR strains)	gp340	cottontop tamarins	Only WR strain derived vaccine could offer protection against malignant lymphoma (24)
1992	Recombinant subunit vaccine/ threonylmuramyl dipeptide adjuvant formulation.	gp340, lack of membrane anchor region, produced using a bovine papillomavirus (BPV) expression vector	cottontop tamarins	3/4 immunized cottontop tamarins showed protection against malignant lymphoma, 1/4 immunized cottontop tamarins developed idiopathic colitis due to low immune responses to gp340 (35).
1993	Replication-defective recombinant adenovirus vaccine	gp340/220	cottontop tamarins	Provided protection against malignant lymphoma despite no detectable neutralizing antibodies in vitro (25).
1994	Subunit vaccine/alum	gp340	Rabbits, cottontop tamarins	3/5 immunized cottontop tamarins showed protection against malignant lymphoma (36).
1996	Recombinant vaccinia virus	gp340	common marmosets challenged with M81	Vaccinated group showed lower virus load compared to control group (26).
1999	Recombinant subunit vaccine/ alum VS Freund's adjuvant	Single chain gp350	rabbits	Elicited high neutralizing antibody titers; three immunizations with MSTOP gp350 elicited neutralizing titers of 3800±5400 in alum and 1,600 ± 3,400 in Freund's adjuvant (27).
2001	Peptide epitopes	HLA A2-restricted epitopes from the latent, lytic and structural proteins	Humanized HLA A2/Kb mice	A maximal response to the epitopes within the structural proteins and low to moderate responses to the latent epitopes, indicating hierarchy of CTL responses between mice and humans (37).
2003	Recombinant poxvirus vaccine	Polyepitope protein comprising 6 HLA A2-restricted epitopes derived from LMP1	Humanized HLA A2/Kb mice	Successfully reversed the out- growth of LMP1-expressing tumors in HLA A2/Kb mice (38).
2009	Epitope/HSP70 and incomplete Freund's adjuvant	Mycobacterial HSP70 and LMP2A (356-364) epitope fusion protein	Humanized HLA-A2.1 mice	Specific CTL more effectively than a single peptide plus incomplete Freund's adjuvant; melanoma tumor cells was suppressed in humanized HLA-A2.1 mice (39).
2009	Recombinant adeno-associated virus/HSP	Latent membrane proteins (LMP1 and LMP2) CTL epitope	BALB/c (H-2d) mice	Specific CTL responses; eliminated tumors in mice (40).
2011	Epitope/ HSP70	Reconstituted complexes of MtHsp70 and LMP2A-peptides	HLA-A2.1 transgenic mice	Specific CTL responses; protective activity and therapeutic efficacy against LMP2A-expressed tumor challenge (41).
2011	EBV-derived VLP	EBV-derived VLP, deleted or function- ally inactivated six viral genes (EBNA2, LMP1, EBNA3A, -B, and -C, BZLF1)	BALB/c mice	Strong $CD8^+$ and CD4+ T cell responses in a preclinical murine model (42).
2011	Combined immunization of DNA, AAV, and adenovirus vector vaccines	LMP2	BALB/c mice	Combined immunization with DNA, AAV, and adenovirus vector vaccines induced specific cellular immunity better than any other combinations (43).
2013	Multimeric subunit vaccine/tetanus toxoid	gp350 (1-470)	BALB/c mice	Tetrameric gp350 induced ~20-fold higher serum titers of specific IgG and >19-fold enhancements in neutralizing titers at the highest dose;tetanus toxoid (TT)-specific CD4+ T-cell epitopes into the tetrameric gp350: no effect on specific antibody responses (44).

TABLE 1 | Continued

Year	Platform/ Adjuvant	Antigen	Animals	Results
2013	Replication-defective chimpanzee-derived adenovirus vectors	Rhesus Lymphocryptovirus EBNA-1 Homologue, rhEBNA-1	rhesus macaques	EBNA-1-specific T cells could be expanded by vaccination (45).
2013	Recombinant subunit vaccine	Truncated EBNA1 (E1ΔGA, codons 390–641), produced from methylotrophic yeast P. pastoris	BALB/c mice	Elicited CD4+ and CD8+ T cell responses (46)
2015	Newcastle disease virus (NDV)-virus-like particle	EBV gp350/220 ectodomain	BALB/c mice	Elicited neutralizing antibody responses, but not better than soluble gp350/220 (47).
2015	Dendritic cells pulsed with recombinant BZLF1	BZLF1	hu-PBL-SCID mice	Elicited specific cellular immunity; improved survival from fatal EBV-LPD (48).
2015	Self-assembling nanoparticles	gp350 D_{123}-ferritin; gp350 D123- encapsulin	BALB/c mice; Cynomolgus Macaques	gp350-nanoparticle elicited 10- to 100-fold higher neutralization titer compared to soluble gp350 (49).
2015	Recombinant subunit vaccine/ TiterMax (CytRx)	native or denatured/alkylated gp350 produced from CHO	Rabbits	Denatured gp350 could induce binding antibodies but no neutralizing antibodies (28).
2015	Designed peptides, coupled with keyhole limpet hemocyanin (KLH), Sigma adjuvant system	Designed gp350 peptides to mimic gp350 amino terminus that interacts with 72A1	BALB/c mice	The gp350 mimetic peptide bound to 72A1 antibody can block gp350 recognition (50).
2016	Multimeric subunit vaccine	trimeric gH/gL; trimeric gB; tetrameric gp350	rabbits	Trimeric and monomeric gH/gL, trimeric gB, and tetrameric gp350 groups induced serum EBV-neutralizing titers >100-, 20-, 18-, and 4-fold higher, respectively, than monomeric gp350 (51).
2016	Multi-epitope vaccine	Chimeric multi-epitope protein referred to as EBV-LMP2m, which is composed of LMP2aa195-232 and LMP2aa419-436	BALB/c mice	Elicited specific antibody and CTL responses (52)
2018	Subunit vaccine	Fc-fused gp350 dimer	BALB/c mice	Elicited higher specific antibody titers than gp350 monomer; elicited potent nAbs (53).
2018	EBV-derived VLP	Viral particle expressed both with lytic and latent proteins by insertion of latent protein epitopes into the major tegument protein BNRF1	Humanized NSG-A2 mice	Provide significant protection against wild-type EBV infection (54)
2019	Self-assembling nanoparticles/ SAS adjuvant	gH/gL-ferritin; gH/gL/gp42-ferritin	BALB/c mice; Cynomolgus macaques	Monkey immunized with gH/gL/gp42-ferritin nanoparticles elicited >40- and ~4-fold higher neutralization titers in B cells in comparison with soluble gH/gL and soluble gH/gL/gp42; in epithelial cells, gH/gL-ferritin and gH/gL/gp42-ferritin nanoparticles showed >25- and ~4-fold higher neutralizing titers than the corresponding soluble glycoprotein vaccines (55).
2020	Newcastle disease virus (NDV)-virus-like particle/ aluminum hydroxide and monophosphoryl lipid A	gp350, gB, gp42, gH, and gL pentavalent complex	rabbits	Elicited specific neutralizing antibodies more robust than soluble gp350 ectodomain (56).
2020	Epitope VLP	Combinations of three gp350 epitopes from receptor-binding domain (aa 16–29/ aa 142–161/ aa 282-301)	BALB/c mice	elicited neutralizing antibodies (57)

TABLE 2 | Epstein-Barr virus prophylactic vaccine clinical trials: past and present[a].

Vaccine	Manufacturer	Clinical trial/publication	Outcome
Recombinant gp350	GlaxoSmithKline Biologicals, Rixensart, Belgium	Phase 1 and phase 2 studies to evaluate safety and immunogenicity of a recombinant gp350 EBV vaccine in healthy adults (58)	Phase 1 (59 subjects evaluated). One severe adverse event. Phase 1 and 2 studies (79 subjects evaluated). One severe adverse event.
Recombinant gp350 and ASO_4 adjuvant system	GlaxoSmithKline Biologicals, Rixensart, Belgium	Recombinant gp350 vaccine for infectious mononucleosis: a phase 2, randomized, double-blind, placebo-controlled trial to evaluate the safety, immunogenicity, and efficacy of an EBV vaccine in healthy young adults. (59)	A total of 178 EBV seronegative subjects were evaluated. No subject discontinued medication for reasons of safety or reactogenicity. In an intention to treat analysis, IM cases were distributed as follows: 9 cases (1 probable and 8 definite) were found in the placebo group and 2 cases (both definite) were found in the vaccine group ($p = 0.03$; $\alpha = 0.05$, by 1-sided Fisher's exact test).
CD8+ T-cell synthetic peptide HLA B*0801-restricted epitope and tetanus toxoid vaccine	Queensland Institute of Medical Research, Australia	Phase 1 trial of a CD8+ T-cell peptide epitope-based vaccine for infectious mononucleosis. (60)	A total of 14 subjects were evaluated. No serious adverse events were reported. Trial too small to estimate vaccine efficacy. Vaccine immunogenic in most individuals.
mRNA-1189	Moderna TX, Inc.	A phase 1, randomized, observer-blind, placebo-controlled, dose-ranging study of an EBV candidate vaccine, mRNA-1189, in 18- to 30-year-old healthy adults. Trial ongoing. Estimated completion date June 2023. NCT05164094	Main outcome is to evaluate the safety and reactogenicity of mRNA-1189 in 18- to 30-year-old healthy adults. Secondary outcome is to evaluate vaccine immunogenicity.
EBV gp350-Ferritin nanoparticle vaccine adjuvanted with Matrix M1	National Institute of Allergy and Infectious Diseases, USA	A phase 1 study of the safety and immunogenicity of an EBV gp350-Ferritin nanoparticle vaccine in healthy adults with or without EBV infection. Trial ongoing. Estimated completion date July 2025. NCT04645147	Main outcome is to evaluate the safety and reactogenicity of gp350-Ferritin nanoparticle vaccine in 18- to 29-year-old healthy adults.

[a]*Numbers in parentheses list relevant references.*

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REVIEW

Human cytomegalovirus (HCMV) infection/re-infection: development of a protective HCMV vaccine

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Table 2 - *Immune response to natural HCMV infection.*

2.1. Innate response	a) CD57 NKG2C^{bright} cells (Lilleri and Gerna, 2017). b) ADCC (Wu *et al.*, 2013; Chung *et al.*, 2014)). c) γ/δ T-cells and, namely, Vδ2^{-} γ/δ T-cells (Pitard *et al.*, 2008; Fornara *et al.*, 2011).
2.2. Antibody response	HCMV-specific antibodies, and namely neutralizing antibodies (NAb), were considered in the past to have a protective effect against vertical HCMV transmission in seronegative mothers (Fowler *et al.*, 1992). However, recently, vertical transmission was also shown to occur in mothers who were seropositive prior to pregnancy (Ross *et al.*, 2006, 2010; Novak *et al.*, 2009).
2.3. T-cell response.	Protective role of HCMV-specific CD4^{+} and CD8^{+} T-cells documented in the 90s following adoptive transfer of *in vitro* expanded CD4^{+} and CD8^{+} T-cells (Walter *et al.*, 1995; Einsele *et al.*, 2002).

ADCC, antibody-dependent cellular cytotoxicity.

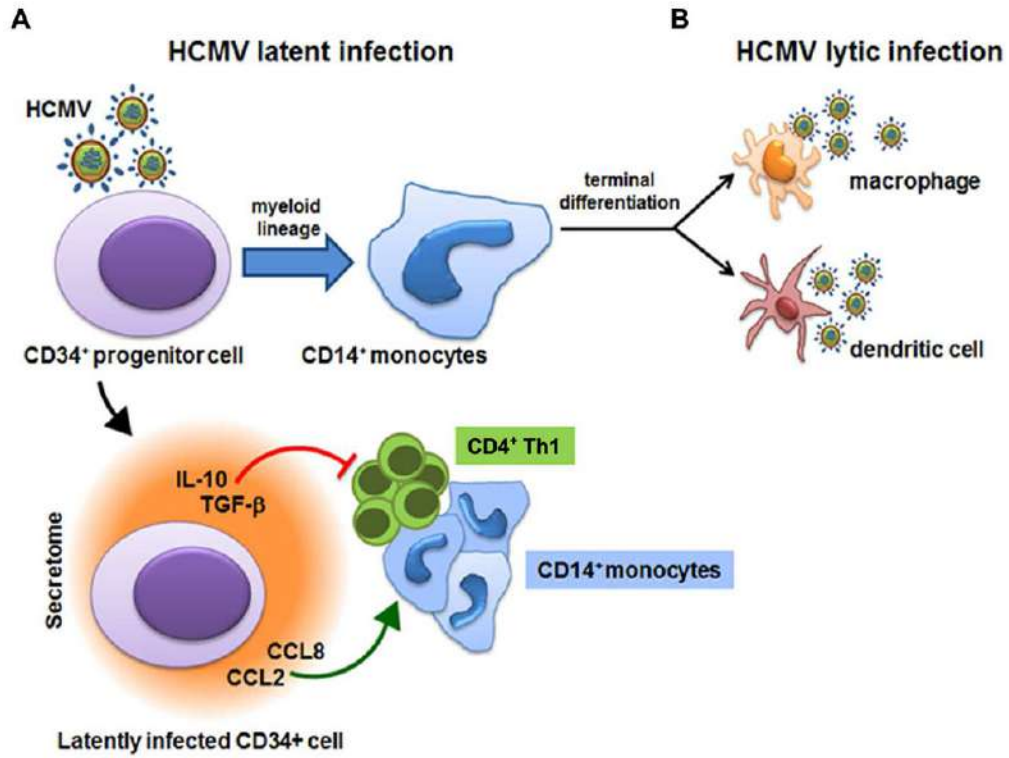
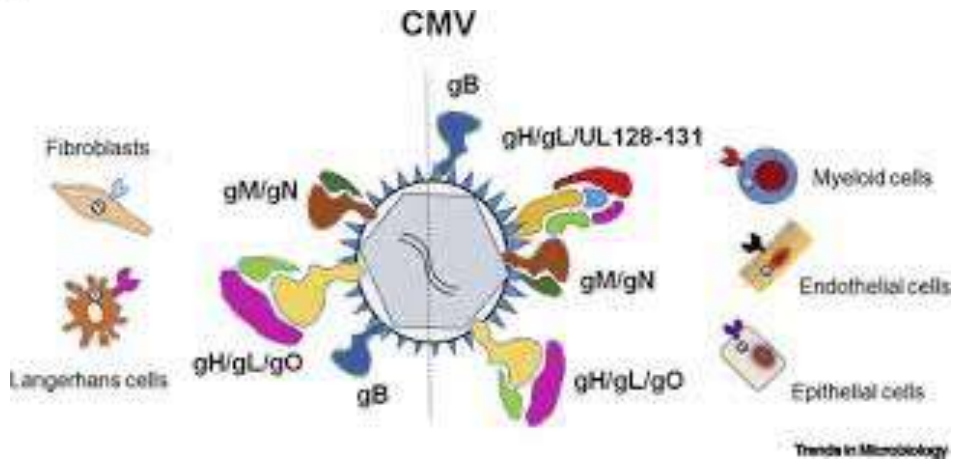


Table 3 - *HCMV major immunogenic antigens.*

3.1. Antibody response	1)	Until 2004, the 3 known glycoprotein complexes (gCs) representing the major targets of the HCMV NAb response were gB (gCI, homotrimer), gH/gL (gCIII), and gM/gN (gCII).
	2)	In 2004-2005, the genomic trimer UL128-130-131 (referred to as UL128L) was identified as indispensable for infection of endothelial (Hahn *et al.*, 2004) and epithelial (Wang & Shenk, 2005) cells. Then, it was shown to be complexed with gH/gL to form the pentamer complex (PC) gH/gL/pUL128L (Ryckman *et al.*, 2008).
	3)	In 2010, a number of potently neutralizing human mAbs were shown to be directed to the 3 components of the trimer pUL128L (Macagno *et al.*, 2010). Murine mAbs with the same potency and specificity were obtained following immunization with PC (Kabanova *et al.*, 2014).
	4)	Finally, early appearance of antibodies to some PC epitopes in pregnant women with primary HCMV infection was associated with a reduced risk of vertical HCMV transmission (Lilleri *et al.*, 2013; Gerna *et al.*, 2015).
3.2. T-cell response	5)	Primary role of T-cell immunity in protection against HCMV infection/disease has been repeatedly recognized with the guiding contribution of HCMV-specific $CD4^+$ T-cells and the secondary intervention of HCMV-specific $CD8^+$ T-cells in both the immunocompetent (Revello *et al.*, 2006; Lilleri *et al.*, 2007, 2008; Fornara *et al.*, 2017) and the immunocompromised host (Sester *et al.*, 2001; Gamadia *et al.*, 2003; Gabanti *et al.*, 2014, 2015).
	6)	The major target for both $CD4^+$ and $CD8^+$ T-cells is pp65, whose $pp65_{495\text{-}503}$ epitope stimulates $CD8^+$ T-cells only in the presence of $CD4^+$ T-cell help (Reiser *et al.*, 2011). The T-cell response to PC has been only preliminarily investigated (Mele *et al.*, 2017).

NAb, neutralizing antibodies; cCMV, congenital HCMV infection; PC, pentameric complex; mAbs monoclonal antibodies.



The Journal of Infectious Diseases

SUPPLEMENT ARTICLE

The Status of Vaccine Development Against the Human Cytomegalovirus

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Table 4 - *Live HCMV vaccines.*

4.1. Towne vaccine	Developed by Plotkin *et al.* (1975) following 125 passages in HELF, the *Towne vaccine* exhibited the following properties in *Phase I/II* clinical studies: 1) no virus excretion; 2) no virus latency; 3) NAb induction; 4) generation of both HCMV-specific $CD4^+$ and $CD8^+$ T-cells; 5) partial protection against virus challenge in both the immunocompetent and the immunocompromised host; 6) The molecular basis of attenuation was later attributed to a 2-bp insertion (TT) causing an aa frameshift mutation in 130 (Murphy *et al.*, 2003).
4.2. AD-169 vaccine	Developed by Elek & Stern in 1974 in London, the *AD-169 vaccine* was abandoned at the end of the 70s (Neff *et al.*, 1979). Later in 2012, the mutated PC in AD-169 was restored by serial passages in endothelial cells (Fu *et al.*, 2012). The revertant AD-169 virus with a restored PC was genetically modified by incorporating a synthetic compound (Shield-1), which allows virus replication only in its presence. This disabled infectious single-cycle (DISC) HCMV vaccine, named V160, was able to induce both humoral and T-cell responses in NHP (Wang *et al.*, 2016) as well as in humans in a *Phase I* study (Fu *et al.*, 2018).
4.3. Towne/Toledo chimera vaccines	Four genetic *Towne/Toledo recombinant chimera vaccine* candidates were constructed by substituting genomic regions of the low-passage Toledo strain with attenuated Towne strain genomic regions (Kemble *et al.*, 1996). In a *Phase I* study, vaccines were well tolerated and not excreted in humans (Adler *et al.*, 2016).
4.4. Viral vectored HCMV vaccines	The following heterologous viral vectors were used to deliver HCMV antigens (clinical trial *Phase/experimental animal* inoculation): 1) *Canarypox virus vector,* delivering either gB (Adler *et al.*, 1999) or pp65 (Berencsi *et al.*, 2001) *(Phase I)*. 2) *Alphavirus vector*, the Venezuelan Equine Encephalitis (VEE) virus delivering extracellular gB and a pp65/IE-1 fusion protein (Reap *et al.*, 2007a, b) *(Phase I)*. 3) *Lymphocyte choriomeningitis virus vector*, delivering pp65 and gB in a bivalent vaccine (Schleiss *et al.*, 2017a) *(Phase I)*. 4) *Modified vaccinia Ankara virus (MVA) vector* expressing: a) pp65, gB, IE-1, IE-2, PC (Wussow *et al.*, 2013); b) pp65, IE-1 (exon-4) and IE-2 (exon-5) in the Triplex vaccine (La Rosa *et al.*, 2017) *(Phase I)*; c) all five PC subunits in HEK cells or following BAC-cloning of MVA vector (Chiuppesi *et al.*, 2017; Wussow *et al.*, 2018) *(mice)*. 5) *Adenovirus type 6 vector* expressing IE-1, IE-2 and pp65 (Tang *et al.*, 2017) *(mice & NHP)*.
4.5. Alphavirus replicon particles (VRPs) vaccines	A *VRP vaccine* is an RNA-based vaccine consisting of an attenuated strain of VEE virus, in which the structural VEE virus protein genes are replaced with HCMV genes encoding for gB or the pp65/IE-1 fusion protein and VEE non-structural genes, while two helper RNAs encode for VEE structural proteins allowing replicon RNA packaging into VRPs. 1) A *bicistronic vaccine (AVX601)* expressing gB and a pp65/IE-1 fusion protein was developed (Reap *et al.*, 2007a, b) and shown to be safe and immunogenic in humans in a *Phase I trial* (Bernstein *et al.*, 2010). 2) Recently, a comparison of VRPs encoding HCMV gH/gL and PC with purified gH/gL and PC complexes showed that PC elicits higher NAb titers than gH/gL *in mice* (Wen *et al.*, 2014).

PC, pentameric complex; VEE Venezuelan equine encephalitis virus; MVA, Modified Vaccinia Ankara virus; VRPs, Virus Replicon Particles; NAb, Neutralizing Antibody; HELF, human embryonic lung fibroblasts; aa, aminoacid; HEK, human embryonic kidney; NHP, non-human primates.

Table 5 - *Non-living HCMV vaccines.*

5.1. gB subunit vaccines	1) The first *gB vaccine* was developed at Chiron (Gonczol *et al.*, 1990) and administered in *Phase II* clinical trials to both adults and toddlers (Pass *et al.*, 1999; Mitchell *et al.*, 2002). However, the antibody response was short-lived. 2) Subsequently, Novartis and then Pasteur modified the gB subunit vaccine by removing the gB transmembrane domain. This vaccine was tested in several *Phase II* clinical trials (Pass *et al.*, 2009; Griffiths *et al.*, 2011; Bernstein *et al.*, 2016), showing a fair degree of protection against primary infection in seronegative and a boosting of immune response in seropositive women.
5.2. DNA-based vaccines	Different types of *plasmid-based DNA* vaccines have been developed, as follows: 1) *ASP0113 bivalent vaccine* consisting of two plasmids, one containing (VCL-6368) a modified pp65 gene, and the other (VCL-6365) a portion of the genomic region encoding the extracellular domain of AD169 gB (Selinski *et al.*, 2005). This vaccine has been clinically tested in *Phase I* (Wlock *et al.*, 2008) and *Phase II* trials in HSCT recipients (Kharfan-Dabaja *et al.*, 2012). In addition, it is being tested in a *Phase III* trial to investigate the therapeutic effect in HSCT recipients (expected to be completed in 2022). *2) Trivalent DNA vaccine (VCL-CT02)* encoding IE1, gB, and pp65 (Jacobson *et al.*, 2009) *(Phase I)* showing the priming effect of DNA vaccine in eliciting the immune response. 3) *SynCon (Synthetic Consensus sequence)* vaccine approach selecting the most conserved aa at each position in the antigen gene sequences, followed by the insertion of the selected sequences into a DNA plasmid (Ramanathan *et al.*, 2009). 4) Vaccine administration using the *Electroporation technique*, which allows the formation of transient pores in the cell membranes near the injection site of plasmids, thus facilitating entry of plasmids into the cytoplasm (Flingai *et al.*, 2013) *(mice & other experimental animals)*.
5.3. RNA-based vaccines	Multiple options have been developed/proposed: *1) synthetic self-amplifying mRNA* expressing a pp65-IE-1 construct and gB was administered to *rhesus macaques* inducing both NAb and T-cell responses (Novartis). *2) mRNA vaccine formulated with lipid nanoparticles* (LNP), encoding gB and PC, was administered to *NHP* and induced potent NAb response (Moderna Therapeutics). *3) mRNA-based multiantigenic vaccine* including pp65, gB and PC inoculated in *mice* elicited potent humoral and cell-mediated immune responses. However, T-cell responses to pp65 mRNA vaccine were inhibited by presence of other HCMV antigens (John *et al.*, 2018).
5.4. Virus-like particle (VLPs) vaccines	VLPs are *enveloped virus-like particles (eVLPs)* simulating wild-type viruses, but lacking viral genome. The following formulations have been proposed: 1) VIB Labs developed an eVLP vaccine expressing the extracellular sequence of gB fused with the transmembrane and the cytoplasmic domains of Vesicular Stomatitis Virus (VSV) G protein (Kirchmeier *et al.*, 2014) (*Phase I*, results expected). 2) VIB developed another eVLP vaccine by transfecting HEK-293 cells with a plasmid encoding the *gag* protein of Moloney murine leukemia virus fused in frame with the HCMV pp65 protein and co-transfecting with a gB plasmid, thus enabling VLP budding from transfected cells. 3) Redvax GmbH (Switzerland), using a baculovirus expression system, developed another eVLP candidate HCMV vaccine (Vicente *et al.*, 2014).
5.5. Dense body (DBs) vaccine	DBs are enveloped dense bodies accumulating inside HCMV-infected cells and containing glycoproteins and tegument proteins, but not DNA. DB inoculation in *mice* induced both NAb and T-cell responses (Becke *et al.*, 2010; Cayatte *et al.*, 2013).
5.6. Peptide vaccines	*Peptide vaccines* have been mostly directed to prevention/protection against HCMV disease in HSCT recipients. 1) In this context, HCMV pp65 has been found to be the most effective viral antigen, and its CTL epitope HLA*201-pp65$_{495\text{-}503}$ was identified as a peptide provided with a high protective potential and fused to a T-helper epitope acting as a stimulus for the immune response (La Rosa *et al.*, 2012). This vaccine, referred to as *PepVax*, was shown in a *Phase I* trial in HSCT recipients to be free of adverse effects. 2) Another peptide vaccine was developed in Australia as a *chimeric vaccine* encoding the extracellular domain of gB and epitopes from 8 different HCMV antigens, both HLA class I- and class II- restricted, expressed as a single fusion protein. Inoculation of *transgenic mice* with this vaccine produced robust antibody and T-cell responses (Dasari *et al.*, 2013).
5.7. Pentameric complex (PC) vaccines	PC was identified in 2008, following the discovery by our group (Hahn *et al.*, 2004) of the UL128-130-131 locus (UL128L) as indispensable for endothelial cell tropism, and the identification of its complex with gH/gL (Ryckman *et al.*, 2008) to form the pentameric gH/gL/pUL128L complex or PC. PC has been shown: a) to be the major HCMV neutralization antigen (Macagno *et al.*, 2010), b) to be required for infection of both endothelial and epithelial cells. Some PC vaccine applications include: 1) *The repair of a frameshift mutation in the UL131 gene* of the *AD169* PC restored PC expression with improved immunogenicity in different animal species (Fu *et al.*, 2012). The revertant AD169 virus with restored PC was genetically modified by incorporating a synthetic compound (V160 vaccine). Several *Phase I* trials have shown that the V160 vaccine is safe and immunogenic inducing both NAb and T-cell responses (Wang *et al.*, 2016; Ha *et al.*, 2017). 2) Several additional PC-based vaccines have been developed, as follows: a) Using an *MVA virus expressing the 5 PC subunits*, a soluble PC was produced in HEK-293 cells and inoculated in *mice* (Chiuppesi *et al.*, 2017). b) A *BAC-cloned MVA vector expressing the 5 PC subunits* was inoculated in *mice* obtaining a potent NAb response (Wussow *et al.*, 2018). c) *VRPs generated with the VEE virus and expressing PC* adjuvanted with MF59 produced high NAb levels in *mice* (Novartis Vaccines). d) *PC vaccine produced in CHO cells* (Kabanova *et al.*, 2014) induced in *mice* a high titer NAb response together with a block of virus dissemination (Gerna *et al.*, 2016).

gB, glycoprotein B; HSCT, Hematopoietic Stem Cell Transplant; PC, Pentameric complex; UL128L, UL128-UL130-UL131 locus; BAC, Bacterial Artificial Chromosome; VRPs, Virus Replicon Particles; MVA, Modified Vaccinia Ankara virus; VEE, Venezuelan Equine Encephalitis virus; CHO cells, Chinese Hamster Ovarian cells; mAbs, monoclonal antibodies.

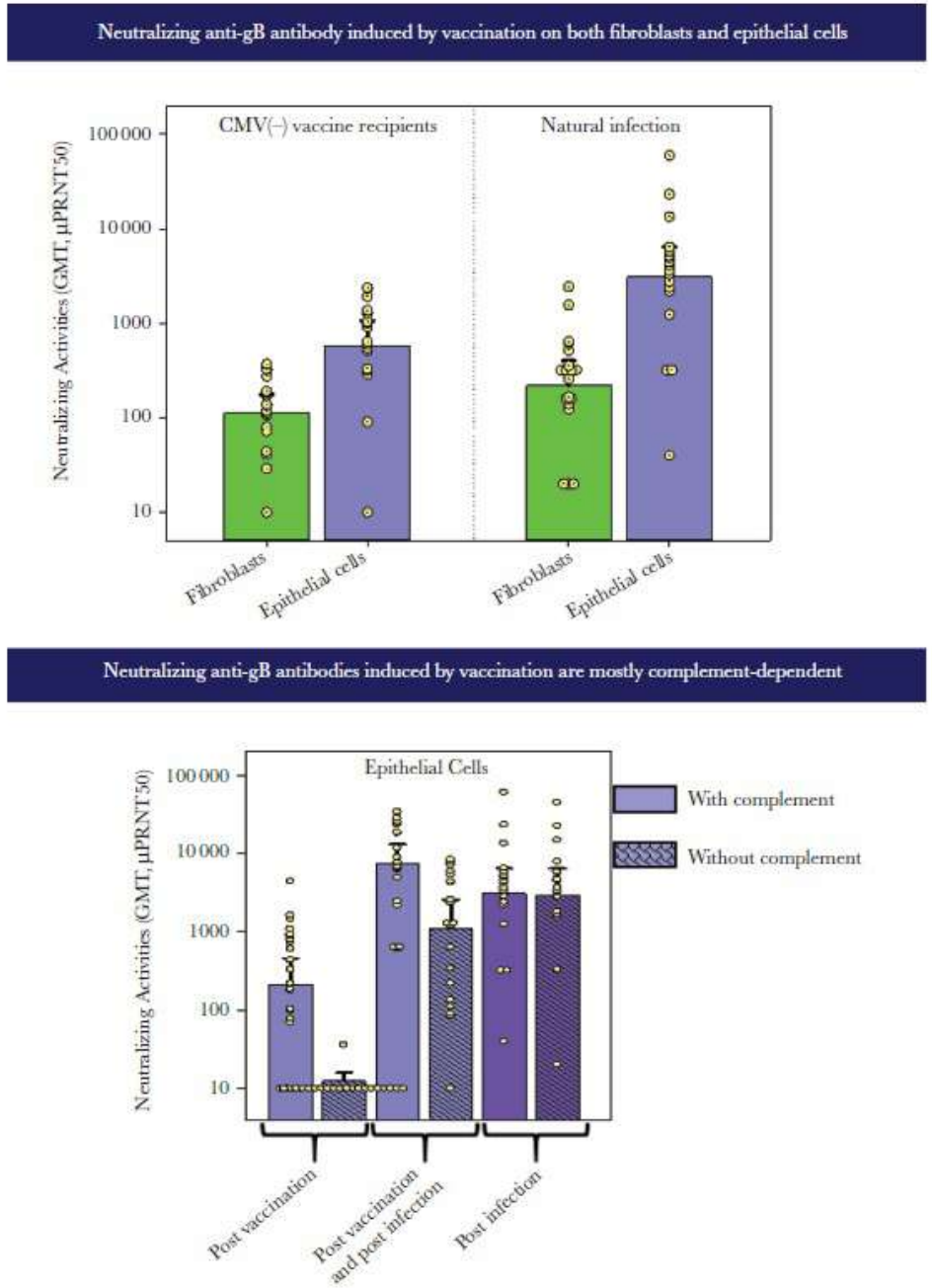


Figure 1. Responses to Sanofi-Pasteur glycoprotein B (gB). CMV, cytomegalovirus; GMT, geometric mean titers.

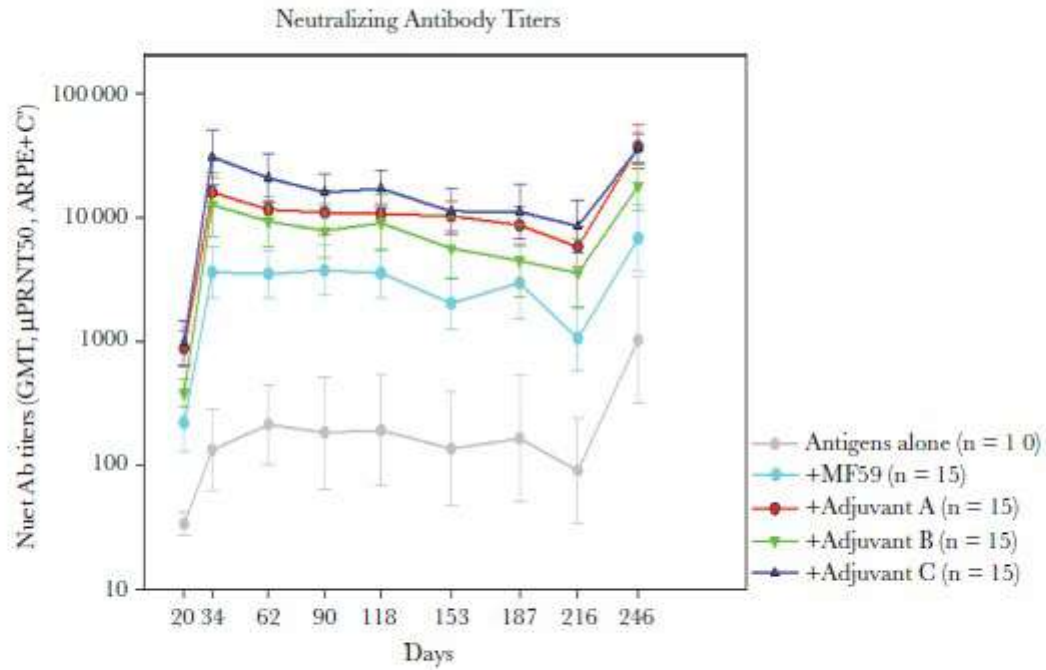


Figure 2. Neutralizing antibody responses to glycoprotein B with various adjuvants. GMT, geometric mean titers.

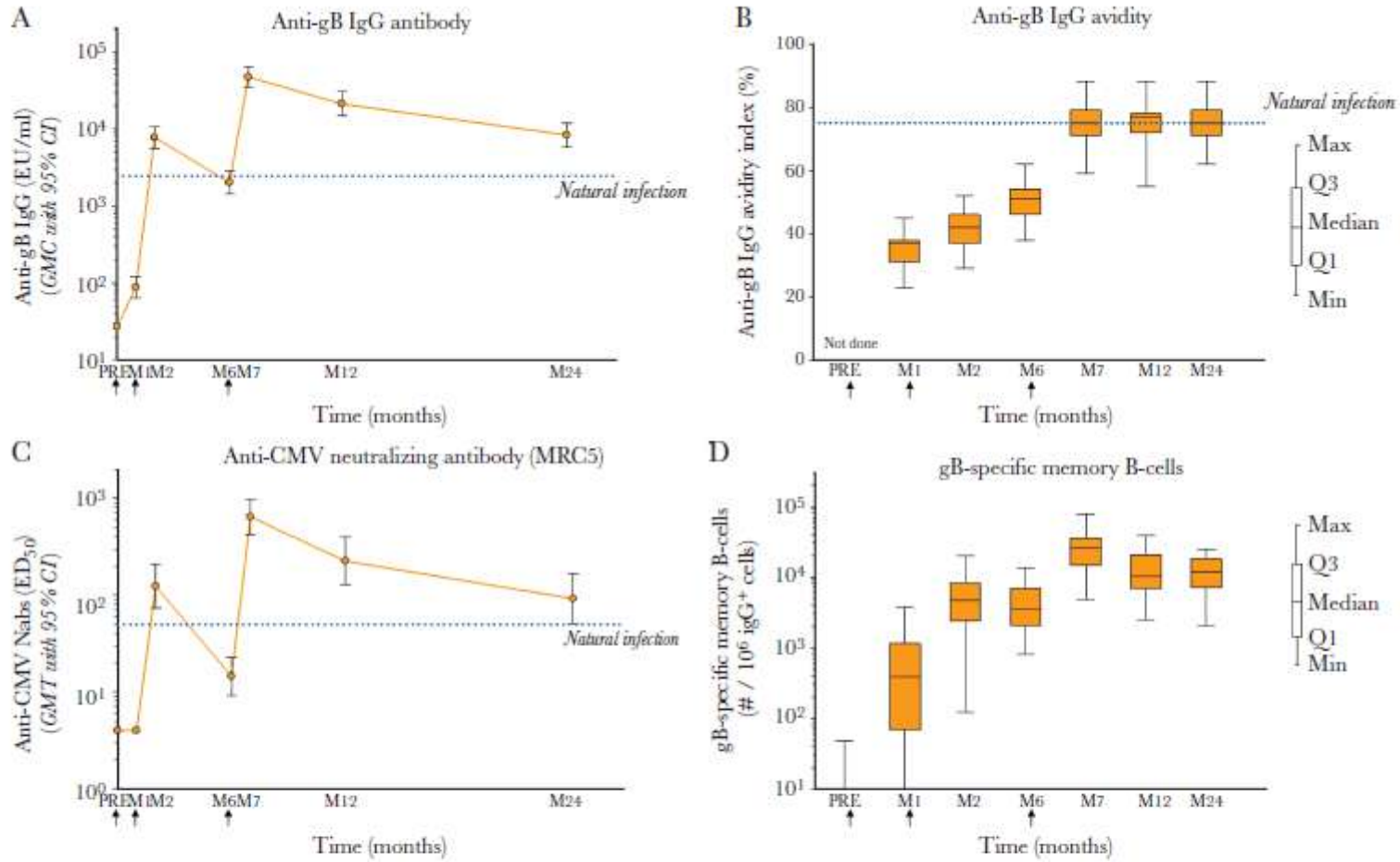


Figure 5. Kinetics of cytomegalovirus (CMV)-specific humoral and B-cell responses to GSK glycoprotein B (gB) combined with AS01E adjuvant. At the indicated time points (A), anti-gB immunoglobulin (Ig)G antibodies were measured by enzyme-linked immunosorbent assay; (B) anti-CMV neutralizing antibodies (Nabs) were measured by microneutralization test using MRC5 fibroblast cells and the AD169 CMV strain; (C) anti-gB IgG avidity indexes were measured by avidity enzyme-linked immunosorbent assay using a chaotropic agent (8 M urea); and (D) gB-specific antibody-secreting B-cells were determined by enzyme-linked immunospot assay. (A) and (B) show geometric mean titer (GMT)/geometric mean concentrations (GMC) and 95% confidence interval (CI). (C) and (D) show boxplots: the box represents the upper and lower quartiles; the horizontal line within the box represents the median value; the whiskers represent the minimum and maximum values. Arrows represent time of vaccine dose administration. Natural infection levels of anti-gB IgG (and avidity index) and anti-CMV neutralizing antibodies were determined at the month 0 timepoint from 39 CMV-seropositive healthy male subjects (dotted line).

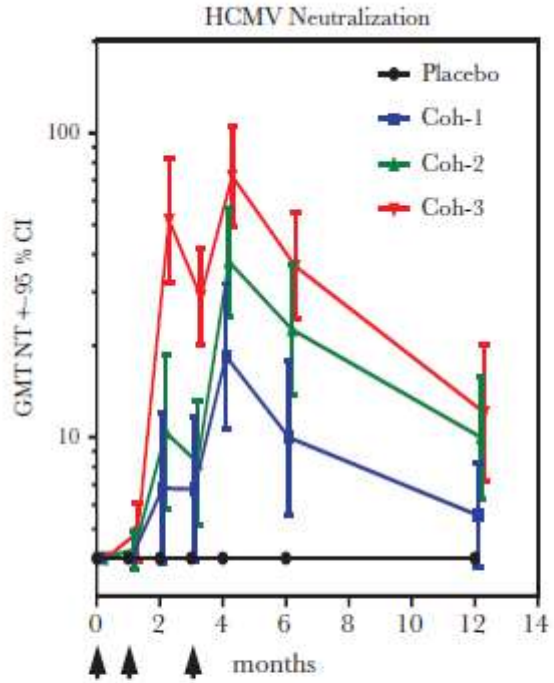
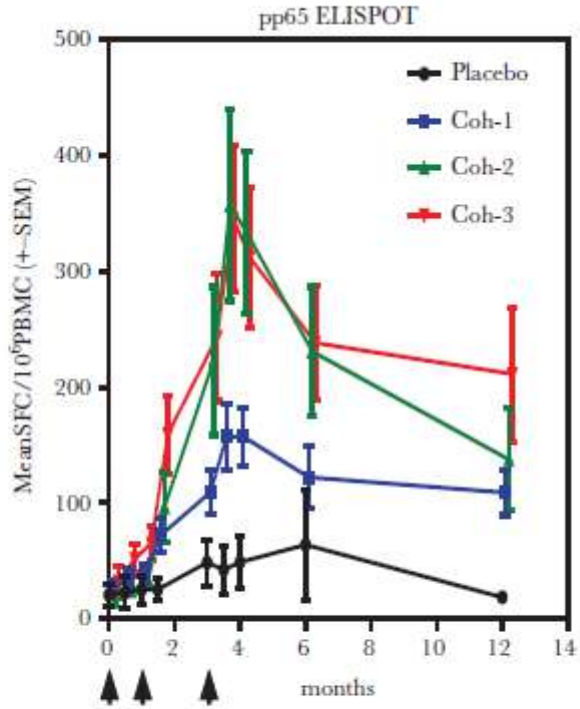


Figure 3. Hookipa HP-101 elicited significant human cytomegalovirus (HCMV)-neutralizing antibody titers compared with placebo: Cohort (Coh) 1, 2.6×10^6 focus-forming units (FFU); Coh 2, 2.6×10^7 FFU; Coh 3, 2.6×10^8 FFU. CI, confidence interval; GMT, geometric mean titers.



Hookipa HB-101 elicited a significant, durable pp65-specific cellular immune response Cohort 1: 2.6×10⁶ FFU; Cohort 2: 2.6×10⁷FFU; Cohort 3: 2.6×10⁸ FFU

Figure 4. Hookipa HB-101 elicited a significant, durable pp65-specific cellular immune response: Cohort (Coh) 1, 2.6×10^6 focus-forming units (FFU); Coh 2, 2.6×10^7 FFU; Coh 3, 2.6×10^8 FFU. ELISPOT, enzyme-linked immunospot; PBMC, peripheral blood mononuclear cells; SEM, standard error of the mean; SFC, spot-forming cells.

Table 7 - *Formulation of an ideal HCMV vaccine.*

Primary endpoint	It should be the prevention of primary HCMV infection in adult seronegative subjects at risk of infection. This seronegative population would include adult women of childbearing age and patients included on the list of SOT and HSCT candidates. However, these patients, once seropositive after vaccination, might not be protected against newly-infecting virus strains, as recently and repeatedly documented.
Secondary long-term endpoint	It should be the achievement of herd immunity, ideally protecting against any new contact with different virus strains. This approach would require vaccination of the entire population, perhaps starting in the first years of life, and administration of booster vaccine doses in the following years/decades. This ideal vaccine would require the acquisition of a lifelong evaluable immune response, both humoral and T-cell mediated.
Schedule	Extended clinical trials should be conducted on the non-immune component of the adult population (both males and females) of one or more developed countries to define the levels of immune protection against re-activation/re-infection episodes of HCMV infection following vaccination. Presumably, levels of protection should refer to cut-off levels (and/or protective activity) of HCMV-specific antibodies and, particularly, NAb, as well as to cut-off levels (and/or protective activity) of HCMV-specific $CD4^+$ and $CD8^+$ T-cells. If immune protection is virus strain-specific, this would make definition of protection more intriguing. However, the follow-up of vaccinees would allow vaccine developers to draw useful conclusions also for seropositive individuals, if they were to receive the same vaccine.
Vaccine composition	It is still unclear whether vaccine components should refer to a single HCMV strain or to multiple strains selected on the basis of consensus sequencing of the major viral antigens. Based on current knowledge, a hypothetical vaccine should contain: 1) gB inducing both antibody and T-cell responses; 2) PC, inducing the most potent NAb response; 3) pp65, inducing the most potent T-cell response.