### 1

### Case

### ADV

**Pharma innovation is doing great now – answers all your warrants.**

Lisa Jarvis, 1-17-2020, (Based in Chicago, Lisa has been covering the biotech and pharmaceutical industries at C&EN since 2006. She writes feature articles that weave together the business and science of developing drugs, while also serving as pharmaceuticals editor for the magazine. She has a particular interest in rare diseases, innovative models for drug discovery, and emerging technologies.) "The new drugs of 2019," Chemical &amp; Engineering News, <https://cen.acs.org/pharmaceuticals/drug-development/new-drugs-2019/98/i3> //Jay

Although pharmaceutical companies last year were unable to top the record-shattering [59 new drugs approved in the US in 2018](https://cen.acs.org/pharmaceuticals/drug-development/new-drugs-2018/97/i3), they were still on a roll. In 2019, the Food and Drug Administration green-lighted 48 medicines, a crop that includes myriad modalities and many new treatments for long-neglected diseases. Taken together, the past 3 years of approvals represent drug companies’ most productive period in more than 2 decades. Still, some analysts caution that the steady flow of new medicines could mask troubling indications about the health of the industry. The year brought several notable trends. The first was an uptick in the number of novel mechanisms on display in the new drugs. Roughly 42% of the medicines were first in class, meaning they had new mechanisms of action; this is a jump over the prior 4 years, when that portion ranged between 32 and 36%. Another trend was the influx of newer modalities. While small molecules continue to account for the lion’s share of new molecular entities (NMEs), making up 67% of overall approvals in 2019, the list also includes several antibody-drug conjugates, an antisense oligonucleotide therapy, and a therapy based on RNA interference (RNAi). Yet another encouraging trend was the influx of innovative therapies for underserved diseases. Standout approvals include two new drugs for sickle cell anemia (Global Blood Therapeutics’ Oxbryta and Novartis’s Adakveo), an antibiotic for treatment-resistant tuberculosis (Global Alliance for TB Drug Development’s pretomanid), and a therapy for women experiencing postpartum depression (Sage Therapeutics’ Zulresso). “The quality of the drugs over the last decade or so has steadily improved since the depths of the innovation crisis 10–12 years ago,” says Bernard Munos, a senior fellow at FasterCures, a drug research think tank. “We’re seeing stuff that frankly would have looked like science fiction back then.” Those futuristic new therapies include [Novartis’s Zolgensma](https://cen.acs.org/articles/97/i22/FDA-approves-second-gene-therapy.html), a gene therapy for spinal muscular atrophy; Alnylam Pharmaceuticals’ Givlaari, the company’s second marketed RNAi-based therapy; and several critical vaccines for infectious diseases, including Ebola, smallpox, and dengue fever. Not all those edgy therapies appear in C&EN’s list. We track approvals granted through the FDA’s main drug approval arm, the Center for Drug Evaluation and Research; drugs like vaccines and gene therapies are generally reviewed through the agency’s Center for Biologics Evaluation and Research. The new-approvals list also doesn’t include several therapies that made their way to patients for the first time, even though the FDA doesn’t consider them new drugs. For example, the agency gave its green light to Johnson & Johnson’s Spravato, making it the first new treatment option for people with major depressive disorder in more than 50 years. The drug is the S enantiomer of ketamine, an N-methyl-D-aspartate receptor antagonist that had been long approved as an anesthetic, gained notoriety as a club drug, and was used for years off label to treat severe depression ([see page 18](https://cen.acs.org/biological-chemistry/neuroscience/Ketamine-revolutionizing-antidepressant-research-still/98/i3)). Also notable in 2019 was a slight dip in the number of cancer drugs, which in recent years typically made up more than a quarter of all new medicines. Last year’s 11 cancer treatments accounted for roughly 23% of approvals.

#### **Reducing IP protections chills future investment – even the perception of wavering commitment scares off companies.**

Grabowski et al. ’15 (Harry; Professor Emeritus of Economics at Duke, and a specialist in the intersection of the pharmaceutical industry and government regulation of business; February 2015; “The Roles Of Patents And Research And Development Incentives In Biopharmaceutical Innovation”; Health Affairs; <https://www.healthaffairs.org/doi/10.1377/hlthaff.2014.1047>; Accessed: 8-31-2021; AU)

Patents and other forms of **intellectual property** **protection** play **essential roles** in encouraging innovation in biopharmaceuticals. As part of the “21st Century Cures” initiative, Congress is reviewing the policy mechanisms designed to accelerate the discovery, development, and delivery of new treatments. Debate continues about how best to balance patent and intellectual property incentives to encourage innovation, on the one hand, and generic utilization and price competition, on the other hand. We review the current framework for accomplishing these dual objectives and the important role of patents and regulatory exclusivity (together, the patent-based system), given the lengthy, costly, and risky biopharmaceutical research and development process. We summarize existing targeted incentives, such as for orphan drugs and neglected diseases, and we consider the pros and cons of proposed voluntary or mandatory alternatives to the patent-based system, such as prizes and government research and development contracting. We conclude that patents and regulatory exclusivity provisions are likely to remain the core approach to providing incentives for biopharmaceutical research and development. However, prizes and other voluntary supplements could play a useful role in addressing unmet needs and gaps in specific circumstances. Technological innovation is widely recognized as a key determinant of economic and public health progress. 1,2 Patents and other forms of intellectual property protection are generally thought to play essential roles in encouraging innovation in biopharmaceuticals. This is because the process of developing a new drug and bringing it to market is **long, costly, and risky**, and the costs of imitation are low. After a new drug has been approved and is being marketed, its **patents protect it** from competition from chemically identical entrants (or entrants infringing on other patents) for a period of time. **For firms** to have an **incentive** to **continue to invest** in innovative development efforts, they must have an **expectation** that they can **charge enough** during this period to **recoup** costs and make a profit. After a drug’s patent or patents expire, **generic rivals** can enter the market at **greatly reduced development cost** and prices, providing added consumer benefit but **eroding** the **innovator drug** company’s revenues. The Drug Price Competition and Patent Term Restoration Act of 1984 (commonly known as the Hatch-Waxman Act) was designed to balance innovation incentives and generic price competition for new drugs (generally small-molecule chemical drugs, with some large-molecule biologic exceptions) by extending the period of a drug’s marketing exclusivity while providing a regulatory framework for generic drug approval. This framework was later changed to encompass so-called biosimilars for large-molecule (biologic) drugs through the separate Biologics Price Competition and Innovation Act of 2009. Other measures have been enacted to provide research and development (R&D) incentives for antibiotics and drugs to treat orphan diseases and neglected tropical diseases. Discussion continues about whether current innovation incentives are optimal or even adequate, given evolving public health needs and scientific knowledge. For instance, the House Energy and Commerce Committee recently embarked on the “21st Century Cures” initiative, 3 following earlier recommendations by the President’s Council of Advisors on Science and Technology on responding to challenges in “propelling innovation in drug discovery, development, and evaluation.” 4 In this context, we discuss the importance of patents and other forms of intellectual property protection to biopharmaceutical innovation, given the unique economic characteristics of drug research and development. We also review the R&D incentives that complement patents in certain circumstances. Finally, we consider the pros and cons of selected voluntary (“opt-in”) or mandatory alternatives to the current patent- and regulatory exclusivity–based system (such as prizes or government-contracted drug development) and whether they could better achieve the dual goals of innovation incentives and price competition. The essential rationale for patent protection for biopharmaceuticals is that long-term benefits in the form of continued future innovation by pioneer or brand-name drug manufacturers outweigh the relatively short-term restrictions on imitative cost competition associated with market exclusivity. Regardless, the entry of other branded agents remains an important source of therapeutic competition during the patent term. Several economic characteristics make patents and intellectual property protection **particularly important** to **innovation incentives** for the biopharmaceutical industry. 5 The R&D process often takes more than a decade to complete, and according to a recent analysis by Joseph DiMasi and colleagues, per new drug approval (including failed attempts), it involves more than a **billion** dollars in out-of-pocket costs. 6 Only approximately one in eight drug candidates survive clinical testing. 6 As a result of the high risks of failure and the high costs, research and development must be funded by the **few successful, on-market products** (the top quintile of marketed products provide the dominant share of R&D returns). 7,8 Once a new drug’s patent term and any regulatory exclusivity provisions have expired, competing manufacturers are allowed to sell generic equivalents that require the investment of only several million dollars and that have a high likelihood of commercial success. **Absent intellectual property protections** that allow marketing exclusivity, innovative firms would be **unlikely** to make the costly and risky investments needed to bring a new drug to market. Patents confer the right to exclude competitors for a limited time within a given scope, as defined by patent claims. However, **they do not guarantee demand**, nor do they prevent competition from nonidentical drugs that treat the same diseases and fall outside the protection of the patents. New products may enter the same therapeutic class with common mechanisms of action but different molecular structures (for example, different statins) or with differing mechanisms of action (such as calcium channel blockers and angiotensin receptor blockers). 9 Joseph DiMasi and Laura Faden have found that the time between a first-in-class new drug and subsequent new drugs in the same therapeutic class has been dramatically reduced, from a median of 10.2 years in the 1970s to 2.5 years in the early 2000s. 10 Drugs in the same class compete through quality and price for preferred placement on drug formularies and physicians’ choices for patient treatment. Patents play an **essential role** in the economic “ecosystem” of **discovery and investment** that has developed since the 1980s. Hundreds of start-up firms, often backed by venture capital, have been launched, and a robust innovation market has emerged. 11 The value of these development-stage firms is largely determined by their proprietary technologies and the candidate drugs they have in development. As a result, the **strength of intellectual property protection** plays a **key role** in funding and partnership opportunities for such firms. Universities also play a key role in the R&D ecosystem because they conduct basic biomedical research supported by sponsored research grants from the National Institutes of Health (NIH) and the National Science Foundation (NSF). The Patent and Trademark Law Amendments Act of 1980 (commonly known as the Bayh-Dole Act) gave universities the right to retain title to patents and discoveries made through federally funded research. This change was designed to encourage technology transfer through industry licensing and the creation of start-up companies. Universities received only 390 patents for their discoveries in 1980, 12 compared to 4,296 in 2011, with biotechnology and pharmaceuticals being the top two technology areas (accounting for 36 percent of all university patent awards in 2012). 13

Park et al 21

Deok Bum Park, Bo-Eun Ahn, Hosun Son, Young-Ran Lee, Yu-Ri Kim, Su Kyoung Jo, Jeong-Hoon Chun, Jae-Yon Yu, Myung-Min Choi & Gi-eun Rhie Construction of a bivalent vaccine against anthrax and smallpox using the attenuated vaccinia virus KVAC103 BMC Microbiology volume 21, Article number: 76 (2021) <https://bmcmicrobiol.biomedcentral.com/articles/10.1186/s12866-021-02121-5> -CAT

Anthrax and smallpox are high-risk infectious diseases, and considered as potential agents for bioterrorism. To develop an effective countermeasure for these diseases, we constructed a bivalent vaccine against both anthrax and smallpox by integrating a gene encoding protective antigen (PA) of Bacillus anthracis to the genome of the attenuated vaccinia virus strain, KVAC103. Results Immunization with this bivalent vaccine induced antibodies against both PA and vaccinia virus in a mouse model. We also observed that the efficacy of this vaccine can be enhanced by combined immunization with immunoadjuvant-expressing KVAC103. Mouse groups co-immunized with PA-expressing KVAC103 and either interleukin-15 (IL-15) or cholera toxin subunit A (CTA1)-expressing KVAC103 showed increased anti-PA IgG titer and survival rate against B. anthracis spore challenge compared to the group immunized with PA-expressing KVAC103 alone. Conclusions We demonstrated that the attenuated smallpox vaccine KVAC103 is an available platform for a multivalent vaccine and co-immunization of immunoadjuvants can improve vaccine performance. Background Bacillus anthracis and Variola virus are causative agents of anthrax and smallpox, respectively, and representative pathogens that can be possibly utilized as bioterrorism or biological weapons. Development of effective medical countermeasures against these pathogens is a national task of high priority [1, 2]. The biological attack in 2001 by B. anthracis spores via the US postal system has prompted the need to develop vaccines and therapeutics against anthrax [1]. Protective antigen (PA) is one of the major component of anthrax toxin, and also a principal ingredient of two licensed anthrax vaccines, Anthrax Vaccine Adsorbed (AVA) and Anthrax Vaccine Precipitated (AVP) [3]. Recently, a recombinant PA protein vaccine is being developed by Korea Centers for Disease Control (KCDC), and clinical trials are in progress [4, 5]. Although endemic smallpox was declared eradicated since the last case observed in 1977, Variolar virus still remains a potential biological weapon [2], and smallpox vaccines have been stockpiled for strategic use in some nations. To reduce side effects of conventional smallpox vaccines, attenuated vaccinia virus strains have been investigated in various ways [6]. KVAC103 is an attenuated vaccinia virus developed by KCDC [7]. Interleukin-15 (IL-15) is a cytokine involved in the proliferation and maintenance of CD8+ memory T cells, and has been suggested as an effective vaccine adjuvant [8, 9]. Previous studies on HIV-1 vaccine demonstrated that co-immunization of IL-15 strongly increased antigen-specific memory T cells and long-term immunity [10, 11]. Smallpox vaccines with integrated IL-15, tested in a mouse model, showed increased and prolonged cellular and humoral immunity [12]. This IL-15-containing smallpox vaccine also has been applied in a multivalent influenza vaccine [13]. Co-administration of IL-15 with staphylococcal enterotoxin B vaccine increased the number of dendritic cells in a mouse model [14]. Cholera toxin (CT) also has long been investigated as an efficient immunoadjuvant. The toxin is composed of subunit A and B, and subunit A contains two fragments, A1 and A2 [15]. The ADP-ribosyltransferase activity of cholera toxin subunit A1 (CTA1) is known to be important for enhancing immune responses [16]. The effect of CTA1 as an immunoadjuvant has been demonstrated against numerous pathogens, such as influenza A virus, HIV, Helicobacter pylori, and Mycobacterium tuberculosis [17,18,19,20]. Vaccinia virus is a popular platform for gene transfer and multivalent vaccine against various diseases [21, 22]. In a previous study, a dual vaccine for smallpox and anthrax has been developed by inserting PA gene of B. anthracis into Wyeth or modified vaccinia Ankara (MVA) strain [23]. A viral vector system that utilizes KVAC103 as a gene delivery system and a multivalent vaccine has been previously invented [7, 24]. In this study, we constructed a bivalent vaccine candidate against both smallpox and anthrax, by integrating a recombinant anthrax PA-encoding gene into KVAC103, using a viral vector pVVT1-EGFP-C7L. We examined the protective efficacy of KVAC103-derived bivalent vaccine in a mouse model. In addition, we observed that the vaccine supplemented with immunoadjuvant-expressing vaccinia viruses can increase immune response against anthrax. Results A human codon-optimized PA was cloned into viral vector pVVT1 to generate smallpox/anthrax dual vaccine candidate. A signal peptide derived from the tissue plasminogen activator was attached to the N-terminal of PA (thPA). We also constructed viral vector clones encoding a human IL-15 (hIL15) or a human codon-optimized CTA1 (hCTA1) gene. The viral vectors were integrated into the KVAC103 genome by homologous recombination at the thymidine kinase (TK) gene site (Fig. 1). Fig. 1 figure1 A diagram of viral vector construction. Human codon-optimized genes encoding PA with a signal peptide (MDAMKRGLCCVLLLCGAVFVSP) derived from the tissue plasminogen activator polypeptide (thPA), IL-15 (hIL15), or CTA1 (hCTA1) were cloned into pVVT1-EGFP-C7L. The GeneBank sequence ID for PA, IL-15, and CTA1 are AAA22637.1, NP\_000576.1, and CAA24995.1, respectively. The expected molecular weight of integrated thPA, IL-15, and CTA1 are 81 kDa (757 aa), 18 kDa (162 aa), and 29 kDa (258 aa), respectively. Viral vector constructs are integrated into KVAC103 genome by homologous recombination at TK gene site Full size image Protein expression of PA and CTA1 in the dual vaccine candidate viruses were confirmed by immunoblot assay (Fig. 2)a. PA and CTA1 were detected in virus-infected cell lysates. This indicates that cells infected by KVAC-thPA-C7L or KVAC-hCTA1-C7L viruses properly express PA or CTA1, respectively. The IL-15 ELISA result shows that cells infected by KVAC-hIL15-C7L also secreted IL-15 in vitro (Fig. 2b). Fig. 2 figure2 Expression of integrated proteins in vitro. a The expression of PA and CTA1 was detected by immunoblot assay in cell lysates. Vero cells were infected with KVAC-thPA-C7L and KVAC-CTA1-C7L for 48 h, and 50 μg of cell lysates from the infected cells were analyzed. The molecular weights from a size marker are indicated on the left. b The expression level of IL-15 was detected by ELISA. The IL-15 expression levels of infected Vero cells with KVAC103 derivatives were determined by the IL-15 ELISA kit (Biolegend). The bars on the graph indicates means of IL-15 expression levels from duplicated results in the same experiments, and the error bars stand for the standard error of the mean (\*\*\*P < 0.001). NS, not significant. One-way ANOVA was applied for analysis Full size image In a preliminary experiment, we observed that repeated vaccination in 3 week interval increased the anti-PA antibody titer around 10-fold compared to single vaccination (data not shown). The in vivo efficacy of the dual vaccine candidate with or without adjuvant-expressing viruses was estimated in a mouse model (Fig. 3). We immunized A/J mice (n = 8) with our vaccine candidate KVAC-thPA-C7L with or without adjuvant expressing viruses 2 times with a 3-week interval. The anti-PA antibody levels of all groups immunized with KVAC-thPA-C7L were increased compared to the groups immunized with the adjuvant only (KVAC-hIL15-C7L or KVAC-hCTA1-C7L). Mouse groups vaccinated with KVAC-thPA-C7L plus an immunoadjuvant-containing strain (KVAC-hIL15-C7L or KVAC-CTA1-C7L) exhibited higher mean values of antibody titers compared to the group immunized with KVAC-thPA-C7L only (Fig. 3a). Except the two outliers which are extraordinarily high in the group immunized with KVAC-thPA-C7L only in Fig. 3a (29,800 and 30,600), the mean values of anti-PA antibody titer are significantly increased in the mouse groups immunized with both KVAC-tPA-C7L and adjuvant-expressing strains (One-way ANOVA, p value < 0.01). Fig. 3 figure3 Immunogenicity and protective efficacy of the bivalent vaccine with or without adjuvant-expressing viruses in a mouse model. a Anti-PA IgG titers of individual mice in 5 groups (n = 8 for each group) were determined by ELISA. The Y-axis represents EC50 values. The horizontal bars indicate mean of individual groups (for KVAC-thPA-C7L, the mean value calculated except the two outliers). The error bars represent standard error of the mean. The asterisks (\*\*) represent significant differences between indicated groups in statistical analysis (\*\*P < 0.01). NMS, normal mouse sera. b Anti-viral antibody titers were determined by PRNT assay. The Y-axis represents PRNT50, the reciprocal of the dilution factor of sera reducing plaque formation in half. The bars represent arithmetic means of results from two independent assays with pooled sera of individual groups (eight mice per group) and the error bars represent standard error of the mean (\*\*\*P < 0.001). NS, not significant. NMS, normal mouse sera. c Immunized mice were challenged with 50 × LD50 of B. anthracis Sterne spores by s.c. injections. Survival rates of 5 groups (n = 8 for each group) were observed for 14 days. The p-value between KVAC-thPA-C7L and KVAC-C7L with immunoadjuvant only (KVAC-hIL-C7L, KVAC-hCTA1-C7L) is lower than 0.001 and indicated as \*\*\*. The p-value between KVAC-thPA-C7L and both KVAC-C7L with PA and immunoadjuvant (KVAC-thPA-C7L + KVAC-hIL-C7L, KVAC-thPA-C7L + KVAC-hCTA1-C7L) is lower than 0.05 and indicated as \* Full size image Neutralizing antibodies against vaccinia virus in mouse sera were measured by PRNT assay. Unlike the anti-PA antibodies, production of neutralizing antibodies against vaccinia virus does not appear to be significantly affected by the presence of immunoadjuvant (Fig. 3b). In a previous study, IL-15 expressing vaccinia virus induced increased neutralizing antibodies compared to the control vaccinia virus in a mouse model [12]. In our experiment, the effect of adjuvants was not observed and all the sera immunized with the constructs induced similar level of neutralizing antibodies. Immunized mice were challenged with B. anthracis Sterne spores 3 weeks after the final vaccination. Survival rates were monitored for 2 weeks. Mice immunized with adjuvant only were all dead within a week. In the group immunized with KVAC-thPA-C7L only, 62.5% of mice survived, while groups immunized with both KVAC-thPA-C7L and immunoadjuvant expressing virus (KVAC-hIL15-C7L or KVAC-CTA1-C7L) were fully protected from the challenge (Fig. 3c). The result indicates that enhanced immunity achieved by co-expression of adjuvant can protect the mice more effectively against anthrax. Discussion Poxviruses have been often used as a vector system for vaccines because of their large DNA genome and convenience in manipulation [21, 22]. In a previous study, engineered vaccinia strains expressing both PA and IL-15 showed enhanced immunogenicity against B. anthracis compared to the conventional anthrax vaccine AVA in animal test [23]. Our result presented that the co-expression of IL-15 in KVAC103 also enhanced protective efficacy of our bivalent vaccine. Co-expression of CTA1 induced immune response against the PA-expressing vaccine in the similar level to IL-15. Our result demonstrated that co-immunization of CTA1, as well as IL-15, was effective enough to enhance the immune responses against PA and reconfirmed that CTA1 is a suitable adjuvant for multivalent vaccines derived from KVAC103. This result is the first observation of the effect of CTA1 as an immunoadjuvant in a viral vaccine system. Conclusion In summary, we explored the possibility of developing a bivalent vaccine using KVAC103, an attenuated vaccinia virus strain. Like other vaccinia virus strains previously utilized, it is confirmed that KVAC103 also can serve as a useful platform for multivalent vaccines. In addition, the vaccine can be further effective with the supplement of cytokines or adjuvants. Methods Cell and virus Vero cell (African green monkey kidney cell) was purchased from the American Type Culture Collection (ATCC, USA). Cells were grown in Opti-Eagle’s Minimum Essential Medium (Opti-MEM, Invitrogen) supplemented with 2% heat-inactivated fetal bovine serum (FBS, Invitrogen), incubated at 37 °C, and humidified with 5% CO2. The attenuated vaccinia virus strain KVAC103 and the viral vector pVVT1-EGFP-C7L were provided by Korea National Institute of Health (KNIH). This vector contains the vaccine virus C7L gene which encodes interferon antagonist, and this is one of the 26 genes defective in KVAC103 compared to its ancestor strain. This gene is required for enhanced viral reproduction [24]. Construction of anthrax/smallpox dual vaccine candidate vectors Viral vector constructs were generated using the pVVT1-EGFP-C7L vector [24] as a template (Fig. 1). Human IL-15 gene and human codon-optimized B. anthracis PA and CTA1 genes were synthesized (Bioneer). The synthesized genes were cloned into the vector using SfiI restriction enzyme site. The constructed vectors were mixed with Lipofectamine (Invitrogen) and transfected into KVAC103-infected Vero cells. Single plaques were isolated from the original infected cells and verified using PCR. The primer sequences used for verification were 5′-TTT GAA GCA TTG GAA GCA ACT-3′ and ‘5’-ACG TTG AAA TGT CCC ATC GAG T-3′. Virus preparation Viruses were infected to mono-layered Vero cells with 0.01 MOI. The virus-infected cell media were harvested when more than 80% of total cells showed cytopathic effect. From the harvested culture supernatant, viruses were collected by ultra-centrifugation. The pellet was resuspended in 1× PBS, pH 7.0 (Gibco). The concentration of viral particles was determined by the standard plaque assay. The viruses were infected the Vero cells overlaid on 6-well plates for 2 days. The plate were staining with crystal violet and the plaque numbers on each well were counted. Western blot analysis Virus-infected Vero cells or their culture supernatants were lysed in 1× RIPA buffer (G-Bioscience) containing 1% PMSF (Theromfisher Scientific) at 4 °C. Fifty μg of protein from each cell lysate was resolved on denaturing polyacrylamide gel electrophoresis (PAGE) and transferred to polyvinylidene difluoride (PVDF) membrane (Amersham). Expression levels of PA and CTA1 proteins were detected using mouse monoclonal antibodies against PA and cholera toxin, respectively (Abcam, 1:1000), and horse radish peroxide (HRP)-conjugated secondary antibodies (Abcam, 1:3000). Mouse immunization and serum collection Female A/J mice (5-week old) were purchased from SLC, Inc. (Japan) and housed in an animal biosafety level 2 (ABL2) facility in KCDC. Mice were immunized with the vaccine candidate virus (5 × 107 pfu/mouse) with or without the adjuvant-expressing virus (5 × 107 pfu/mouse) 2-times at 3-week intervals subcutaneously (s.c.) with 8 mice as a group. Mice sera were collected 20 days after final immunization to measure anti-PA IgG and vaccinia virus plaque reduction neutralizing antibody titers. The schematic view of mouse immunization and serum collection is in Fig. 4. Fig. 4 figure4 A Schematic view of the animal experiment. Mice were immunized two times with 3 weeks interval and sera were collected by bleeding 20 days after the last vaccination. One day after bleeding, mice were challenged with anthrax spores. Survival was observed for 14 days Full size image Enzyme-linked immunosorbent assay (ELISA) The anti-PA IgG titers of mice sera were determined by ELISA as previously described with some modifications [25]. Briefly, 96 well plates were coated with 1 μg/ml of recombinant PA (Green Cross, Korea). Mouse sera were diluted from 1:100 to 1:204800 and loaded to each well, incubated for 1 h at 37 °C. Horseradish peroxidase-conjugated anti-mouse IgG goat antibody (Invitrogen) and 3,3`,5,5`-tetramethylbenzidine (TMB) substrate were used for detection. The optical density of each well was measured at 450 nm and the half maximal effective concentration (EC50) was calculated by 4-parameter logistic equation regression using SoftMaxPro5.3 (Molecular Device, USA). The data were analyzed and visualized using GraphPad Prism 5. The IL-15 expression level of KVAC103 derivatives were determined by the IL-15 ELISA kit (Biolegend) according to the manufacturer’s protocol. Vero cells were infected with viruses of 0.01 MOI. Cell lysates were collected 2 days after infection and analyzed. Plaque reduction neutralization test (PRNT) Serial two-fold dilutions of heat-inactivated mouse sera were mixed with vaccinia virus Lister strain of approximately 50 plaque forming units (PFU). After 2 h incubation at 37 °C, the serum and virus mixtures were inoculated onto monolayered Vero cells. After two days incubation at 37 °C with 5% CO2, cells were fixed and stained using a mixture of crystal violet and formalin for 10 min. Stained plates were dried in air at room temperature and the plaque numbers were counted. The neutralizing antibody titer was defined as the reciprocal of dilution factor that reduced plaque numbers in half (50%) compared to a serum-free control (PRNT50). B. anthracis spore challenge Immunized mice were challenged with 50-fold of lethal dose 50 (LD50) of B. anthracis Sterne spore by s.c. injections. Survival of the mice was monitored for 14 days as described in Fig. 4. Spores were prepared according to a previous study [26]. The LD50 determined by Reed-Muench method [27] in A/J mice model via s.c. route was 1794 spores. Survived animals were euthanized using CO2 gas. Animal study protocols (KCDC-102-16-2A and KCDC-039-17-2A) were approved by the Institutional Animal Care and Use Committee (IACUC) of Korea Centers for Disease Control and Prevention (KCDC). All procedures involved in the housing and care of animal strictly followed guidelines and requirements of the IACUC. Statistical analysis The Statistical analysis was performed using GraphPad Prism 5. To analyze the anti-PA ELISA titer, One-way ANOVA followed by Tukey’s post hoc test were used to evaluate the difference between groups. To analyze the survival rate, Kaplan-Meier survival plots were evaluated with the log-rank test. Availability of data and materials The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. References 1. Russell PK. Project BioShield: What It Is, Why It Is Needed, and Its Accomplishments So Far. Clin Infect Dis. 2007;45(Supplement\_1):S68–72. Article Google Scholar 2. Cieslak TJ, Christopher GW, Kortepeter MG, Rowe JR, Pavlin JA, Culpepper RC, Eitzen EM Jr. Immunization against potential biological warfare agents. Clin Infect Dis. 2000;30(6):843–50. CAS Article Google Scholar 3. Cybulski RJ Jr, Sanz P, O'Brien AD. Anthrax vaccination strategies. Mol Asp Med. 2009;30(6):490–502. CAS Article Google Scholar 4. Rhie GE, Park YM, Chun JH, Yoo CK, Seong WK, Oh HB. Expression and secretion of the protective antigen of Bacillus anthracis in Bacillus brevis. FEMS Immunol Med Microbiol. 2005;45(2):331–9. CAS Article Google Scholar 5. Kang CK, Kim NH, Kim CJ, Rhie GE, Jo SK, Ahn M, Kang J, Choe PG, Park WB, Kim NJ, et al. Immunogenicity and safety of a novel recombinant protective antigen anthrax vaccine (GC1109), a randomized, single-blind, placebo controlled phase II clinical study. Vaccine. 2019;37:3820–4. CAS Article Google Scholar 6. Kennedy RB, Lane JM, Henderson DA, Poland GA. 54 - Smallpox and Vaccinia. In: Plotkin SA, Orenstein WA, Offit PA, Edwards KM, editors. Plotkin's Vaccines (Seventh Edition): Elsevier; 2018. p. 1001–30. e1012. https://doi.org/10.1016/B978-0-323-35761-6.00054-7. https://www.sciencedirect.com/science/article/pii/B9780323357616000547. 7. Lee YJ, Son HS, Yeo SG, Yoo JS, Lee SW. Development of viral vector using attenuated Vaccinia virus strain, KVAC103. Public Health Weekly Report, KCDC. 2016;9(38):755–9. Google Scholar 8. Tovey MG, Lallemand C. Adjuvant activity of cytokines. Methods Mol Biol. 2010;626:287–309. CAS Article Google Scholar 9. Perera PY, Lichy JH, Waldmann TA, Perera LP. The role of interleukin-15 in inflammation and immune responses to infection: implications for its therapeutic use. Microbes Infect. 2012;14(3):247–61. CAS Article Google Scholar 10. Calarota SA, Dai A, Trocio JN, Weiner DB, Lori F, Lisziewicz J. IL-15 as memory T-cell adjuvant for topical HIV-1 DermaVir vaccine. Vaccine. 2008;26(40):5188–95. CAS Article Google Scholar 11. Oh S, Berzofsky JA, Burke DS, Waldmann TA, Perera LP. Coadministration of HIV vaccine vectors with vaccinia viruses expressing IL-15 but not IL-2 induces long-lasting cellular immunity. Proc Natl Acad Sci U S A. 2003;100(6):3392–7. CAS Article Google Scholar 12. Perera LP, Waldmann TA, Mosca JD, Baldwin N, Berzofsky JA, Oh S. Development of smallpox vaccine candidates with integrated interleukin-15 that demonstrate superior immunogenicity, efficacy, and safety in mice. J Virol. 2007;81(16):8774–83. CAS Article Google Scholar 13. Valkenburg SA, Li OT, Mak PW, Mok CK, Nicholls JM, Guan Y, Waldmann TA, Peiris JS, Perera LP, Poon LL. IL-15 adjuvanted multivalent vaccinia-based universal influenza vaccine requires CD4+ T cells for heterosubtypic protection. Proc Natl Acad Sci U S A. 2014;111(15):5676–81. CAS Article Google Scholar 14. Saikh KU, Kissner TL, Nystrom S, Ruthel G, Ulrich RG. Interleukin-15 increases vaccine efficacy through a mechanism linked to dendritic cell maturation and enhanced antibody titers. Clin Vaccine Immunol. 2008;15(1):131–7. CAS Article Google Scholar 15. Agren L, Lowenadler B, Lycke N. A novel concept in mucosal adjuvanticity: the CTA1-DD adjuvant is a B cell-targeted fusion protein that incorporates the enzymatically active cholera toxin A1 subunit. Immunol Cell Biol. 1998;76(3):280–7. CAS Article Google Scholar 16. Agren LC, Ekman L, Lowenadler B, Nedrud JG, Lycke NY. Adjuvanticity of the cholera toxin A1-based gene fusion protein, CTA1-DD, is critically dependent on the ADP-ribosyltransferase and Ig-binding activity. J Immunol. 1999;162(4):2432–40. CAS PubMed Google Scholar 17. Eliasson DG, El Bakkouri K, Schon K, Ramne A, Festjens E, Lowenadler B, Fiers W, Saelens X, Lycke N. CTA1-M2e-DD: a novel mucosal adjuvant targeted influenza vaccine. Vaccine. 2008;26(9):1243–52. CAS Article Google Scholar 18. Sundling C, Schon K, Morner A, Forsell MNE, Wyatt RT, Thorstensson R, Hedestam GBK, Lycke NY. CTA1-DD adjuvant promotes strong immunity against human immunodeficiency virus type 1 envelope glycoproteins following mucosal immunization. J Gen Virol. 2008;89(Pt 12):2954–64. CAS Article Google Scholar 19. Akhiani AA, Stensson A, Schon K, Lycke N. The nontoxic CTA1-DD adjuvant enhances protective immunity against helicobacter pylori infection following mucosal immunization. Scand J Immunol. 2006;63(2):97–105. CAS Article Google Scholar 20. Andersen CS, Dietrich J, Agger EM, Lycke NY, Lovgren K, Andersen P. The combined CTA1-DD/ISCOMs vector is an effective intranasal adjuvant for boosting prior Mycobacterium bovis BCG immunity to Mycobacterium tuberculosis. Infect Immun. 2007;75(1):408–16. CAS Article Google Scholar 21. Altenburg AF, Kreijtz JH, de Vries RD, Song F, Fux R, Rimmelzwaan GF, Sutter G, Volz A. Modified vaccinia virus Ankara (MVA) as production platform for vaccines against influenza and other viral respiratory diseases. Viruses. 2014;6(7):2735–61. CAS Article Google Scholar 22. Gomez CE, Najera JL, Krupa M, Esteban M. The poxvirus vectors MVA and NYVAC as gene delivery systems for vaccination against infectious diseases and cancer. Curr Gene Ther. 2008;8(2):97–120. CAS Article Google Scholar 23. Merkel TJ, Perera PY, Kelly VK, Verma A, Llewellyn ZN, Waldmann TA, Mosca JD, Perera LP. Development of a highly efficacious vaccinia-based dual vaccine against smallpox and anthrax, two important bioterror entities. Proc Natl Acad Sci U S A. 2010;107(42):18091–6. CAS Article Google Scholar 24. Son HS, Yeo SG, Lee SW: Recombinant Vaccinia Virus Derived From Kvac103 Strain. In. US patent 9,879,281: Korea centers for disease control and prevention; 2016. 25. Chun JH, Choi OJ, Cho MH, Hong KJ, Seong WK, Oh HB, Rhie GE. Serological correlate of protection in Guinea pigs for a recombinant protective antigen Anthrax vaccine produced from Bacillus brevis. Osong Public Health Res Perspect. 2012;3(3):170–6. Article Google Scholar 26. Ivins B, Fellows P, Pitt L, Estep J, Farchaus J, Friedlander A, Gibbs P. Experimental anthrax vaccines: efficacy of adjuvants combined with protective antigen against an aerosol Bacillus anthracis spore challenge in Guinea pigs. Vaccine. 1995;13(18):1779–84. CAS Article Google Scholar 27. Reed LJ, Muench H. A simple method of estimating fifty per cent endpointS12. Am J Epidemiol. 1938;27(3):493–7.

They don’t solve – don’t do most of the necessary solvency

1AC Arnold Ventures, RECUT, READS GREEN

9-24-2020 "'Evergreening' Stunts Competition, Costs Consumers and Taxpayers" <https://www.arnoldventures.org/stories/evergreening-stunts-competition-costs-consumers-and-taxpayers/> (Arnold Ventures is focused on evidence-based giving in a wide range of categories including: criminal justice, education, health care, and public finance)//Elmer -recut CAT

What Can Be Done As the Evergreen Drug Patent Search makes clear, the positive impact of Hatch-Waxman has been steadily and severely eroded by a regulatory system vulnerable to increasingly sophisticated forms of manipulation. “You might say that the patent and regulatory system has been weaponized,” Feldman said. “When billions of dollars are at stake, there’s a lot of money available to look for ways to exploit the legal system. And companies have become adept at this, as our work has found.” There are several key steps that Congress could take to restore the balance between innovation and competition that is the key to a successful prescription drug regulatory process. These may include: Imposing restrictions on the number of patents that prescription drug manufacturers can defend in court to discourage the use of anticompetitive patent thickets. Limiting the patentability of so-called secondary patents — which don’t improve the safety or efficacy of a drug — through patent and exclusivity reform. Reforming the 180-day generic exclusivity, which can currently be abused to block other competitive therapies. “The Evergreen Drug Patent Search provides the publicly available, evidence-based foundation that defines the extent of the problem, and it can be used to develop policies that solve the problem of anti-competitive patent abuses,” said Kristi Martin, VP of Drug Pricing at Arnold Ventures. “Our incentives have gotten out of whack,” Martin said. “The luxury of monopoly protection should only be provided to innovations that provide meaningful benefits in saving lives, curing illnesses, or improving the quality of people’s lives. It should not be provided to those gaming the system. If we can change that, we can save consumers, employers, and taxpayers many billions of dollars while increasing the incentives for pharmaceutical companies to achieve breakthroughs."

#### Critical innovation STARTS At the NIH.

NIH COVID-19 Research 20, 12-18-2020, "National Institutes of Health," <https://covid19.nih.gov/treatments-and-vaccines/covid-19-vaccines> //Jay

Understanding Vaccine Studies What are the differences between the vaccines that are available?  Vaccine syringe These vaccines have received emergency use authorization from the FDA:   ModernaTX, Inc., mRNA-1273: On December 18, 2020, the FDA authorized emergency use of this NIH-funded COVID-19 vaccine in the United States for people 18 years and older. View the infographic to see what’s in the Moderna COVID-19 vaccine.  Pfizer, Inc., and BioNTech BNT162b2: On December 11, 2020, the FDA authorized emergency use of the first COVID-19 vaccine in the United States for people 16 years and older. View the infographic to see what’s in the Pfizer COVID-19 vaccine.  Janssen Pharmaceutical Companies of Johnson & Johnson: On February 27, 2021, the FDA authorized emergency use of this single-shot vaccine for people 18 years and older. Developed with support from NIH funding, this vaccine does not require special refrigeration.  Women younger than 50 years old should be aware of the rare risk of blood clots after vaccination. How do I know the vaccines are safe and effective? Vaccine vial and syringe being held Vaccines have very high safety standards, and the vaccines available to prevent COVID-19 are no exception. COVID-19 vaccines are authorized  by the FDA for use only if they have proven safe and effective in a large group of people. Although the search for and development of the COVID-19 vaccines seemed to move fast, decades of existing research on coronaviruses gave the scientific community a head start on understanding COVID-19 and developing a vaccine. Researchers, the federal government, and drug companies came together like never before to cooperate and share resources, making the testing process more efficient. Also, the FDA has made the safety standards and approval process even tougher than usual. The FDA set minimum requirements for the effectiveness of products to approve only those vaccines that could offer immunity to the majority of the population.   Find more questions and answers about a COVID-19 vaccine How long will my immunity last after I get the vaccine?  Vaccine being administered to individual Researchers don’t know for sure how long immunity produced by vaccination lasts. You are considered fully protected two weeks after your second dose of the Moderna or Pfizer-BioNTech vaccine, or two weeks after the single-dose Johnson & Johnson’s Janssen vaccine.   However, because we do not know how long the vaccine protects you, you should still take precautions to protect yourself and others by following current CDC guidelines.   Do the vaccines work against the virus variants?  COVID molecule We know that the COVID-19 vaccines available now are effective at preventing COVID-19. So far, studies suggest that these vaccines should also work against the variants that have emerged. Current data show that the antibodies our bodies make after vaccination may recognize and protect against the variants. This is being closely investigated, and more studies are underway.   The rise of these variants is a reminder that as long as SARS-CoV-2 continues to spread, it has the potential to evolve into new variants. Viruses can’t mutate if they can’t replicate. The more people who get vaccinated, the harder it is for the virus to replicate. Widespread vaccination will help reduce the rise of additional variants.  Read about NIH research to study the variants When can children get a vaccine?  Children and teens ages 12 years and older can now get the Pfizer vaccine. COVID-19 vaccines are not yet approved for use in people younger than 12 (Pfizer) or 18 (Moderna and Johnson & Johnson vaccines). However, clinical trials are now underway to test the safety and effectiveness of currently available vaccines in younger children. Experts hope children and adolescents will be approved to receive the vaccines by fall 2021. Will I need a booster shot after being fully vaccinated against COVID-19? Person signing up for treatment NIH is researching whether people who have been fully vaccinated against COVID-19 will require additional shots for continued immunity.

