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Vaccine Genome Researchers Report 99.7% Colinear Identity Between a U.S. Civil War Era Smallpox Vaccine and Horsepox Virus

Findings Published in the Current Issue of the Journal Genome Biology

Horsepox is the Vector for Tonix's Experimental TNX-1800 COVID-19 Vaccine

Horsepox Has Been Used as a Smallpox Vaccine Since at Least the 1860's

Smallpox is the Only Viral Disease Ever Eradicated

CHATHAM, N.J., Dec. 04, 2020 (GLOBE NEWSWIRE) -- Tonix Pharmaceuticals Holding Corp. (Nasdaq: TNXP) (Tonix or the Company), a clinical-stage biopharmaceutical company, announced today that independent researchers reported 99.7% colinear identity between a circa 1860 U.S. smallpox vaccine and horsepox virus. Tonix's TNX-801 is a horsepox-based live virus vaccine being developed as a potential vaccine to prevent smallpox and monkeypox. Tonix's TNX-801 is also the vector on which Tonix's COVID-19 experimental vaccine is based. Tonix was not involved in the research reported.

The publication in the Journal *Genome Biology*, entitled "Re-assembly of 19th-century smallpox vaccine genomes reveals the contemporaneous use of horsepox and horsepox-related viruses in the United States"[1] analyzed sequences of U.S. smallpox vaccines that had been published recently by a different group also in *Genome Biology*, entitled "The origins and genomic diversity of American Civil War Era smallpox vaccine strains"[2]. The first paper applied modern techniques of sequencing old DNA to smallpox vaccine specimens stored in the Mütter Museum in Philadelphia, and identified that vaccines with horsepox core viral sequences were used in the 1860's and referenced sequence information from several vaccines that the authors posted in GenBank, the National Institute of Health genetic sequence database. The new report re-assembled certain genomes from the GenBank sequences associated with the first paper and found a remarkable degree of identity with the circa 1860 U.S. smallpox vaccine VK05 and the 1976 Mongolian horspox isolate called MNR-76. Tonix's TNX-801 was synthesized[3] based on the sequence of the Mongolian horsepox isolate MNR-76[4].

"The extent of colinear identity, approximately 99.7%, is remarkable between the circa 1860 U.S. smallpox vaccine VK05 and the 1976 Mongolian horsepox genome," said, José Esparza, MD, PhD, Adjunct Professor at the Institute of Human Virology, School of Medicine of the University of Maryland, who was an author of the Genome Biology letter. "This finding

indicates that a true horsepox virus was used as a smallpox vaccine in the U.S. in the 1860's. It is astonishing that there are so few differences between these viruses despite the separation of their isolation by over 100 years and from different continents."

"This recent discovery is another step in establishing that what is called 'horsepox' today was used to vaccinate against smallpox in the 19th century," said Seth Lederman, M.D., President and Chief Executive Officer of Tonix Pharmaceuticals. "Dr. Edward Jenner invented vaccination in 1798 and the procedure was called vaccination because the inoculum material was initially obtained from lesions on the udders of cows affected by a mild disease known as cowpox. 'Cow' is 'vacca' in Latin. However, Dr. Jenner suspected that cowpox originated from horsepox.[5] Subsequently, Dr. Jenner and others immunized against smallpox using material directly obtained from horses. The use of vaccines from horses was sometimes called 'equination' from the Latin 'equus' which means 'horse'. Equination and vaccination were practiced side-by-side in Europe[6]. Several years ago, we recognized that the 2006 genome sequence of the 1976 Mongolian horsepox[4] may be more like the vaccines that Jenner used than modern vaccinia vaccines, because the modern vaccinia vaccines appear to have undergone both divergent and convergent evolution since Jenner's time. To recreate a vaccine similar to Jenner's, together with scientists at the University of Alberta, Canada, we synthesized[3] a live horsepox virus based on the 1976 Mongolian horsepox virus sequence and we are developing it as a potential vaccine for smallpox and monkeypox, called TNX-801. Horsepox is also the vector for our COVID-19 vaccine, TNX-1800, which has been modified to direct the expression of CoV-2 spike protein."

"The new findings are particularly significant, because they confirm that horsepox has been used as a safe and effective smallpox vaccine since at least 1860, and probably since Edward Jenner's time in 1798," continued Dr. Lederman. "The new report confirms that modern 'vaccinia'-based smallpox vaccines from around the world have lost, apparently convergently, two genomic segments in evolution: an 11 Kilobase (kB) segment on the left side and a 6 kB Segment on the right side relative to the progenitor horsepox-like vaccines. The presence of these genetic elements in horsepox may contribute to the decreased virulence of horsepox relative to vaccinia in mice and the smaller plaque size in tissue culture. Later, after approximately 1875, when vaccinia propagation was moved from 'arm to arm' to growth on cows in 'vaccine farms'[8], the core viral sequences of modern vaccinia vaccines appear to have diverged away from the horsepox core viral sequences. A standard for modern vaccine development and manufacturing is to make a 'master virus bank' and tie vaccine production to the exact properties of the original master virus bank. Edward Jenner did not keep a master virus bank, because his work pre-dated understanding of viruses by more than 100 years. However, the recent work on sequencing and analyzing archaic smallpox vaccines gives us a clearer understanding of the structure and features of Jenner's vaccines. The more we learn, the more we are led to believe that Jenner's vaccinia was either horsepox or more like horsepox than modern vaccinia vaccines."

The recent report is the latest in a series from a team composed by Dr. Esparza, Clarissa R Damaso, PhD at the Carlos Chagas Filho Biophisics Institute of the Federal University of Rio de Janeiro, Brazil, and Andreas Nitsche, Ph.D. at the Robert Koch Institute in Berlin, Germany. They have previously reported[7] that the core viral sequence of a 1902 U.S. smallpox vaccine manufactured by Mulford, a vaccine manufacturer subsequently acquired by Merck, was closely related to the Mongolian horsepox MNR-76 on which TNX-801 is

based. The latest report shows that the circa 1860 VK05 vaccine also contains a high degree of colinear identity through the ends of the viruses, including regions where all previously characterized smallpox vaccines including Mulford 1902 contained deletions of approximately 11 kB on the left side and 6 kB on the right side.

Dr. Lederman continued, “Smallpox was eradicated using single-dose live replicating vaccines that induce durable T cell immunity, prevent serious illness after infection and block forward transmission. Our hope and our goal is to produce vaccines that will provide long term immunity with a single dose using a proven technology that can be readily scaled up for manufacturing and that does not require a costly and cumbersome cold chain for distribution and storage.”

About TNX-801*

TNX-801 is a live virus vaccine based on synthesized horsepox[3,4]. Horsepox and vaccinia are closely related orthopoxviruses that are believed to share a common ancestor. Molecular analysis suggests that TNX-801 has relatively “complete” left and right inverted terminal repeats (ITRs) while different vaccinia isolates have a variety of deletions adjacent to the left and right ITRs including a characteristic approximately 11 kB deletion on the left side and an approximately 6 kB deletion on the right side. Therefore, TNX-801 has additional genes, relative to vaccinia vaccines, that may play roles in host immune interactions and one or more of such proteins may serve as antigens for protective immunity. Molecular analysis also shows that horsepox is closer than modern vaccines in DNA sequence to the vaccine discovered and disseminated by Dr. Edward Jenner[3,4,5,7,8]. The small plaque size in culture of TNX-801 appears identical to the U.S. Centers for Disease Control publication of the natural isolate[10]. Relative to vaccinia, horsepox has substantially decreased virulence in mice[3]. Tonix’s TNX-801 vaccine candidate is administered percutaneously using a two-pronged, or “bifurcated” needle. The major cutaneous reaction or “take” to vaccinia vaccine was described by Dr. Edward Jenner in 1796 and has been used since then as a biomarker for protective immunity to smallpox, including in the World Health Organization’s (WHO) accelerated smallpox eradication program that successfully eradicated smallpox in the 1960’s. The “take” is a measure of functional T cell immunity validated by the eradication of smallpox, a respiratory-transmitted disease caused by variola. Tonix’s proprietary horsepox vector is believed to be more closely related to Jenner’s vaccinia vaccine than modern vaccinia vaccines, which appear to have evolved by deletions and mutations to a phenotype of larger plaque size in tissue culture and greater virulence in mice. TNX-801 vaccinated macaques showed no overt clinical signs after monkeypox challenge[11].

About TNX-1800**

TNX-1800 is a live modified horsepox virus vaccine for percutaneous administration that is designed to express the Spike protein of the SARS-CoV-2 virus and to elicit a predominant T cell response. TNX-1800 is based on a horsepox vector, which is a live replicating, attenuated virus that elicits a strong immune response. Live replicating orthopoxviruses, like vaccinia or horsepox, can be engineered to express foreign genes and have been explored as platforms for vaccine development because they possess; (1) large packaging capacity for exogenous DNA inserts, (2) precise virus-specific control of exogenous gene insert expression, (3) lack of persistence or genomic integration in the host, (4) strong immunogenicity as a vaccine, (5) ability to rapidly generate vector/insert constructs, (6) readily manufacturable at scale, and (7) ability to provide direct antigen presentation.

Relative to vaccinia, horsepox has substantially decreased virulence in mice[3]. Horsepox-based vaccines are designed to be single dose, vial-sparing vaccines, that can be manufactured using conventional cell culture systems, with the potential for mass scale production and packaging in multi-dose vials. Like TNX-801, Tonix's TNX-1800 vaccine candidate is administered percutaneously using a two-pronged, or "bifurcated" needle. Tonix recently reported that immunization with a single dose of TNX-1800 induced "takes" and neutralizing anti-SARS-CoV-2 antibodies in non-human primates.

*TNX-801 is in the pre-IND stage and has not been approved for any indication.

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[1]Brinkmann A et al, Genome Biology (2020) 21:286 https://doi.org/10.1186/s13059-020-02202-0

[2]Duggan A et al. Genome Biology (2020) 21:175 https://doi.org/10.1186/s13059-020-02079-z

[3]Noyce RS, et al. (2018) PLoS One. 13(1):e0188453

[4]Tulman ER, et al. (2006) J Virol.80(18):9244-58.PMID:16940536

[5]Jenner E. "An Inquiry Into the Causes and Effects of the Variole Vaccinae, a Disease Discovered in Some of the Western Counties of England, Particularly Gloucestershire and Known by the Name of the cow-pox." London: Sampson Low, 1798.

[6]Esparza E, et al Vaccine. (2017) 35(52):7222-7230.

[7]Schrick L et al N Engl J Med (2017); 377:1491-1492

[8]Esparza J et al. Vaccine. (2020); 38(30):4773-4779.

[9]Qin et al. J. Virol. 89:1809 (2015).

[10]Trindale GS et al. Viruses (2016) (12). pii: E328 PMID:27973399

[11]Noyce, RS, et al. Synthetic Chimeric Horsepox Virus (scHPXV) Vaccination Protects Macaques from Monkeypox Presented as a poster at the American Society of Microbiology BioThreats Conference - January 29, 2020, Arlington, VA. (https://content.equisolve.net/tonixpharma/media/10929ac27f4fb5f5204f5cf41d59a121.pdf)*

About Tonix Pharmaceuticals Holding Corp.

Tonix is a clinical-stage biopharmaceutical company focused on discovering, licensing, acquiring and developing small molecules and biologics to treat and prevent human disease and alleviate suffering. Tonix's portfolio is primarily composed of central nervous system (CNS) and immunology product candidates. The immunology portfolio includes vaccines to prevent infectious diseases and biologics to address immunosuppression, cancer and autoimmune diseases. The CNS portfolio includes both small molecules and biologics to treat pain, neurologic, psychiatric and addiction conditions. Tonix's lead vaccine candidate, TNX-1800*, is a live replicating vaccine based on the horsepox viral vector platform to protect against COVID-19, primarily by eliciting a T cell response. Tonix expects data from animal studies of TNX-1800 in the fourth quarter of this year and the first quarter of 2021. TNX-801*, live horsepox virus vaccine for percutaneous administration, is in development to protect against smallpox and monkeypox. Tonix is also developing TNX-2300* and TNX-2600*, live replicating vaccine candidates for the prevention of COVID-19 but using bovine parainfluenza as the vector. Tonix's lead CNS candidate, TNX-102 SL**, is in Phase 3 development for the management of fibromyalgia. The Company expects topline data in the Phase 3 RELIEF study in the fourth quarter of 2020. Tonix is also currently enrolling participants in the Phase 3 RALLY study for the management of fibromyalgia using TNX-102

SL, and the results are expected in second half of 2021. TNX-102 SL is also in development for PTSD, agitation in Alzheimer's disease (AAD) and alcohol use disorder (AUD). The PTSD program is in Phase 3 development, while AAD and AUD are Phase 2 ready. The AAD program has FDA Fast Track designation. Tonix's programs for treating addiction conditions also include TNX-1300* (T172R/G173Q double-mutant cocaine esterase 200 mg, i.v. solution), which is in Phase 2 development for the treatment of life-threatening cocaine intoxication and has FDA Breakthrough Therapy designation. TNX-601 CR** (tianeptine oxalate controlled-release tablets) is another CNS program, currently in Phase 1 development as a daytime treatment for depression while TNX-1900**, intranasal oxytocin, is in development as a non-addictive treatment for migraine and cranio-facial pain. Tonix's preclinical pipeline includes TNX-1600** (triple reuptake inhibitor), a new molecular entity being developed as a treatment for PTSD; TNX-1500* (anti-CD154), a monoclonal antibody being developed to prevent and treat organ transplant rejection and autoimmune conditions; and TNX-1700* (rTFF2), a biologic being developed to treat gastric and pancreatic cancers.

*TNX-1800, TNX-801, TNX-2300, TNX-2600, TNX-1300, TNX-1500 and TNX-1700 are investigational new biologics and have not been approved for any indication.

**TNX-102 SL, TNX-601 CR, TNX-1600 and TNX-1900 are investigational new drugs and have not been approved for any indication.

This press release and further information about Tonix can be found at www.tonixpharma.com.

Forward Looking Statements

Certain statements in this press release are forward-looking within the meaning of the Private Securities Litigation Reform Act of 1995. These statements may be identified by the use of forward-looking words such as "anticipate," "believe," "forecast," "estimate," "expect," and "intend," among others. These forward-looking statements are based on Tonix's current expectations and actual results could differ materially. There are a number of factors that could cause actual events to differ materially from those indicated by such forward-looking statements. These factors include, but are not limited to, risks related to failure to obtain FDA clearances or approvals and noncompliance with FDA regulations; delays and uncertainties caused by the global COVID-19 pandemic; risks related to the timing and progress of clinical development of our product candidates; our need for additional financing; uncertainties of patent protection and litigation; uncertainties of government or third party payor reimbursement; limited research and development efforts and dependence upon third parties; and substantial competition. As with any pharmaceutical under development, there are significant risks in the development, regulatory approval and commercialization of new products. Tonix does not undertake an obligation to update or revise any forward-looking statement. Investors should read the risk factors set forth in the Annual Report on Form 10-K for the year ended December 31, 2019, as filed with the Securities and Exchange Commission (the "SEC") on March 24, 2020, and periodic reports filed with the SEC on or after the date thereof. All of Tonix's forward-looking statements are expressly qualified by all such risk factors and other cautionary statements. The information set forth herein speaks only as of the date thereof.

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