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# Enhancing viral vaccine production using engineered knockout vero cell lines – A second look

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ABSTRACT

The global adoption of vaccines to combat disease is hampered by the high cost of vaccine manufacturing. The work described herein follows two previous publications (van der Sanden et al., 2016; Wu et al., 2017) that report a strategy to enhance poliovirus and rotavirus vaccine production through genetic modification of the Vero cell lines used in large-scale vaccine manufacturing. CRISPR/Cas9 gene editing tools were used to knockout Vero target genes previously shown to play a role in polio- and rotavirus production. Subsequently, small-scale models of current industry manufacturing systems were developed and adopted to assess the increases in polio- and rotavirus output by multiple stable knockout cell lines. Unlike previous studies, the Vero knockout cell lines failed to achieve desired target yield increases. These findings suggest that additional research will be required before implementing the genetically engineered Vero cell lines in the manufacturing process for polio- and rotavirus vaccines to be able to supply vaccines at reduced prices.


## 1. Introduction

Vaccines have a profound impact on global health, preventing illness, death, and improving the quality of life across the globe. However, the current costs of vaccine manufacturing and distribution often prevent the poorest segments of the world's population from accessing these critical medicines. To address this problem, the identification and adoption of new technologies that lower costs and make vaccines affordable is an important objective.

Vaccine manufacturing processes are typically low yielding and production for global distribution regularly requires large and expensive manufacturing facilities that result in high vaccine prices and impede developing countries from initiating and/or expanding in-country manufacturing capabilities. To address this, new manufacturing technologies are being explored, including the development of optimized cell culture media, novel bioreactor designs that boost virus production by increasing cell densities, and innovative purification resins and membranes that result in higher recoveries and shorter process times (Barrett et al., [3]; Jacquemart et al., [4]; Tapia et al., [5], Rajendran et al., [6]). Another area for exploration is the engineering of manufacturing cell lines to improve virus propagation and vaccine yield. Viral vaccines are manufactured on a range of mammalian cell substrates including Vero, MRC-5, PER.C6 that are capable of supporting propagation and production of the vaccine virus strain. These cell substrates are a critical factor in the manufacturing process as they

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determine to a large extent overall vaccine yield. In general, approved manufacturing cell lines have remained unchanged for vaccine production. Several manufacturers have approached production and pricing challenges by attempting to modify the properties of the cells used to grow the virus. In some cases, adherent cells can be transitioned into suspension growth, thereby increasing virus production through increase in cell densities (Sanders et al., [7]). In other instances, clonal selection, the selection of a (sub)-clonal population within a parental cell population, has been used to improve the manufacturing properties of a cell substrate. Specifically, studies have shown that within a homogeneous population of cells, variants demonstrating improved vaccine production can be selected (Davies et al., [8]). In such cases, care must be taken in the screening process to ensure that the selected cell populations do not contain any traits that may negatively impact on the manufacturing process.

While clonal selection offers a proven opportunity to enhance virus yields, the underlying molecular basis for a cell's improved properties remains unclear and the long-term stability of clones with enhanced traits remains a key challenge (Hou et al., [9]; Feng et al., [10]). To address these issues, researchers in both academic and industrial settings have begun to combine the vast wealth of knowledge generated during the genomics revolution with a new generation of synthetic biology tools (e.g., RNAi, and CRISPR/Cas9 gene editing). While such modified cell lines will need to undergo extensive testing to address questions regarding (1) genetic stability, and (2) the compatibility of modified cell line traits (e.g. doubling time, cell viability) with current vaccine manufacturing processes, this amalgam of technologies may enhance the production of both vaccines and biotherapeutic molecules.

The work presented here is a follow-up to a study performed by Van der Sanden et al. in 2016 [1]. In that report a genome-wide RNA interference (RNAi) screen identified multiple host gene knockdown events that enhanced the production of Sabin and wild type poliovirus (PV). These gene knockdown-mediated increases were dramatic, with 20- to 60-fold increases in viral titers observed in two unrelated cell lines (Vero and Hep-2C). Moreover, the overall effects (i) varied with virus serotype, (ii) were demonstrated to exhibit additive properties, and in some cases, (iii) facilitated the production of closely related viruses (e.g., EV-71). Importantly, the authors created stable Vero knockout cell lines of the top gene candidates using clustered regularly interspaced short palindromic repeat (CRISPR; Ran et al., [11]) technology and, using plaque assays, demonstrated that stable KO clones could dramatically improve PV vaccine strain production. In a separate study that examined gene targets that enhanced rotavirus (RV) production, the same group recently reported that 7- to 18-fold increases could be achieved through knockout of a single Vero cell host gene (Wu et al. [2]). With the reported dramatic yield increases for multiple viral vaccines, these discoveries could address the challenges currently facing governments and vaccine manufacturers.

In this manuscript, we investigated this approach further by evaluating the gene targets identified in the van der Sanden and Wu publications to determine whether stably engineered single and double knockout cell lines with greatly increased viral production of PV and RV could be created in the WHO 10-87 GMP Vero cell line currently employed in industry. Focusing attention on these vaccines is essential to address the economic and disease burden these two pathogens impose on the developing world. While significant progress has been made towards eradication of polio since the introduction of the Sabin live oral polio vaccine (OPV) in the 1950′s and the Salk inactivated polio vaccine (IPV) in the 1960′s, complete eradication requires that OPV, because it can result in rare cases of vaccine associated paralytic poliomyelitis (VAPP), be phased out and replaced with IPV. Such a change has a challenging price and supply impact. OPV is typically sold for less than $0.20 per dose (compared to IPV, which is sold in different price tiers based on the financial resources of the country; from under $1 per dose for the Global Alliance for Vaccines and Immunization (GAVI) countries up to $2.40 per dose for middle-income countries[web 1, web 2]. In addition, since IPV is an inactivated virus formulation, it requires nearly ten times greater amount of viral antigen to achieve equivalent levels of protection. Phasing out the OPV vaccine in favor of IPV would thus require a considerable increase in virus production capacity. For this reason, development of new cell lines, as well as new manufacturing technologies, enabling increased IPV production at reduced costs are paramount for achieving the global health goal of eradicating PV.

Similar issues surround RV infections and vaccines. RV infections have remained the most common cause of severe gastroenteritis among children under 5 years of age, leading to an estimated 215,000 deaths per year and millions of hospitalizations (Atherly et al., [12]; Tate et al. [13]). As almost all RV-related deaths occur in less developed countries where access to medical care is limited, the RV pathogen places an enormous burden on the healthcare resources of economically-strained geographies (Rheingans et al. [14]; Rheingans et al. [15]). Introduction of the Rotarix vaccine (GSK, Belgium) and RotaTeq vaccine (Merck, USA) have shown that immunization can significantly reduce RV-related hospitalizations in developed and developing countries (Leshem et al. [16]). However, the current RV vaccines are costly. Prices in developed countries such as the US and EU range from $50-$100 per dose and even in these countries, price has been cited as a barrier to the introduction of the vaccine with, for example, the UK, France and Germany delaying introduction of the vaccine into their childhood vaccination campaigns[web 3].

Both Rotarix and RotaTeq are made available at reduced prices to low- and middle-income countries. For example GAVI prices in 2016 were $2–3.50 per dose[web 4] while for PAHO in 2014 it was $5.50–$6.50 per dose. Despite this however, lower prices would be more conducive to the widespread adoption and use of these vaccines in poorer countries. New RV vaccines are coming on to the market, for example RotaVac (Bharat Biotech, India) and RotaSiil (Serum Institute of India) are in late stages of clinical development, while inactivated RV vaccines are also under development (Wang et al. [17]), which may improve vaccine pricing for the future through reduced manufacturing and infrastructure costs[web 5]. Regardless of the formulation, there is clearly a need for new technologies that increase the production of more affordable RV vaccines.

Engineered Vero cell lines capable of greatly increased production of IPV and RV vaccines would have an enormous impact on global health. In this study, we describe our efforts to generate single knock-out cell lines from the WHO Vero 10–87 cell line, capable of enhancing the production of PV and RV vaccines. In parallel, we summarize a novel study designed to combine the best knockout targets from the van der Sanden and Wu studies to create a double-knockout Vero GMP cell bank capable of enhancing production of both viral vaccines. For each program, a target titer amplification goal of 30-fold or greater was set, based on the previous publications indicating such a yield increase should be achievable (Van der Sanden et al. [1], Wu et al. [2]). In addition, a 30-fold increase in production can significantly alter the cost and capacity of current vaccine manufacturing platforms thereby making vaccines accessible and affordable to a greater portion of the global population. Small-scale models of the current industry manufacturing systems were developed and adopted to assess the increase in output by knockout cell lines. These procedures allowed for the testing of lead clones that could proceed rapidly into GMP Master Cell Bank manufacturing to dramatically increase vaccine supply at significantly reduced prices.

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