

# Rapid-response manufacturing of adenovirus-vectored vaccines

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To the Editor:

The Coalition for Epidemic Preparedness Innovations (CEPI) has proposed a '100-day mission', or 'moonshot' aspiration of compressing the time taken for launch of a new vaccine to 100 days from pathogen identification <sup>2</sup>. Alongside this, there is recognition of the importance of 'the second hundred days', i.e. rollout of a new vaccine at large scale. Vaccine platform technologies with known safety, immunogenicity, manufacturing and distribution characteristics will be critical to meet these challenges.

Many features of pre-clinical and clinical development of new vaccines are independent of the platform technology. Manufacturing and distribution are therefore the major points of divergence in the pathways to deployment of vaccines based on different platforms. Here, we show that 'rapid response' manufacturing of adenovirus-vectored vaccines can ~~be highly competitive with mRNA vaccine manufacturing~~ enable compressed development timelines competitive with other platforms, and discuss the implications of improved vaccine manufacturing for future outbreak response and equity of access to vaccines.

Adenoviral vectors offer a rapidly adaptable and deployable platform with proven safety and efficacy, and particular advantages in achieving equitable access. The 'Oxford - AstraZeneca' COVID-19 vaccine (ChAdOx1 nCoV-19 / *Vaxzevria*), based on the chimpanzee adenovirus platform technology, is estimated to have saved around 6 million lives in 2021, more than any other COVID-19 vaccine <sup>3</sup>. A review of 79 real-world effectiveness studies ~~indicated~~ confirmed its high efficacy against death or severe disease ~~was high and similar to that of mRNA vaccines~~ <sup>4</sup>. Thrombosis with thrombocytopenia syndrome has occurred very rarely in recipients of adenovirus-vectored vaccines and appears even rarer in data sets from outside Europe and North America <sup>5,6</sup>. In many contexts – including emergency response to pathogens with high mortality and no existing vaccine – the frequency of this syndrome would have little impact on the risk : benefit balance of vaccination.

With AstraZeneca and other industrial partners, we developed a simple manufacturing process which enabled production of more than 3 billion doses across a network of facilities in 12 countries on five continents <sup>7</sup>. The cost of production is low, and suitability of the platform for refrigerated (rather than frozen) storage enabled distribution to hard-to-serve communities, notably in low and middle income countries <sup>8</sup>.

Alongside these positive features, however, adenovirus manufacturing has had two notable disadvantages ~~as compared to mRNA vaccines~~. Firstly, time taken to prepare viral seed as a starting material for production has delayed the availability of initial batches for clinical trials. In 2020, the first-in-human use of an mRNA SARS-CoV-2 vaccine was 63 days after the publication of the pathogen sequence<sup>9</sup>. The first clinical batch of ChAdOx1 nCoV-19 was not released until a month later. Secondly, volumetric productivity (number of doses per litre of bioreactor capacity), has been estimated to be at least an order of magnitude higher for mRNA vaccines than the c. 2000 doses/L achieved for ChAdOx1 nCoV-19<sup>7,10</sup>. This has necessitated a greater ‘footprint’ for manufacturing of adenovirus-vector drug substance (bulk vaccine).

We recently published work seeking to address these disadvantages<sup>1</sup>. We showed that streamlining viral seed production could enable release of a first vaccine batch for clinical trials within 60 days of availability of the sequence of a new pathogen, and release of a first large-scale commercial batch within 100 days (Figure 1A shows a simplified development timeline). We showed also that intensification of the upstream process (i.e. cell culture and viral replication) could quadruple volumetric productivity as compared to the process used for commercial production of ChAdOx1 nCoV-19. Productivity of the improved process is approximately  $8 \times 10^{14}$  VP of purified drug substance per litre of bioreactor culture, sufficient for >10 000 doses of drug product<sup>1</sup>.

We now describe techno-economic modelling of commercial-scale implementation of this improved manufacturing process (Figure 1B). Models were constructed and evaluated using Biosolve [Process 8](#) software (Biopharm Services). We modelled a facility with a cell expansion seed train using a 200 L ‘n–2’ bioreactor for alternating seeding of each of two 200 L perfusion-capable ‘n–1’ bioreactors, in turn servicing two 2000 L production bioreactors, and a single downstream purification train. Assumptions made were as follows, based upon our published data<sup>1,7</sup>: cell doubling time 36 hours; upstream process productivity  $1.5 \times 10^{12}$  viral particles per mL (VP/mL); benzonase nuclease concentration 100 units/mL during harvest; clarification filter loading up to  $1 \times 10^{12}$  cells/m<sup>2</sup>, corresponding to 75 L/m<sup>2</sup> with peak cell density  $1 \times 10^7$  cells/mL and a 33% safety factor; clarification product recovery 66%; anion exchange membrane binding capacity  $1 \times 10^{16}$  VP/L; anion exchange recovery 90%; tangential-flow filtration filter loading  $2 \times 10^{16}$  VP/m<sup>2</sup>. Concentration in the tangential-flow filtration stage was limited to  $0.33 \times$  the bioreactor volume, corresponding to an expected product concentration of  $2.2 \times 10^{12}$  VP/mL. Costs were based upon [Sartorius and](#) Biosolve’s proprietary databases. [The resulting model, after redaction of the proprietary itemised cost information, is provided as Supplementary Table 1.](#)

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97 This bioreactor configuration could provide a batch around every 3.5 days (Figure 1C). This more than  
98 doubles facility output as compared to a facility lacking perfusion in the seed train and using a single  
99 production bioreactor, while requiring a proportionately small increase in footprint. Use of perfusion  
100 for additional cell expansion at 'n-1' reduces the cycle time of the production bioreactors, while a  
101 single purification train remains sufficient to process the output from both production reactors. The  
102 downstream process could be executed with moderate modification to the equipment used for global  
103 production of ChAdOx1 nCoV-19. Although the volumes of buffer required for AEX are large as  
104 compared with other process stages, these are likely to be manageable for many facilities by making  
105 use of buffer concentrates and in-line dilution technology. We have not, to date, optimized the final  
106 tangential-flow filtration to minimize the required membrane area, and our model here used cautious  
107 assumptions based upon our (unoptimized) experience. The projected tangential-flow filtration  
108 membrane area of 84 m<sup>2</sup> / batch is large, highlighting the need for such optimization, but could  
109 nonetheless be achieved either using a custom tangential-flow filtration skid or multiple cycles on off-  
110 the-shelf skids.

111

112 Assuming 70% facility utilization, a single such facility is predicted to be capable of producing  
113 approximately 1.3 billion doses per year, with a cost of goods of drug substance below 0.10 EUR/dose  
114 (lower than that of the fed-batch process used to produce ChAdOx1 nCoV-19). This corresponds to  
115 output of c. 900 doses per L of installed bioreactor capacity per day. Capital expenditure to construct  
116 and equip such a facility would be c. 40m EUR, with operating expenditure of <100m EUR/yr.

117

118 Under an alternative assumption of short-term maximum-capacity operation for emergency response,  
119 we estimate that eight such facilities (i.e. total installed bioreactor capacity of 32 000 L) could provide  
120 a total of one billion doses per month. Although substantial, this would be smaller than the network  
121 of facilities which produced ChAdOx1 nCoV-19 in 2021. We believe several existing facilities (including  
122 a number in low- and middle-income countries) would be suitable.

123

124 These results have a number of implications for preparedness for future pandemics.

125

126 In our work to improve the adenovirus manufacturing platform, we focused initially on three time-  
127 based metrics: time from pathogen-sequence availability to release of the first clinical trial batch, the  
128 first commercial batch, and the billionth dose. In contrast to the situation in 2020, we believe that  
129 both adenovirus manufacturing and ~~mRNA manufacturing platforms — and probably~~ other platform

technologies —are now capable of meeting the CEPI ‘100-day’ aspiration. Completion of pre-clinical and clinical development in substantially shorter periods than this seems unlikely. Within the second hundred days, our modelling suggests that a well-prepared ‘all out’ global effort using a realistically-sized manufacturing network could release over 3 billion doses. This would be sufficient to provide a first dose to a large proportion of the global population, and represents a level of output which took over two years for any COVID-19 vaccine programme to achieve.

Between 2020 and 2022, there has thus been a step change in the speed of vaccine manufacturing which is technically feasible. Rather than being a question of technical feasibility, ability to deliver such manufacturing speed is now mostly a question of finance and preparation, including work to mitigate risks of failure and to prepare template regulatory filings in advance. A fully validated version of the process we have described, preferably including an independent parallel ‘back up campaign’ for seed production and supported by rapid-turnaround platform analytics (~~notably easily accessible nucleic-acid based adventitious agent assays~~), would in our view provide high confidence of success.

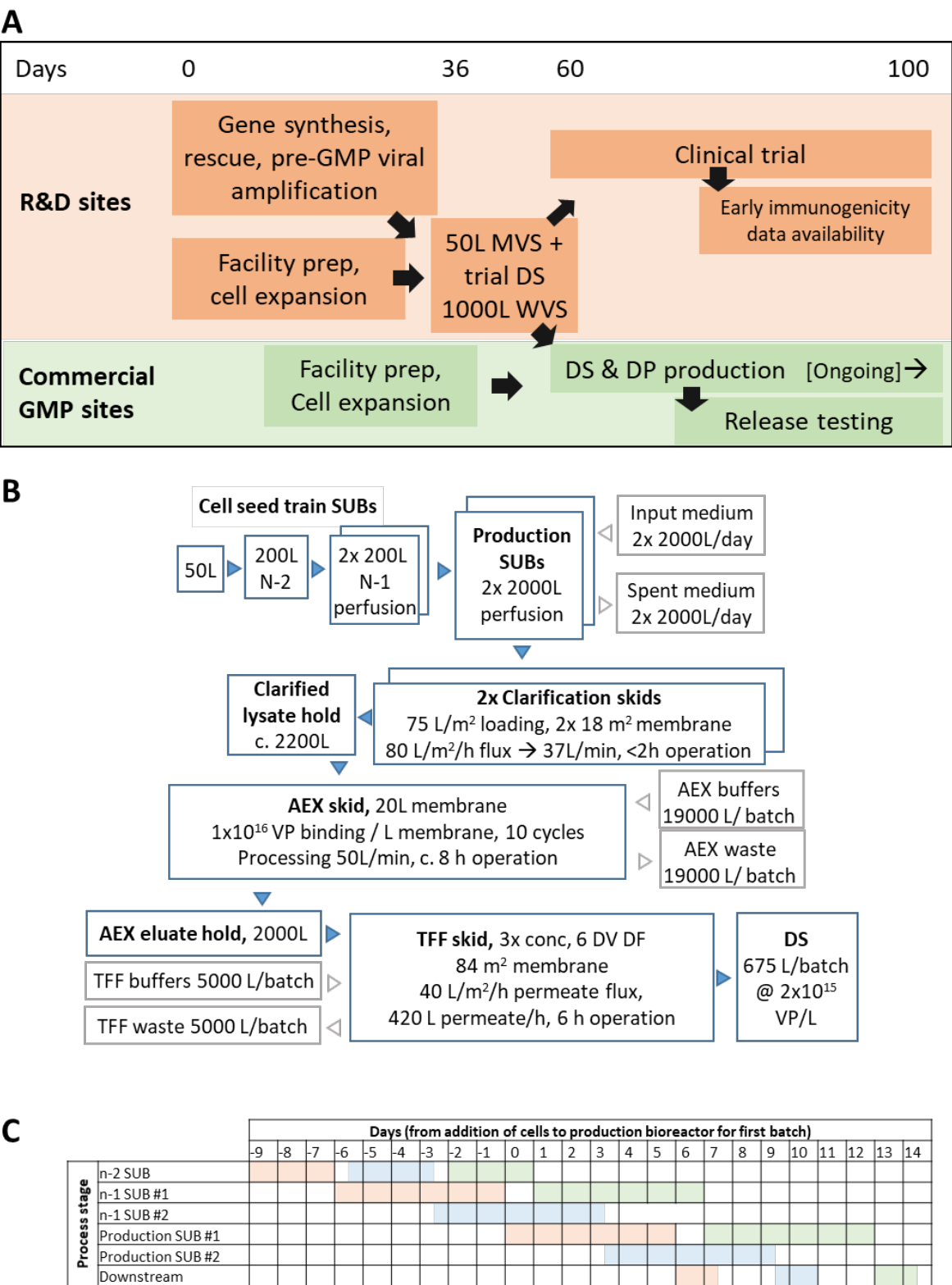
Quality control testing is ‘rate limiting’ for vaccine availability. The timeline we have suggested (Figure 1A) allows 16 days for release testing of the first clinical trial batch and 35 days for testing of the first commercial batch. The difference is due to an assumption of performing 28-day *in vitro* culture-based assays for adventitious viral agents and replication competent adenovirus for commercial material, while nucleic-acid based assays (supported by 14-day *in vitro* assays) are used for clinical trial material. Full validation of PCR- or next-generation-sequencing-based methods for detection of such contaminants could bring forward the release of the first commercial batch to day 80 or sooner.

Further improvement is probably possible. However, any vaccine platform which can achieve 100 days to the first commercial batch, productivity of 1000 doses per litre of bioreactor capacity per day and cost of <1 EUR per dose of drug substance is likely to be competitive from a manufacturing perspective. Further improvement may provide relatively marginal benefits. Leading vaccine platforms have now attained levels of manufacturing speed and facility productivity at which other factors are probably more important. Such considerations include safety, tolerability, efficacy, stability, cost, programmatic suitability, individual recipient preference, and manufacturer willingness to support technology transfer beyond their own facilities. There are likely to be valid scientific reasons for selection of different vaccines in different contexts.

Future epidemics are inevitable. Prompt availability of diverse vaccines, including adenoviruses, is necessary to protect public health and prosperity. It would in our view be economically attractive to maintain a network of regional facilities 'warm-lit' (pre-staffed, pre-stocked with materials, and pre-financed) for manufacturing of any adenovirus-vectored vaccine on billion-dose scale, as a global public good. Based on our modelling, it might cost ~~around~~ less than 200m EUR/yr to finance a global facility network capable of providing a billion doses of an adenovirus-vectored vaccine to a wide range of countries in well under 200 days from identification of a novel pathogen. This estimate is based upon holding a sufficient stockpile of materials and consumables to produce 1 billion doses (55 batches, each providing c. 18m doses, with materials and consumables costing c. 1.2m EUR/batch), and, renewing stocks every 12 months. Some materials and consumables will have shelf lives greater than 12 months, reducing costs, but warehousing costs are not included., and Rapid response requires the use of the pre-existing facilities, for instance those of contract manufacturing organisations which for makingmake other vaccines or biologicals between epidemics, and the negotiation of (with contracts in place for emergency 'walk-in' rights in the event of a pandemic). Use of existing facilities would reduce capital and labour costs, although these are predicted to constitute only a minority of the total cost of vaccine production. A key uncertainty is the cost of 'walk-in' emergency availability of a facility. Between pandemics, this would require maintenance of staff training and process validation, perhaps requiring intermittent execution of the process, and – critically – would preclude use of the facility to make products for which output interruption of a few months could not be tolerated. Our estimate assumes that such walk-in capacity could be procured for c. 10-15m EUR per facility per year which, over five years, is close to the total capital and labour cost of a purpose-built facility. Greater cost-effectiveness might be achieved at a facility which used a similar process to make another viral vector for a non-pandemic indication, or by concentrating the eight production trains in a smaller number of facilities.

Costs of establishing such 'warm-lit' networks are significant but correspond to only c. 0.002% of the USD 12.5 trillion global cost of the COVID-19 pandemic (as estimated by the International Monetary Fund <sup>11</sup>), or c. 0.4% of global spending on COVID-19 vaccines in 2021 <sup>12</sup>. Which entity might fund and commission such a network for adenoviruses or indeed any other vaccine platform is an open question for global policymakers, as this extends beyond CEPI's core role of supporting research and development.

195      Figure 1: Accelerated high-productivity adenovirus manufacturing



197 **Figure legend**

198 **Figure 1**

199 (A) High-level overview of development campaign combining seed production, supply of vaccine to  
200 clinical trial, execution of clinical trial, and large-scale manufacturing, enabling large-scale product  
201 release at day 100.

202

203 (B) Equipment, product, and materials flow in modelled facility.

204

205 (C) Illustrative production schedule, showing use of two 'n-1' reactors, two production bioreactors  
206 and single downstream purification train.

207

208 AEX, anion exchange; DP, drug product; DS, drug substance; GMP, Good Manufacturing Practice;

209 MVS, master virus seed; SUB, single-use bioreactor; TFF, tangential-flow filtration.

210 WVS, working virus seed.

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