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Current Opinion in Biotechnology

Speed and need: twin development challenges in rapid response for a SARS-CoV-2 antibody cocktail

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Neutralizing antibodies are one available tool for the treatment of infectious diseases. Speed in developing monoclonal antibody treatments is an understood requirement for emerging infectious diseases, and need for COVID-19 treatments during the worldwide pandemic has provided additional urgency. Process development (at Regeneron) and technology transfer (within Regeneron and to Genentech) of casirivimab and imdevimab (REGEN-COV™ or Ronapreve™) manufacturing processes have addressed speed and need with selected purification and cell culture examples provided, respectively, for these two development challenges. This was achieved through three key pillars: (1) Regeneron's proprietary Velocisuite® technologies, (2) deep monoclonal antibody process and manufacturing knowledge at both companies, and (3) Regeneron's and Genentech's commitment to deliver therapeutics to patients in need. Combined with business processes and risk management, these pillars rapidly allowed casirivimab and imdevimab to move to clinical manufacturing and to production at Genentech in a first-time process transfer under compressed timelines between the companies.

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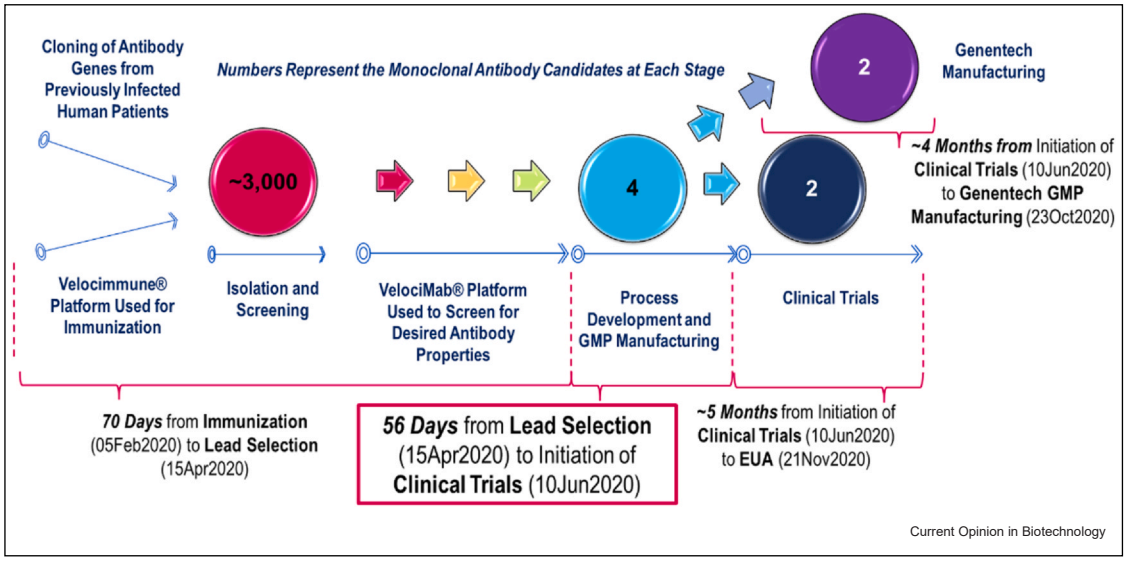
Introduction

Neutralizing antibodies have been used successfully in the treatment of infectious diseases [1]. The COVID-19 pandemic has specifically demonstrated the necessity to both accelerate the antibody development process (speed) and increase the demand for antibody treatments (need). Regeneron's platform approach to the expedited development of neutralizing antibodies to treat infectious diseases, previously demonstrated during the West Africa Ebola outbreak [2] and now incorporated into the first-time collaboration with Genentech to expand production capacity, has addressed speed and need requirements. The result has been the REGEN-COV™ cocktail consisting of the casirivimab and imdevimab monoclonal antibodies currently with emergency use authorization (EUA) or commercial approval in various countries.

The Regeneron platform using VelociSuite™ technologies allowed the identification of candidate antibodies in an accelerated fashion. As a historical example [2], these technologies were used to generate the now approved treatment for Ebola (Inmazeb®, a cocktail of three monoclonal antibodies). In the case of the COVID-19 pandemic, monoclonal antibody candidates against the spike protein of severe acute respiratory syndrome coronavirus 2 also used a cocktail approach [3] and resulted in obtaining an EUA for casirivimab and imdevimab in less than 10 months (Figure 1). More specifically, candidate antibodies were derived both from the VelocImmune® platform through immunization of VelocImmune® mice and by cloning from cells of previously infected human patients [4]. The VelociMab™ technology platform has an extensive history and brings these candidates into a platform allowing accelerated screening and production of these antibodies with high-level Chinese hamster ovary expression systems.

Process development and technology transfer, the focus of the current review, emphasized accelerated timelines (speed) and were performed on four candidate monoclonal antibodies, which included casirivimab and imdevimab that went from lead selection to initiation of clinical trials in 56 days. To accommodate potential demand (need), the platform process was simultaneously transferred to both the typical intermediate-scale single-use bioreactor platform (2000 L) and, for casirivimab and

Figure 1



Milestone event timelines. Timeline of key events in the generation of casirivimab and imdevimab from the time of initiation of monoclonal antibody generation using the VelocImmune® platform or cloning of antibody genes from previously infected patients through EUA. Numbers in the circles represent the antibody candidates available at the indicated stage in the process. Four candidates were taken into process development and Good Manufacturing Practices (GMP) Manufacturing. Casirivimab and imdevimab represent the final two antibodies that entered clinical trials and were produced at the largest scales available at Regeneron and Genentech. The red box emphasizes development and manufacturing activities that are further detailed in Figure 3.

imdevimab, the larger stainless-steel manufacturing scale (10 000 L) available at Regeneron. These transfers were the first instance in which the current version of the production platform was used at 10 000 L scale. After successful process performance qualification (PPQ) of the batches at the two scales at Regeneron, the process was rapidly transferred to an even larger stainless-steel manufacturing scale (25 000 L) at Genentech's Vacaville manufacturing facility as part of the Genentech collaboration. The process transfer for these antibodies was the first between the two companies. This transfer required building process transfer teams and understanding different manufacturing systems while working virtually as pandemic travel restrictions prevented in-person support. Use of key raw materials, during a period of supply chain disruptions, from Regeneron inventories for the initial batches at Genentech was a further demonstration of the collaborative effort. Collaborations and process transfers between biopharmaceutical companies do not routinely occur at this level of speed and need.

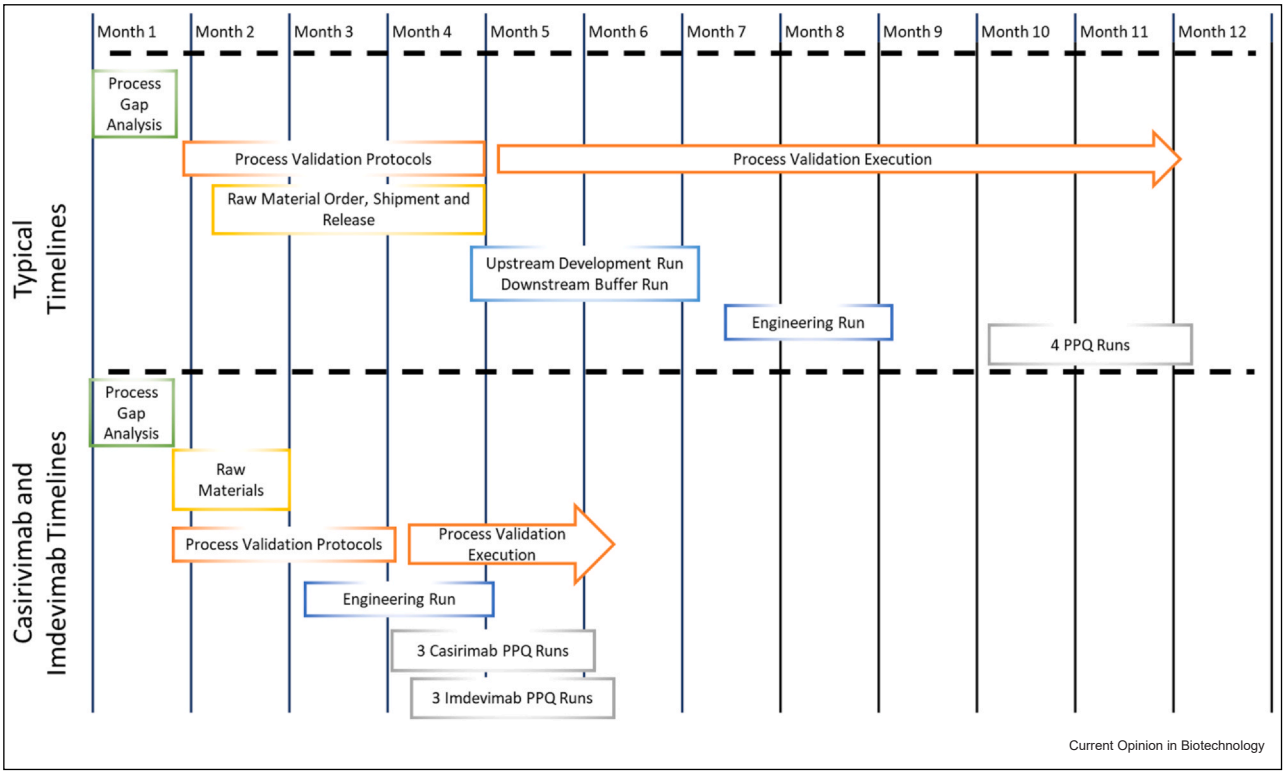
Genentech continued the focus on accelerated timelines by executing multiple steps of the typical transfer process in parallel (Figure 2). Site functional teams instilled the Regeneron platform process requirements by developing aligned process descriptions, batch records, and facility modifications concurrent with the project strategy deliverables such as transfer gap and risk analysis and master transfer plans.

Multiple entry points existed for optimizing the speed and need during development and transfer, and this paper describes a subset of the process development aspects for the first time. Downstream examples illustrate the speed obtained through minimizing the development timelines at Regeneron and performing at-scale resin carryover studies at Genentech in parallel with the manufacturing operations. Upstream examples address the need through successful production scale-up that focuses on appropriate production bioreactor gassing strategies at both companies.

Downstream process development and transfer emphasize speed

Development of the downstream process presented opportunities for speed, as, using prior knowledge of the well-characterized platform, some purification chromatography steps were rapidly optimized for casirivimab and imdevimab (Figure 3). The development window consisted of several days occurring between the harvest of the first-ever research batch (preview production) and the harvest of the material used in nonhuman primate toxicology studies (toxicology production). The screening efforts for the purification chromatography steps focused on defining setpoints and exploring the

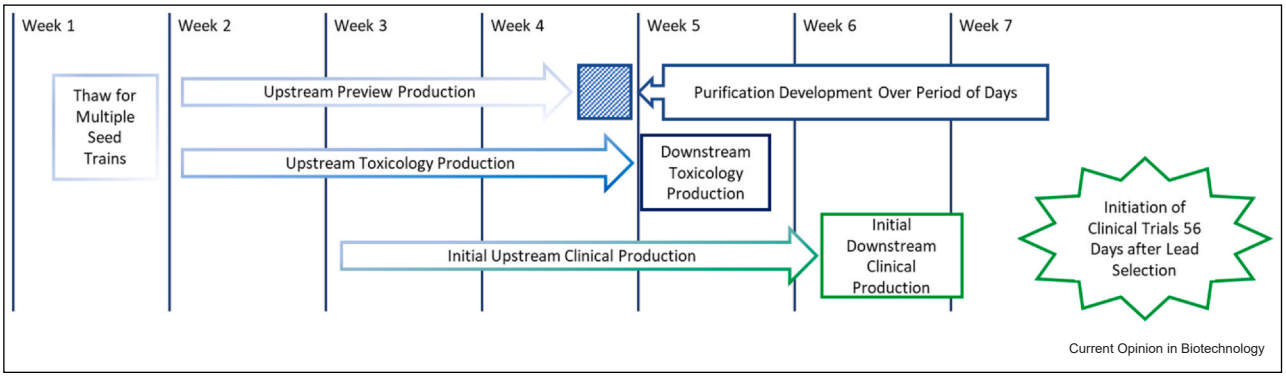
Figure 2



Typical Genentech timeline versus the casirivimab and imdevimab timelines. Typical timelines are approximately one year for one antibody and were shortened to just under six months by conducting standard activities in parallel and eliminating the batch prior to the engineering runs for casirivimab and imdevimab.

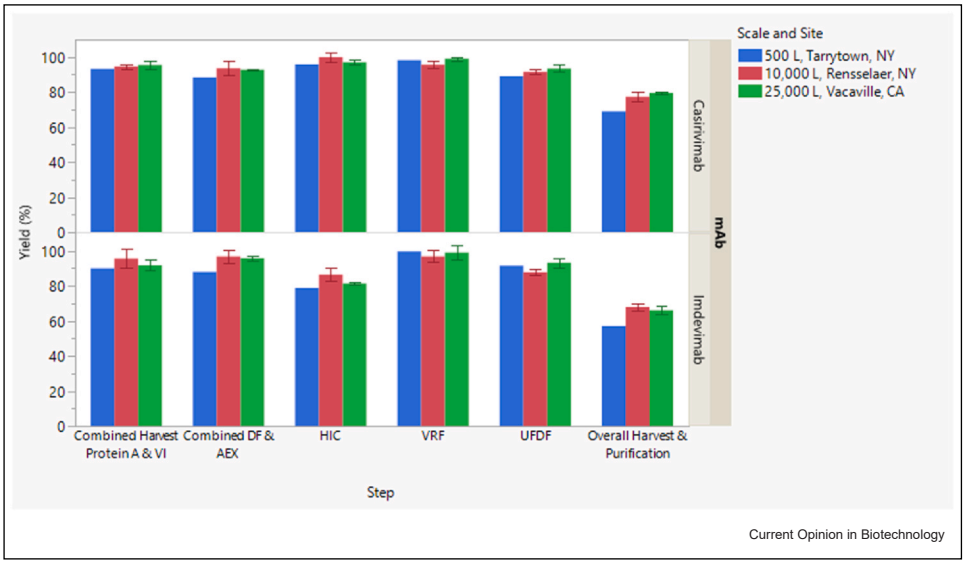
impact of variability in select process parameters. These efforts required careful coordination with groups providing analytical data that informed the final process setpoint decisions. In addition to providing material used in the toxicology studies, these production batches served as the only process verification for the initial clinical manufacturing, which was performed approximately one week later. Overall, these activities

Figure 3



Regeneron process development timelines. Upstream and downstream timelines are shown for the preview production batches used for process development, production batches for nonhuman primate toxicology studies (toxicology production), and initial clinical production batches. Speed is notably emphasized by the rapid purification development timelines, indicated by the hatched box, conducted in a matter of days, and the overlap of the timelines for the upstream toxicology batch, which also served as the final process verification, and the initial clinical production batch that followed by approximately one week.

Figure 4



Purification yields by unit operation. Purification step yields for the indicated downstream process unit operations for casirivimab and imdevimab, with the overall yield indicated by bars at the far right. The 500 L scale consists of a single batch for each mAb, while 10 000 L (N=4) and 25 000 L (N=3) scale manufacturing batches show the validation batches. Error bars are one standard deviation.

supported a timeline between lead selection and the start of clinical trials of 56 days.

This rapid development led to consistent product quality (data not shown) while optimizing the yields that transferred across scales (Figure 4).

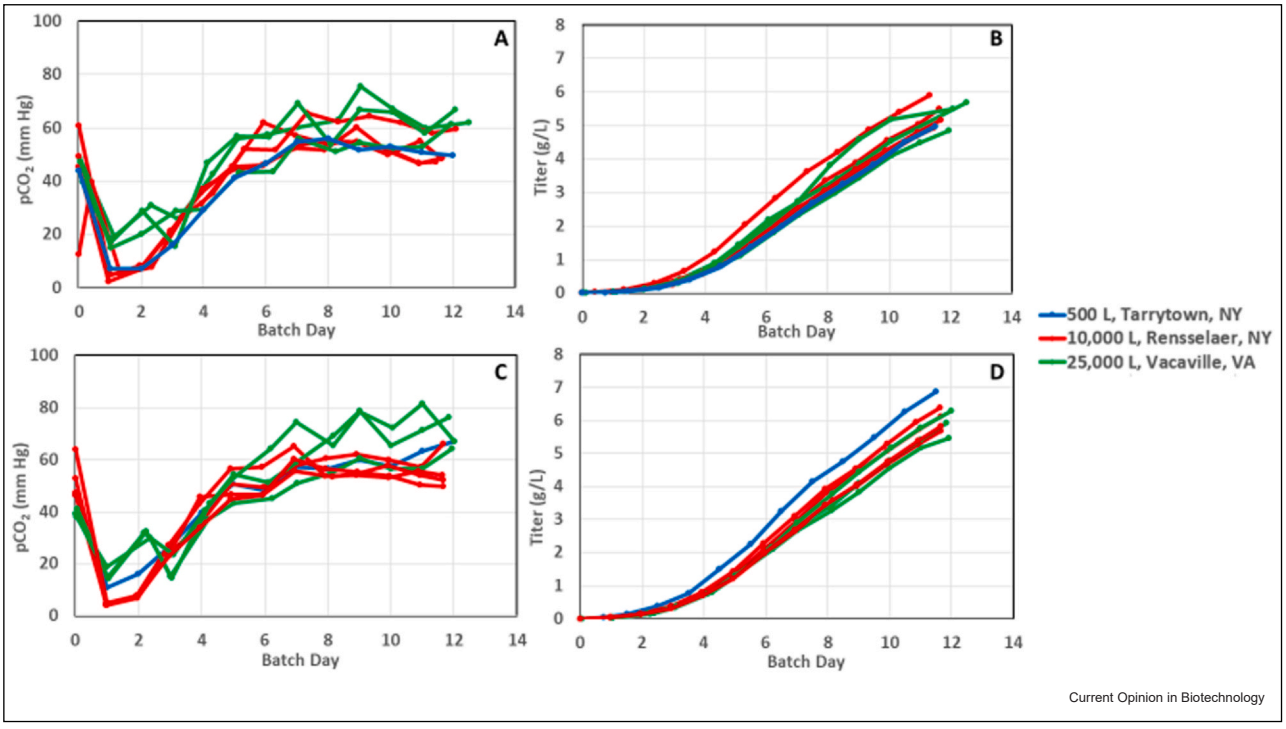
Speed via parallel approach to process implementation and characterization continued during technology transfer to 25 000 L scale at Genentech. The departure from the traditional linear technology transfer structure increased the risk to the project, but the project teams mitigated these risks by implementing modular capabilities for REGEN-COV™ production, creating flexibility in the operation of the facility (e.g. scalable depth filtration distribution manifold in line with the second chromatography step, enabling the option to perform virus filtration as the third or fourth purification step) instead of the traditional permanent or fixed modifications that often have increased scopes of work and the potential to affect existing processes. As previously shown in Figure 2, adoption of this approach supported a reduction of the transfer timeline by 57%, mainly by decoupling traditional stage gates and accelerating the implementation of master batch records and facility modifications.

A similar approach was taken with at-scale process validation activities. The importance of this project challenged the teams to look for innovative opportunities to integrate validation into routine operations, minimizing the production time lost due to validation and ultimately increasing the speed of transfer completion and availability of the product to patients. Demonstration of consistent and robust resin regeneration and sanitization processes is an important aspect of process scale-up and transfer. These studies often involve the preparation of a full suite of chromatography buffers and execution of nonproduction chromatography sequences to generate a 'mock pool' for carryover analysis, taking as long to complete as the time required to produce a full commercial batch for patient use. The joint transfer teams identified an opportunity to eliminate the dedication of plant time for nonproduction activities by integrating carryover validation into routine operations. The REGEN-COV™ chromatography precycle and Regeneron carryover strategy permitted sample collection and analysis of resin carryover in triplicate for each mAb during the qualification batch production. Capitalizing on this validation approach saved at least two commercial run slots in Genentech Vacaville for additional REGEN-COV™ production, which translated to an additional 100 000 doses.

Upstream process development and transfer emphasize need

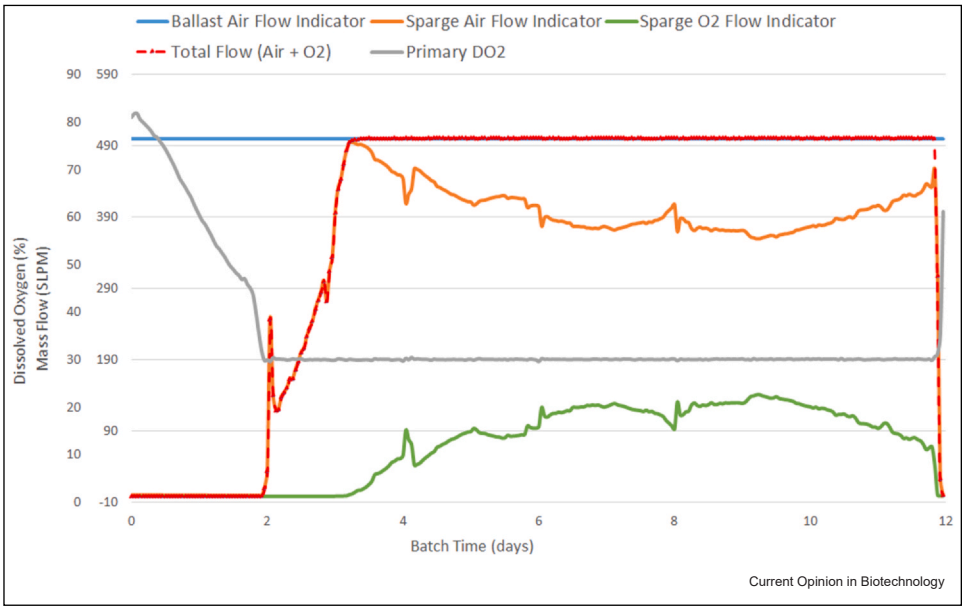
The movement to increased scales happened rapidly for the REGEN-COV™ program. Prior knowledge and platform aspects of the upstream process were critical to achieving rapid and successful process transfers. Historical data at Regeneron and previous literature findings [5,6] indicated that maintaining CO_2 profiles

Figure 5



Scale comparison of key upstream performance measures. Stainless-steel Regeneron bioreactor pilot (500 L, blue), Regeneron Manufacturing (10 000 L, red), and Genentech Manufacturing (25 000 L, green) profiles for (A) pCO_2 and (B) titer for casirivimab and (C) pCO_2 and (D) titer for imdevimab. The manufacturing batches shown represent validation batches.

Figure 6



Genentech dissolved oxygen manufacturing control strategy. Maintenance of the dissolved oxygen setpoint (30%; gray) required the addition of a constant 500 SLPM air sparge (blue) to the normal 25 000 L bioreactor strategy of increasing the air sparge to 500 SLPM (orange) and then replacing air with oxygen (green), maintaining a 500 SLPM total sparge (red) as part of the normal dissolved oxygen control system.

was an indicator of successful scale-up. This was initially shown across Regeneron scales and ultimately upon transfer to Genentech (Figure 5).

As previously mentioned, production at 10 000 L stainless-steel scale to satisfy material need had not been done with the platform process leveraged for imdevimab and casirivimab. To facilitate successful process transfer and scale-up, additional work considered oxygen supply for the production bioreactors and was conducted in a targeted, risk-based manner, leading to a successful technology transfer to 10 000 L scale without the use of engineering batches.

For speed of technology transfer to 25 000 L scale at Genentech, mechanical bioreactor modifications were restricted to those that were necessary. The standard dissolved oxygen (DO) strategy and equipment configuration in the 25 000 L bioreactor were not sufficient to match the process oxygen demand. The standard 25 000 L production bioreactor DO is controlled by both air and oxygen (O_2) sparge with a maximum combined sparge rate of 500 SLPM (standard liter per minute). The air sparge is used initially until a maximum flow of the air sparge is reached, and then the O_2 sparge replaces the air flow to maintain the DO setpoint. To accommodate the oxygen demand for casirivimab and imdevimab in the production bioreactor, an existing alternate ballast line sparger with sprayballs was repurposed with a fixed air flow rate of 500 SLPM (Figure 6).

Conclusions

The rapid launch of casirivimab and imdevimab as a COVID-19 treatment was initially enabled with Regeneron's Velocisuite® of technologies. Speed and ultimately production need were subsequently supported by a production platform with deep process knowledge and experience, which led to rapid development and process transfer at new scales within both Regeneron and Genentech. Both companies used their significant historical manufacturing expertise to accomplish successful process transfer in a first-time collaborative effort. The speed of this transfer, occurring under significant pandemic limitations, was a further confirmation of the culture at both companies of using science to address the need for timely treatments for the COVID-19 pandemic at both Regeneron and Genentech.

Author contributions

Aimee Lehman: Writing — original draft; Writing — review & editing. **Virgnia A. Muñiz**: Writing — original draft; Writing — review & editing. **Ryan Chaney**: Writing — original draft; Writing — review & editing. **Joseph Pimentel**: Writing — original draft; Writing — review & editing; Visualization. **John Mattila**: Writing — original draft; Writing — review & editing; Visualization. **Shawn Lawrence**: Conceptualization; Writing — original draft; Writing — review & editing.

Conflict of interest statement

Nothing declared.

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