EVUSHELD: Delivering Bioassay Methods and Critical Reagents Under Highly Accelerated Timelines from Clinical to Commercial phases

CASSS BIOASSAYS 2023

LeeAnn Machiesky

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AZD7442 is monoclonal antibodies (mAb) combination therapy comprised of 2 antibodies targeting non-overlapping epitopes on the RBD of the SARS-CoV-2 spike protein.

• The product is a co-pack of the 2 mAb and route of administration is intermuscular injection. Coformulation was investigated but never launched.

• AZ received Emergency Use Approval (EUA) for EVUSHELD in Dec 2021 and was the paused from the market in Jan 2023.

• The target population is immuno-compromised patients and is the only mAb authorized for pre-exposure treatment.

• Five mAbs authorized for EUA for treatment of mild to moderate COVID-19 disease include: ACTEMRA® (tocilizumab), Bamlanivimab, Bebtelovimab, REGEN-COV® (casirivimab and imdevimab), Sotrovimab.
**EVUSHELD**

**MOA: mechanism of action and engineering**

**MOA:** Binding to RBD of SARS-CoV-2 spike protein, blocking viral entry and attachment

**Molecular engineering:**
- **YTE mutation** to increase binding affinity to the FcRn protein which increasing product half-life and thus reduces dosing frequency for patients.
- **TM (triple mutation)** to reduce unwanted Fc effector function, thus increasing safety profile.

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# Overview of Regulatory Approvals

**Last Modified: 15 Feb 2023 09:31 AM US ET**

<table>
<thead>
<tr>
<th>Market</th>
<th>Area</th>
<th>Group</th>
<th>Sub-Group</th>
<th>Pathway</th>
<th>Status</th>
<th>Indication(s)</th>
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<td>INT</td>
<td>MEA</td>
<td>GCC</td>
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</table>

- **55** Authorizations
- **22** Pathway MA
- **29** Pathway EUA
- **4** Pathway Import
- **2** Indications
**Overall Analytical Strategy**

*Delivery of full panel of lot release methods in 8-10 weeks.*

<table>
<thead>
<tr>
<th>Analytical Release Panel</th>
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<tbody>
<tr>
<td>Appearance - clarity</td>
</tr>
<tr>
<td>Appearance - color</td>
</tr>
<tr>
<td>Appearance – particles</td>
</tr>
<tr>
<td>Bioburden</td>
</tr>
<tr>
<td>Capillary isoelectric focusing (cIEF)</td>
</tr>
<tr>
<td>Endotoxin</td>
</tr>
<tr>
<td>High performance size exclusion chromatography (HPSEC)</td>
</tr>
<tr>
<td>Host cell proteins</td>
</tr>
<tr>
<td>Host cell DNA</td>
</tr>
<tr>
<td>Non-reducing CE-SDS</td>
</tr>
<tr>
<td>Osmolality</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Protein A</td>
</tr>
<tr>
<td>Polysorbate 80</td>
</tr>
<tr>
<td>Sterility</td>
</tr>
<tr>
<td>Sub-visible particles</td>
</tr>
<tr>
<td><strong>SARS-COV-2 RBD binding DELFIA</strong></td>
</tr>
<tr>
<td>Total protein</td>
</tr>
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</table>
Accelerated Delivery of Potency Methods

Weekly Trends in Number of COVID-19 Cases in The United States Reported to CDC

- April-May: Binding Method DEV
- May-June: PV Neut Qual/TT
- June: Antibody Discovery to clone selection
- June 20': IND Filed
- July 20'-Mar 21': Pseudovirus Generation, Qual
- July 21': Bridging Study
- Jul 21': Emergency Use Authorization
- Jan 17, 2022: Spike: 6M weekly cases

https://covid.cdc.gov/covid-data-tracker/#trends_weeklycases
Bioassay Strategy
Guidance Documents for Anti-viral Products

Pre-pandemic – Dec 2020:

January 2021:

March 2023:
“Guidance for Industry: Potency Assay Considerations for Monoclonal Antibodies and Other Therapeutic Proteins Targeting Viral Pathogens.”
Regulatory Guidance released from FDA, in January 2021

**COVID 19: Potency Assay considerations for monoclonal antibodies and other therapeutic proteins targeting SARS-CoV-2 infectivity**

## A. Methods

Although a binding assay is generally sufficient to serve as a potency assay at the IND stage of development, because it demonstrates binding between the mAb or therapeutic protein and its target, a binding assay assesses only one aspect of the potency of a product; therefore, sponsors should subsequently develop methods that more comprehensively monitor the proposed mechanism(s) of action of the products. These methods should be incorporated into drug substance and drug product release testing and stability protocols. Potency assays should be described, justified, qualified, and validated to support a BLA.

## 2. Viral Neutralization Assays

In comparison to binding assays, in vitro viral neutralization assays more comprehensively confirm a mAb’s or therapeutic protein’s mechanism of action and potency in blocking viral entry into susceptible cells. Because of the potential importance to evaluating these products, the Agency recommends establishing an in vitro viral neutralization assay early in development. This type of assay can be useful for advancing development, quality control, and characterization of neutralizing mAbs and other products targeting viral entry. The SARS-CoV-2 cellular entry includes four steps:

1. Binding to the cell surface receptor ACE2
2. Proteolytic cleavage of the spike (S) protein (by TMPRSS2 and/or Cathepsin L)
3. Six-helix bundle formation leading to virus-cell fusion
4. Release of the viral capsid into the cytosol

Assays that assess the ability of the protein(s) to inhibit any of these steps are predominantly cell-based assays and typically involve the use of wild-type (wt) virus, pseudotyped virus, or pseudotyped virus-like particles (VLP). When considering which method to use, sponsors
Proactively engaged with FDA for approval of our strategy.
Characterization Strategy

Biological Characterization Methods

- Live Virus Neutralization
- Target binding
- FcRn binding
- FcγR binding

Primary cell w/live virus*

- mAb specific RBD Binding
- AlphaLISA®
- SPR

* External lab
Lot Release
Binding Assay
Utilized DELFIA® binding method for rapid development

- 1 plate assay design
- Established incubation times
- Critical reagents main point of development
  - Binding protein (Spike, S1, RBD?)
  - EU-IgG secondary mAb
  - Product mAb concentration and dilution interval

- Quick transfer to GMP lab due to analyst familiarity with DELFIA assay format
- Ability to initiate automation early

From initial clones to qualification in 2 months
Method Qualification

- Qualification was conducted according to ICH guidelines Q2 R1, R2.
- All assays passed criteria for **linearity, intermediate precision, repeatability, range (50-150%), specificity and stability indicating properties**.
- Performed method co-qualification with GMP testing group.
- Also qualified method on Hamilton Robot for DEV and GMP testing.

*Also tested emerging strains of SARS-CoV-2 for product characterization.*
Virus Neutralization Assays
Virus Neutralization Assays

**Live Virus Killing Assay – BSL3**
- Vero cells
- Cells fixed
- Immunoassay with Fluorescence readout

**Pseudovirus Neutralization**
- SARS-CoV-2 Pseudovirus (Luc)
- ACE2
- AD293 cells
- Infection
- Luc
- Mab A^*
- Mab B^*
“Making a Pseudovirus small scale <3L”

Research process in place.

Transfection in 293X cell
Harvest (spin and filter)
Concentrate/purify
Resuspend pellets
Aliquot
Test for activity

~ 50 Individual use aliquots
“But how do we Scale-up?”

Major Hurdles found during Scale-up and need for GMP quality reagents

Major Hurdles for Scale up:
1. Low virus Titer
2. Concentration Step:
   - Large volumes 3L to 10 L
   - Equipment availability
3. Speed of the process (viral activity matters)

Solution:
1. Changed the plasmid ratio
2. Moved from using an ultracentrifuge to floor centrifuge + sucrose cushion
3. Moved purification step from research to development labs

Updated plasmid ratio & Concentration Step!

New process production was successful.
“How many scientists does it take to make Pseudovirus?”

Day 1
- 7 liters of harvested virus media
  - Add into 24 bottles
  - Add sucrose to bottles, balance
  - Load 4 centrifuges

Day 2
- Unload 4 centrifuges
- Resuspend 24 pellets, pool together
- Aliquot into 300 pre-labeled vials
- Freeze at -80°C

Sequence# | #vials | Dilution in assay
--- | --- | ---
-Lot #5 | 290 | 1:50
-Lot #6 | 320 | 1:50
-Lot #7 | 324 | 1:50

After moving the concentration step to the development lab, we were able to make 3 active lots in sequence.
PV Method Development and Qualification

Development:
Investigated many parameters: reagent volumes, pre-plating cells, use of dextran, incubation temperature, media components; however, no major changes were made. Found that the activity of the PV was the most impactful component for method performance and variability of the upper asymptote.

Qualification:
Qualification was executed in April 2021 according to ICH guidelines Q2 R1,R2. Assays passed the qualification targets; however, results for accuracy, intermediate precision, repeatability were close to the limits of the acceptance criteria.

Methods for AZD1061 and AZD8895 were successfully transferred to the GMP team, commercial team and eventually to CROs in China and South Korea.
Bridging Study: DELFIA binding vs PV Neutralization

Equivalence Across Potency Range:

<table>
<thead>
<tr>
<th>Potency Levels</th>
<th>Mean % Recovery Target Binding</th>
<th>Mean % Recovery PV Neut</th>
<th>Mean Difference</th>
<th>90% CI of Mean Difference</th>
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</thead>
<tbody>
<tr>
<td>50%</td>
<td>100.4</td>
<td>93.1</td>
<td>-7.3</td>
<td>-17.4, 2.8</td>
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<tr>
<td>75%</td>
<td>102.1</td>
<td>92.8</td>
<td>-9.4</td>
<td>-19.5, 0.8</td>
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<tr>
<td>100%</td>
<td>100.1</td>
<td>92.8</td>
<td>-7.3</td>
<td>-17.4, 2.8</td>
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<td>125%</td>
<td>101.5</td>
<td>105.0</td>
<td>3.5</td>
<td>-6.6, 13.7</td>
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<tr>
<td>150%</td>
<td>105.5</td>
<td>100.9</td>
<td>-4.6</td>
<td>-14.7, 5.6</td>
</tr>
</tbody>
</table>

Comparison of DS Release result across multiple lots:

- 2 x Process 1, 2000L scale @ site 1
- 3 x Process 2, 2000L scale @ Site 1
- 6 x Process 2, 15K scale @ site 2

Comparison of stressed samples and stability samples.

No significant different in %RP between methods for samples stressed with UV or 40°C.
Remaining Hurdles for use of PV Neutralization Method

- Assay variability is higher than desired.
- Production of additional lot of virus requirement significant time resources.
- Significant time and required to meet shipping requirements for sending PV to China and possibly other countries.
- No commercial manufacturer of the PV, therefore not the most viable option for commercial lot release.
- Major re-work required to manufacturer new strains of PV.

What should be our path forward for our next generation of anti-COVID mAbs?
2. **Viral Neutralization Assays**

In comparison to binding assays, in vitro viral neutralization assays more comprehensively confirm a mAb’s or therapeutic protein’s mechanism of action and potency in blocking infection of susceptible cells. Because of the potential importance to evaluating these products, the Agency recommends establishing an in vitro viral neutralization assay early in development. This type of assay can be useful for advancing development, quality control, and characterization of neutralizing mAbs and other products targeting viral attachment and entry. Given the diversity of mechanisms for viral attachment and entry into host cells, the assay should reflect that virus’s mechanisms for attachment and entry.

Assays that assess the ability of the mAbs or other therapeutic proteins to inhibit any of the binding or entry steps are predominantly cell-based assays and typically involve the use of wild-type (wt) virus, \[^{19}\] pseudotyped virus, or pseudotyped virus-like particles (VLP). When considering which method to use, sponsors should select a method that best monitors the binding/entry step the product is expected to target in the virus replication cycle. Although wt virus neutralization assays are considered the gold standard for in vitro potency assays, alternative methods may be acceptable. For example, a potency assay could be designed to characterize the effect of the product on a specific entry step (e.g., virus-cell fusion). Additionally, accessibility to appropriate BSL laboratories, as well as challenges to qualifying critical reagents and validating the overall assay performance, should be considered in assay selection. For methods using transfected cell lines, sponsors should also address target cell viability and variability. Whichever method is ultimately used, sponsors should observe all provisions of the select agent regulations\[^{20}\] (if applicable) and other applicable governmental and institutional biosafety and biosecurity provisions.
VLP Assay System from Promega

Assay Design:

1. HiBiT-tagged VLPs pseudotyped with SARS-CoV-2 Spike protein are added to SARS-CoV-2 Target Cells
   - HiBiT is packaged inside the PsVLPs

2. In the absence of inhibitors or neutralizing antibodies (nAbs), SARS-CoV-2 HiBiT-PsVLPs bind to target cells via Spike/ACE2 interaction and undergo membrane fusion mediated by cellular proteases. HiBiT is released into target cells and binds to LgBiT to generate a luminescent signal in the presence of substrate.

3. In the presence of inhibitors or nAbs of SARS-CoV-2 entry, the entry/fusion processes of PsVLPs are blocked, thereby preventing HiBiT release. No luminescent signal is produced.

https://www.promega.com/applications/infectious-diseases/viral-research/sars-cov2-therapeutic-vaccine-discovery
VLP Neutralization Results (omicron strain)

Repeatability

Linearity

<table>
<thead>
<tr>
<th>sample</th>
<th>% RP</th>
<th>% accuracy</th>
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<td>NA</td>
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<tr>
<td>AC</td>
<td>101</td>
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<tr>
<td>(50%)</td>
<td>54</td>
<td>108</td>
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<td>(75%)</td>
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<td>(125%)</td>
<td>128</td>
<td>102</td>
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<tr>
<td>(150%)</td>
<td>147</td>
<td>98</td>
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Average accuracy = 95%

Average accuracy = 102%

Assay system produces very accurate results with little optimization required.
PV vs VLP Neutralization Assay (omicron strain)

**PV Neutralization**

```
mAb 1
IC50 8.4
mAb 1+2
IC50 18
```

**VLP Neutralization**

```
mAb 1
IC50 76
mAb 1+2
IC50 136
```

**Similar shifts in neutralization between antibodies indicates methods are comparable and representing virus neutralization using similar system kinetics.**
PV vs VLP neutralization method:

<table>
<thead>
<tr>
<th></th>
<th>Pseudovirus method</th>
<th>VLP method</th>
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<tr>
<td>Assay incubation time?</td>
<td>20-22 hrs</td>
<td>4 hrs</td>
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<tr>
<td>Commercial source of CR?</td>
<td>No</td>
<td>yes</td>
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<tr>
<td>Inhouse plasmids needed?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Biosafety concerns for shipping?</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>New strains can be made rapidly?</td>
<td>Yes, but not in bulk. Scale up can take months of optimization and testing.</td>
<td>Likely yes. Would take 1-2 months for new lots. Multiple strains are available.</td>
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<td>Method variability?</td>
<td>Medium</td>
<td>Low</td>
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<td>Recommended by FDA as surrogate for live virus?</td>
<td>Yes</td>
<td>yes</td>
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**VLP method is preferred method for testing of future anti-SARS-CoV-2 IgGs**
Critical Reagents purchased/made, vialed & qualified

**2020-22 for EVUSHELD**

<table>
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<tr>
<th>Reagent</th>
<th># of Vials</th>
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<tr>
<td>Wuhan RBD</td>
<td>1600</td>
<td>External</td>
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<tr>
<td>EU-IgG</td>
<td>1600</td>
<td>External</td>
</tr>
<tr>
<td>RBD mutant (1061)</td>
<td>500</td>
<td>AZ</td>
</tr>
<tr>
<td>RBD mutant (8895)</td>
<td>500</td>
<td>AZ</td>
</tr>
<tr>
<td>Pseudovirus Lot#1,2</td>
<td>580</td>
<td>AZ</td>
</tr>
<tr>
<td>Pseudovirus Lot#5,6,7</td>
<td>950</td>
<td>AZ</td>
</tr>
<tr>
<td>ACE-2 (ARCB) inhouse</td>
<td>390</td>
<td>AZ</td>
</tr>
<tr>
<td>ACE-2 (ARCB) contract lab</td>
<td>1000</td>
<td>External</td>
</tr>
</tbody>
</table>

6,120 vials
2000 Samples Tested in 18 months

1,400 - DEV
600 - GMP
Thank-you!
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