Elranatamab: Bispecific mAb Bioassay Strategy from R&D to Commercialization

Peter Richard

Principal Scientist

Pfizer Biotherapeutics Pharmaceutical Sciences

Analytical Research and Development

Pfizer

Tuesday, 08-April-2025

Agenda

1. Introduction to Elranatamab
2. Initial Bioassay Strategy for Early Clinical Phases
3. Later Bioassay Strategy for Clinical Development
4. Bioassay Strategy for Commercialization
5. Conclusion

Elranatamab

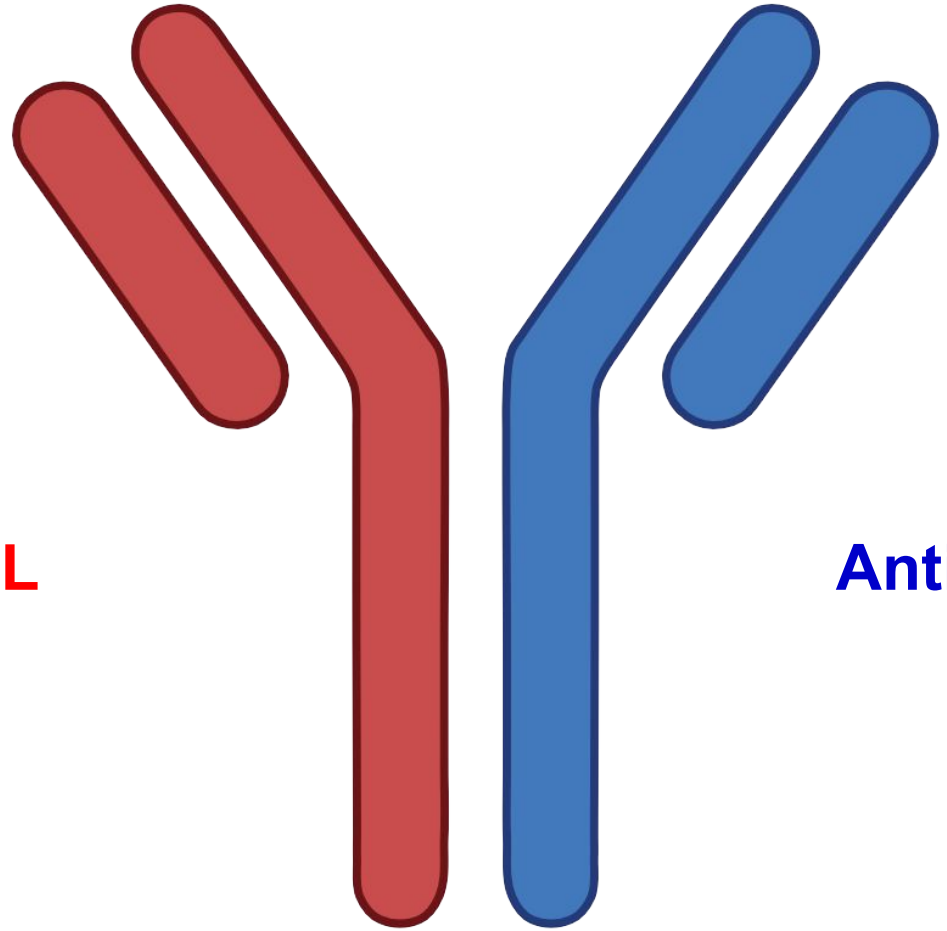
Elranatamab is a recombinant human bispecific antibody treatment for relapsed and refractory multiple myeloma.

Elranatamab is derived from two separate monoclonal antibodies (mAbs):

1. **Anti-B-cell maturation antigen (BCMA) mAb**
2. **Anti-cluster of differentiation 3 (CD3) mAb**

Each mAb contributes one distinct heavy (H) chain and one distinct light (L) chain to the bispecific elranatamab.

Elranatamab's Bispecific Structure



Anti-BCMA H and L Chains

Anti-CD3 H and L Chains

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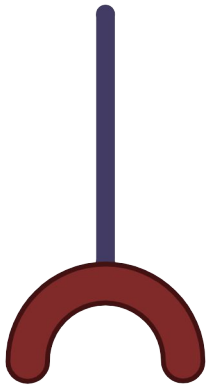
Elranatamab's Bispecific Targets

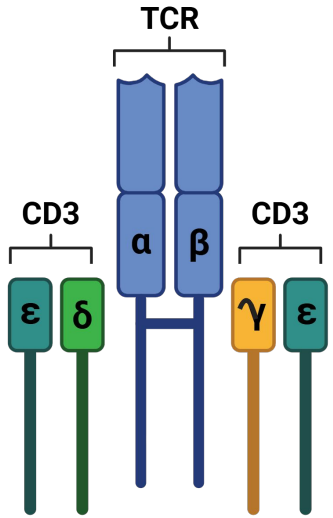
1. B Cell Maturation Antigen (BCMA)



- B cell surface receptor
  - Overexpressed on the surface of myeloma cells

2. Cluster of Differentiation 3 (CD3)

- T cell co-receptor
  - Prominent role in T cell activation



TCR/CD3 Complex

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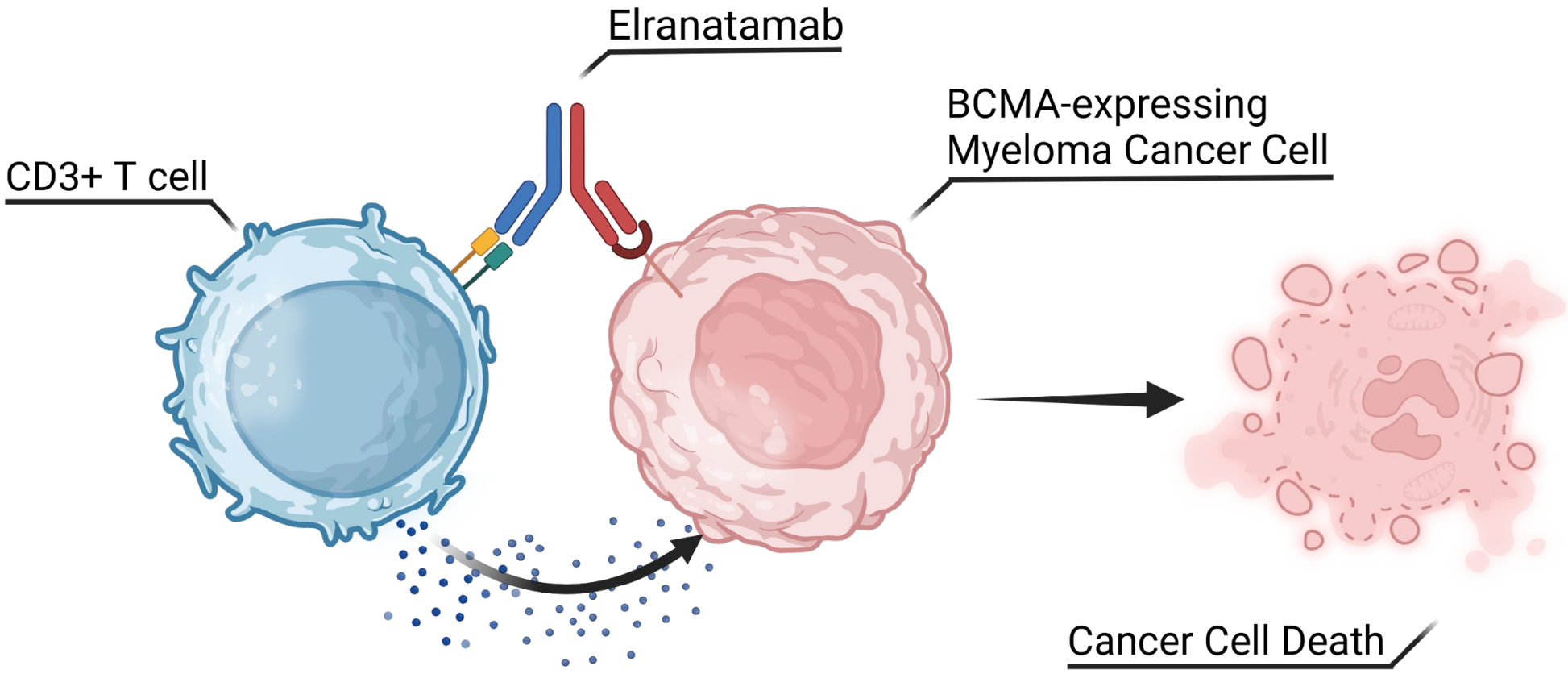
Elranatamab's Mechanism of Action

Elranatamab's dual binding process transiently tethers the BCMA-expressing myeloma cell and the CD3-expressing T cell, activating the T cell to destroy the myeloma cell.



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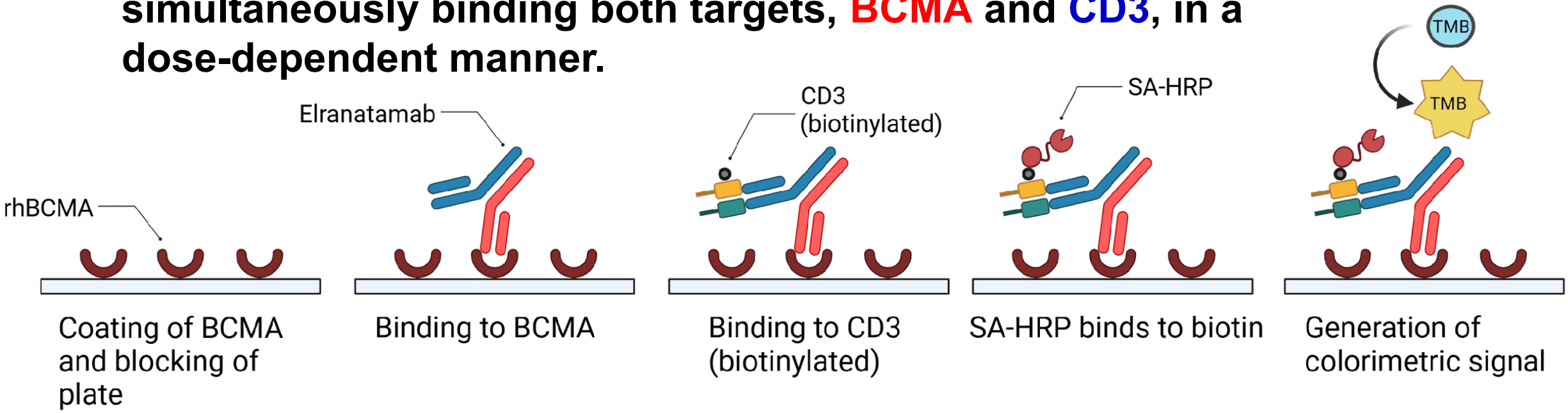
Elranatamab's Initial Bioassay Strategy

Launch with Phase 1 and carry us into Phase 2

A Bispecific Target Binding ELISA was developed and qualified to evaluate the in vitro biological activity of elranatamab.

- **This ELISA demonstrates that elranatamab is capable of simultaneously binding both targets, BCMA and CD3, in a dose-dependent manner.**



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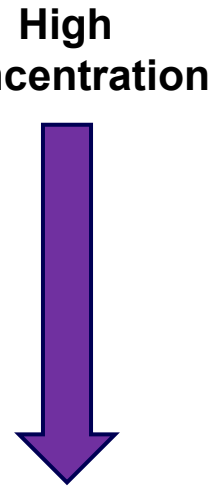
Elranatamab Target Binding ELISA Plate Format

The Target Binding ELISA is a nonautomated single plate assay with each test article loaded in triplicate across the plate.

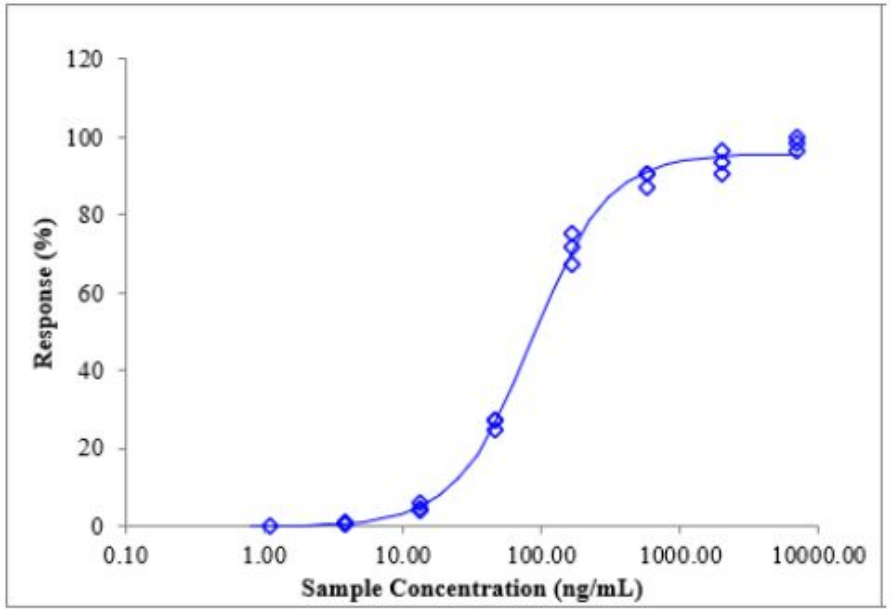
	1	2	3	4	5	6	7	8	9	10	11	12
A	Reference Material	Test Sample 1	Test Sample 2	Test Sample 3	Reference Material	Test Sample 1	Test Sample 2	Test Sample 3	Reference Material	Test Sample 1	Test Sample 2	Test Sample 3
B												
C												
D												
E												
F												
G												
H												

Internal Control sample may be loaded into any Test Sample Position



Data Analysis for the Elranatamab Target Binding ELISA

- Non-linear curve fitting
- Assessment of parallelism between the standard (reference material) and sample curves.
- Sample potency is determined from the shift in EC50 between the parallel curves.
- The relative potency (%) of the sample is reported vs. the reference material



Qualification of the Elranatamab Target Binding ELISA

Qualification at Target		Result
Sample at Target (Reference 100%)	Repeatability (% RSD)	2.0
	Intermediate Precision (% RSD)	4.9
	Accuracy (% Accuracy)	99.4

Qualification across Range		Result
Sample across Range (Reference 50-150%)	Intermediate Precision (% RSD)	≤ 6.7
	Accuracy (% Accuracy)	± 2.5

Elranatamab Target Binding ELISA is Filed as the Potency Test Method in the Initial IND

Regulatory Request for Information:

- Establish an appropriate cell-based potency method that reflects the mechanism of action of your product in the intended clinical population, as clinical development proceeds.

Pfizer's Commitments

- Pursue development of a cell-based potency assay
  - Reflects the primary presumed in vivo mechanism of action
  - Includes appropriate acceptance criteria
- Submit an IND amendment prior to initiating Phase 3 trials
  - Outcomes of assay development and evaluation
  - Drug substance and drug product release and stability testing
- Conserve sample retains from non-clinical and clinical
  - Qualification/validation of any future potency assay
  - Ensure lot-to-lot consistency of potency

Elranatamab Cell-Based Bioassay

Functional Potency Method to take us into Phase 3 and Beyond

Development

T Cell Activation (Reporter Gene) Assay

Target Cell Line: MM-1S

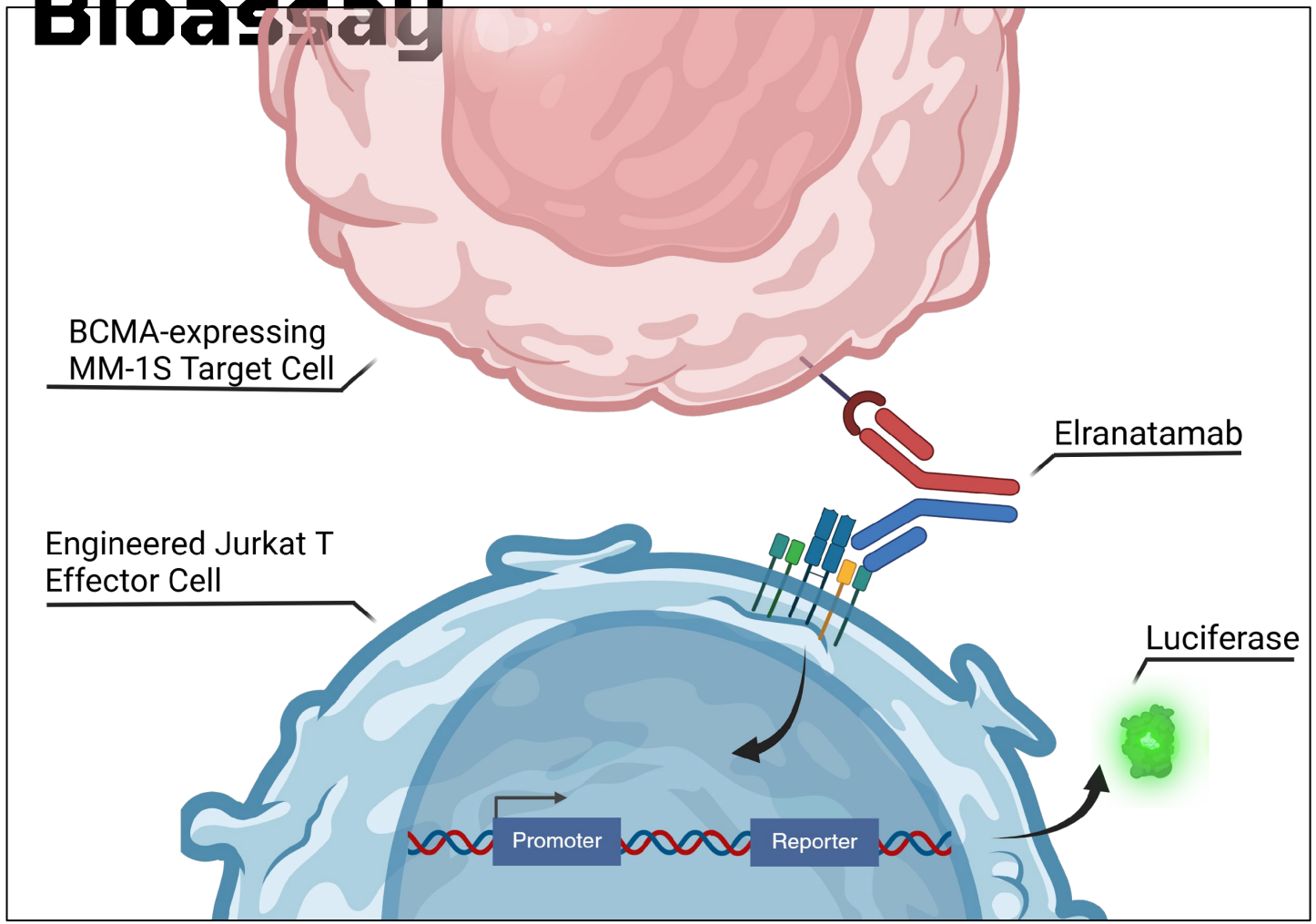
BCMA-presenting target tumor cells

Effector Cell Line: Jurkat T Effector Cell Line

Endogenous TCR/CD3 complex
and engineered with a luciferase reporter gene
expressed upon T cell activation

Both commercially available

Mechanism of the Elranatamab Cell-Based Bioassay



T cell activation: Engagement of the T cell's TCR/CD3 complex activates intracellular signaling events and the expression of the luciferase

Image Created in https://BioRender.com

Elranatamab Cell-Based Bioassay Plate Format

The Cell-Based Bioassay is a nonautomated single plate assay with each test article loaded in triplicate across the plate.

	1	2	3	4	5	6	7	8	9	10	11	12
A	Reference Material	Test Sample 1	Test Sample 2	Test Sample 3	Reference Material	Test Sample 1	Test Sample 2	Test Sample 3	Reference Material	Test Sample 1	Test Sample 2	Test Sample 3
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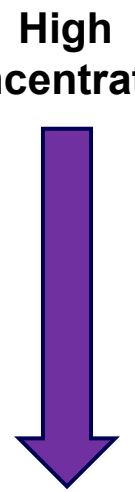
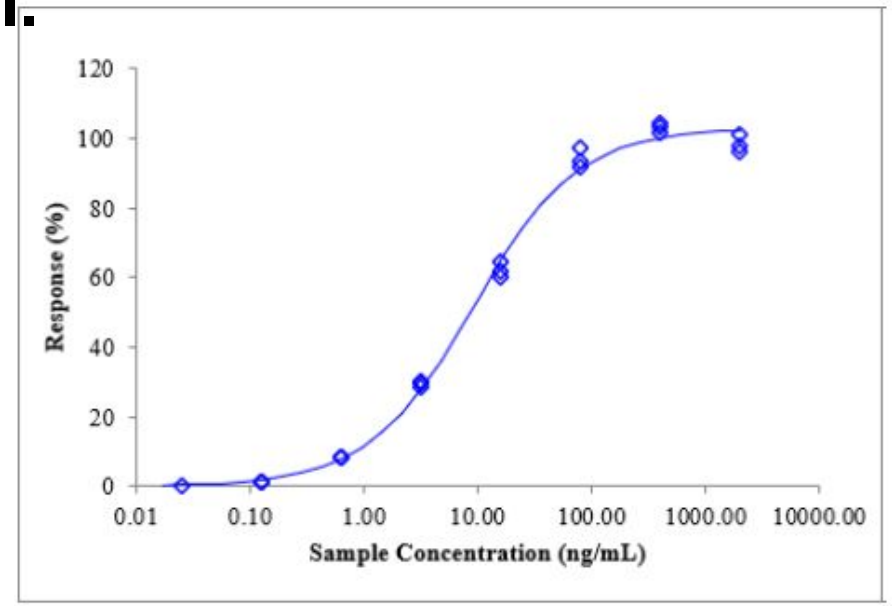
Internal Control sample may be loaded into any Test Sample Position



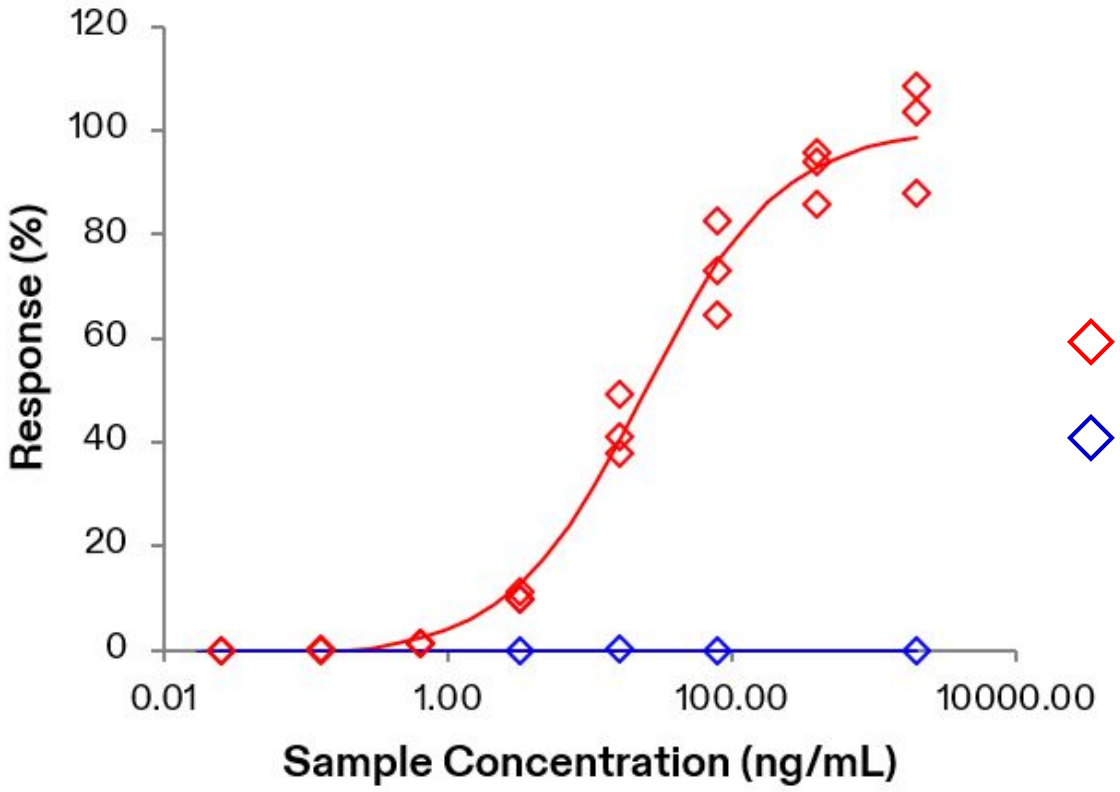
Elranatamab Cell-Based Bioassay Data Analysis

- Non-linear curve fitting
- An assessment of parallelism between the standard (reference material) and sample curves.
- Sample potency is determined from the shift in EC50 between the parallel curves.
- The relative potency (%) of the sample is reported vs. the reference material.



Binding Specificity of the Cell-Based Assay

- T cell activation does not occur in the cell-based assay without co-engagement of both **BCMA** and **CD3** on their respective cell lines.



Qualification of the Elranatamab Cell-Based Bioassay

Qualification at Target		Result
Sample at Target (Reference 100%)	Repeatability (% RSD)	12
	Intermediate Precision (% RSD)	9.7
	Accuracy (% Accuracy)	98.6

Qualification across Range		Result
Sample across Range (Reference 50-150%)	Intermediate Precision (% RSD)	≤ 8.9
	Accuracy (% Accuracy)	± 10

Two Elranatamab Potency Test Methods

In addition to the target binding ELISA, the qualified cell-based bioassay is established for drug substance and drug product release and stability testing of pivotal clinical batches.

Both the binding ELISA and the cell-based bioassay test methods were successfully validated and transferred to commercial laboratories in advance of Process Validation.

Bioassay Strategy for Commercialization

Transition from the Target Binding ELISA to the Cell-Based Bioassay as the single potency test method for commercial testing.

The Cell-Based Bioassay more closely demonstrates the mechanism of action of elranatamab

- **Binding to BCMA and CD3 cellular targets**
- **Cellular co-engagement**
- **T cell activation**

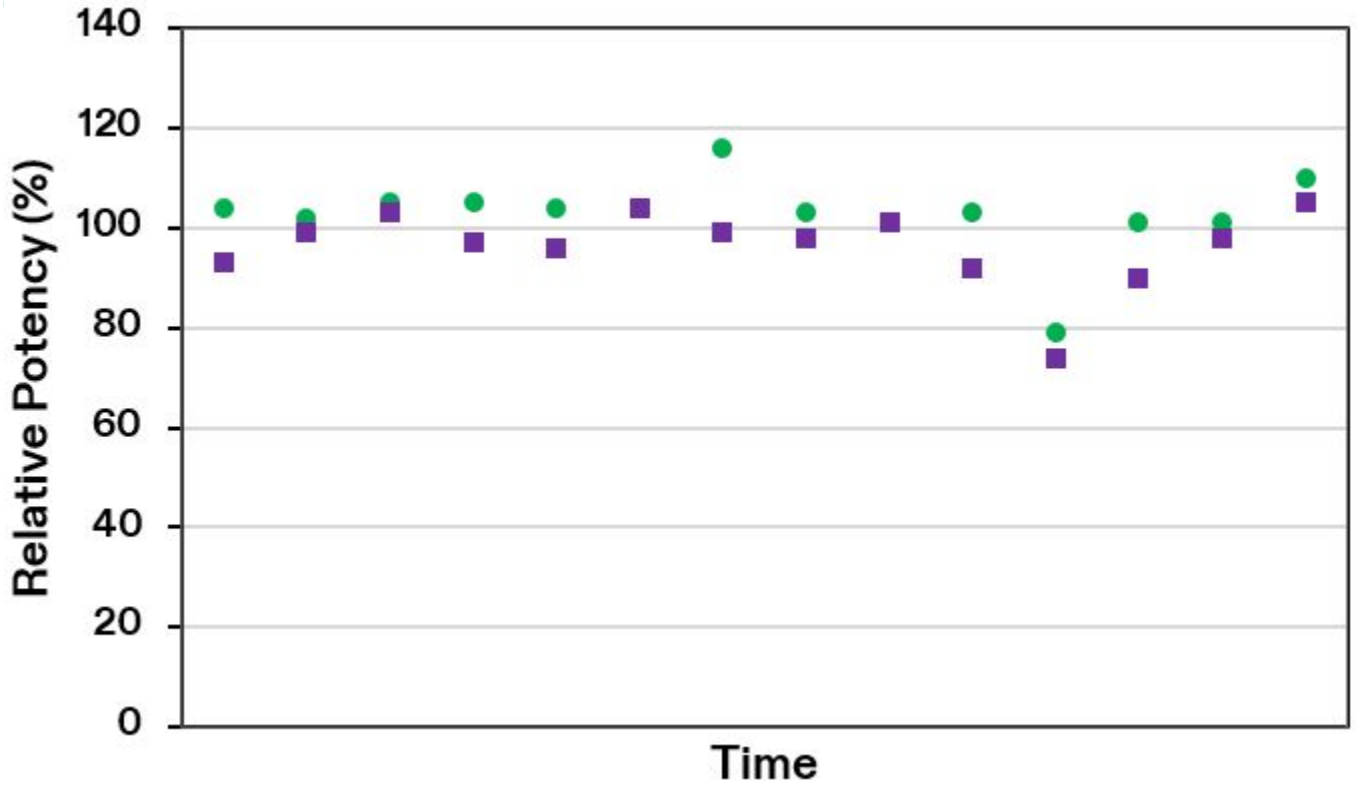
Justification of Bioassay Strategy for Commercialization

Data used to support the transition from the Target Binding ELISA to the Cell-Based Bioassay as the single potency test method for commercial testing:

- Drug Substance Batch Release and Stability
- Drug Product Lot Release and Stability
- Spiked Clipped Species
- Forced Degradation

Elranatamab Drug Substance Primary Stability Studies:

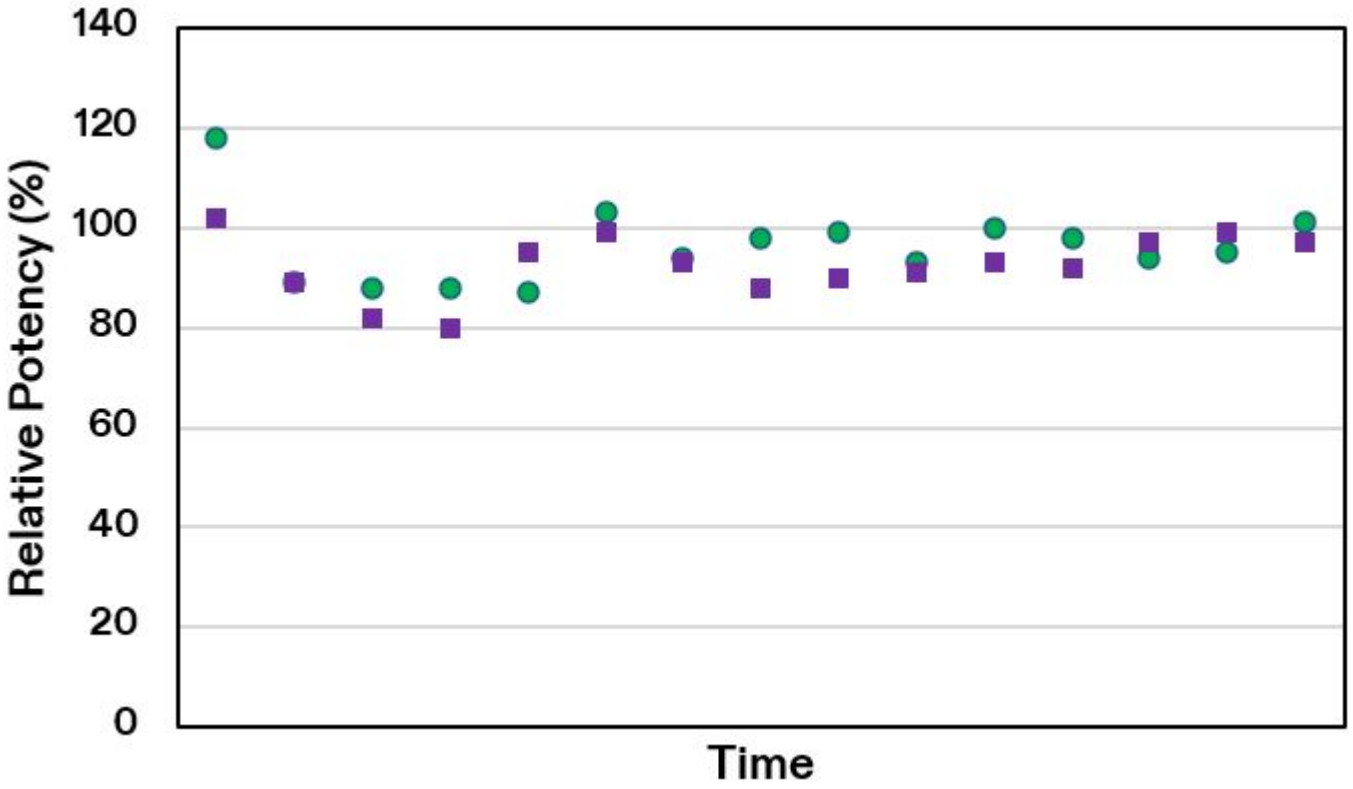
Target Binding ELISA and Cell Based Bioassay Results



Both sets of results correlate across 3 Drug Substance batches.

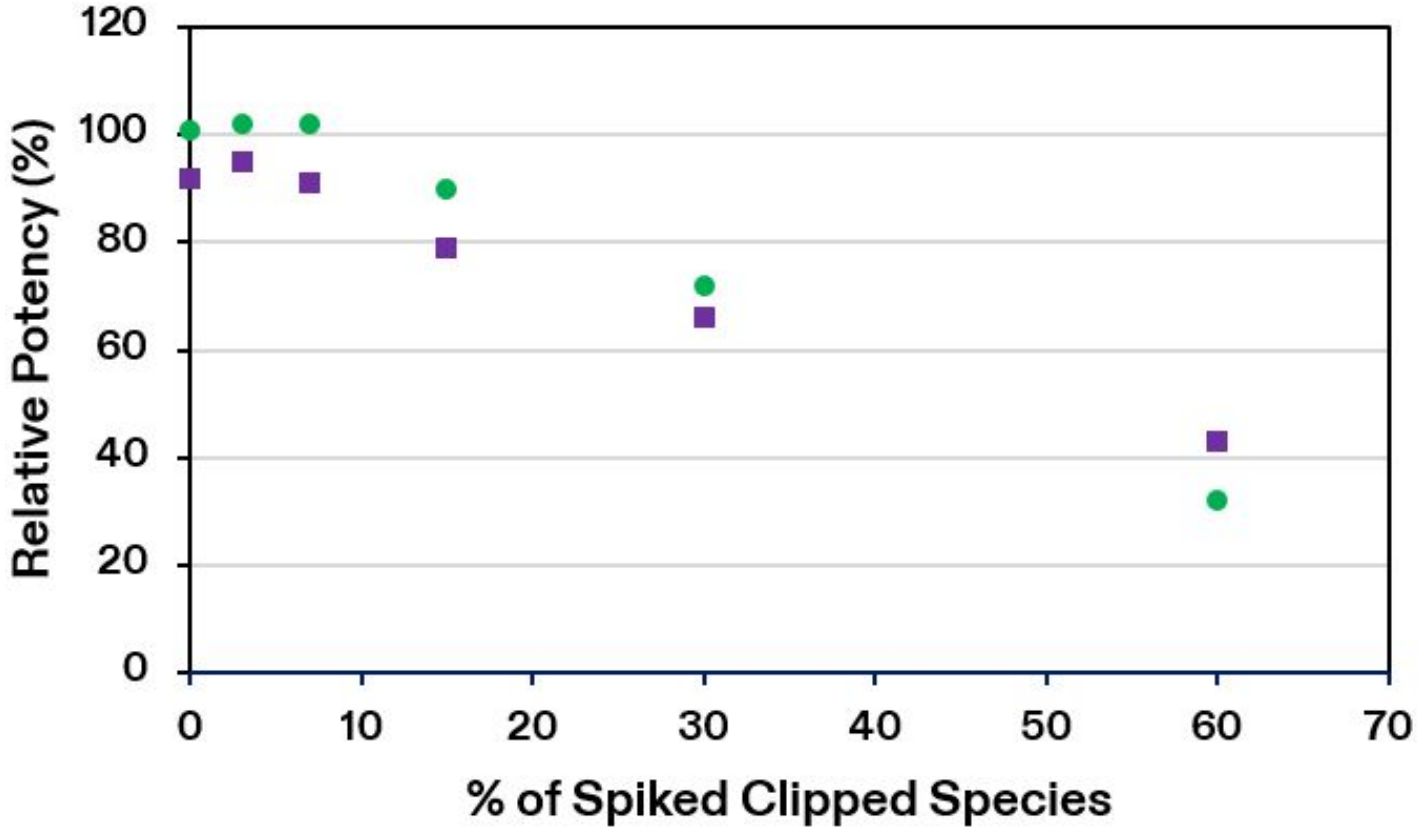
Elranatamab Drug Product Primary Stability Studies:

Target Binding ELISA and Cell Based Bioassay Results



Both sets of results correlate across 5 Drug Product batches.

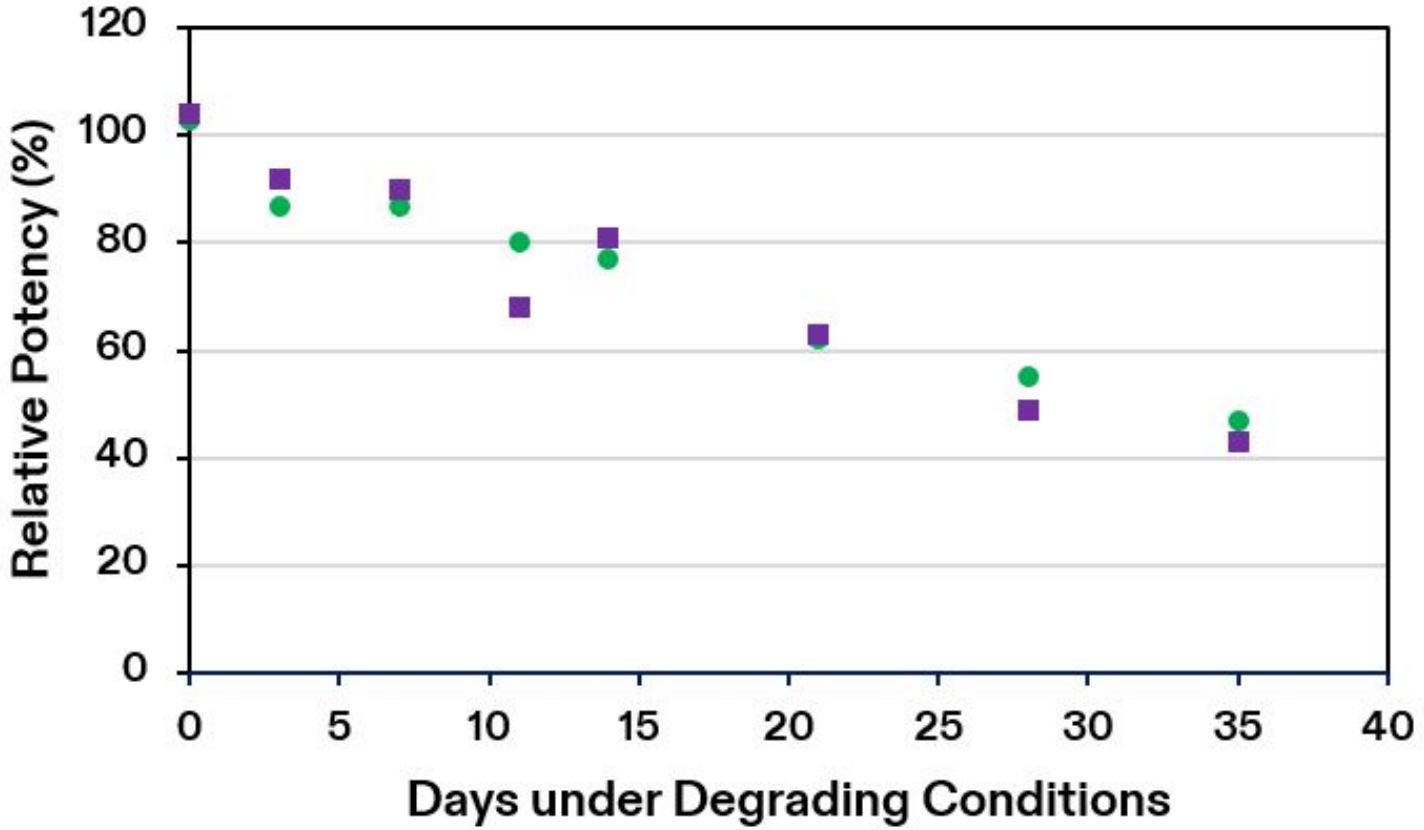
Elranatamab Spiked Clipped Species Study: Target Binding ELISA and Cell-Based Bioassay Results



Both sets of results correlate across an increase of clipped species.

Elranatamab Potency Test Method Stability Indication:

Target Bir assay Results



Both sets of results correlate across an increase of forced degradation.

License Applications Filed with Bioassay Strategy for Commercial Testing

The following regulatory request for information was received:

- Justify how T cell activation in the cell-based bioassay reflects and correlates to the target cell death of BCMA-positive malignant plasma cells.

T Cell Killing Assay

A flow cytometry-based T cell killing assay was developed to demonstrate the correlation of T cell killing with the validated potency test methods.

Target Cell Line: MM-1S (target cell of the cell-based bioassay)

Effector Cell Line: CD8+ primary T cells

Relative Potency (%) Results of Elranatamab Bioassays Correlate with those of the T Cell Kil

Photostability Sample	Target Binding ELISA	Cell-Based Bioassay (T Cell Activation)	T Cell Killing Assay
Dark Control	95	99	98
Light Exposed	65	64	63

Thermal Degradation Sample	Target Binding ELISA	Cell-Based Bioassay (T Cell Activation)	T Cell Killing Assay
0 Days	103	104	85
7 Days	87	90	79
21 Days	62	63	66
35 Days	47	43	46

Bioassay Strategy is Accepted by Regulators!

Elranatamab's commercial bioassay strategy is included in multiple approved license applications.

Elranatamab is now used to treat adults with multiple myeloma so that they may have more special moments.

Conclusions

- **Target Binding ELISA was acceptable as the single potency test method for release and stability of DS and DP until Phase 3.**
- **Cell-Based Bioassay introduced as an additional potency test method for release and stability of DS and DP for the Phase 3 clinical supplies.**
- **The cell-based assay was authorized as the single, commercial potency test method.**

Acknowledgements:

Biotherapeutics Pharmaceutical Sciences:

Analytical Research and Development (ARD)

- ARD Bioassay and Impurity Testing

Oncology Research Unit

Drug Safety Research and Development

Pharmacokinetics, Dynamics and Metabolism

Pfizer Regulatory Chemistry and Manufacturing Control

Pfizer Regulatory Oncology Strategy

Pfizer Global Supply

Thank You!