

IMPLEMENTATION & QUALIFICATION OF **CELLULAR BIOASSAYS** FOR POTENCY, QC & RELEASE TESTING FOR BIOLOGICS UNDER GMP ENVIRONMENT

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Congress**

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WHEN YOU NEED TO BE SURE

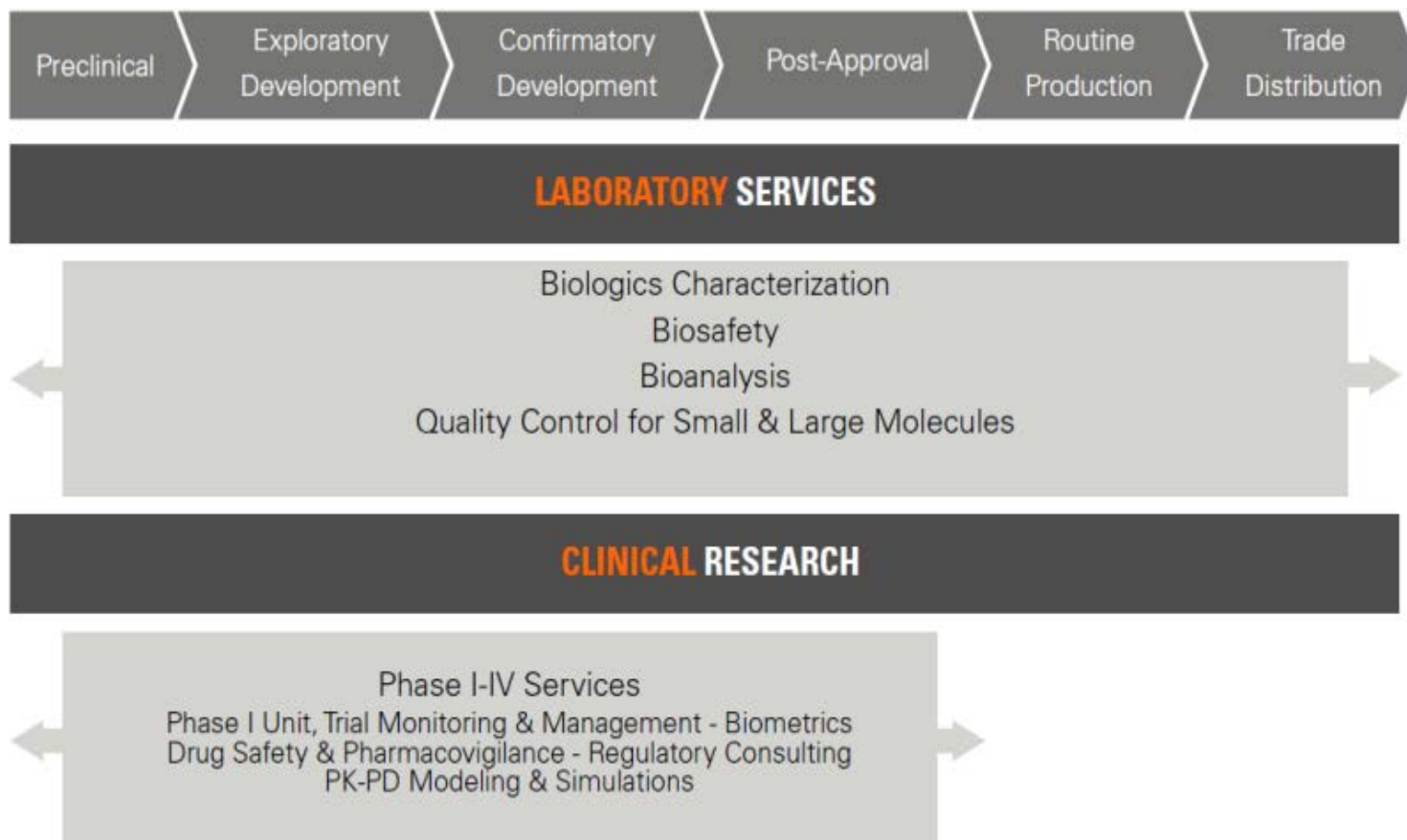


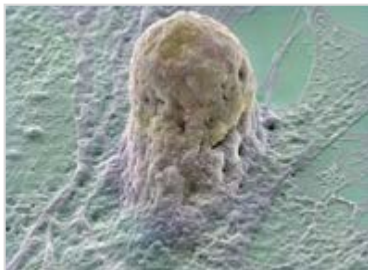
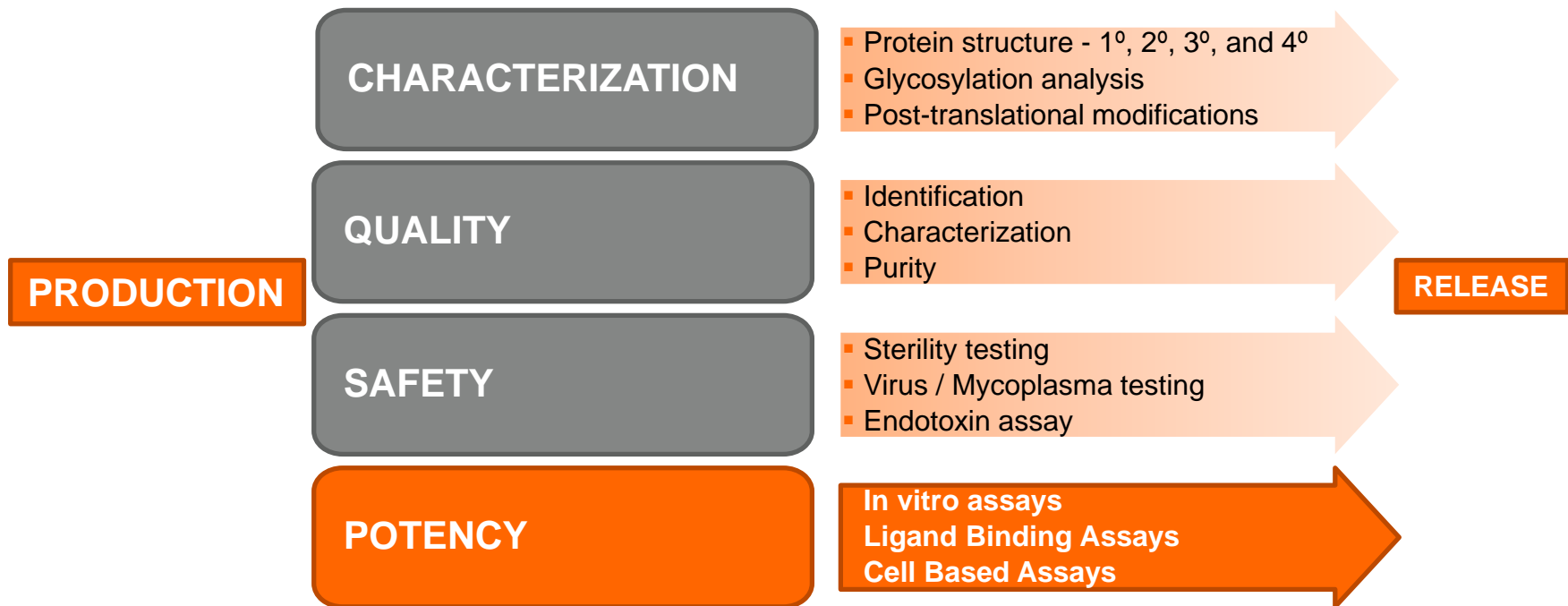
SGS MEANS
EXPERIENCE

- Over 35 years experience
- 1.600 full time employees (of which 1.000 within Laboratories)
- 27 facilities in 14 countries
- Leader with unique international analytical laboratory network with 21 laboratories
 - across America, Europe, Asia
- Global drug development partner from Molecule to Market
 - Wide-range of laboratory infrastructure, size and diverse testing capabilities matching Biopharmaceutical and Small molecules needs
- Strong commitment to laboratory Quality and Operational Excellence in many areas
 - Harmonized QMS and Validation & Transfer methods, LIMS, Lean

DRUG DEVELOPMENT PARTNER - FROM MOLECULE TO MARKET -

**SGS MEANS
FULL SERVICE**





- Clinical Monitoring & PreClinical Assessment
 - ✓ Immunogenicity : Neutralizing Ab (**NAb**) Monitoring
 - ✓ By Immunoanalysis or Cell Based Assays
 - ✓ ImmunoPhenotyping (FACS)
 - ✓ Receptor Occupancy Testing (FACS)
 - ✓ Eosinophil Shape Monitoring
- Biocomparability Studies of BioSimilar compared to Originators
- Potency Assay : Set up & Batch Testing for NBEs & BioSimilar
 - ✓ ADCC : Antibody Dependent Cell-mediated Cytotoxicity (Biosimilars)
 - ✓ CDC : Complement Dependent Cytotoxicity
 - ✓ Cell Proliferation & Differentiation Monitoring
 - ✓ Receptor Phosphorylation (e.g. Insulin analogs)
 - ✓ Chemotaxis & Chemokinesis Assay
 - ✓ Proliferation Assay (e.g. MCF-7 cell line, WEHI, ...)
 - ✓ Lipogenesis Assay (3T3-L1 cell line)
 - ✓ Receptor Binding Assay (Cultured cells & radioactive method)

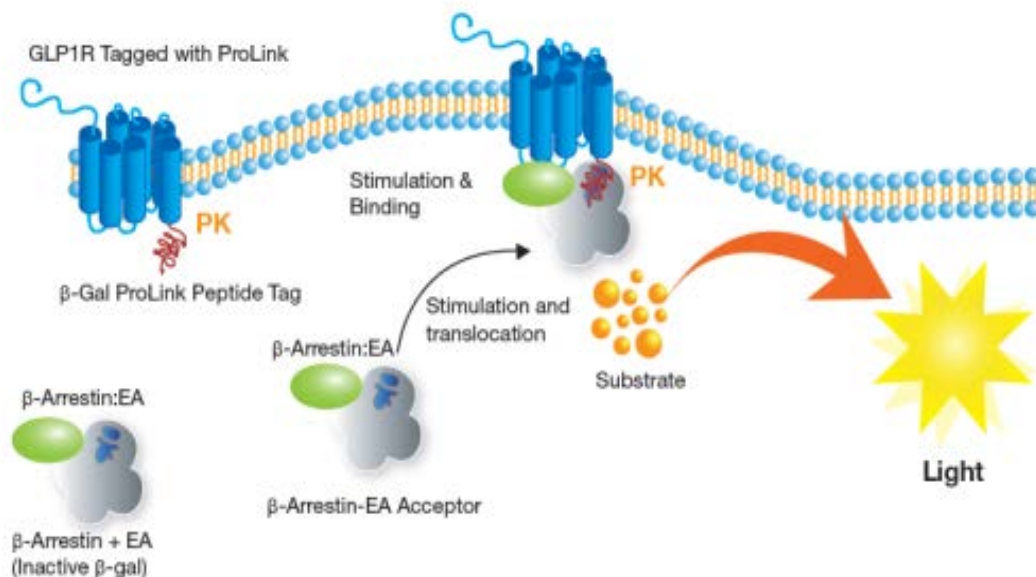


- Under the supervision of the **Qualified Person**
- Biological products (Ab, Therapeutic Recombinant Proteins, ...)
- **GMP Method Development & Validation for Batch Release**
 - ✓ Conformity, Impurities, Concentration, Biological Activity, ...
- **Potency Assay & other techniques supporting Batch Release**
 - ✓ Cell-Based Assays Potency Assays
 - ✓ Immunoassays Potency Assays, Impurity Testing, HCP
 - ✓ Western Blot Integrity & Impurity Testing
 - ✓ Validated Methods following ICH Guidelines
- Release of **Certificate of Analysis (CoA)**

New EU GMP Annex 16 - Certification by
Qualified Person and Batch Release



FIT-FOR-PURPOSE QUALIFIED ASSAYS FOR BIOSIMILAR DEVELOPMENT



- Qualification for:
 - Intra-plate/Inter-plate Precision
 - Inter-Day Precision
 - Preliminary assessment of relative Accuracy
 - Evaluation of the Robustness (edge effect)
- High Precision
- Accuracy
- Intermediate precision
- PathHunter bioassays for:
 - Exendin-4
 - Bevacizumab
 - Cetuximab
 - In Partnership with:

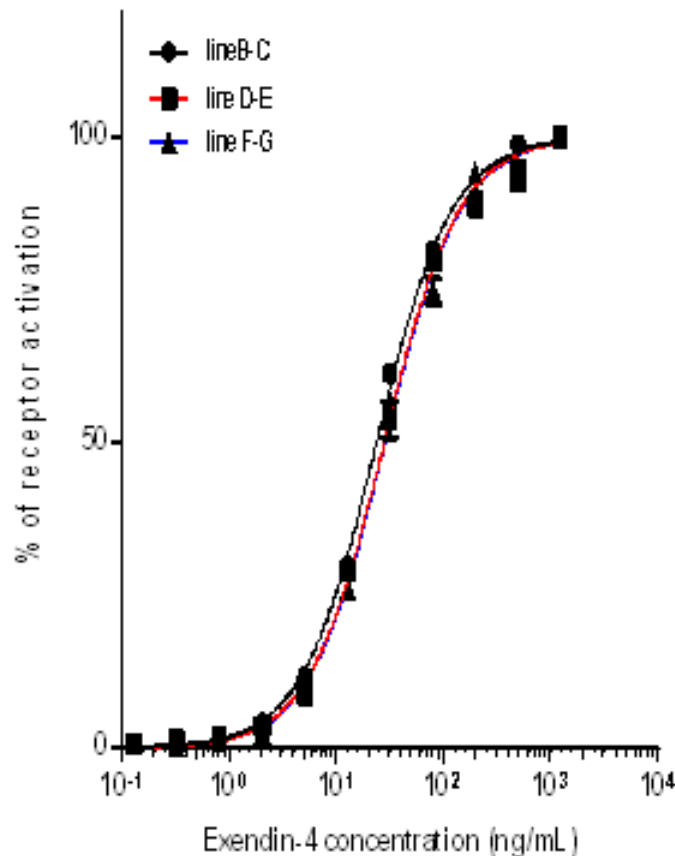
- Regulatory authorities worldwide require head-to-head comparisons to demonstrate that a proposed biosimilar is “similar or highly similar” to the reference product.
- Step-wise regulatory pathways call for structural and functional characterization to demonstrate biosimilarity.
- The objective of the present study was to perform qualification of commercially available cell-based assays to establish biological activity and potency of Exendin-4, Bevacizumab and Cetuximab.

SOME VALIDATED CELLULAR BIOASSAYS

- Exendin-4: Glucagon-like peptide-1 receptor agonist
- Bevacizumab: anti-VEGF
- Cetuximab: EGFR inhibitor
- PathHunter® bioassay kits & associated reporter cell lines were supplied by DiscoverX Corporation.
- Exendin-4 was supplied by DiscoverX
- Cetuximab and Bevacizumab were purchased from Bionical Ltd..
- Bioassay kits and reporter cell lines were used following manufacturer's recommendations. All assays were performed in 96-well plate.

- Dose response curve
- Evaluation of the robustness (edge effect)
- Intra-plate/inter-plate precision
- Inter-day precision
- Preliminary assessment of relative accuracy

- Curve fitted with 4-PL model and determination of EC50 or IC50
- Parallelism assessment using F test and $p < 0.05$ for rejection of null hypothesis (slopes of DR curve are not significantly different)
- Calculation of relative potency or relative accuracy



- Low CV% on replicates
- Concentration range (0.131 to 2500 ng/mL)
- 4-PL dose-response pattern
- $R^2 > 0.9$
- Top/Bottom ratio > 10

	Global (shared)
Comparison of Fits	
Null hypothesis	HillSlope same for all data sets
Alternative hypothesis	HillSlope different for each data set
P value	0.6039
Conclusion (alpha = 0.05)	Do not reject null hypothesis
Preferred model	HillSlope same for all data sets

- Intra-plate precision at 8% and 6%
- Inter-plate precision at 6%

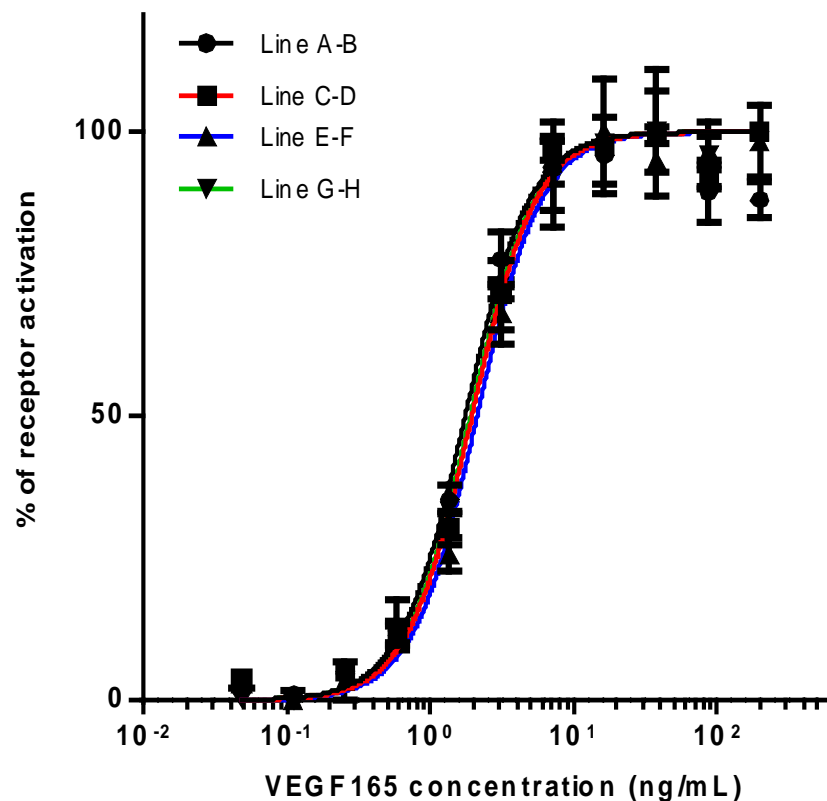
		Preparation No.	EC50 (ng/mL)	R2	T/B	parallelism	Intra run precision			Inter-run precision		
							Intra-run EC50 Mean	SD	CV %	Inter-run EC50 Mean	SD	CV %
Run 06 09-Oct-2015 An.SaD	Curve #1	1	10.77	0.995	19	0.08	10.18	0.84	8	10.25	0.64	6
	Curve #2	2	9.58	0.998	19							
Run 07 09-Oct-2015 An.SaD	Curve #1	1	NA	NA	NA	0.40	NA	NA	NA			
	Curve #2	2	9.731	0.998	11							
Run 08 09-Oct-2015 An.SaD	Curve #1	1	10.1	0.998	20	0.34	10.58	0.68	6			
	Curve #2	2	11.06	0.999	19							

■ Inter-day precision at 13%

	Preparation No.	EC50 (ng/mL)	R2	T/B	parallelism	Intra day EC50 Mean (ng/mL)	SD	CV %	Inter-day EC50 Mean (ng/mL)	SD	CV %
Run 05 08-Oct-2015 An.SaD	1	12.52	0.998	9	0.09	12.33	0.47	4	11.29	1.47	13
	2	12.8	0.995	9							
	3	12.3	0.997	9							
	4	11.7	0.997	9							
Run 06 09-Oct-2015 An.SaD	1	10.77	0.995	19	0.08	10.25	0.64	6			
	2	9.58	0.998	19							
Run 07 09-Oct-2015 An.SaD	1	NA	NA	NA	0.40						
	2	9.731	0.998	11							
Run 08 09-Oct-2015 An.SaD	1	10.1	0.998	20	0.34						
	2	11.06	0.999	19							

- 50% Sample
- Inter-run accuracy of 109% with 7% precision

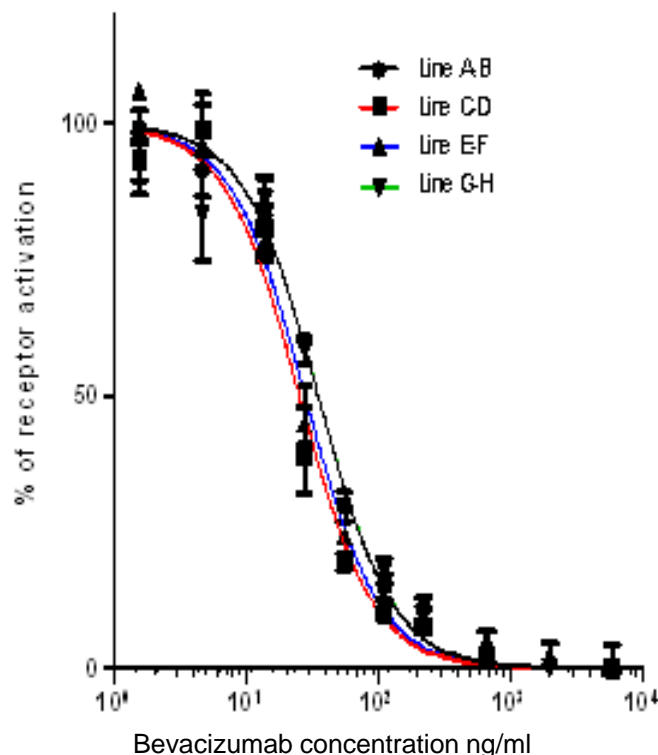
	Preparation No.	EC50 (ng/mL)	R2	T/B	parallelism	Intra run precision			Inter-run precision			Relative potency			Intra-run Relative Accuracy			Inter-run Relative Accuracy						
						Intra-run EC50 Mean (ng/mL)	SD	CV %	Inter-run EC50 Mean (ng/mL)	SD	CV %	RP	Mean RP (%)	CV%	Relative accuracy (%)	Mean intra- run RA (%)	CV%	Mean inter- run RA (%)	CV%					
Run 06 09-Oct-2015 An.SaD	1	18.29	0.999	17	0.08	19.02	1.03	5	18.84	1.75	9	56	54	5	111	107	5	109	7					
	2	19.74	0.999	18								52			103									
Run 07 09-Oct-2015 An.SaD	1	NA	NA	NA	0.40	16.72	NA	NA				NA	NA	NA	NA	NA	NA			NA	NA	NA	109	7
	2	16.72	0.997	11								58			116									
Run 08 09-Oct-2015 An.SaD	1	18.15	0.999	18	0.34	19.74	2.24	11				58	54	11	117	108	11			99				
	2	21.32	0.999	17								50												

Stage 1: Activation of VEGF-R by VEGF₁₆₅

- Low CV% on replicates
- 4-PL dose-response pattern
- $R^2 > 0.9$
- T/B ratio > 3
- VEGF165 Mean EC80 = 3.93 ng/mL

Stage 2: Neutralization by Bevacizumab of VEGF-R activation induced by VEGF₁₆₅

- Low CV% on replicates
- 4-PL dose-response pattern
- $R^2 > 0.9$
- T/B ratio > 2
- Bevacizumab IC₅₀ = 31.2 ng/mL



	Global (shared)
Comparison of Fits	
Null hypothesis	HillSlope same for all data sets
Alternative hypothesis	HillSlope different for each data set
P value	0.2333
Conclusion (alpha = 0.05)	Do not reject null hypothesis
Preferred model	HillSlope same for all data sets

■ Relative accuracy with 50% Sample

	Preparation	Lines	EC50 (ng/mL)	R2	T/B	parallelism	Intra-run								
							IC50 Mean (ng/mL)	SD	CV %	RP (%)	Mean RP	CV %	RA (%)	Mean RA	CV %
Run 06 24Nov2015 An.OW	Preparation No.1 at 50%	B and F	112	0.96	2.2	0.2	103	12.9	13	53	58	13	105	115	13
	Preparation No.2 at 50%	D and H	93.6	0.92	2.2					63			126		
Run 07 24Nov2015 An.OW	Preparation No.1 at 50%	B and F	61.6	0.97	2.4	0.8	71.4	14.0	20	65	57	20	129	114	20
	Preparation No.2 at 50%	D and H	81.3	0.91	2.4					49			98		
Run 08 24Nov2015 An.OW	Preparation No.1 at 50%	B and F	73.1	0.97	2.9	0.4	66.8	8.95	13	52	58	13	105	115	13
	Preparation No.2 at 50%	D and H	60.4	0.96	2.8					63			126		

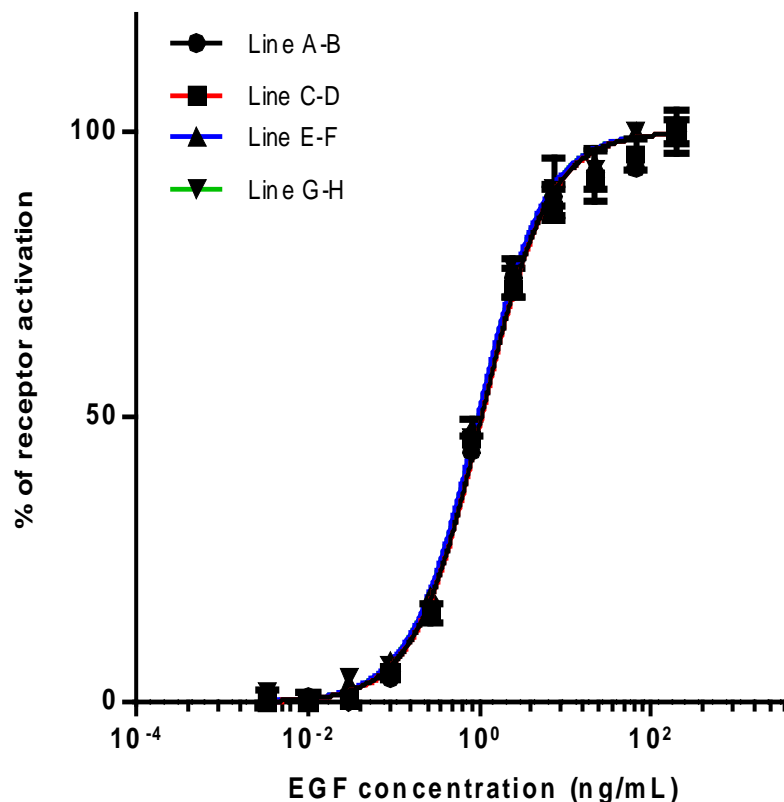
Inter-run IC50 precision	Mean (ng/mL)	80.3
	SD	19.6
	CV%	24

Inter-run RP precision	Mean (%)	57
	SD	7
	CV%	12

Inter-run RA precision	Mean (%)	115
	SD	14
	CV%	12

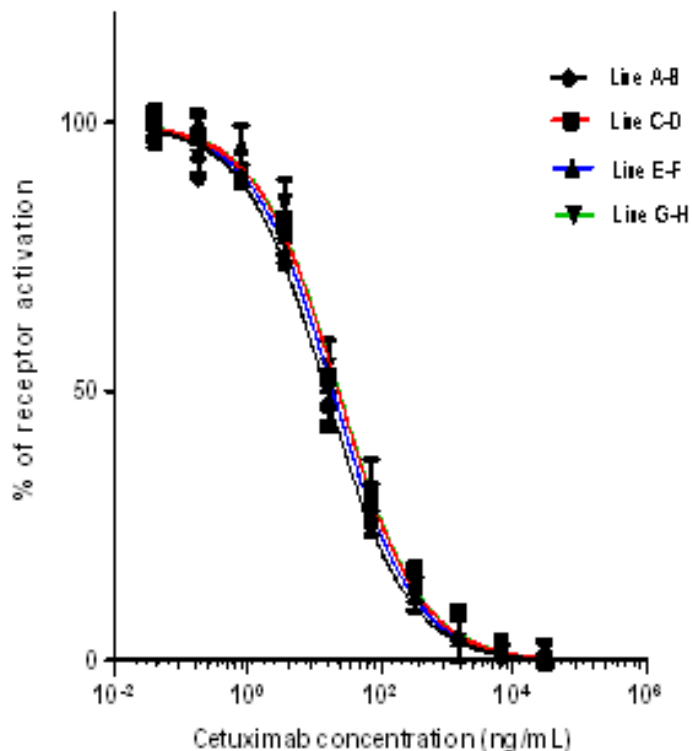
■ Inter-run Precision at the level of IC50 values: 24%

■ Relative Accuracy and Relative Potency Inter-run precision : 12%

Stage 1: EGF-R Activation by EGF

- Low CV% on replicates
- 4-PL dose-response pattern
- $R^2 > 0.9$
- T/B ratio > 5
- EGF EC90 = 9.42 ng/mL

Stage 2: Cetuximab induced Neutralization of EGF-R Activation



- Low CV% on replicates
- 4-PL dose-response pattern
- $R^2 > 0.9$
- T/B ratio > 3
- Cetuximab IC₅₀ = 20.5ng/mL

	Global(shared)
Comparison of Fits	
Null hypothesis	HillSlope same for all data sets
Alternative hypothesis	HillSlope different for each data set
P value	0.2169
Conclusion (alpha = 0.05)	Do not reject null hypothesis
Referred model	HillSlope same for all data sets

■ Relative accuracy with 50% Sample

		Lines	IC50 (ng/mL)	R2	T/B	parallelism	Intra run IC50 Mean	SD	CV %	RP (%)	Mean RP (%)	CV%	Relative Accuracy	Mean RA (%)	CV%
Run 09	Preparation No.1	F and G	40.3	0.99	3.79	0.30	43.7	4.92	11	55	51	11	110	102	11
29-Oct-2015	Preparation No.2	A and H	47.2	0.99	3.56					47			94		
Run 10	Preparation No.2	A and H	52.84	0.99	3.80	0.81	52.8	NA	NA	46	NA	NA	93	NA	NA
Run 11	Preparation No.1	F and G	49.9	0.99	3.32	0.73	45.7	5.96	13	51	56	13	102	112	13
30-Oct-2015	Preparation No.2	A and H	41.5	0.99	3.30					61			122		
Run 12	Preparation No.1	F and G	72.4	0.99	3.08	0.81	64.4	11.29	18	54	61	18	108	123	18
30-Oct-2015	Preparation No.2	A and H	56.4	0.99	3.05					69			138		
Run 13	Preparation No.1	F and G	89.3	0.99	2.63	0.55	95.0	8.05	8	50	47	8	101	95	8
30-Oct-2015	Preparation No.2	A and H	100.7	0.98	2.63					45			89		

Cetuximab at 50% strenght	Inter Day IC50 Mean (ng/mL)	61.2
	SD	21.6
	CV %	35

Cetuximab at 50% strenght	Inter Day RP	53
	SD	8
	CV %	15

Cetuximab at 50% strenght	Inter Day RA Mean (%)	106
	SD	16
	CV %	15

■ Intermediate precision at 35% (for IC50)

■ Intermediate precision at 15% (Rel. Accuracy and Rel. Potency)

- Optimal dilution ranges :
 - 0.131 to 2500 ng/L (Exendin-4);
 - 0.131 to 2500 ng/L (Bevacuzimab),
 - 0.0396 to 30000 ng/mL (Cetuximab).
- A 4-PL model was used to fit the dose repose curve with a R2 greater than 0.9 and a wide Top/Bottom ratio greater than 2 - 10.
- In all cases, no edge effect was observed.
- The mean IC50 was around 12 ng/mL (Exendin-4) and 31 ng/mL (Bevacuzimab and Cetuximab).
- Intra-run precision was from 5% to 20% and inter-run precision from 6% to 16%, with intra-run relative accuracy in the range of 93% to 123% and inter-run relative accuracy >106%.

SUITABILITY OF DISCOVERX MODELS FOR BIOCOMPARABILITY STUDIES

- Demonstration of high accuracy and intermediate precision for the following bioassays:
 - Bioassay for Exendin-4
 - Bioassay for Bevacizumab
 - Bioassay for Cetuximab
- Suitable for functional comparability assessment required by regulators.
- Assays are easy-to-use, commercially available, and highly reproducible,
 - significant reduction in assay development time, overall cost savings in a biosimilar development programme.
- **All bioassays showed good performance and can be used for the assessment of biosimilar Potency**



ANALYTICAL AND CLINICAL TESTING NETWORK

GMP, GLP, GCP –
FROM MOLECULE TO MARKET

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Agriculture, Food and Life

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