



CQA process modulation of high producing biosimilar Mab CHO^{BC}® cell lines

Some examples

BioProduction Congress 19-20 October, 2016, Dublin

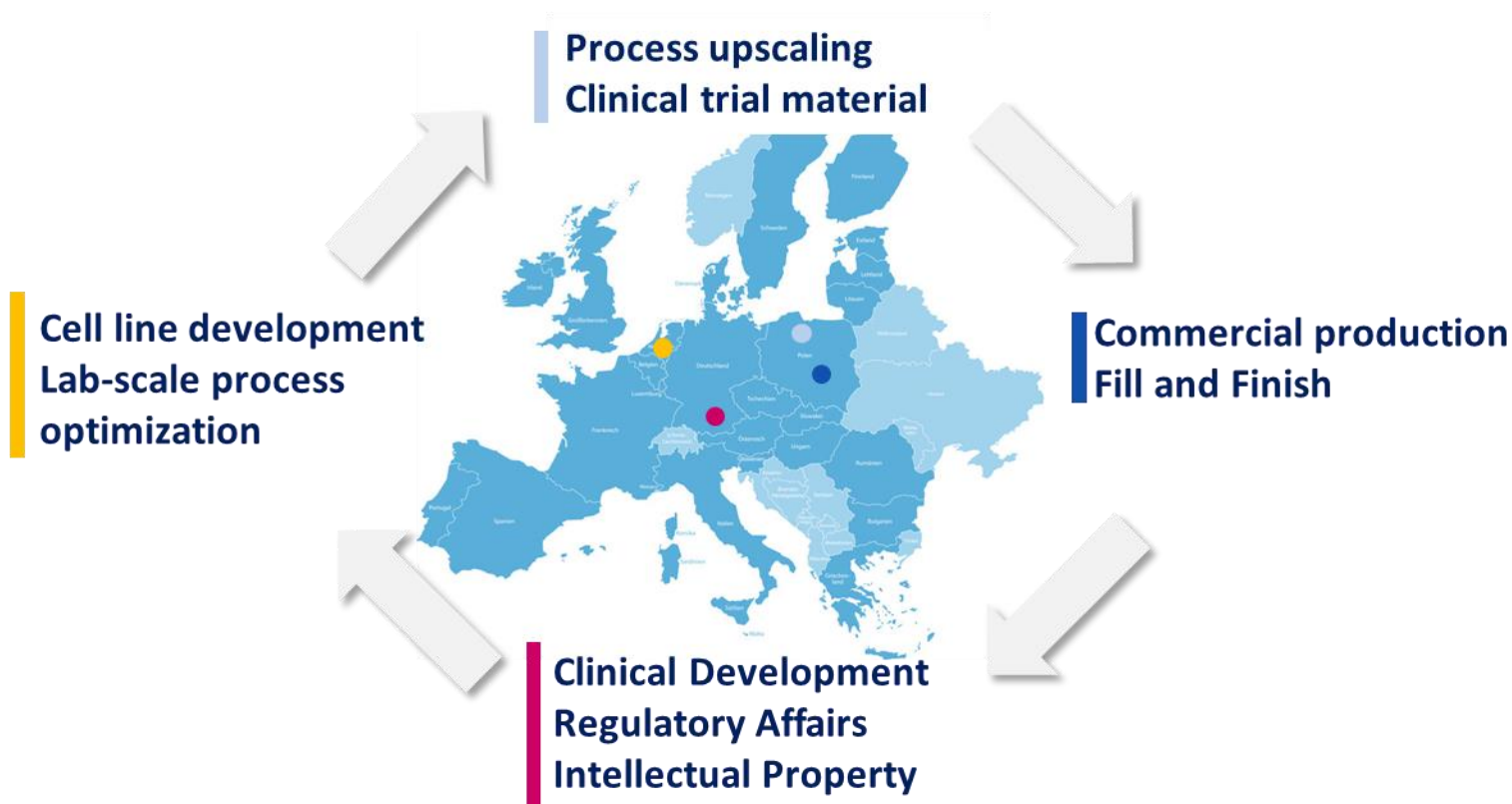
Louis Boon
CSO

- Founded in 2003 and current member of the Polpharma Biologics group
- Early development of monoclonal antibodies & other proteins
 - Generation of monoclonal antibodies in rodents
 - Humanization
 - Cell Line Development in its proprietary CHO^{BC}[®] cell line
 - For NME development
 - For biosimilar development
 - Upstream process modulation to meet CQAs
 - Upstream and Downstream Development
 - Analytical- and Bio-assays



One Stop Shop

Polpharma Biologics offering all elements in development and production of biopharmaceuticals

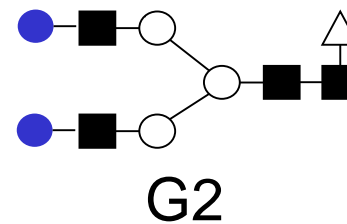
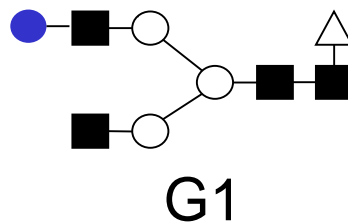
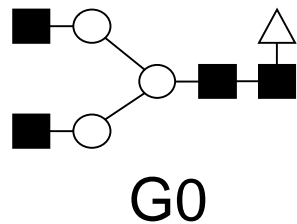


Not only about machines but all about expertise

Bioceros: CHO^{BC} based biosimilar lines

Identity	Designation	Biosimilar to	Antibody	Highest producing cell line	Status
#1	BC002REEM	Herceptin	Trastuzumab	2.4 g/L	Finished
#2	BC004	Humira	Adalimumab	4.0 g/L	Finished
#3	BC005	Erbitux	Cetuximab	1.5 g/L	Optimizing for higher titers and fingerprint biosimilarity
#4	BC006	Avastin	Bevacizumab	2.0 g/L	Optimizing for higher titers and fingerprint biosimilarity
#5	BC007	Rituxan/MabThera	Rituximab	3.0 g/L	Optimizing for higher titers and fingerprint biosimilarity
#6	BC008	Enbrel	Etanercept	3.0 g/L	Finished
#7	BC009	Synagis	Palivizumab	2.5 g/L	Optimizing for higher titers and fingerprint biosimilarity
#8	BC011NPI	Xolair	Omalizumab	2.8 g/L	Modulation for fingerprint biosimilarity and DSP development
#9	BC012	Myozyme	Alglucosidase a	Available	Parental clones available

Expected major N-linked glycan on heavy chain for a Mab



■ N-acetylglucosamine

△ Fucose →

○ Mannose

● Galactose →

Absence important for ADCC

Presence important for CDC

Current dogma: glycosylation  cell line dependent

Overview USP Process Development Platform

Screening

Modulation

Scaling up

Lock

3 months

3 months

3 months

2 months

CLG provides 5-10 stable high productivity clones

Infors
Eppendorf
Small scale reactors (2 L):
Clone screening

Select 1-3 lead clones

SF: Media/feed screening CLG

Medium	Supplier	Cells	Feed	Supplier	Feed
ProCHO-5	Lonza	BE 12-7892	None		
PowerCHO-2	Lonza	BE 12-7712	BC feed Basal Tarts EX-CELL Feed 1	In house HyClone Sigma	N/A SP10287 24367C
AdPS	HyClone	SP10287	BC feed Basal Tarts EX-CELL Feed 1	In house HyClone Sigma	N/A SP10287 24367C
EX-CELL Advanced CHO	Sigma	14386C	BC feed Basal Tarts EX-CELL Feed 1	In house HyClone Sigma	N/A SP10287 24367C
Dynasys	ThermoFisher	A208101	BC feed Basal Tarts EX-CELL Feed 1	In house HyClone Sigma	N/A SP10287 24367C
CDMPERMA5	HyClone	SP10287	BC feed Basal Tarts EX-CELL Feed 1	In house HyClone Sigma	N/A SP10287 24367C
CDMCHO	HyClone	SP10287	BC feed Basal Tarts EX-CELL Feed 1	In house HyClone Sigma	N/A SP10287 24367C

8x 2L systems: Modulation

Select final clone

SF: Elaborate titrations for detailed
information on modulation

Eppendorf/Hyclone
50L

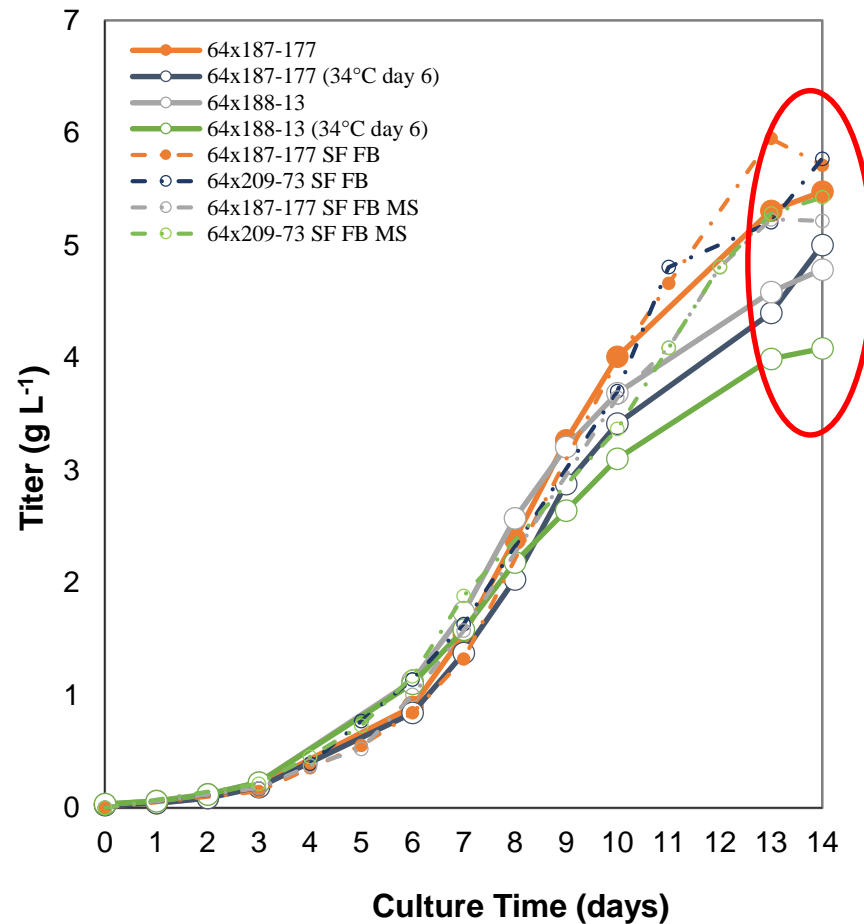
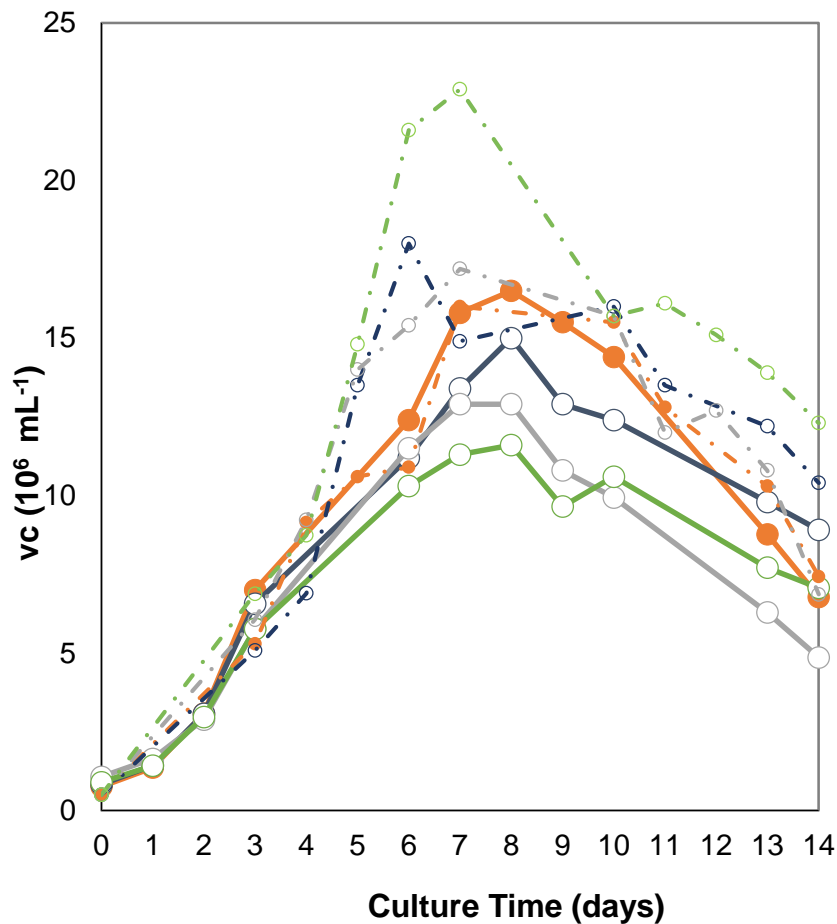
Eppendorf
10L
Eppendorf
Infors
SF

Select final
process
conditions

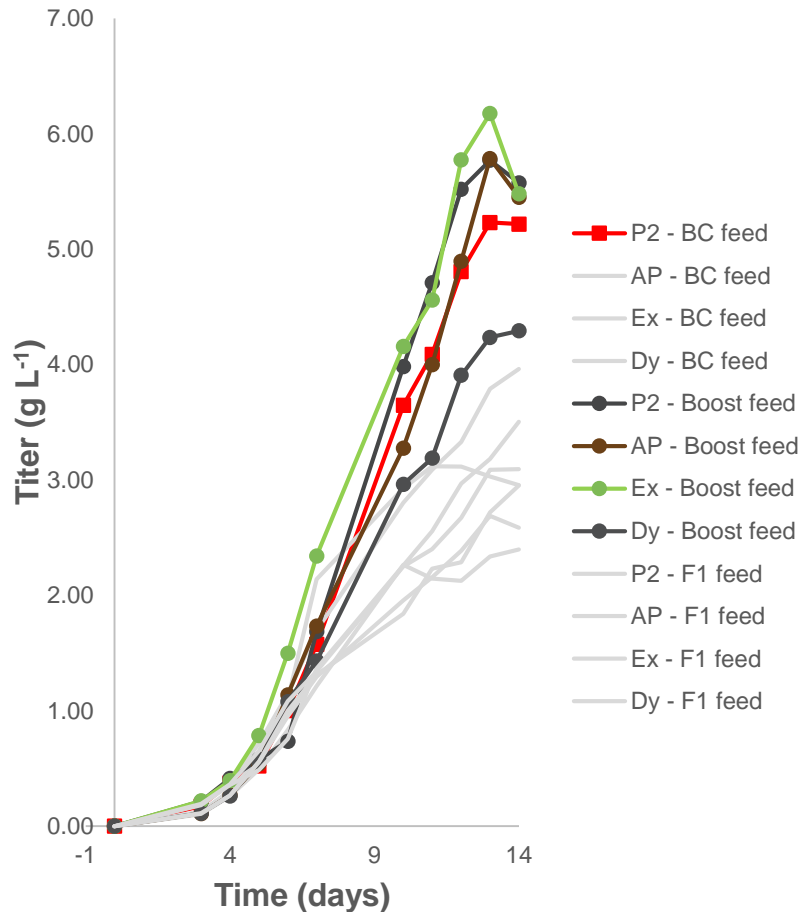
Eppendorf/Hyclone
50L

Run 3x 50L for
Process Lock

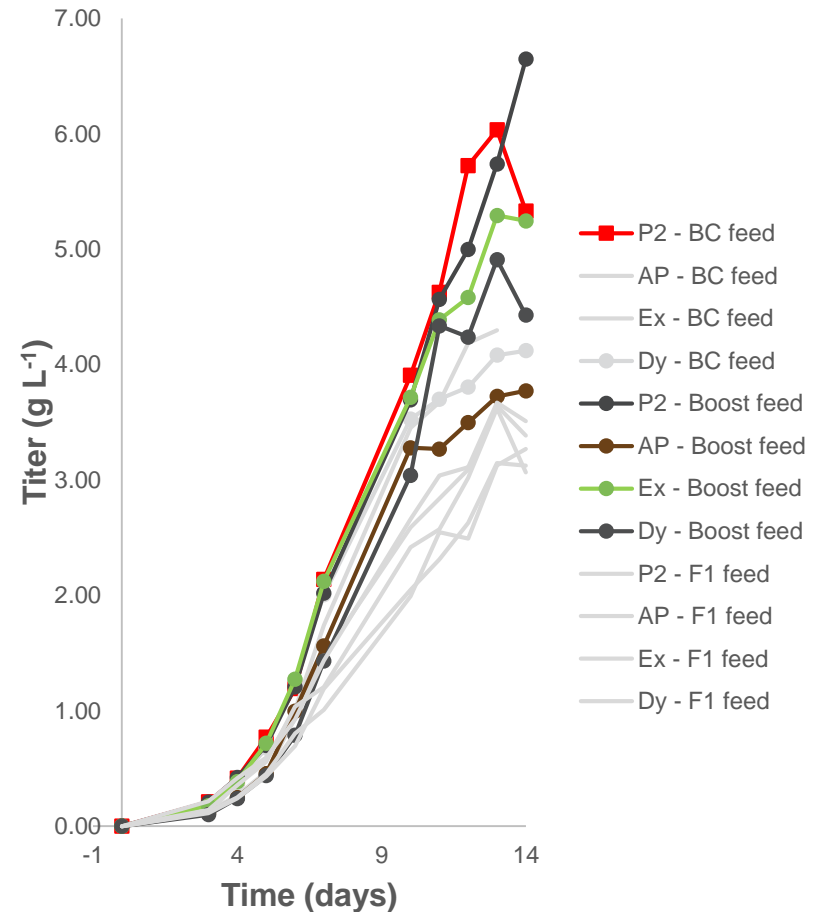
Example clone screening in bioreactors versus shakers



64x187-177

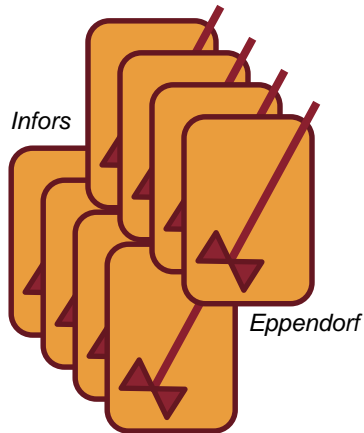


64x188-13



Modulation studies to address Quality Attributes

3 months

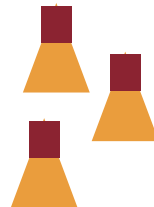
8x 2L systems: Modulation



Select 1-3 lead clones

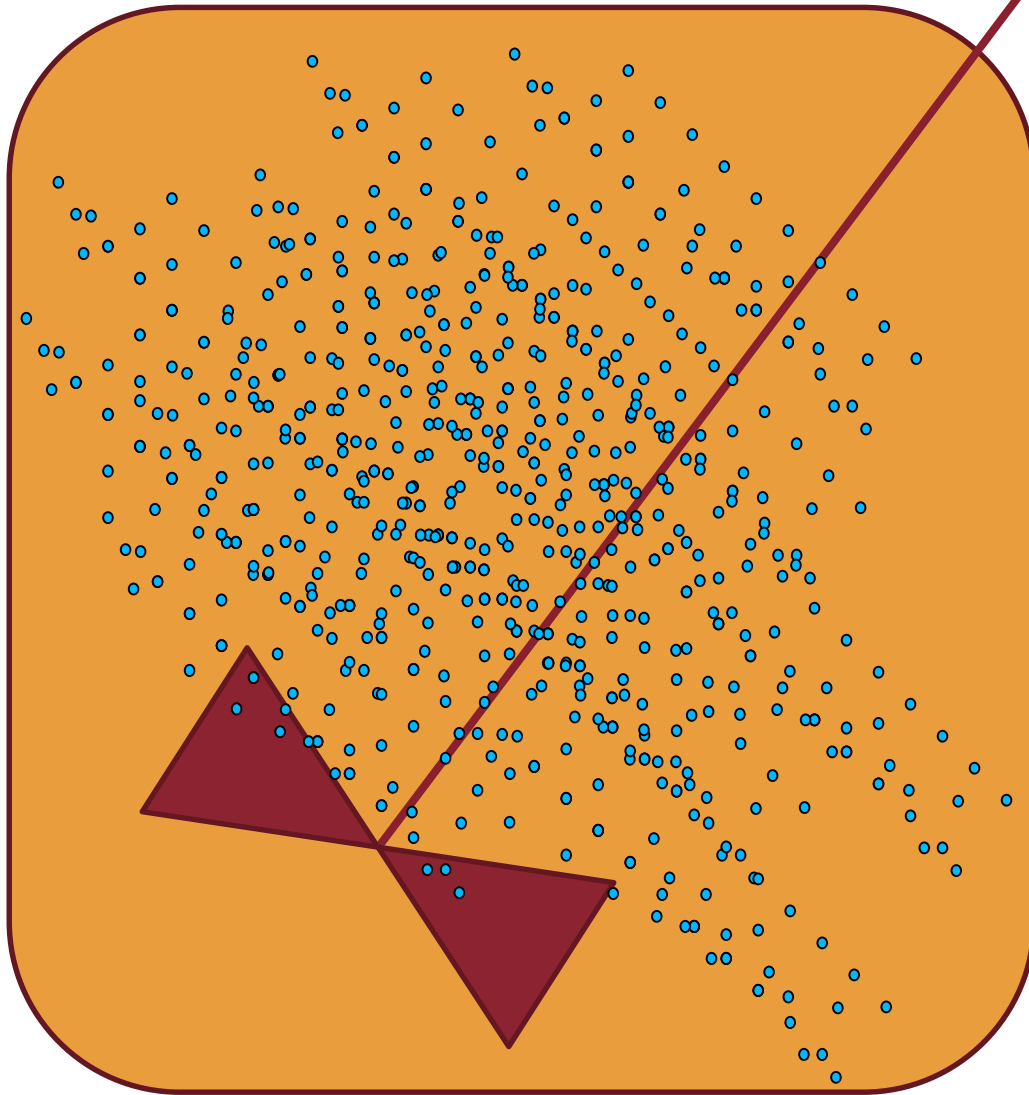


Select final clone



SF: Elaborate titrations for detailed information on modulation

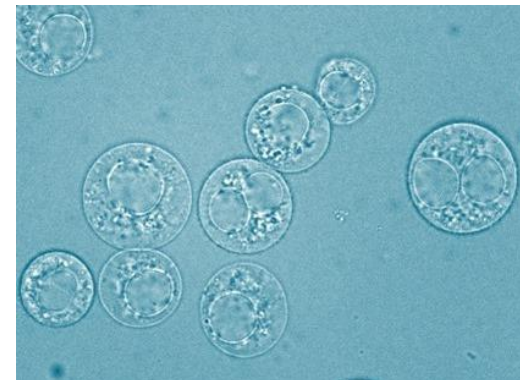
Modulation is not keeping the cells alive only



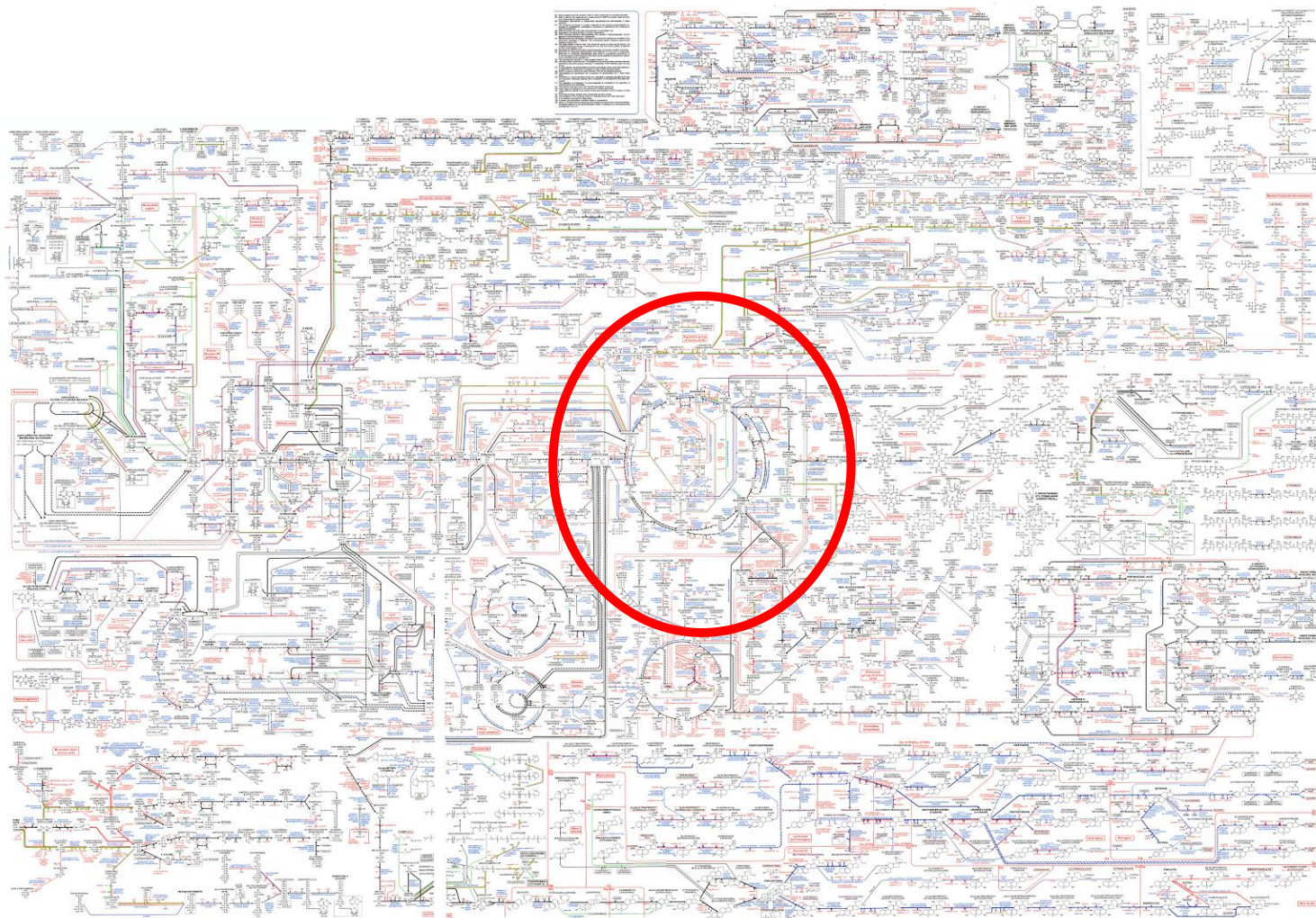
A process is more than:

- VCD, viability
- Product
- Lactate, glucose, ammonia, glutamate, glutamine.....

There is much complexity

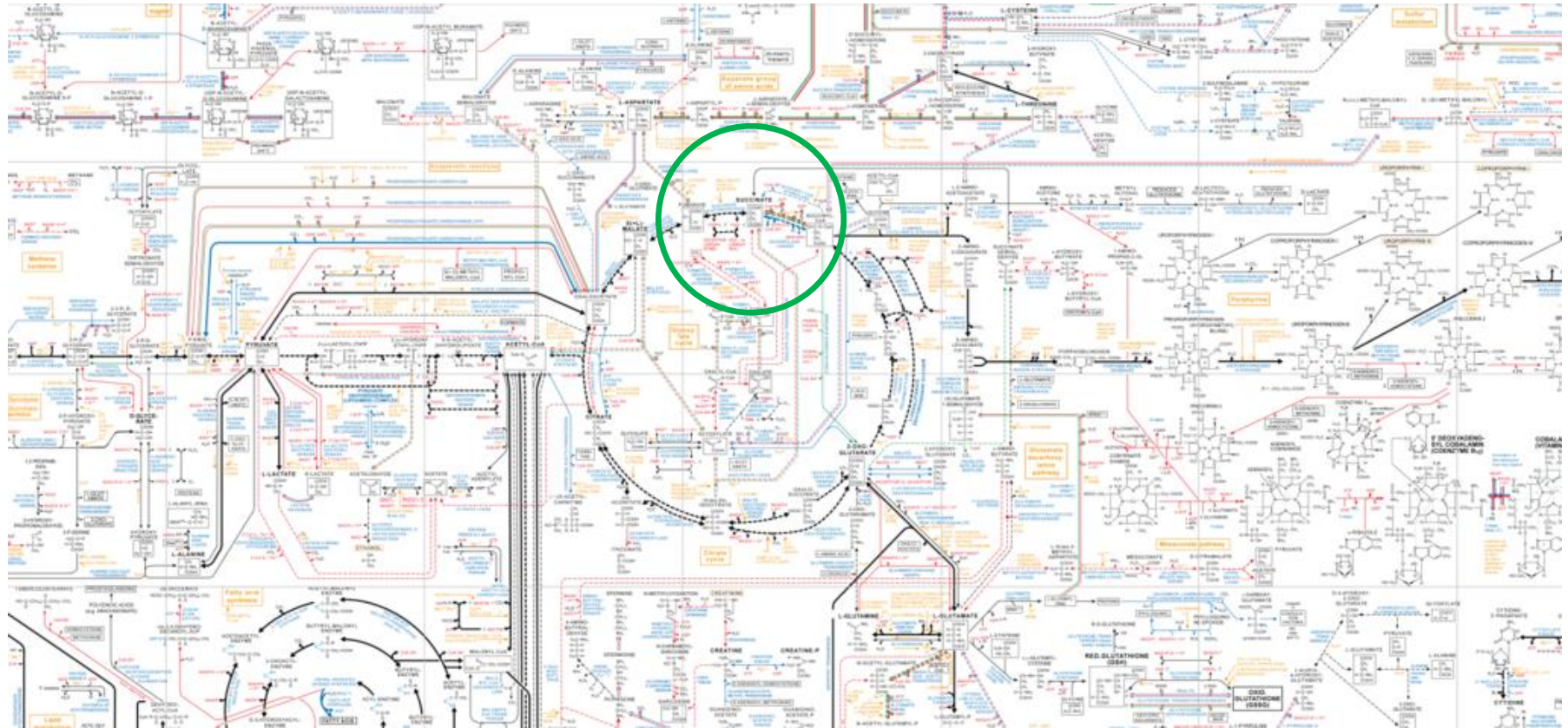


Intracellular metabolism is complex



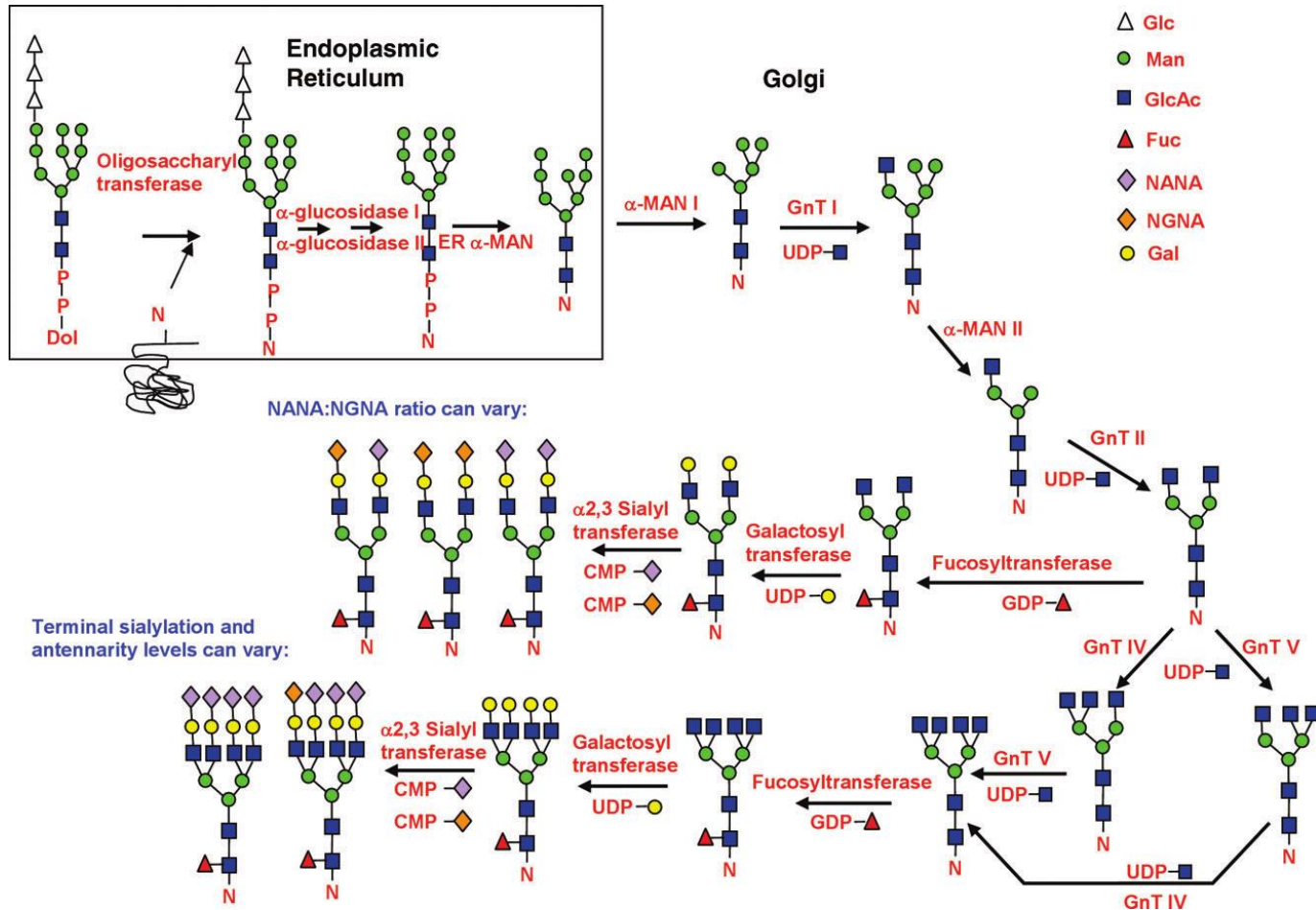
Intracellular metabolism is complex

Zooming in





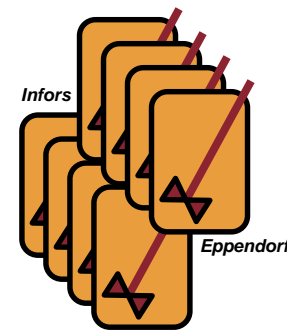
N-Linked glycosylation is an interplay between enzymes in two different intracellular compartments



For modulation a scientific strategy is obligatory

Modulation experiments gave us the ability to **modulate** and **control**

- **Basic species** ↓ ↑
- **Acidic species** ↓ X n.n.
- **Galactosylation** ↓ ↑
- **Fucosylation** ↓ X n.n.
- **Mannosylation** n.n. X ↑



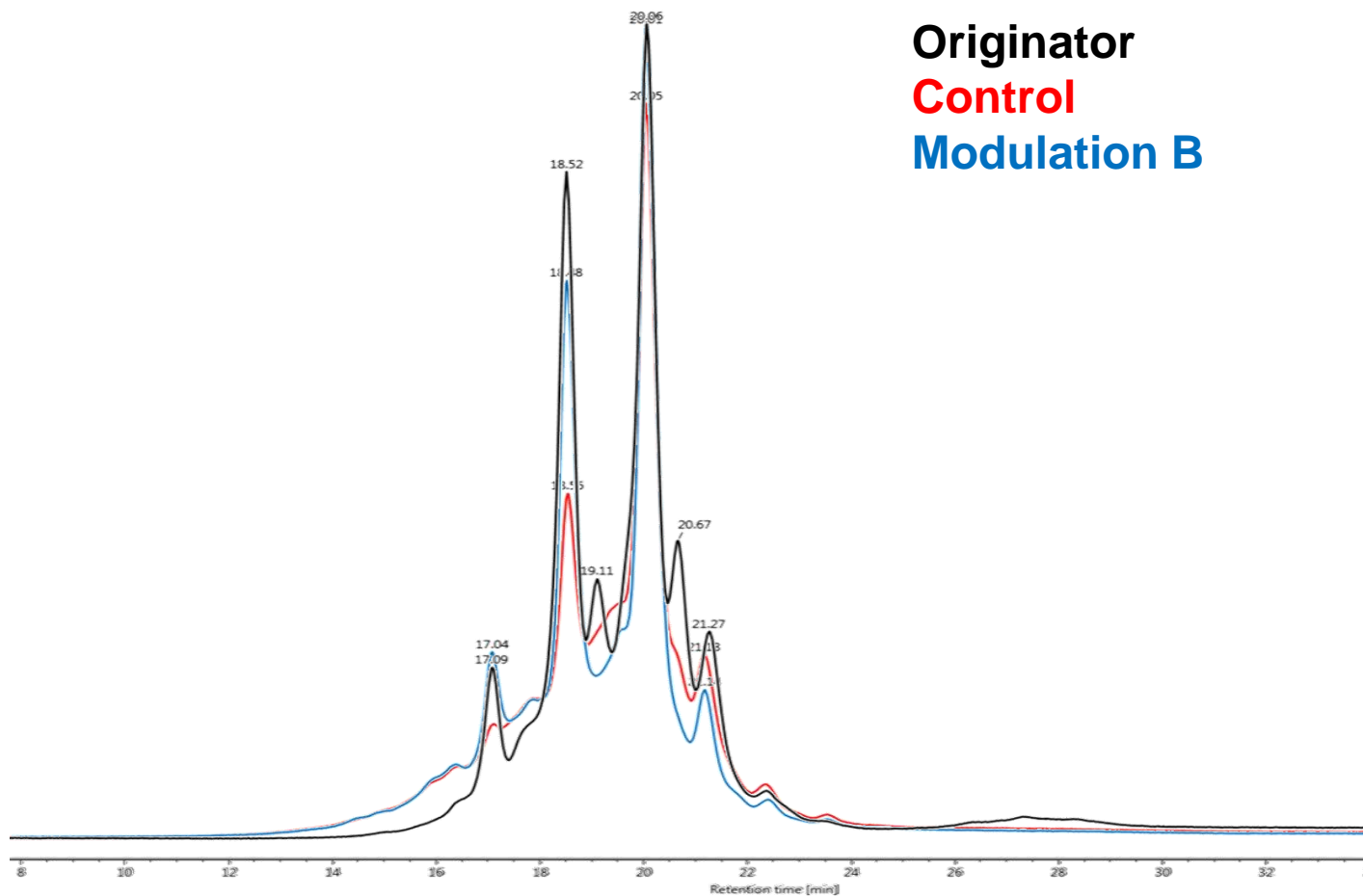
n.n. = not needed

Decreasing galactosylation by USP PD

Sample description		Man5+ (%)	G0-GlcNAc (%)	G0F-GlcNAc (%)	G0 (%)	G0F (%)	G1 + G1' (%)	G1F (%)	G1'F (%)	G2F (%)	*(%)
81-35-123	Control	7.1	1.21	2.12	5.14	44.38	1.97	22.74	7.74	5.09	98.56
	Modulation B	6.18	0.93	1.98	4.56	45.32	1.75	23.74	7.95	5.21	99.21
	+ 2x A	7.12	1	3	4.44	53.29	0.81	17.71	6.61	3.09	99.31
	+ 5x A	6.39	0.78	3.2	4.01	55.71	0.67	15.69	6.27	2.42	98.92
81-35-230	Control	5.52	0.83	1.48	4.38	37.18	2.32	28.47	9.22	7.12	98.72
	Modulation B	4.28	0.52	1.24	3.72	38.67	2.1	29.6	9.53	7.3	98.67
	+ 2x A	5.69	0.68	2.63	3.98	51.12	1.18	19.61	7.28	3.21	98.45
	+ 5x A	5.58	0.58	2.76	3.96	56.26	0.66	16.06	6.38	2.21	98.84
Originator		6.36	1.95	7.87	2.75	65.61	<0.5	9.03	3.85	0.82	99.99
Originator before Protein A		6.28	2.03	8.14	2.81	65.68	<0.5	8.85	3.69	0.73	99.68

- **Modulation A:**
 - Higher concentration of A decreases galactosylation
 - Higher concentration of A increases GOF-GlcNAc
 - More A needed to further decrease galactosylation and increase GOF-GlcNAc
 - Experiments ongoing

Example of charge variants modulation



Improving galactosylation by USP PD

Glycoforms	Originator 1	Originator 2
% G0F-GlcNac	3.2	3.1
% G1F-GlcNac	3.8	4.0
% G0F	27.5	27.5
% G1F	46.9	46.9
% G2F	18.6	18.5

Glycoforms	CL 1 (0x)	CL 1 (4x)	CL 1 (8x)
% G0F-GlcNac	2.7	2.6	1.7
% G0	6.9	7.6	8.0
% G0F	50.7	39.2	32.5
% G1F	30.3	37.0	42.2
% G2F	9.4	13.6	15.6
Glycoforms	CL 2 (0x)	CL 2 (4x)	CL 2 (8x)
% G0F-GlcNac	2.0	2.0	1.6
% G0	2.8	2.8	2.5
% G0F	47.0	34.5	32.2
% G1F	37.9	47.0	46.8
% G2F	10.2	16.7	16.9
Glycoforms	CL 3 (0x)	CL 3 (4x)	CL 3 (8x)
% G0F-GlcNac	2.2	2.0	1.8
% G0	2.3	2.6	2.6
% G0F	50.7	40.5	38.2
% G1F	34.9	40.2	43.0
% G2F	9.9	14.6	14.4

Titration of galactosylation modulation



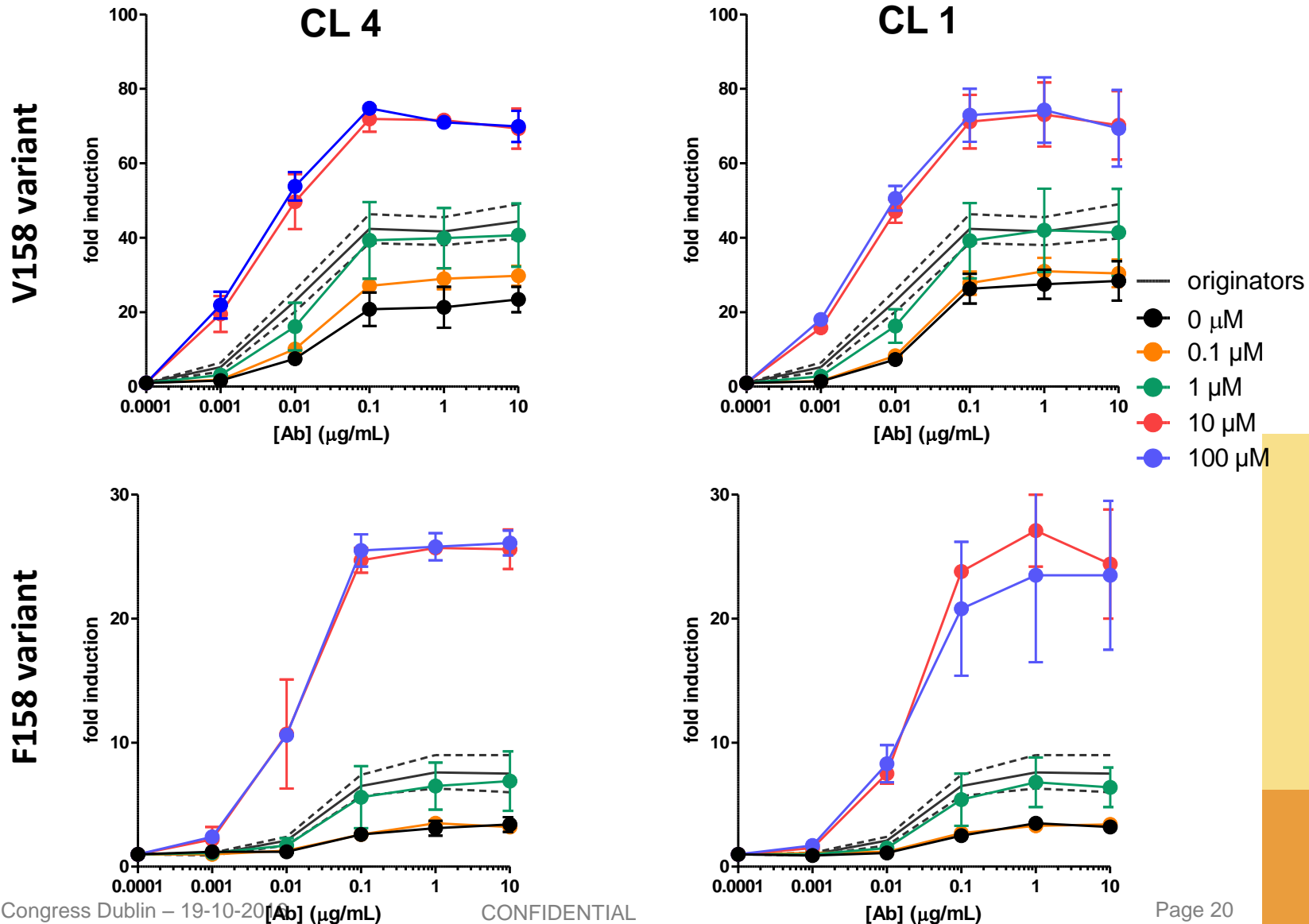
Improvement of galactosylation

Glycoforms	Originator 1	Originator 2	Originator 3	Originator 4
% G0F-GlcNac	2.8	4.2	3.9	3.6
% G0	7.7	7.2	5.8	6.9
% G0F	36.1	44.9	49.2	47.5
% G1F	42.1	36.1	34.1	35.7
% G2F	11.3	7.7	6.8	6.3

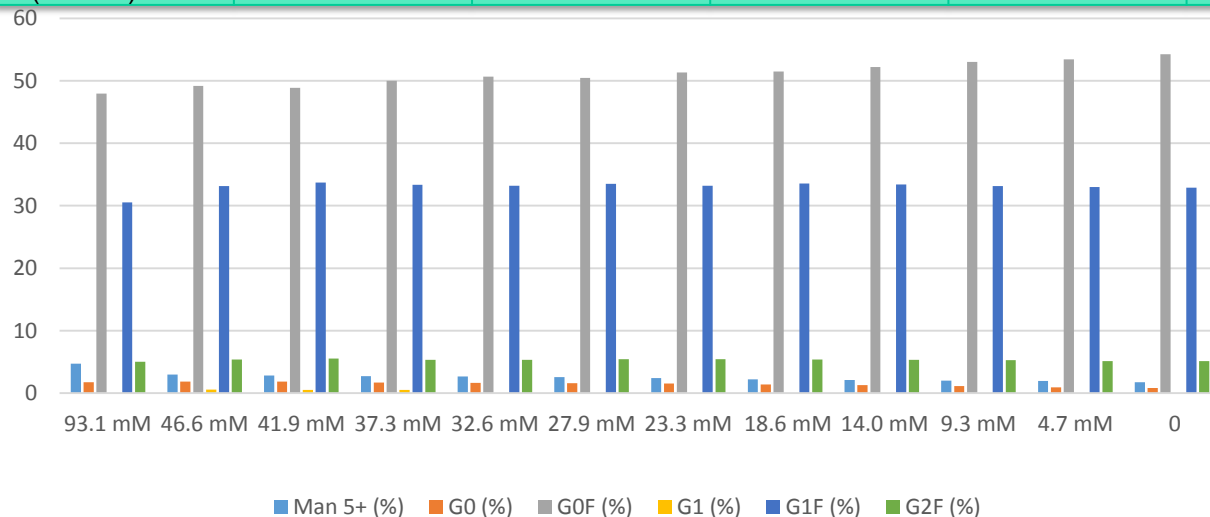
Glycoforms	CL 4 control	CL 4 1 uM X	CL 4 10 uM X
% G0-GlcNac	n.d.	n.d.	2.8
% G0F-GlcNac	3.6	3.1	4.5
% G0	3.5	8.4	48
% G0F	51.3	46.5	10.6
% G1	n.d.	n.d.	22.2
% G1F	34.5	31.8	8.5
% G2	n.d.	n.d.	trace
% G2F	7.3	10.2	3.6

Total non-fucosylated: 8.4 % 70.2 %

ADCC activity of modulated mab

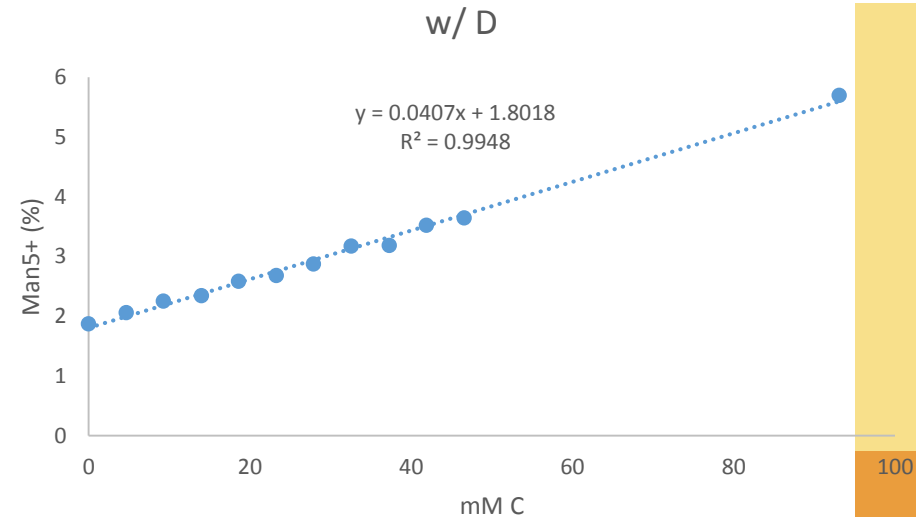
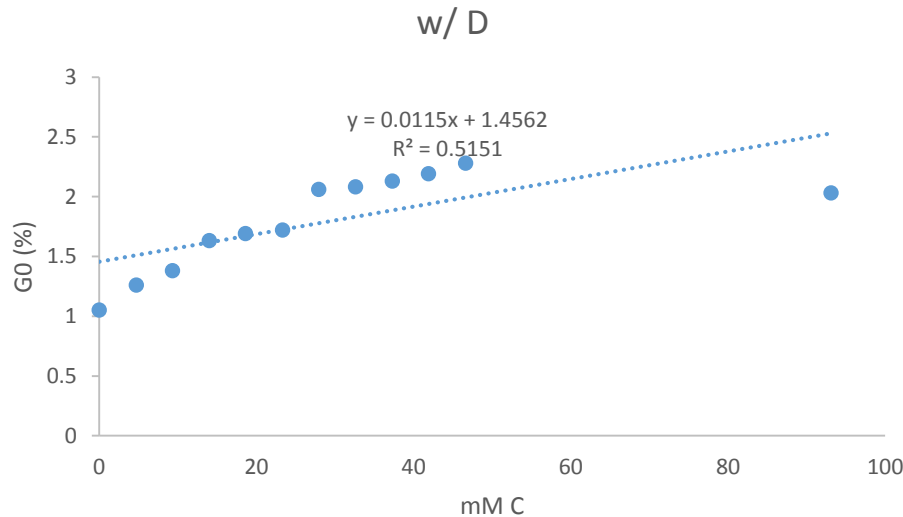
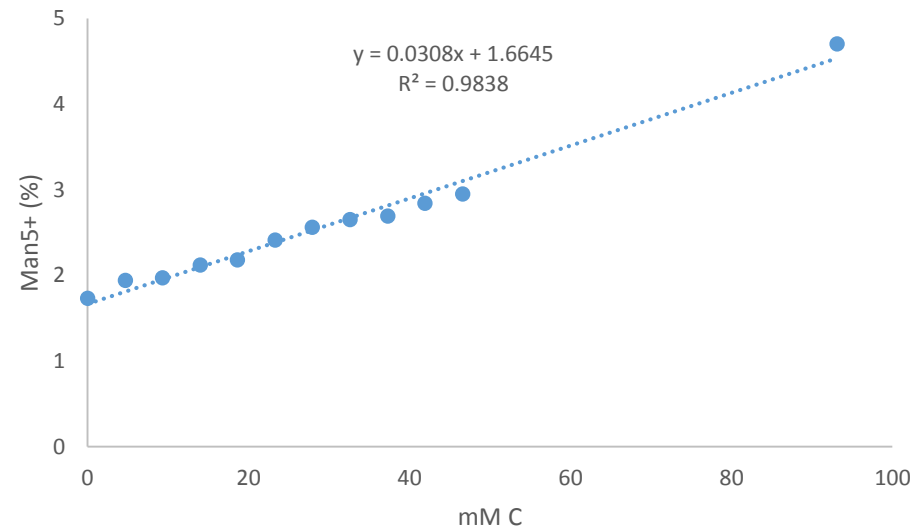
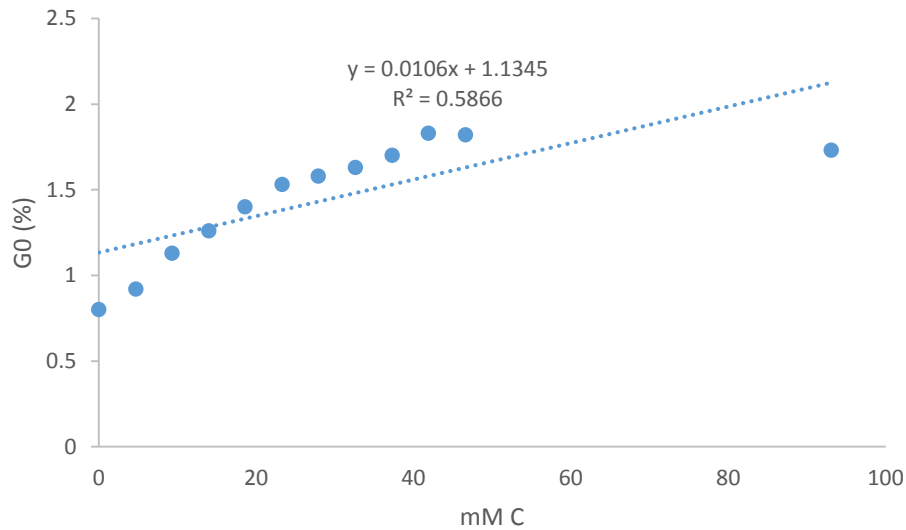


ID	Man 5+ (%)	G0 (%)	G0F (%)	G1 (%)	G1F (%)	G2F (%)
Originator	5.54	0.67	68.45	<0.5	15.31	1.14
93.1 mM	4.7	1.73	47.95	<0.5	30.51	5.02
46.6 mM	2.95	1.82	49.19	0.56	33.16	5.4
41.9 mM	2.84	1.83	48.89	0.52	33.72	5.51
37.3 mM	2.69	1.7	49.97	0.5	33.37	5.34
32.6 mM	2.65	1.63	50.66	<0.5	33.21	5.34
27.9 mM	2.56	1.58	50.46	<0.5	33.5	5.44
23.3 mM	2.41	1.53	51.35	<0.5	33.2	5.41
18.6 mM	2.18	1.4	51.47	<0.5	33.57	5.39
14.0 mM	2.12	1.26	52.18	<0.5	33.38	5.35
9.3 mM	1.97	1.13	53.03	<0.5	33.16	5.28
4.7 mM	1.94	0.92	53.45	<0.5	32.98	5.13
0 (control)	1.73	0.8	54.27	<0.5	32.89	5.11



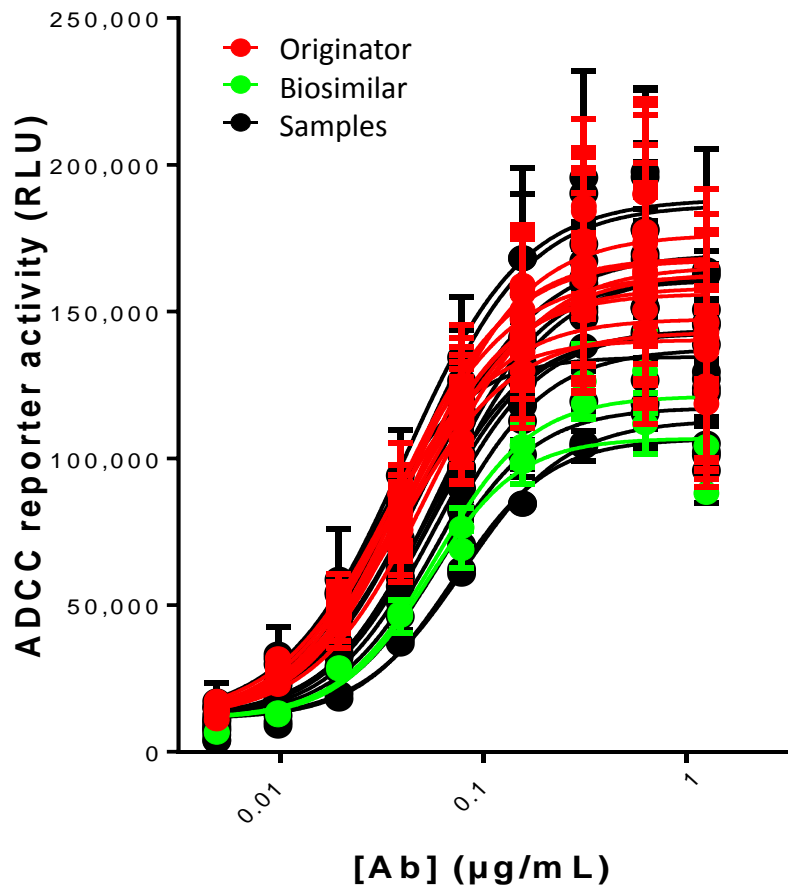
**Addition of C
enhances Man5+
and G0**

Relation Man5+ (%) and G0 (%) vs C

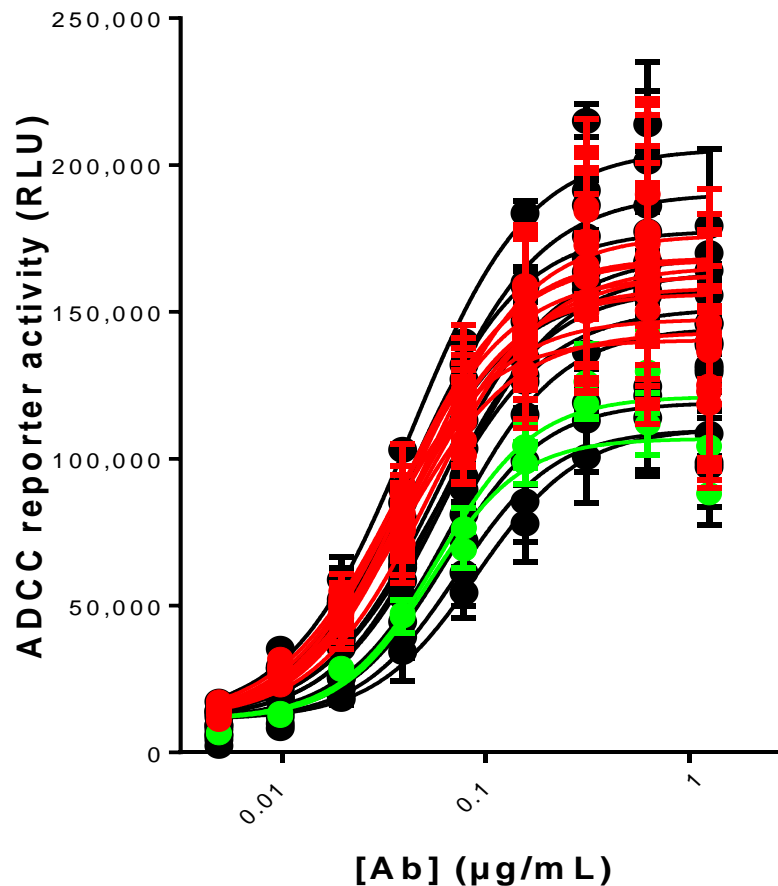


Modulation by C and D of ADCC activity

C

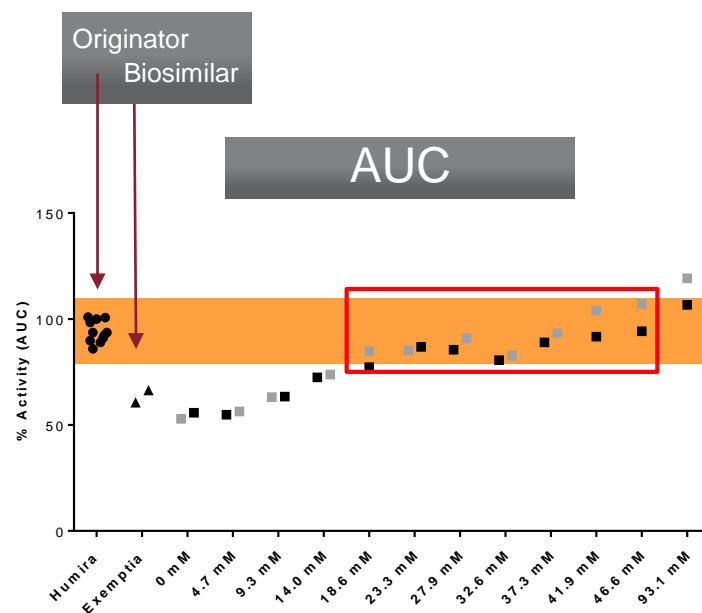
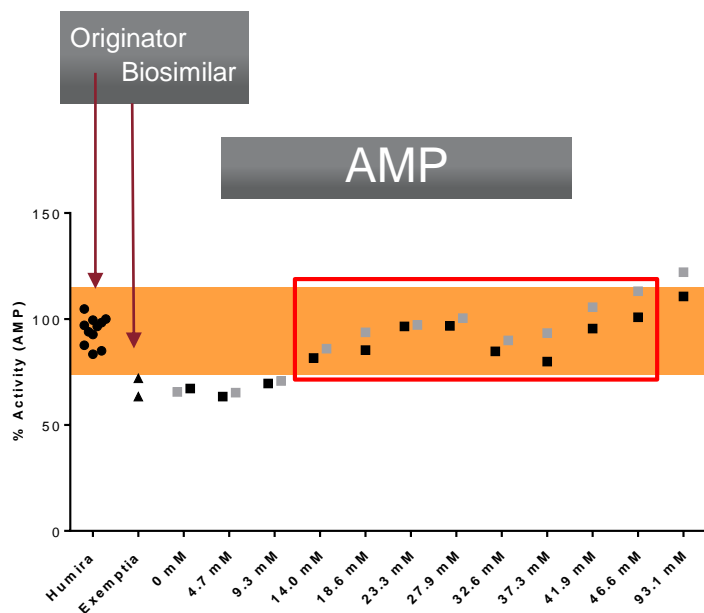


C + D

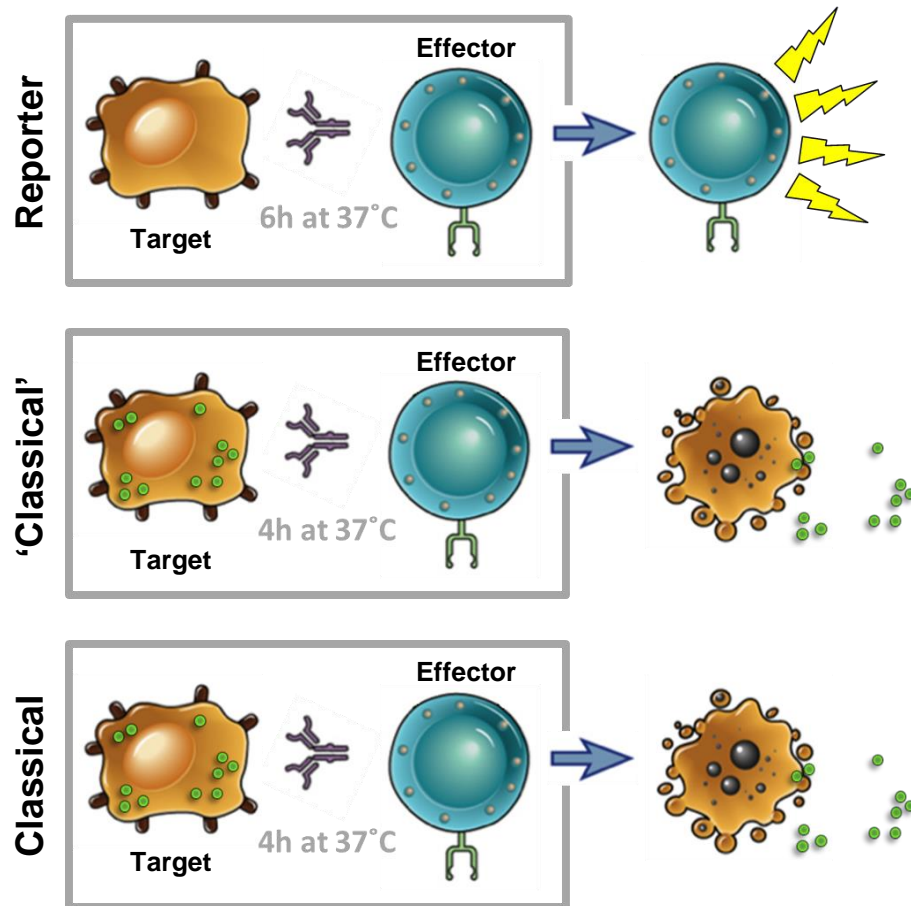


ADCC activity modulated within the TIER 2 quality range

Samples are within the Tier 2 Quality Range



■ C
■ C + D



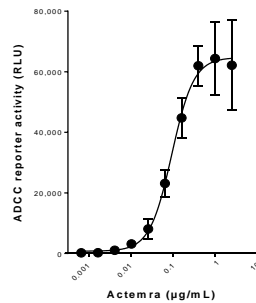
Target cells	Effector cells	Read out
Unlabeled	Reporter cells	Reporter activation
Calcein AM labeled	NK cell line (CD16+ both F and V variants)	Target cell lysis
Calcein AM labeled	Primary NK cells (negative selection)	Target cell lysis

Target cells

HEK IL-6R

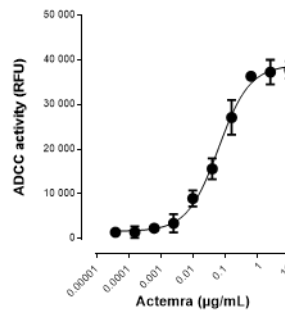
Reporter

CD16V

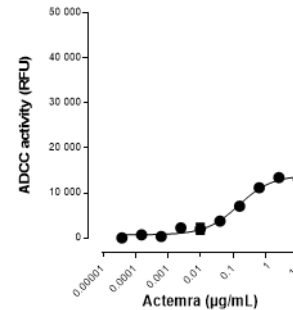


'Classical'

CD16V

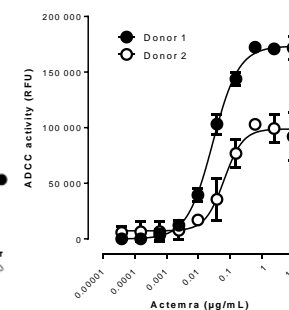


CD16F

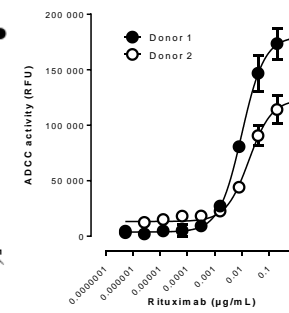
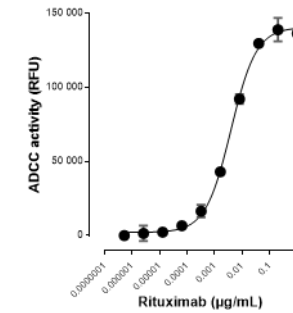
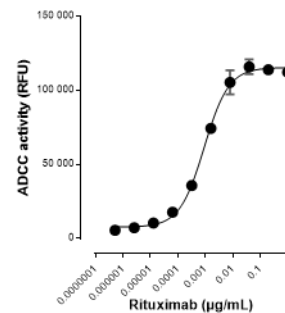
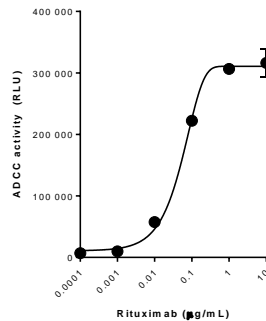


Classical

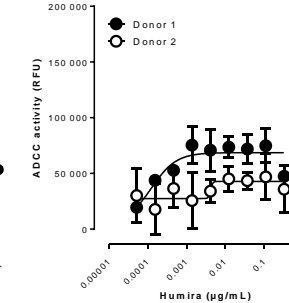
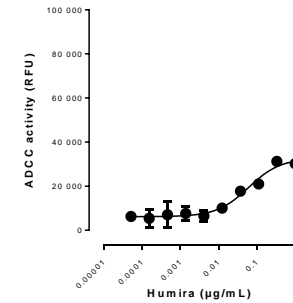
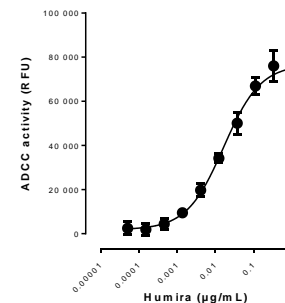
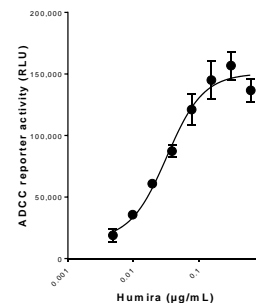
CD16V/F?

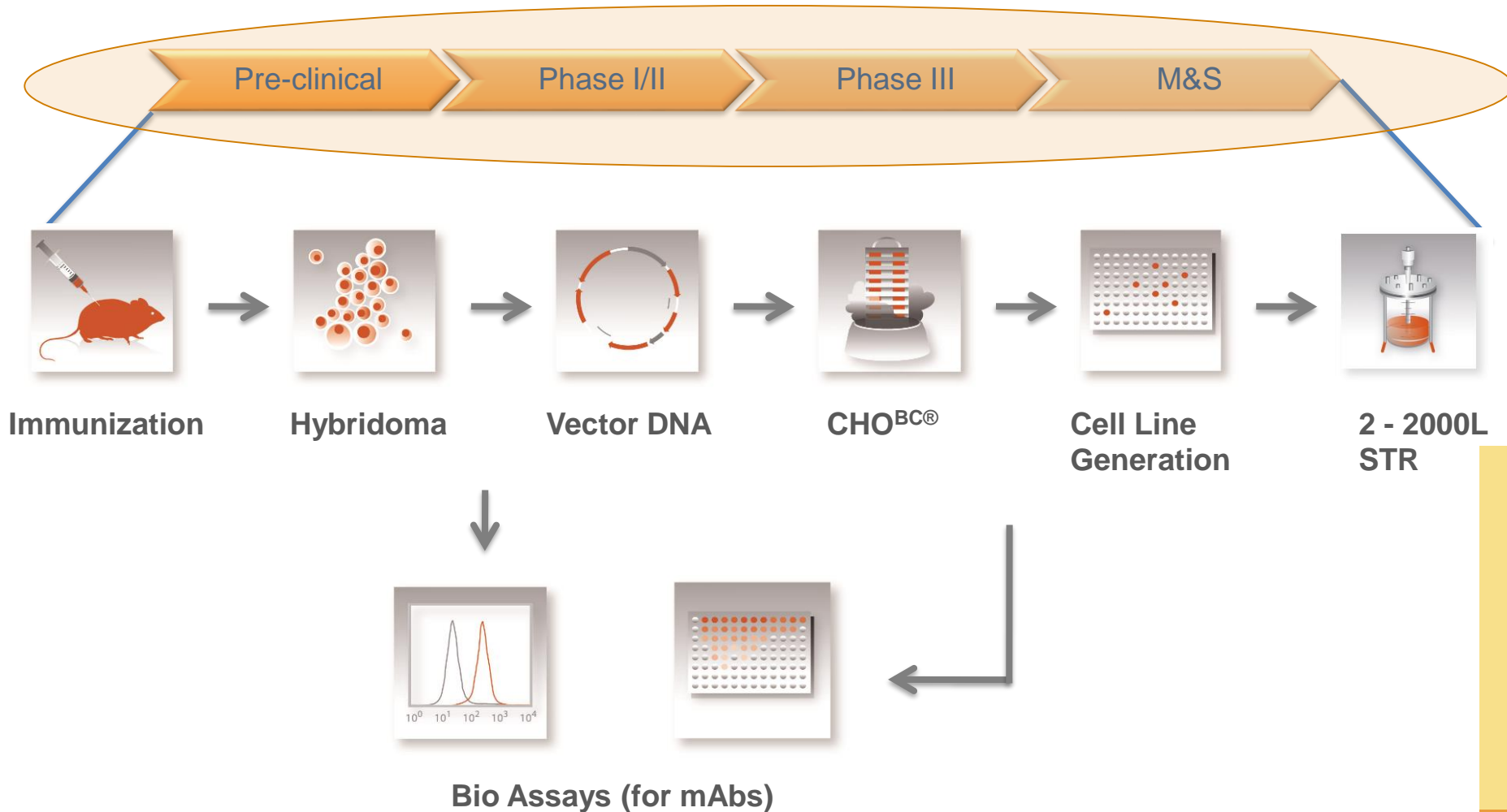


Daudi CD20



HEK TNF α





- Modulation of galactosylation, fucosylation and charged variants can be targeted by different methods
- Procedures are without effect on titer or VCD
- Select for high producers and adapt the process as needed
- Glycosylation and charged heterogeneity is mainly process dependent
- Classical cell line based ADCC bioassay developed
- Integrated vertical approach from cDNA to market supply