Afucosylation: Potency Evaluation Strategies

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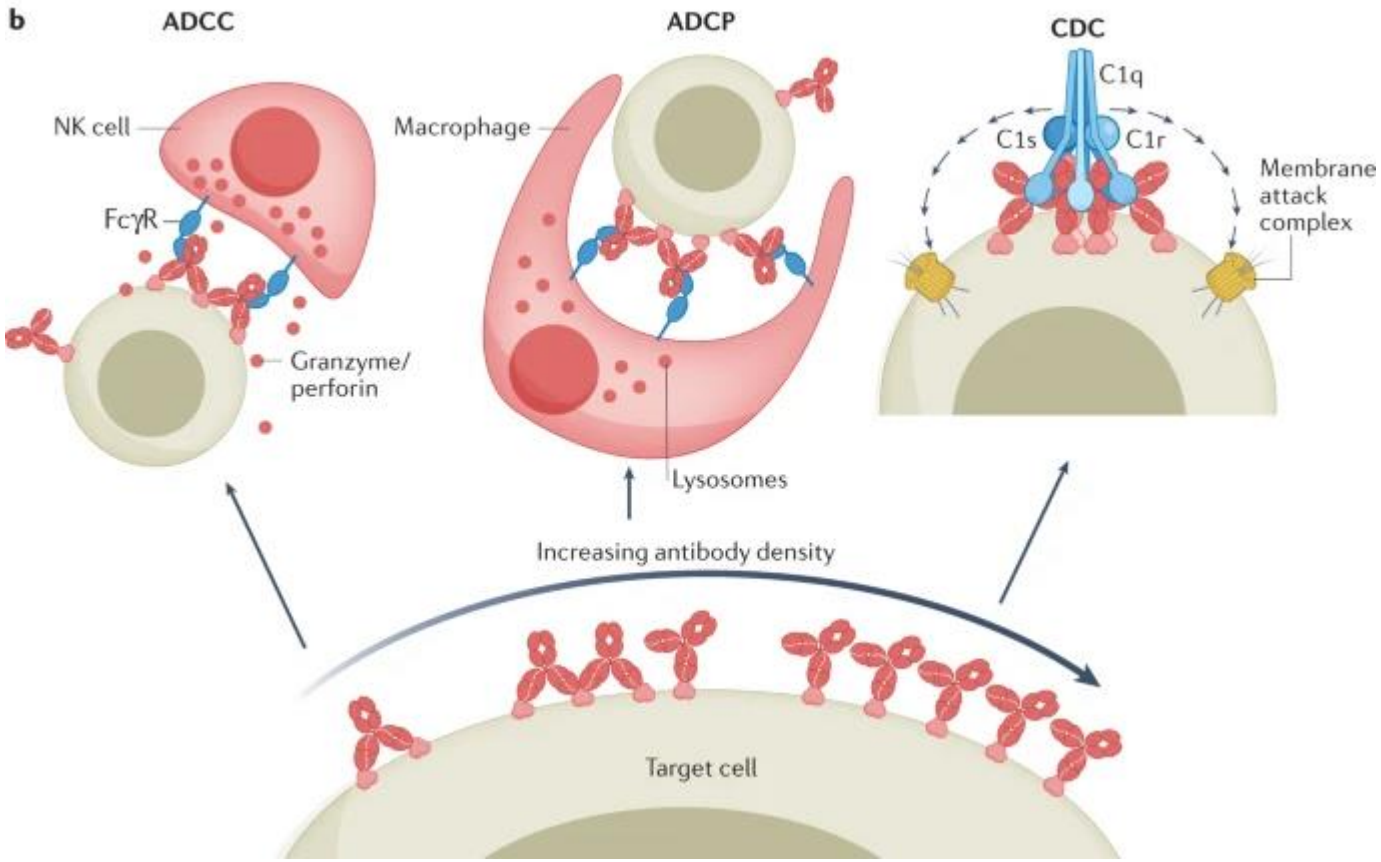
Victoria Swiss Ph.D.

Bristol Myers Squibb™

Effector Function:
Biological Activity of mAbs beyond target binding

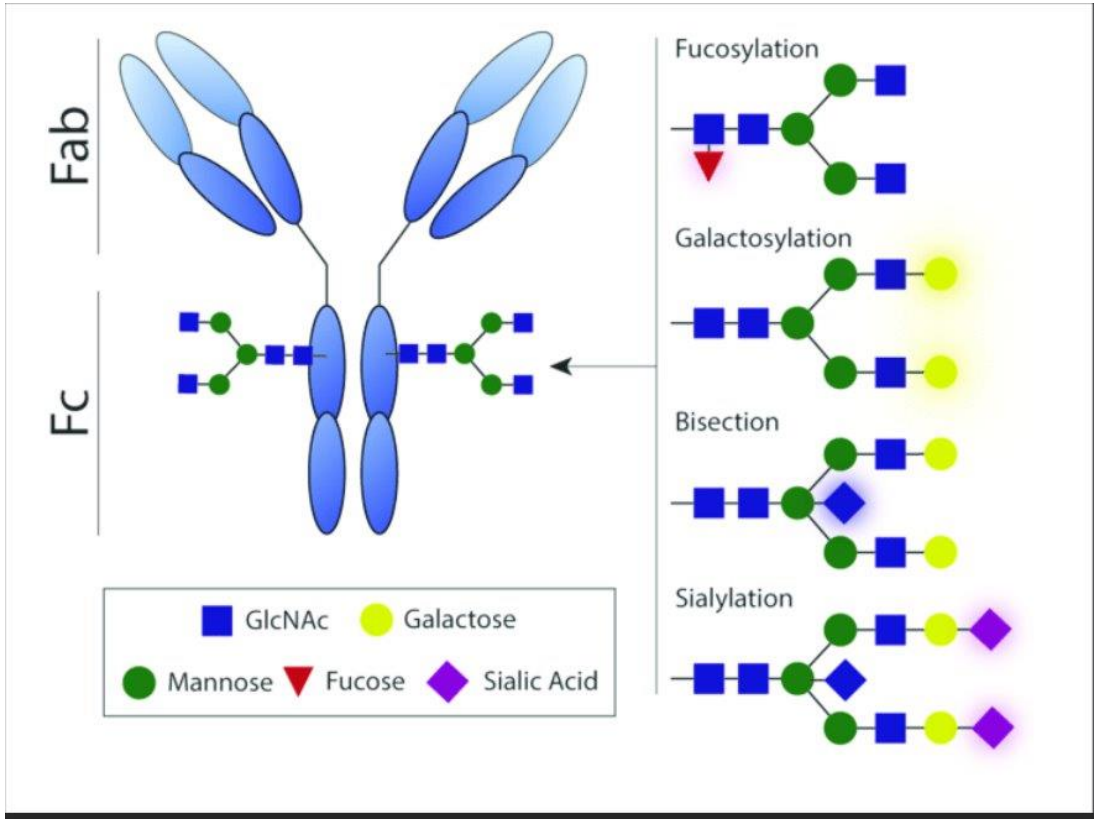
- Effector function response may consist of antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), or complement-dependent cytotoxicity (CDC).
- Effector function activity is influenced by several factors, including the subtype of the immunoglobulin, the Fc gamma receptor type (FcγR) type, and the Fc-glycan composition.



1.Oostindie, S. C.; *et al*. Avidity in antibody effector functions and biotherapeutic drug design. *Nature Reviews Drug Discovery*. 2022, 21(10): 715-735.

Afucosylation of IgG Impacts Structure and Function

- Changes in the ~N297 glycosylation can impact Fc receptor binding, leading to changes in effector function-mediated biological activity
  - Wild-type IgG1 CH2 domains bind CD16a
    - Two allotypes of FcγRIIIa are prominent in the human population
    - CD16a-V allotype has higher affinity than CD allele for mAb-Fc
    - CD16a-F allotype is most prevalent (~90% of population)
  - Afucosylation at ~N297 increases binding of CD16 by up to 50-fold
  - Binding to CD16a (FcgRIIIa) is a critical component and the first step mediating Antibody-Dependent Cellular Cytotoxicity (ADCC)
  - Impact of afucosylation on antibody-dependent cellular phagocytosis (ADCP) not as well-understood or documented, but may be important



van Erp, Liz & Luytjes, Willem & Ferwerda, Gerben & van Kasteren, Puck. (2019). Fc-Mediated Antibody Effector Functions During Respiratory Syncytial Virus Infection and Disease. Frontiers in Immunology. 10. 548. 10.3389/fimmu.2019.00548.

Difference in Afucosylation can lead to Clinical Differences Example: Rituximab and Obinutuzumab

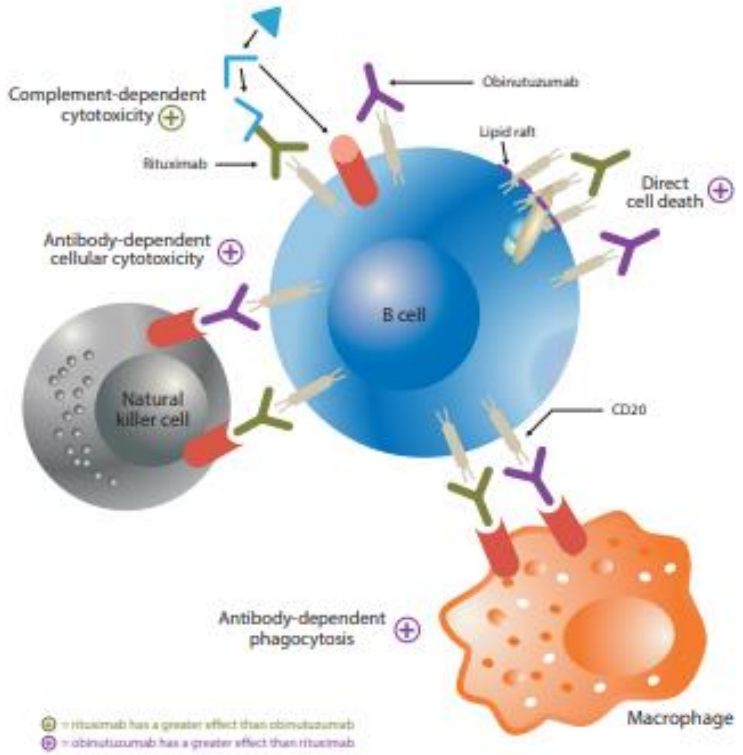


Figure 1: Differences in the proposed mechanisms of action of rituximab and obinutuzumab. Rituximab is a type I antibody that functions by the stabilisation of CD20 on lipid rafts, resulting in strong complement-dependent cytotoxicity. Obinutuzumab is a glycoengineered type II antibody that leaves CD20 distributed across the surface of the B cell and has much lower complement-dependent cytotoxicity, but greater antibody-dependent cellular cytotoxicity, antibody-dependent phagocytosis and direct cell death.

Hernandez et. Al. Nature Portfolio Sponsor Feature

- Rituximab (anti-CD20)
  - Class I antibody
  - Mechanism of Action includes: CDC/ADCC/ADCP Effector Functions

- Obinutuzumab (anti-CD20)
  - Class II antibody (different CD20 binding site)
  - **Glycoengineered to reduce fucosylation**
  - Compared to Rituximab:
    - **Enhanced ADCC activity**
    - Reduced CDC activity (due to CD20 binding site)

Obintuzumab has superior effects in clinical trials

- Chronic Lympohcytic Leukaemia (CLL)
- Follicular Lymphoma (FL)
- But Not Diffuse Large B-Cell Lymphoma

Afucosylation % is a Critical Quality Attribute for IgG1 molecules

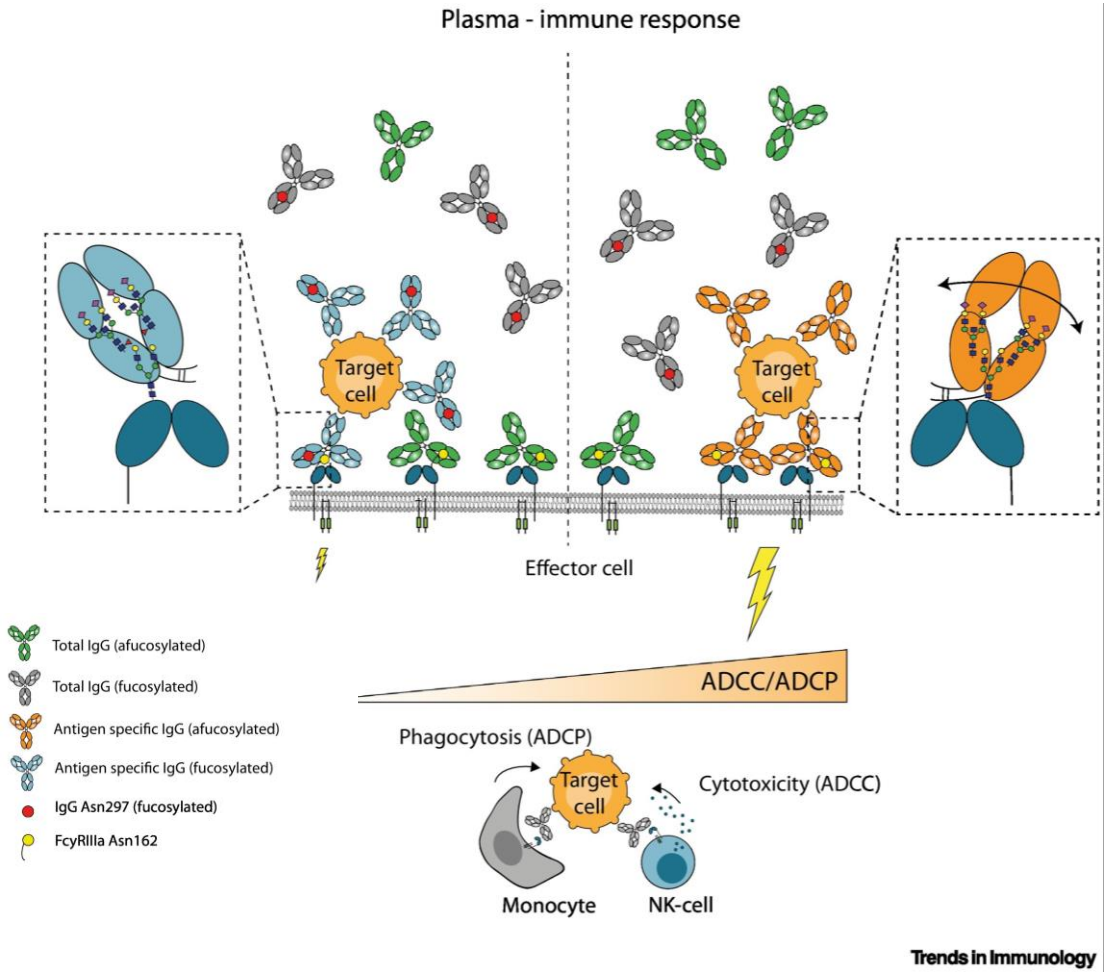
- Total Afucosylation may be measured as the aggregate of several components
  - Antibodies with higher Man5 / afucosylatied glycoforms have been demonstrated to have increased ADCC activity *in vitro*

- Molecules may be designed for enhanced ADCC (NF), or to be Fc-inert. Afucosylation impact to biological function for these molecules is controlled *via* molecular sequence or manufacturing cell mutation.

- For the molecules where binding to CD16 is a key component of the intended MoA *and may vary as a result of post-translational modifications*, **afucosylation identified as a critical attribute to understand and monitor**

- **Control of Afucosylation is critical to Patients**

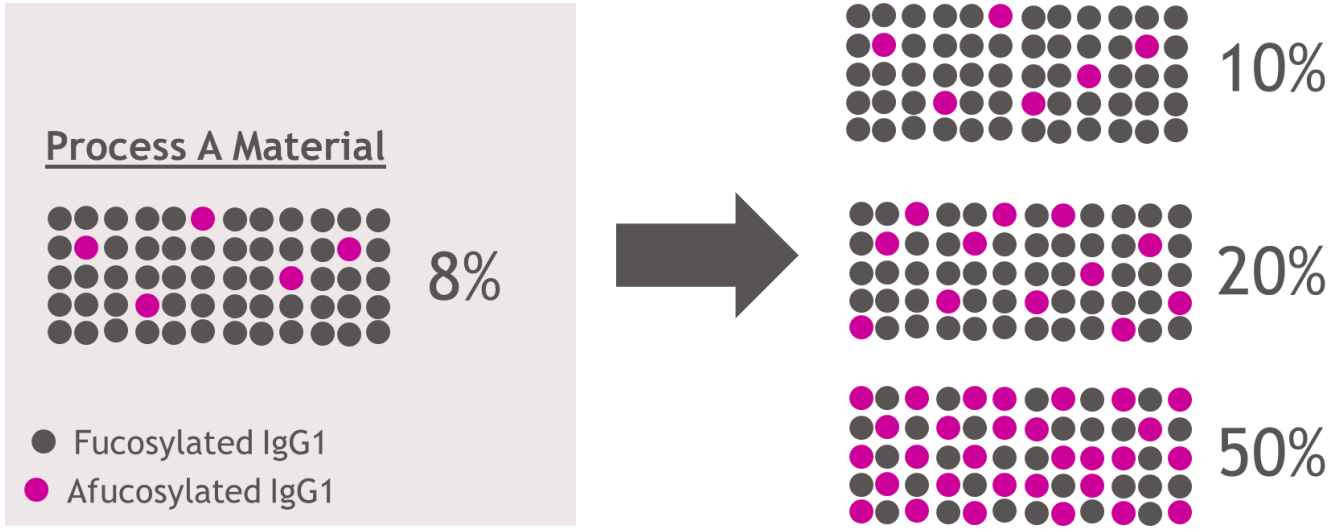
Afucosylation% Low **Afucosylation% High**



Ensuring Comparability of Processes

- ICH Q5E – Comparability
  - The goal of the comparability exercise is to ensure the quality, safety and efficacy of drug product produced by a changed manufacturing process, through collection and evaluation of the relevant data to determine whether there might be any adverse impact on the drug product due to the manufacturing process changes
  - The demonstration of comparability does not necessarily mean that the quality attributes of the pre-change and post-change product are identical, but that they are highly similar and that the existing knowledge is sufficiently predictive to ensure that any differences in quality attributes have no adverse impact upon safety or efficacy of the drug product.

Which of these Materials are comparable to Process A?



How do we define ranges of suitable Afuocosylation levels for CMC?

- Understanding Manufacturing Process Capabilities
- Specification setting

Workflow to Define Impact of Afucosylation Change

Create Samples

Assess Activity

Analyze

Define Risk

Overview:
Workflow to Define Afucosylation Impact to a Biologic

- Create Samples
  - Create sample with varying Afucosylation
  - Measure Afucosylation Level
- Assess Activity
- Analyze
- Define Range/Risk

- Create Samples with Varying Afucosylation levels by mixing of Enriched Afucosylated and Non-Enriched mAb
  - Sources of Afuocosylated Materials:
    - Use 100% (Non-Fucosylated)
    - Isolate Afucosylated species with chromatography

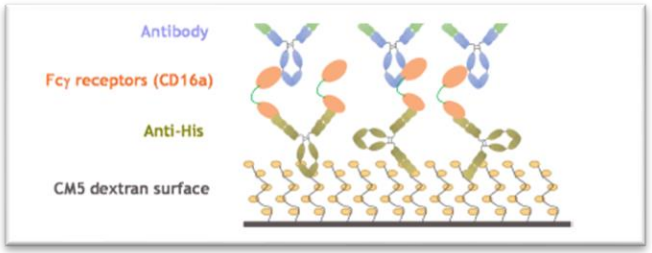
- Measure Afucosylation level of each samples (N-glycan method)
  - Provides direct measurement of % afucosylation
  - Afucosylation % =

$$\frac{\text{SUM of all non-fucosylated species}}{ALL\ Species}$$

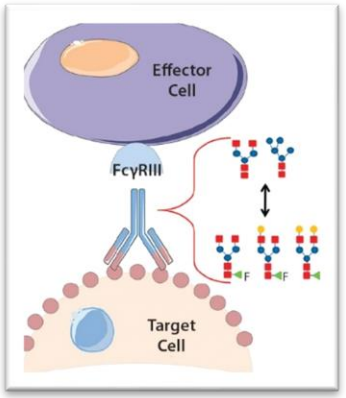
Overview:
Workflow to Define Afucosylation Impact to a Biologic

- Create Samples
- Assess Activity
  - Assess CD16a binding activity
  - Assess ADCC activity
- Analyze
- Define Range/Risk

- **CD16a Kinetic analysis - Quantitative Analysis**
  - CD16a-F allotype AND CD16a-V allotype
  - Utilize SPR analysis for Quantitative Data



- **ADCC Bioassay - usually Qualitative Analysis**
  - TARGET specific Assay
  - Direct Lysis Assay
    OR
  - CD16a Reporter

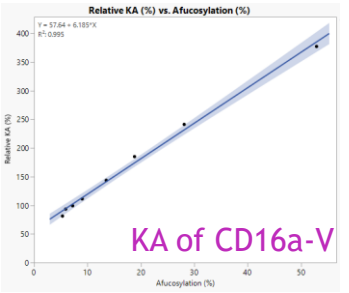


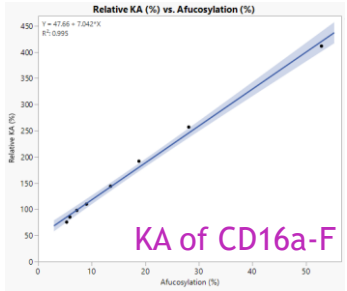
Overview:
Workflow to Define Afucosylation Impact to a Biologic

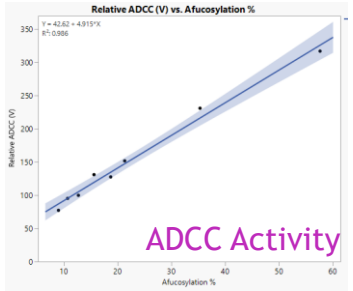
- Create Samples
- Assess Activity
- Analyze — Understand Correlation Between Afucosylation and Activity
- Define Range/Risk

- Plot Afucosylation and Activity
  - Activity plotted as relative measurement relative to reference standard
- Ensure strong linear correlation
- Utilize Slope to define Afucosylation Impact

$$\text{Slope} = {}^{\Delta\, Activity}/_{\Delta\, Afucosylation}$$







Defining Afucosylation ranges to de-risk manufacturing change

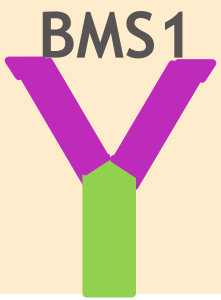
Overview: Define Impact of Afucosylation change to BMS1

Background:

- BMS1 is an mAb with an IgG1 backbone
- MoA includes ADCC

- BMS1, Process A manufacturing process
  - afucosylation levels 18.5 ± 0.5% (n=6)

- What is suitable level of afucosylation in new manufacturing process?

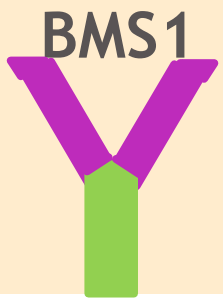


Create Samples	Create sample with varying afucosylation by mixing BMS1 Process A with 100% afucosylated BMS1
Assess Activity	Assess CD16a binding activity Assess ADCC activity
Analyze	Understand correlation between Afuocsylation level and Activity
Define Risk	Ranges for low and high risk

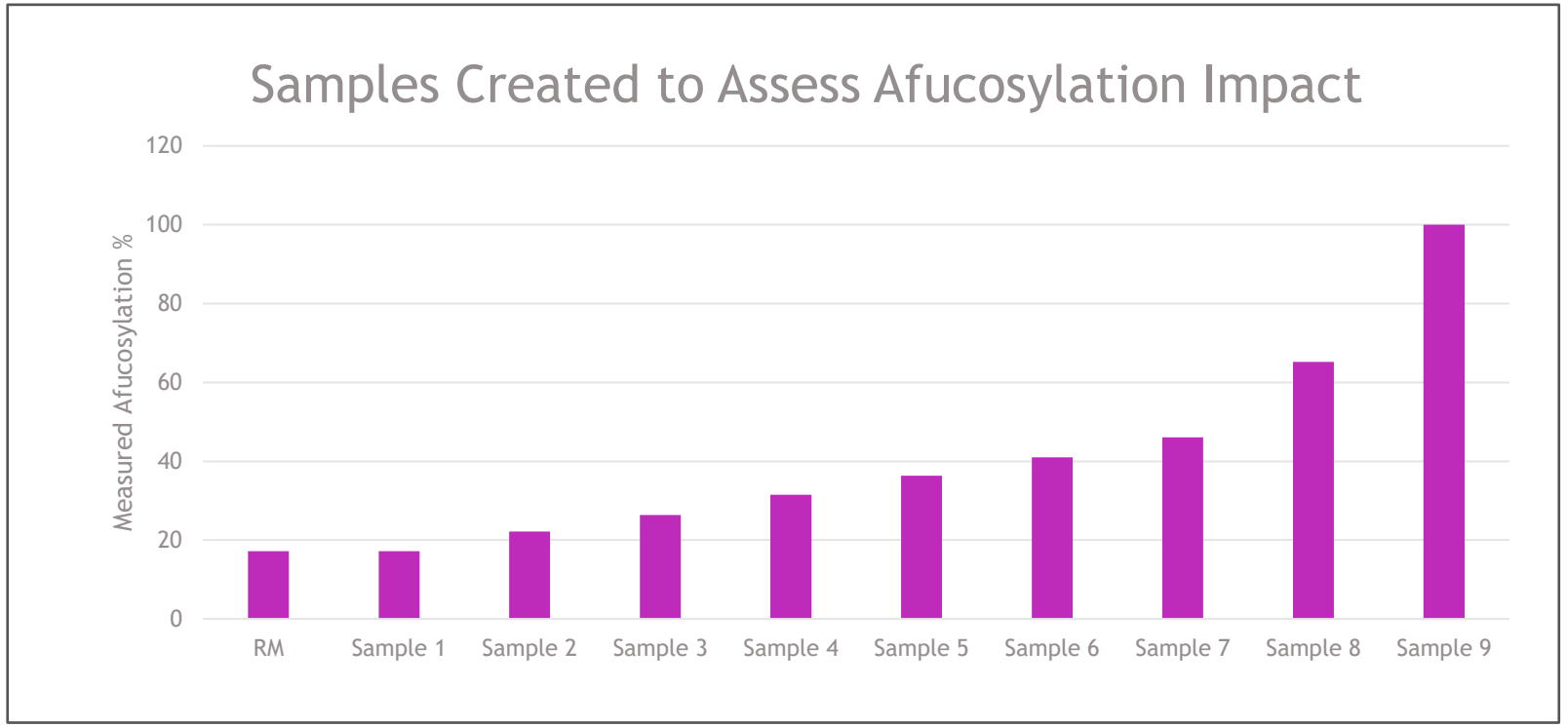
Define Impact of Afucosylation change to BMS1
Create samples with a range of Afuocosylation

Background:

- BMS1 is an mAb with an IgG1 backbone
- MoA includes ADCC
- BMS1, Process A manufacturing process
  - afucosylation levels 18.5 ± 0.5% (n=6)
- What is suitable level of afucosylation in new manufacturing process?



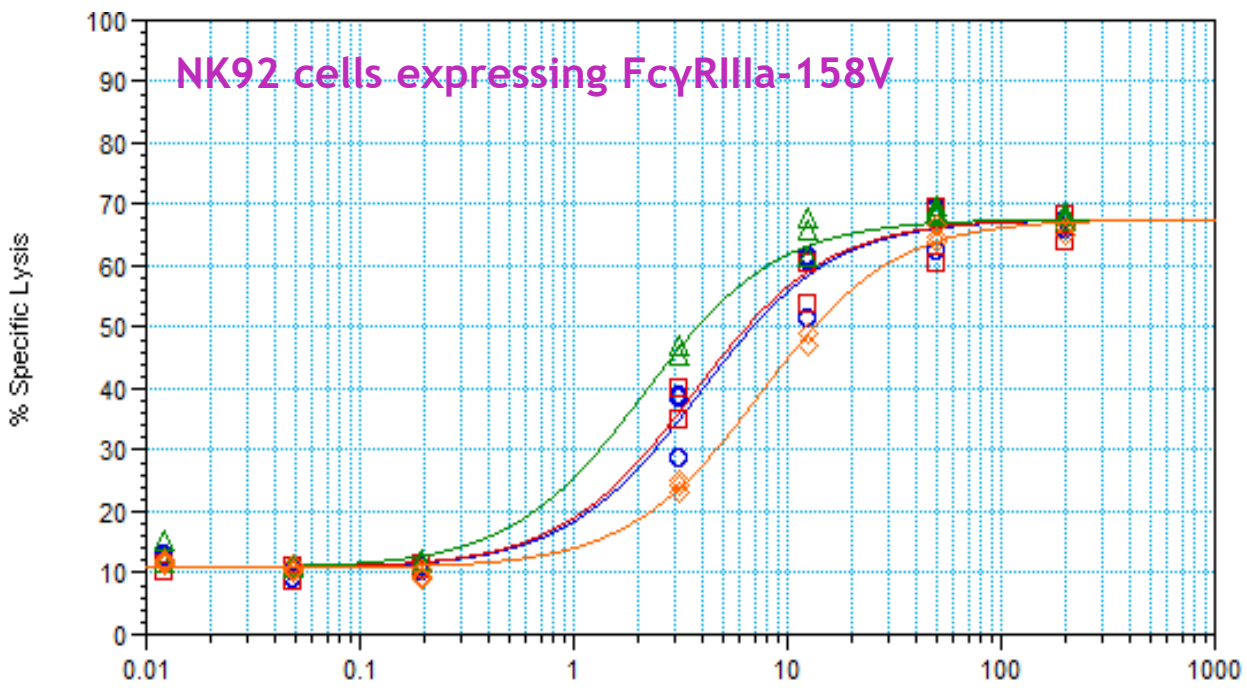
✓ Create samples by
Mixing BMS1 Process A with 100% Afucosylated BMS1

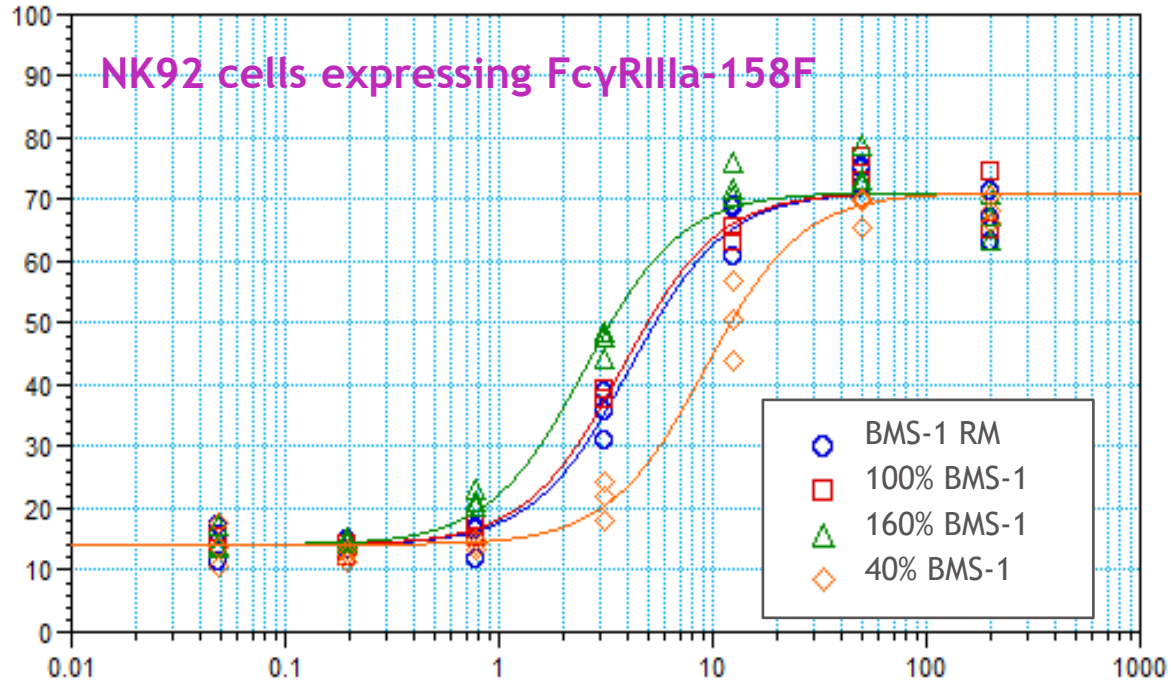


Define Impact of Afucosylation change to BMS1 Develop ADCC assays to assess activity

NK Lysis ADCC Bioassays Developed for BMS1

- Target cells loaded with BATDA dye
- NK92 effector cells expressing CD16a (either V or F variant)





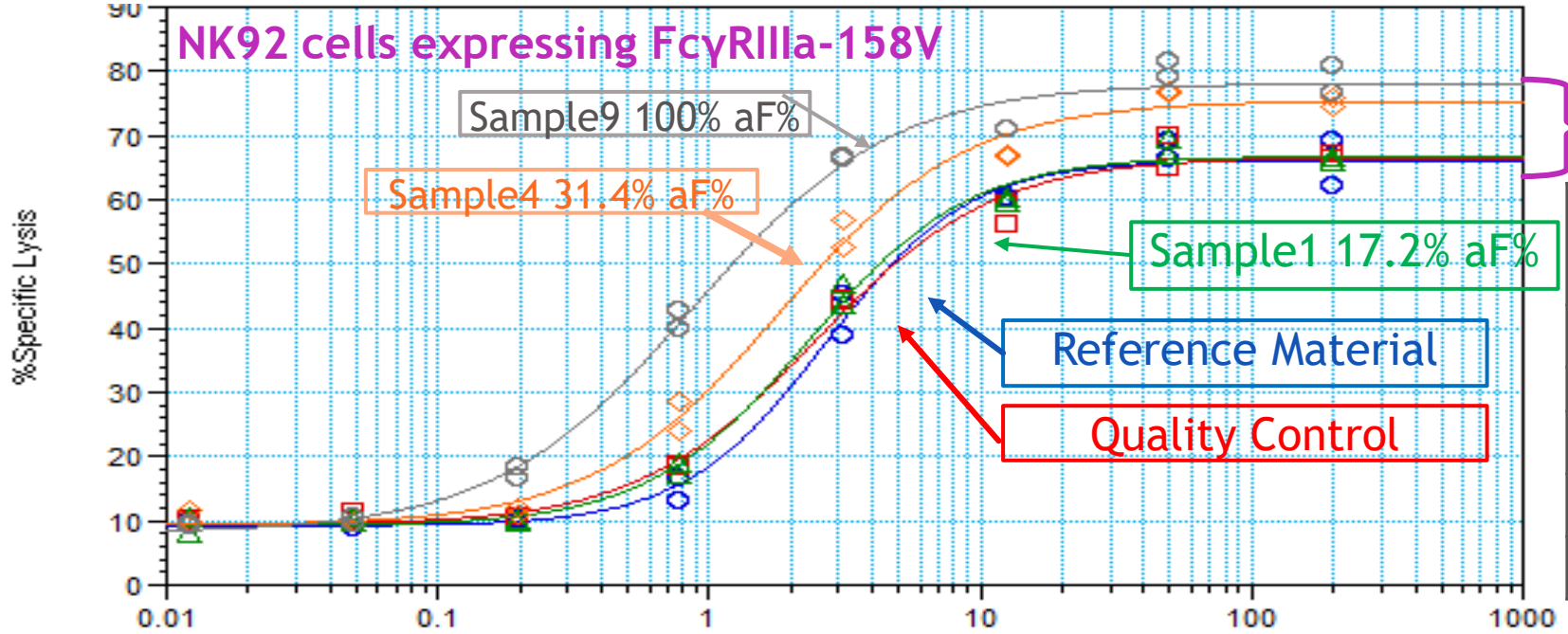
Each Assay system demonstrates parallelism of curves for recovery samples ranging from 40%-160%.

Define Impact of Afucosylation change to BMS1 Assess activity with ADCC Bioassays

BMS1

ADCC Bioassay provides a qualitative description of functional impact

NK92 cells expressing FcγRIIIa-158F show similar response (data not shown)



Dose response curves DO NOT demonstrate parallelism to RM

	R2	Parallelism: Lower Asymptote Difference	Parallelism Upper Asymptote Difference
REF	0.996		
QC	0.993	0.35	0.88
Smp1 17.2% aF%	0.999	0.11	1.05
Smp4 31.4% aF%	0.998	0.19	16.30
Smp9 100% aF%	0.997	1.79	21.03

Samples demonstrate shifts in dose response curve, EC50 ratio gives semi-quantitative understanding.

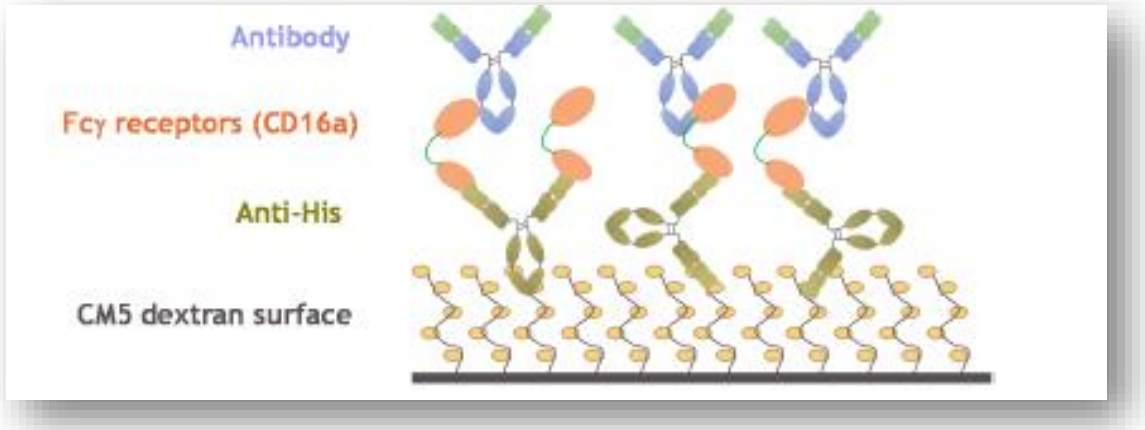
Define Impact of Afucosylation change to BMS1 Assess Activity with CD16a SPR analysis

BMS1

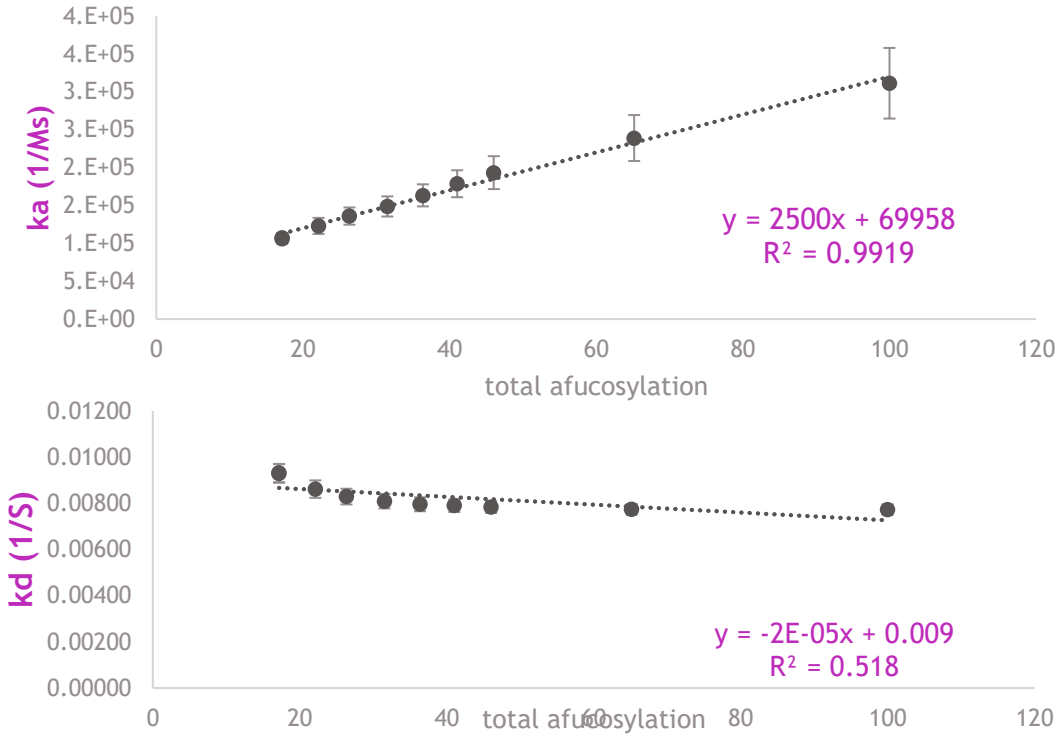
CD16a interaction analysis (via SPR) provides a quantitative readout

CD16a-V and CD16a-F analysis show similar response (data not shown)

Measure CD16a kinetics using SPR analysis



CD16a-V ka correlated with afucosylation



Define Impact of Afucosylation change to BMS1
Analyze Correlation of Afucosylation with Activity

BMS1

ADCC EC50 (relative to Reference Standard) correlation plot

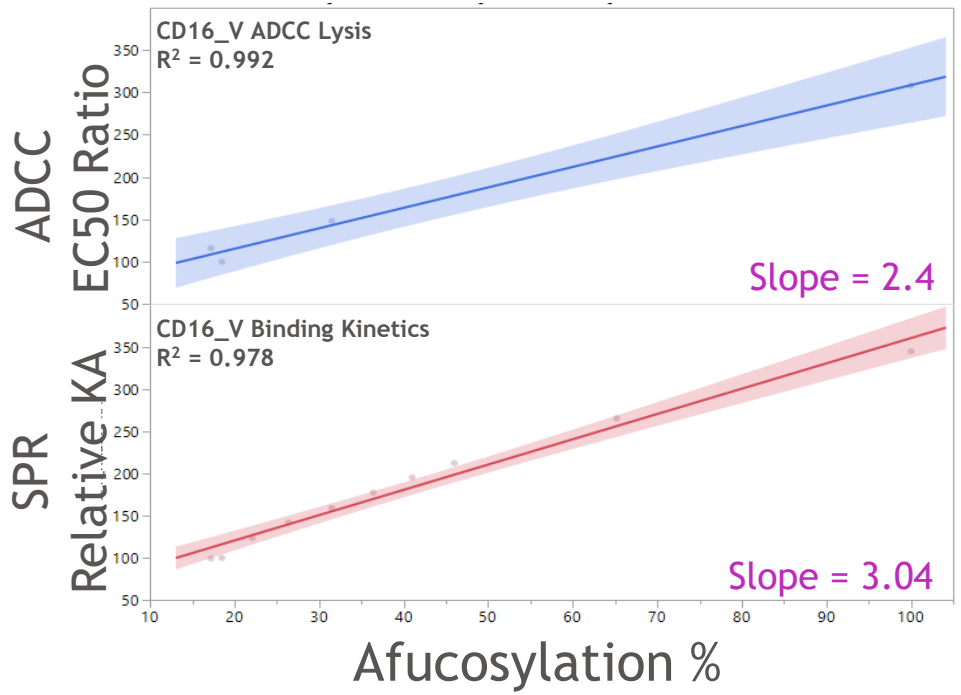
SPR Relative KA (relative to Reference Standard) correlation plot

A strong linear correlation of afucosylation to:

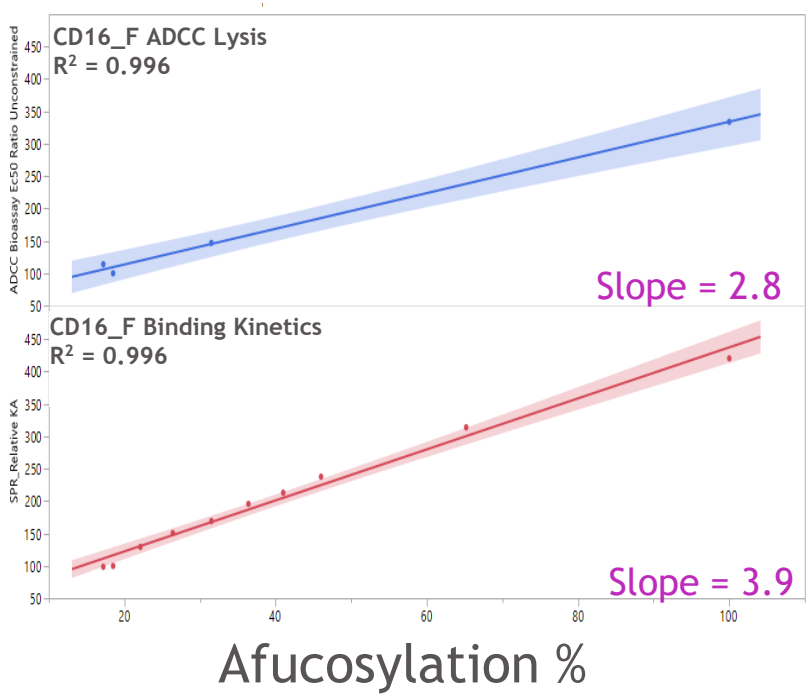
- KA% (R^2=0.978)
- ADCC Activity (R^2=0.992)

For BMS-1: an increase of 1% afucosylation leads to an increased equilibrium association constant (KA) of CD16a-V of ~3% relative to reference material.

CD16a-V



CD16a-F

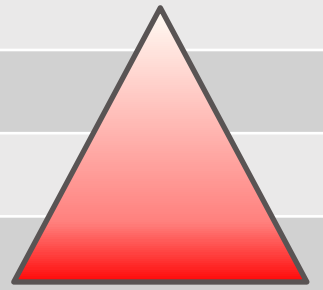


Define Impact of Afucosylation change to BMS1
Define Afuocosylation Range of low risk to comparability

BMS1

Utilize linear regression of SPR analyses to predict afucosylation levels and change from current RM

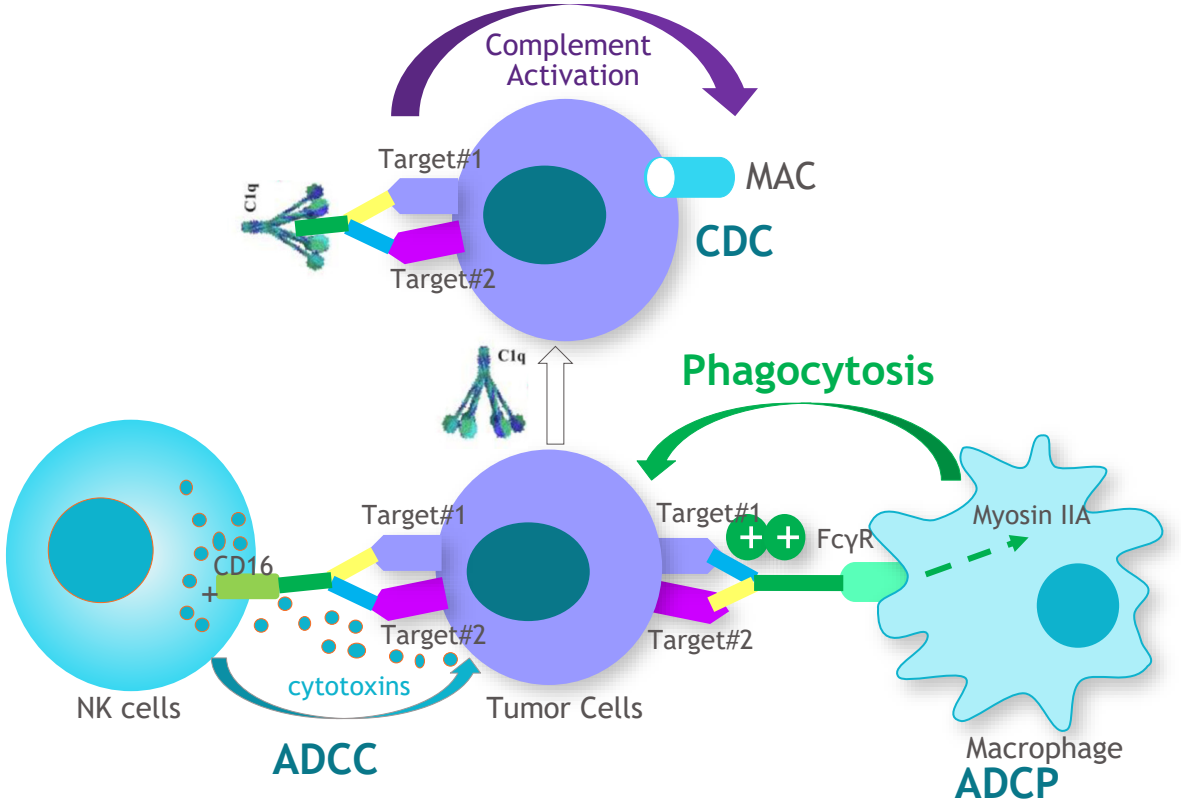
Afucosylation CD16_V SPR analysis	Afucosylation CD16_F SPR analysis	% CD16 KA Relative to RM	Risk to Comparability
17	17	100	None
20	19	110	
22	21	120	
25	23	130	
27	25	140	
30	27	150	
> 30	> 27	>150	



Overview: Define Impact of Afucosylation change to BMS2

Background:

- BMS2 is an bispecific mAb with an IgG1 backbone
- MoA includes ADCC AND ADCP
- BMS2, Process A manufacturing process
  - afucosylation levels 10 ± 0.5% (n=3)
- What is suitable level of afucosylation in new manufacturing process?

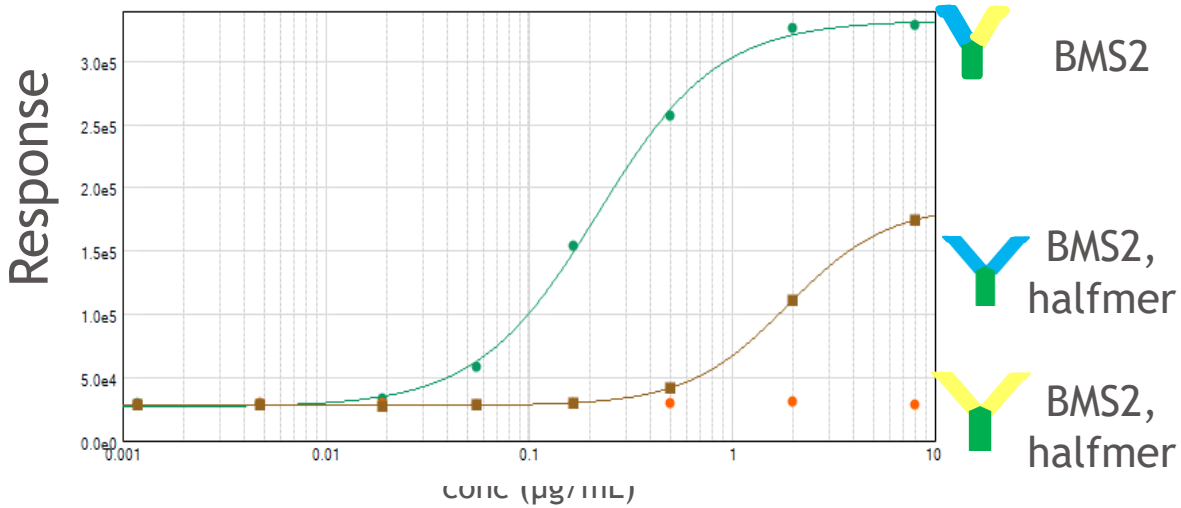


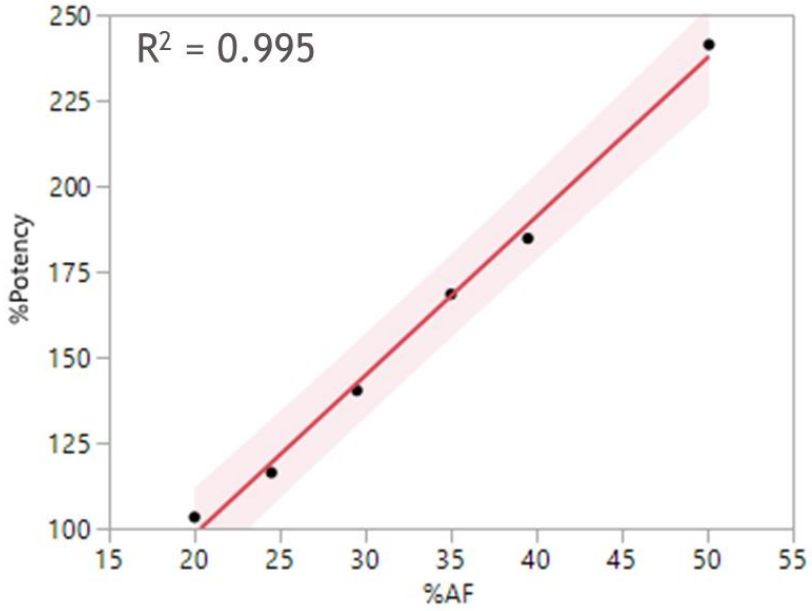
Overview: Define Impact of Afucosylation change to BMS2

BMS2

✓ Create Samples	Mixing BMS2 Process-A with Enriched Afucosylated BMS2 (isolated via chromatography)
Assess Activity	Assess ADCC activity with NK Reporter assay

ADCC NK Cell Activation - Target cell binding





Effector cells infected with lentivirus containing NFAT Luciferase (**N**uclear **F**actor of **A**ctivated **T** cells) show

- Shows little/no response to 'parent' halfmers

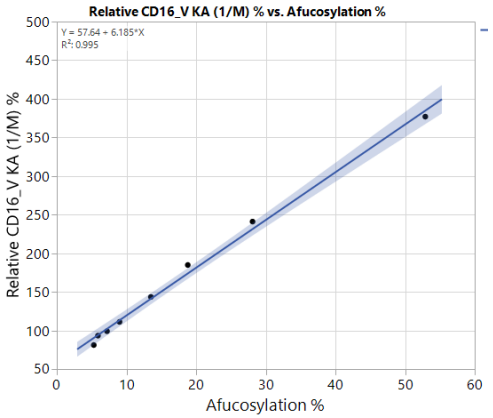
Overview: Define Impact of Afucosylation change to BMS2

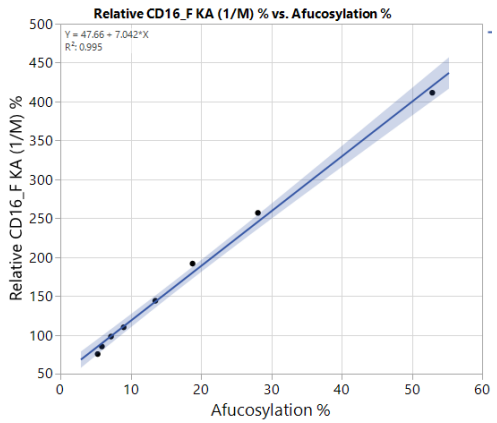
BMS2

Analyze — Understand correlation between Afucosylation level and Activity

Define Risk — Define Ranges for low and high risk

Afucosylation Impact to CD16 interaction





Afucosylation (Δ RM)	Predicted %KA relative to RM			
	CD16a-V	CD16a-F	CD32a	CD32b/c
7 (0)	100	100	Not Correlated	Not Correlated
8 (+1)	110	105		
9 (+2)	115	110		
10 (+3)	120	120		
11 (+4)	125	125		
12 (+5)	130	130		
13 (+6)	140	140		
14 (+7)	145	145		
15 (+8)	150	150		

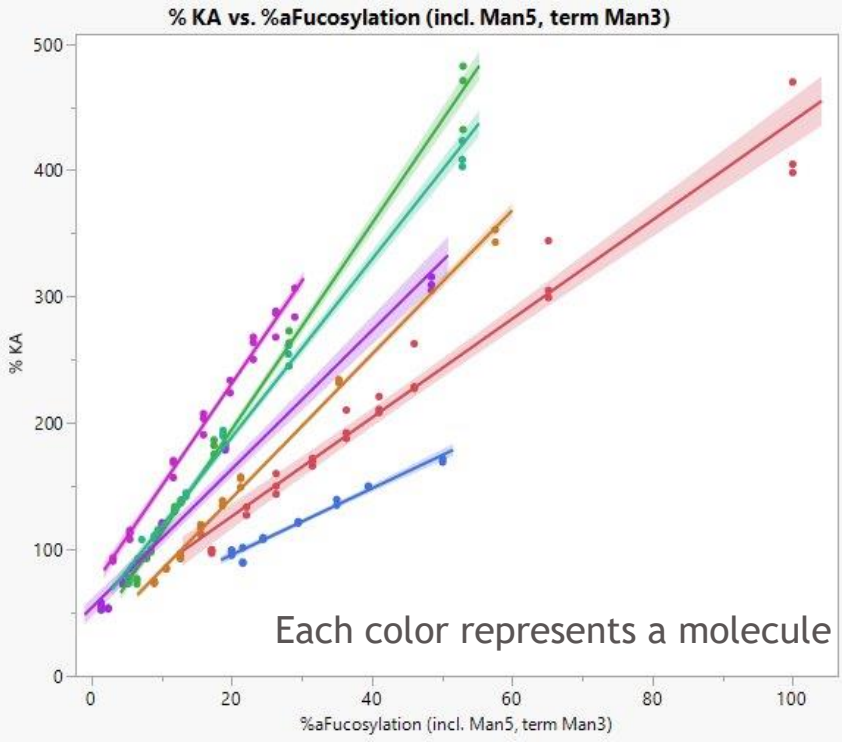
Comparability Risk Scale

CD16a is most sensitive to changes in afucosylation and represents the worst-case risk scenario.

CD32 receptors are most often implicated in phagocytosis.

Workflow has been used to understand > 10 molecules Degree of Afucosylation Impact is Molecule Dependent

Molecule	CD16-V Binding		CD16-V Cell Based Assay		CD16-F Binding		CD16-F Cell Based Assay	
	R^2	Slope	R^2	Slope	R^2	Slope	R^2	Slope
BMS1	0.978	3.0	0.993	2.4	0.986	3.9	0.996	2.8
BMS2	0.998	2.6	0.995	4.6	0.998	2.6	ND	ND
BMS3	0.995	6.2	ND	ND	0.995	7.0	ND	ND
BMS4	0.999	5.2	0.998	8.3	0.994	5.4	0.979	6.6
BMS5	0.995	5.0	0.931	4.0	0.998	5.7	0.951	4.2
BMS6	0.960	12.2	ND	ND	ND	ND	ND	ND

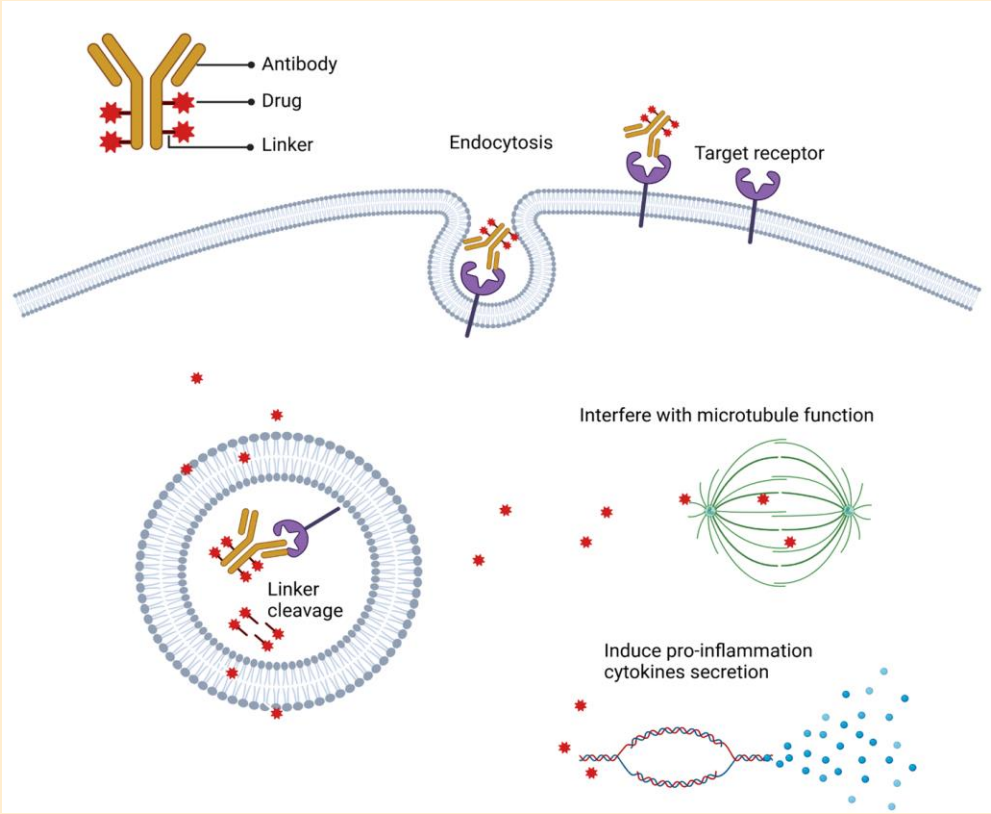


- Correlation of affinity due to afucosylation
  - Is observed for IgG1 molecules regardless of intended MoA
  - CD16-F binding is more sensitive than CD16-V
  - Slope of correlation varies depending upon molecule (2.6 – 12.2 % affinity/afucosylation)

BMS10

Antibody Drug Conjugate (ADC) –

Interaction of Afucosylation and Payload on ADCC activity



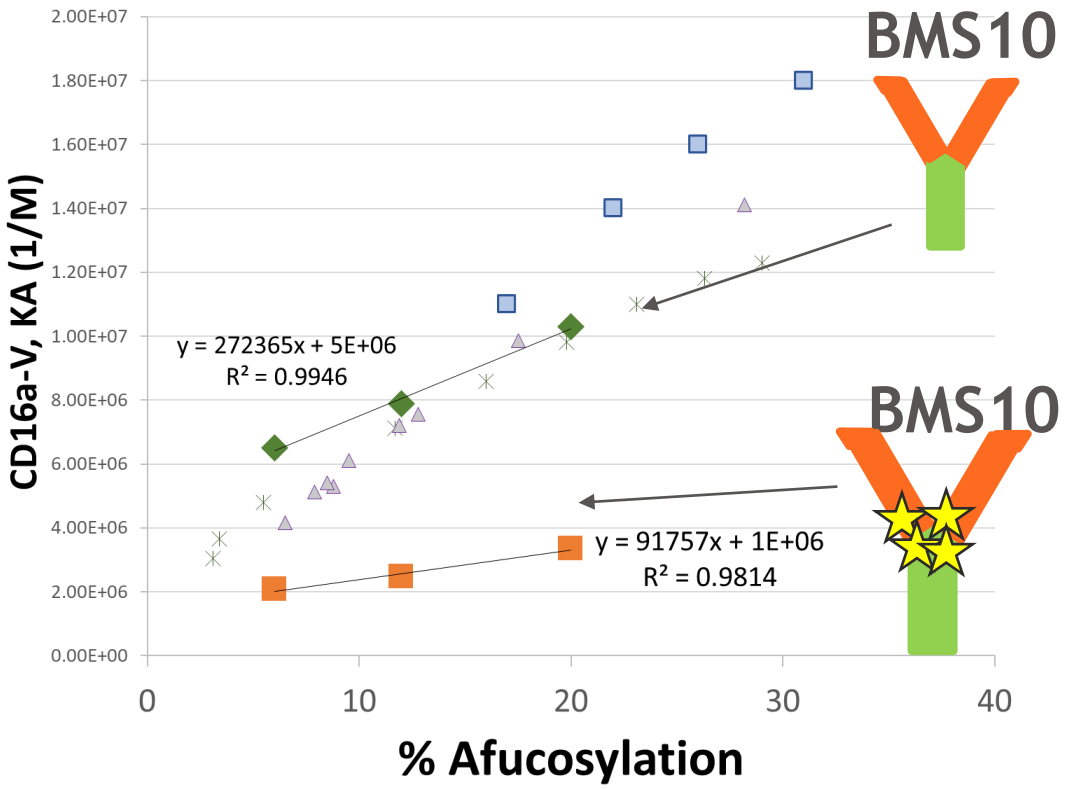
Considerations for ADC molecules with IgG1 backbone

BMS-10 has a wild type IgG1 backbone

- BMS-10 (no payload) demonstrates CD16-V binding activity sensitive to afucosylation level
- BMS-10 (with payload ★) demonstrates CD16-V binding activity sensitive to afucosylation level BUT with reduced overall affinity
- ADCC Cell Based Activity demonstrated minimal activity of BMS-10 (with payload)

Conclusions:

- Afucosylation of IgG1 mAb used for ADCs should be evaluated during development
- Consideration of the primary MoA should also be considered part of the risk assessment.



Summary

- ❑ Afucosylation may be a CQA based on it known correlation with CD16a receptor interactions
- ❑ A workflow for assessing impact of afucosylation was developed and implemented across different modalities
- ❑ SPR analysis provides a quantitative readout to precisely define magnitude of change in CD16 interaction relative to change in afucosylation level
- ❑ Cell Based Assays (Direct Cell lysis, NK cell reporter) provide semi-quantative data to define impact of afuoscylation change which is orthogonal SPR analysis.
- ❑ MetaAnalysis of >10 molecules indicates that Afucosylation levels influence ADCC activity differentially depending upon the molecule.
- ❑ Assessing risk must consider not only the molecular subtype but also the holistic understanding of biological activity profile and propensity for ADCC activity.

Thank you!


BMS Potency and Impurities Analytical Development Group
• Tara Stauffer
• Victoria Swiss
• Colleen Santoro
• Patrick Kuehne
• Bryan Mottley