Current experiences with ADA isotype characterization

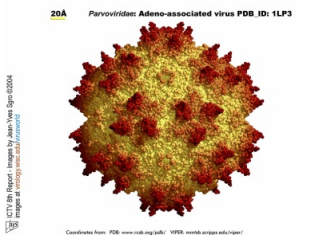
Boris Gorovits

Pfizer, Andover MA USA

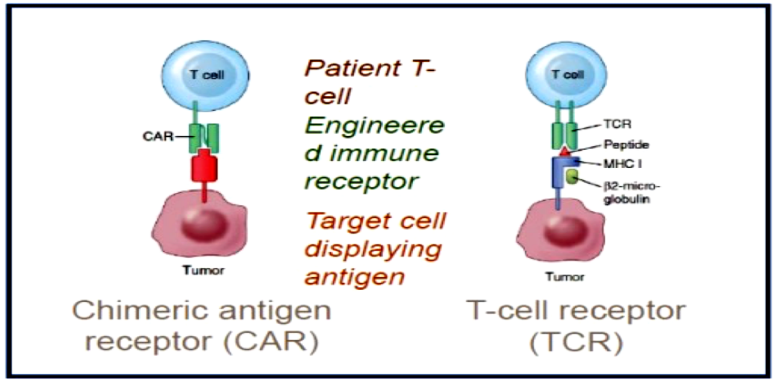
2020

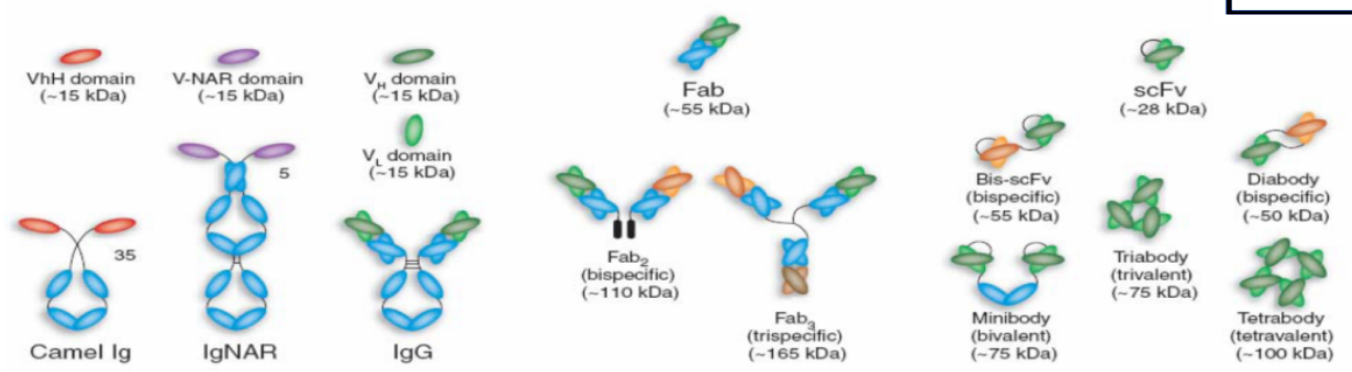
Growing diversity of Biotherapeutic modalities. From mAbs to bi-Abs to GTx and CAR-Ts

Gene therapy



Cellular therapy

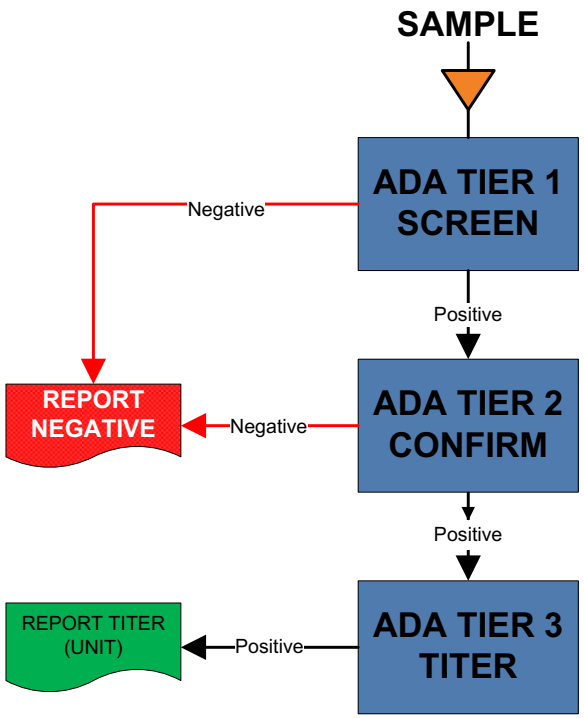




Holliger & Hudson, Nat. Biotech 23(9)

ADA Testing Uses a Tiered Strategy

Tiered testing strategy



Assay formats

Tier 1: Screen
- Screening at MRD

Tier 2: Confirmation of specificity
- At MRD with excess drug

Tier 3: Titer
- At multiple dilutions to ID which dilution crosses at the cut point

Additional options to characterize ADA response:

- **(main) ADA isotype**
- ADA domain specificity
- Affinity of ADA-drug interaction

Currently there is no regulatory or safety drivers outside of IgE identification in some cases

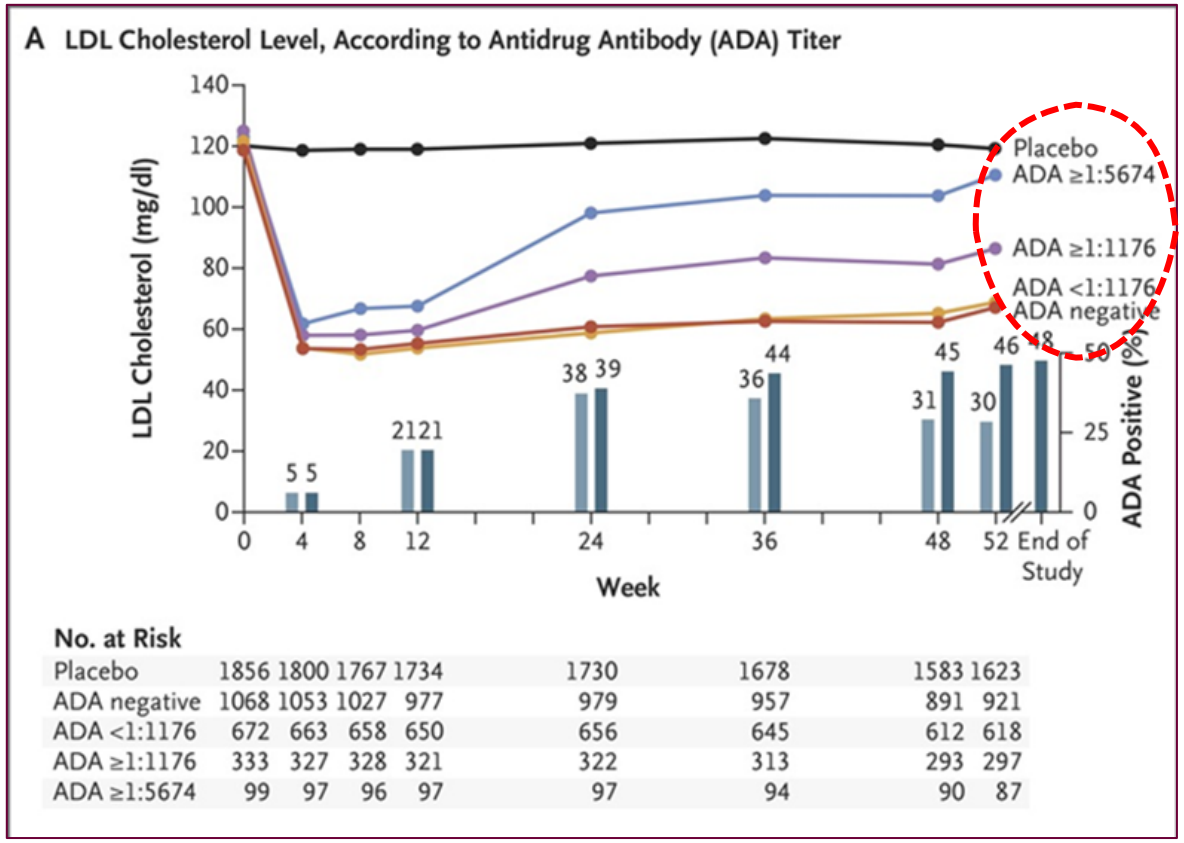
T1, T2, and T3 are typically all variations of the same underlying methodology

Dan Baltrukonis

Characterization of the ADA Response to Understand Clinical Relevance

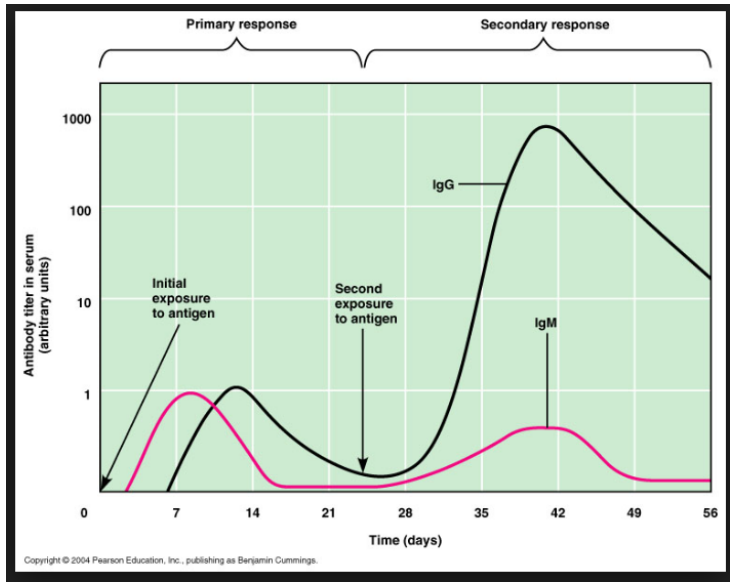
Bococizumab: Titer dependent impact on efficacy

Ridker, 2017



Isotype Response diversity

- Stimulation of adaptive immunity results in high affinity, highly specific antibodies with potentially long-lasting lymphocyte ‘memory’ of response
- **Response is often polyclonal**
- Isotypes detected is based on stage and maturity of immune response
- IgM: primary immune response, recent exposure, not mature
- IgG: secondary immune response, repeat exposure, mature
- IgE: hypersensitivity Type I
- IgA: mucosal immunity



	IgG1	IgG2	IgG3	IgG4	IgA1	IgA2	IgM‡	IgE	IgD
Molecular weight†	150,000	150,000	150,000	150,000	150,000 – 600,000	150,000 – 600,000	900,000	190,000	150,000
Heavy-chain component	γ1	γ2	γ3	γ4	α1	α2	μ	ε	δ
Normal serum level (mg/ml)	9	3	1	0.5	3.0	0.5	1.5	0.0003	0.03
In vivo serum half-life (days)	23	23	8	23	6	6	5	2.5	3
Activates classical complement pathway	+	+/–	++	–	–	–	++	–	–
Crosses placenta	+	+/–	+	+	–	–	–	–	–
Present on membrane of mature B cells	–	–	–	–	–	–	+	–	+
Binds to Fc receptors of phagocytes	++	+/–	++	+	–	–	?	–	–
Mucosal transport	–	–	–	–	++	++	+	–	–
Induces mast cell degranulation	–	–	–	–	–	–	–	+	–

Properties and biological activities of classes and subclasses of human serum immunoglobulins.
Kuby, Immunology, Freeman 2007

Prevalence and Isotypic Complexity of the Anti-Chinese Hamster Ovary Host Cell Protein Antibodies in Normal Human Serum.
Xue et al AAPS J v.12, 2010

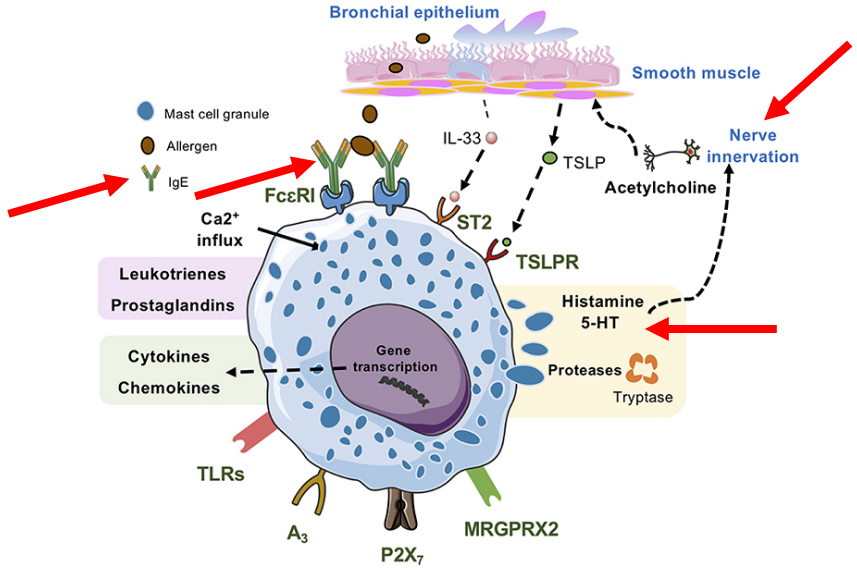
Anti-CHO-HCP-positive samples	Anti-CHO-HCP isotypic reactivity						
	IgG1	IgG2	IgG3	IgG4	IgG	IgM	IgE
HS-39	+++	–	–	–	+++	–	–
HS-44	–	–	–	–	+	+++	–
HS-53	+	++	–	–	+++	–	–
HS-13	+++	–	–	–	+++	–	–
HS-35	–	+	–	–	+++	–	–
HS-40	+++	+++	+	–	+++	–	–
HS-43	+	+++	–	+[b]	+++	+	–
HS-8	–	–	+++	+++[b]	+++	+	–
HS-14	–	–	–	–	++	–	–
HS-B3	+++	+	–	–	+++	+	–
HS-B2	++	+++	+	–	+++	–	–
HS-B8	+	+	+	–	+++	++	–
HS-B17	++	–	+	–	+++	+	–
HS-27	–	+	–	–	+++	–	–
HS-3	+++	+	–	–	+++	+++[a]	–

Let's keep IgE detection out of scope

- IgE-mediated Hypersensitivity Reactions may occur
- Mast cells can be activated by many stimuli, including IgE/antigen-mediated activation of FcεRI triggered exocytosis of granular compounds leading to a release of leukotrienes and prostaglandins, synthesis and release of cytokines and chemokines
- Skin testing can be performed, even if not routinely
- Skin tests are not standardized
- Regulators expect pg/mL sensitivity for IgE detecting assays

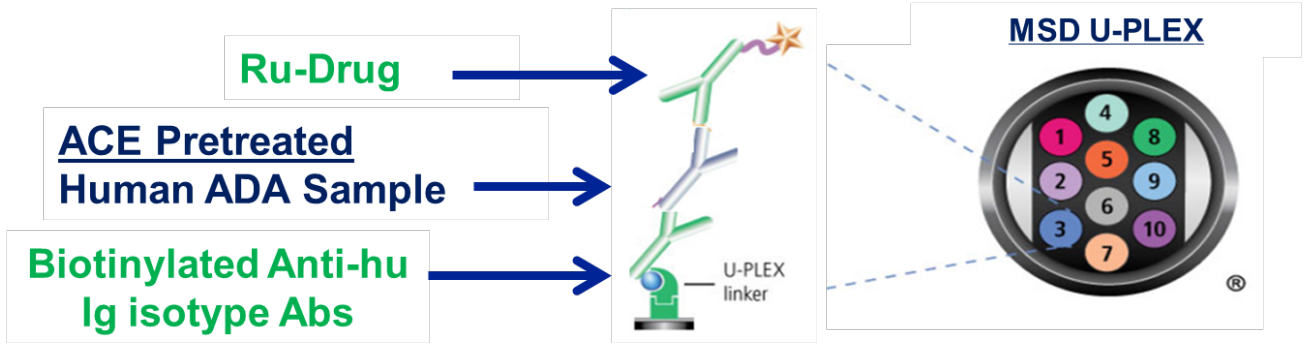
Méndez-Enríquez Front Immunol. 2019
Vultaggio, J INTERF & CYT RES V34, N 12, 2014
Leach et al. Tox Pathol. 2014;42(1):293

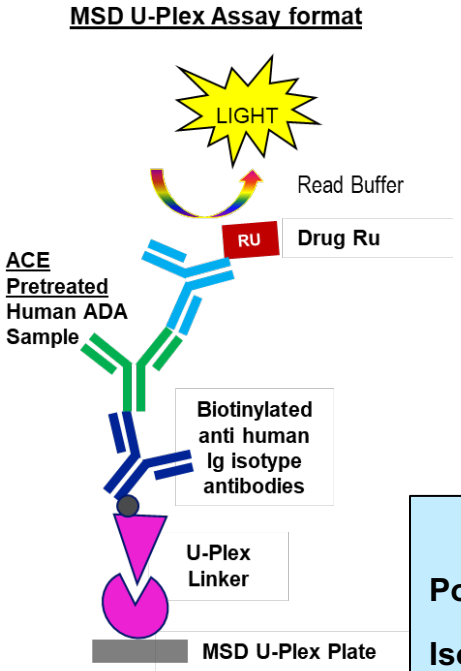
	Hypersensitivity reaction type		
	Type 1	Type 2	Type 3
Immune reactant	IgE	IgG1, IgG3, IgM	IgG
Antigen	Soluble Ag	Cell or matrix associated Ag	Soluble Ag
Effector cells	Mast cells	Complement, FCR+ cells	Complement, FcR+ cells

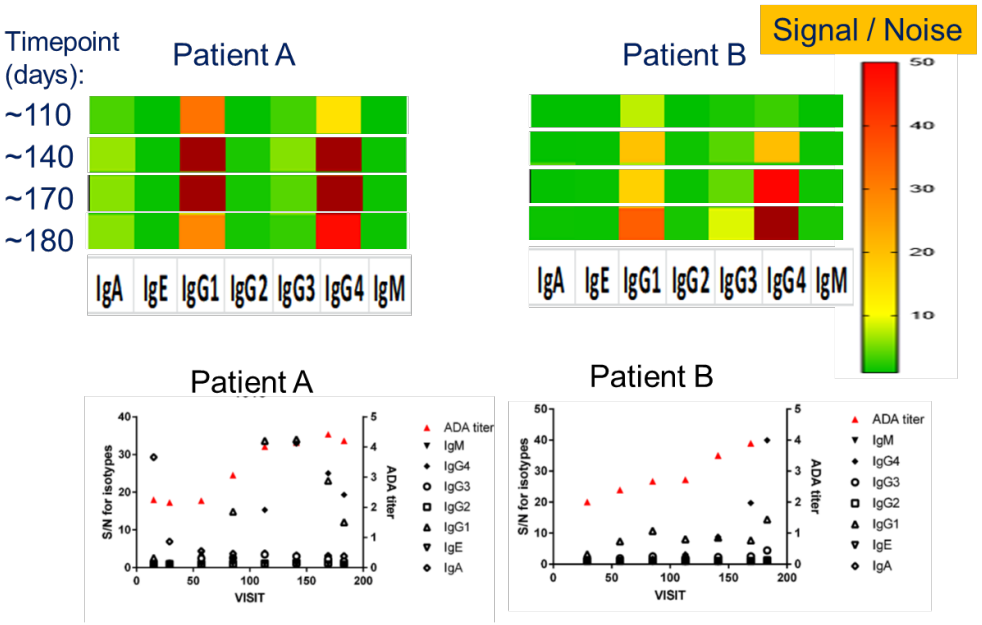


Methods: MSD U-PLEX
Isotyping of Anti-Drug X ADA response in Human Serum

Lisa Dyleski







Key Assay Characteristics

Positive Control: Rabbit anti-Drug Ab

Isotypes detected:
IgM, IgG 1,2, 3 and 4, IgA, IgE

Cut point Determination:
Standard stat data analysis

Separate Anti Rb Cut Point to monitor assay performance

Sensitivity: <20 ng/mL

Drug Tolerance:
Up to 500 µg/mL drug @ 100 ng/mL PC

Methods: IC-LCMS
Isotyping of Anti-Drug Y ADA response in Human Serum

Jason Walsh
Nick Psychogios
Hendrik Neubert

1. Acid dissociation of ADA-drug complexes
2. Pull-down by biotin-drug on strep-beads
3. Elution / neutralization, add stable isotope labeled IS (unique to each Ig)
4. Tryptic digestion of proteins and IS SIL peptide mix
5. Peptides by nanoflow LC-MS, multiplexing via MRM

- Human isotype panel for:
  IgG1 / 2 / 3 / 4, IgA1, IgA2, IgE, IgM, IgD
- Isotype specific peptides, absent in Drug Y
- Need to define background signal for each peptide
- Need to define cut-point for each peptide

Acid dissociation (pH: 2.5)
Neutralization (biotin-drug, pH: 9.5)

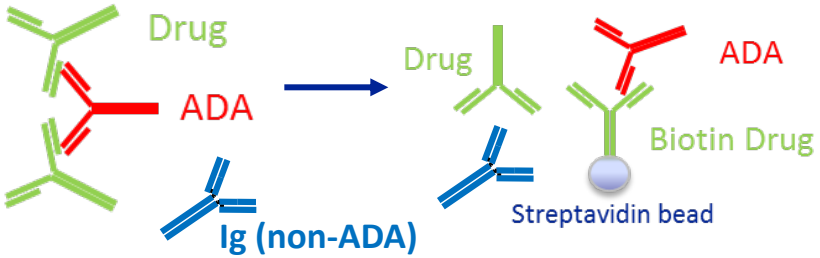
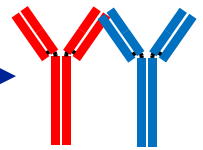


Plate washing
Elution
Neutralization
Spike IgG SIL mix

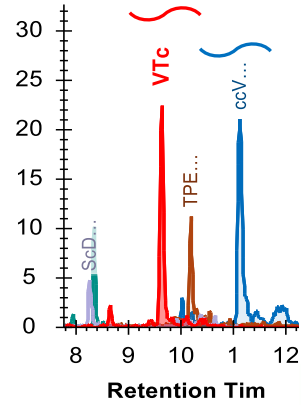
SIL: Stable Isotope Labeled



SIL Mix

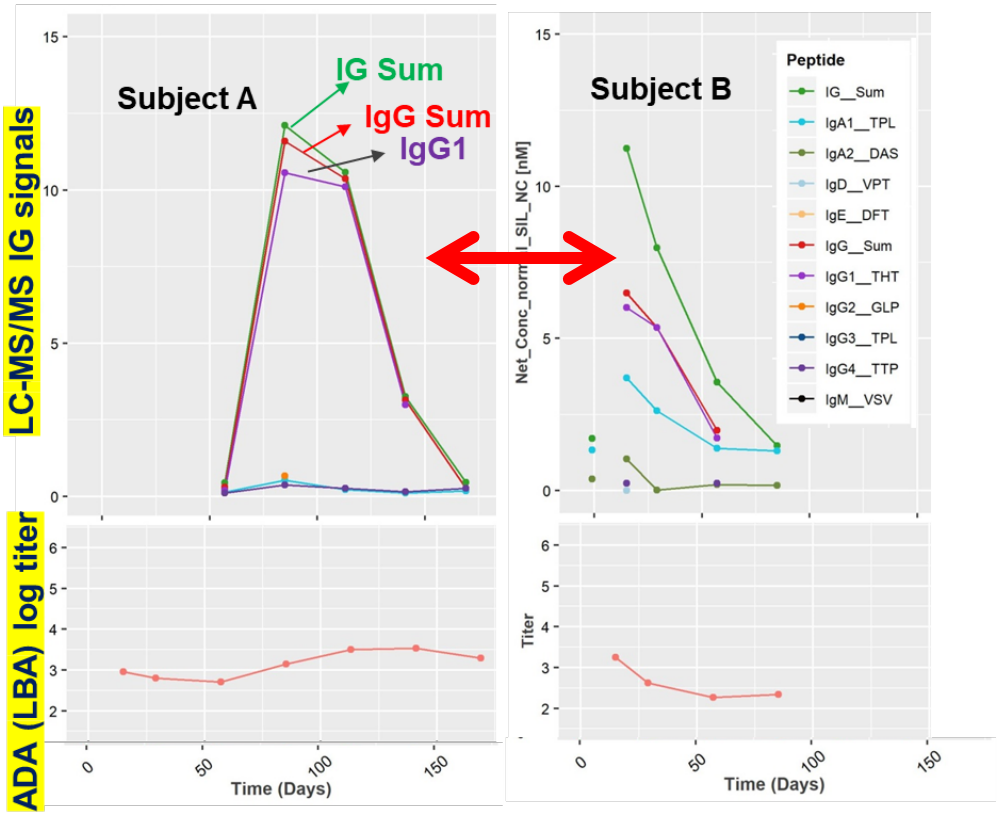
Reduction, alkylation Digestion of IGs, ADAs

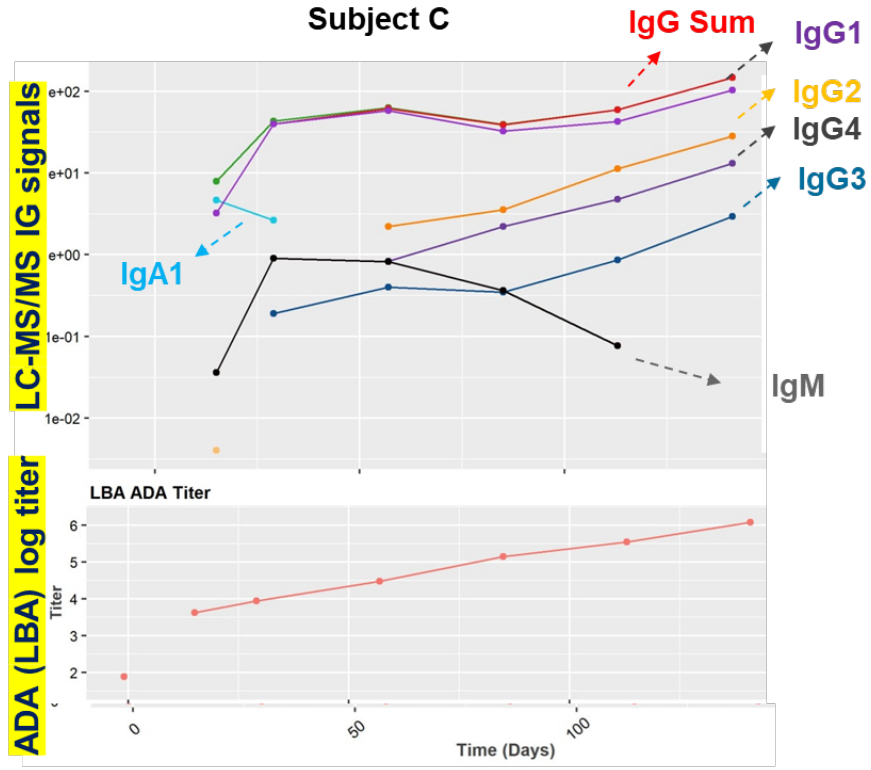
ADA peptides
Residual Ig (non-ADA) peptides
SIL, peptides



Subject Specific anti-Drug Y ADA Isotype Composition

Jason Walsh
Nick Psychogios
Hendrik Neubert

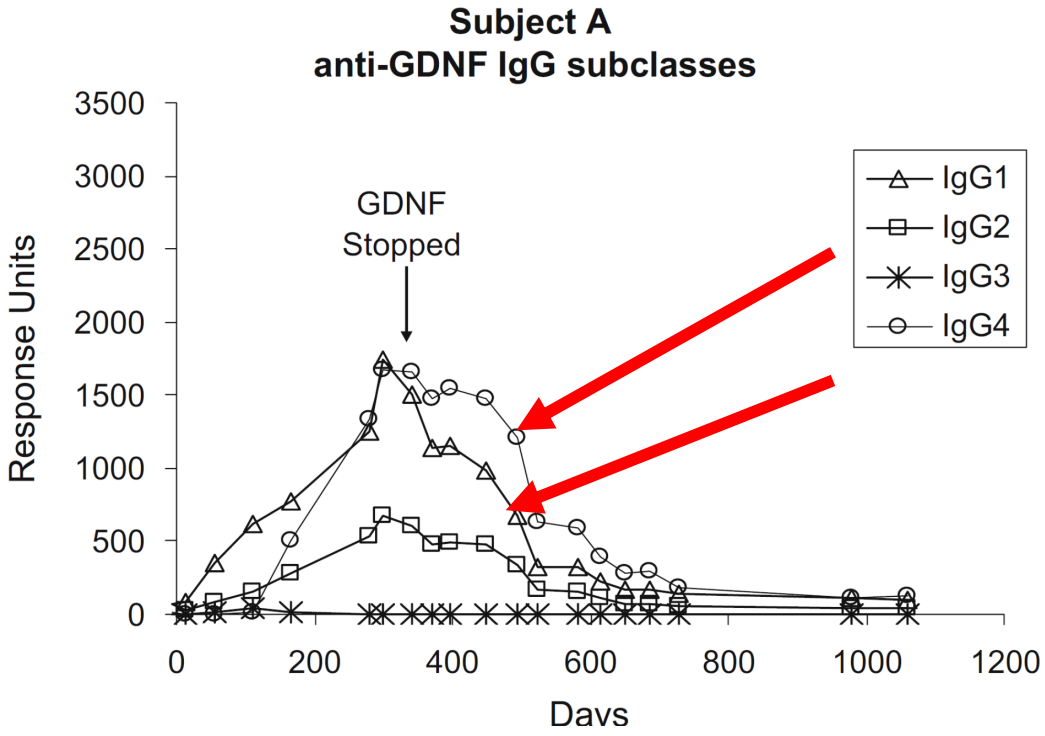




Example 1: huGDNF (Human Glial-Derived Neurotrophic Factor) ADA isotype characterization

Tatarewicz et al. J clin immune 2007;27(6):620
Hovland et al. Tox Pathol. 2007;35(7):1013

- Development of anti-r-metHuGDNF IgG subclasses for subject A
- Administered via continuous bilateral intraputaminal infusion
- Early timepoints: IgG1 >> IgG2 and, no detectable levels of IgG3 or IgG4
- At 340 days: concentrations of IgG4 increased beyond IgG1 and IgG2 levels
- Antibody concentrations decreased when treatment was stopped at 340 days
- Relationship of IgG subclass concentrations remained at levels of IgG4 > IgG1 > IgG2



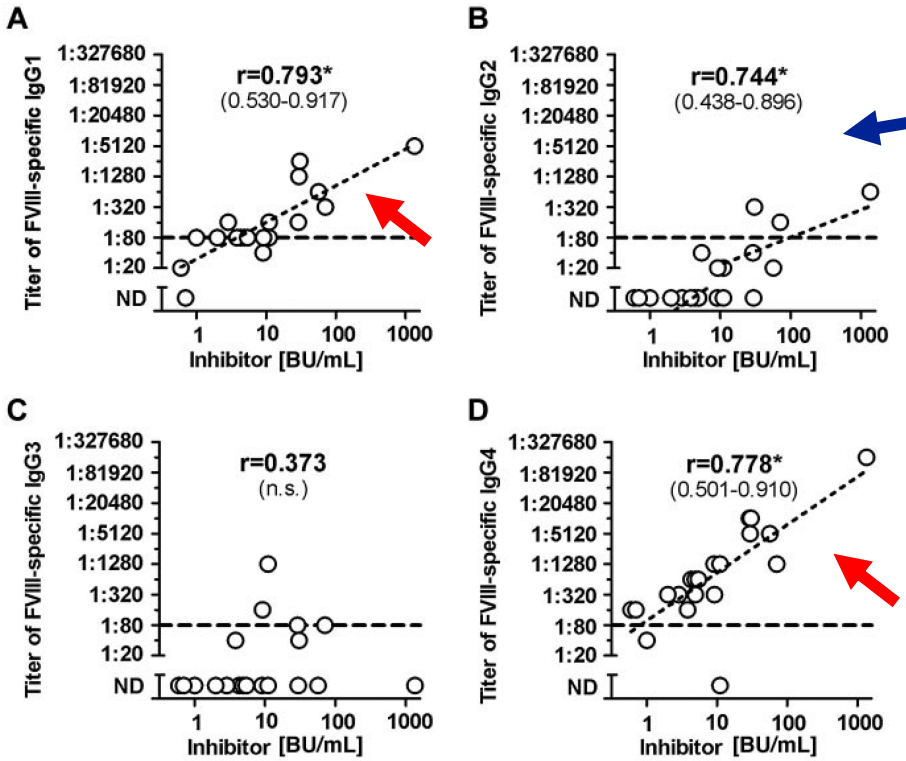
huGDNF case study represents an example of a T-cell mediated immunity that resulted in formation of a mixed IgG4 and IgG1 dominant subclass response

Example 2: Antibody responses to Factor VIII in healthy individuals, acquired (AHA) & congenital hemophilia A patients

Whelan et al. Thrombosis & Hemostatis 2013
Tiere et al. Blood 2016

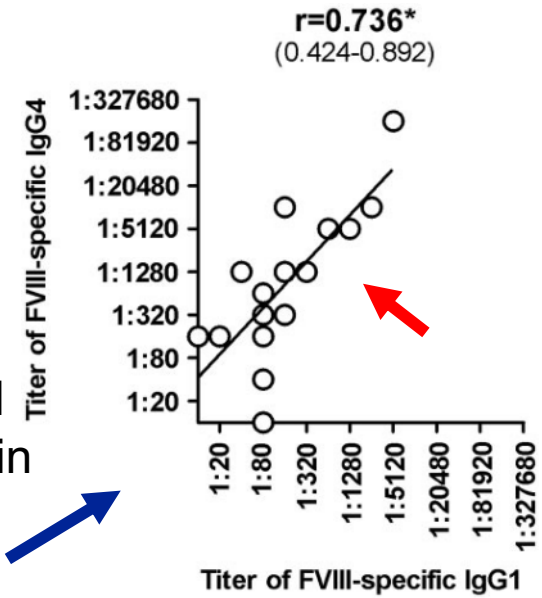
Table 3. Estimated prevalence of Ig isotypes and IgG subclasses of FVIII-binding antibodies found in healthy individuals and in different cohorts of hemophilia A patients

Study cohort	Sample size	IgG1, %*	IgG2, %*	IgG3, %*	IgG4, %*	IgA, %*	IgM, %*
Healthy	600	6	1	6	0	6	1
Hemophilia A without inhibitor (HA-noINH)	77	19	1	13	0	4	3
Hemophilia A after successful ITI (HA-ITI)	23	30	9	4	0	0	0
Hemophilia A with inhibitor (HA-INH)	20	95	35	25	95	10	5
Acquired hemophilia A (Acqu-HA)	9	100	67	22	100	11	11



Ab titer correlation with inhibitor scores in hemophilia A patients

Correlation between a-FVIII IgG4 and IgG1 in patients with inhibitors



Distinctly different a-FVIII Ab isotype composition was identified in healthy individuals, acquired Hem A and congenital Hem A patients with or w/o FVIII inhibitors

a-FVIII IgG1 & IgG4 Abs strongly associated with development of AHA or development of FVIII inhibitors in congenital hem A

Example 3: IgG4 Production Against Adalimumab During Long Term Treatment of RA Patients

Schouwenburg et al. J Clin Immunol 2012; 32:1000
Aalberse et al. Clin Exp Allergy. 2009;39 (4):469
Vultaggio et al. Allergy 2010

- Induction of ADA in RA patients treated with Adalimumab assessed for total ADA and IgG4
- 32% of patients developed ADAs
- IgG4s detected in 29% of the patients (90% of ADA+ patients)
- Development of IgG4s was associated with Neutralization of drug activity and reduction of clinical response

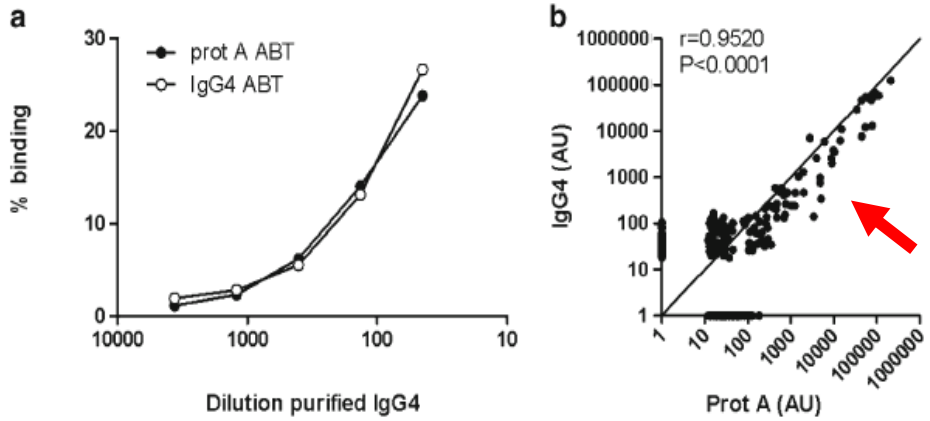
IgG4s have

- limited ability to trigger immunological effector functions,
- form small immune complexes,
- have low affinity to Fc receptors –

Hence - IgG4s are presumed to be “harmless” or have a protective effect against complement induced damage

But - IgG4 presence indicates mature ADA response with ability to compete for the target – drug binding, i.e. neutralizing drug activity and clinical non-response.

Correlation between total ADA (prot A) and IgG4 ADA against adalimumab



Total ADA & IgG4 ADA vs. time of treatment in patients with undetectable adalimumab levels.

Conclusions

- Assessment of ADA isotype composition was conducted for high immun. risk biotherapeutics
- FDA 2019: assays that discriminate between ADA isotypes may be considered
- IgE detection: for therapeutics with a high risk of anaphylaxis or where anaphylaxis has been observed
- Information about ADA isotype composition **may be helpful or critical** when determining treatment strategy or trying to explain observed rate of HAEs
- ADA isotype composition may help to predict disease progression
- Methods are available to identify ADA isotypes, individually or in multiplexed approach
- More information will be needed before a broader ADA isotype assessment can be recommended
- Can Do ≠ Must Do

For more information: Gorovits, AAPS J, Nov. 2020

Thank you

Q&A

Biography and Contact Information

1. Boris Gorovits is a Senior Director in the BioMedicine Design group, Pfizer
2. Boris leads a bioanalytical team focused on development of PK and Immunogenicity monitoring methods for a wide range of biotherapeutics
3. Most recently, his team has been closely engaged in support of GTx and other novel modality biologics
4. Boris can be contacted at boris.gorovits@pfizer.com