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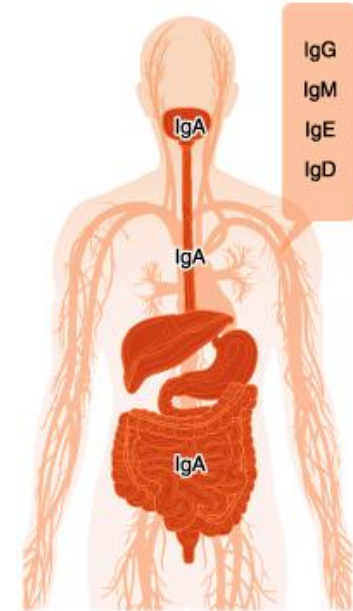
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*In-depth characterization and
quasi-quantification of ADAs
using hybrid LC-MS methods to
support clinical development
of biotherapeutics*

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EIP symposium
Lisbon, 26 Feb 2024

Human immunoglobulins isotypes

Isotype	2D Représentation	MW (kDa)	Function	Proportions in human (serum)
IgM		970	Produced first upon antigen invasion	10 %
IgG		IgG1: 150 IgG2: 150 IgG3: 170 IgG4: 150	Highest opsonization and neutralization activities	75 % IgG1 : 65 % IgG2 : 20 % IgG3 : 10 % IgG4 : 5 %
IgA		320	<ul style="list-style-type: none"> Expressed in mucosal tissues Form dimers after secretion 	15 % IgA1 / IgA2
IgE		190	Involved in allergy	0.002 %
IgD			Function not completely understood	0.2 %



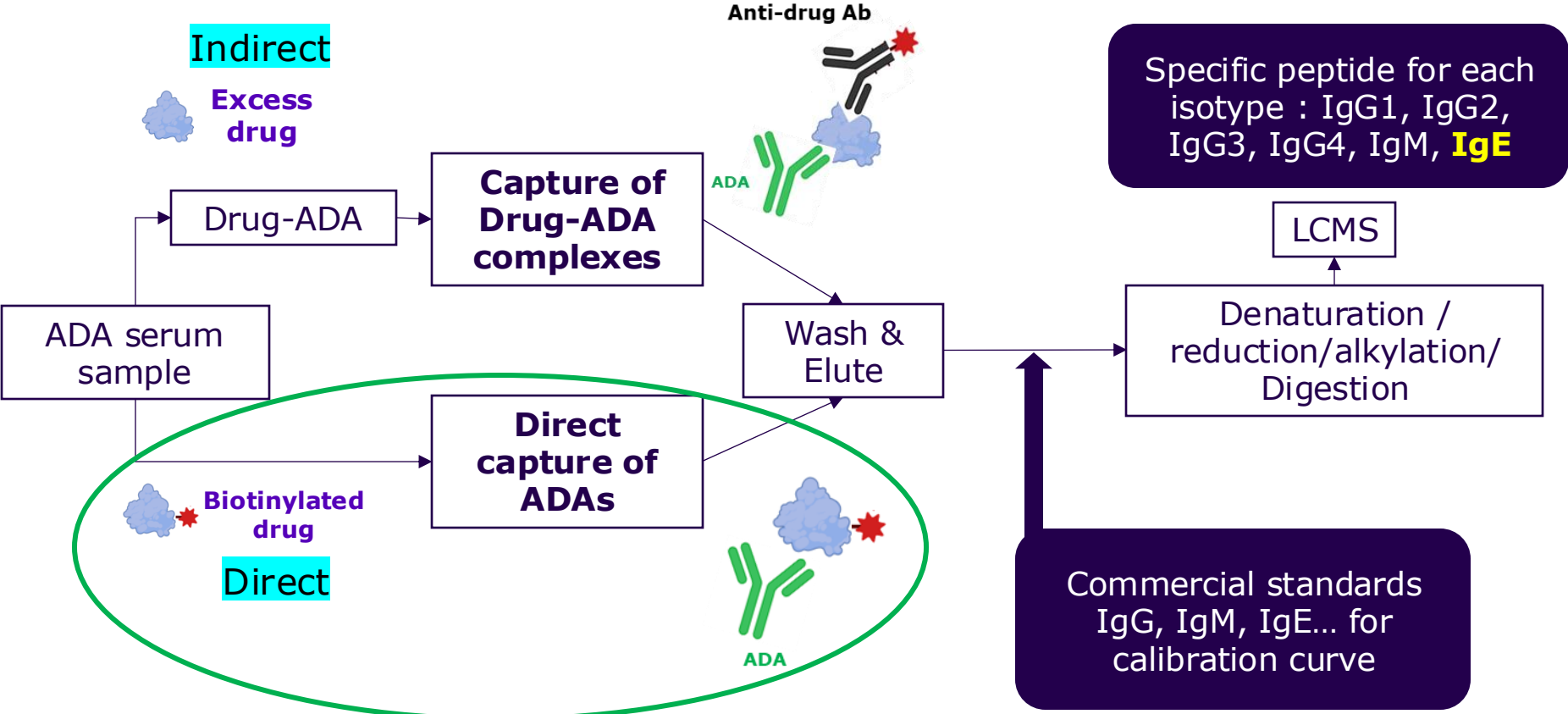
ADA isotyping requirements

- **FDA and EMA require the isotype characterization of ADA response for therapeutics with a high risk of (or high proven) immunogenicity:**
 - **FDA / Janvier 2019: Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection**

"In some circumstances the sponsor should develop assays that discriminate between antibody isotypes. For example, for therapeutic protein products where there is a high risk for anaphylaxis or where anaphylaxis has been observed, results from antigen-specific IgE assays may be informative."
 - **EMA / December 2017: Guideline on Immunogenicity assessment of therapeutic proteins**

"In certain circumstances, it may be feasible to further characterize the ADA response, e.g., in case of anaphylactoid reactions and follow up of the maturity of emerging immune response. In these cases, determination of the isotype and IgG-subclasses or even T cell reactivity may be useful."
- **LBA assays (Gold standard) can detect antibodies of most isotypes but don't provide information on which isotypes are being detected**

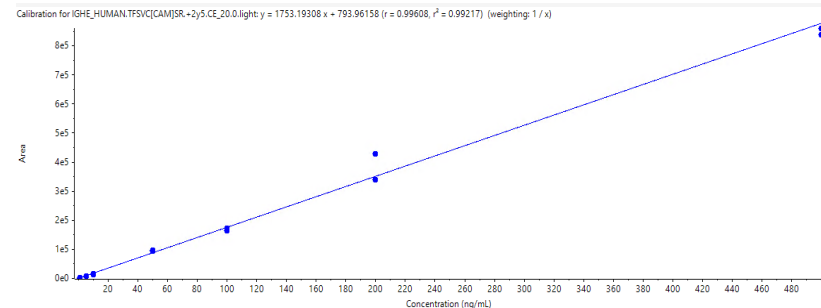
Hybrid IC-LC-MS approaches for ADA quantification in serum



Quasi-quantitative method

- **Calibration curves: spiked isotype control standards in immunocapture eluent of pooled selected blank human serum (5 donors)**
- **Dynamic range and lower limit of quantification in pooled blank human serum**

Isotype	Calibration range (ng/mL)
IgG1	100 – 50 000
IgG2	100 – 50 000
IgG3	100 – 50 000
IgG4	10.0 – 500
IgE	5.00 – 500
IgM	500 – 50 000



IgE calibration curve

**LLOQ required : 100 ng/mL total ADA
low ng/mL IgE**

Clinical study

Case study



Compound administered at 0, 5, 20, 60 mg/kg by subcutaneous (SC) single ascending dose



24 healthy volunteers
Sampling at Pre-dose, Day 15, 29 and 60



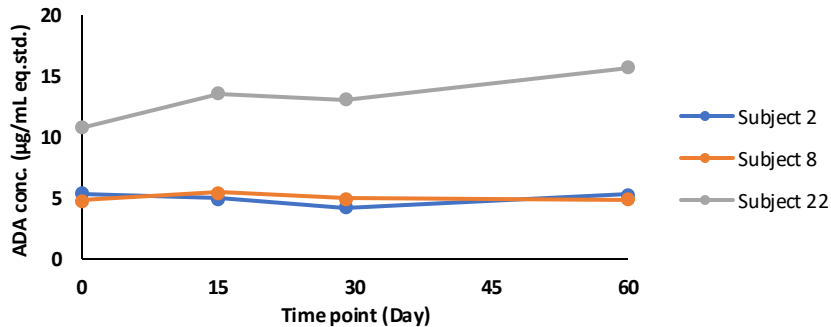
Samples analysed by LBA and Hybrid LC-MS/MS

ADA isotyping: IgM detection

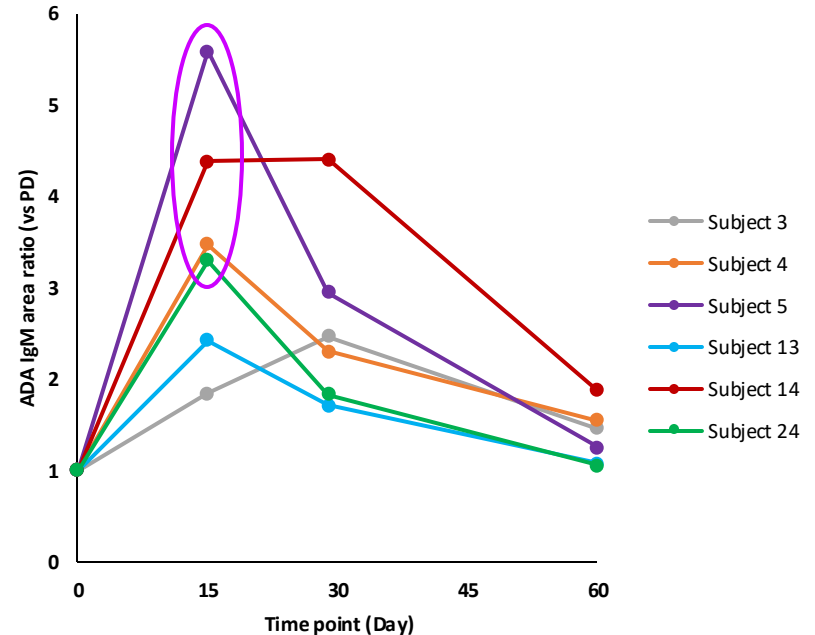
- Generally early onset of IgM after 1-2 weeks (primary immune response)
- Onset of IgM at day 15 for 5 subjects
- High level of Pre-Existing ADA (PEA) in placebo subjects:



IgM ADA levels (IC-LC-MS) in placebo serum samples



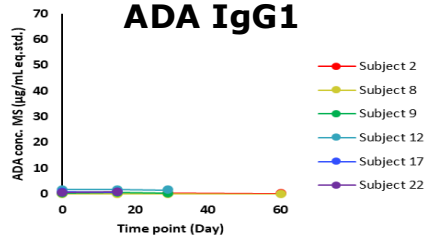
ADA IgM –Ratio D15-D29-D60 vs Pre-Dose



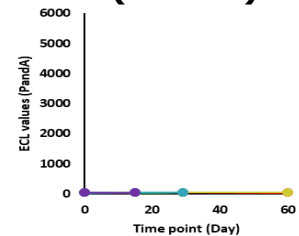
Observations

- **IgG1 major ADA isotype**
- **Correlation between hybrid and LBA assay:**
 - Good correlation between IgG1 ADA levels by hybrid IC-LC-MS and total ADAs by LBA assay
- **Few discrepancies (subject 11) probably due to Pre-existing ADA measurement by hybrid assay**

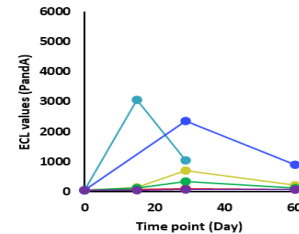
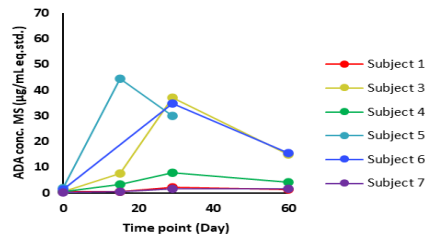
IC-LC-MS/MS ADA IgG1



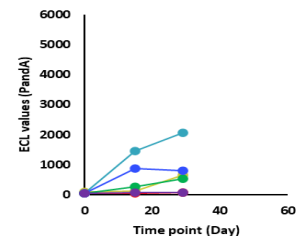
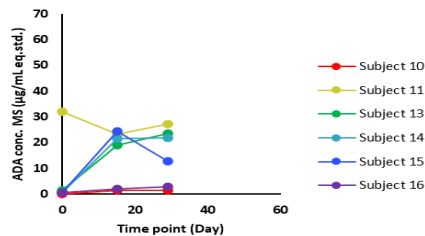
LBA (Panda)



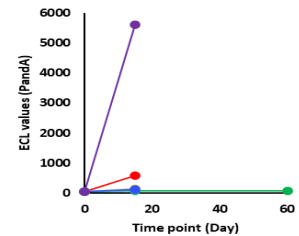
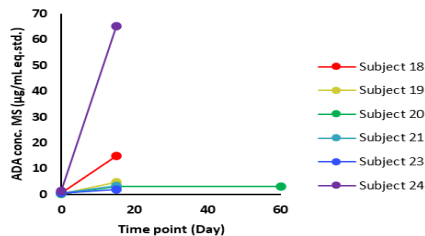
0 mg/kg



5 mg/kg



20 mg/kg



60 mg/kg

ADA : Impact on PK

Clinical samples

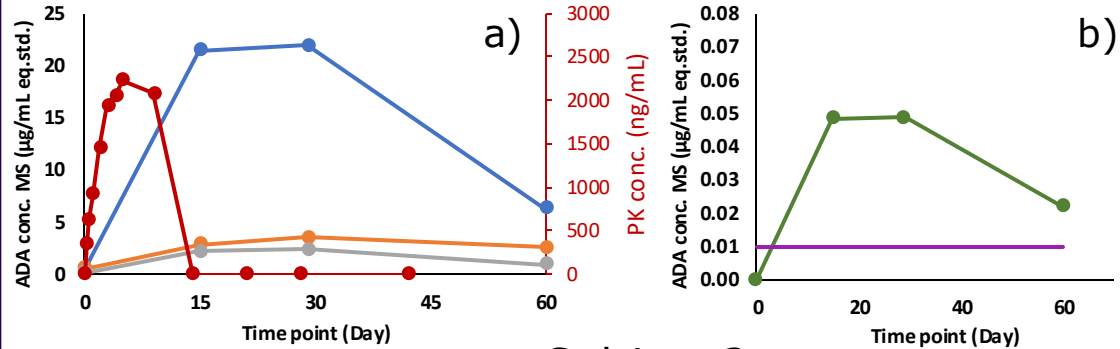
Focus on 2 subjects (treated with the same dose) presenting different ADA isotypes proportions:

- a) IgG1 major isotype, b) zoom IgG4
- c) IgG3 major isotype, d) zoom IgG4

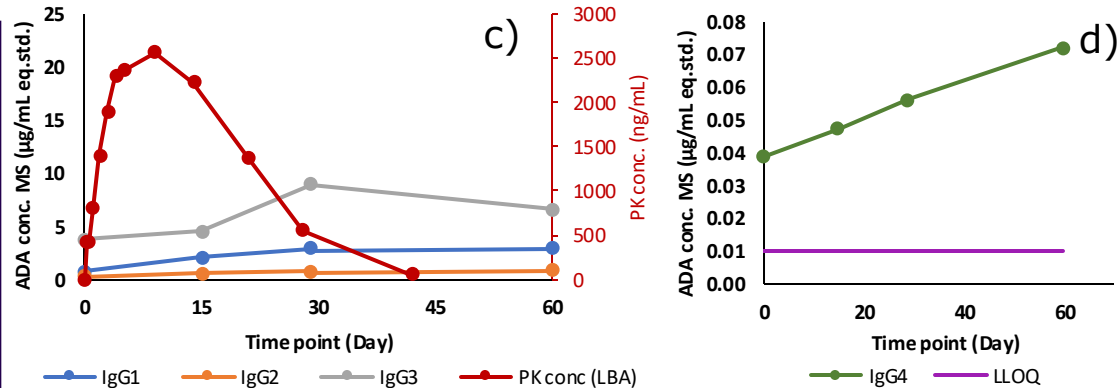
Observations

- Possible higher clearing capacity for IgG1 than other isotypes
- Low levels of IgG4 known to have neutralizing capabilities

Subject 1

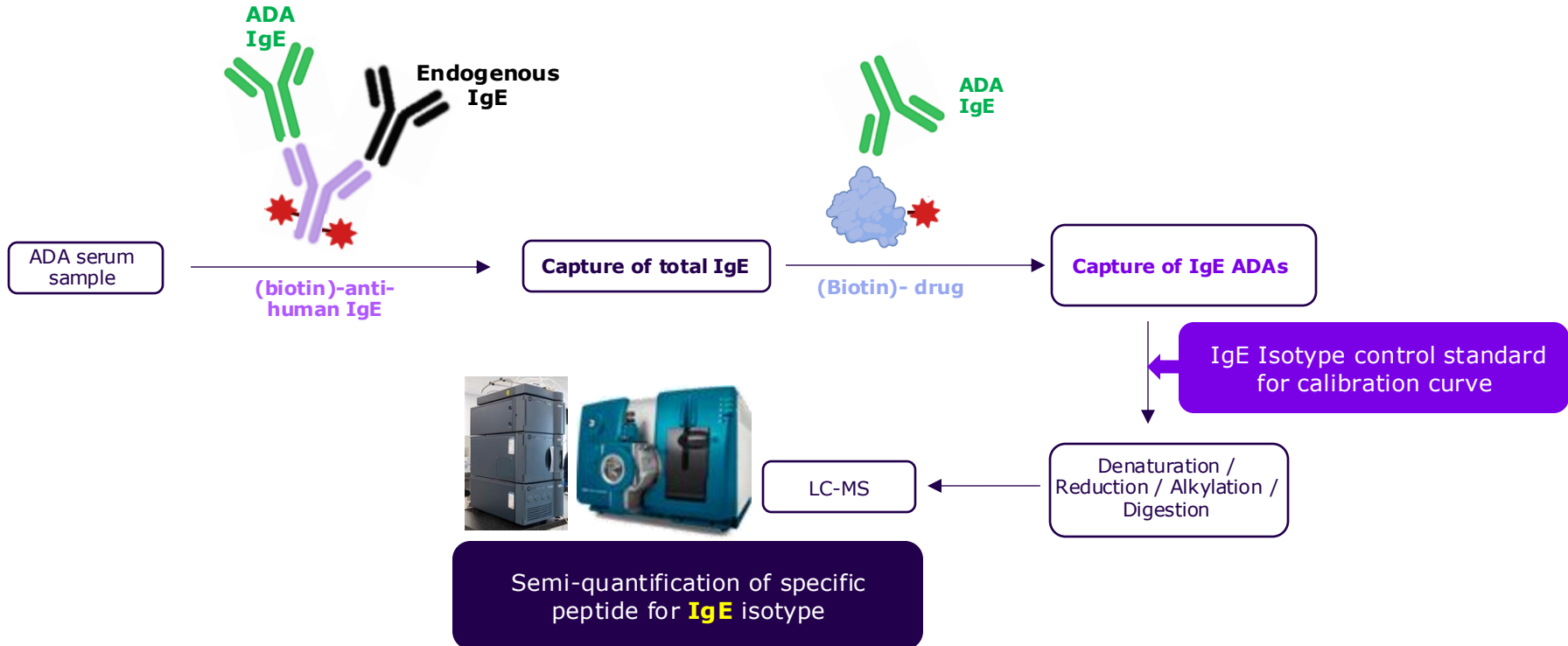


Subject 2



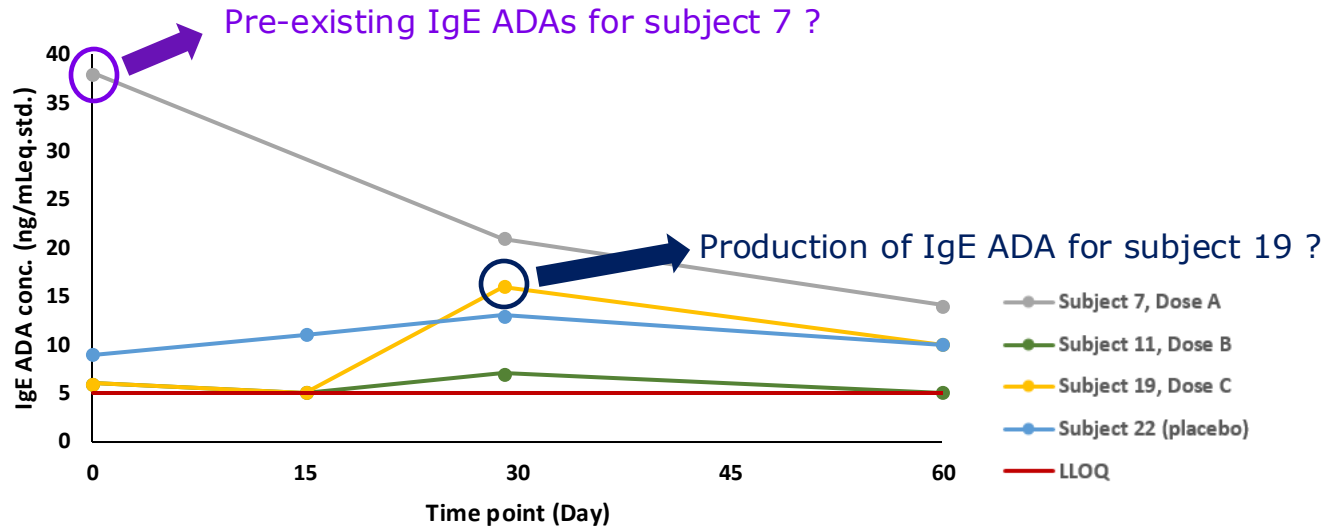
Hybrid LC-MS strategy for IgE ADA quantification in human serum

Sequential immunocapture



Capture with sequential immunocapture: IgE results

- **3 out of 18 treated subjects with detected IgE ADA:**



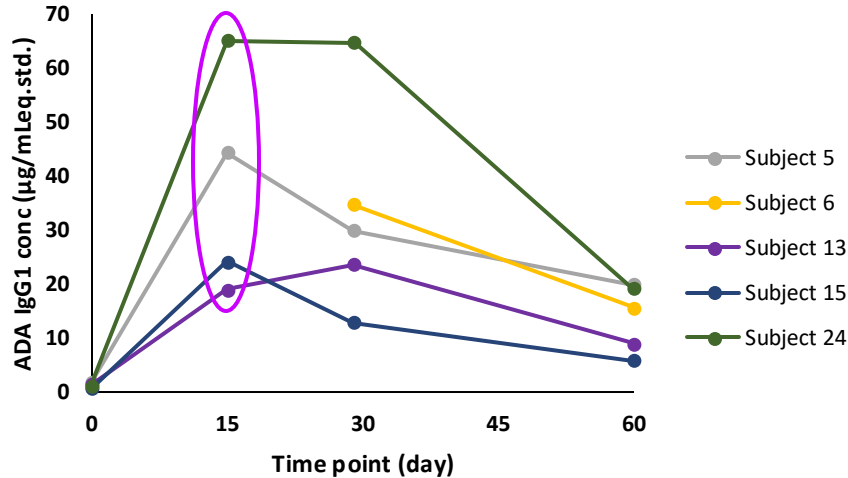
Hypersensitivity reactions observed for subjects 5, 6, 13, 15 and 24 between D13 and D15
No reaction observed in subjects 7 & 19...

IgE mediated hypersensitivity response requires prior sensitization

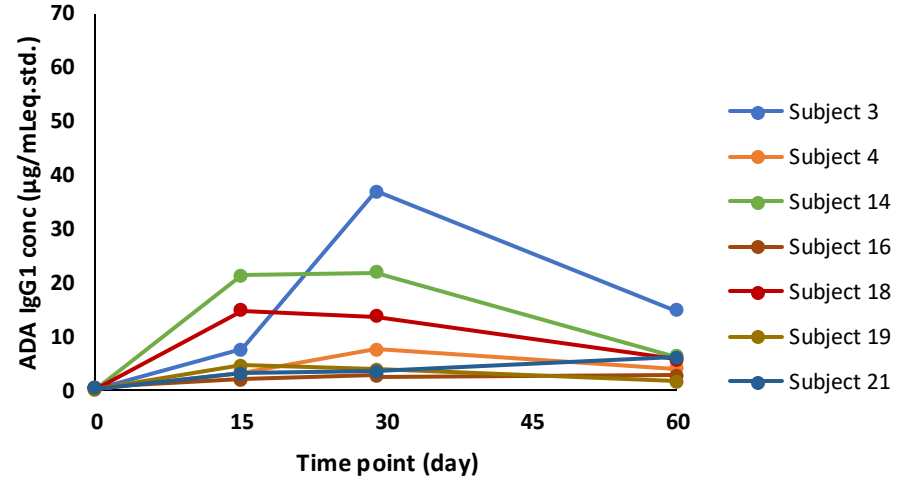
Come back to IgG response...

IgG1

Subjects with hypersensitivity reactions



Other subjects



**Early onset of IgG1 at day 15:
IgG 1 mediated hypersensitivity reaction, "pseudo-allergy" ?**

Conclusion

Multiplexed hybrid IC-LC-MS/MS Methods

ADA IgG subclasses
ADA IgM



New hybrid IgE method

ADA IgE

Need more samples



ADA isotyping by hybrid IC-LC-MS/MS

Complement to
LBA assay

Understand the
impact of ADAs on
the PK

Better characterize
hypersensitivity
reactions

Generic method
(except
immunocapture
step)

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Thank you
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