Validation of an immunoassay to selectively quantify the naked antibody of a new Sanofi Antibody Drug Conjugate: an additional tool for improvement of PK interpretation

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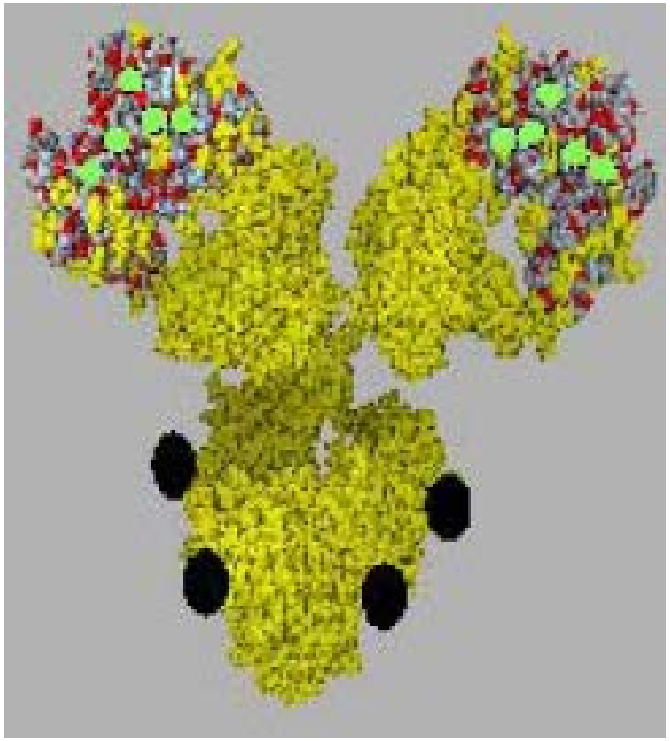
Outline

- Antibody Drug Conjugate : SAR566658
- Naked Ab assay: context, interest and challenge
- Presentation of the naked Ab assay
- Key results obtained during the development and the validation of the naked Ab assay
- Application of the naked Ab assay in the first in human study of SAR566658
- Conclusion

ADC SAR566658

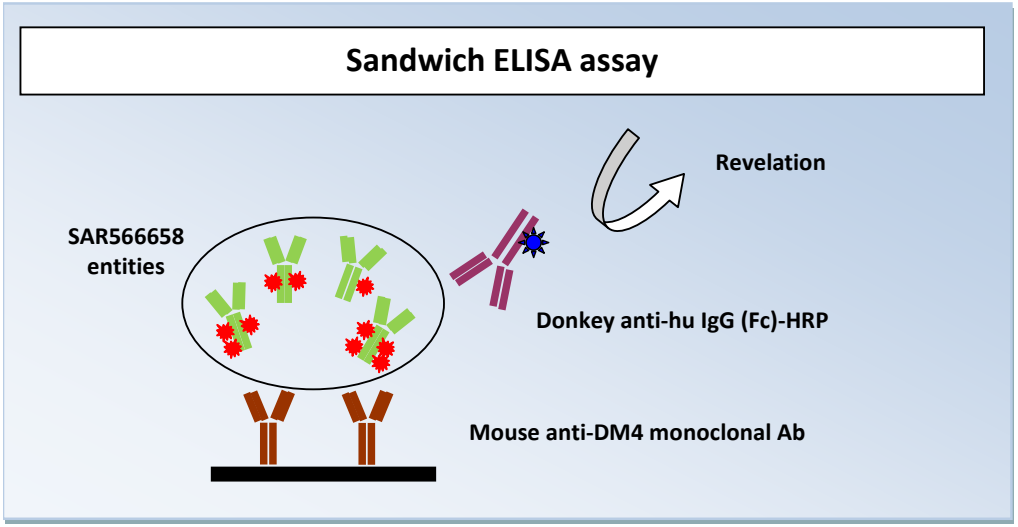


- HuDS6 is a humanized IgG1 monoclonal antibody targeting the antigen CA6, part of the extracellular domain of MUC1 and that belongs to the mucin protein family. Its aberrant overexpression (~60% of tumors) contributes to oncogenesis.
- HuDs6 has no functional activity.
- HuDS6 is covalently conjugated with a potent cytotoxic maytansinoid derivative, DM4, that inhibits tubulin polymerization and microtubule assembly
- This conjugation is done through an optimized linker SPDB (N-succinimidyl-4-(2-pyridyldithio) butyrate) classified as cleavable (hindered disulfide bond)

SAR566658 is the 1st immunoconjugate targeting solid tumors in sanofi portfolio

Naked Ab assay: context

- To support the PK clinical package of this project, three types of assays have been developed and validated:
  - **the unconjugated cytotoxic DM4 and its derivatives are quantified by LC/MS/MS**
  - **The first immunoassay measures the conjugated Ab (SAR566658) carrying at least 1 cytotoxic molecule (i.e. any entities with Drug Antibody ratio or DAR equal or greater than 1)**



- Calibration: SAR566658

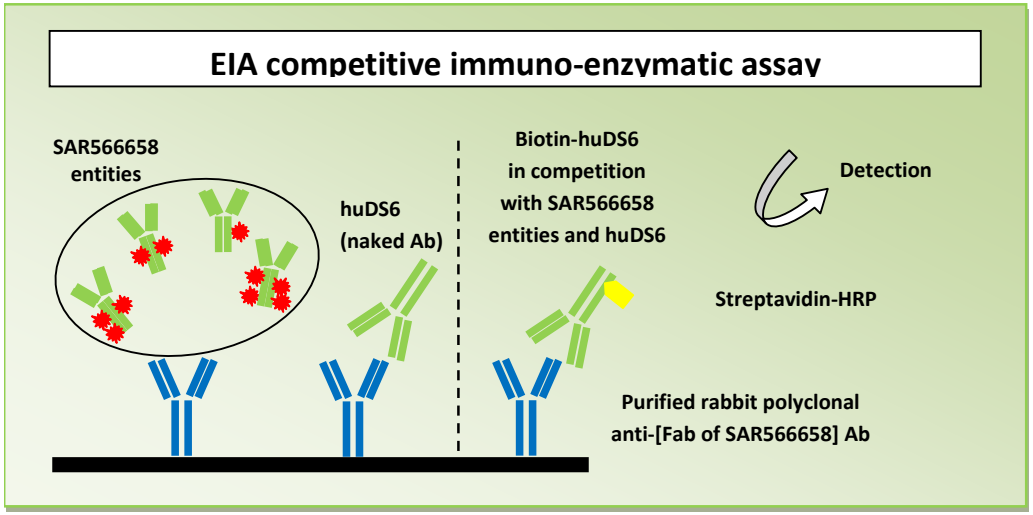
- CA6 target antigen not available as a purifed and characterized protein

- Measures: free SAR566658 and complex [SAR566658:target] with at least one cytotoxic molecule

- Quantitation range: 500 (LLOQ) - 6000 ng/mL

Naked Ab assay: context

- **The second immunoassay measures the total Ab defined as the ADC irrespective of the drug payload, i.e., all entities with or without cytotoxic molecule (DAR ≥ 0)**



- **Calibration**: SAR566658
- **CA6 target antigen not available as a purifed and characterized protein**
- **Measures**: free SAR566658, free huDS6 and complexes [SAR566658:target, huDS6:target] whatever the cytotoxic loading
- **Quantitation Range:** 400 (LLOQ) to 8000 ng/mL

Analytical limitation of the total antibody assay:

- difficulty to adequately calibrate the assay due to the lack of a reference standard representative for the different circulating entities which changed in proportion and characteristics with time following ADC dosing.
- amplified for ADC with a cleavable linker

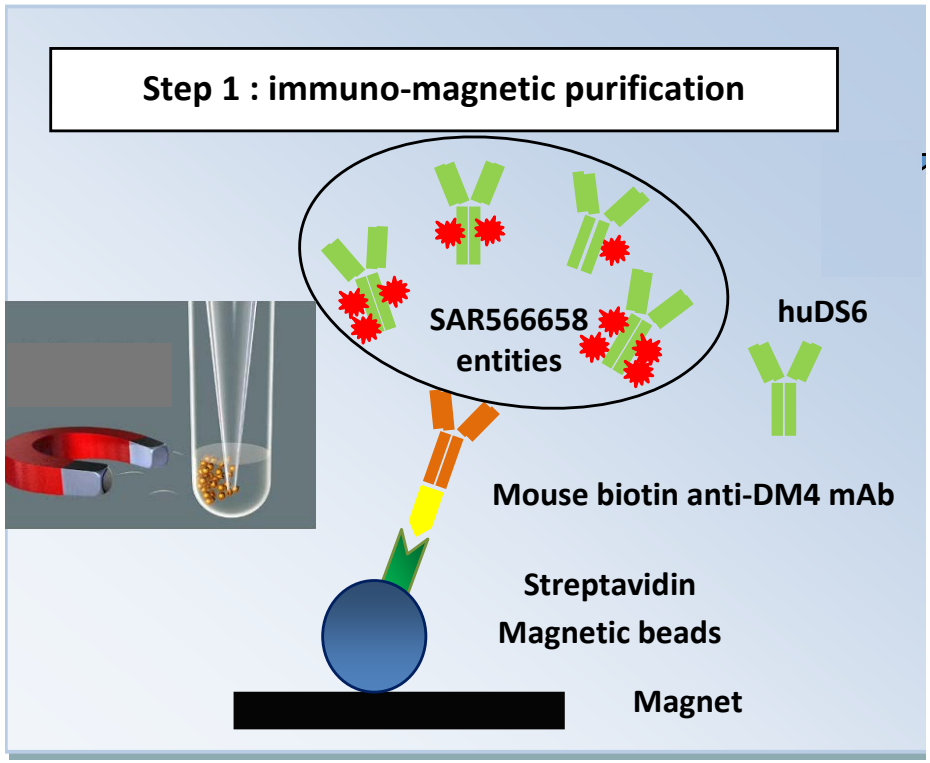
Naked Ab assay: interest and challenge

- **Develop an assay that selectively quantify the naked Ab → new analytical approach to get round the highlighted limitation of the total Ab assay**

- **Interest of the naked Ab assay**:
  - **Analytical conditions used for assaying naked Ab are the most specific biological assay**
    - no dependent of DM4 loading
    - method uses the appropriate analytical Reference Standard for the calibration
  - **Follow the naked Ab along the SAR dosing**
    - is more relevant than the Total Ab from an bioanalytical point of view (remind: SAR566658 is used for the calibration).
    - will allow to better monitor the non active circulating entity that may compete on the target binding sites with the active ADC

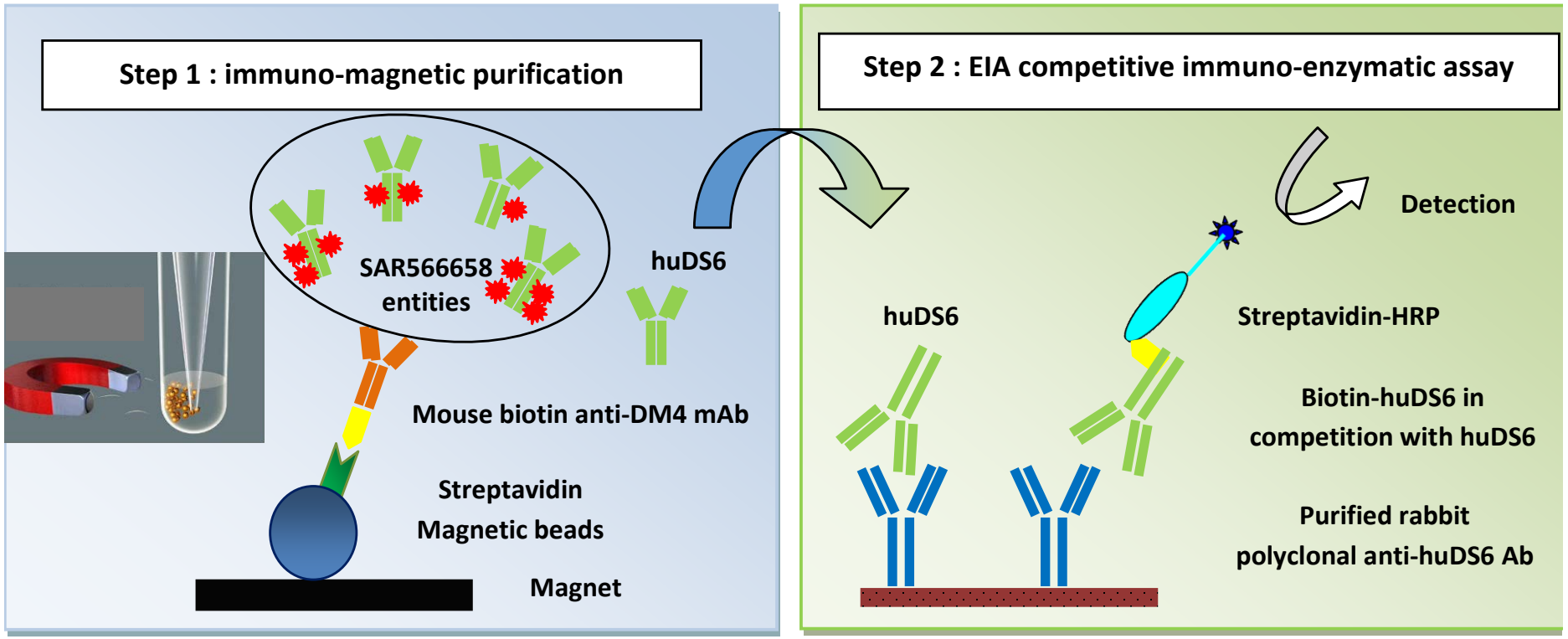
- **Main challenges**
  - **Find a way to measure the naked Ab in the presence of elevated concentrations of SAR566658**
  - **Provide enough sensitivity for pharmacokinetic valuable comparison with other immunoassays**

Naked Ab assay: principle



- Use of biotin mouse anti-DM4 monoclonal Ab coated on streptavidin beads with the objective to capture the maximum DM4 conjugated antibody forms (DAR 1 to n)
- Use of magnet for the separation

Naked Ab assay: principle



Naked Ab assay: method development phase

- **Main objective:** optimization of the immuno-purification step to remove as much as possible SAR entities from plasma sample

Evaluation of SAR interference on the quantitation of the naked Ab at two anticipated LLOQ levels prepared in individual human matrices

Plasma Id	Nominal concentrations of huDS6 (ng/mL)					
	LLOQ tested at 200 ng/mL				LLOQ tested at 400 ng/mL	
	without ADC	with ADC at 3.00 µg/mL	without ADC	with ADC at 2.00 µg/mL	without ADC	with ADC at 2.00 µg/mL
Individual female 1	206	~283	204	~245	393	438
Individual female 2	236	~350	199	~258	423	455
Individual female 3	171	~252	198	~250	428	461
Individual male 1	163	223	230	~263	370	397
Individual male 2	215	~298	210	231	382	392
Individual male 3	215	~435	163	212	354	388
Number outside ±20% nominal	0	5	0	4	0	0
%Diff from nominal (min - max)	-19 to 18%	11 to 117%	-18 to 15%	6.2 to 32%	-12 to 6.9%	-3.0 to 15%

Each determination is the mean result of a duplicate analysis
~: > ±20% theoretical

- **LLOQ of the naked Ab to be validated : 400 ng/mL**
- **Maximal "SAR566658 tolerance" of the naked Ab assay: 2.00 µg/mL**

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Naked Ab assay: experiments performed during the method validation

- **Method validated according to FDA and EMA guidances for drug quantitation**

Quantitation range	Validation samples (VS)
- 400 to 3000 ng/mL of naked Ab prepared in human LiHe plasma	- 6 levels : LLOQ, Low, Mid, High, ULOQ, Super High (10.0 µg/mL) prepared in human LiHe plasma
Core study or Performance characterization	
Between-run assay variability	between-run assay precision and accuracy and assessment of LLOQ
Within assay variability (or large batch size evaluation)	- within-run assay precision and accuracy - size equivalent to a prospective run of study samples (2 microplates)
Effect of matrix variability or selectivity	Impact of individual matrices on quantitation at LLOQ and ULOQ levels in presence of SAR566658
Dilution effect	1 (hook effect), 5, 10 and 20-fold with Super High VS
Satellite study or Assay characterization	
SAR566658 stability in matrix under different conditions	- On bench and at +4°C up to 24 hours and at 37°C for 15 min - After freeze – thaws cycles at -20°C and -80°C - In frozen matrix in anticipated storage tube at -20°C and -80°C (short and long term)
Hemolysis effect (3% blood)	
Specificity: evaluation of in vitro interference from unconjugated DM4 and anti-SAR566658 antibodies	
Interaction with the circulating target antigen using a surrogate antigen	

Experiments highlighted in red were done with VS analyzed with the full process including the immuno-purification step with magnetic beads followed by the competitive immuno-enzymatic assay.

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Naked Ab method validation : between-run assay variability

- **6 different occasions**
- **3 determinations per level per occasion**
- **Full process**

Nominal concentrations of huDS6 (ng/mL) in plasma					
Validation sample levels	LLOQ	Low	Mid	ULOQ	Super High VS
	400	500	1000	3000	10000
Mean	387	478	999	3268	10404
CV%	8.1	9.3	10	13	10
%Diff from nominal	-3.2	-4.3	-0.14	8.9	4.0
Number of determinations	18	18	18	18	17
Number outside ±20% nominal	0	0	0	4	2 + 1 PR

Each determination is the mean result of a duplicate analysis
PR: poor replicate (CV% duplicate > 20%)

- **between-run assay precision : < 13%**
- **between-run accuracy: from -4.3 to +8.9%**
- **Total error (%) : at most 22% (ULOQ level)**
- **No additional variability brought by the immuno-purification step**

Naked Ab method validation : effect of matrix variability or selectivity

- **10 different individual plasma spiked at LLOQ and ULOQ levels and tested with and without the SAR compound**
- **3 determinations per individual plasma with the full process**

Mean (n=3 determinations) of measured concentrations of huDS6 in plasma

Plasma Id	Plasma at LLOQ level (400 ng/mL)		Plasma at ULOQ level (3000 ng/mL)	
	Without ADC	In presence of ADC 2 µg/mL	Without ADC	In presence of ADC 2 µg/mL
Individual female 1	393	438	2949	2910
Individual female 2	423	455	2734	3295
Individual female 3	428	461	3422	3538
Individual female 4	359	333	2980	2741
Individual female 5	394	427	3214	3181
Individual male 1	370	397	2938	3156
Individual male 2	382	392	3259	3134
Individual male 3	354	388	2699	2749
Individual male 4	437	477	2843	2516
Individual male 5	397	*~314*	2831	2646
Total number of determinations	30	30	30	29 (1 PR)
Number outside ±20% nominal	0	3	0	1
CV% (min – max)	2.9 - 8.6	1.1 – 29%	0.56 – 7.4	2.1 - 10
%Diff from nominal (min - max)	-12 to 9.2	-22 to 19	-10 to 14	-16 to 18

Each determination is the mean result of a duplicate analysis; Descriptive statistics are calculated with the whole data set (n=30);
PR: poor replicate (CV% duplicate > 20%)
~: > ±20% theoretical

- **Individual plasma did not interfere in the naked Ab quantitation at LLOQ and ULOQ levels under the assay conditions (with and without presence of SAR566658)**
- **The presence of SAR566658 up to 2.00 µg/mL did not affect the precision and the accuracy of the assay over the full range of quantitation: 9 matrices out of 10 within acceptance criteria**
- **LLOQ definitively validated at 400 ng/mL**

Naked Ab method validation : in vitro interference from the unconjugated DM4

- **VS at LLOQ level spiked with 2.00 µg/mL of SAR566658 and 100 ng/mL of DM4 (10-fold higher than DM4 Cmax observed in clinical studies of other ADCs)**
- **3 determinations per VS**
- **Full process**

Mean (n=3 determinations) of measured concentrations of huDS6 at LLOQ level (400 ng/mL) in plasma

Conditions	In presence of ADC (a)	In presence of ADC (a) and unconjugated DM4 (b)
Mean	375	411
CV%	3.5	4.0
%Diff	-6.3	2.8

Each determination is the mean result of a duplicate analysis
(a) tested at 2.00 µg/mL
(b) tested at the concentration 10-fold higher as observed in clinical studies from other ADCs (100 ng/mL)

- **The unconjugated DM4 up to 100 ng/mL did not interfere in the naked Ab quantitation at the LLOQ level under the assay conditions (in presence of SAR566658 at 2.00 µg/mL)**

Application in the first in human study after SAR566658 dosing

- **Preliminary performance in production**
  - 30 runs performed – only 1 rejected for QC acceptance criteria
  - About 450 samples analyzed → 29 patients
  - QCs performance :

QC level	Low 500 ng/mL	Mid 1000 ng/mL	High 2400 ng/mL
Mean	493	959	2180
CV%	8.44	9.70	9.82
%Diff	-1.40	-4.10	-9.17
n	60	59 + 1 PR	58 + 2 PR

PR: poor replicate (CV% duplicate > 20%)

- **ISR**
  - 41 incurred samples reanalyzed
  - %Diff between repeat and original value > ± 30%: 2 out of 41

Application in the first in human study after SAR566658 dosing

- **Parallelism testing**
  - Incurred samples analysed after 3 serial dilutions (with blank plasma)
  - dilutions performed to have concentration at low, mid and high levels of the standard curve

Sample Id	Concentrations of naked Ab (ng/mL)						Mean	CV%
1	17433	*(1:4)*	19567	*(1:10)*	19200	*(1:20)*	18733	6.1
2	21933	*(1:10)*	22600	*(1:20)*	25850	*(1:50)*	23461	8.9
3	10860	*(1:6)*	11424	*(1:12)*	12440	*(1:20)*	11575	6.9
4	5258	*(1:2.5)*	5110	*(1:5)*	4970	*(1:10)*	5113	2.8

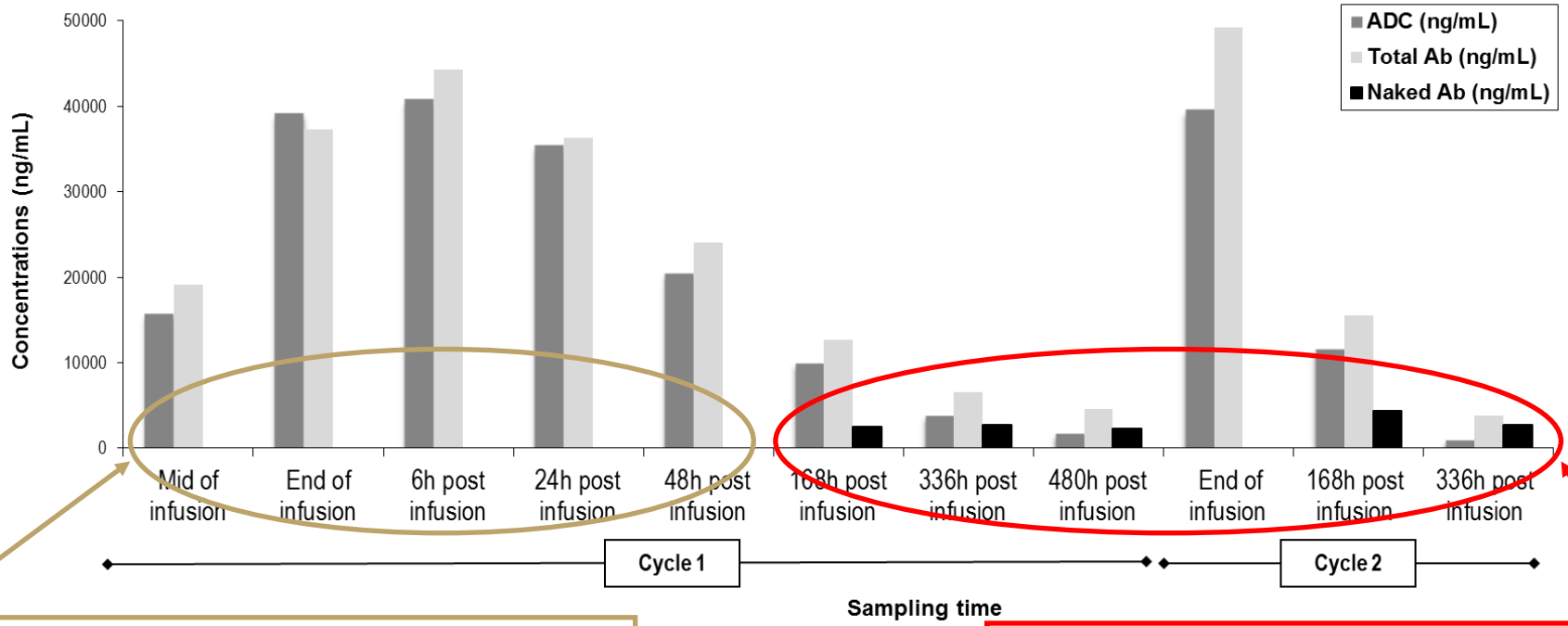
- CV% : at most 9%
- No issue on parallelism anticipated for this assay

- **Check before release of naked Ab data**

  Due to the known interference of SAR566658 at concentrations > 2.00 µg/mL, verify that the sample was appropriately pre-diluted before the immuno-magnetic purification step to bring the SAR566658 concentrations below 2.00 µg/mL→ based on concentration results of SAR566658 first analysed

Application in the first in human study after SAR566658 dosing

- **Preliminary data concentrations of ADC, total Ab and naked Ab in LiHe plasma samples collected in one patient after 2 cycles of SAR566658 dosing**



- No or low concentrations of naked Ab few days after the SAR dosing
- The need to dilute sample to remove SAR566658 above 2.00 µg/mL prevents the ability to detect the naked Ab in this period of time

- Naked Ab was quantifiable from Day 7 after the first dosing of SAR566658
- Naked Ab was quantifiable at the same time as the dissociation of SAR566658 and total Ab concentration curves was observed

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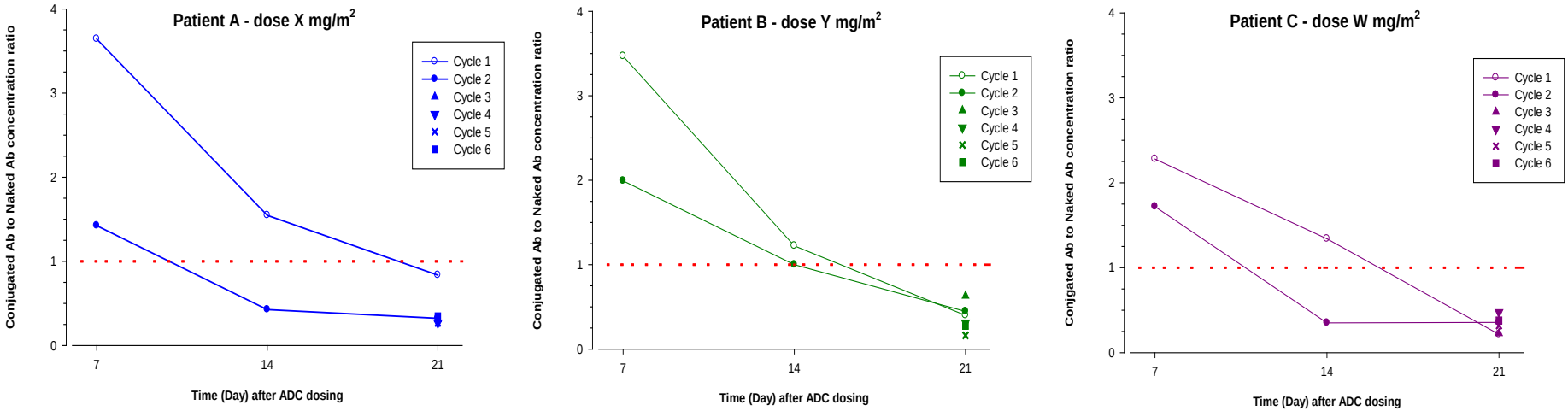
Application in the first in human study after SAR566658 dosing

- **Individual ratio of ADC versus naked Ab concentrations observed at several cycles of dosing and calculated from 3 different patients having received 3 different doses**



- **At Cycle 1: SAR levels remained higher or similar to the naked Ab levels up to Day 14 after the SAR dosing**
- **From Cycle 2 and subsequent cycles: SAR levels remained always below naked Ab levels from Day 14 after the SAR dosing**

Additional tool to support dose and regimen selection (non-active Ab)

Naked Ab assay: Conclusion

- Assay successfully validated for the selective quantitation of the naked Ab of our ADC in human LiHe plasma even in the presence of SAR566658 up to 2.00 µg/mL.
- This assay may be highly valuable for a better assessment of the PK behavior of the dosed ADCs especially for those with cleavable linker and non-active Ab. Indeed, in the context of this project, the measure of the naked Ab allowed to directly monitor the non-active circulating entity that may compete on the target binding sites with the active ADC.
- This assay may also provide an additional tool to document in vivo plasma stability of ADC and potentially to support the dose and regimen selection.
- Today, in the on-going first in human study of the SAR566658, in addition to the ADC analyzed in priority, the naked Ab assay has been prioritized to the total Ab assay.
- The naked Ab assay has also been added to the PK clinical package for further ADCs that will enter in Phase I provided that the naked Ab has no functional activity.
- Complementary information regarding the development and validation of the naked Ab assay are published in the Journal of Immunological Methods 396 (2013), pp. 140-146

http://authors.elsevier.com/sd/article/S0022175913001981

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