A Bioactive PK Assay as Surrogate for Detecting Neutralizing Antibodies

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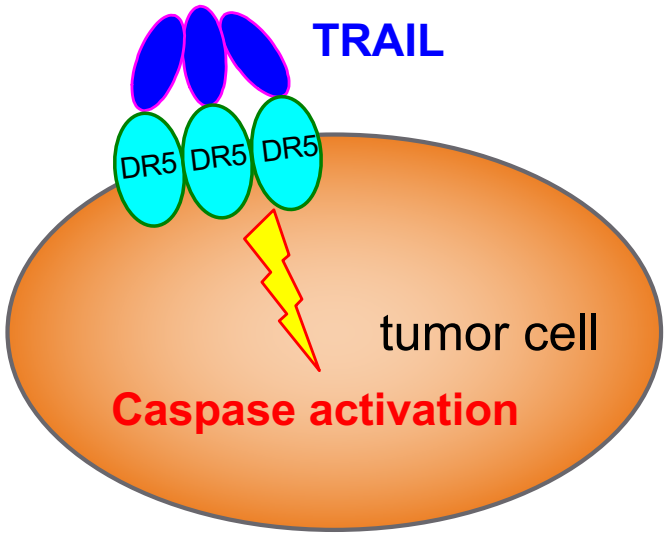
DR5 Mediates Apoptosis in Cancer Cells

- TRAIL is a homo-trimer that clusters DR5 to activate apoptotic signaling
- DR5 is significantly expressed on tumor cells but has limited expression in normal tissue.



Therapeutic hypothesis:
AGONISTS of DR5 will activate death pathways in human cancers resulting in anti-tumor activity.

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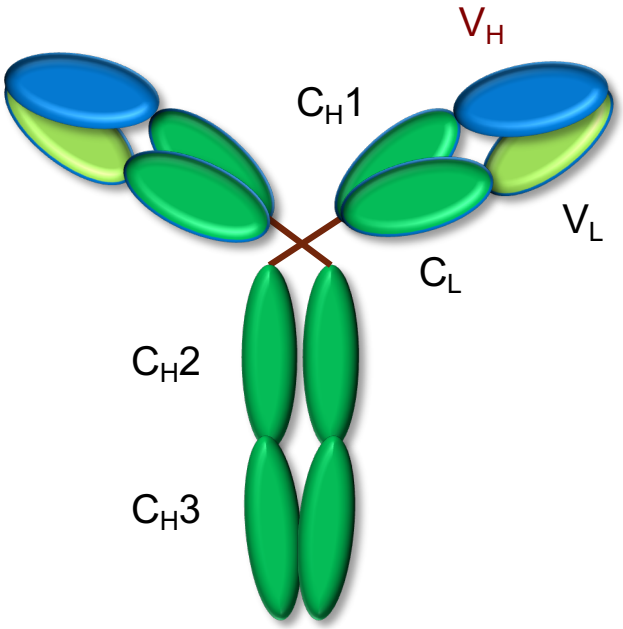
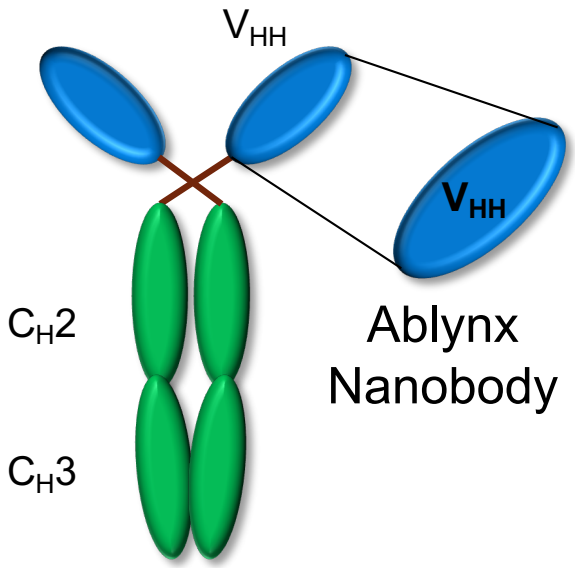
Ablynx Nanobody Technology offers Advantages over Conventional Antibodies for Certain Targets

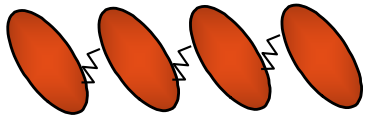
Conventional Antibody



Heavy Chain Antibody (Camelids)



DR5 Nanobody TAS266



- Four DR5 specific nanobodies separated by peptide linker
- DR5 affinity ~10^{-12} M

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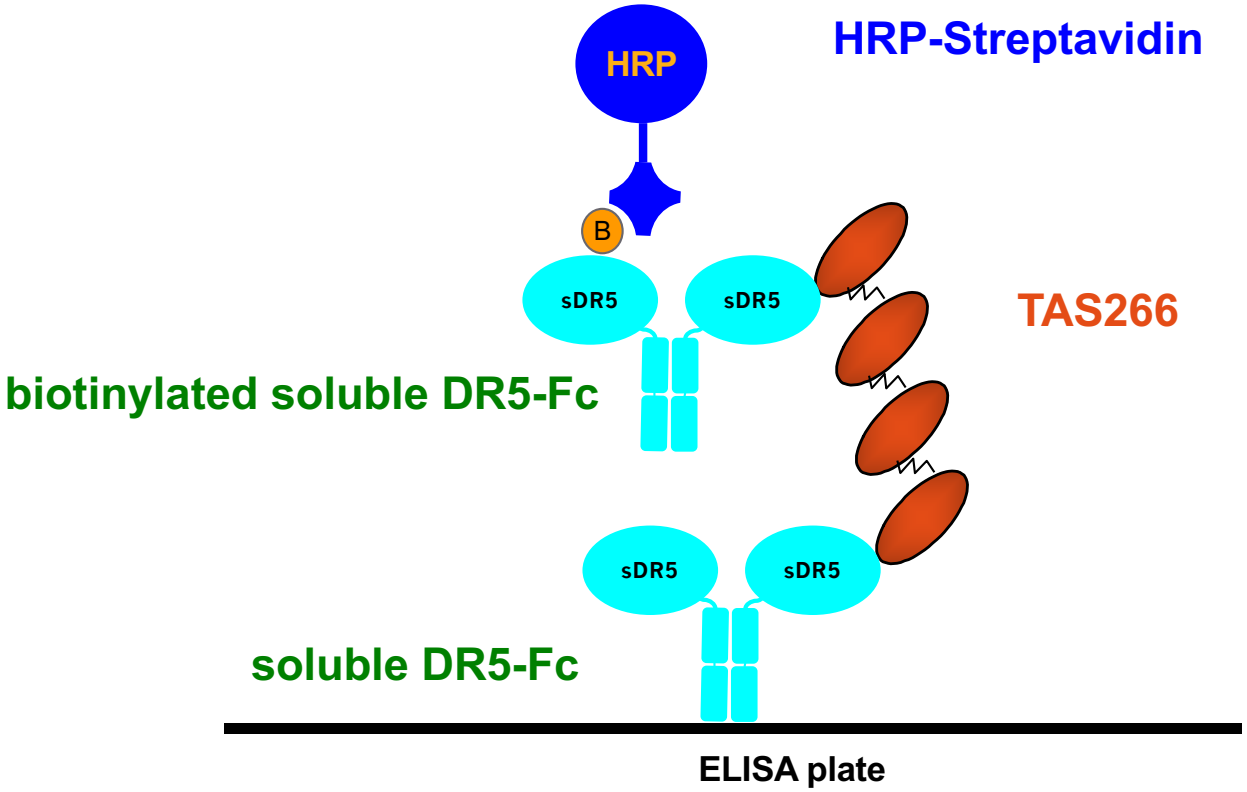
Bioanalytical Strategy

- PK: free drug PK assay
- IG: explain potentially unusual PK profiles
- PD vs. nAb assay?

PK assay format: sequential bridging ELISA

LLOQ = 15 ng/mL



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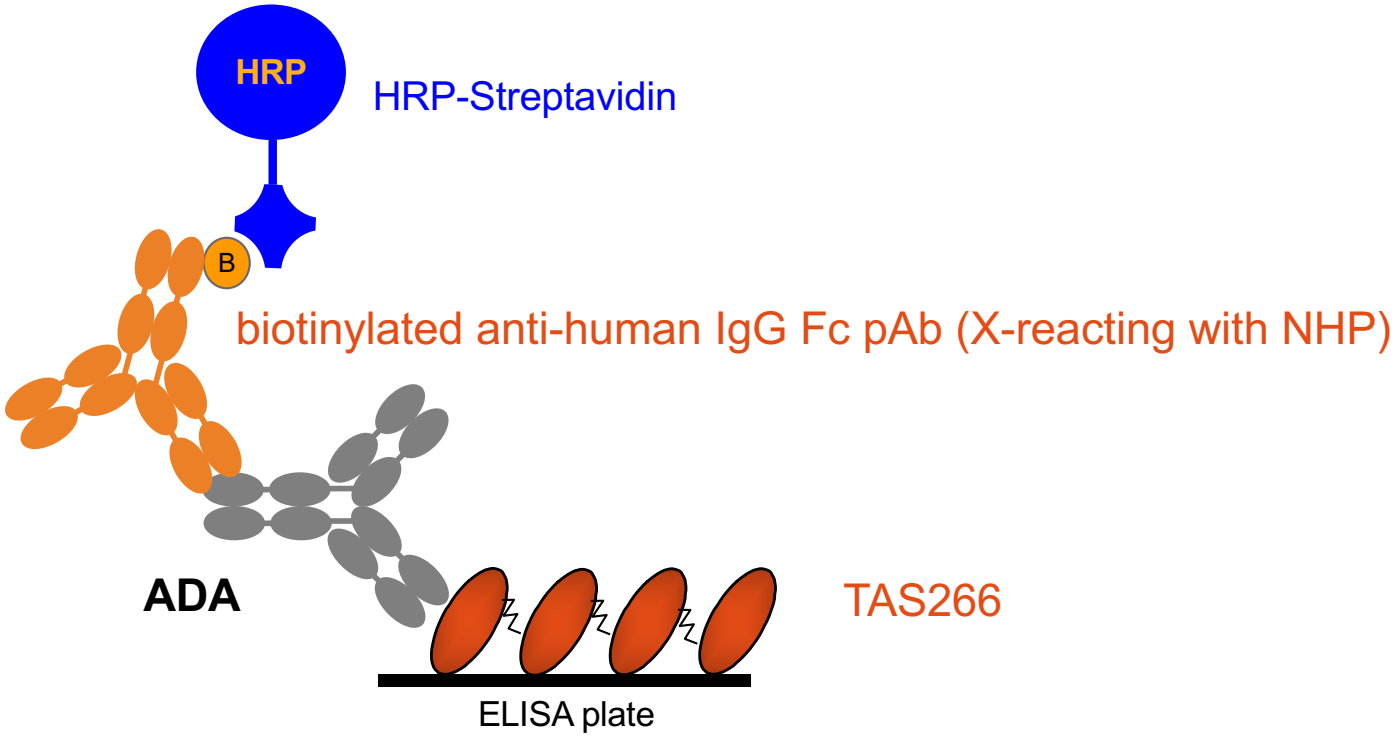
IG Assay format: IgG specific sandwich ELISA



- Sensitivity 300 ng/mL of mAb PC
- Drug tolerance: 15 x molar excess

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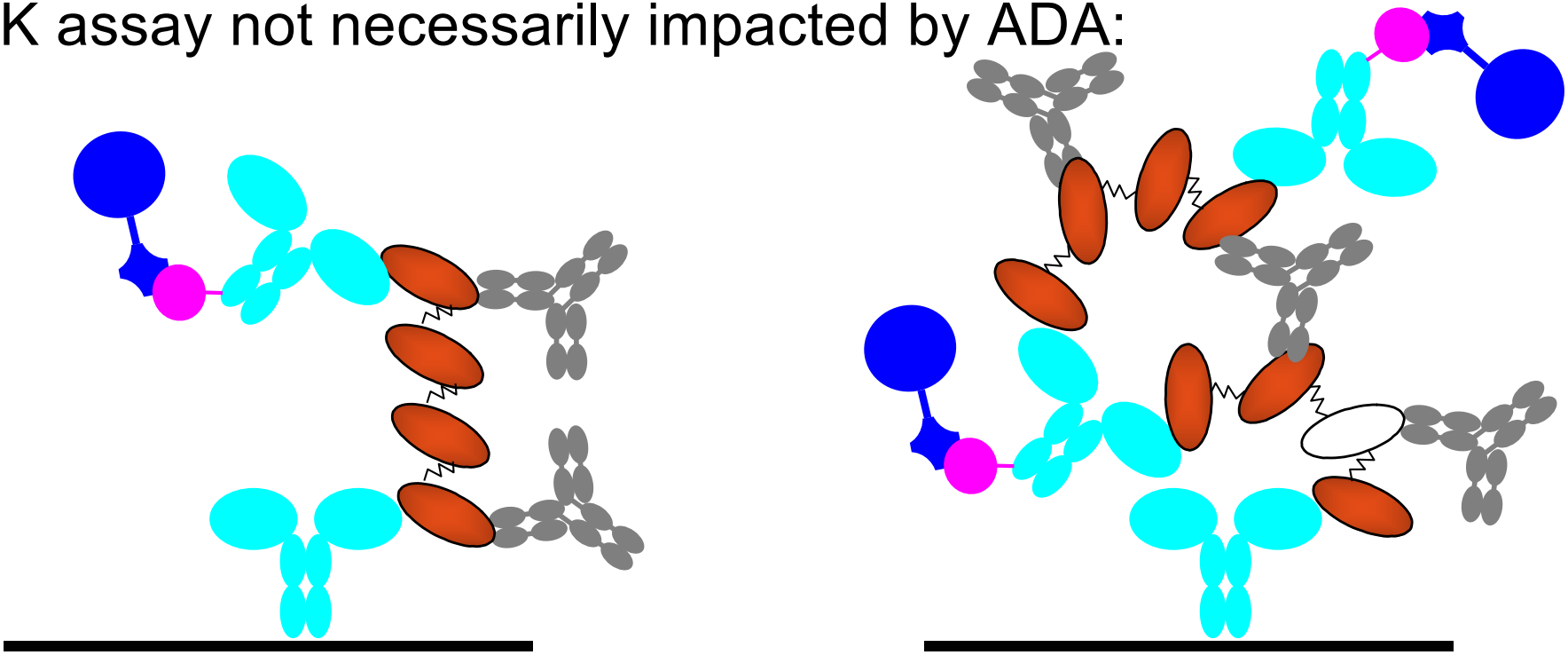
Efficacy of the drug in the presence of ADA?

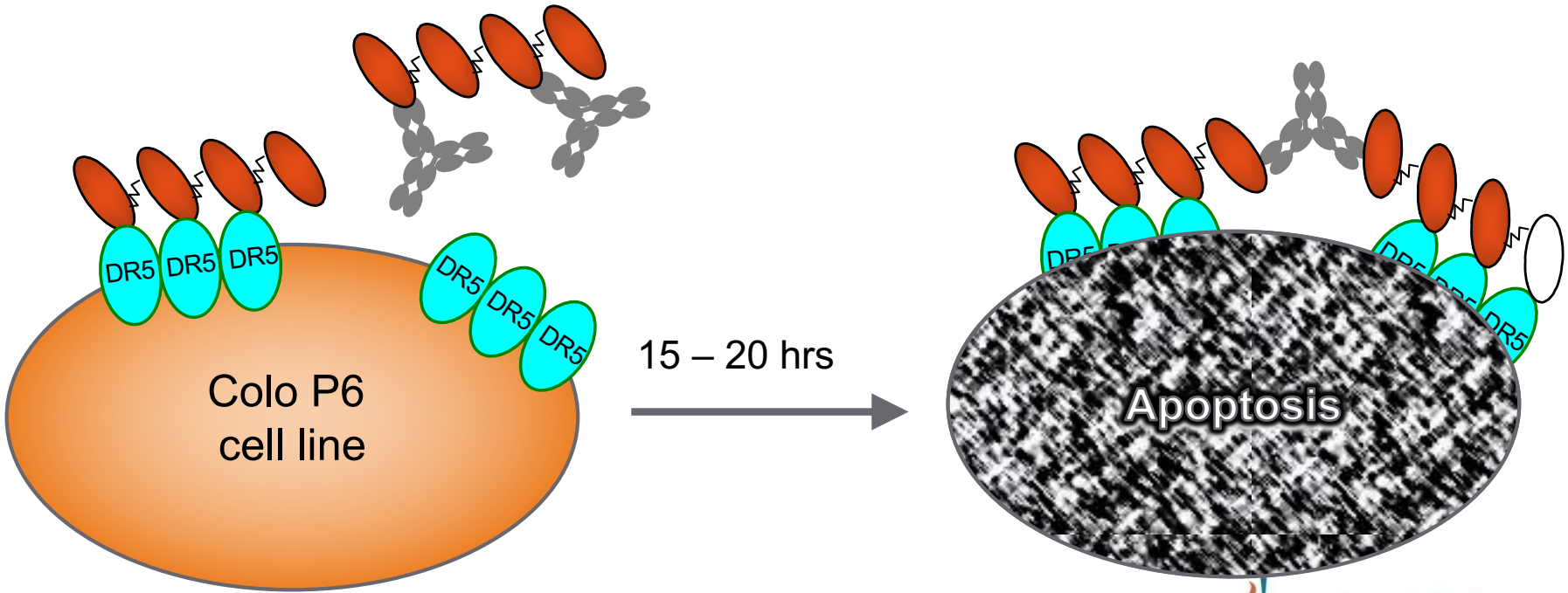
- PK assay not necessarily impacted by ADA:



- nAb assay as binding assay: Suboptimal, as inhibition of agonistic drug effect would not be demonstrated.

Efficacy of the drug in the presence of ADA?

- nAb assay as cell based assay: Addition of drug at constant concentration – drug tolerance might be problematic if only low amounts of drug need to be added.
- Discrimination of inhibitory and potentially super-agonistic effects not possible



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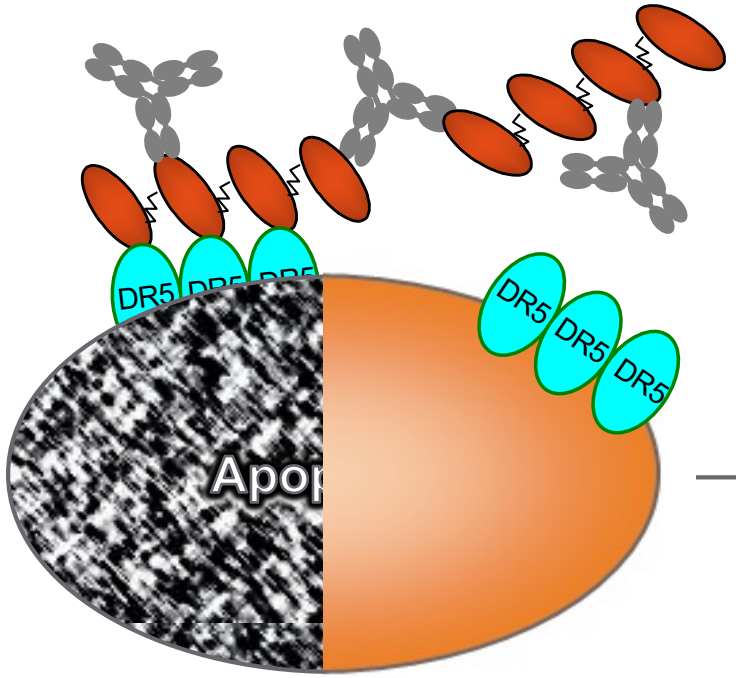
Cell-based free bioactive PK assay

- Detects only bioactive drug in the sample: Efficacy is monitored.
  - Not a neutralizing Ab assay
  - Avidity of multi-valent drug considered
  - Integrates superagonistic and neutralizing effects



15 – 20 hrs

Quantification of viable cells by cellular ATP content

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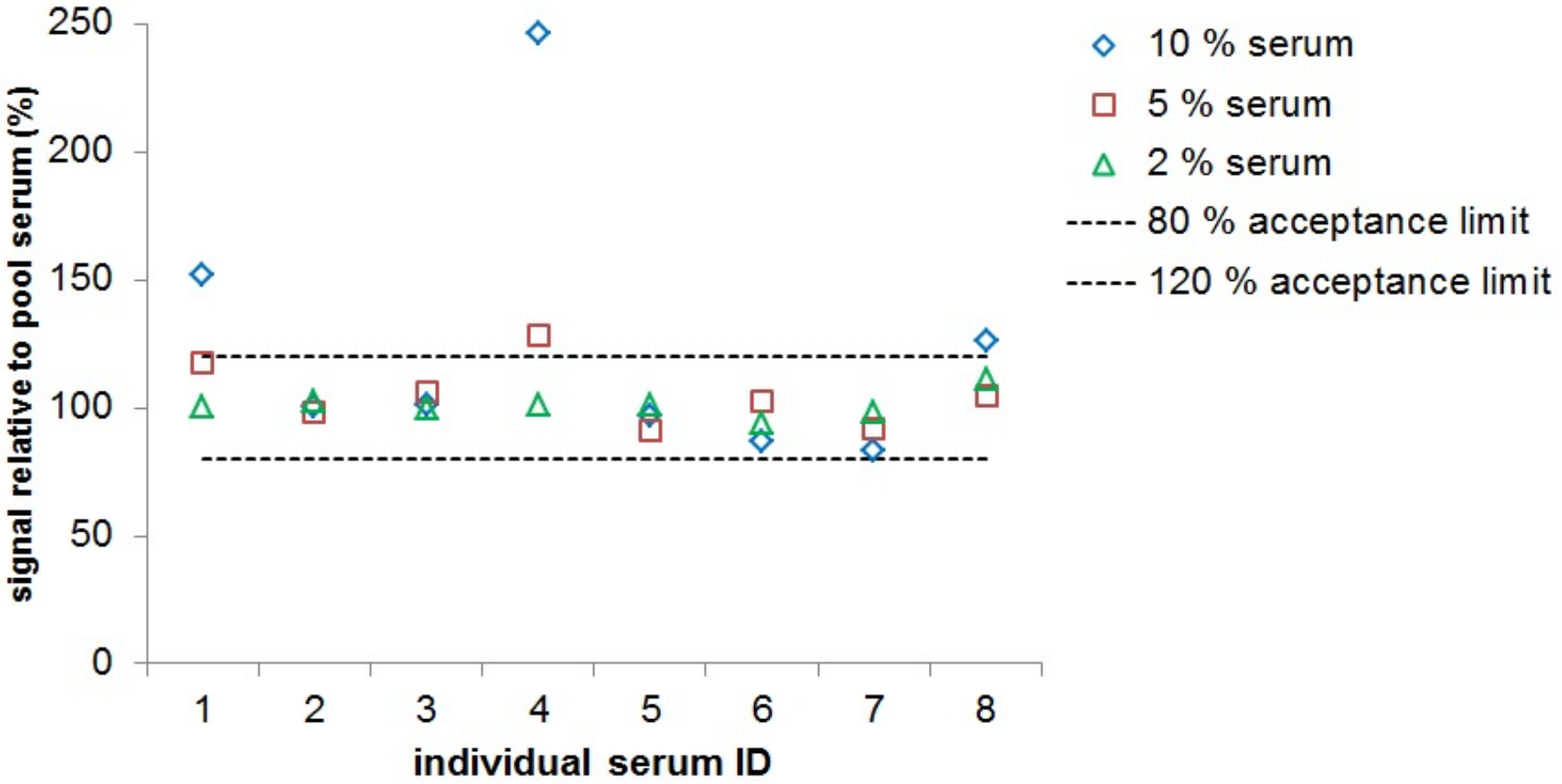
Assay development

- Based on potency assay transferred from CMC
- Basic assay parameters checked upon assay transfer by DoE
  - Source of cells
  - Days in culture
  - Cell number/well
  - Incubation time (h)
  - MRD
  - Source of FCS
  - Necessity of addition of cynomolgus pool serum
- Initial focus on stable assay performance with pool serum

Reduction of background noise by high dilution

- Reduction of individual serum fraction important for reproducible assay performance



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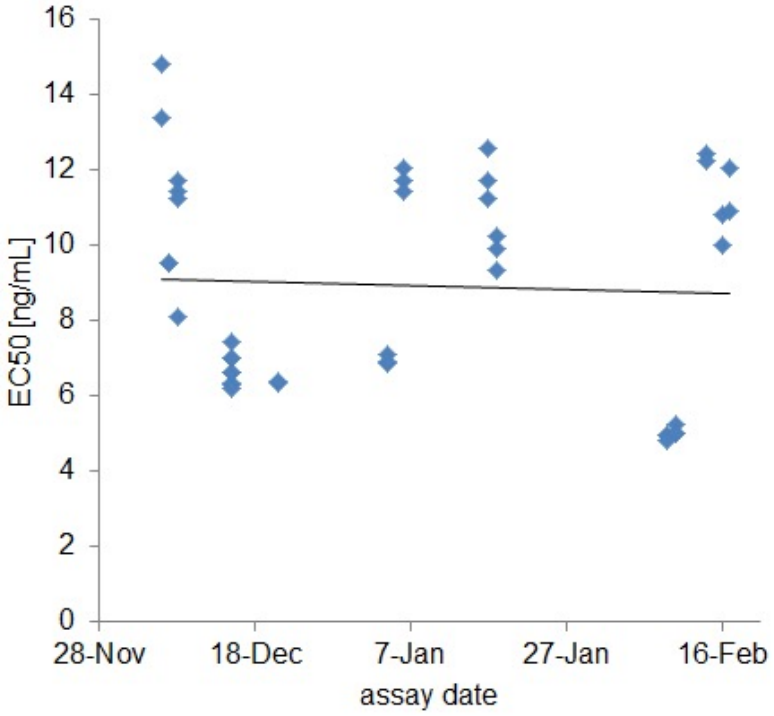
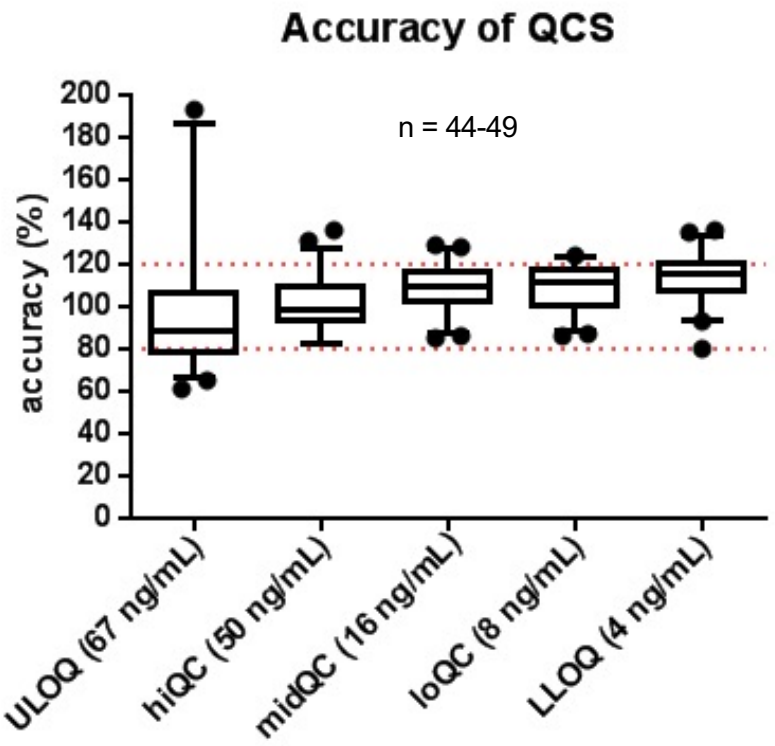
Assay development - Trending





No obvious correlation of EC50 with passage number, days after cell split, incubation time, S/B

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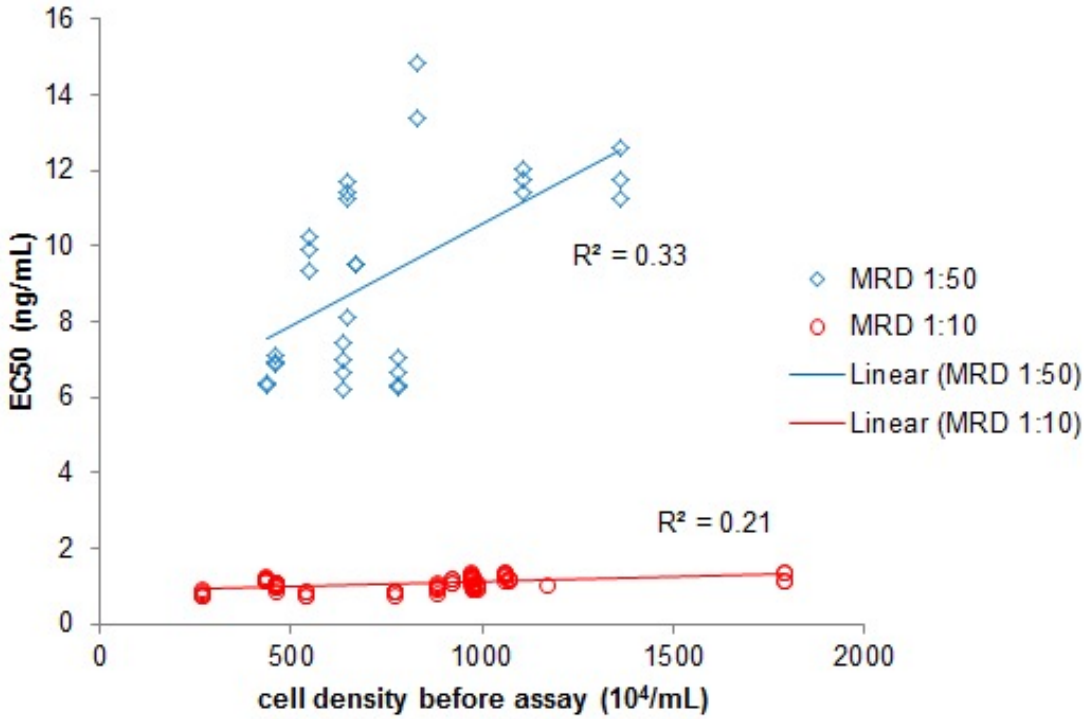
Effect of cell density at higher MRD on calibration curve position

- Calibration Standard Curve:
  - Stable EC50 important for stable working range & correctly set QCs
  - High likelihood for dependency of EC50 on cell status, i.e. density in the culture flask before use. Detailed cell culture conditions are described in the method.



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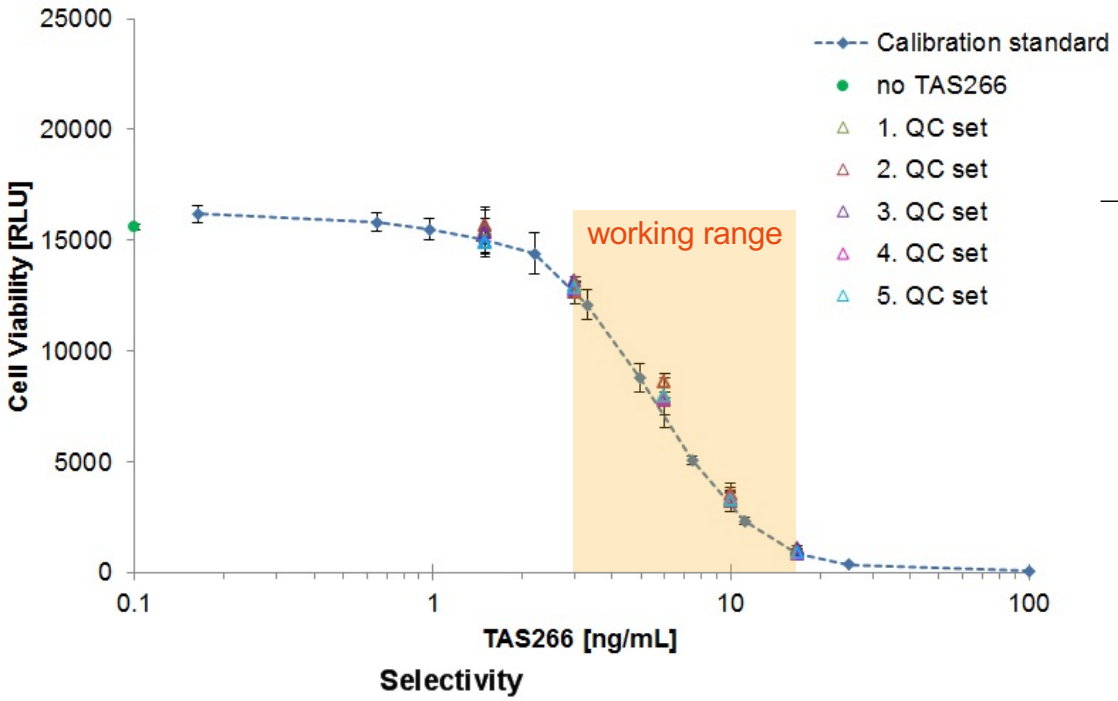
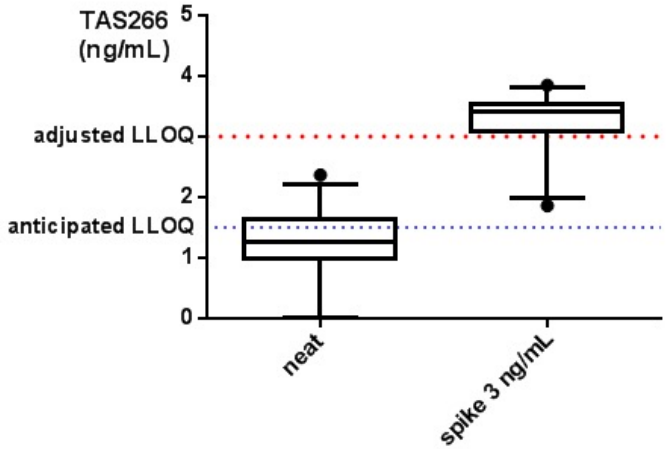
Cell-based free bioactive PK assay: Validation



QCS	Precision (%)			Accuracy
(ng/mL)	intra-run	inter-run	total	Bias (%)
1.5	14	19	24	11
3	7	6	10	-5
6	6	4	7	-11
10	5	3	6	-7
16.7	3	4	5	0

Selectivity



- MRD: 1:50
- Working range 3 – 16.7 ng/mL
- Dilution linearity 1:1000

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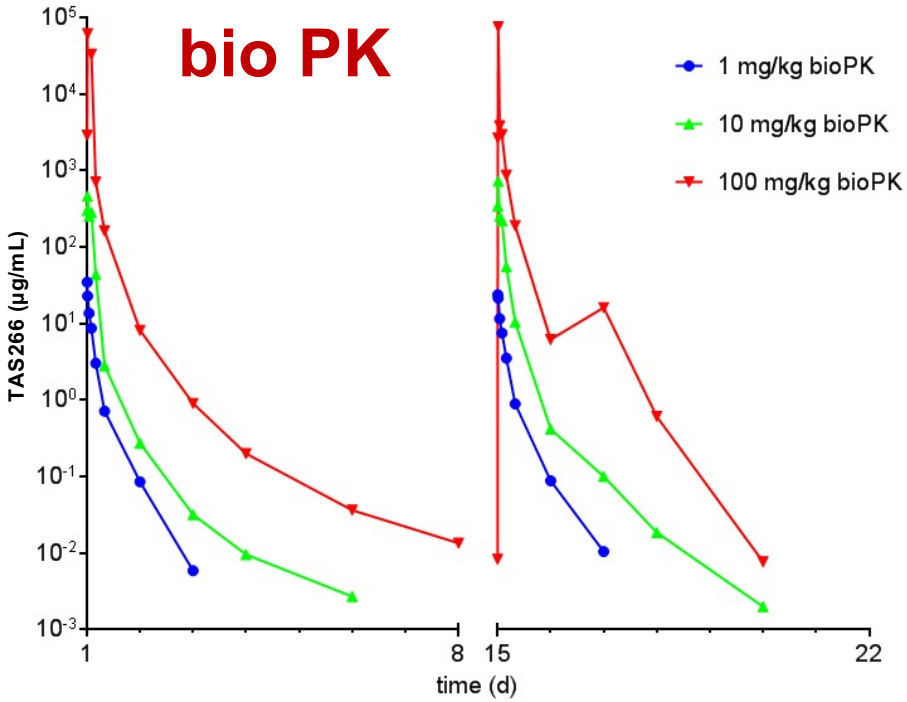
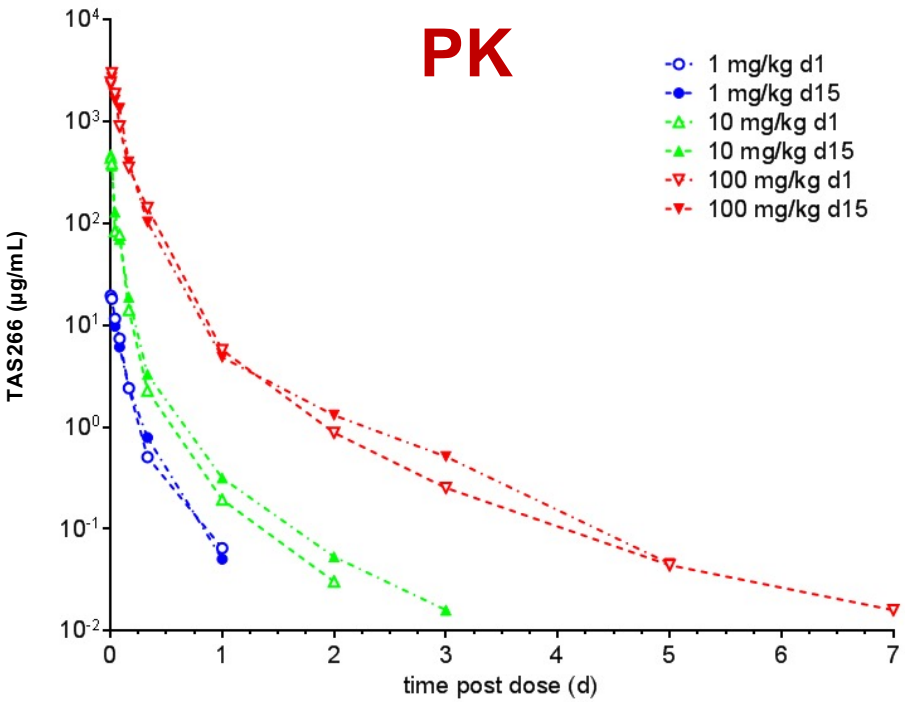
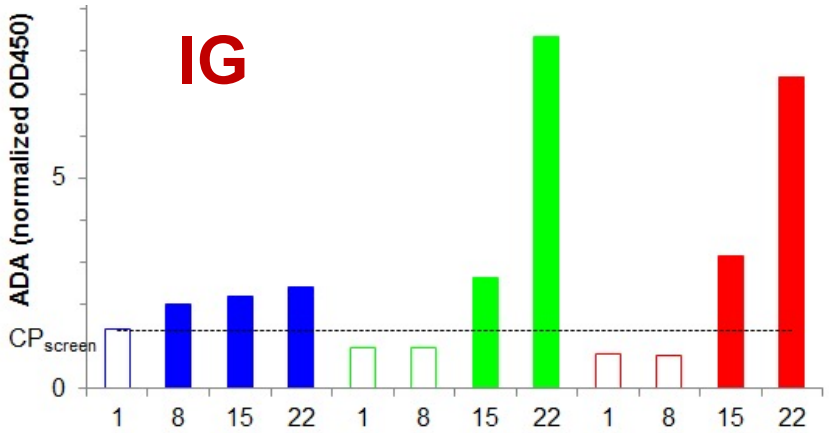
DRF Study Design & TK/IG sampling

- 3-week study, weekly dosing (4x) – necropsy day 24
- Dosing groups: 1, 10, 100 mg/kg & vehicle, 3 animals per group
- TK profiles taken for 1st & 3rd (pen-ultimate) dose
- IG samples taken pre-dose on days 1, 8, 15, 22
- Bioactive PK analyzed retrospectively from frozen PK samples after assay was available.
  - One animal per group was analyzed (2x in 100 mg/kg)

DRF study results (PK & bioactive PK, IG)







- PK and bioPK profiles overlaying.
- Day 1 and 15 profiles overlaying, despite IG in all animals on day 15.

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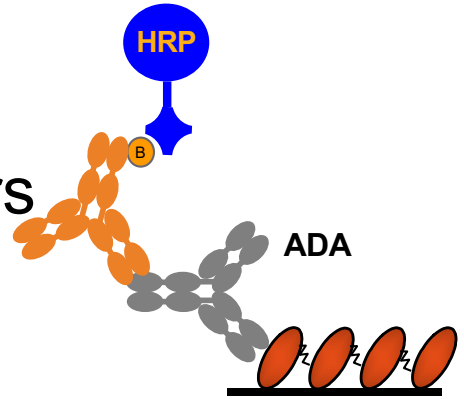
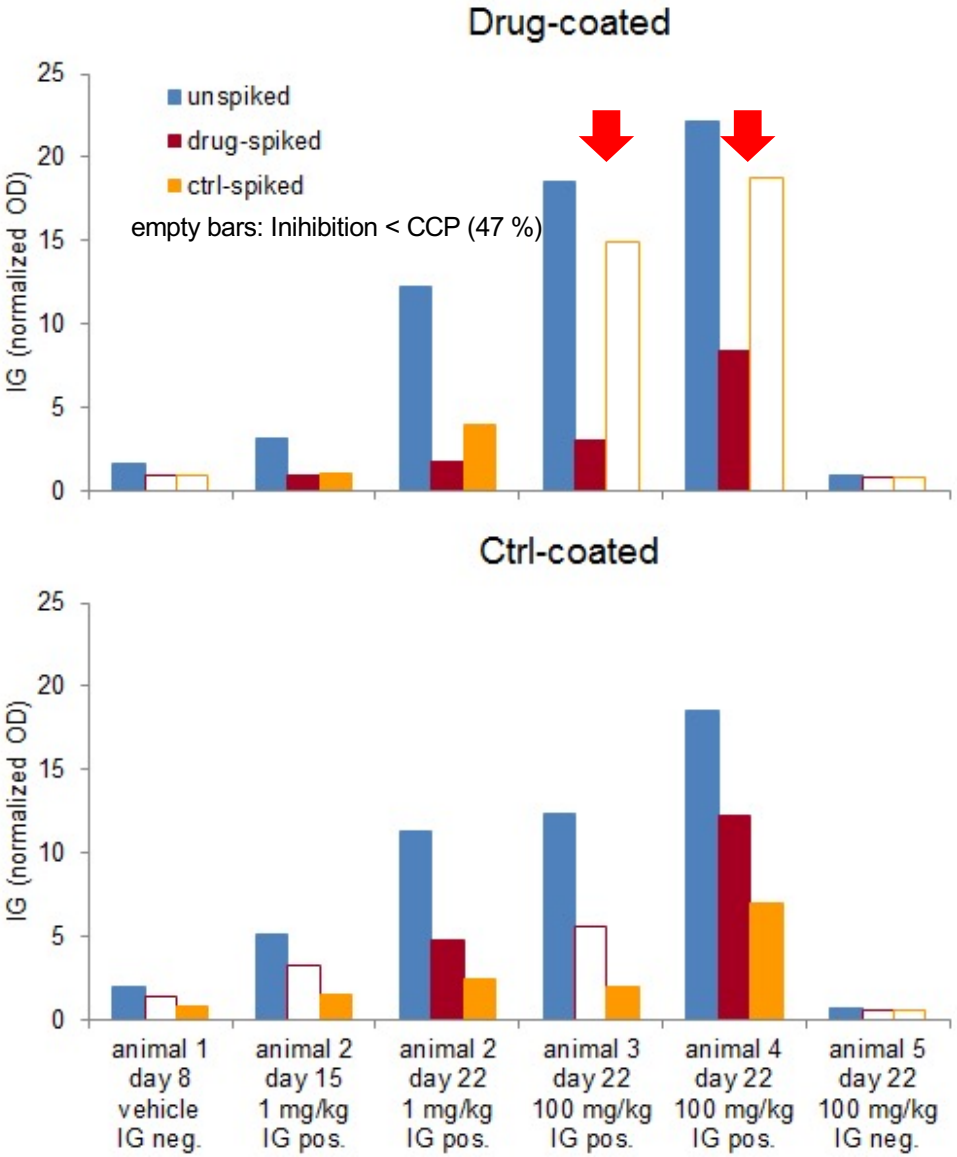
What is the target of the ADAs – CDR or backbone?

- Utilize IG assay
- Competition assay with control Nb tetramers
  - Backbones of the 2 controls differ by 3 aa each
  - Mixture of 2 ctrl Nb tetramers
  - Coating with either TAS266 or control



- Anti-backbone depletion assay
  - Sequentially deplete anti-backbone ADAs by incubating the sample with ctrl tetramer coated plates
  - Thereafter analyze on drug-coated plate

Competition assay



Pattern of IG signal inhibition by both, drug and control, suggests presence of ADA against antigen-binding site and backbone.

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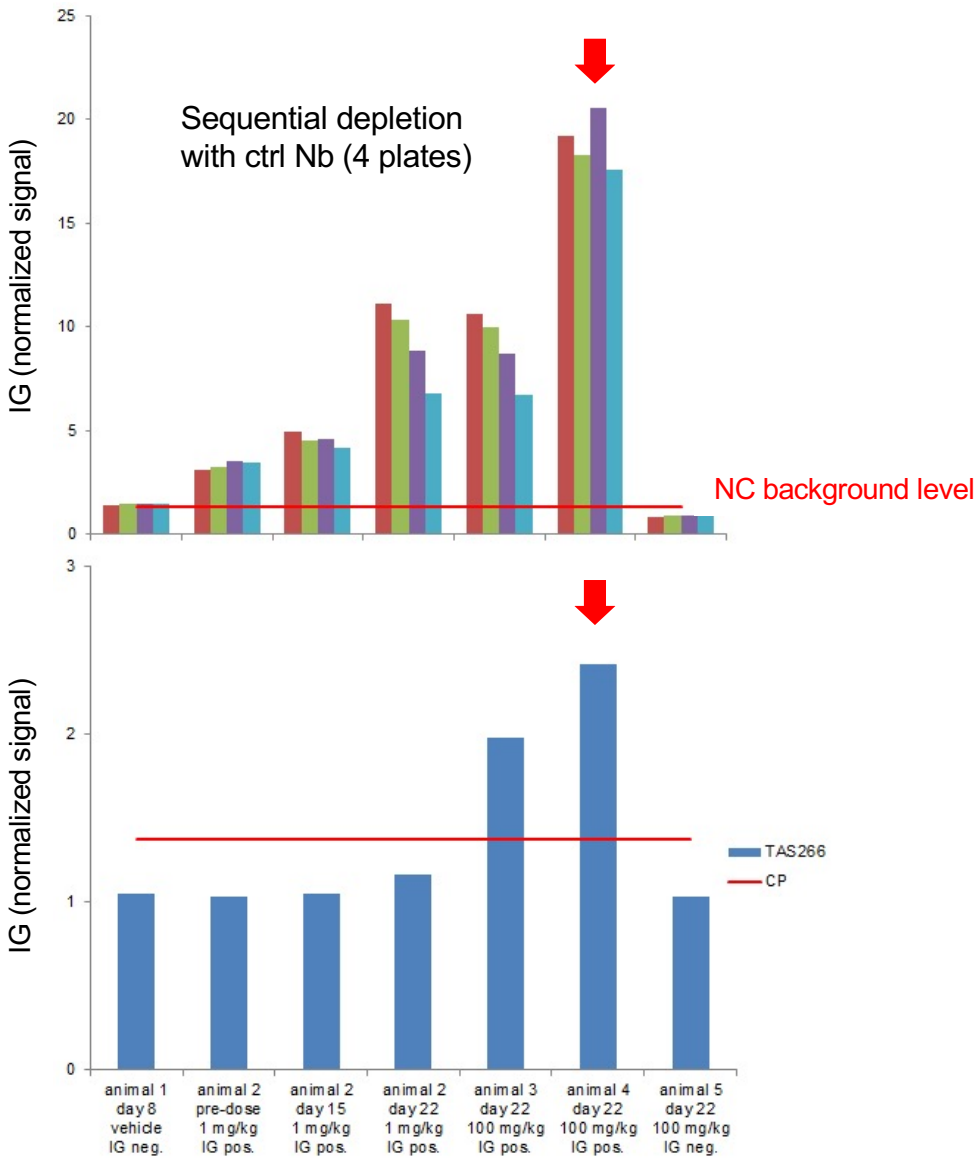
Depletion assay



- Continued reduction of signal upon repeated sample incubation on ctrl-coated plates
- Thereafter, a strong IG signal upon sample incubation on drug-coated plate could indicate anti-CDR antibodies
- Since different coatings were used, a direct comparison of signal intensities does not give quantitative information, i.e. initial signal decrease to background on the ctrl-coated plates would have been a better indicator.

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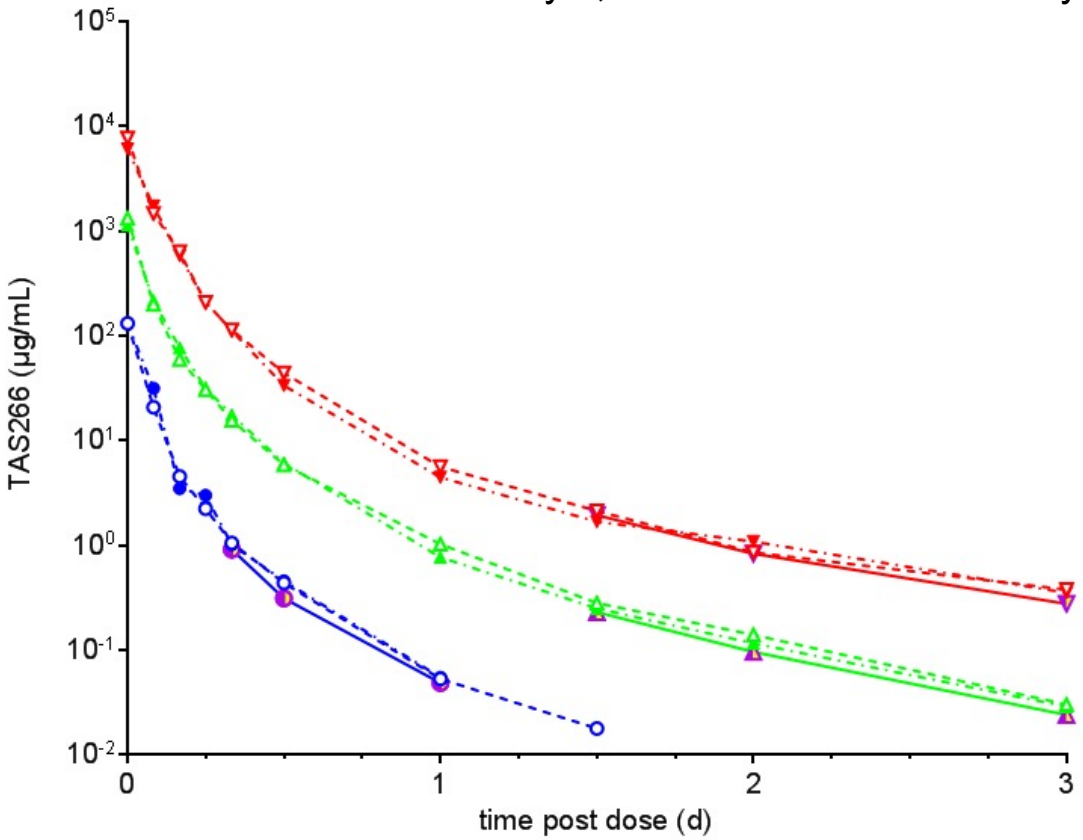
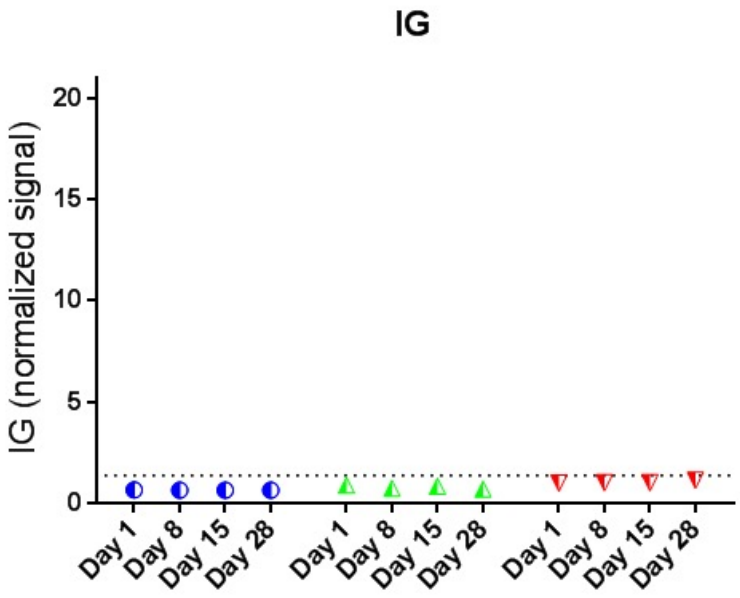
Toxicology Study Design & TK/IG sampling

- 4-week study, bi-weekly dosing (8x) – necropsy day 28
- Dosing groups: 5, 50, 200 mg/kg & vehicle, 6 animals per group plus 4 recovery animals (200 mg/kg & vehicle)
- TK profiles taken for 1st & 8th (ultimate) dose (day 25)
- IG samples taken pre-dose on days 1, 8, 15, 28
- Bioactive PK analyzed from last three PK sampling time points with quantifiable result after the last dose (day 25).

Exemplary PK / bioPK / IG results

blue 5 mg/kg, green 50 mg/kg, red 200 mg/kg
broken line: PK day 1, broken/dotted line: PK day 25, solid line/purple symbols: bioPK day 25





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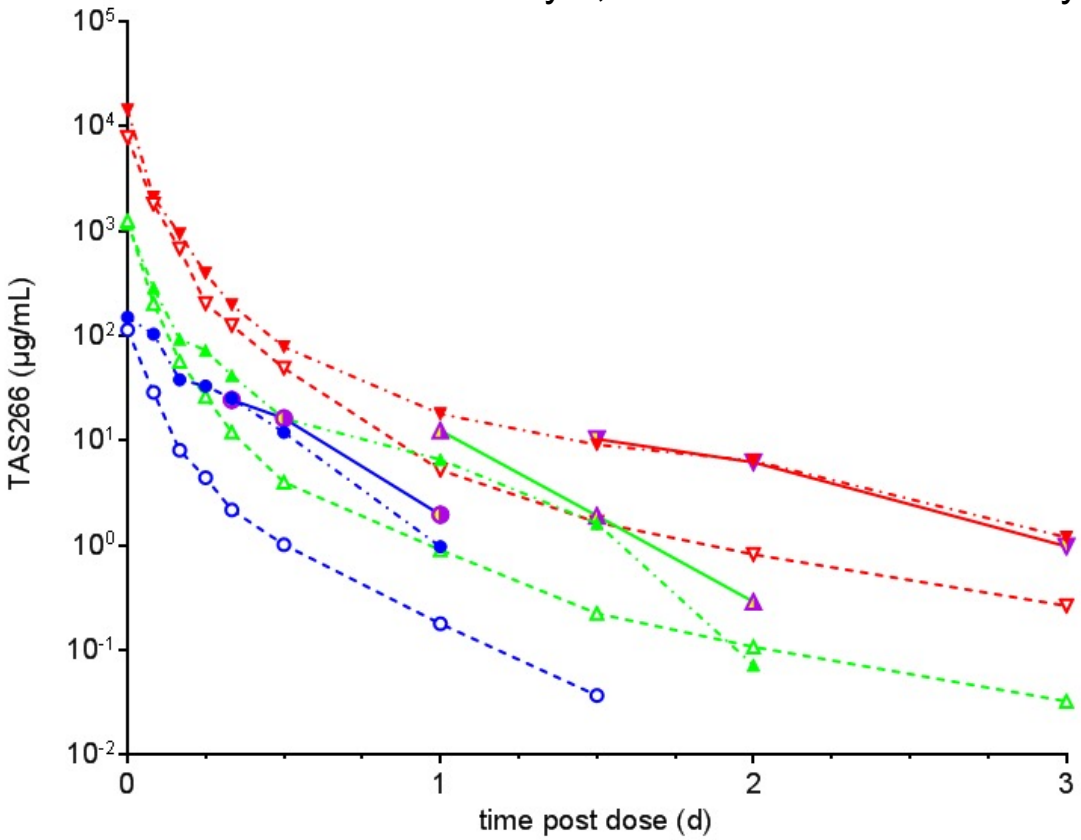

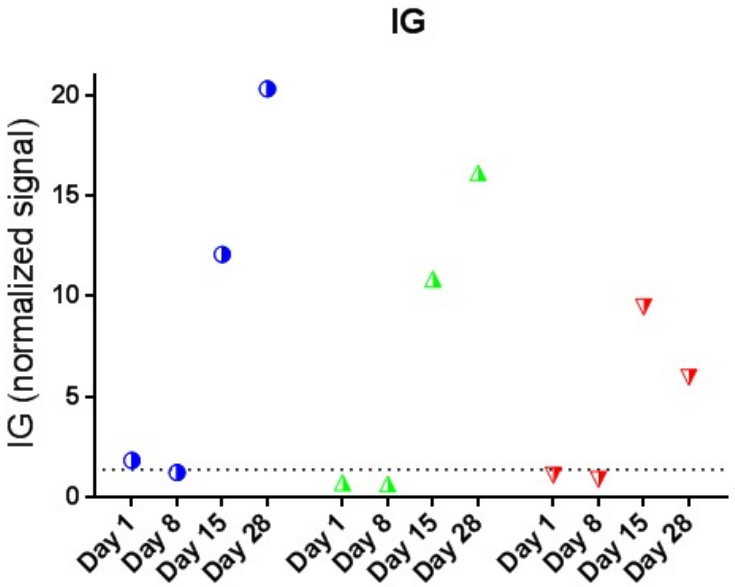

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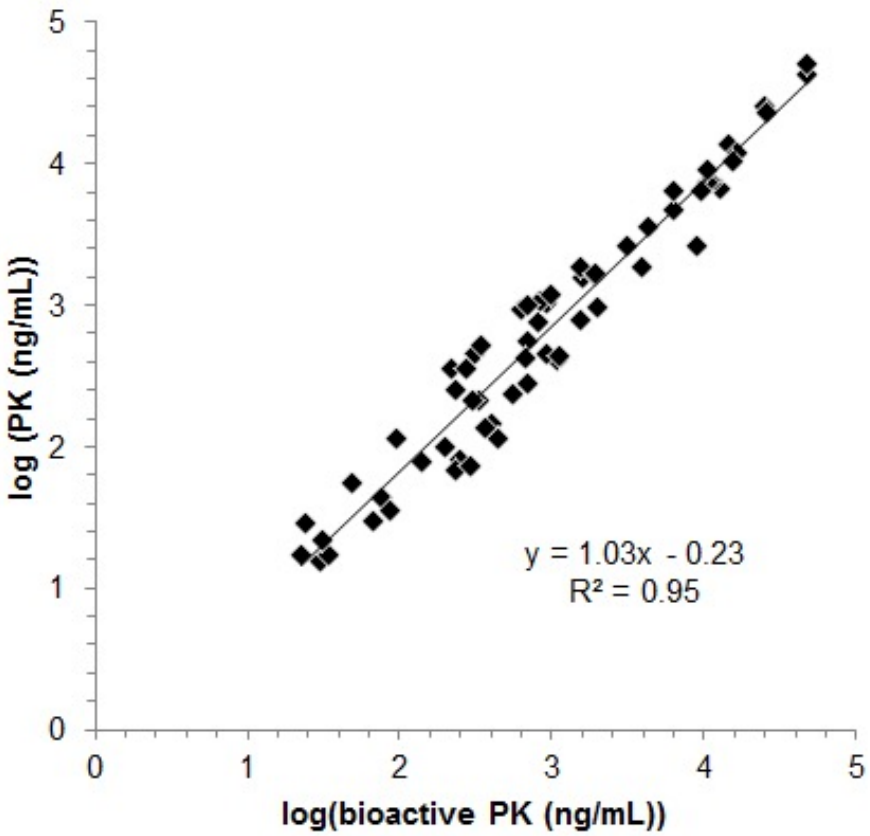
Bioactivity correlates with PK, with and without IG developing during the study

IG	Animal number
No IG observed during study	5 (1M, 4F)
Develop IG during dosing phase of the study – clearance impacted	13 (8M, 5F)
Other: High background, develop IG late during study, ...	4 (2M, 2F)



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Conclusion

- Bioactivity is not impacted by IG
  - Despite development of a strong IG response, probably targeting the Ag-binding site, no impact on bioactivity of drug was detected.
  - Higher affinity?
  - Higher avidity of tetravalent drug to trimeric receptor than to bivalent ADA?
- Clearance profile after repeated administration might be caused by
  - initially prolonged halflife mediated by ADA,
  - later formation of ICs.
- Bioactivity / nAb assay not foreseen for Phase I.

Clinical outcome

- Phase I study: 3 of 4 solid tissue tumor patients experienced unexpected drug-related hepatotoxicity
- Underlying mechanism is not fully elucidated
  - Correlated with IG
    - potentially due to enhanced crosslinking of target by ADA
  - Target might be upregulated after preceding chemotherapy
    - potentially enhanced DR5 clustering and activation of hepatocyte apoptosis
- Reinforces the need for exploration of the potential impact of pre-existing antibodies on the safety of biotherapeutics.

Acknowledgements

- Alexandra Fuchs
- Emilie Bossuge
- Sebastian Spindeldreher
- Tim-Robert Soellick
- Kerstin Kentsch
- Peter Lloyd
- Jennifer Sims
- Heather Huet
- Ursula Jeffry
- Sanela Bilic
- Shefali Kakar
- Varsha Iyer
- Randi Isaacs
- Stefan de Buck
- Sabine Meyenburg
- Jens Lohrmann
- Kapil Gupta
- Isabelle Weigert