**Background: Nanobodies**
- The isolated variable domains (VHH) of camelid heavy-chain only antibodies are stable and fully functional
- Nanobodies represent the next generation of antibody-derived biologics
- > 45 programmes, 6 Nanobodies in clinical development

**Background: Immuneogenicity testing**
- Neutralizing antibodies (Nama) are defined as antibodies (Ab) able to affect the function of the Nanobody by blocking its ability to bind to target, including Ab binding to complementary determining region (CDR)
- Development of a NAb assay based on drug target interaction is challenging and often requires extensive pre-treatment steps in order to obtain the required drug and target tolerance for proper detection of NAB during clinical trials
- Such an assay set up often introduces a sensitivity difference between the ADA assay and the NAb assay
- The sensitivity gap complicates ADA data interpretation as a discrepancy between ADA and NAb results can either reflect presence of non-neutralizing antibodies only or reflect neutralizing antibodies that are left undetected in the NAb assay
- An alternative NAb assay format was developed based on the conventional bridging ADA assay format which allows unambiguous comparison of the levels of total ADA and NAb

**The alternative NAb assay**
- An excess amount of the null variant of therapeutic Nanobody, i.e. a variant of the Nanobody which is non-functional for target binding and which is identical to the Nanobody with the exception of altered CDR’s of the target binding Nanobody domain, is added to the reagent mastermix
- Positve assay signals reflect antibodies with neutralizing potential only

**ADA and alternative NAb assay qualification data**
- Similar sensitivity and drug tolerance characteristics as compared to the ADA assay
- Target tolerance characteristics similar as the ADA assay. In case of a monomeric target, this NAb format is target tolerant
- Intra-run and inter-assay precision ≤ 20%

**Conclusion**
- The alternative NAb assay format is based on the conventional bridging ADA assay format. The assay is performed in presence of excess of a null variant of therapeutic drug added to the reagent master mix
- The null variant of the Nanobody is non-functional for target binding and is identical to the Nanobody with the exception of altered CDR’s of the target binding Nanobody domain
- Fit for purpose of the assay format was demonstrated using an established panel of neutralizing and non-neutralizing Ab for which the (non)neutralizing potential is based on their (in)ability to block target interaction via a CLBA
- Signals generated in this assay reflect antibodies that bind to the CDR region and have a potential for neutralization
- Non-neutralizing are left undetected as compared with the null variant, in a way comparable to the conventional drug displacement set-up (confirmatory assay)
- Allows unambiguous immuneogenicity data interpretation as potential NAb are detected with same assay characteristics as ADA assay
- While these assays represent a significant advance in methodology, the clinical relevance of the results must be viewed in relation to effects on drug PK, PD, efficacy and safety