

Correlation of ADA with Clinical and Non-Clinical Study Endpoints: A Case Study and Lessons Learned

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Tanezumab: An Introduction

- Humanized IgG₂ monoclonal antibody for the treatment of chronic pain
 - Tanezumab:
 - binds with high affinity and specificity to nerve growth factor - NGF ($K_D < 10$ pM)
 - prevents interaction of NGF with its receptors (TrkA and p75)
 - NGF inhibition using tanezumab (or its murine precursor, MuMab911) has been associated with improvements in pain in nonclinical animal models of pathological pain including osteoarthritis (OA), fracture, bone cancer and post-surgical pain models
 - NGF exists as a dimer

Background

- During development, nerve growth factor (NGF) is required for neuronal survival and growth
- However, in adulthood, NGF is involved in sensitization of nociceptors following tissue injury:
 - Increased NGF expression is observed in inflamed tissues in conditions such as arthritis, pancreatitis, and prostatitis and in animal models of inflammatory pain
 - NGF blockade has been shown to improve symptoms of pain and hyperalgesia in animals (but does not compromise normal nociceptor function or cause the detrimental loss of sympathetic or sensory neurons)
- NGF has thus become a target of strategies to develop novel analgesic therapeutics

Tanezumab: Clinical Development Program Status

- Tanezumab is being investigated for a variety of chronic pain conditions including:
 - OA
 - chronic low back pain
 - metastatic bone pain
 - post-herpetic neuralgia
 - interstitial cystitis
- Tanezumab is currently in Phase III trials for OA of the knee
 - Includes 10 studies and approximately 7000 patients

Why do Immunogenicity Testing?

- The development of anti-drug antibodies (ADA) to protein therapeutics can result in:
 - rapid clearance, altering the pharmacokinetic (PK) and/or pharmacodynamic profile of the drug
 - loss of efficacy due to neutralization of drug
 - generalized immune effects, such as anaphylaxis, serum sickness or hypersensitivity
 - neutralization of the endogenous human protein, causing deficiency syndrome, with fatal outcomes
- ADA status of the animal model is required for interpretation of the pharmacology and toxicology data
- Monitoring of immunogenicity is required at all stages of biotherapeutic drug development for evaluation of safety and efficacy and is a key component of regulatory filings

The Immunogenicity Assay

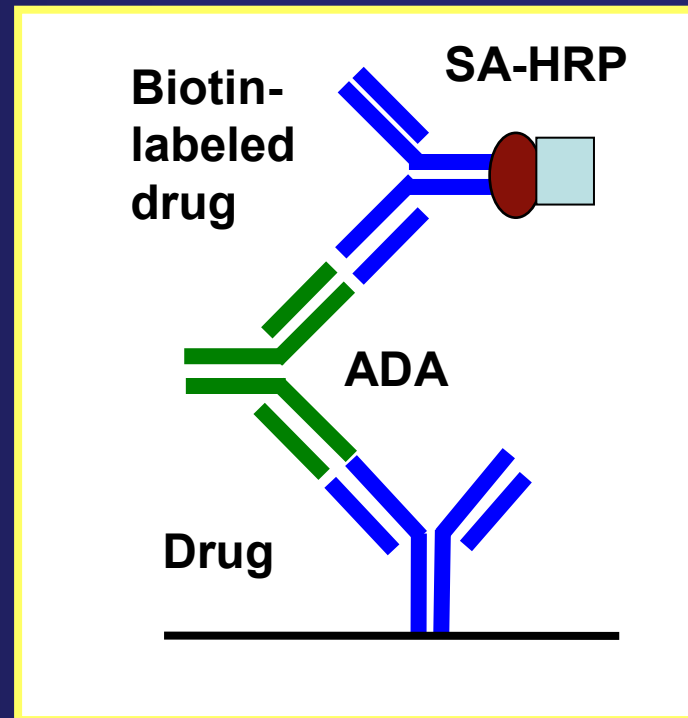
- The immunogenicity assay should have:
 - Sensitivity
 - Reproducibility/precision
 - Specificity
 - An appropriate cut-off
 - And be based on an understanding of relative drug and ligand tolerance limits

Assessing the Immunogenicity of Tanezumab

- Clinical
 - Total ADA
 - Neutralizing antibody (Nab)
 - (competitive ligand binding assay)
- Toxicology (Embryo-Fetal Development [EFD] Study)
 - Total ADA
 - Maternal serum
 - Maternal breast milk
 - Newborn serum

Detection of Tanezumab Anti-Drug Antibodies

- Initial studies (monkey toxicology and First-In-Human [FIH] clinical studies) used a Bridging ELISA



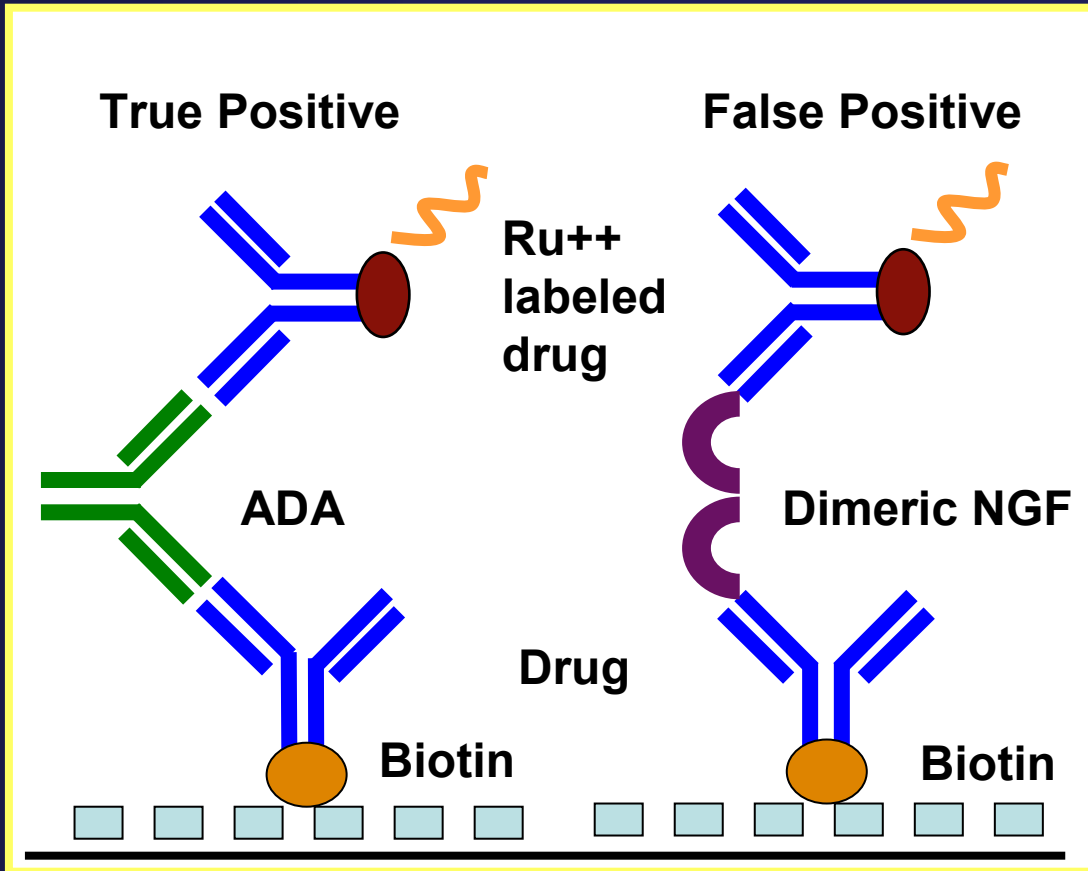
Results with Bridging ELISA

- In monkey toxicology studies, ADA was detected with corresponding changes in PK parameters (reduced tanezumab plasma concentrations)
- In FIH clinical studies bridging ELISA did not detect ADA. Was this:
 - Real?
 - Due to inadequate assay sensitivity?
 - Or due to drug interference?
- Efforts were undertaken to develop a more sensitive and drug tolerant assay

Development of a Bridging Electrochemiluminescence Assay

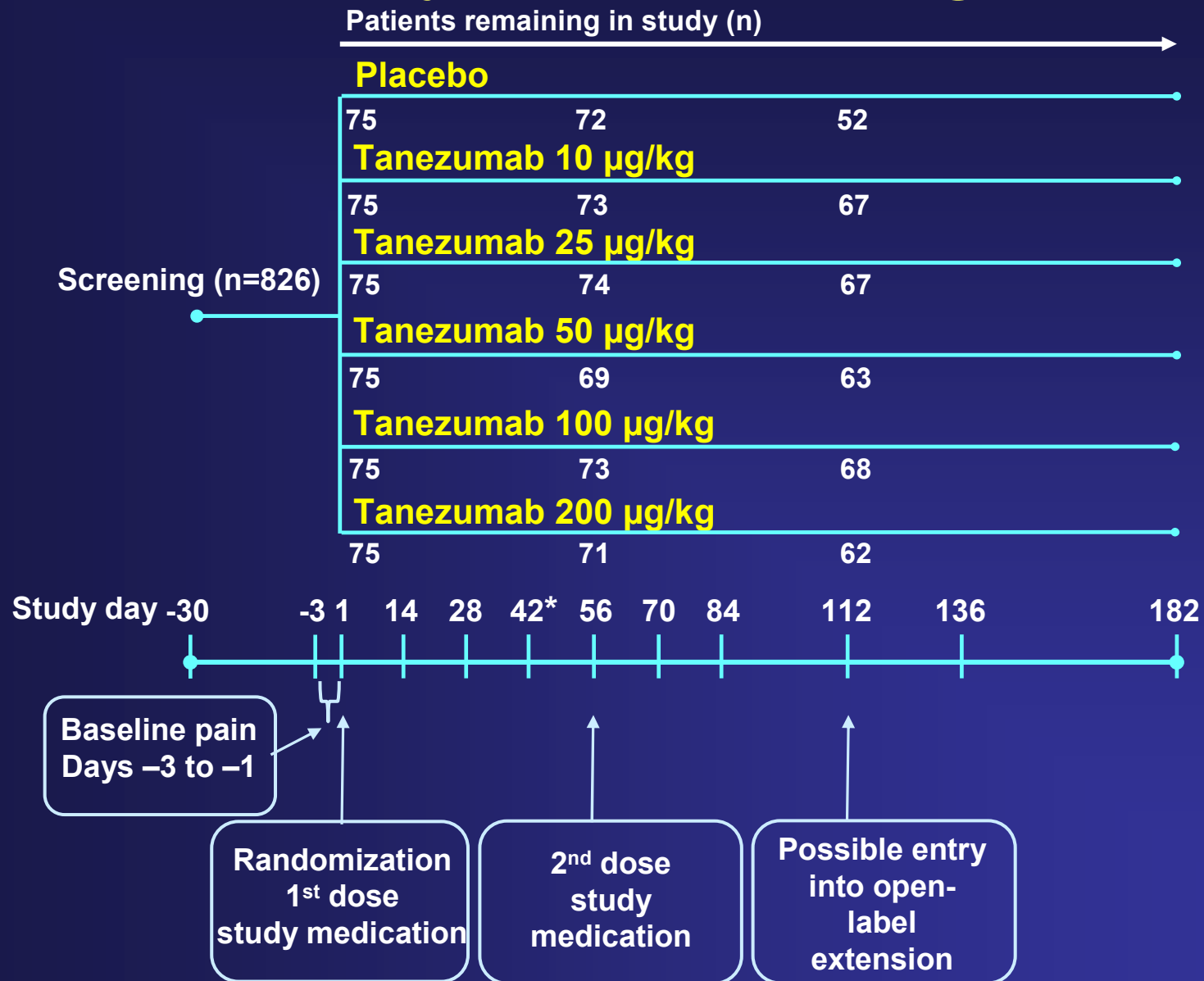
- In the bridging electrochemiluminescence (ECL) assay:
 - Drug is conjugated with a ruthenium complex that emits light upon application of an electric potential
 - A separate sample of drug is conjugated with biotin
 - Sera is incubated with equimolar concentrations of ruthenium and biotin-conjugated drug and the mixture added to streptavidin-coated plates equipped with electrodes to capture biotin-drug-analyte-ruthenium drug complexes
- The bridging ECL assay has been shown to be ~50 times more sensitive and ~20 times more tolerant of free drug than bridging ELISA

Results with Bridging ECL



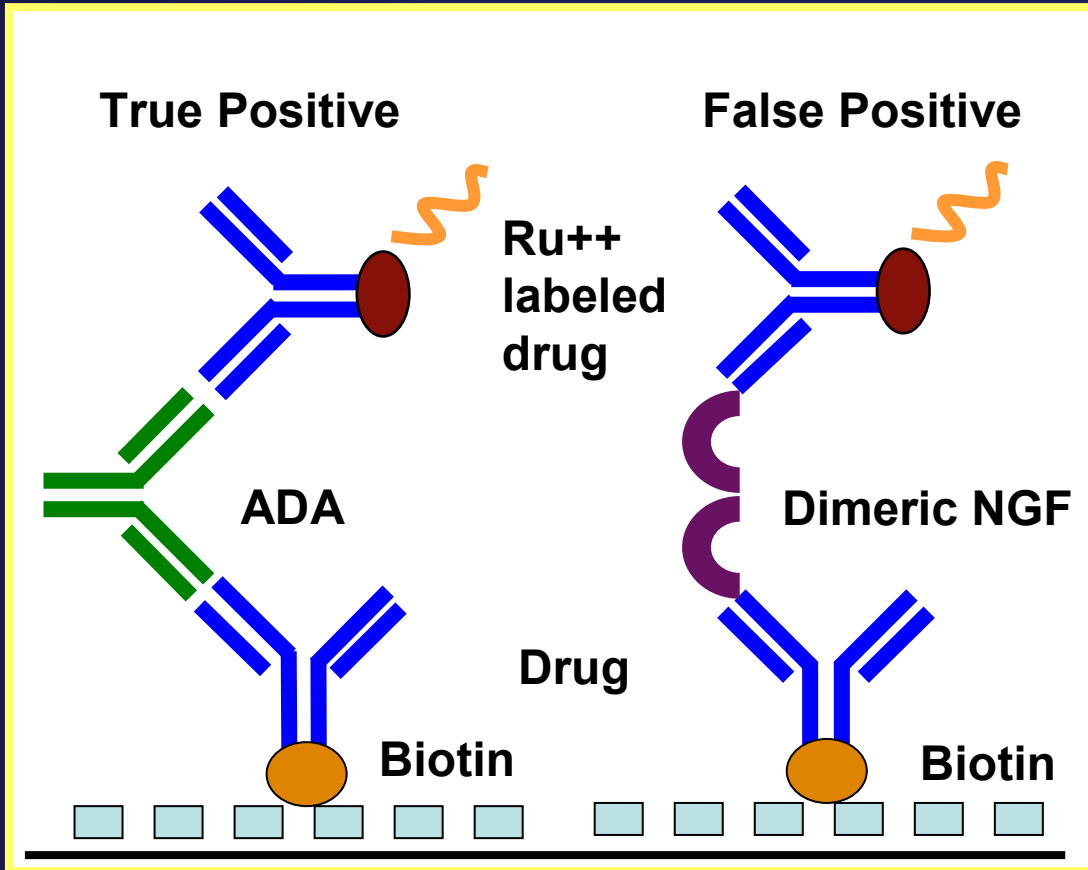
- Since the target molecule can exist as a dimer, experiments were conducted to determine the impact of NGF with no ADA present
- NGF at levels higher than physiological caused false positives with this assay (Since levels of drug were going to be 100x+ higher than NGF levels, interference due to free NGF in the assay was not perceived to be a problem)

Study 1008[†]: Design



*Telephone contact; [†]Phase II study in patients with OA of the knee

Results with Bridging ECL

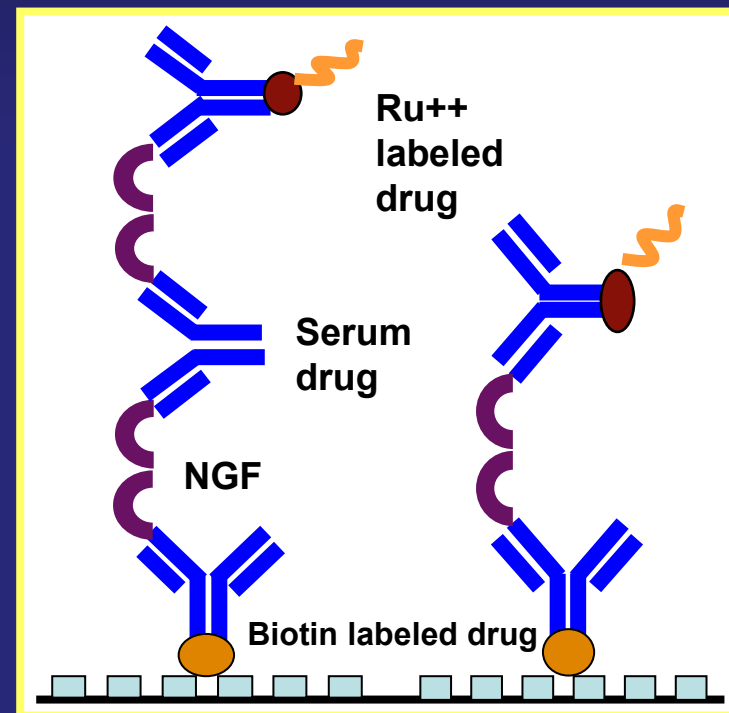


- In Study 1008, 50% of samples tested positive for ADA, with signals 2-20 times higher than normal serum matrix.

Results with Bridging ECL

- Are the positive results obtained with the bridging ECL assay real?
- To answer, look at the specificity of the response by:
 - Addition of excess drug
 - Ig depletion methods

However, neither of these methods are conclusive in the case of a false signal.



Further Evaluation of ADA Results

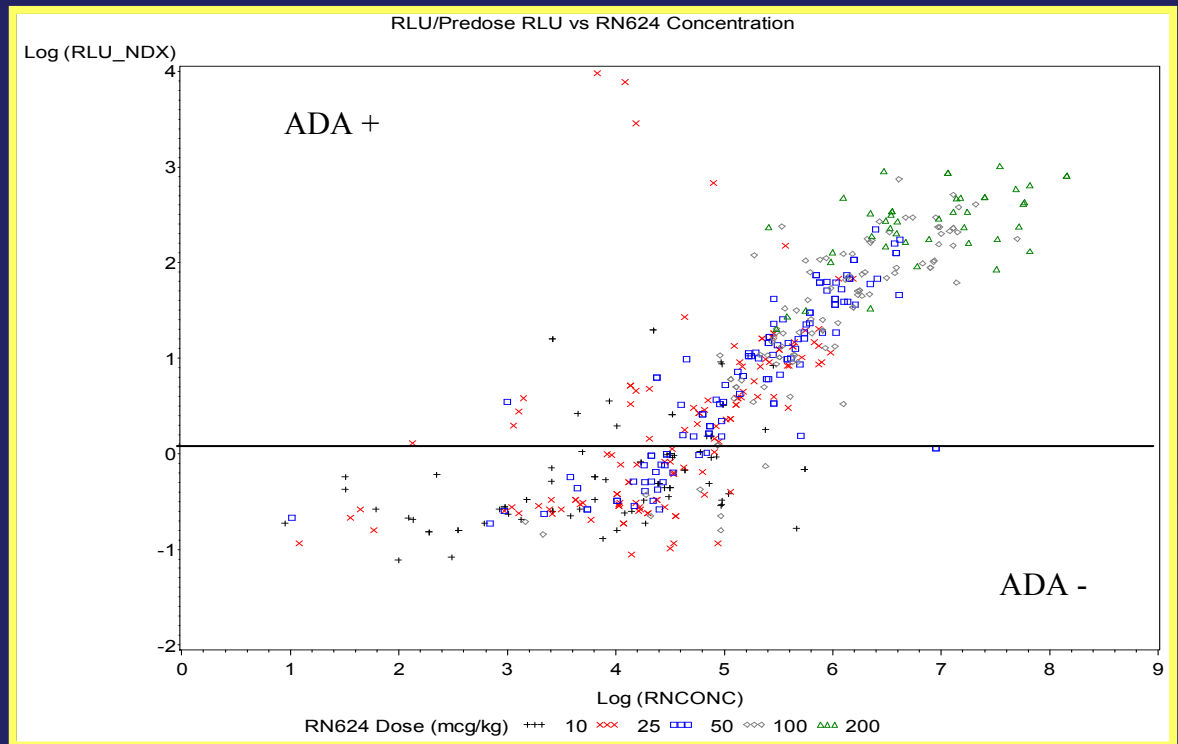
To test whether the observed results are true, or are false positives, further analyses were conducted:

1. Correlation of ADA with PK data
2. Analysis of PK samples collected prior to Study Day 5 (i.e. before one would expect an ADA response)
3. Evaluation of whether NGF alone or NGF-drug complexes cause false positives
4. Use of a non-bridging ELISA format
5. Addition of soluble TrkA receptor (to bind NGF) to representative pooled samples from Study 1008 that produced a positive response

Study 1008: Comparison of ADA and PK Data

- For Study 1008, a comparison of ADA response and PK data revealed an increase in tanezumab plasma concentration with increased ADA.

This implies that the ADA positive results seen in Study 1008 are not real or, at least, not entirely due to ADA.



RN624 = tanezumab

Analysis of PK Samples Collected Prior to Day 5

- Day 5 samples from 2 patients in Study 1008 were pooled and tested in the ECL assay in order to determine whether NFG-drug complexes formed could cause false positives in the ECL assay
 - Results were negative. However:
 - 5 days may be too soon after dosing to allow the formation of NGF-drug complexes
 - High concentrations of drug in the sample may have caused interference in the assay

NGF and NGF-Drug Complex Evaluation (Mock Samples)

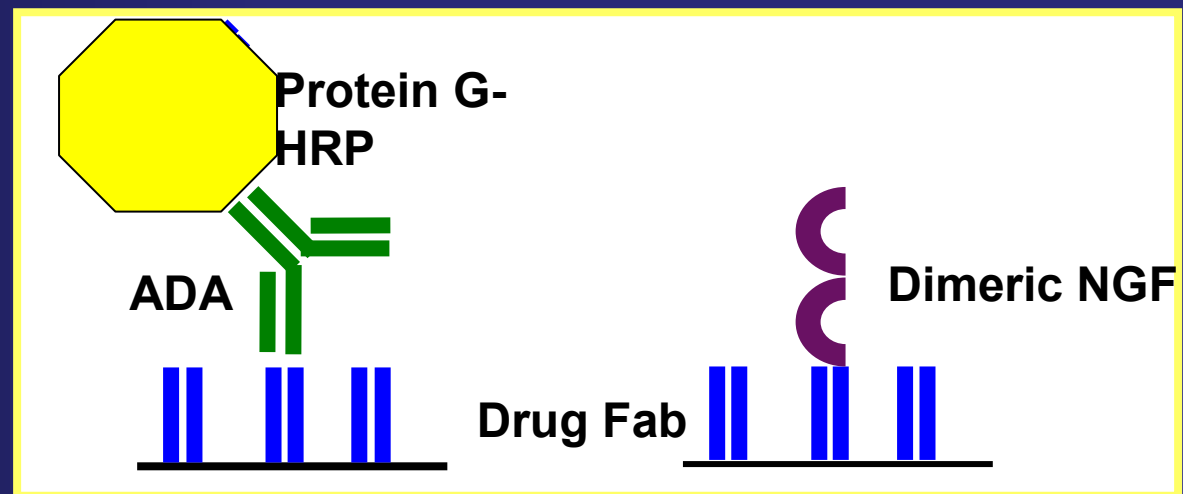
- NGF alone:
 - at concentrations 10x physiological levels gave false positives which increased with NGF concentration
- NGF-drug complex:
 - gave a lower response than NGF alone
 - however, at 100-1000x NGF physiological levels, NFG-drug complex caused false positives
 - the response decreased with increasing drug concentration

Thus, it is possible that NGF or NGF-drug complex causes a false positive result at NGF levels greater than physiological

Non-Bridging ELISA Assay

- Assay utilized Fab of tanezumab and protein G-HRP as detection reagent
- Results were negative for ADA (n =4) and there were no false negatives in the presence of NGF at 100x physiological levels (spike in)
- However, the assay was ~100x less sensitive than ECL

Therefore the results are not conclusive that we do not have ADA in our samples



Addition of TrkA to Presumptive ADA Positive Samples

- TrkA binds to NGF and competes with drug for NGF binding
- Addition of TrkA to pooled samples from 4 patients who tested ADA positive in Study 1008 resulted in a $\geq 50\%$ decrease in assay signal with no effect on the assay positive control

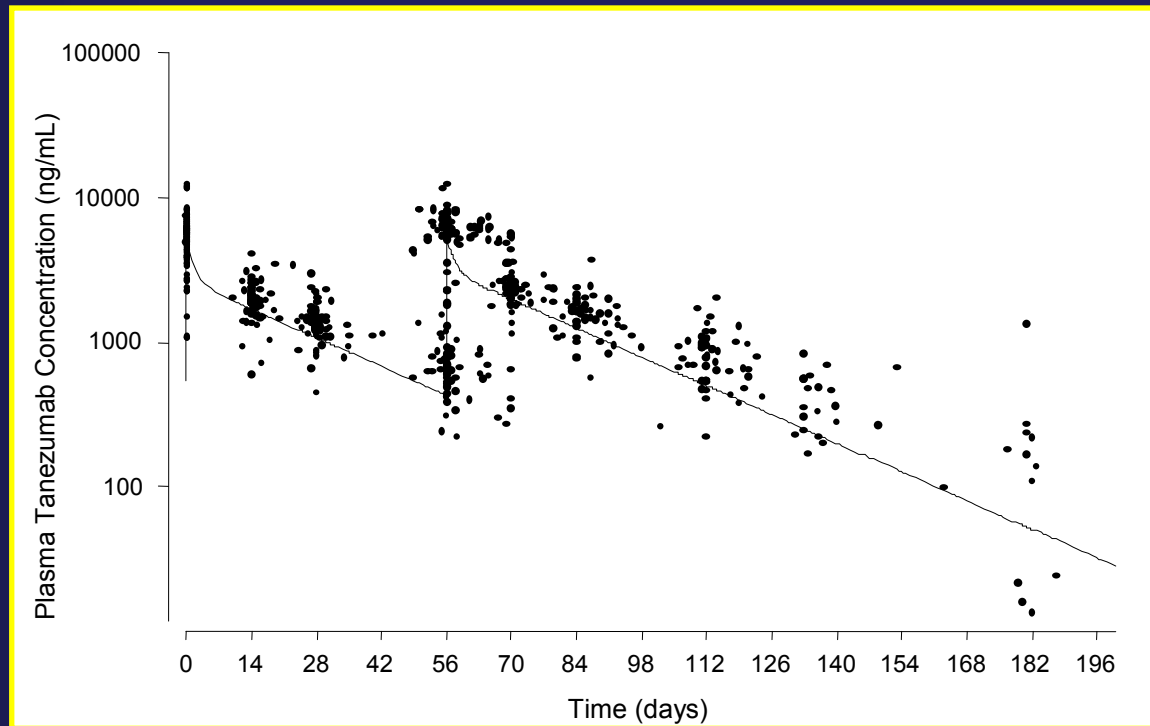
Thus, the presumptive positive ADA results were likely due to NGF and/or NGF-drug complexes

	TrkA (ng/mL)			
Sample	40000	20000	10000	0
1	980	1231	1580	4252
2	982	1218	1599	4388
3	1128	1410	1805	5408
4	1019	1242	1554	4028
Control	15711	15604	15617	13720

Plate cut point = 574

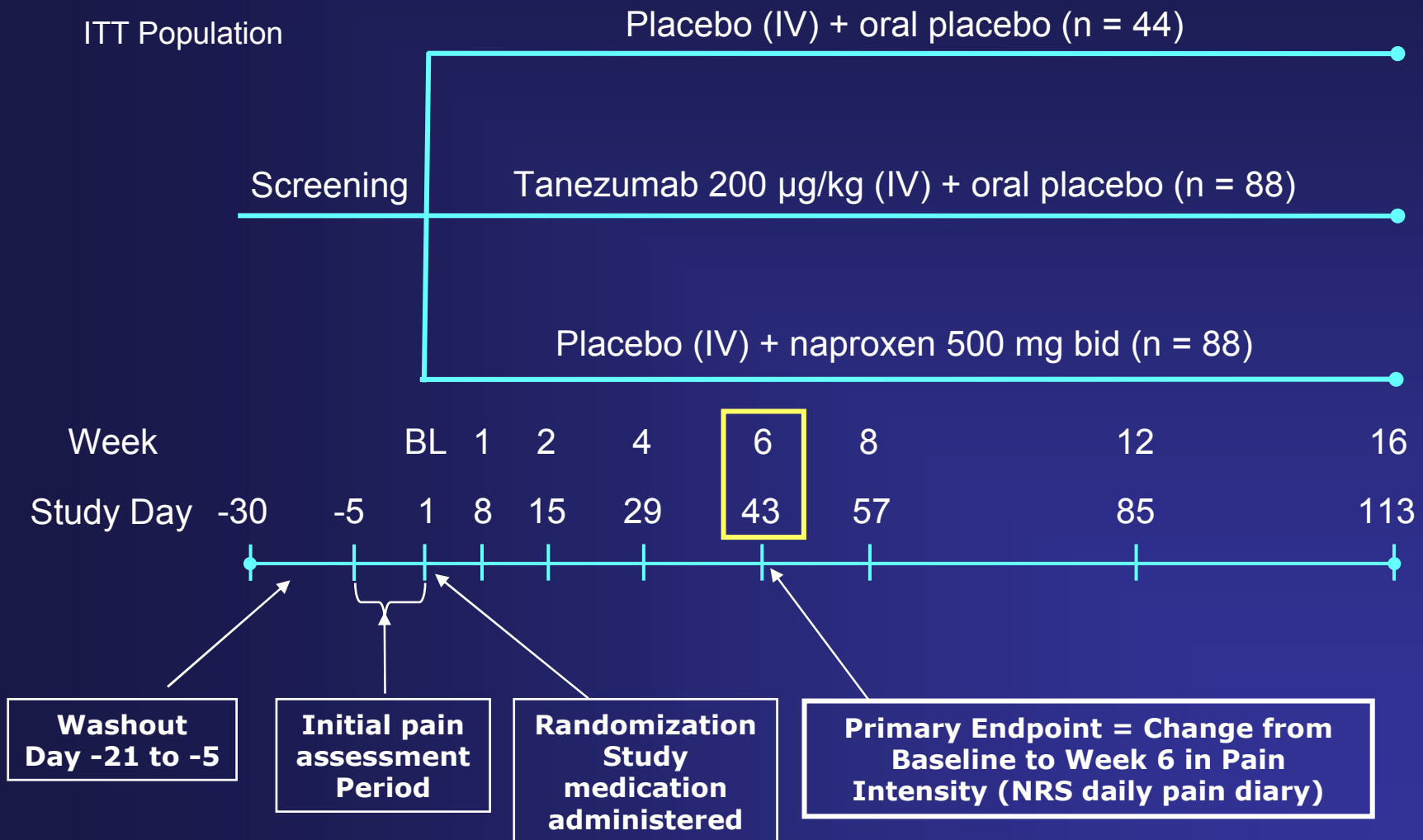
Study 1008: PK Results

Tanezumab plasma concentration vs. time following single doses of 200 $\mu\text{g}/\text{kg}$ on Days 1 and 56



Using the technique of nonbridging ELISA with FAB capture, no ADA was detected in Study 1008, in contrast with the bridging ECL assay

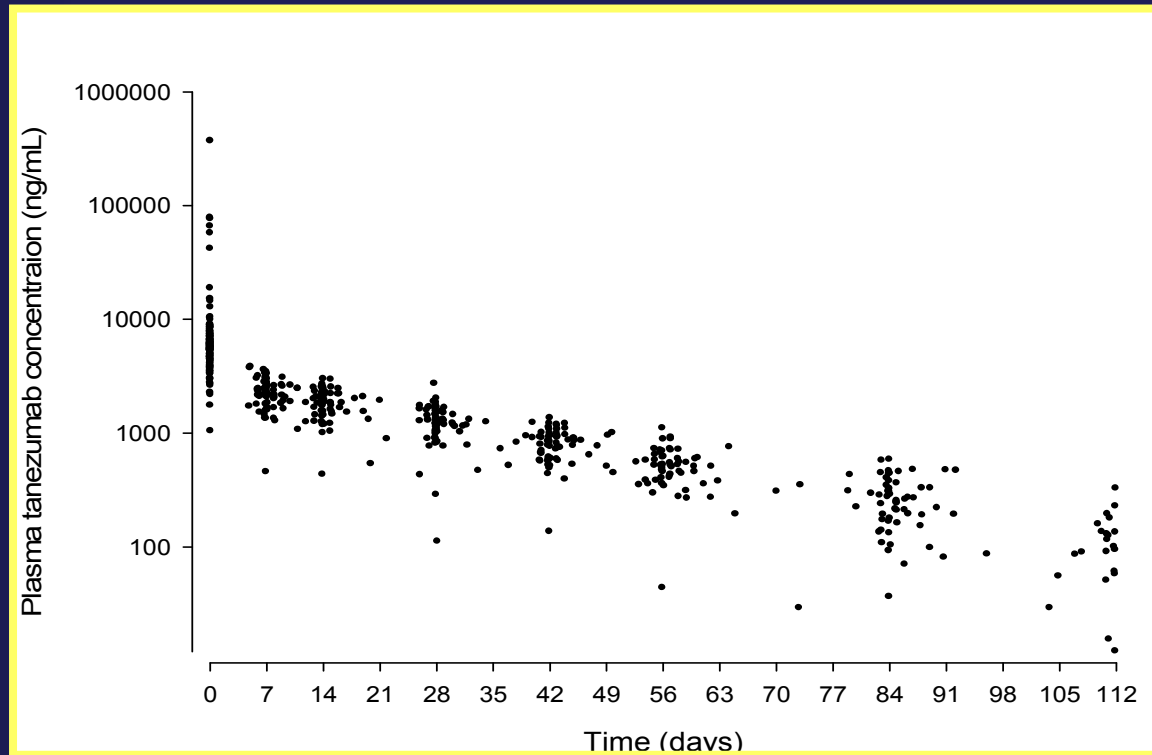
Study 1004[†]: Design



[†]Phase II study in patients with chronic lower back pain

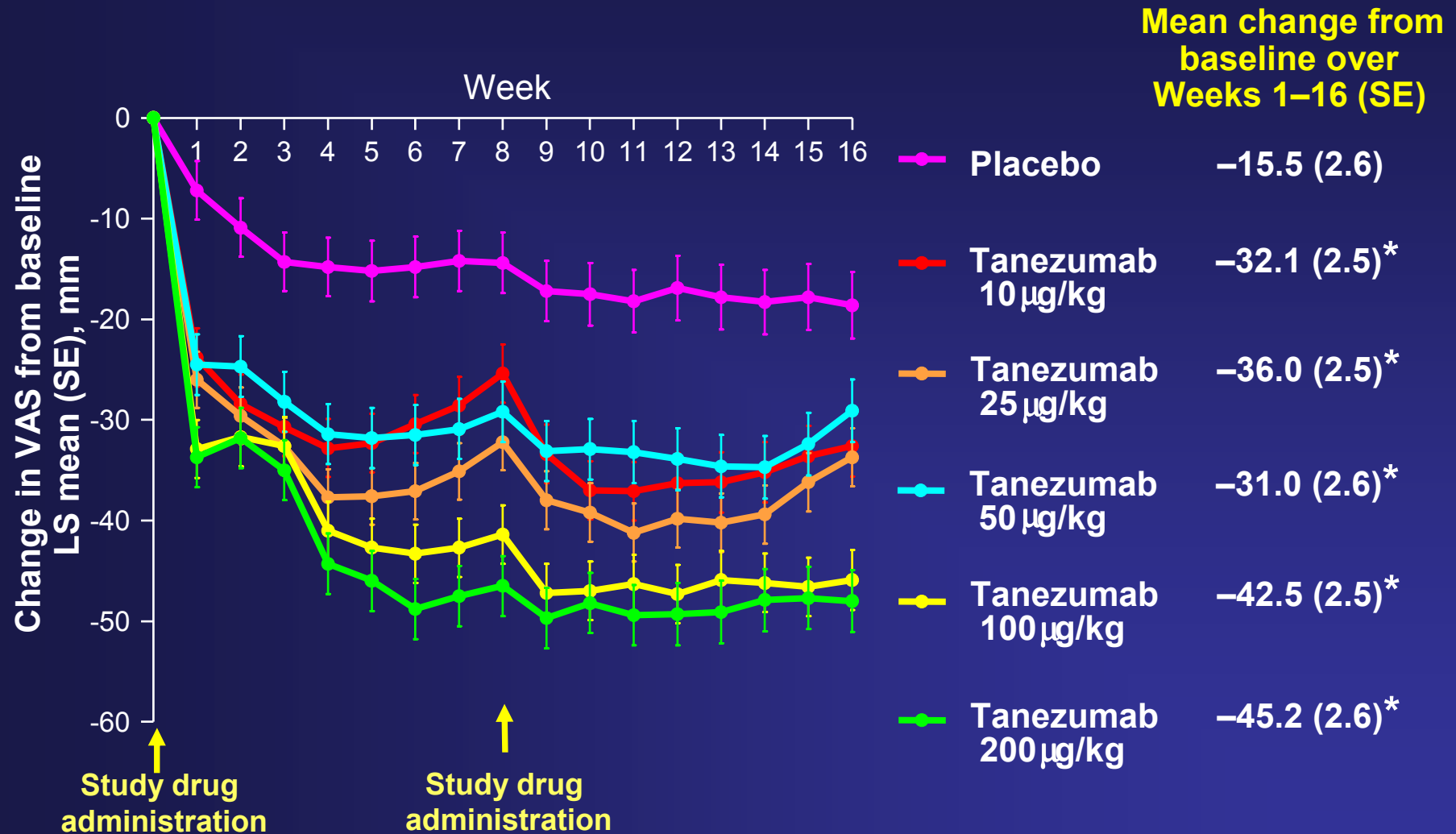
Study 1004: PK Results

Tanezumab plasma concentration vs. time



- PK results are consistent with what is typically observed for an endogenous IgG₂ antibody
- Systemic clearance in the range of 1-4 mL/kg/day; a terminal half-life of approximately 21 days; and a small steady-state volume of distribution imply no ADA formation
- No ADA was detected in Study 1004 using the technique of nonbridging ELISA with FAB capture

Study 1008: Efficacy Results



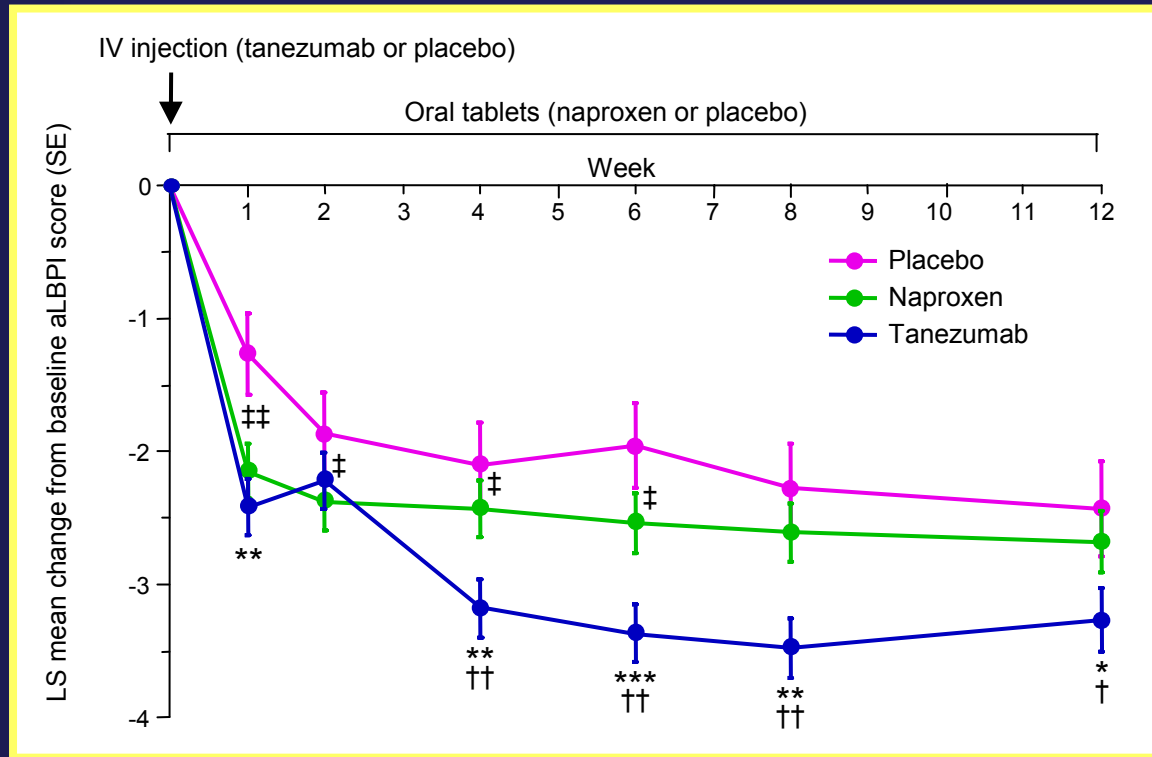
*P<0.001 vs. placebo

LS, least squares; SE, standard error; VAS, visual analog scale

Knee pain range = no pain (0) to extreme pain (100)

Study 1004: Efficacy Results

Least Squares Mean Change from Baseline[¶] in Average Low Back Pain Intensity by Study Week



Tanezumab vs. placebo: *P<0.05; **P<0.01; ***P<0.001

Tanezumab vs. naproxen: †P<0.05; ††P<0.01

Naproxen vs. placebo: †P<0.2; ††P<0.01

aLBPI, average Low Back Pain Intensity; IV, intravenous; LS, least squares; SE, standard error

[¶]Mean score at baseline derived from aLBPI scores over the 5 days prior to randomization and those at all time points post-baseline derived from aLBPI scores over the preceding week

Clinical Studies: Safety Results

- In both Studies 1008 and 1004 tanezumab was generally well tolerated with a safety profile that was consistent with no ADA formation
- No generalized immune effects, characteristic of ADA formation, such as hypersensitivity were observed
- The most common adverse events among patients who received tanezumab were:

Study 1004

arthralgia (14.8%)

headache (11.4%)

myalgia (8.0%)

Study 1008

headache (8.9%),

upper respiratory tract infection (7.3%)

paresthesia (6.8%)

Summary

- Positive ADA results obtained using the bridging ECL assay in Study 1008 are not real as demonstrated by:
 - No correlation with PK data
 - False positives due to NGF or NGF-drug complexes
 - Attenuation of the ADA signal in the presence of TrkA

Therefore false positive results were due to complex formation ⇒ a bridging assay format cannot be used

- When study samples were re-analyzed using the non-bridging Fab capture ELISA format
 - 7% presumptive positive
 - All samples were confirmed negative
 - Moreover, the efficacy and safety results from the clinical studies are consistent with the conclusion that positive ADA results are not real

Peri/Postnatal Study

- **Objective**

- To test for ADA in breast milk post delivery and in serum during gestation and post delivery in mother and offspring

- **Methods**

- A bridging ELISA assay that had been used for all toxicology programs with no issues with false positives as seen in human studies

- **Results**

- Pregnant Control monkeys had higher signals (false positives) with assay as gestation progressed
- Increased levels of NGF were suspected (there are conflicting reports in the literature regarding pregnancy and NGF levels) and thus gestation specific cutoff points had to be set in order to analyze samples
- Using gestation specific cutpoints the data correlated well with PK data but without this adjustment the data was hard to interpret

Conclusions

Clinical

- Non-bridging ELISA with Fab capture has been shown to produce results consistent with the clinical data
 - It is therefore sufficient for future ADA analysis
 - However, it could potentially miss ADA generated against the Fc portion of the drug
 - The FDA could request that an Fc assay be developed

Nonclinical

- Assays used for toxicology studies may not be appropriate during pregnancy if the target has the potential to change during pregnancy
 - Assays may need to be modified for gestation day-specific cutpoints in order to assess generation of ADA versus false positives
 - These false positives may be due to NGF; however since NGF was not measured this is a hypothesis at this point

Conclusions (continued)

- The challenges encountered in this program demonstrate the difficulty of understanding the true physiological matrix one will receive with actual study samples
- In particular, knowledge of target levels in different pain indications or in pregnant animals may not be well known
- In this particular program an assay for target was not part of the program so levels of total/free target were not known. An NGF assay has since been developed

Thus, even if mock samples are made to try to mimic what one might expect, it is only when actual study samples are received that one may encounter unexpected issues.