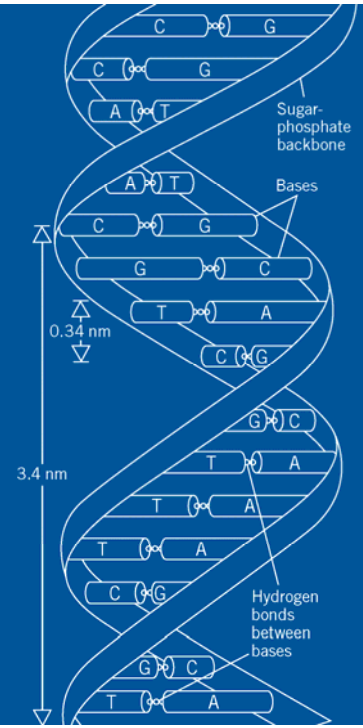

Target/drug interference considerations in immunogenicity assessment

Eric Wakshull, PhD
Senior Scientist
March 9, 2016
EMA Workshop on Immunogenicity

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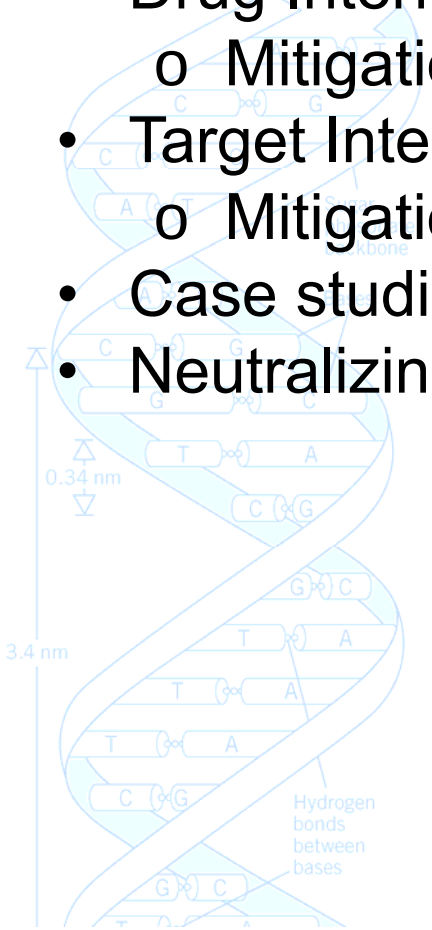
Outline

- Basic Immunogenicity screening assay
- Drug Interference
  - Mitigation strategies
- Target Interference
  - Mitigation Strategies
- Case studies
- Neutralizing antibody assays (time permitting)



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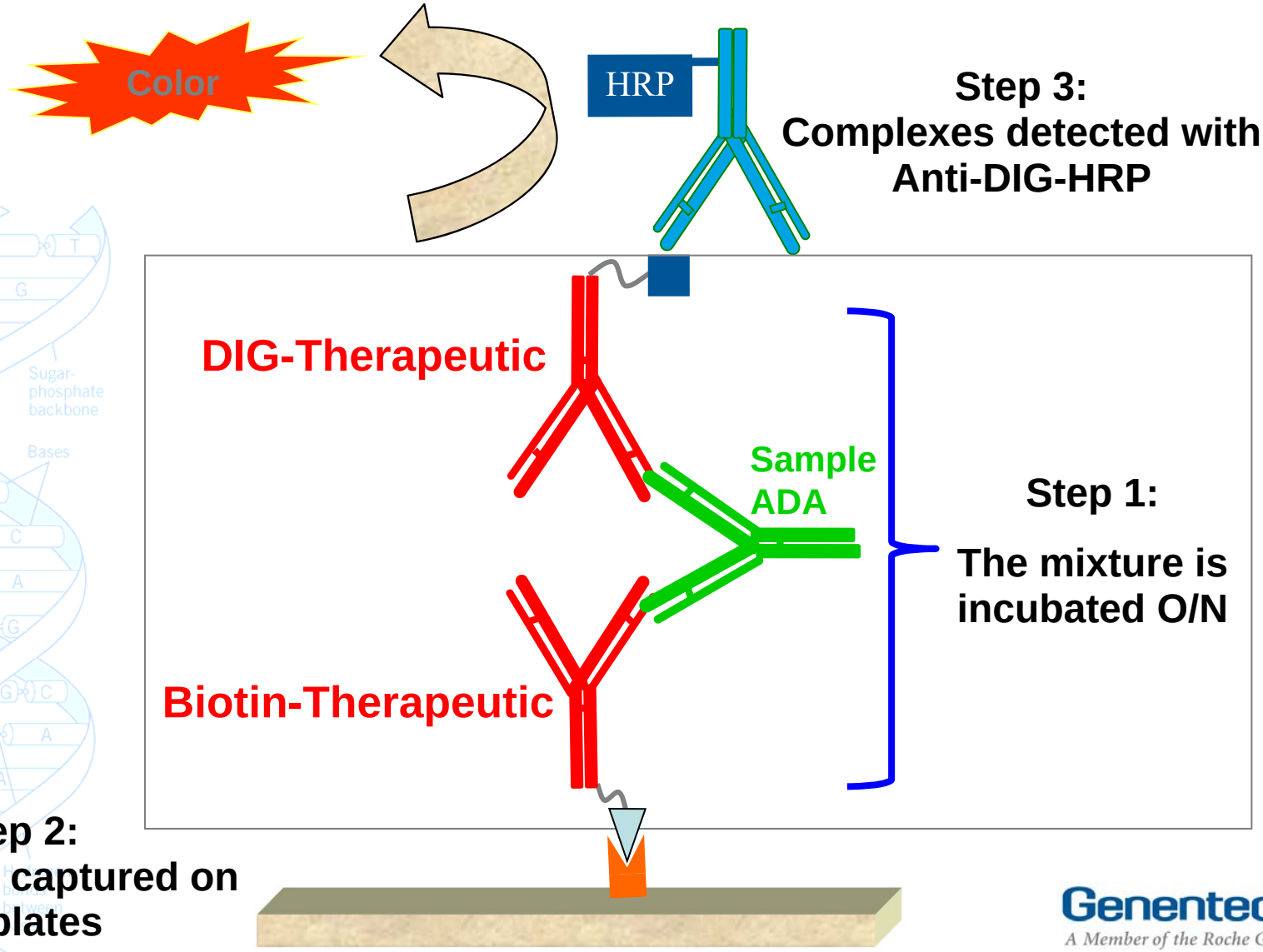
Biotin/DIG-Based Homogenous Bridging ELISA



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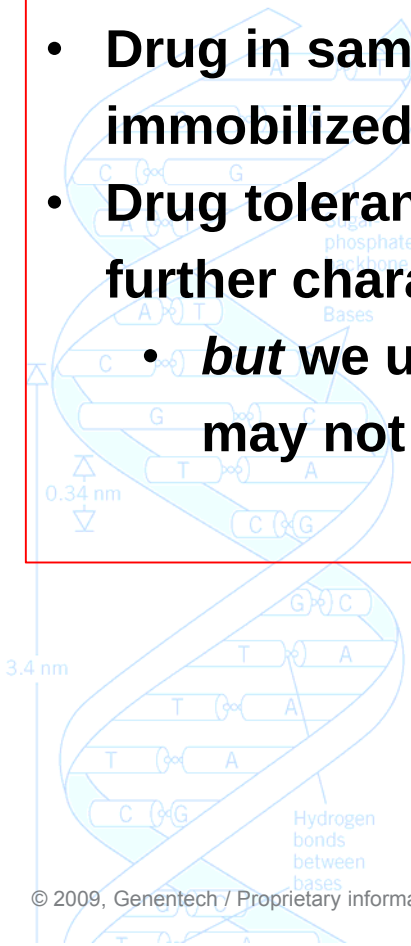
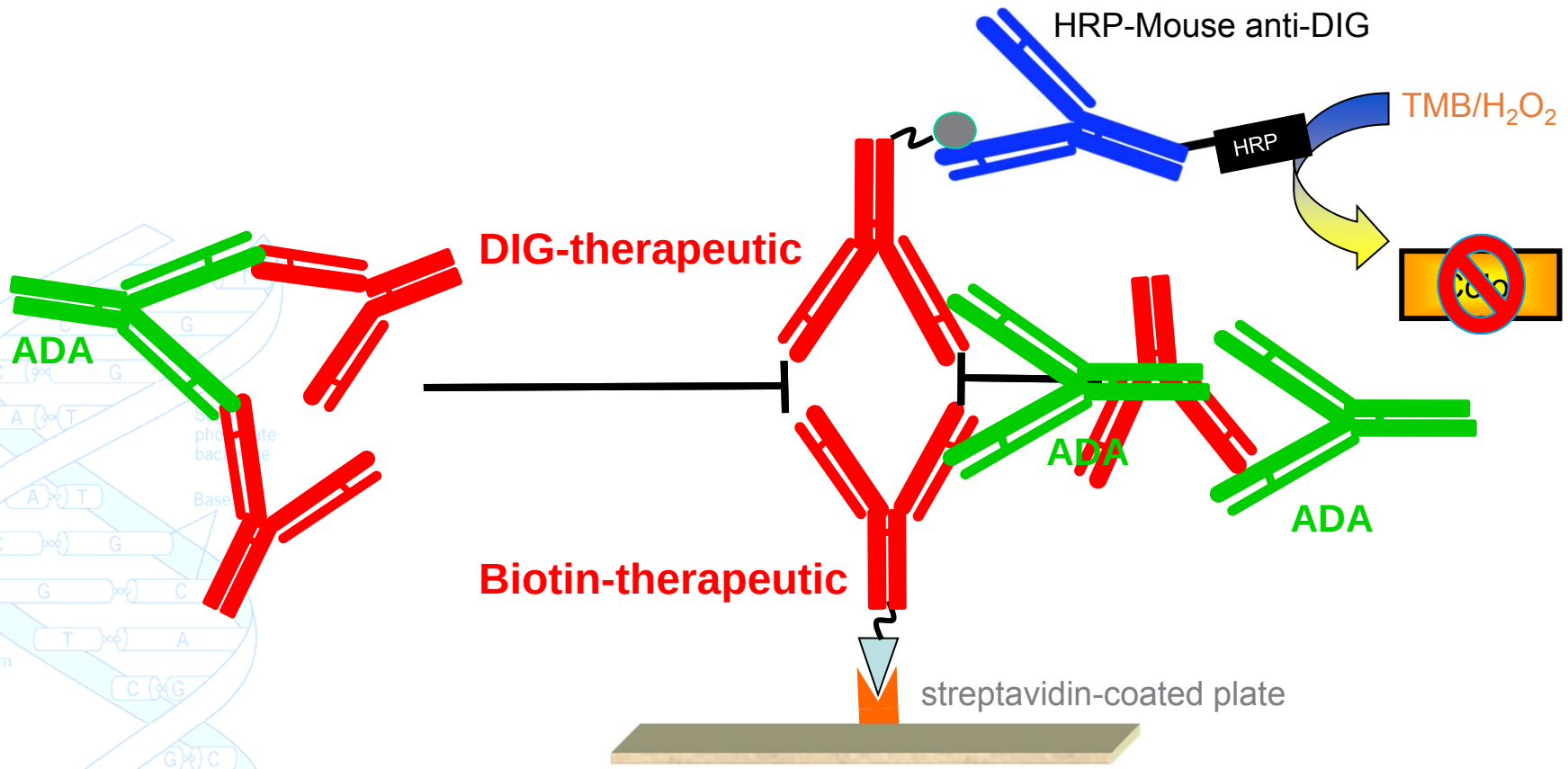
Drug Interference

Drug interference:

- **Drug as capture and/or detection reagent in either bridging or sandwich format**
- **Drug in sample will *compete* for ADA binding with labeled (or immobilized) drug→False negative**
- **Drug tolerance commonly evaluated in assay development and further characterized in validation**
  - ***but* we use a surrogate, usually high affinity, positive control—may not be representative of a patient immune response**



Drug Interference in ADA Assay



Unconjugated drug from samples can interfere in detection of ADA by competition with labeled (or immobilized) drug

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Drug Interference

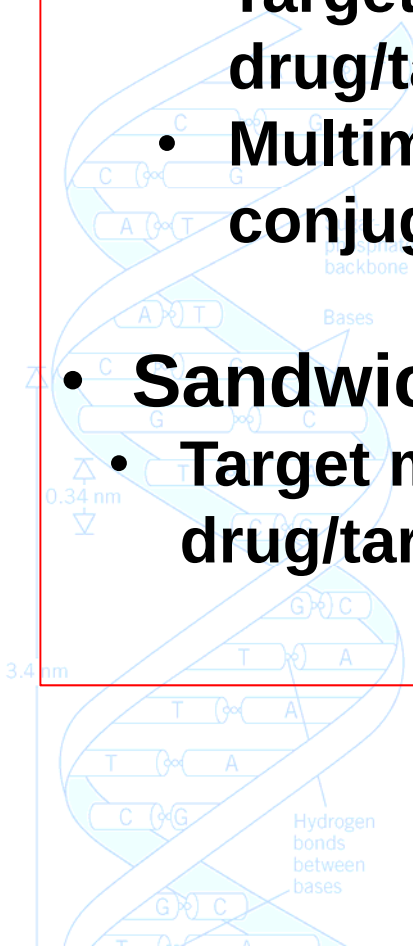
Mitigation Strategies

- Obtain samples with low/no drug concentrations (washout)
  - Not always feasible
- Optimize assay for drug tolerance
  - For bridging assays, generally increase sample dilution and/or conjugate concentration: Have achieved >100-fold up to 900-fold molar excess in drug tolerance ([drug]/[ADA]}
    - Often a trade-off with assay sensitivity
  - Longer incubation times (allow “assay” drug to outcompete “sample” drug)
- Acid dissociation
  - Basic method (with permutations): Low pH dissociates ICs
  - Provides some increased drug tolerance
  - Unknown effect on patient sample ADAs (low pH denaturation) which may not be apparent using PC to develop optimal conditions
  - May release sol Target from Drug/Target complex→interference

Target Interference

Soluble Target interference:

- **Bridging immunoassays**
  - **Target may inhibit ADA binding at or near the drug/target interaction domain→False negative**
  - **Multimeric target may form bridge with drug conjugate(s)→False positive**
- **Sandwich immunoassays**
  - **Target may inhibit ADA binding at or near the drug/target interaction domain→False negative**



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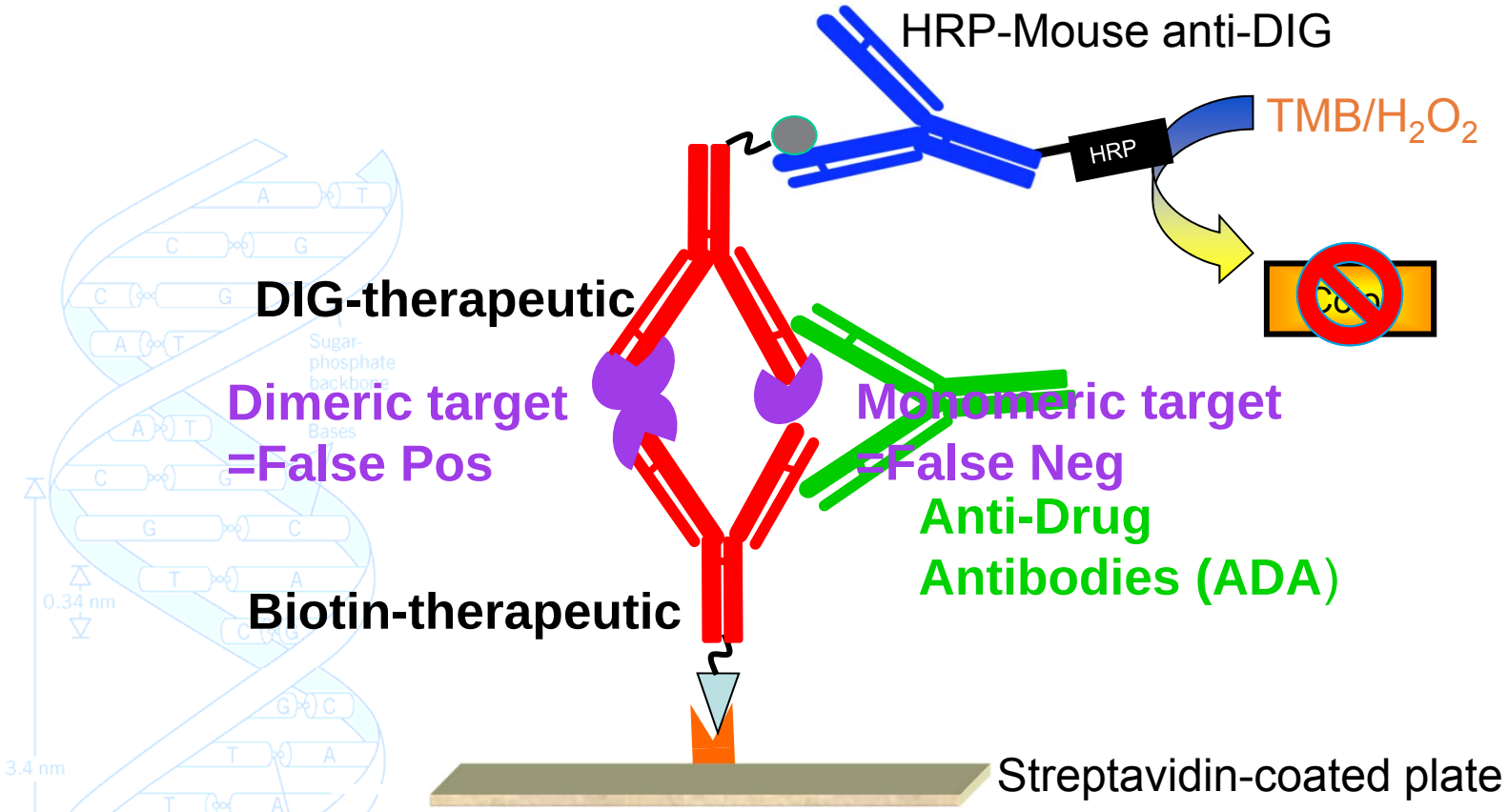
Target Interference: Bridging ELISA



Mitigation Strategies Considered

- Deplete sTarget
  - –Dissociate drug/sTarget complexes (if necessary)
  - –Affinity capture/remove sTarget and/or complexes
  - –Operationally complex

- Block drug/sTarget interaction
  - – Murine anti-drug Ab with different CDR
  - – Soluble recombinant Receptor
  - – Lectin
  - – Operationally simple—>add to diluent buffer
  - – Caveat: Reagent may not be readily available, often needed in large quantitites.

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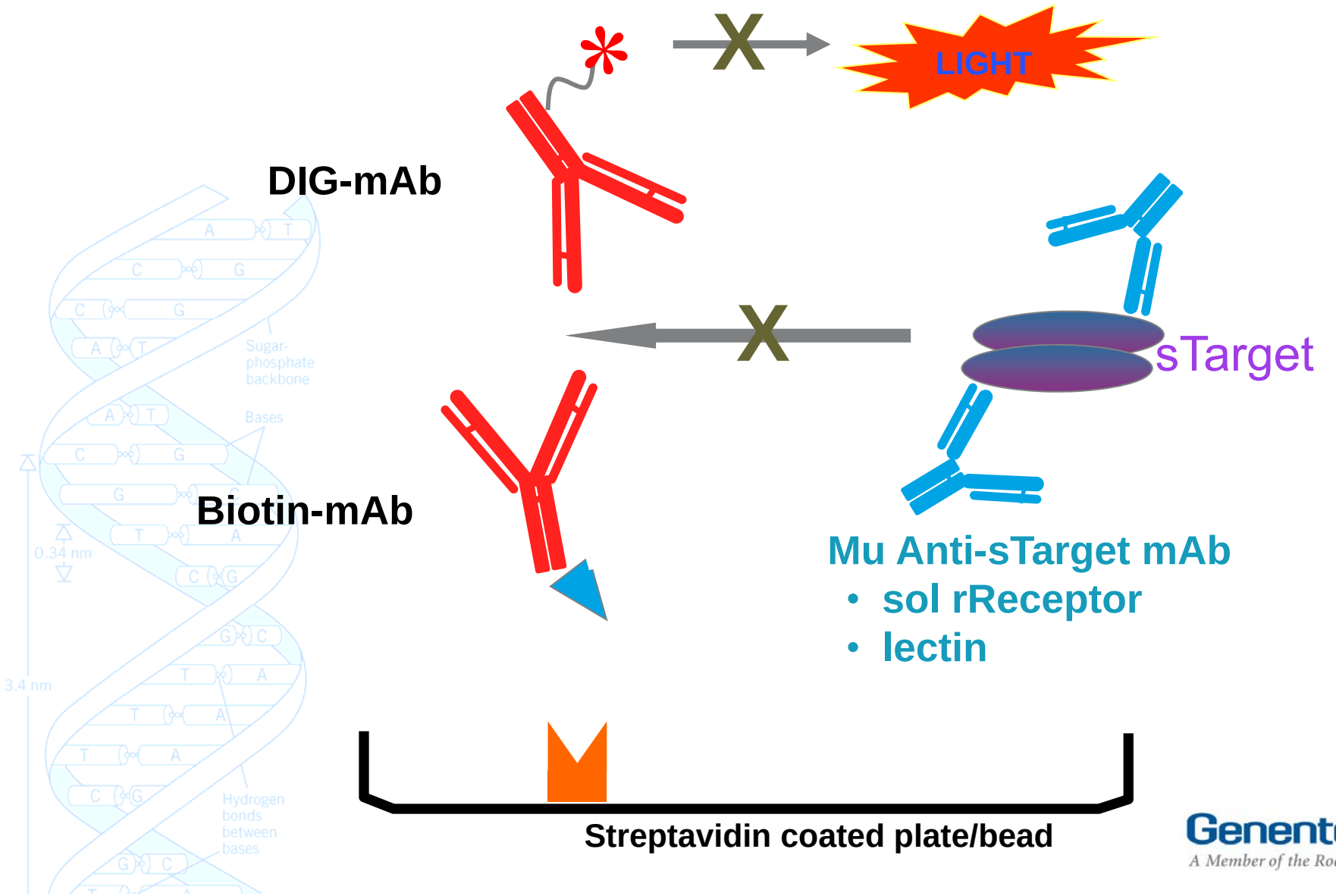
sTarget Blocking Eliminates False Positives (or False Negatives)



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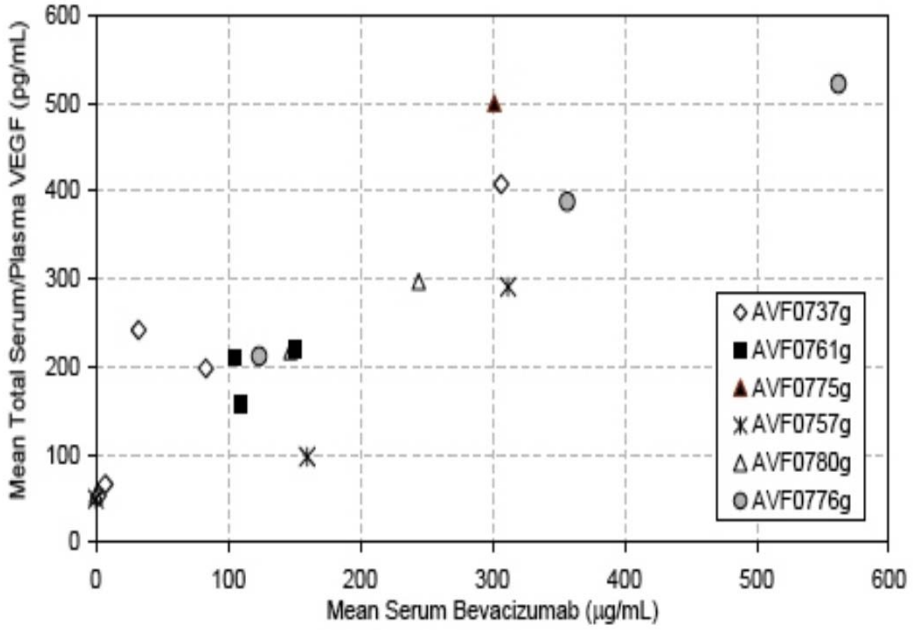
Example 1: Interference from VEGF, a dimeric vascular growth factor

- VEGF is a dimeric vascular growth factor targeted by Bevacizumab
- **Initial clinical results: 94% ADA+ (??)**



- Baseline VEGF levels vary by disease & severity: ~100-1500 pg/ml.
- Post-bevacizumab total VEGF levels can increase ≥ 10x, PK effect
- Cause of high incidence due to VEGF interference?

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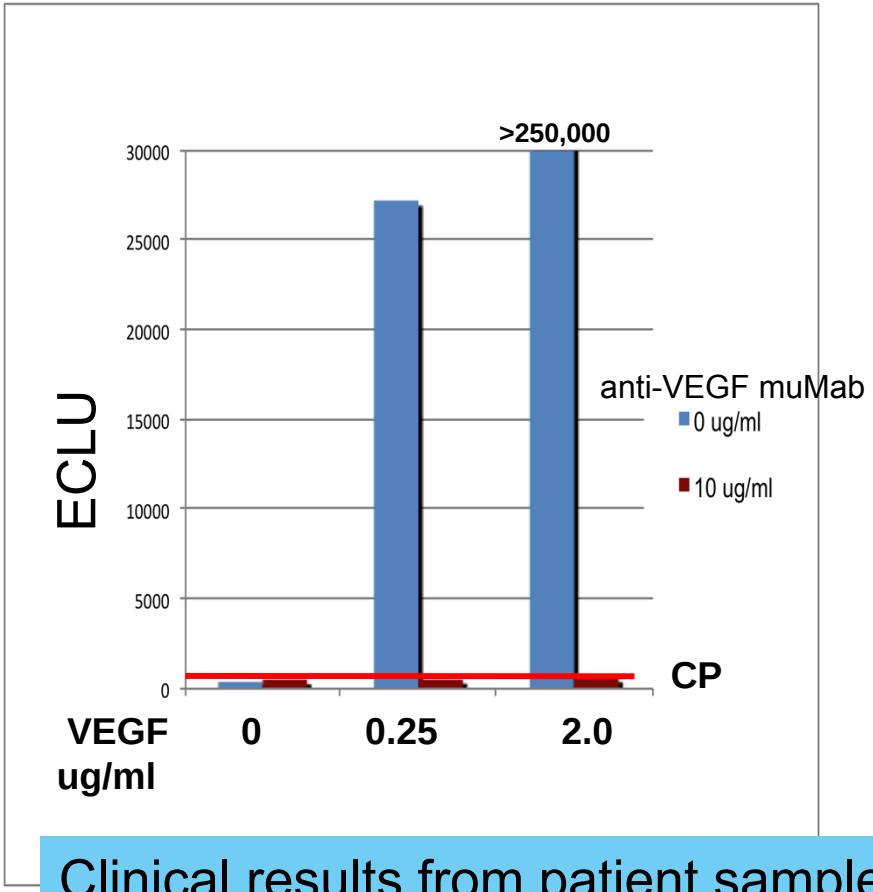
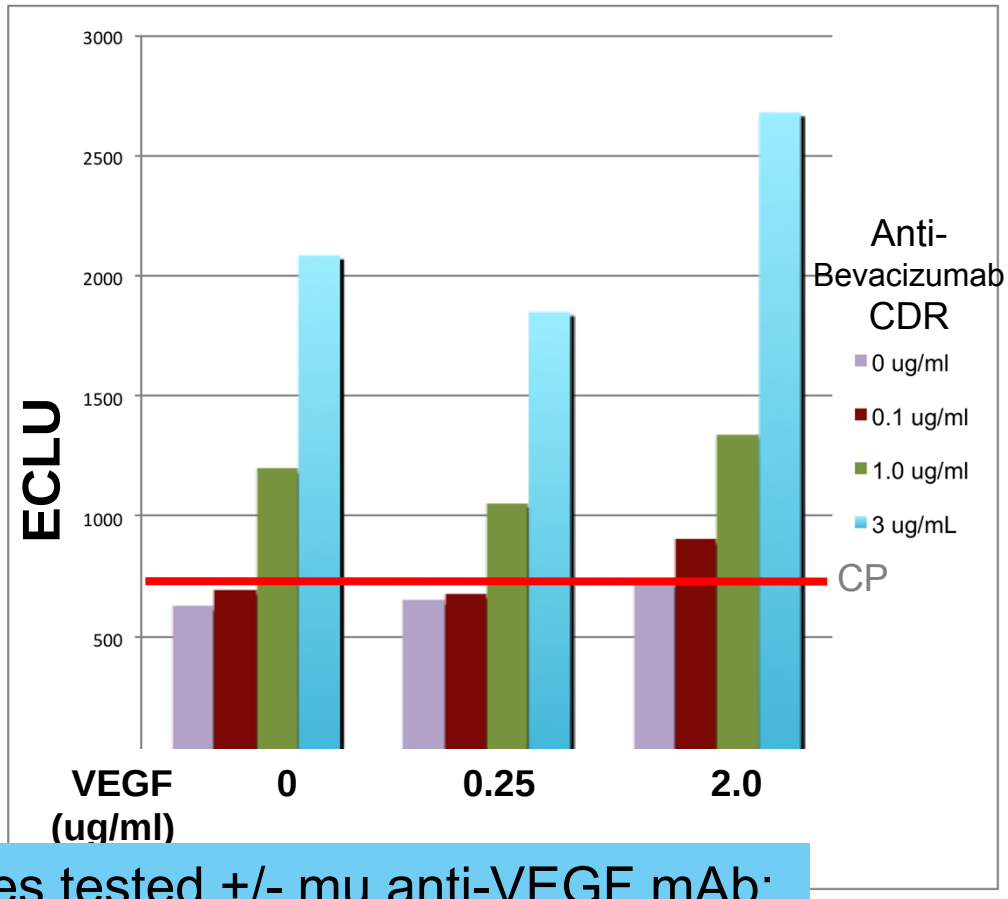
Murine anti-VEGF mAb blocks VEGF False Positives but not ADA

VEGF-induced signals are ↓ by anti-VEGF muMab



ADAs are detected in the presence of anti-VEGF muMab



Clinical results from patient samples tested +/- mu anti-VEGF mAb:

- No blocker: 9/18 patients (50%) Ab+
- With blocker: 1/18 patients (5.5%) Ab+

➢ Final ADA incidence from 2 pIII trials: ≤1%(6/761; 8/1472)

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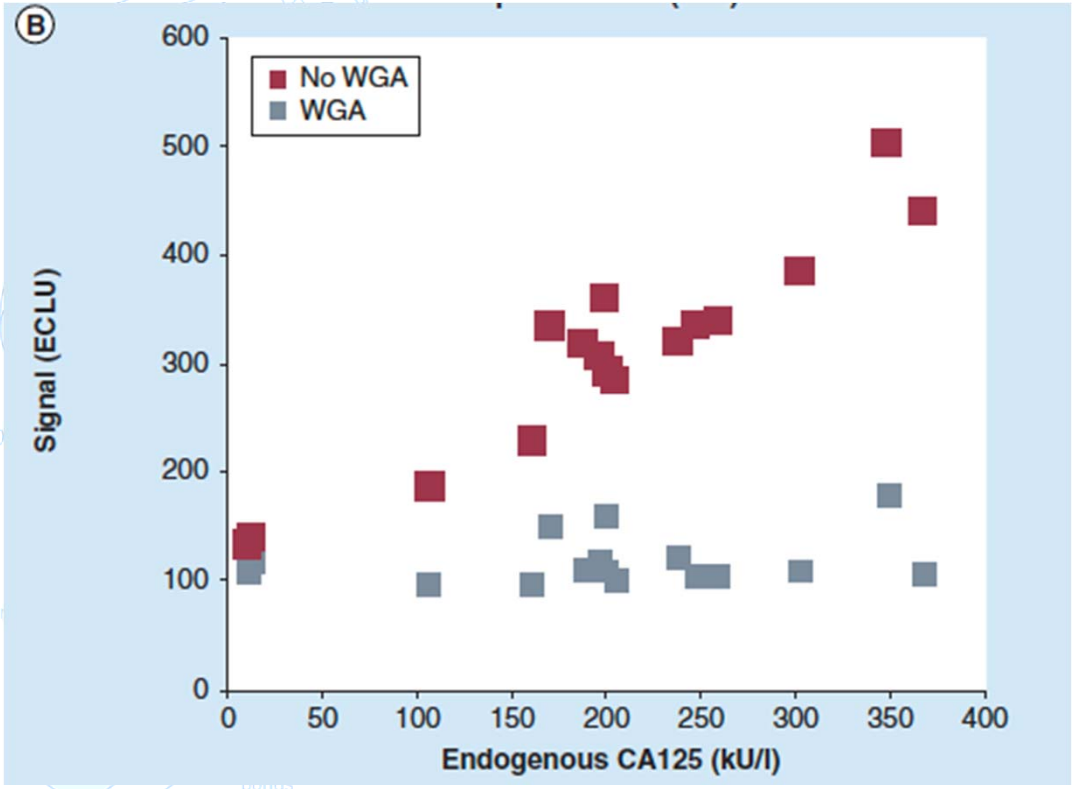
Example 2: Interference from multimeric CA125, Soluble fragment from MUC16

- Anti-MUC16 targets membrane bound MUC16
- CA125 is a multimeric soluble proteoglycan fragment of membrane MUC16
- Biomarker for OvCa
- Present in serum at levels >11,000 kU/L from clinical samples



Carrasco-Triguero etal 2012

- ADA assay signal is proportional to CA125 in samples, potentially producing False Positives
- CA125-induced signal inhibited by the lectin WGA.

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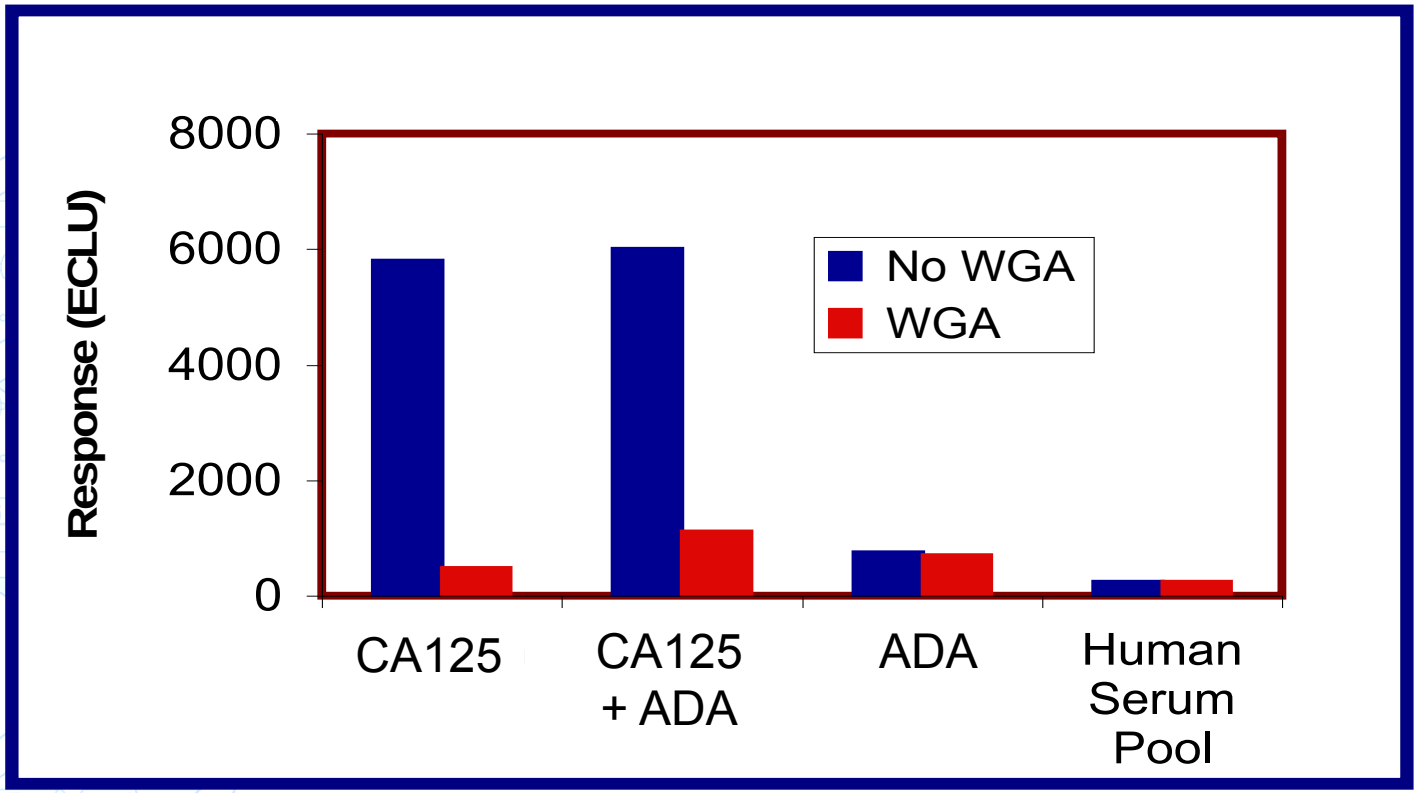
WGA Lectin Does Not Impact on the Detection of ADA

5000 kU/L* CA125 ± ADA were incubated ± WGA in the ADA assay

*source: patient-derived ascites



Drug/Target Interference in NAb Assays: En Brief

Nab assays: Cell-based and ligand binding-based
***Significant additional complexities* depending on drug MOA and signaling pathway**
For example:

MOA: Drug targets ligand for cell receptor or targets cell receptor, inhibiting ligand/receptor interaction

- **Potential interacting molecules**
  - **$Ligand_{Assay}$**
  - **$Cell\ Receptor_{Assay}$**
  - **$Drug_{Assay}$**
  - **NAb_{Sample}**
  - **$Drug_{Sample}$**
  - **$Soluble\ Receptor_{Sample}$**
  - **$Ligand_{Sample}$**

Assay Components

Derived from Samples

➢ **Outcomes (True/False, Negative/Positive) depends on the relative concentration and affinity of the interacting molecules**

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