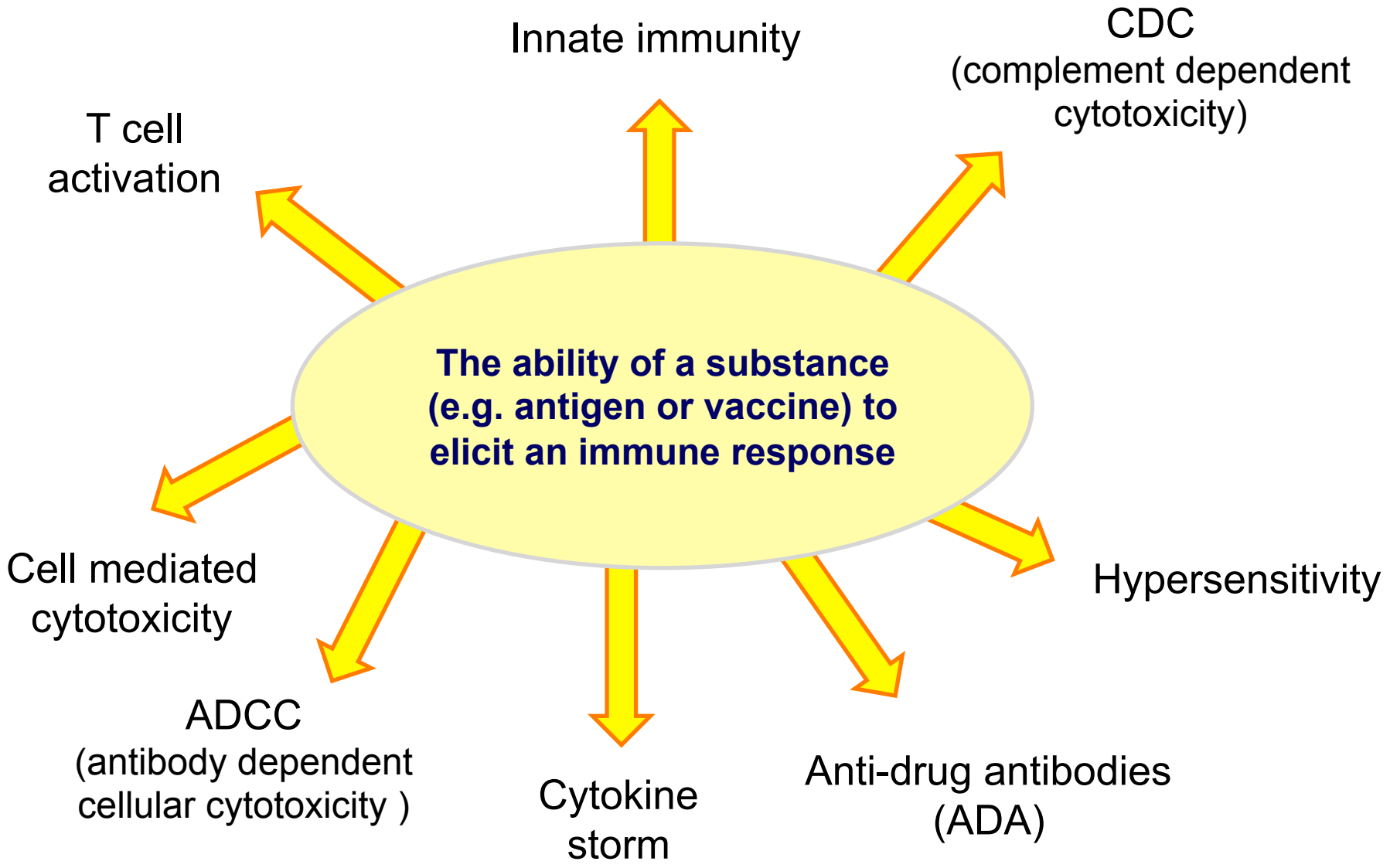
Neutralizing anti-drug antibodies

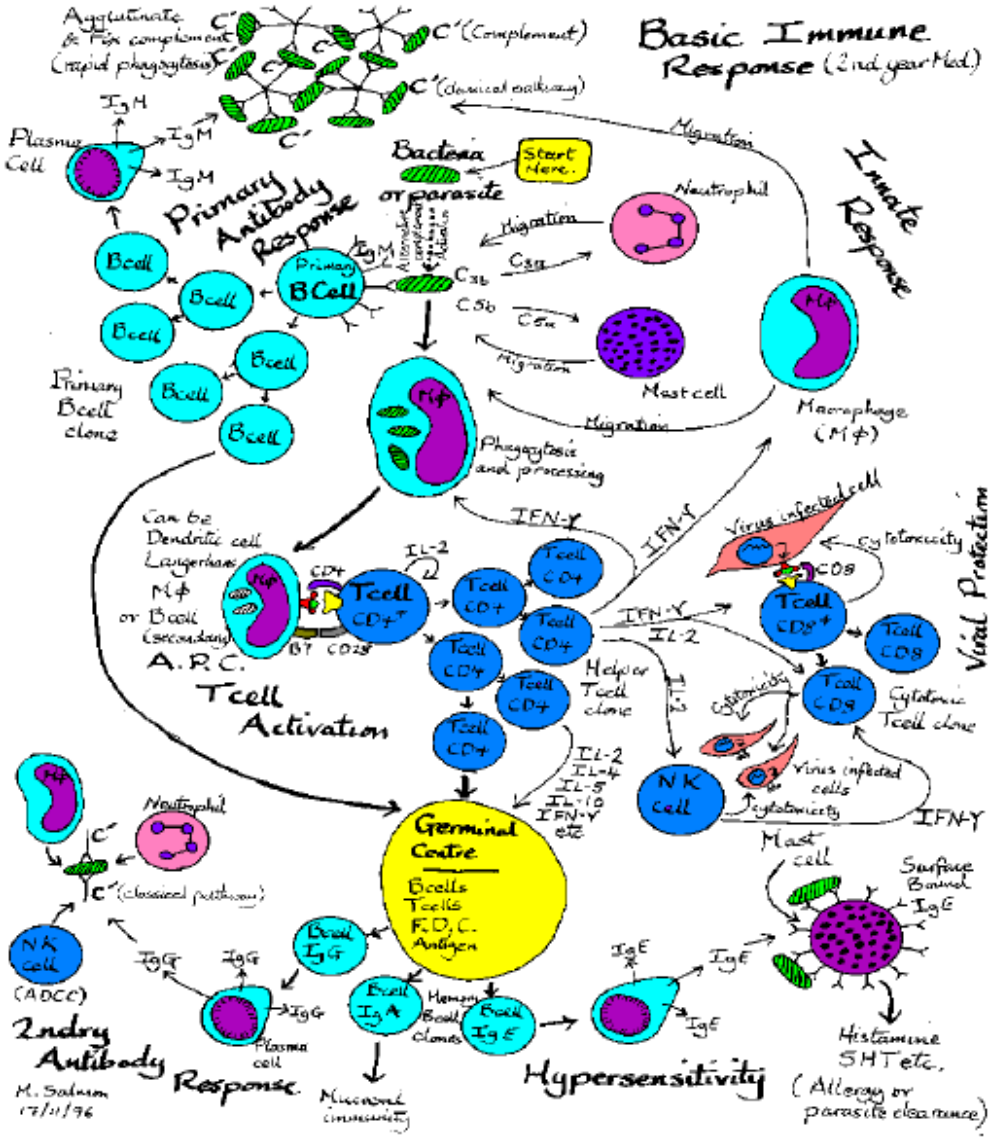
Emerging Trends and Clinical Impact

A. Kromminga

What is immunogenicity?



Complexity of the immune response



FDA says:

Because of the size of some clinical trials and the necessity of testing patient samples at several time-points, FDA recommends a multi-tiered approach to the testing of patient samples. ….

Neutralizing antibodies (NAB) are generally of more concern than binding antibodies (BAB) that are not neutralizing, but both may have clinical consequences.

Questions

Why are neutralizing antibodies (NAB) of more concern than binding antibodies (BAB) that are not neutralizing if both may have clinical consequences?

Do NAB assays more realistically reflect the situation in the body?

FDA says:

Generally, bioassays have significant variability and a limited dynamic range for their activity curves. Such problems can make development and validation of neutralization assays difficult and FDA understands such difficulties. Nonetheless, we will recommend such assays because they are critical to understanding the importance of patient immune responses to therapeutic proteins.

ADA Analysis

Screening ADA Assays

- ELISA
- ECL
- DELFIA
- Gyros
- FEIA
- RIPA
- SPR

Homogeneous

Heterogeneous

NAB assays

Cell based Assays

- Cell proliferation
- Biomarker
- Gene expression
- Gene reporter
- ADCC
- CDC

Non-cell based Assays

- CLBA
- SPR

Purpose of ADA vs NAB assay

Screening ADA assays

- Analytical sensitivity: < 500 ng/ml
- Clinical sensitivity: 100 %
- Clinical specificity: 95%
- Drug interference: n.d

NAB assays

- Analytical sensitivity: n.d.
- Clinical sensitivity: n.d.
- Clinical specificity: 100 %
- Drug interference: n.d.

Cell-based versus non-cell based NAB detection

IPM Biotech

FDA immunogenicity guideline, 2009:

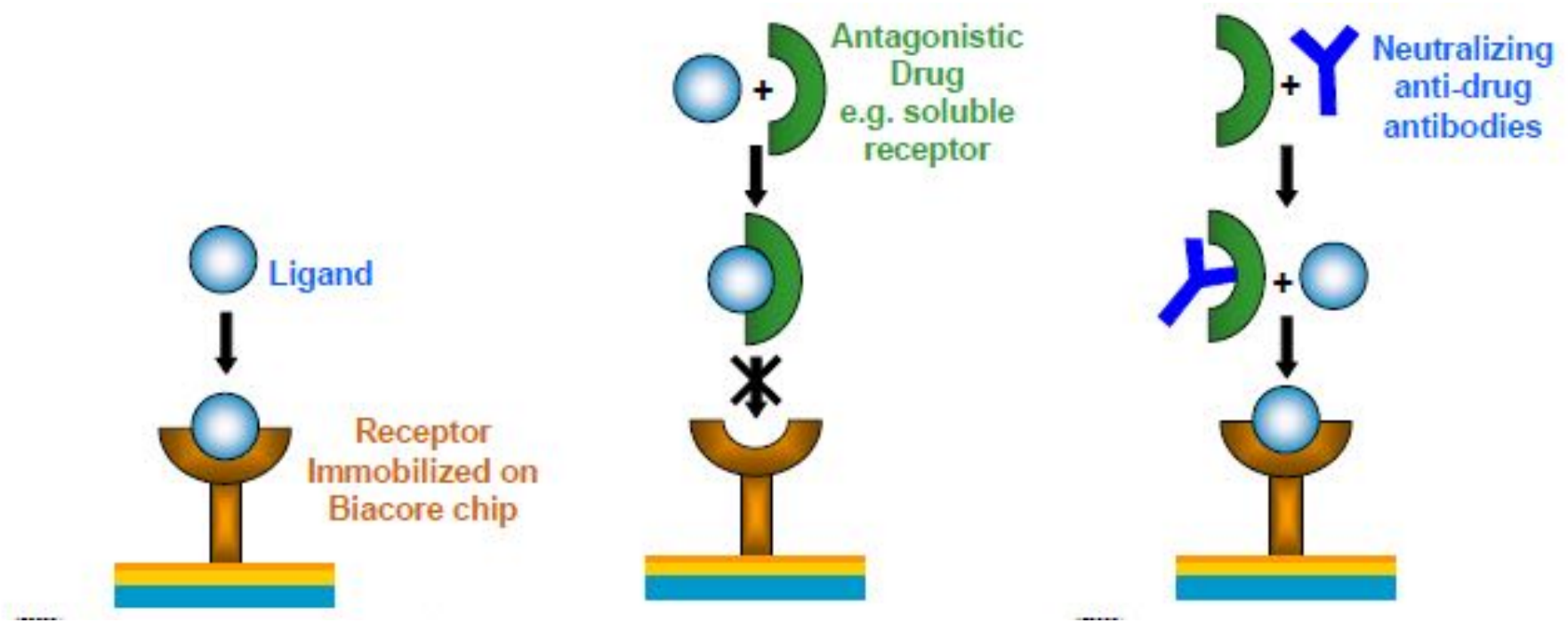
- Generally FDA considers that bioassays are more reflective of the in vivo situation and are recommended.
- For NAB assays, the bioassay should be related to product mechanism of action, otherwise the assay will not be informative as to the effect of NAB on clinical results.
- The development and validation of neutralization assays may be difficult and FDA understands such difficulties. Nonetheless, we will recommend such assays because they are critical to understanding the importance of patient immune responses to therapeutic proteins.

Cell-based versus non-cell based NAB detection

EMA immunogenicity guideline, EMEA/CHMP/BMWP/14327/2006:

- If neutralising cell-based assays are not feasible/available competitive ligand binding assays or alternatives may be suitable.
- However, when these are used, it must be demonstrated that they reflect neutralizing capacity/potential in an appropriate manner.

NAB analysis
by a competitive ligand binding assay



Validation data of CLB

	Run1		Run 2		Run 3		Run 4		Mean	
NAB	**Signal**	**%CV**	**Signal**	**%CV**	**Signal**	**%CV**	**Signal**	**%CV**	**Signal**	**%CV**
125	0.476	4.20	0.802	6.10	0.951	3.1	0.687	13.6	**0.729**	0.2
31	0.923	2.20	1.056	0.40	1.353	2.8	1.141	6.6	**1.118**	0.2
16	0.980	2.00	1.066	0.60	1.382	2.7	1.215	3.1	**1.161**	0.2
8	1.029	1.00	1.121	0.70	1.376	4.0	1.228	3.9	**1.189**	0.1
2	1.069	0.90	1.194	0.00	1.485	1.0	1.266	3.8	**1.254**	0.2
0	1.051	1.90	1.154	3.90	1.512	1,1	1.304	2.5	**1.255**	0.2
NC	1.142	2.60	1.212	1.80	1.476	2,9	1.134	1.2	**1.241**	0.1
blank	0.012	-	0.015	-	0.016	9.4	0.017	-	**0.015**	-

Requirements for cellular assays

- Suitable cell line
- Linearity
- Interference
- Cut point
- Sensitivity
- Specificity
- Precision
- Robustness
- Ruggedness

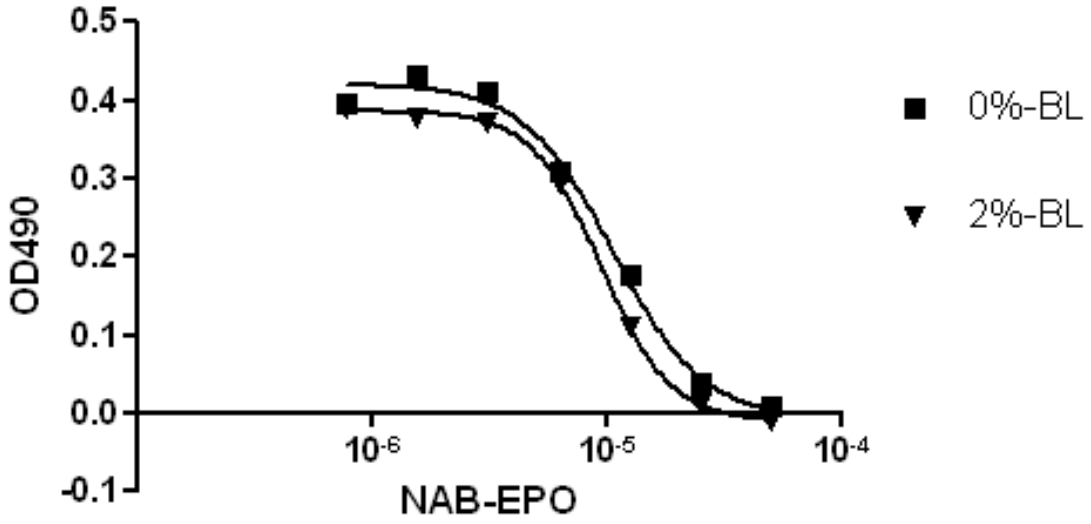
Example: Erythropoietin

- recombinant human protein drug with a non-redundant endogenous counterpart
- used for the treatment of renal and non-renal anemia

Antibodies against EPO

1. Pure red cell aplasia (PRCA) after initial successful erythropoietin therapy
   - Progressive, transfusion-dependent anemia
   - Almost total lost of erythroid progenitor cells with normal BM
2. Antibodies against erythropoietin
3. No endogenous erythropoietin detectable

NAB-EPO Detection



Based on the inhibition of drug-specific proliferation in the presence of ADA.

Interference w/ IL-3

	IL-3 [pg/mL]				
	0	**50**	**100**	**500**	**1,000**
% inhib	95	96	94	82	68
Diff [%]	13%	14%	10%	14%	13%
AC	≤ 30%	≤ 30%	≤ 30%	≤ 30%	≤ 30%

There is no interaction with IL-3 leading to a significant change of inhibitory effect of anti-EPO antibodies. Average IL-3 level in normal healthy subjects is 27 pg/ml

Sensitivity/LLOD

Monkey anti-EPO (ng/ml)	%-Inhibition				SD	%CV	AC
	1	2	3	mean			
200	50.1	52.2	55.3	52.5	2.1	4.1	≤ 30%
100	71.9	70.6	71.9	71.5	0.6	0.9	≤ 30%
50	48.5	52.7	56.8	52.7	3.4	6.4	≤ 30%
25	21.0	17.1	28.3	22.2	4.6	20.9	≤ 30%
12.5	7.3	6.0	13.0	8.7	3.0	34.8	≤ 30%
6.25	6.5	0.3	7.3	4.7	3.1	67.1	≤ 30%
3.13	3.9	6.2	4.9	5.0	1.0	19.0	≤ 30%
1.55	-0.1	4.2	8.0	3.8	3.6	94.9	≤ 30%
0	-5.2	2.4	2.9	0.0	3.7	-	≤ 30%

Precision

Intra-Assay

	1	2	3	4	mean	SD	%CV	AC
PC1	98.6	95.1	100.8	98.0	**98.1**	2.6	2.1	≤ 30%
PC2	37.2	35.5	37.7	37.1	**36.9**	0.8	2.6	≤ 30%

Inter-Assay

	1	2	3	mean	SD	%CV	AC
PC1	98	100	99	**99**	0.9	0.9	≤ 30%
PC2	46	39	37	**41**	3.7	9.1	≤ 30%

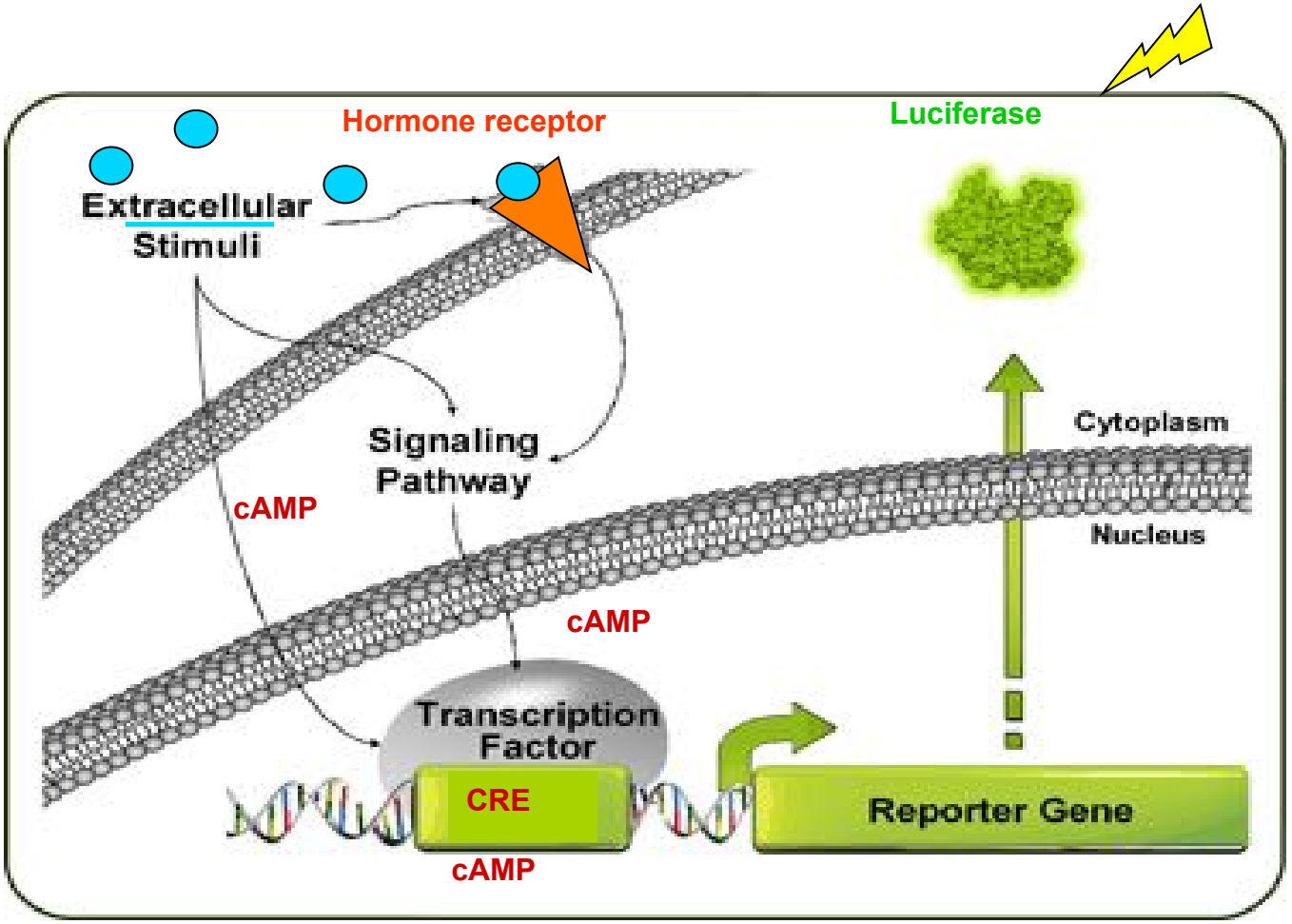
Summary: NAB EPO

Validation characteristics	Data
Challenging concentration of EPO	20 pM
Intra-assay precision	≤ 2 % CV
Inter-assay precision	≤ 9 % CV
Stability for 3 days at +2-8°C	≤ 8 % deviation
Stability for 3 weeks at -20°C	≤ 12 % deviation
Stability at ≤ -15 °C after 3 Freeze/Thaw cycles	≤ 11 % deviation
Stability at ≤ -70 °C after 3 Freeze/Thaw cycles	≤ 14 % deviation
Drug tolerance	250 mIU/ml
Clinical Specificity	100 %
Cross reactivity against IL-3	none
Screening cut point (% inhibition)	17 %
Sensitivity in 2% serum	25 ng/mL
Sensitivitiy in undiluted serum	1250 ng/mL
Minimum required dilution (MRD)	2 % serum

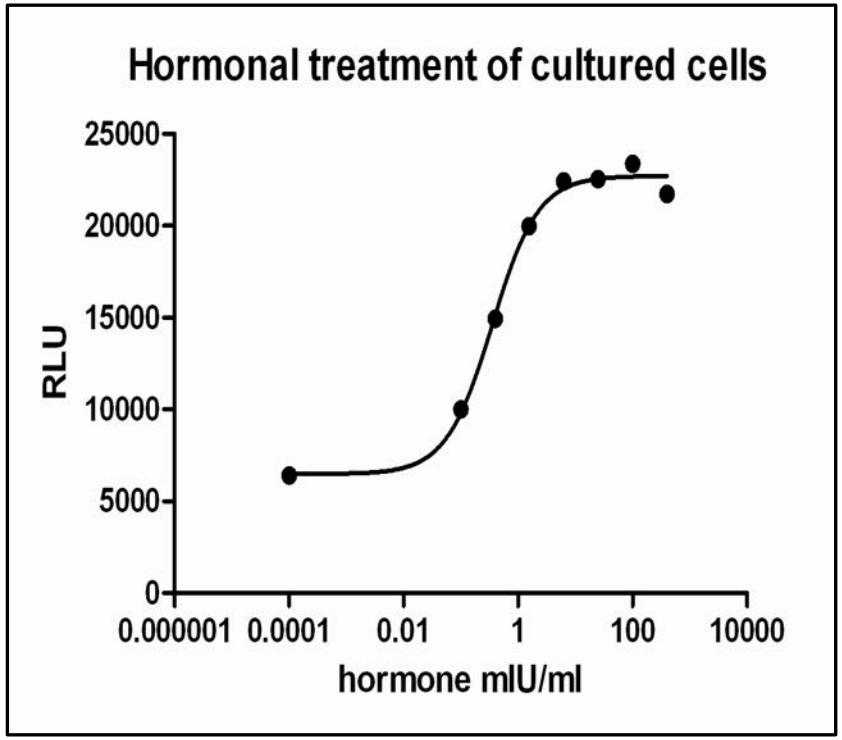
Example: FSH

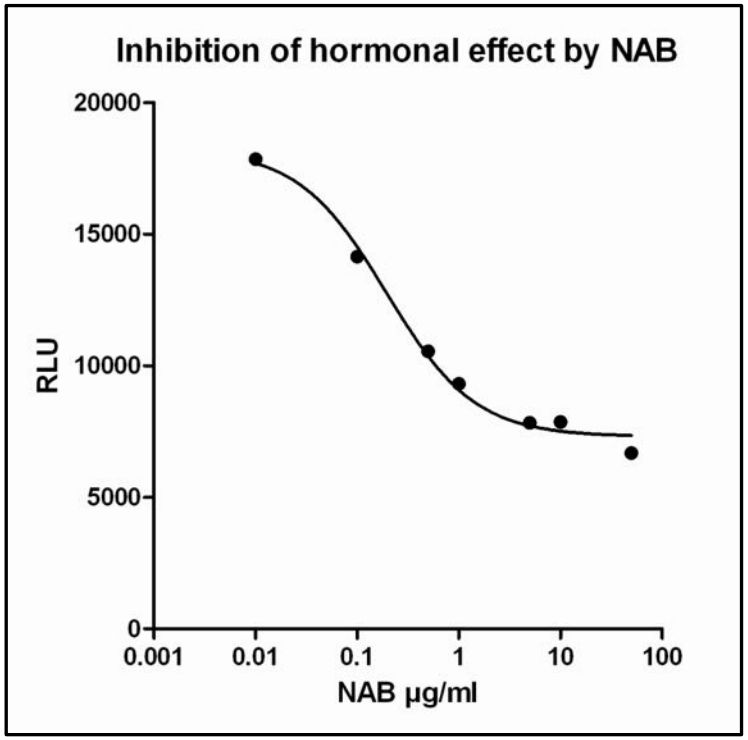
- recombinant human protein drug with an endogenous counterpart
- used for the treatment of induction of ovulation/pregnancy and for the development of multiple follicles.

NAB against FSH



NAB FSH


Hormonal treatment of cultured cells
RLU
25000
20000
15000
10000
5000
0
0.000001
0.0001
0.01
1
100
10000
hormone mIU/ml


Inhibition of hormonal effect by NAB
RLU
20000
15000
10000
5000
0
0.001
0.01
0.1
1
10
100
NAB µg/ml

NAB FSH

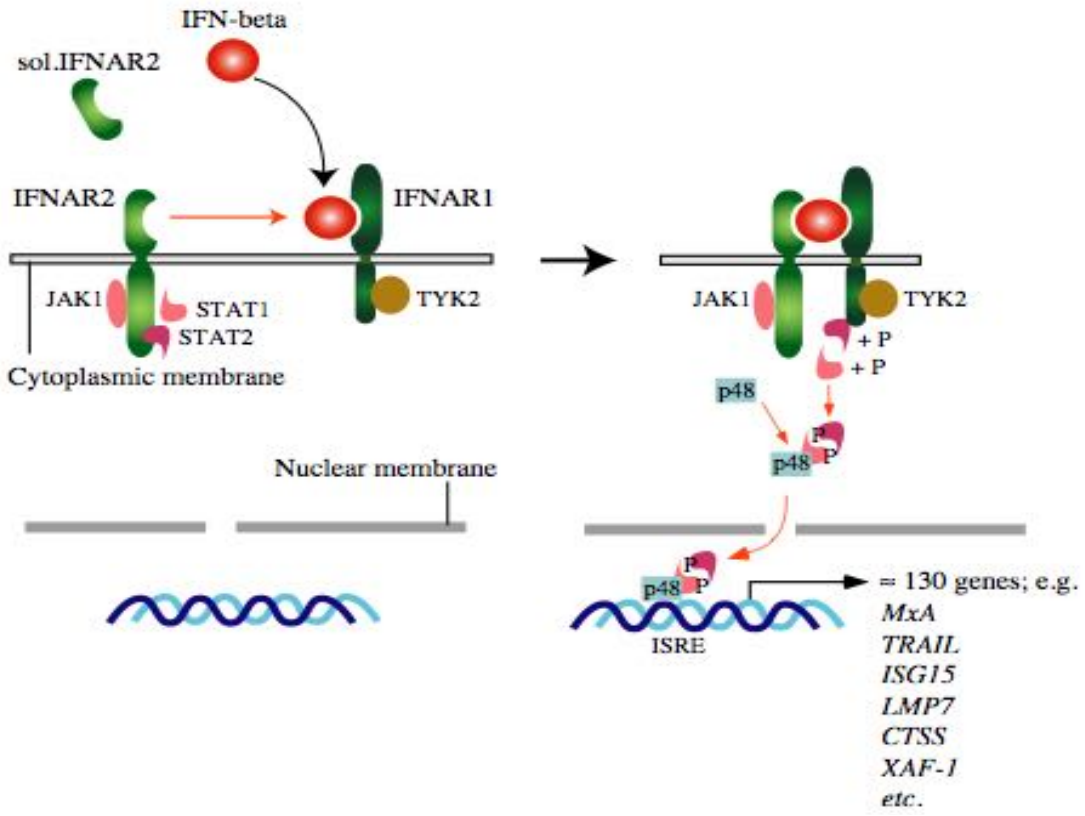
Validation characteristics	Data
Challenging concentration of FSH	1 mIU/ml
Intra-assay precision	≤ 4 % CV
Inter-assay precision	≤ 14 % CV
Stability for 3 days at RT	≤ 11 % deviation
Stability for 3 days at +2-8°C	≤ 5 % deviation
Stability at ≤ -15 °C after 3 Freeze/Thaw cycles	≤ 11 % deviation
Stability at ≤ -70 °C after 3 Freeze/Thaw cycles	≤ 14 % deviation
Drug tolerance at 150 µg/ml	7.5 ng/mL
Drug tolerance at 15 µg/ml	0.75 ng/mL
Clinical Specificity	99 %
Cross reactivity against LH, TSH, CGalpha	None
Screening cut point (% inhibition)	23 % inhibition
Sensitivity	100 ng/ml
Minimum required dilution (MRD)	2 % serum

Example: Interferon

- recombinant human protein drug with an endogenous counterpart
- used for the treatment of
  - Multiple Sclerosis (IFN-β) and
  - Hepatitis virus infection (IFN-α)

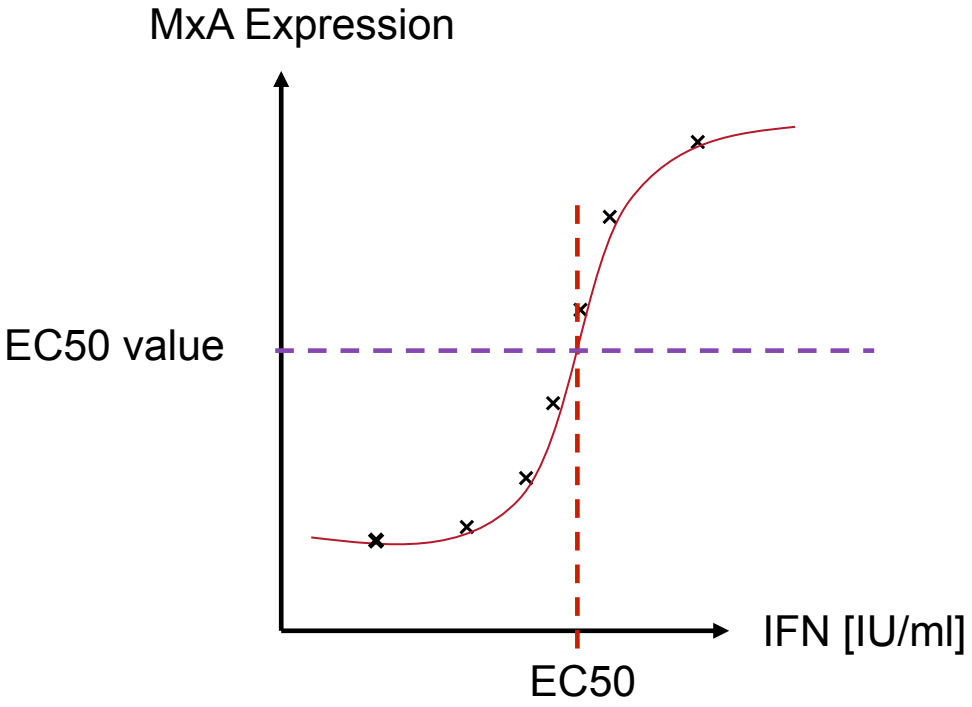
Gene expression assay

NAB against inferferon



Gene expression assay

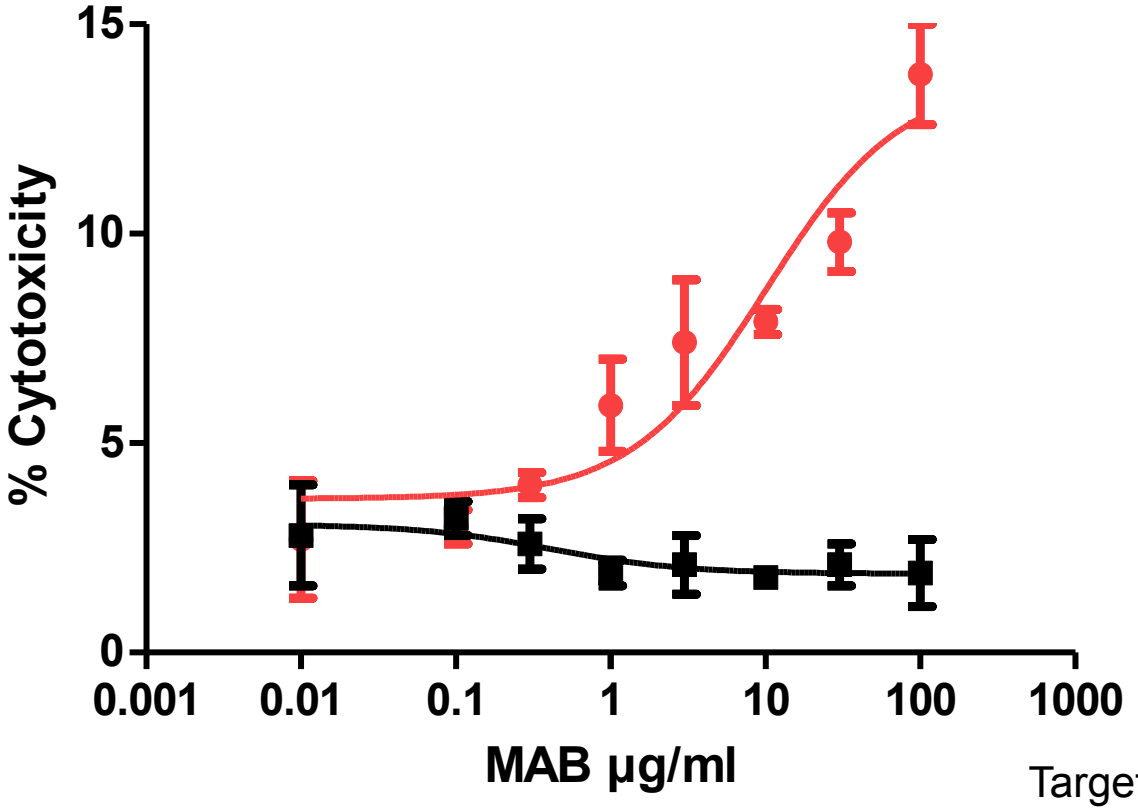
NAB against inferferon by MxA analysis



Positive sample: sample signal < EC50

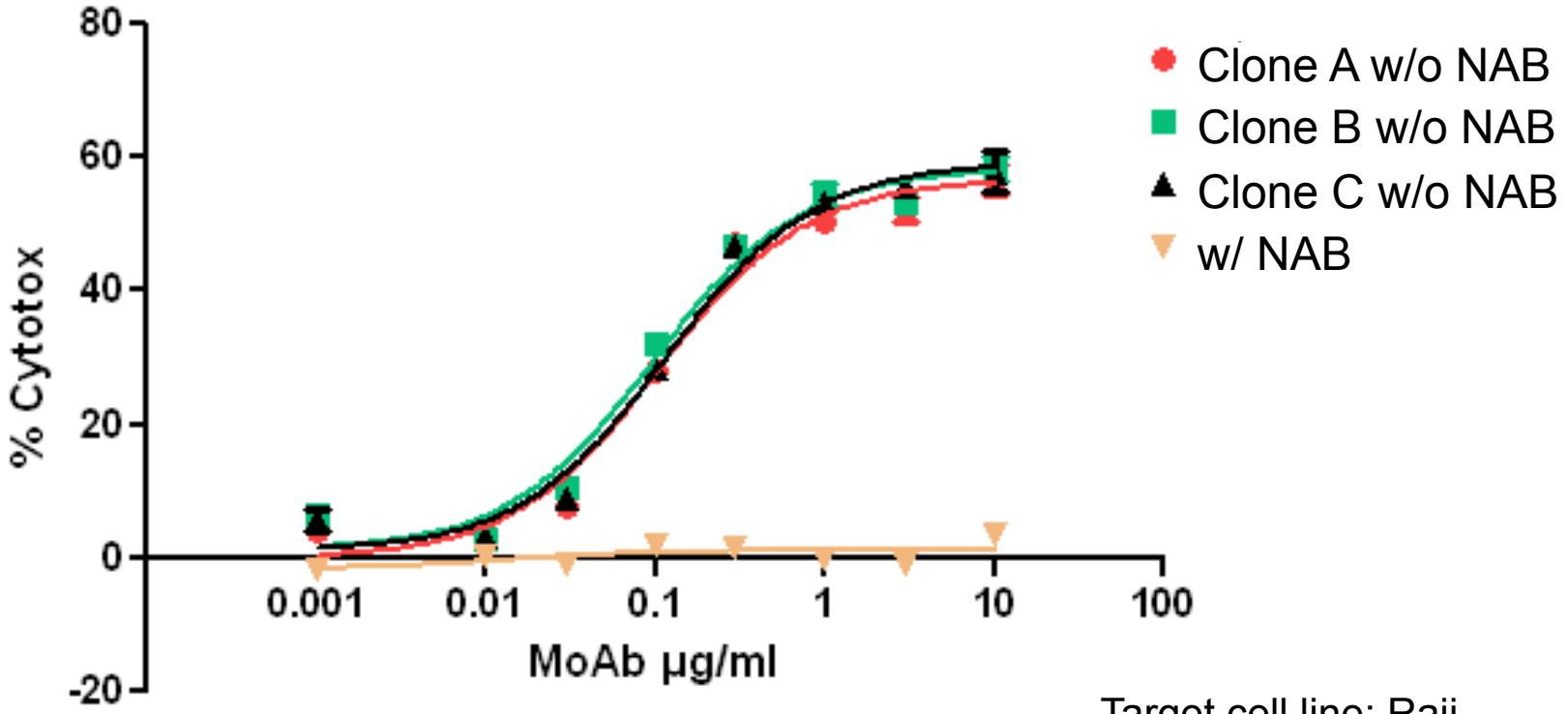
Example: mab

Inhibition of ADCC by anti-IS



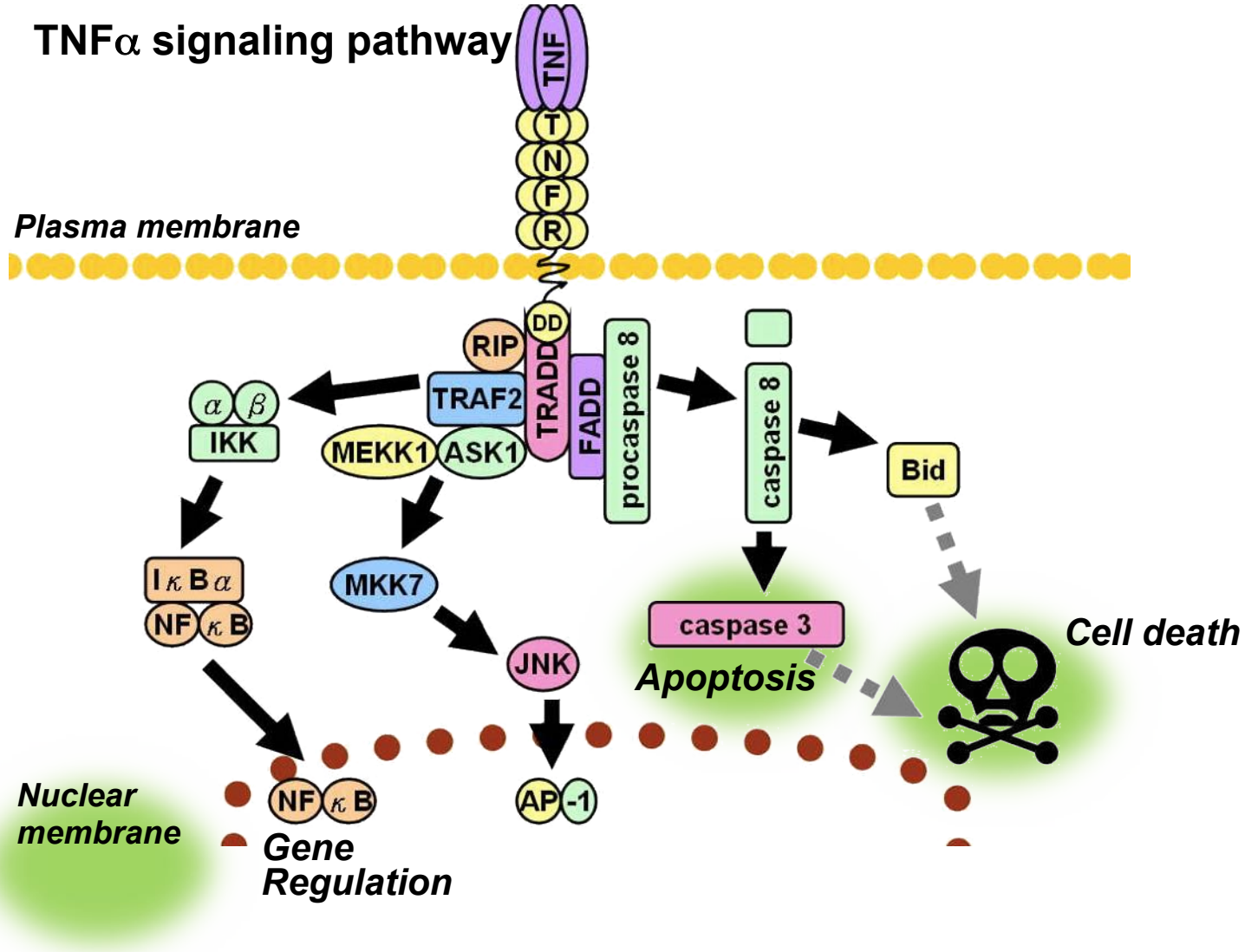
Target cell line: SK-BR3i
MRD: 2 %
Effector cell: CD16-NK
Drug: anti-Her2/neu

Inhibition of CDC by anti-IS



Target cell line: Raji
MRD: 2 %
Effector: complement (10:1)
Drug: anti-CD20

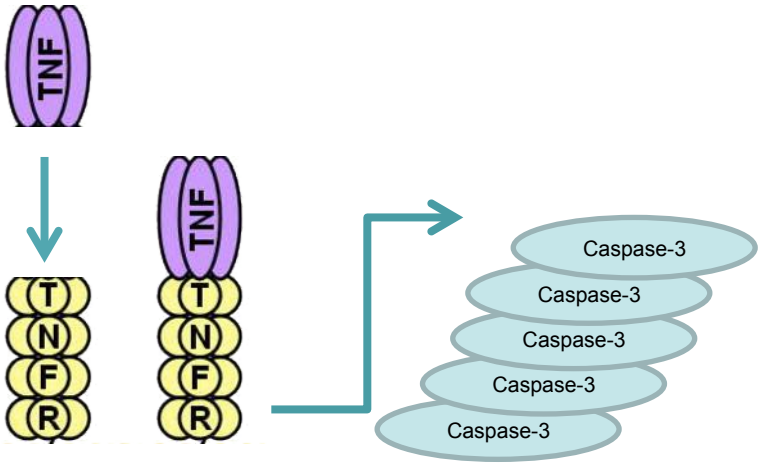
Mechanism of action via TNFα signaling

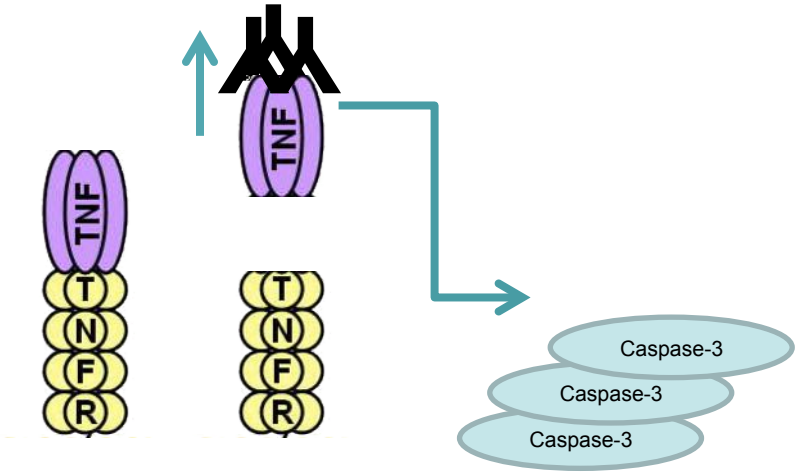


TNFα blocker cell-based bioassay based on caspase 3 activity

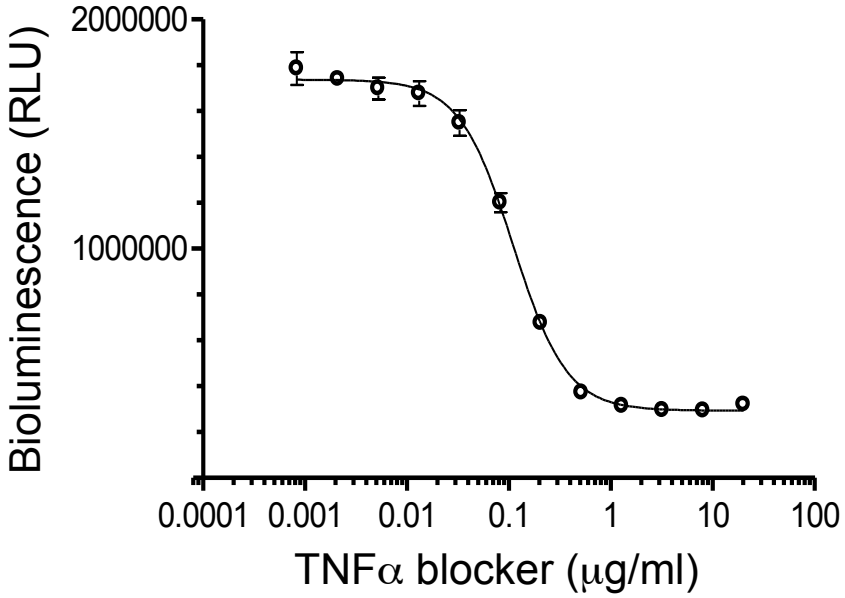
TNFα / TNFα receptor signaling via the apoptosis pathway increases caspase 3 activity

A TNFα blocker drug dose-responsively lowers caspase 3 activity of TNFα by blocking TNFα binding to receptors





Bioluminescent caspase-based bioassay of TNFα blocker drug activity on TNFα signaling

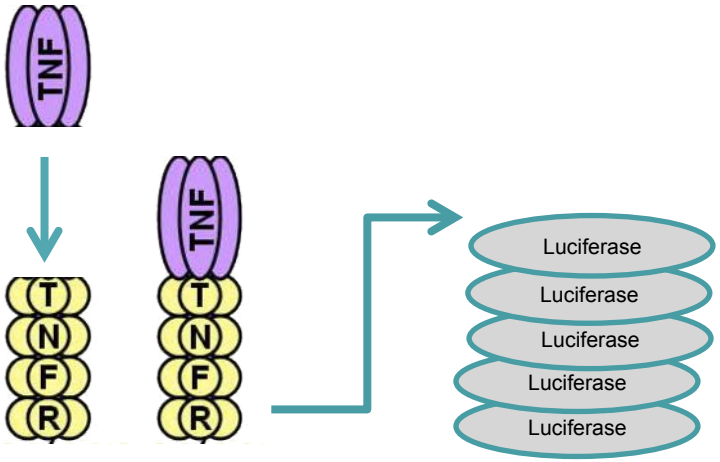


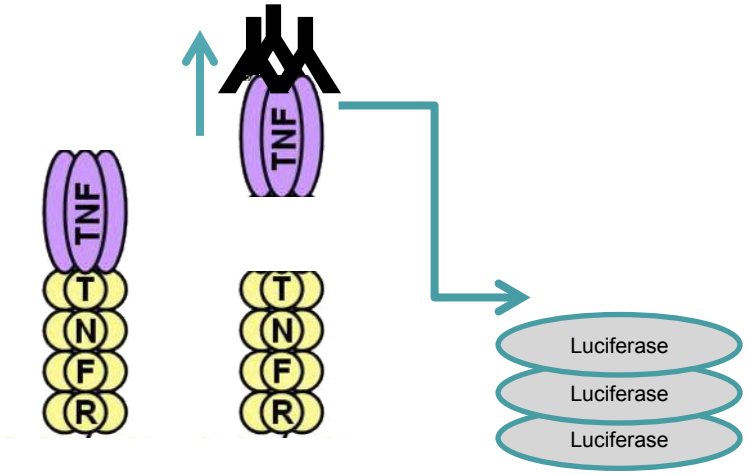
- Rapidly responsive human U937 cells in provide bioassay high consistency
- Bioluminescence readout provides excellent bioassay sensitivity and dynamic range
- Fast assay (2.5 hr response)

TNFα blocker cell-based bioassay based on NF-κB luc reporter activity

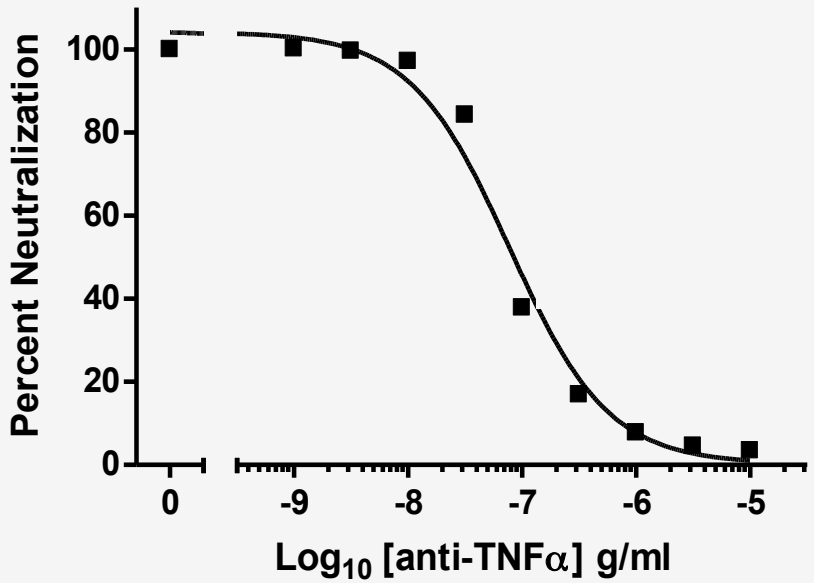
TNFα / TNFα receptor signaling via the NF-κB pathway increases gene expression driven by the NF-κB response element.

A TNFα blocker drug dose-responsively lowers NF-κB driven luciferase activity of TNFα by blocking TNFα binding to receptors



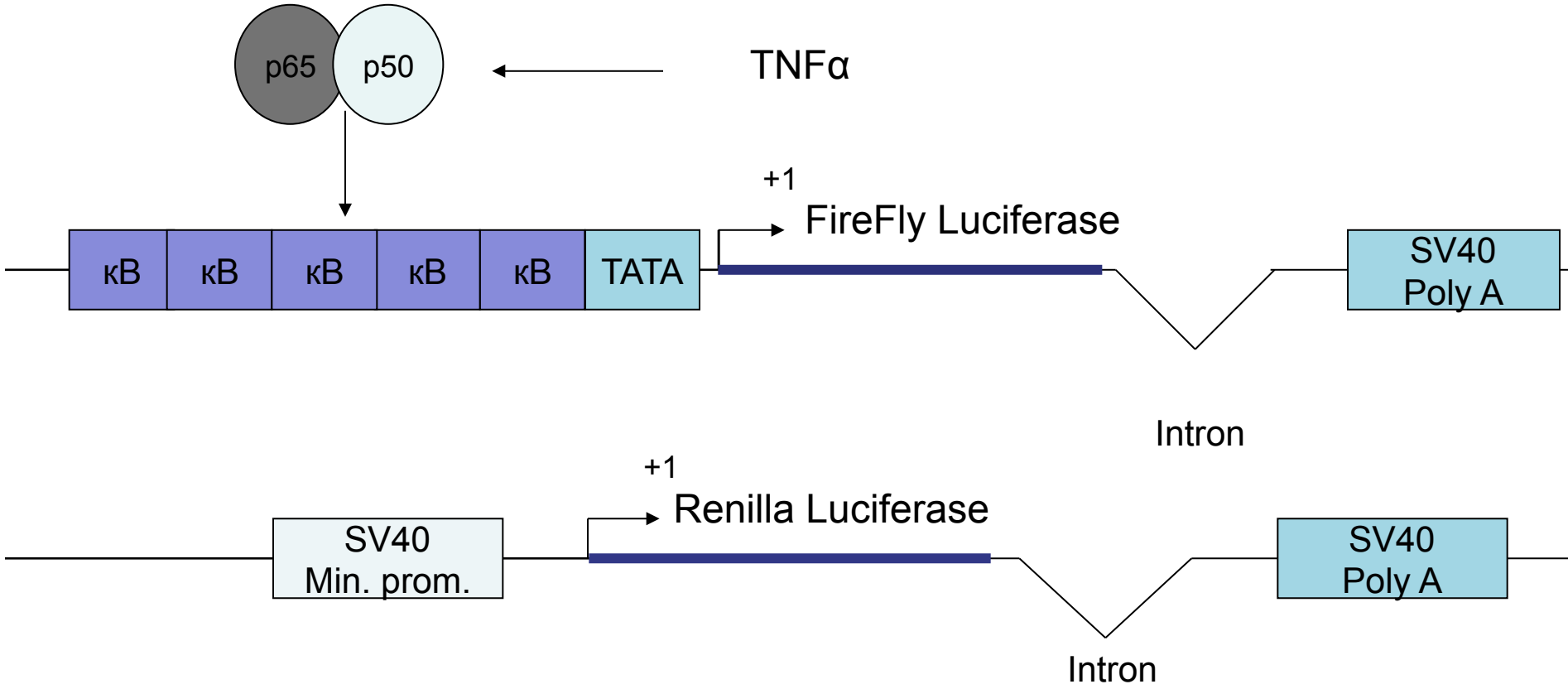


Bioluminescent NF-κB reporter gene bioassay of TNFα blocker drug activity on TNFα signaling



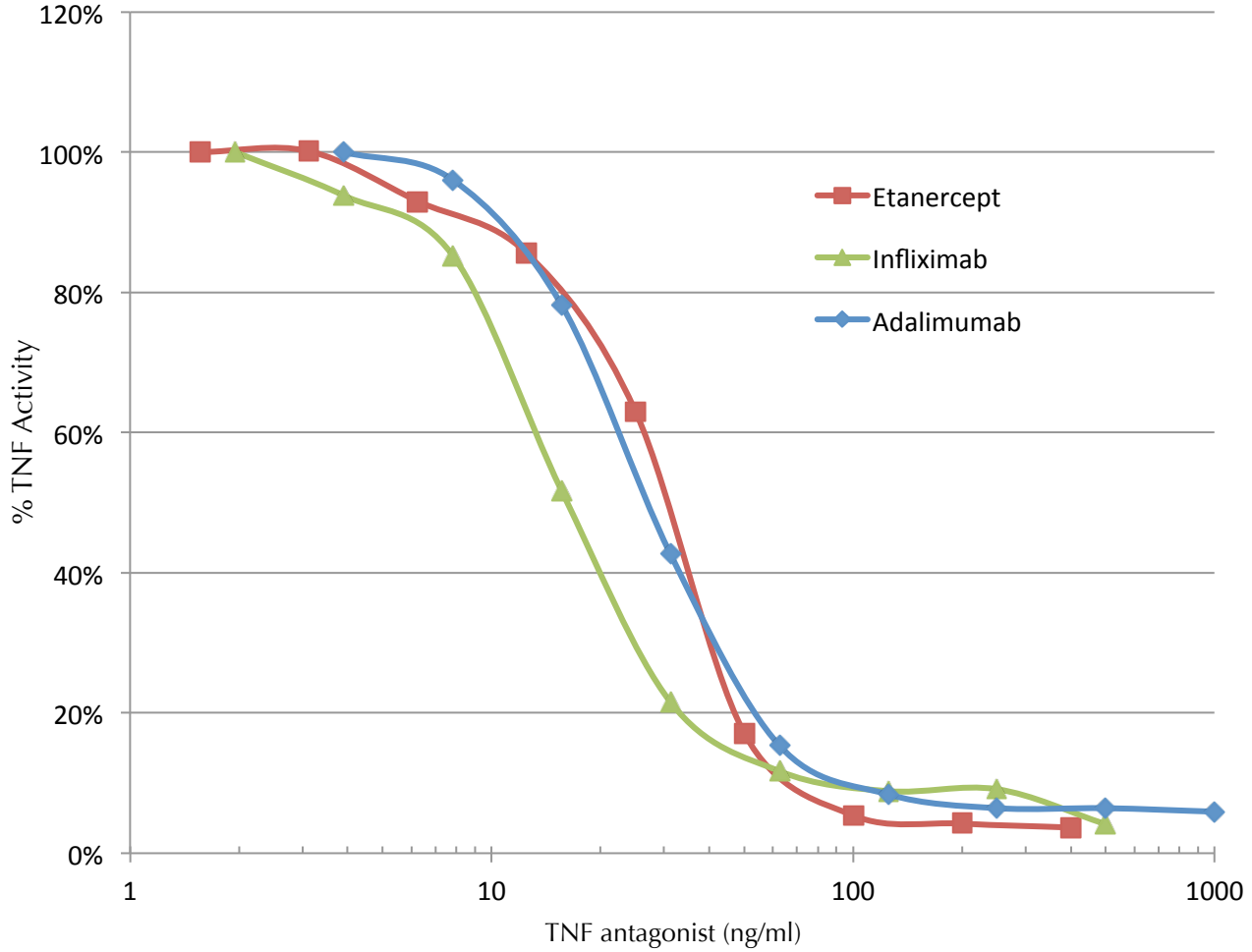
- Stably transfected human NF−κB HEK-293 cells provide high consistency
- Bioluminescence readout provides excellent bioassay sensitivity and dynamic range
- Fast assay (4 hr induction of NF-κB driven luciferase expression)

TNFα Resp Reporter Gene Construct



Lallemand C, et al, **Tovey** MG. J Immunol Methods. 2011

Anti-TNFα-NAB analysis



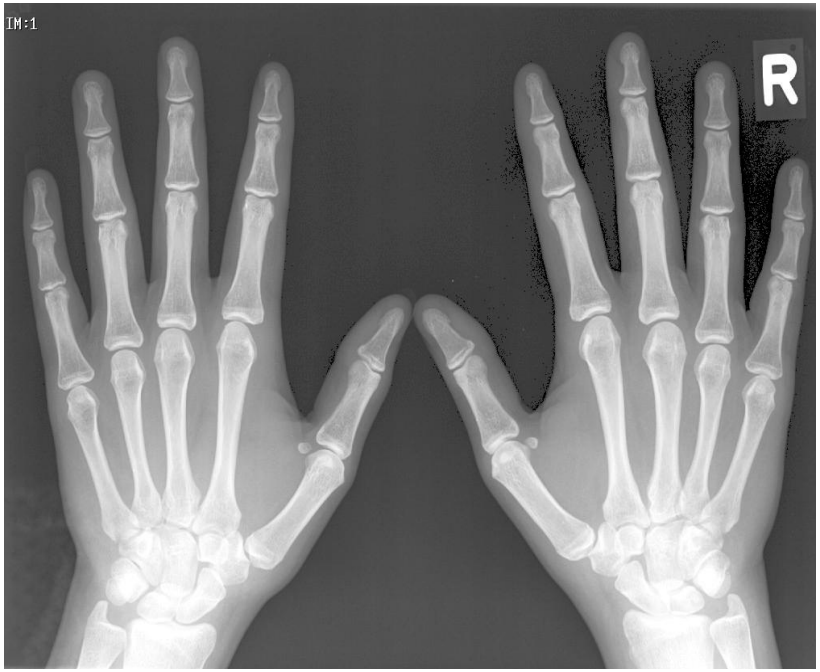
Lallemand C, et al, **Tovey** MG. J Immunol Methods. 2011

Clinical consequences

Correlation with clinical responses is usually necessary to determine the clinical relevance of both binding and neutralizing antibody responses.

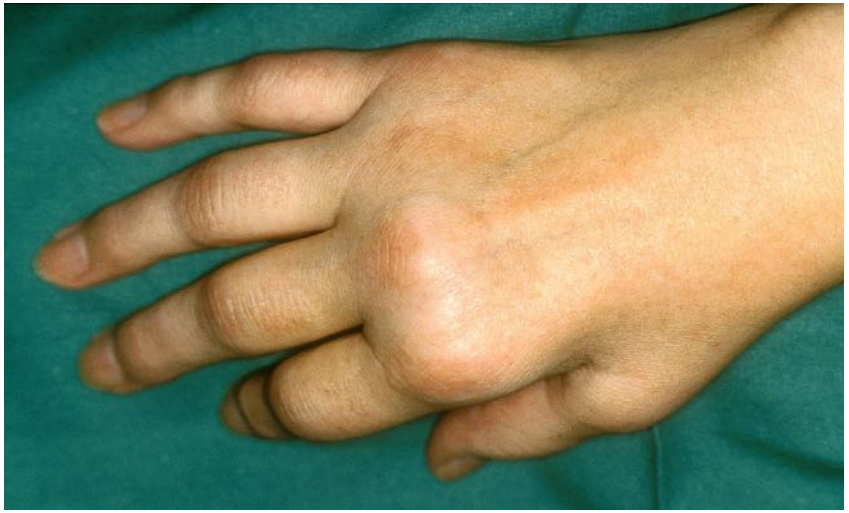
FDA Guidance, 2013

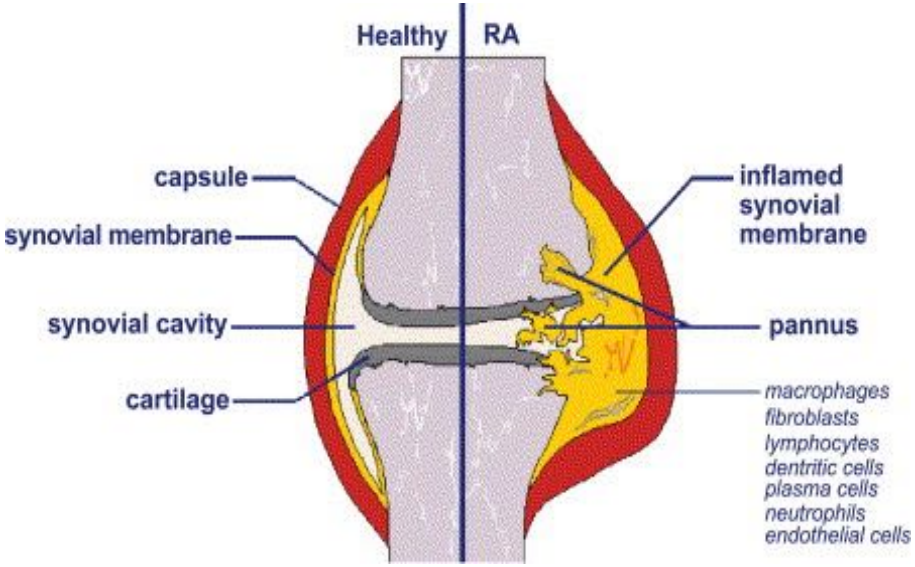
Left and right hand



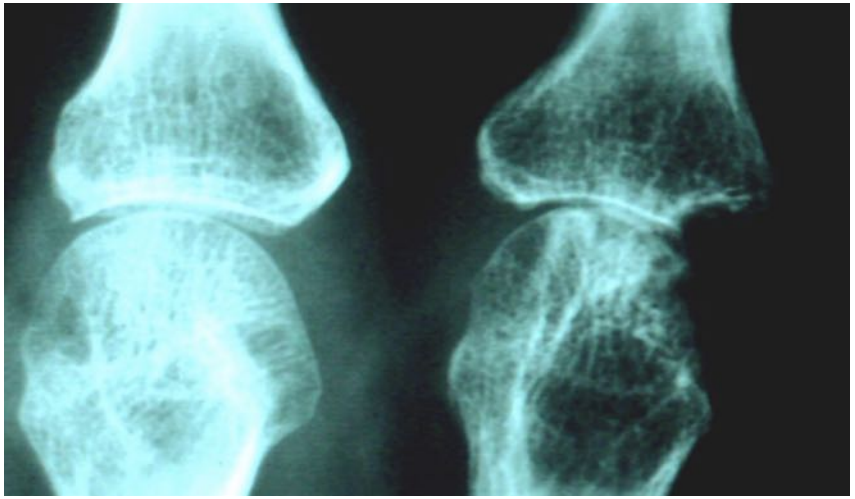
Rheumatoid Arthritis

- Prevalence: 1.0%
- f/m: 2.5/1
- Age: 43 (± 40)
- Chronic synovialitis
- Anti-CCP antibodies (CCP)

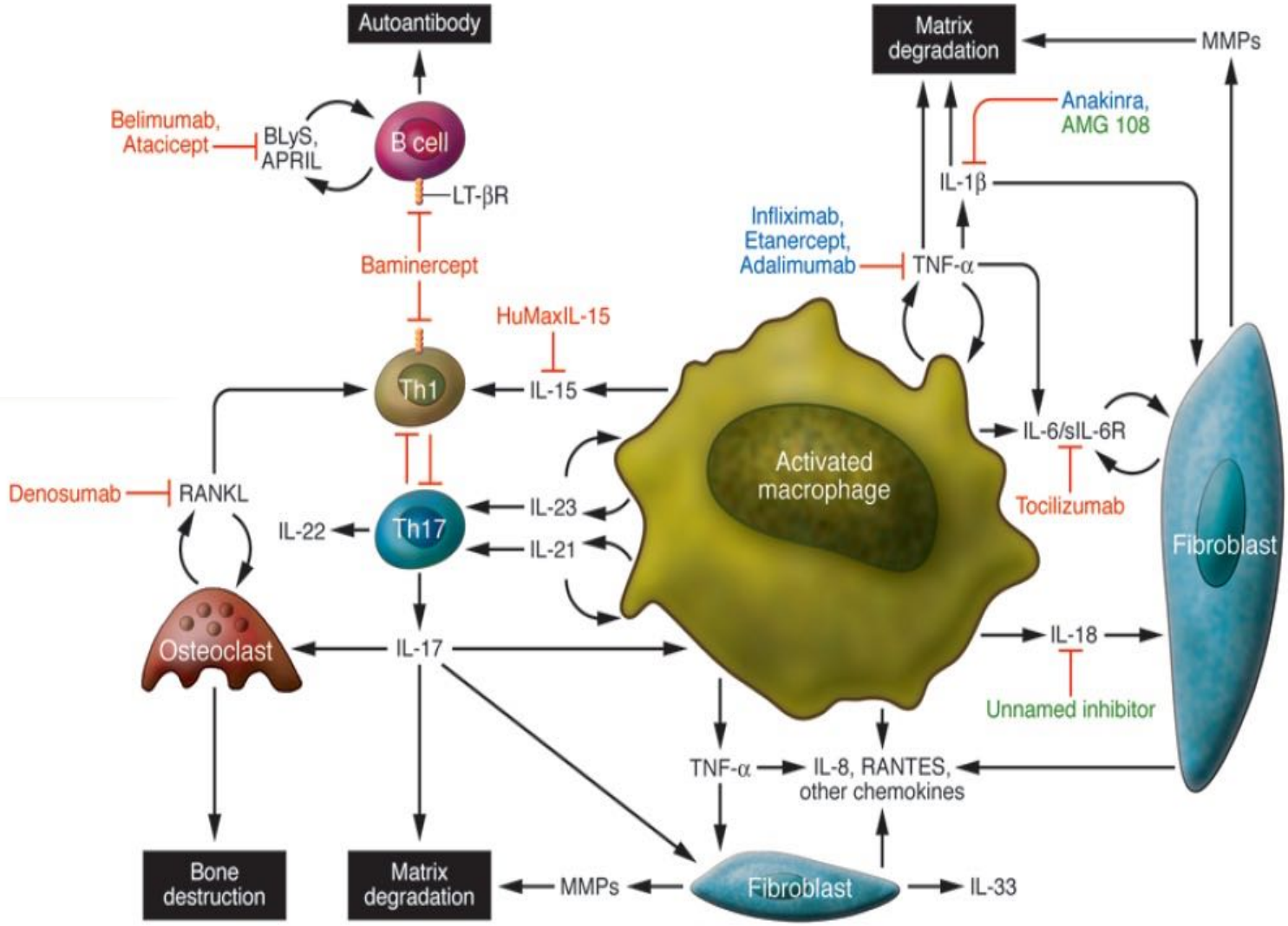




Nijenhuis S et al Clin Chim Acta, 2004



Targets of treatment in RA



Case 1

Patient	Geb.-Datum	Kasse	Labor-Nr./Eingang	
[redacted] w	03.07.1987	BMÄ		Labor Lademannbogen MVZ GmbH Professor Rüdiger Arndt Haus Laboratoriumsmedizin und Mikrobiologie Tel. (0 40) 5 38 05-0

	Ergebnis	Einheit	Referenz	Methodik
AUTOIMMUN-DIAGNOSTIK				
AK gg. Infliximab	negativ	µg/ml	negativ	EIA

Sonic Healthcare Labor Lademannbogen

Case 2

Patient	Geb.-Datum	Kasse	Labor-Nr./Eingang	Labor Lademannbogen MVZ GmbH Professor Rüdiger Arndt Haus Laboratoriumsmedizin und Mikrobiologie Tel. (0 40) 5 38 05-0
[redacted] w	01.09.1971	BMÄ		

Seite 1

	Ergebnis	Einheit	Referenz	Methodik
AUTOIMMUN-DIAGNOSTIK				
AK gg. Infliximab	2.8	µg/ml	negativ	EIA
Infliximab	<4.0	µg/ml		EIA

Leicht erhöhte Antikörper gegen Infliximab nachweisbar. Bei klinischem Hinweis auf einen Therapieverlust kurzfristige Verlaufskontrolle empfehlenswert.
Ggf. tel. Rücksprache unter 040/53805 514, PD Dr. Kromminga.

Slightly increased antibodies against infliximab detectable. In case of clinical signs of loss of efficacy or therapeutic non-responsiveness, consider a change of treatment.

Sonic Healthcare Labor Lademannbogen

Case 3

Patient	Geb.-Datum	Kasse	Labor-Nr./Eingang	Labor Lademannbogen MVZ GmbH Professor Rüdiger Arndt Haus Laboratoriumsmedizin und Mikrobiologie Tel. (0 40) 5 38 05-0
[redacted] m	03.12.1963	BMÄ		

Seite 1

	Ergebnis	Einheit	Referenz	Methodik
AUTOIMMUN-DIAGNOSTIK				
AK gg. Infliximab	>80.0	µg/ml	negativ	EIA

Erhöhte Antikörper gegen Infliximab nachweisbar. Bei klinischem Hinweis auf einen Verlust der Therapieeffizienz Umstellung der Therapie empfohlen.

Ggf. tel. Rücksprache unter 040/53805 514, PD Dr. Kromminga.

Increased antibodies against infliximab detectable. In case of clinical signs of loss of efficacy or therapeutic non-responsiveness, consider a change of treatment.

Sonic Healthcare Labor Lademannbogen

Case 4

Patient		Geb.-Datum	Kasse	Labor-Nr./Eingang	
[redacted]	w	23.10.1966	BMÄ		Labor Lademannbogen MVZ GmbH Professor Rüdiger Arndt Haus Laboratoriumsmedizin und Mikrobiologie Tel. (0 40) 5 38 05-0

Seite 1

	Ergebnis	Einheit	Referenz	Methodik
AUTOIMMUN-DIAGNOSTIK				
AK gg. TNF-alpha-Blocker	s.unten		negativ	EIA

The measurement of antibodies against TNF-alpha inhibitors were performed using different assay formats.

*Antibodies against infliximab were detectable on **08.01.2010** by both an ELISA-based method and a cell-based bioassay. These results were confirmed on **22.03.2011**. The cell-based assay could not be used at that time, possibly due to circulating infliximab levels.*

*The same results were obtained on **19.04.2011** (positive by ACE-ELISA and not evaluable by bioassay.*

In case of clinical signs of loss of efficacy or therapeutic non-responsiveness, consider a change of treatment.

Conclusion

- Assays for the detection of neutralizing antibodies are to be included in the cascade of immunogenicity assessment.
- Neutralizing antibodies (NAB) are generally of more concern than binding antibodies (BAB).
- The detection of NAB can be performed by cell-based assays (CBA) or by non-cell-based competitive ligand binding assays (CLBA).
- FDA prefers CBA because these more realistically reflect the in vivo situation.
- Sometime cell-based assays are more difficult and tedious to establish. Recombinant cell lines / reporter gene readouts may be an alternative for the NAB analysis if other cell-based assay are not available.
- A **therapeutic ADA/NAB monitoring** should be mandatory in all patients treated with Biologicals.

Future Hamburg