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Applied Translational Medicine

The Road to Functional Bioanalysis: Development and Validation of a Cell-Based Assay for Neutralizing Anti-Drug Antibody Analysis

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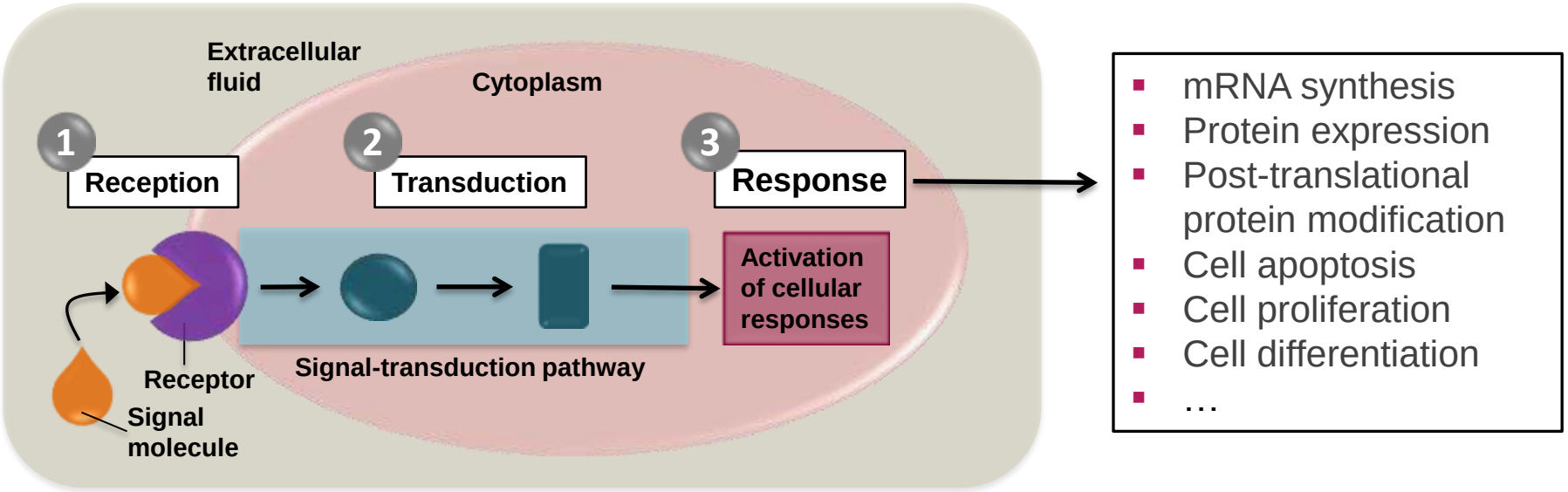
Immunogenicity and Functional Bioanalysis

- Immune response to therapeutic proteins ► patient safety and drug efficacy issues
- Major safety concern: **neutralizing antibodies (NAbs)**, able to neutralize the biological activity of bound Ag
  - ► loss of product efficacy by binding to the product active site (highly critical if the product is a lifesaving drug)
  - ► cross-reactivity to and inhibition of the endogenous counterpart of the therapeutic protein (highly critical if the endogenous protein is nonredundant)
- The demand for drug immunogenicity assessment and characterization is growing (2014 FDA Guidance on "Immunogenicity Assessment for Therapeutic Protein Products")
- Key safety data that impact critical decisions on the continuation of a drug development project
- **Cell-based assays (CBA)** (also called bioassay) are recognized by regulatory authorities as the gold standard to measure drug propensity to generate NAbs

Basics of Cell-based Assays

The 3 stages of the cell-signaling process



- The types of response are highly diverse, all measurable
- The 3 different steps of cell-signaling are individually measurable
- **The cell is a test system that allows a vast number of assay possibilities measuring a biological activity**
  - ► **Functional Bioanalysis**

Use of Cell-based Assays

- Determination of biologics potency for lot release
- Screening drug candidate targeting specific biological process during drug discovery
- **Functional bioanalysis for neutralizing antibody testing**

	CBA	Non-CBA
PROs	▪ Test for the functionality/biological activity ▪ Closer to physiological conditions ▪ Recommended by authorities for NAbs assessment ▪ More complex: more assay format and readout possibilities	▪ Less variability ▪ Easier to develop ▪ Faster to develop
CONs	▪ Higher variability ▪ More complex: experienced assay designer required, time-consuming	▪ No functional characterization

Development of a CBA for NAb Testing: Challenges and Outcomes

Development of a CBA for NAb Testing

1. Choose the relevant assay format and endpoint

- The assay format is derived from the drug molecular mechanism
- Understanding the biological activity of the drug is essential to selecting the most appropriated cell-based assay format and endpoint
- What are the critical biological pathways involved and can they be exploited to develop a bioassay?

► *Development of a CBA to detect NAb against a PEGylated form of human G-CSF*

Biological Activity and Molecular Mechanism of G-CSF

- **G-CSF = G**ranulocyte **- C**olony **S**timulating **F**actors
- In vivo, G-CSF stimulates the bone marrow to induce differentiation into and proliferation of neutrophil granulocytes (aka neutrophils)
- Neutrophils: most abundant white blood cells (70%), essential part of the innate immune system, one of the first cells to migrate towards the site of infection to digest bacteria, major component of pus
- In clinical setup, recombinant human G-CSF (Filgrastim) is used to stimulate neutrophils production in patients suffering from neutropenia (low neutrophil counts, congenital or chemotherapy-induced)

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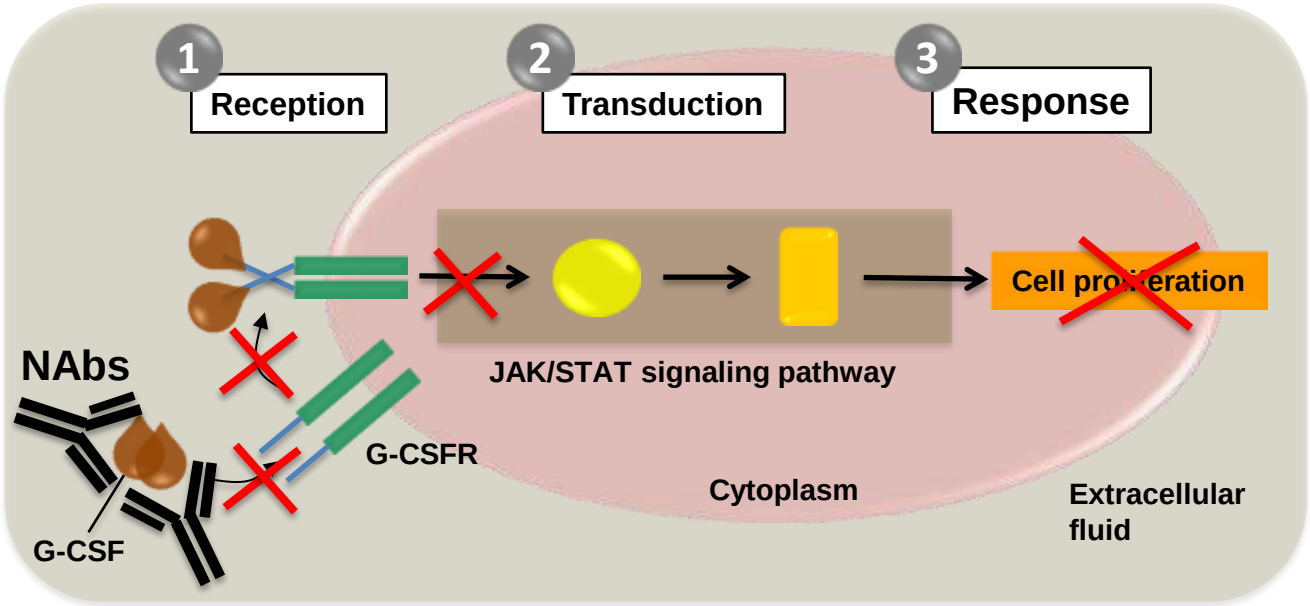
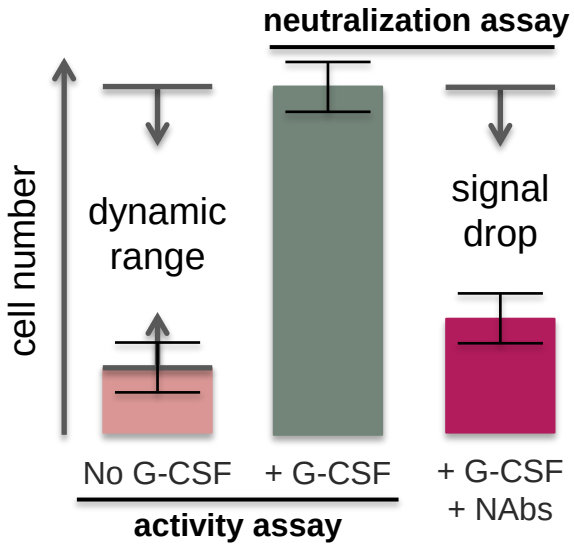
Biological Activity and Molecular Mechanism of G-CSF



► *Cell proliferation assay to detect NAbs against G-CSF*



► *Direct neutralization assay (inhibition of stimulation), measures the inhibition of the drug activity*

Development of a CBA for NAb Testing

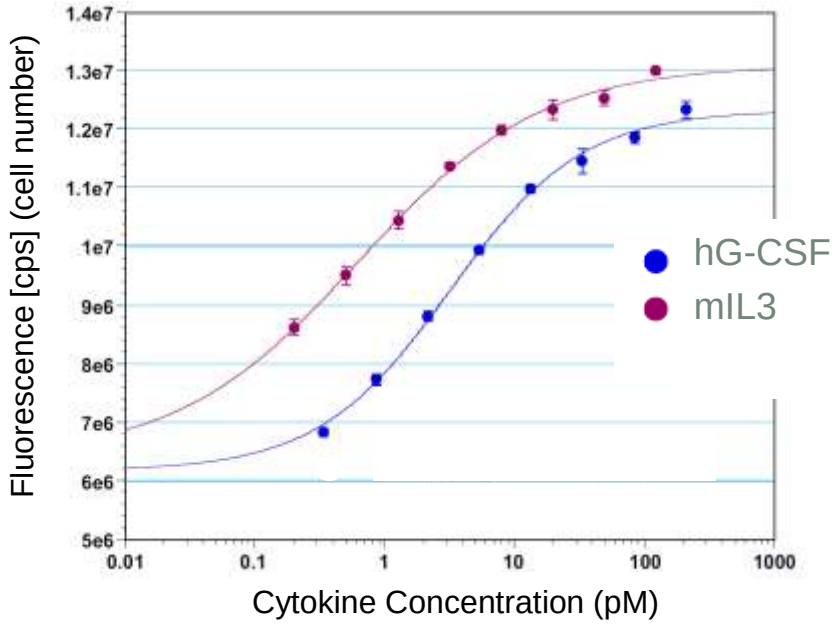
2. Choose the relevant cell line and readout

- The assay format conditions the choice of the cell line and readout

► *NFS-60 cell line with alamar blue test as readout for cell proliferation (measure of the cell metabolic activity, proportional to number of cells at a defined time-point, fluorescence readout)*

Confirmation of NFS-60 phenotype

- NFS-60 cells express G-CSFR and IL3R
- Proliferation in response to hG-CSF and mIL3



Cell Bank Establishment

Origin/Source
- Cell background/species
- History, genetic modifications
- Number of passages
- Cell culture conditions

Biosafety
- BSL2 conditions
- Bacterial contamination free

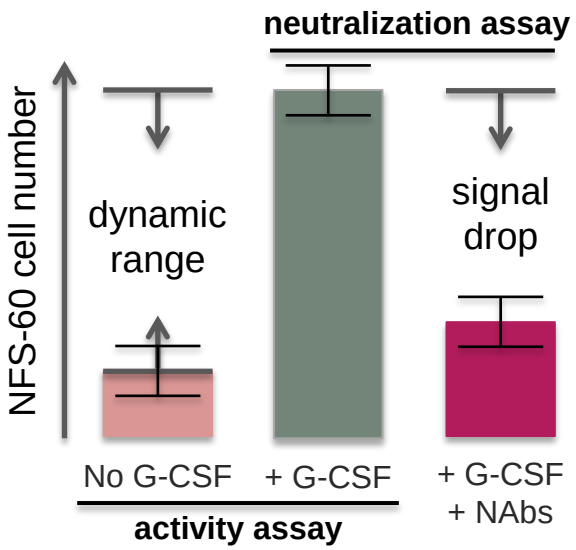
Documentation
- Cell background/species
- History, genetic modifications
- Number of passages
- Cell culture conditions
- Biosafety

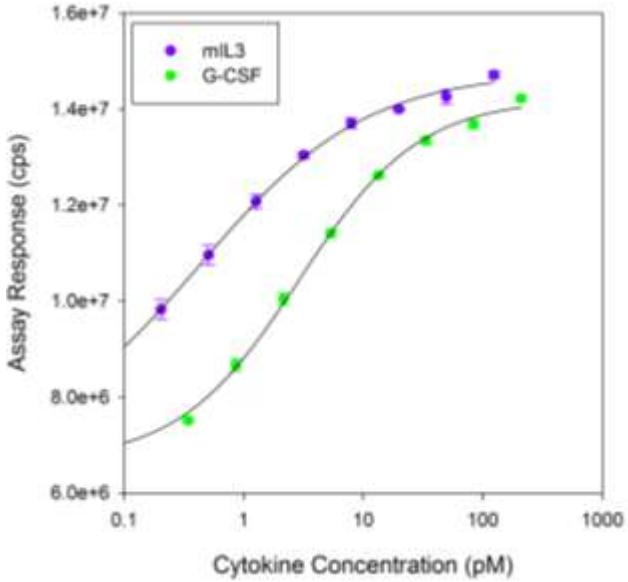
Cell bank (CB)
- Tiered cell bank system
- (Initial CB → Master CB → Working CB)
- (identical passage number at each level)
- Qualified storage container
- (vapor phase of liquid N_2)

Characterization of CB
- Viability
- Growth curve

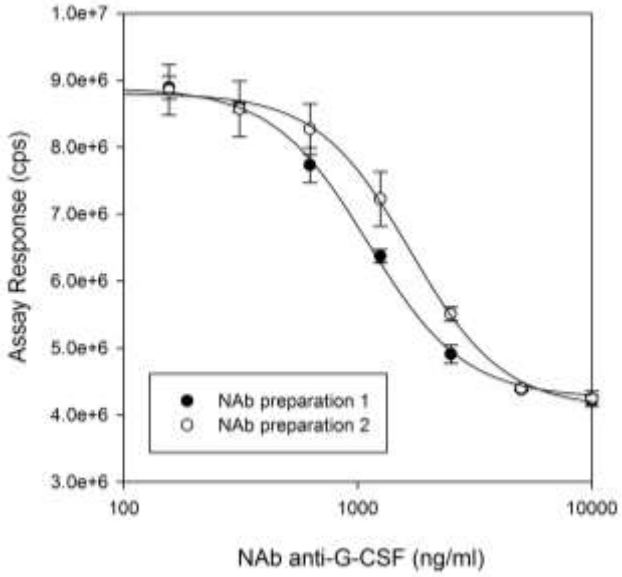
G-CSF Activity and Neutralization Assay

Assay principle





Cells
+
G-CSF
72h
cell number measurement



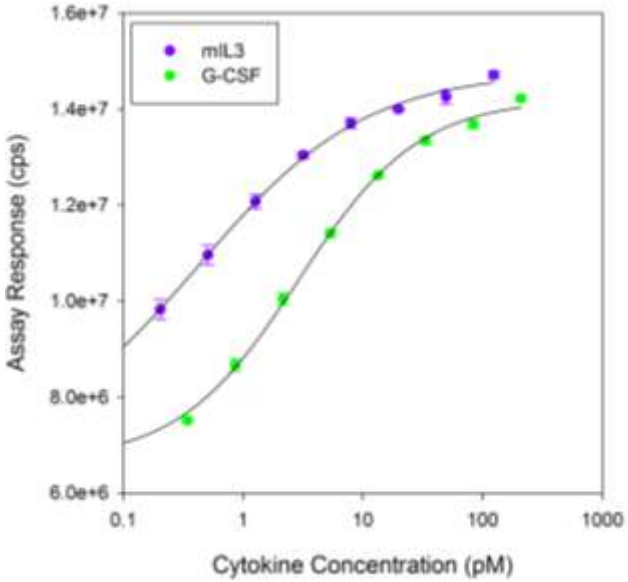
Cells
+
G-CSF/NAb anti-G-CSF
72h
cell number measurement

Matrix Interference Assay – Specificity Assay

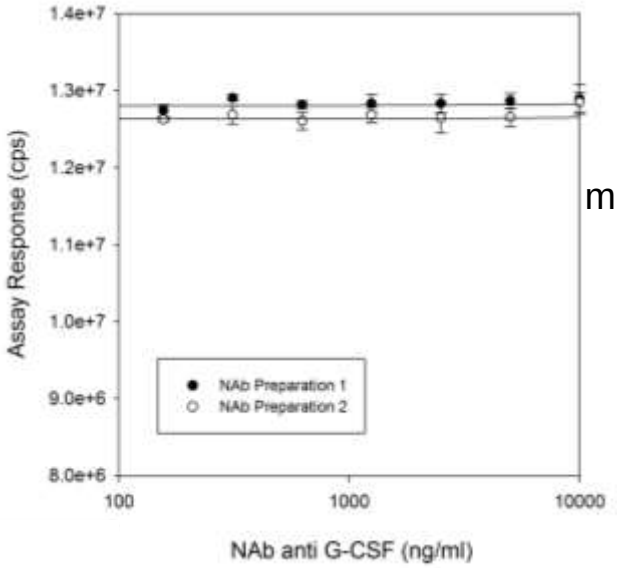
- More than any other method, CBA performance can be affected by factors present in matrix
- Matrix interference assay is recommended in NAb testing
- **Differentiation between drug-specific NAbs and other non-specific inhibitory factors**

► *Alternative stimulus assay*

- *Induction of cell proliferation using another stimulus: mIL3*
- *Anti G-CSF NAbs are not able to inhibit mIL3-induced NFS-60 cell proliferation* ► ***assay specificity***



Cells
+
mIL3
72h
cell number measurement



Cells
+
mIL3/NAb anti-G-CSF
72h
cell number measurement

Validation Steps of a Cell-based NAb Assay

- No regulatory guidelines, but industry standard:
  - Gupta S. et al., Recommendations for the design, optimization, and qualification of cell-based assays used for the detection of neutralizing antibody responses elicited to biological therapeutics, Journal of Immunological Methods (321) 1-18, 2007

- Cut point calculation (10-20 drug naïve healthy individuals, statistical approach)
- Sensitivity
- Selectivity in individuals
- Specificity/matrix interference assay
- Free drug tolerance
- Precision
- Stability of PC antibodies
- Robustness (determination of acceptance criteria for cell passage number)
- Hemolysis

Conclusion

- Validation steps for cell-based NAb assay are similar to those for binding ADA assay

- **Living cells** as test system
- "Reagent" more difficult to control as compared to chemicals
- Large contributor to assay variability
- Requirement for a controlled process to ensure continuous availability of a consistent and reliable cell source ► cell banking
- Requirement for specificity assay performed in parallel to the neutralizing assay ► matrix interference assay

- Understanding the drug's mode of action as well as some of the clinical features (study population) is critical for cell-based method development

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Thank you for your attention