Unexpected Effects of Large Molecules on Circulating Blood Cells

Nancy Everds, DVM, Dipl ACVP
Amgen Inc
neverds@amgen.com
Why blood cells as a target of large molecules?

- Fluid tissue allowing extensive interactions with other cell types and biological cascades
- Exposed to relatively high concentrations of drug
  - mAbs distribute to the plasma/blood compartment
  - Acute effects on large numbers of cells
  - New cells constantly being released and exposed
- Express large numbers of surface receptors
  - Cross-linking of receptors (platelets, WBCs) results in activation
  - Coating with antibodies results in removal (platelets, RBC)

Unexpected Hematologic Effects of Biotherapeutics in Nonclinical Species and in Humans

Nancy E. Everds\textsuperscript{1} and Jacqueline M. Tarrant\textsuperscript{2}

\textsuperscript{1}Amgen Inc., Seattle, Washington, USA
\textsuperscript{2}Genentech, Inc., South San Francisco, California, USA
Mechanisms for unexpected effects of large molecules on circulating blood cells

- **Direct unexpected effects**
  - On-target or off-target binding
  - To blood or other cell types (e.g., endothelial cells)

- **Effects secondary to other processes**
  - Complexes or cascades
    - Complement, cytokines, coagulation
  - Immune-mediated
    - Activation/suppression of immune cells
    - Induction of autoimmunity (esp platelets and RBCs)
    - Antidrug antibodies (ADA) / drug complexes

- **Low incidence or high incidence finding, depending on mechanism**
About platelets

- Large number and variety of surface receptors
  - Binding / crosslinking of receptors leads to activation

- Express largest pool of FcγRIIa (CD32) in circulation
  - 200,000-600,000 platelets/uL blood
  - ~5000 FcγRIIa/platelet
  - Low affinity receptor for monomeric IgG

- Activated by large molecules, especially Abs
  - Binding of mAb via Fab and Fc portions to same or different platelets
  - ADAs against a drug that associates with platelets (heparin)
  - Multimeric IgGs (aggregates, ADA/drug immune complexes)
Sequelae of acute platelet activation

- Acute post-dosing clinical signs
- Release of vasoactive mediators
- Intravascular platelet aggregates
- Activation of complement and clotting cascades
  - Thrombosis, thromboemboli
- Decreased platelet counts and functionality

Adapted from Current Opinion in Cardiology 2008, 23:302–308
Three case studies

- AMG X: Decreased platelets
- mAb Z: Decreased platelets, neutrophils, +/- monocytes
- mAb-Y.1: Decreased platelets and RBCs
AMG X causes species-specific off-target decrease in platelets

- **Human IgG2 MAb**
  - Soluble target not expressed by platelets

- **One month cyno study**
  - 30, 100, 300 mg/kg SC; 300 mg/kg IV weekly
  - Red face and transient loss of consciousness (300 mg/kg IV)
  - Minimally decreased PLT one week after 4th dose

- **Follow-up single dose study**
  - Red face and loss of consciousness after IV dosing, but not after SQ
  - Acute profound decreases in platelet counts

1Santostefano et al, ToxPath 2012
AMG X induces aggregation of macaque but not human or baboon platelets

Cynomolgus

Similar results for rhesus and pigtail macaques

Human

Similar results for baboon
AMG X at ≥1 mg/mL strongly activates macaque but not human or baboon platelets

Activation by AMG X at ≥1 mg/mL

Similar effects for rhesus and pigtail macaques

Activation correlated with binding to platelets

Similar effects for baboons

Representative data from analysis of 16 human donors and 13 cyno donors
Other mAbs against the target do not aggregate or activate cyno or human PLTs

AMG A, B, and C competed with AMG X for the same target
Other mAbs against target have no effect on platelet counts in cynomolgus monkeys in vivo.

- Clinical signs* with AMG X only

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*Separate CV study showed blood pressure changes consistent with serotonin release from platelets
AMG X activates macaque platelets

- AMG X caused platelet activation which resulted in
  - Profound decreases in platelet counts
  - Red skin and transient loss of consciousness due to serotonin release from activated platelets

- Effects on platelets
  - Were genus-specific
  - Required binding of Fab portion to platelets, and crosslinking via the Fc portion to FcγRIIa

- Likely off-target

- Ongoing efforts to identify pathway or binding site
Infusion reactions and blood cells

- Infusion reactions occur by multiple mechanisms
  - Type III Hypersensitivity = immune complex; serum sickness
    - IgG-mediated process (vascular, other)
  - Type I Hypersensitivity = anaphylaxis
    - Antigen-induced IgE-mediated degranulation of mast cells and basophils (e.g. cetuximab)
  - Anaphylactoid reactions
    - Non-immunologic release of mediators from basophils and mast cells

- Other causes of infusion reactions
  - Complement activation (CARPA)
  - Tumor lysis
  - Cytokine storm

- Decreases in peripheral blood cell counts sometimes associated with infusion reactions
Acute immune complex-related infusion reactions

- Generally after IV dosing
- Non-specific clinical signs
  - Vomiting
  - Fainting
  - Shortness of breath
- Pathology
  - Decreased platelets, neutrophils, +/- monocytes
  - Vasculitis, microthrombi, thromboemboli
mAb-Z: IV Dosing

- Human monoclonal antibody against a cell membrane target

- Uneventful 4-week study
  - Once weekly IV doses of 20, 100, 300 mg/kg
  - No non-pharmacologic effects

- Eventful 14-week study
  - Once weekly IV doses at 10, 50, and 300 mg/kg
  - Acute post-dosing clinical signs in 3 mid-dose females only
    - Animal 1: Week 5
    - Animal 2: Weeks 8, 9, and 10
    - Animal 3: Weeks 13 and 14
Mid-dose Clinical Signs and Pathology Changes Consistent with Immune Complex Formation

- **Clinical pathology**
  - Post-dose decreased platelets and neutrophils with or without decreased monocytes
  - No effect on lymphocytes

- **Histology**
  - Vasculitis, pulmonary thromboemboli, hemorrhage

- **Collected blood for hematology, ADAs, and ICs pre/post dose from all animals**
  - Included MPC (mean platelet component--platelet activation)
Platelet Counts: Change in Platelet Counts Day 92 (Post-Pre Dose)

Change in PLT count (Post-Pre) Day 92

Animals with attenuated pharmacologic effects

Animal with pharmacologic effects

15 minutes post-dose

Mid-dose female with post-dose clinical signs

Change (x10^3 /uL)

mg/kg/wk
Mean Platelet Component: Day 92 Post-Dose

- Measure of platelet activation
  - Inversely correlated
  - Marker of granularity

- Complexed IgG activates platelet via FcγRIIa

- Correlated with immune complexes

Animal with evidence of pharmacologic effects

Mid-dose female with post-dose clinical signs

Animal with evidence of ADA+ pharmacologic effects

Animal with evidence of ADA- pharmacologic effects
Conditions promoting acute post-dosing effects due to immune complexes

- 3 conditions responsible (but not sufficient)
  - Animals with free ADAs
  - Drug near or below limit of quantitation at time of dosing
  - IV bolus administration

- IC-related findings not relevant for human dosing

- Not considered in determining the NOAEL
mAbY.1 causes marked decreases in platelet counts and red cell mass in cynos

- **Doses**
  - 10, 50, and 300 mg/kg SC
  - 300 mg/kg IV

- **Decreased platelets and red cell mass at 40 hr post dose**
  - Occurred after subsequent doses

- **Enlarged spleen with prominent macrophages after multiple doses**

- **Unexpected**
  - Not anticipated based on tissue expression
  - Not observed with small molecule inhibitors of intended target

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*Everds et al, ToxPath 2013*
Platelet counts in cynos after first dose of mAbY.1

Increasing Dose

Large platelets at 300 mg/kg

Vehicle
10 mg/kg SC
50 mg/kg SC
300 mg/kg SC
300 mg/kg IV
HCT in cynos after first dose of mAbY.1

Hct as % of pretest

Increasing Dose

Spherocytes and reticulocytes at 300 mg/kg

Subcutaneous

0 mg/kg 10 mg/kg 50 mg/kg 300 mg/kg
Hypotheses considered

- mAbY.1 binds to epitope on RBCs and PLTs
  - mAbY.1 binds to something expressed on platelets and/or red cells
  - Coated cells phagocytosed by macrophages

- mAbY.1 stimulates processes that activate macrophages
  - Acquired hemophagocytic syndrome
  - Platelets and RBCs are innocent bystanders

- Combination of above

- However...
  - No binding of mAbY.1 to cyno or human blood cells
  - No activation of cyno or human platelets by mAbY.1
Hemophagocytic syndromes

- **Congenital disease**
  - Defect in perforin pathway (NK cell function)

- **Acquired disease**: Dysregulation of immune system
  - Herpes viruses, other viral, bacterial, and parasitic infections, cancer, autoimmune disease
  - Biologicals (numerous interleukins, hematopoietic growth factors, mAbs)

- **Can cause secondary life-threatening cytopenias**
  - RBCs only
  - Platelets only
  - Multiple blood cell types, including WBCs
**Hemophagocytic syndromes**

- **CD4+**
  - Activated MΦ
  - IFNγ, M-CSF
  - IL-2

- **CD8+**
  - IFNγ, M-CSF
  - Uncontrolled T cell activity

- **NK**
  - Defective NK cell activity: no cytotoxic control over T cells or activated macrophages

- **Activated MΦ**
  - TNFα
  - IL-1, IL-6, IFNγ
  - sCD163

**No in vitro cytokine release by mAbY.1 in cynomolgus PBMCs**
mAbY.1 induced platelet phagocytosis in cyno but not human monocytes

No induction of phagocytosis in cyno monocytes by isotype controls, other mAbs against target, modified molecules (F(ab’)2 or aglyco IgG1) or by mAbY.1

No induction of phagocytosis in human monocytes by isotype controls, other mAbs against target, modified molecules (F(ab’)2 or aglyco IgG1) or by mAbY.1
Cyno study with mAbY.1, 4 other molecules against same target, and aglyco IgG1 mAbY.1

- Single dose 72-hour study
- Spleen enlarged at 72 hrs post dose after mAbY.1
  - RBC morphology suggested removal of red blood cells by macrophages
- No changes in cytokines, lymphocyte subsets, etc.

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All molecules dosed at 300 mg/kg IV
Splenic cytology: macrophage activation / proliferation, and erythrophagocytosis

Cyno: Stock animal

Cyno: 72 hours post-mAbY.1
Increased mitoses/phagocytosis in spleen at 72 hr post-dose

Phagocytosis within macrophage population (arrows)

Mitotic figures (arrowheads)

HE, 40x
mAbY.1 Hematologic Effects

- Associated with activation of splenic macs / peripheral blood monocytes
- Unique for mAbY.1 and not observed with other molecules against target
- Dependent on functional Fc (evaluated in vitro only)
- Effects are likely not relevant to humans
- Further investigations ongoing to isolate the specific mechanism
Summary

- Unexpected effects of large molecules can include dramatic effects on peripheral blood cells
  - Direct or indirect
  - Single or multiple cell types
  - Can be species-specific

- Platelets are particularly vulnerable
Acknowledgments

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Important of Fc and Fab for AMG X induced toxicity

- In vitro activation of macaque platelets by AMG X
  - Correlates with binding to platelets
  - Requires interactions with platelets through both the Fab and Fc
    - Fc required: No activation if Fc binding blocked or with F(ab’)2
    - Fab required: No activation if preincubation with intended target or preincubation of platelets with AMG X F(ab’)2

- In vitro incubation of macaque platelets with AMG X
  - Caused release of serotonin
Immune complex-related infusion reactions in macaques after repeated immunization

- **Experimental paradigm:** immunize animals with IM or SQ doses of antigen
- **Immunized cynos challenged with IV antigen**
  - Clinical signs similar to humans with antidextran antibodies
  - Complement activation
  - Decreased platelets, neutrophils, and monocytes
  - Likely platelet activation
  - Lymphocytes relatively spared

Smedegard et al 1980; Revenas et al 1980; Birmingham et al 1999; Rojas et al, 2005