Cardiovascular Safety of Biologics: Challenges and Opportunities

Rakesh Dixit, Ph.D., DABT
Vice President, R & D
Head, Biologics Safety Assessment
Translational Sciences
CV toxicities, including TDP are the major contributor to drug marketing withdrawals costing billions in lost revenue.

Adapted from Watkins, P. B.: Clinical Pharmacology & Therapeutics: 89 (6), 788. 2011
About 25% of all approved biological agents have been issued significant safety warnings

- Only one therapeutic mAb (efalizumab) has been voluntarily withdrawn by Genentech from the market in 2009 due to increased number of progressive multifocal encephalopathy (PML) cases

The major concern with biologics is the toxicities observed after long-term chronic use. The probability of a first safety-related regulatory action, derived from Kaplan-Meier analyses, was:

- 14% (95% confidence interval [CI], 9%-19%) 3 years after approval
- 29% (95% CI, 20%-37%) 10 years after approval.

Most common adverse events consistent with MOA:

- Infusion and hypersensitivity reactions (generally acute in nature)
- Infections and infestations (e.g., TB, PML)
- Immune system disorders (e.g., autoimmune diseases)
- Cancer benign, malignant, and unspecified
Key Safety Concerns with Monoclonal Antibodies

**Infusion, Hypersensitivity Reactions and Immunogenicity**

**Cytokine Release Syndrome**
- Muromomab-CD3
- TGN-1412 induced cytokine storm leading to multiple organ failure

**Infections and Infestations**
- Reactivation of respiratory infections: Tuberculosis (e.g., anti-TNF mAbs)
- Progressive Multifocal Encephalopathy (e.g., natalizumab, efalizumab)

**Platelet and Thrombotic Disorders**
- Thrombocytopenia (e.g., infliximab, efalizumab, alemtuzumab)
- Bevacizumab induced HTN & arterial thromboembolic events

**Autoimmune Diseases and Cancer**
- Lupus-like syndromes (e.g., anti-TNFs)
- Autoimmune colitis (CTLA-4 specific ipilimumab)
- Autoimmune thyroid disease (e.g., alemtuzumab)
- Increased malignancies seen with anti-TNF antibodies

Biologics and Cardiovascular Toxicities

- Generally class-MOA based direct toxicity, type II injury (potentiation of previous injury) and maybe secondary to hemodynamics and other toxicities (e.g., electrolyte and fluid imbalance, proteinuria, arterial thrombosis)

  - Trastuzumab (anti-Her2) induced left ventricular dysfunction, cardiomyopathy, CHF (increased in combination with anthracyclines)
  - Bevacizumab (anti-VEGF) induced hypertension, arterial thromboembolism, cardiac ischemia, CHF
  - Rituximab (anti-CD20)- arrhythmias, hypotension, angioedema: rare cardiac failure
  - Alemtuzumab induced hypotension
  - Cetuximab- hypotension in setting of severe infusion reactions
  - Interleukins IL-2 : hypotension, arrhythmias
  - Denileukin diftitox: hypotension
  - Interferon-alpha: hypotension, ischemia
CV Toxicities with Biologics: Differences and Similarities with Small Molecule Pharmaceuticals

Small Molecule Pharmaceuticals

◆ The major MOA for QT prolongation, proarrhythmia and TDP is through highly promiscuous hERG channel binding (generally an off-target effect due to high promiscuity of synthetic drugs and their metabolites)

◆ Cellular and Molecular Mechanisms of CV toxicities (e.g., anthracyclines, tyrosine kinase Inhibitors, her-2 inhibitor like Tykerb)
  – MOA related to blockage of both extracellular and intracellular signaling pathways
  – Myocardial apoptosis, necrosis and adaptation
  – Signaling (extracellular and intracellular) pathways and mitochondrial toxicities
  – Cytokines and other factors leading to myocardial death signaling

Large Molecule Biologics

◆ hERG channel inner pore is not readily accessible by large protein Mabs and the high target antigen specificity of mAbs is exceptionally differential to extracellular ERG toxin-binding site of hERG channel

◆ Mabs targeting HER-2 and angiogenesis pathways
  – MOA related to blockage largely limited to extracellular signaling targets, including soluble targets
  – Type II toxicities of Her-2 inhibition leading to impairment of cardiomyocyte repair mechanisms (e.g., trastuzumab vs. Tykerb)
  – Cytokine release syndrome associated mechanism of myocardial injury
  – Anti-angiogenesis
    • Hypertension, edema, electrolyte imbalance
### Challenges

- Increasing concerns with biologics alone or in combination with small molecule drugs
- Non-hERG cardiac adverse effects, including effects on heart rate, blood pressure, ECG are not easily identified by standard GLP toxicology studies of biologics
- Selection of appropriate tox species appropriate for toxicity evaluations (e.g., monkeys (limited experience and data base) vs. dogs) still remains a major limitation for biologics
- Pleiotropic MOA of biologics is not often attainable in toxicology species
  - Makes it difficult to evaluate MOA based toxicities
- Safety biomarkers have not been appropriately qualified in biologics cv assessment in preclinical settings (In vitro or in vivo)

### Potential Opportunities

- Identify MOA based cv toxicities or potential risk factors (e.g., changes in heart rate, blood pressure etc.) early in the selection of molecules
  - Address MOA based cv safety separate from general safety assessment studies
  - Safety biomarkers enriched CV jacketed telemetry study
- Develop robust data base on cv events in monkeys to identify subtle changes in blood pressure, heart rate and ECG parameters
- Develop robust cardiac clinical safety plans based on cardiac dose-PK-PD-safety biomarkers
  - Use of cardiac troponin I successfully applied in reducing doxorubicin-trastuzumab toxicity in combination settings
Case study of Cardiac Toxicity of Trastuzumab: Value of MOA based toxicity evaluation vs. Standard GLP Nonclinical Safety Assessment
HER2 Inhibitors (Efficacy)

◆ Unprecedented benefits

◆ Adjuvant
  – Adding trastuzumab to chemotherapy reduces relative risk of recurrence by 50% and death by 33%
  – Usually concurrent with taxane-based chemotherapy

◆ Metastatic
  – Revolutionary in terms of improving survival when added to chemotherapy
  – Combination of trastuzumab + chemotherapy for 4–6 months, then trastuzumab alone
  – If tumor ER+ and HER2+, letrozole + lapatinib may be best option for many patients
  – Lapatinib + either chemotherapy or trastuzumab improves outcomes in multiply refractory metastatic breast cancer

Cardiovascular Toxicity with Trastuzumab

Cardiac dysfunction: cardiomyopathy characterized by a decrease in LVEF, either global or more severe in the septum; symptoms of CHF; associated signs of CHF; and decline in LVEF of at least 5% to less than 55% with signs and symptoms of CHF or 10% to less than 55% without signs or symptoms of CHF.

• 2% to 8% of patients undergoing therapy with trastuzumab
• 27% of patients receiving trastuzumab and doxorubicin
• 13% of patients treated with trastuzumab and paclitaxel

www.fda.gov (trastuzumab BLA filing)
A Single-Photon-Emission Computed Tomographic Image Showing a Sagittal Slice through the Lower Chest and Upper Abdominal Region of a Woman with Metastatic Breast Cancer and a Large Hepatic Metastasis in the Left Lobe of the Liver.

Strong uptake is seen in the liver metastasis, which has almost replaced the left hepatic lobe (arrow), as well as in the horseshoe-shaped myocardial wall (arrowheads) located above. A denotes anterior, and P posterior.

Seven of the 20 patients with myocardial up take developed cardiotoxicity. None of the 13 patients without myocardial up take developed any evidence of cardiac toxicity.

NEJM 345, 995, 2001
Trastuzumab TCR using frozen human and cyno tissues

High degree of homology in Her-2 between cynomolgus monkeys and humans

Positive membrane staining in a subset of epithelial cells, including exocervix, skin, esophagus, urothelium of bladder and tonsil.

Epithelial cells in breast acinar and ductal cells, endocervical glands, renal tubules, gi tract, pancreas, salivary gland also showed positive staining.

No cardiac tissue staining in cynos heart or normal healthy human heart.

www.fda.gov (trastuzumab BLA filing)
Nonclinical Toxicology studies with Trastuzumab (alone) and in combination with Doxorubicin

◆ **Multiple dose i.v. toxicity in rhesus (4 weeks only) and cynomolgus monkeys (12 and 26-weeks)**
  - Up to 25 mg/kg (~ 16X AUC margin over maximal human exposures)
    - Well tolerated with no evidence of cardiac toxicity
      - Sporadic very mild reduction in heart rate and very slight (5 sec) prolongation in QT interval
      - Considered not adverse and thought to be within the normal variation seen in cynos
    - No evidence of immunogenicity

◆ **Cardiotoxicity studies**
  - Lack of binding to cardiac tissue (no positive TCR) from monkeys and humans
  - Single dose studies in rhesus monkeys with trastuzumab-doxorubicin (both at 1.5 mg/kg) showed no evidence of cardiac toxicity
  - Rat c-erbB2 surrogate antibody showed no potentiation of doxorubicin cardiotoxicity in the rat model

[www.fda.gov (trastuzumab BLA filing)]
Role of ErbB2/Her-2 in Cardiac Homeostasis

◆ T-tubules (contains essential components of excitation-contraction coupling machinery) in cardiac muscles are rich in ErbB2 and ErbB4 receptors.

◆ ErbB2 receptors activate various signaling pathways, including the activity of K⁺ channels, nonselective cation channels, and G-protein coupled receptors in the heart. ErbB2 functions as a coreceptor in neuregulin pathway.

◆ Conditional (cardiac restricted) mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy in mice (Ozcelik et al., PNAS 99 (13), 8880, 2002)
  - Enhanced QT interval with delayed ventricular repolarization, severely compromised pumping capacity, sudden death
  - Enlarged ventricular chambers and myofiber hypertrophy; increased heart to body weight ratio
  - Enhanced susceptibility to anthracycline-induced cardiac damage
Mitochondrial dysfunction, energy compromise, and cytochrome c release.

Worsening of cardiac contractibility, left ventricular function.

Mitochondrial dysfunction, energy compromise, and cytochrome c release.

Essential for cardiomyocyte proliferation and contractile function in the adult.

Potential Mechanism of Cardiotoxicity Of Trastuzumab

Comparison of Doxorubicin vs. Trastuzumab Cardiac Toxicity

<table>
<thead>
<tr>
<th>Type I</th>
<th>Type II</th>
</tr>
</thead>
<tbody>
<tr>
<td>(eg, Doxorubicin)</td>
<td>(eg, Trastuzumab)</td>
</tr>
<tr>
<td>Cellular death</td>
<td>Cellular dysfunction</td>
</tr>
<tr>
<td>Damage starts with the first administration</td>
<td></td>
</tr>
<tr>
<td>Biopsy changes (typical of anthracyclines)</td>
<td>No typical anthracycline-like biopsy changes</td>
</tr>
<tr>
<td>Cumulative dose-related</td>
<td>Not cumulative dose related</td>
</tr>
<tr>
<td>Permanent damage (myocyte death; bad prognosis)</td>
<td>Predominantly reversible (myocyte dysfunction; good prognosis)</td>
</tr>
<tr>
<td>Risk factors: Combination CT</td>
<td>Risk factors: Prior/concomitant anthracyclines or paclitaxel</td>
</tr>
<tr>
<td>Prior/concomitant RT</td>
<td>Age</td>
</tr>
<tr>
<td>Age</td>
<td>Previous cardiac disease</td>
</tr>
<tr>
<td>Previous cardiac disease</td>
<td>Obesity (BMI &gt; 25 kg/m²)</td>
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</table>

CT = computed tomography; RT = radiotherapy; BMI = body mass index. Barrett-Lee et al, 2009; Ewer et al, 2005; Ewer, 2007; Rahman et al, 2007.
Cardiovascular Injury: Dangerous Liaisons between Anthracyclines and Trastuzumab

Type I
Cell death injury

Type II
Cellular dysfunction, impairment of repair

Anthracyclines  Trastuzumab

Augmentation of Type I Injury

Myocardial Remodeling is Progressive

Type II insult dissipates, (trastuzumab t1/2 ≈ 25 days; it may persist in circulation for up to 18 weeks) sometimes leaving an augmented Type I injury with all the late sequelae and late expression of any injury that results in cellular death

modified from: Mann DL; Circ 1999
• The loss of cardiac ErbB2 can lead to heart failure and increased susceptibility to anthracycline cardiotoxicity.

• Clinical studies support the concept that trastuzumab (type II cardiotoxin) acts as a molecular modifier of anthracycline-induced cardiotoxic effects and associated heart failure.

• The irreversible loss of viable cardiac muscle is normally prevented by the concomitant activation of cardiomyocyte-survival pathways, including gp130 cytokines and neuregulin.

• Trastuzumab, by inhibiting the ErbB2 receptor, leads to a loss of the neuregulin-dependent pathways needed for the survival of cardiac myocytes.

• In most patients, the activation of other survival pathways is sufficient to prevent the loss of viable muscle cells and the onset of heart failure. However, in a subgroup of patients, the loss of the ErbB2-dependent survival pathways promotes the cardiotoxic effects of anthracyclines.
MEDI0639 (anti-DLL4 IgG1 mAb, a potent angiogenesis inhibitor): Cardiovascular Safety Evaluation in Cynomolgus Monkeys
DLL-4: Notch1 Signaling and Angiogenesis

A

DLL4 (ECs-restricted)
- Anti-DLL4
- EGF-like repeats
- DSL
- DLL4-Fc
- Notch (Broad Expression)

ADAM
- LN
- RAM
- ANK
- TAD
- NLSs
- PEST

NICD

Target genes

B

- Sprouting
- EC proliferation
- EC survival
- Vascular organization
- Tumor vascular density
- Tumor vessel perfusion
- Tumor growth

DLL4 blockade

DLL4/Notch

VEGF/VEGFR

Sprouting
EC proliferation
Vessel lumen size
Vascular organization
Tumor vascular density
Tumor vessel perfusion
Tumor growth

VEGF blockade

Clin Cancer Research (2007): 13, 7243
MEDI0639 is a potent modulator of angiogenesis

- MEDI0639 is a human antibody that binds to DLL4 and potently inhibits Notch signaling.

- ProQinase human endothelial cell matrigel plug model: HUVEC spheroids in matrigel/fibrin plug implanted s.c. in SCID mice. Abs dosed i.p. 2/week for 3 weeks.

KEY: CD34 αSMA

Vehicle control MEDI0639

<table>
<thead>
<tr>
<th>Vessel number</th>
<th>Mural cell coverage</th>
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<tbody>
<tr>
<td>% of hCD34⁺ve vessels covered with mural cells</td>
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- Treatment with MEDI0639 causes expected phenotype - increased vessel density with less mural cell coverage.
Cardiovascular Safety Pharmacology Study in Cynomolgus Monkeys (Jacketed Telemetry)

Study Outline:

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/kg IV q2wk)</th>
<th>Volume (mL/kg)</th>
<th>Animals Males</th>
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<tbody>
<tr>
<td>1</td>
<td>Control</td>
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<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2</td>
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<td>4</td>
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<td>10</td>
<td>5</td>
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</table>

- **Objective:** To identify any potential adverse functional effects on the cardiovascular system associated with repeat dosing of MEDI0639 in animals surgically implanted with radiotelemetry transmitter.

- **Repeat IV (slow bolus) injections Q2 weeks dosing for 7 total doses over 3 months.**
  - Monitor CV safety endpoints (ECG, bp, heart rate, etc) continuously via telemetry in conscious animals; TK, ADA, PD by sVEGFR2, cardiac biomarkers (e.g. cardiac Troponin I, CK isozymes, NT-pro-BNP, CRP) and clinical pathology at various time points.
  - Monitor animals over additional 8-week dose-free recovery phase
Dose-related increases in HR and BP in CV Safety Study
Echocardiogram Results Weeks 5 and 14

- Findings indicate wall thickening, decreased chamber diameter, and reduced cardiac output

<table>
<thead>
<tr>
<th>Prestudy Group Averages</th>
<th>LVPWd (cm)</th>
<th>LVPWs (cm)</th>
<th>LVIDd (cm)</th>
<th>LVIDs (cm)</th>
<th>EDV (mL)</th>
<th>ESV (mL)</th>
<th>EF (%)</th>
<th>CO (L/min)</th>
<th>HR (bpm)</th>
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<tbody>
<tr>
<td>Group 1</td>
<td>0.38</td>
<td>0.55</td>
<td>1.61</td>
<td>0.94</td>
<td>7.39</td>
<td>1.75</td>
<td>75.62</td>
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<td>0.37</td>
<td>0.56</td>
<td>1.64</td>
<td>1.01</td>
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<td>2.18</td>
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<td>Group 3</td>
<td>0.36</td>
<td>0.55</td>
<td>1.46</td>
<td>0.92</td>
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<td>1.68</td>
<td>69.62</td>
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<td>Group 4</td>
<td>0.38</td>
<td>0.58</td>
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<th>LVPWs (cm)</th>
<th>LVIDd (cm)</th>
<th>LVIDs (cm)</th>
<th>EDV (mL)</th>
<th>ESV (mL)</th>
<th>EF (%)</th>
<th>CO (L/min)</th>
<th>HR (bpm)</th>
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<td>0.51</td>
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<td>0.63</td>
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<td>0.45</td>
<td>0.64</td>
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<th>Day 96 Group Averages</th>
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<th>LVPWs (cm)</th>
<th>LVIDd (cm)</th>
<th>LVIDs (cm)</th>
<th>EDV (mL)</th>
<th>ESV (mL)</th>
<th>EF (%)</th>
<th>CO (L/min)</th>
<th>HR (bpm)</th>
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<tbody>
<tr>
<td>Group 1</td>
<td>0.37</td>
<td>0.54</td>
<td>1.60</td>
<td>0.95</td>
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<td>Group 4</td>
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<td>1.17</td>
<td>72.86</td>
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</table>

LVPWd = Left ventricular posterior wall thickness in diastole
LVPWs = left ventricular posterior wall thickness in systole
LVIDd = Left ventricular internal diameter in diastole
LVIDs = Left ventricular internal diameter in systole
EDV = End diastolic volume
ESV = End systolic volume
EF = Ejection fraction
CO = Cardiac output
HR = Heart rate
CV Safety Study Results Summary

- Slight increase in CRP without any changes in cTi or CPK or BNP
- QA interval decreased at 10 mg/kg suggesting increase in cardiac contractility.
- Echocardiogram results are suggestive of left ventricular wall thickening, decreased internal diameter and reduced cardiac output.
- Reduced CO consistent with decreased chamber volume and could explain increased workload (HR increase).
- Increased RPP reflects increased heart workload or oxygen demand.
- Highest non-severely toxic dose (HNSTD) was 1 mg/kg and provided generally acceptable safety margins for FTIH doses.

Cardiac Pathology

- Dilated capillaries;
- Myofiber degeneration;
- Inflammation;
- Necrosis
CRP Changes in CV Safety Study

Mean CRP Levels in CV Safety Study

- Days: -20, 30, 80, 130, 180
- Percent Hct: 0, 100, 200, 300, 400, 500, 600, 700, 800, 900

Lines represent:
- Vehicle
- 1 mg/kg
- 3 mg/kg
- 10 mg/kg
Cardiac Safety and Potential Mechanisms

◆ MEDI0639 cardiac safety profile as identified by preclinical tox studies in NHPs:
  - Hypertension and Cardiac toxicity – monitorable (BP/HR/ECHO) & reversible
  - Threshold dependent with greater than 10X safety margin for overtly cardiotoxic exposures
  - Human clinical safety assessment of nonclinical findings is uncertain
    • No evidence of cardiac toxicities in cancer clinical trials with very similar MOA- Oncomed’s anti-DLL4 mab PMP-21M18 (Smith D et al http://www.oncomed.com/news/pr/Study1posterfinalNov10.pdf)

◆ DLL4-Notch1 Signaling and CV Homeostasis
  - Epithelial-mesenchymal transition as a potential source of mesenchymal stem cells in vascular and cardiac valves (Rusamescu et al (2008) Current Cardiology Reviews 4, 148-156)
  - Notch 1 inactivating mutations in humans cause an early defect in the aortic valve leading to progressive aortic valve disease (Garg et al)

J. Dupont (2011), OncoMed Pharmaceuticals
Conclusions

- Although the hERG or other ion channels interaction associated QT prolongation is not the major concern for the vast majority of biologics, significant CV toxicities through variety of MOAs can occur.
  - Lack of TCR in normal cardiac tissue is not sufficient to discount cardiac toxicity
  - The MOA of biologics (e.g., RTK, EGFR, angiogenesis, CRS etc) is critical to understanding cardiovascular safety characteristics
  - Renal and vascular adverse effects should be considered

- Specialized CV safety pharmacology (jacketed telemetry) should be conducted only in relevant animal model that can reproduce desired pharmacodynamics and anticipated drug exposures for the investigational biologic.
  - Safety biomarkers (e.g., cTi, BNP, CPK isoenzymes, CRP), and PD endpoints should be appropriated included
Acknowledgements

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