Safety Assessment of Antibody Drug Conjugates

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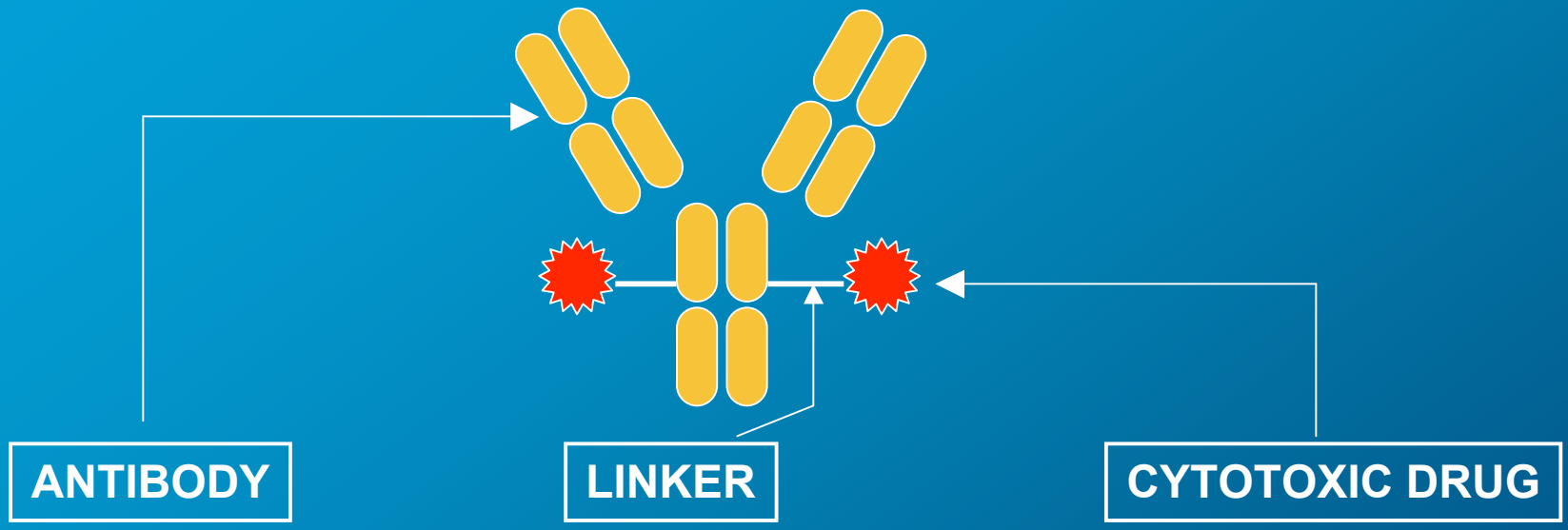
Overview

- Background for Antibody Drug Conjugates (ADCs)
- Designing an ADC Toxicology Program
- Trastuzumab-MCC-DM1 Toxicology Program

An ADC is a Highly Complex Pro-drug



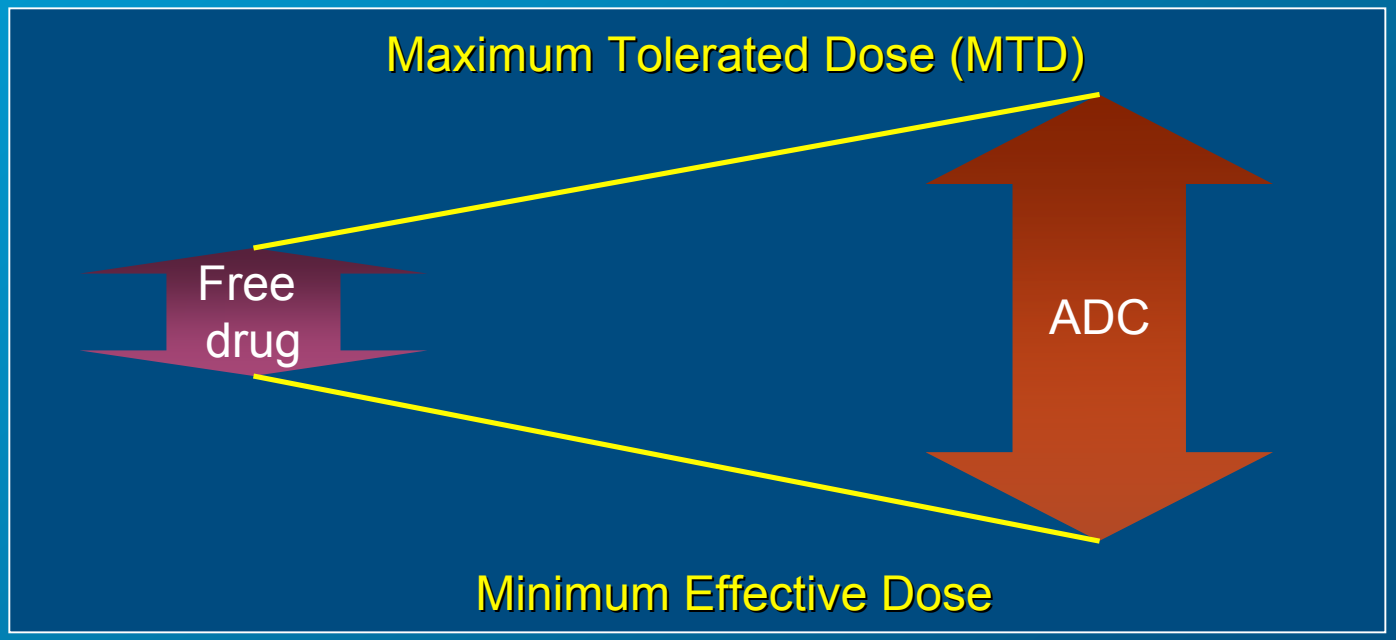
- MAbs
- THIO-MAbs

- Acid labile (hydrazone)
- Enzyme cleavable dipeptides
  - cleavable
- Thioether
  - uncleavable
- Hindered disulfide
  - uncleavable

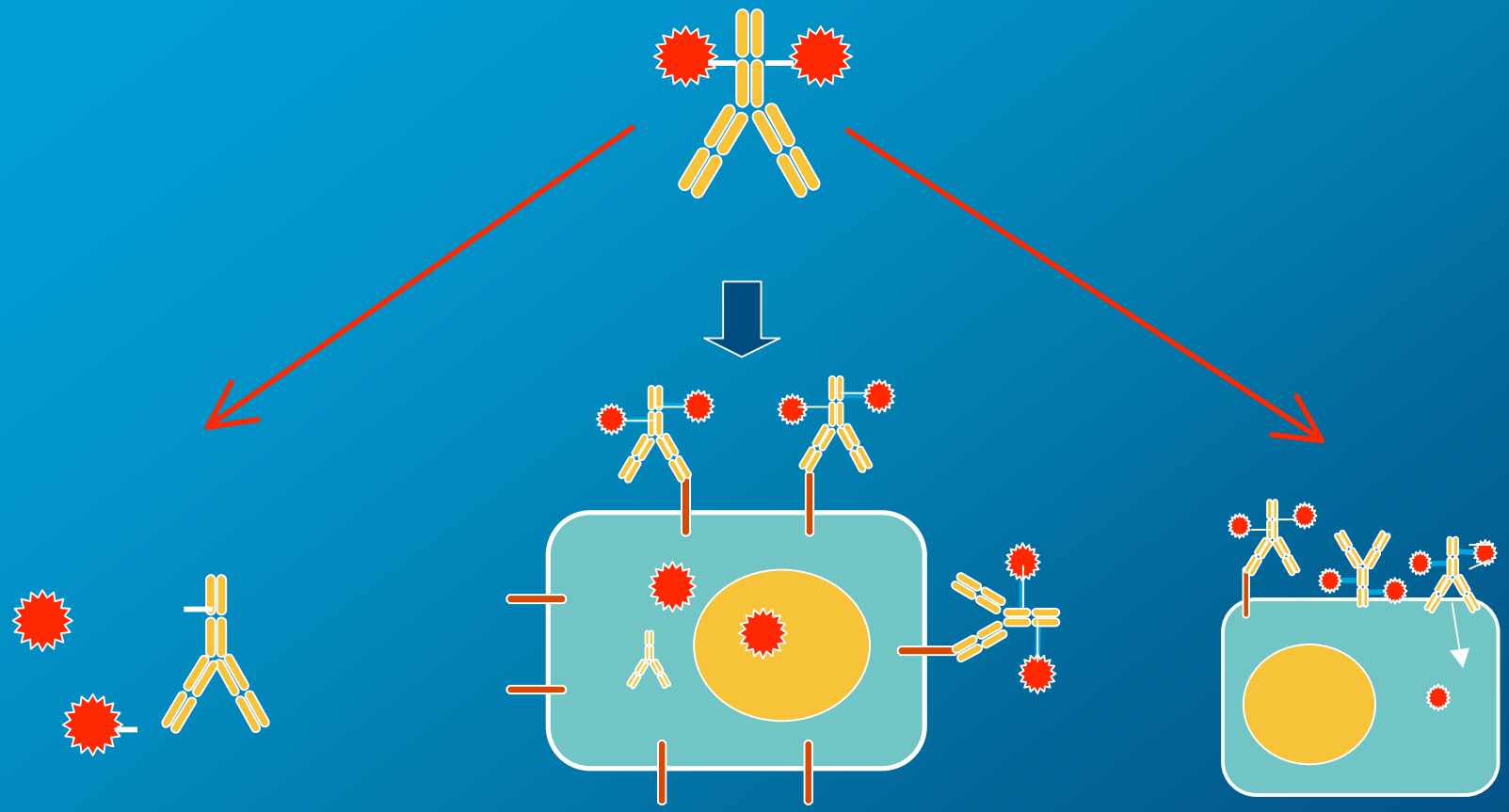
- Tubulin polymerization inhibitors
  - maytansines (DM1, DM4)
  - auristatins (MMAE, MMAF)
- DNA damaging agents
  - calicheamicin, duocarmycin
  - doxorubicin

Improving Therapeutic Window

- ADCs target delivery of a potent cytotoxic drug to tumor cells via tumor-specific and/or over-expressed antigens
  - Increase drug delivery to tumor
  - Reduce normal-tissue drug exposure



Antibody Drug Conjugate Processing



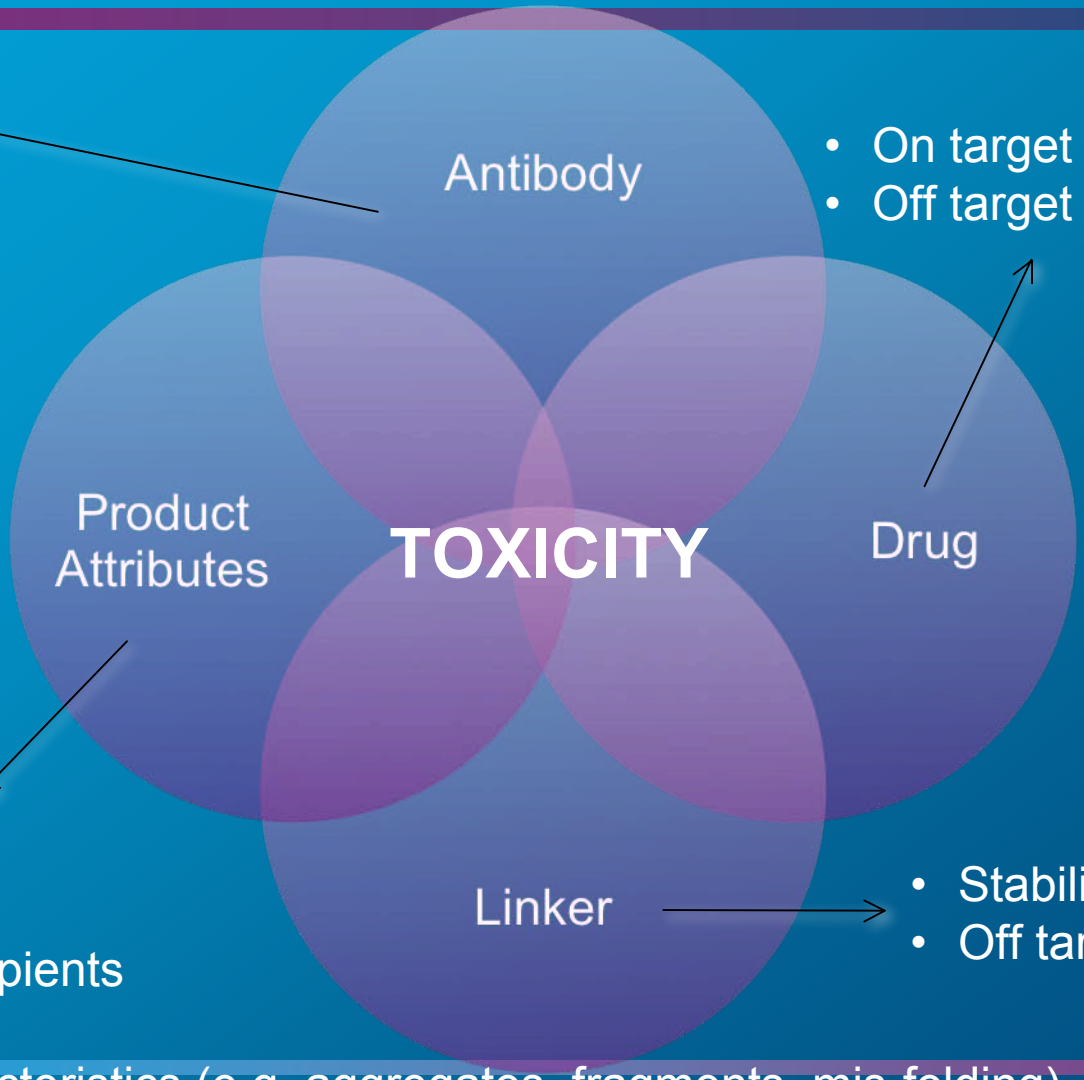
Target-independent toxicity
- Linker instability

Antigen-specific cytotoxicity
- Tumor
- Normal tissue

Target-independent toxicity
- Non-antigen mediated uptake (Fc, pinocytosis)

Determinants of Toxicity

- Antigen
- FcγRs
- FcRN



- On target (i.e. MOA-related)
- Off target

- Stability
- Purity, DAR
- Free drug
- Impurities, excipients
- Formulation
- Antibody characteristics (e.g. aggregates, fragments, mis-folding)

- Stability
- Off target

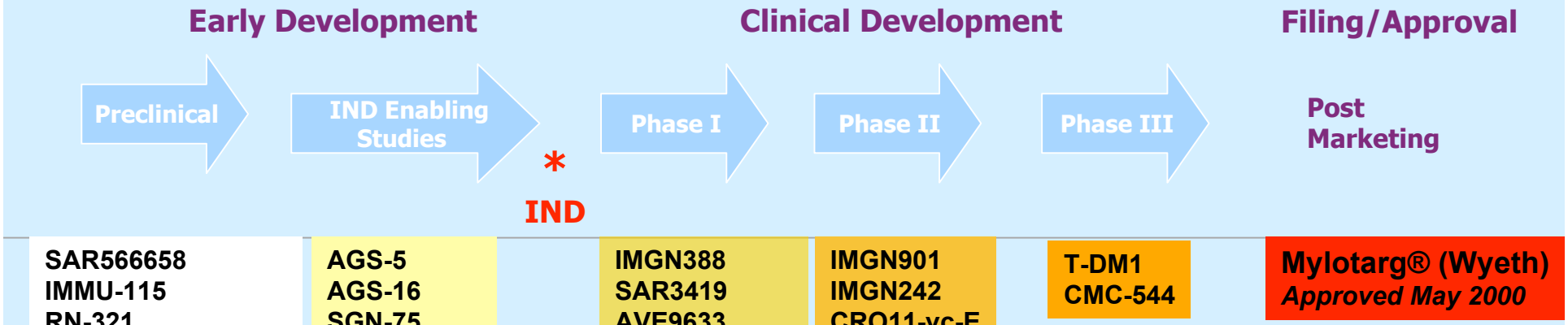
Designing an ADC Toxicology Program

- No specific guidance for ADCs
- Principles same as with other molecules; should be case-by-case and scientifically and data driven
- ADCs, the debate in a nutshell:
  - What is the most appropriate/relevant toxicology species for a hybrid molecule?
  - What is the test article?
  - Should we test the components?
  - Should we measure to define PK/TK?
  - Should we or should we not assess developmental and genetic toxicity?

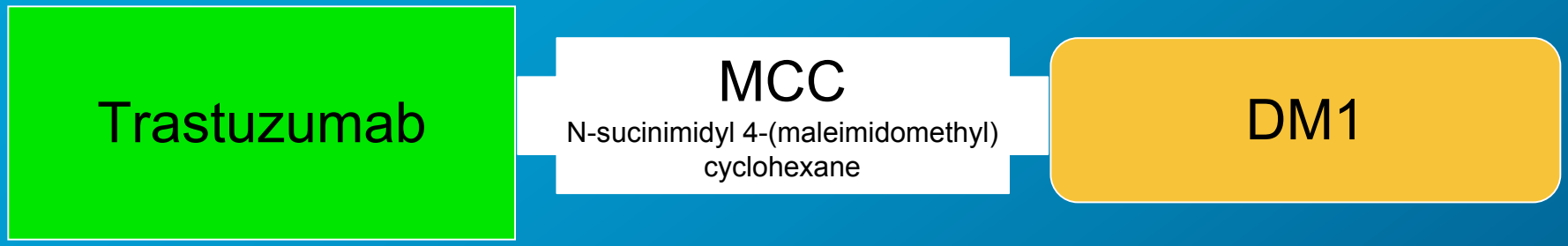
Designing an ADC Toxicology Program

POINTS TO CONSIDER	SMALL MOLECULE	BIOLOGICS	ADCs
Species Selection	2 species	Often 1 species	1 or 2 species
Test Article	Chemical	Protein	Conjugate (protein-chemical)
Manufacturing	Synthesized	Culture-derived	Both + conjugation
Toxicity	On- and off-target	Typically exaggerated pharmacology	Typically antigen-independent
PK	Less specific binding	Specific antigen binding	Specific antigen binding; non-specific for released SM
	Short t½	Long t½	Long t½ (antibody); sustained delivery of SM and rapid clearance
	High Vd	Lower Vd, target dependent	Lower Vd, mainly target dependent
PK Assays	Drug product, metabolites (if applicable)	Total antibody	Total antibody, ADC, un-conjugated toxin
Immunogenicity Assays	No	Yes	Yes

ADC Pipeline in 2009



Trastuzumab-MCC-DM1 (T-DM1)



Antibody – trastuzumab

- IgG1 MAb, targets HER2 expressed in ~25% of patients with breast cancer
- Trastuzumab binding: monkey HER2 = human HER2 (relevant species)

Linker (N-sucinimidyl 4-(maleimidomethyl) Cyclohexane

- Thioether linkage via lysine residues
- Stable, non-reducible

Cytotoxin – DM1

- Highly potent anti-mitotic agent
- Maytansine derivative; methyl group replaced with thiol
- Toxicity of maytansine well characterized

T-DM1 Nonclinical Program: Pivotal Studies

Toxicology Studies Conducted (GLP):		
Type of Study	**Species**	**ADC/Mab/Cytotoxic Drug**
Single Dose Tox	Rat, Monkey	T-DM1
Repeat Dose Tox (q3w x 4 dose)	Monkey	T-DM1
CV Safety Pharmacology	Monkey	T-DM1
Tissue Cross-Reactivity	Monkey, Human	T-DM1
Hemolytic Potential/Blood Compatibility	Monkey, Human	T-DM1
Single Dose Tox *(requested by FDA)*	Rat	DM1
Chronic Tox (6 mo., q3w x 8 dose)	Monkey	T-DM1
hERG	In vitro	DM1
Genotoxicity: Bacterial Reverse Mutation Assay & Rat Micronucleus	In vitro, Rat	DM1

Repeat Dose IV Toxicity Study of T-DM1 in Cynomolgus Monkeys

Test Article	Dose mg/Kg (ug DM1/m ^{2})	# Animals	Terminal Necropsy (Day 66) # animals	Recovery Necropsy (Day 85) # animals	Recovery Necropsy 2 (Day 106) # animals
Vehicle	0 (0)	7M/7F	3M/3F	2M/2F	2M/2F
T-DM1	3 (600)	7M/7F	3M/3F	2M/2F	2M/2F
T-DM1	10 (2000)	7M/7F	3M/3F	2M/2F	2M/2F
T-DM1	30 (6000)	7M/7F	3M/3F	2M/2F	2M/2F

- Regimen: 4 doses, q3wk, (Days 1, 22, 43, and 64)
- Route: IV

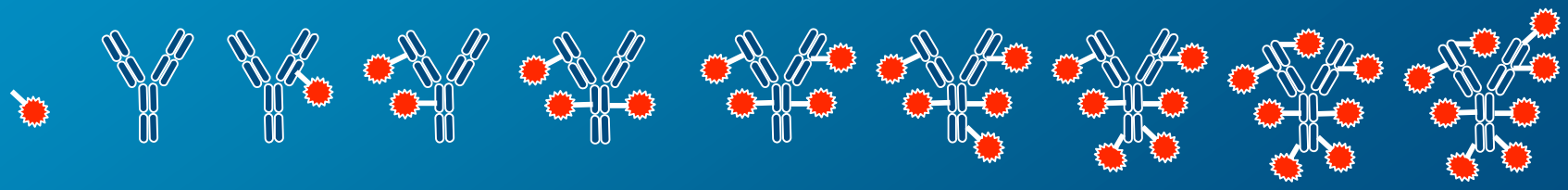
Endpoints:

- Body weights
- Clinical observations
- Food consumption
- Clinical pathology
- Ophthalmology examinations
- Physical examinations
- Neurological examinations
- ECGs
- Toxicokinetic evaluation
- Immunogenicity
- Gross necropsy observations
- Organ weights
- Histopathology

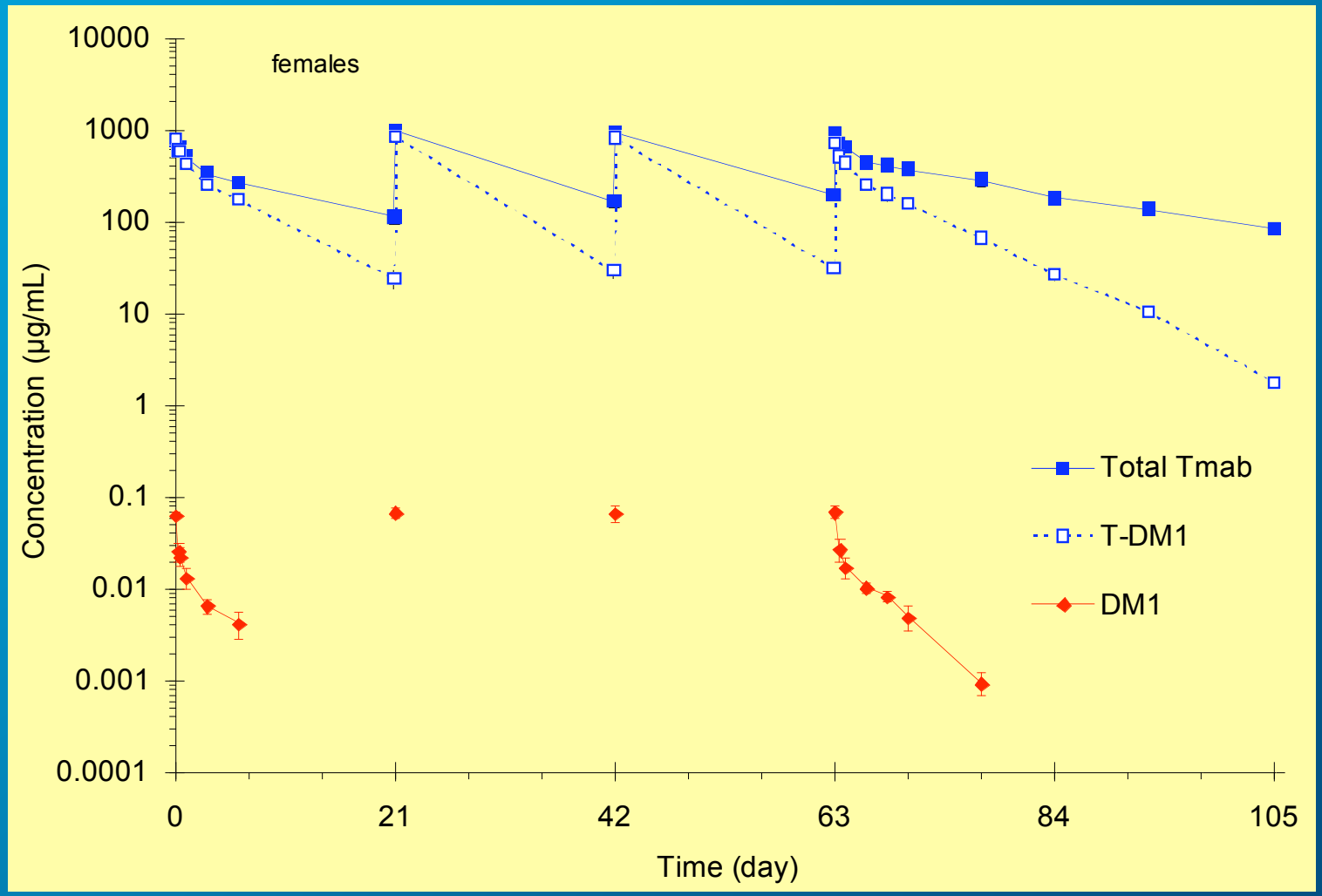
Toxicokinetic Assays

Serum/plasma concentrations measured for different components of T-DM1:

- T-DM1 conjugate ELISA
- Total trastuzumab ELISA
- Unconjugated DM1 LC/MS/MS assay
- Immunogenicity (anti-T-DM1antibodies), ECLA



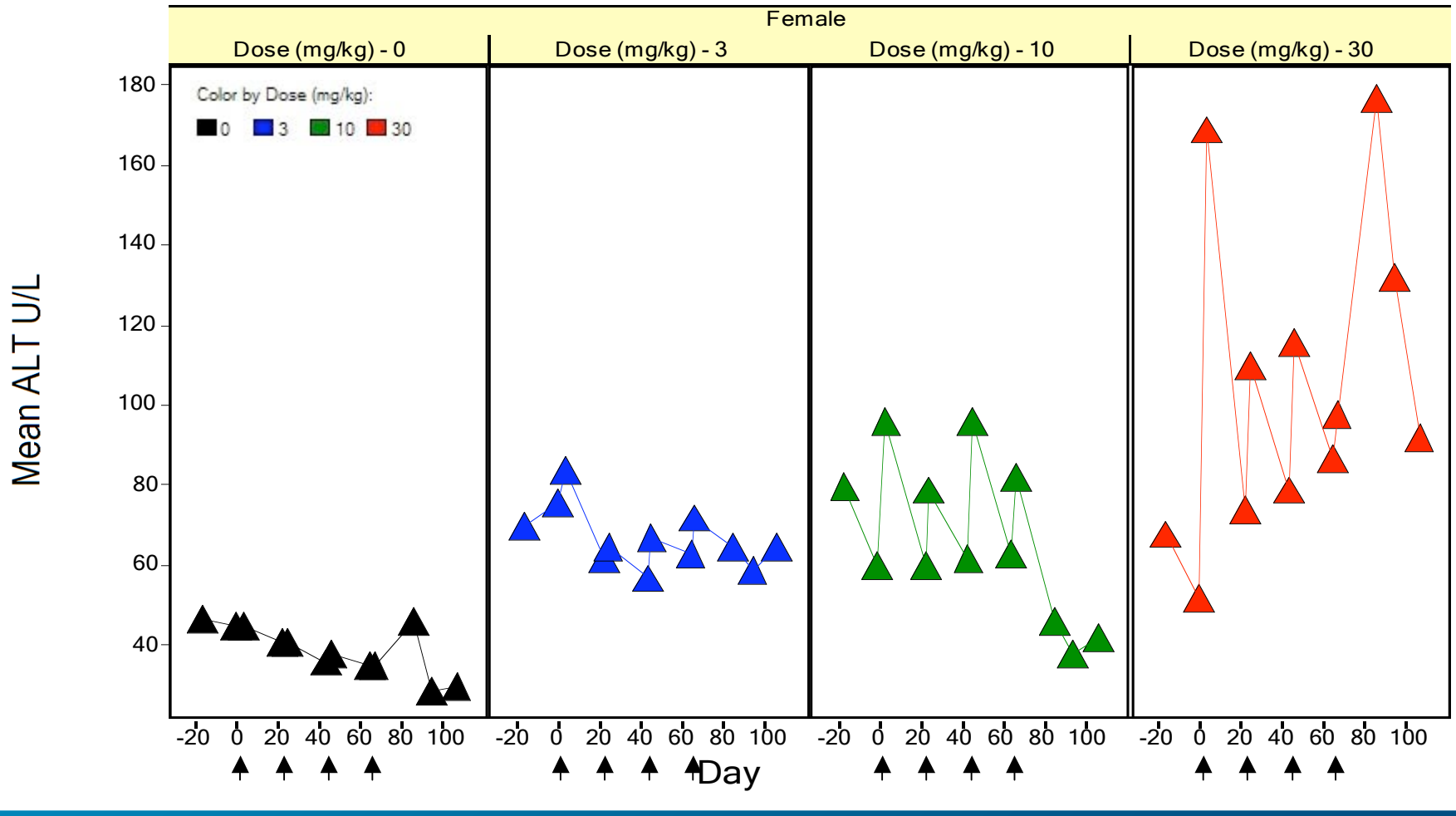
Concentrations of measured analytes following a 30 mg/kg dose of T-DM1 to cynomolgus monkeys



T-DM1 Clinically Well-Tolerated in Monkeys

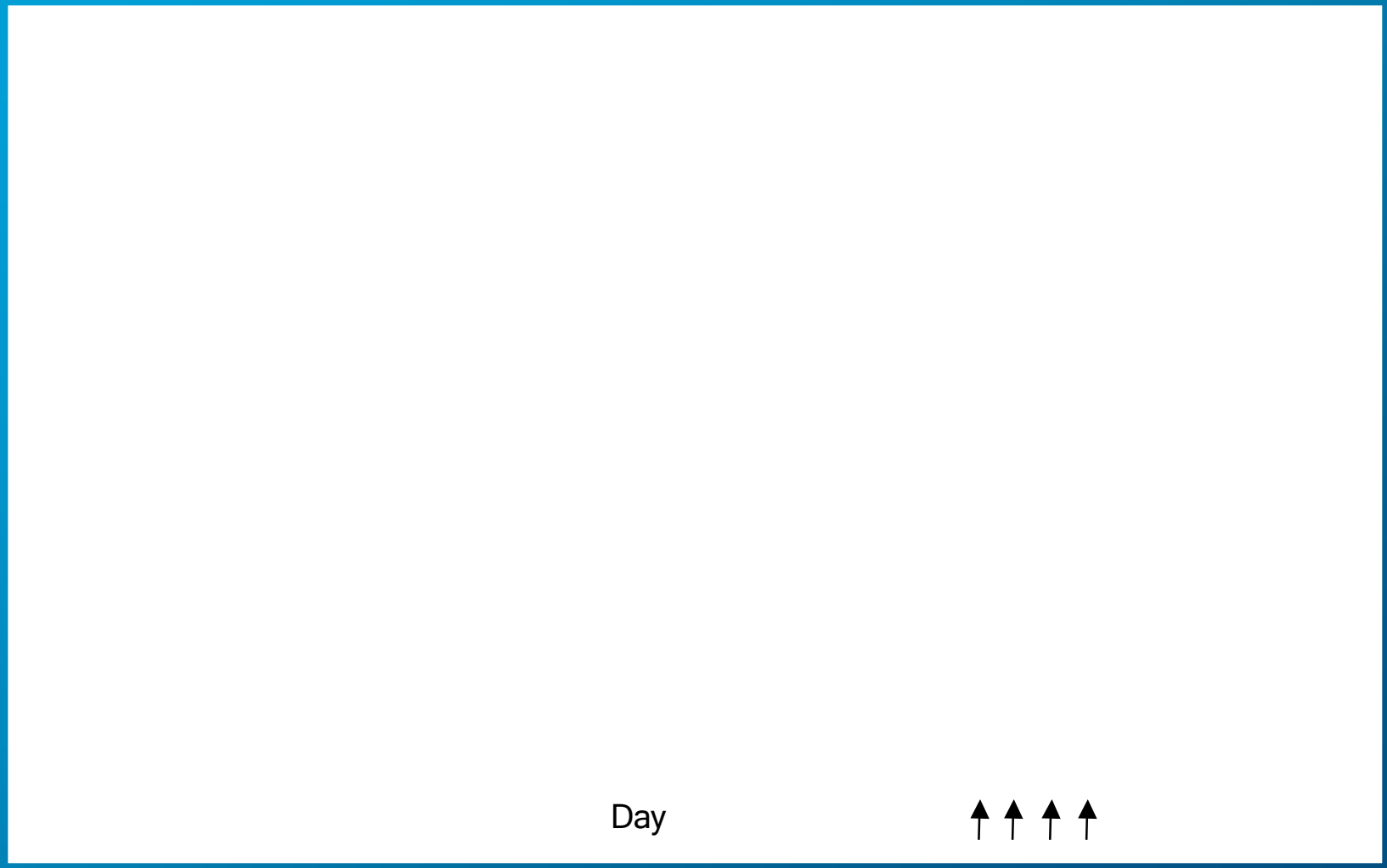
- No significant changes in animals treated with 3 mg/kg T-DM1
- 10 and 30 mg/kg, test article-related findings limited to:
  - Clinical pathology
  - Histopathology

Elevations in liver transaminases were transient and reversible at 10 and 30 mg/Kg



- similar changes observed for AST
- peak changes noted 2 days post-dose

Mild decreases in platelets were transient and reversible at 30 mg/Kg



- peak changes noted 2 days post-dose

Histopathology

- ***Mild and reversible hepatotoxicity***
  - Hypertrophy of Kupffer cells w/presence of increased mitotic figures
  - Increased sinusoidal leukocytes
  - Increased #'s of multinucleated hepatocytes

- ***Increased mitotic figures in multiple cells of epithelial or phagocytic origin***
  - Noted in spleen, tongue, skin, injection site, kidney, mandibular salivary gland, gallbladder, heart, prostate, seminal vesicle, oviduct, uterus, sciatic nerve histiocytes
  - Low magnitude, reversible

Non-reversible axonal degeneration observed in sciatic nerve, spinal cord (dorsal funiculus)

Incidence

- 6 of 14 animals (10 mg/kg)
- 14 of 14 animals (30 mg/kg)

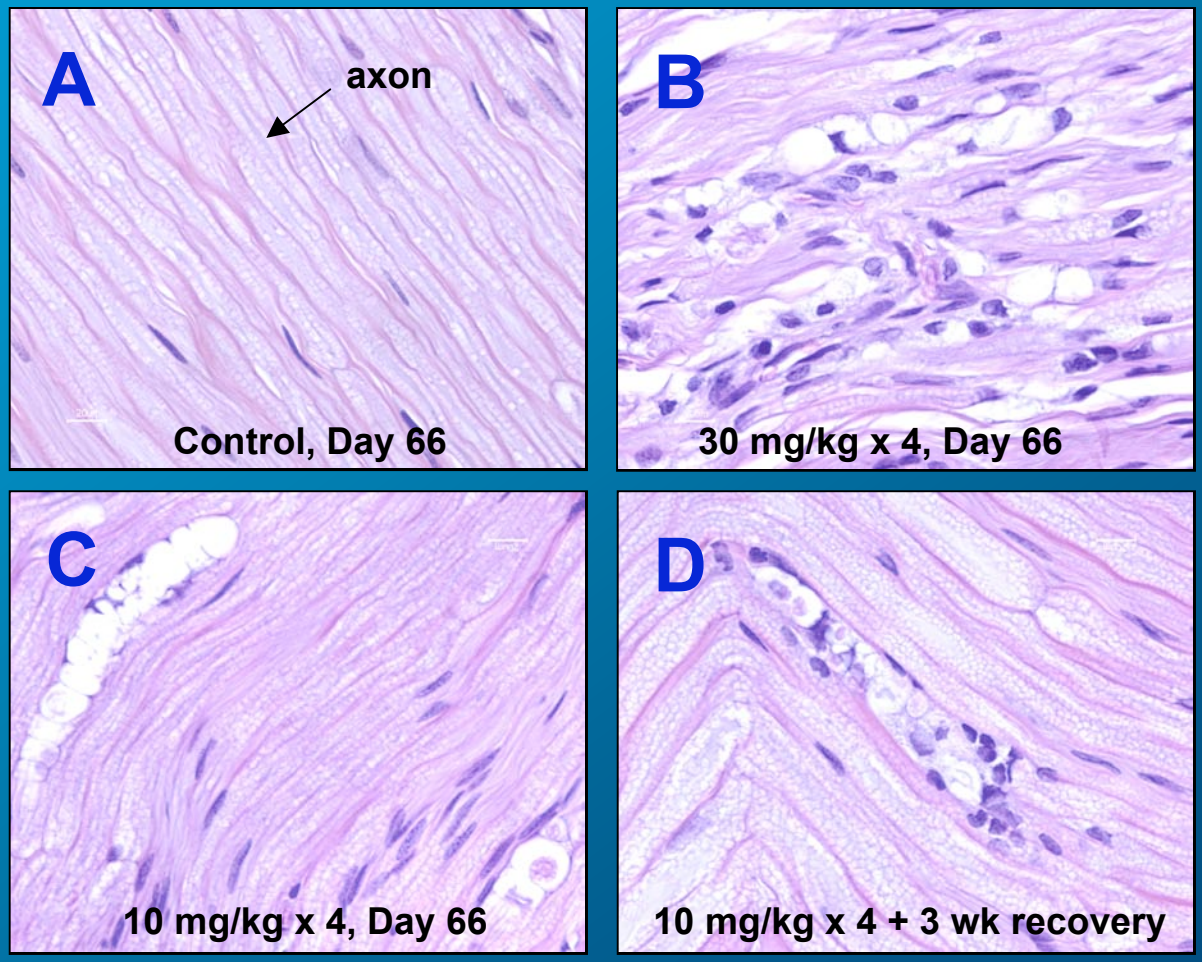
Severity

- Minimal (10 mg/kg)
- Slight to severe (30 mg/kg)

HNSTD

10 mg/kg

(2000 µg DM1 /m^2)



Similar reversible toxicities seen in single dose studies

Study	Findings	HNSTD
Monkey T-DM1	• ↑ AST, ALP, ↓ platelets • Increased mitotic figures • Kupffer cell hypertrophy	30 mg/Kg (6000 µg/m^2)
Rat T-DM1	• Morbidity at 60 mg/kg (6000 µg/m^2) • ↑ ALT, ALP, AST, GGT, T. Bili, ↓ platelets • Increased mitotic figures • Kupffer cell hypertrophy/hepatocellular degeneration/necrosis	20 mg/Kg (2000 µg/m^2)
Rat DM1	• Tolerated at all doses • Comparable findings to rat T-DM1	0.2 mg/Kg (1200 µg/m^2)

- Single and repeat dose monkey dose levels: 3, 10, 30 mg/kg or 600, 2000, 6000 µg DM1/m^2
- Rats received equivalent µg DM1/m^2 doses as monkeys (600, 2000, 6000 µg DM1/m^2)

Summary of Results for T-DM1 Preclinical Safety Assessment Studies

- Comparable cross reactivity of T-DM1 for human and monkey tissues
- No cardiovascular changes noted
- Concordance of reversible toxicities across all monkey and rat studies w/ T-DM1 and DM1:
  - Decreased platelets
  - Hepatotoxicity
  - Selected tissues with increased numbers of mitotic figures
- Irreversible axonal degeneration seen after repeat dosing of T-DM1 (monkeys only)

- Highest Non-Severely Toxic Dose (HNSTD) = 10 mg/kg
- Starting dose in humans = 1/12 HNSTD (0.3 mg/kg)

Chronic Toxicity Assessed in Cynomolgus Monkeys

Test Article	Dose, mg /kg (μg DM1/m^2)	N	Terminal Necropsy (D155)	Recovery Necropsy (D190)
Vehicle	0	6M/6F	3M/3F	3M/3F
T-DM1	1 (200)	6M/6F	3M/3F	3M/3F
T-DM1	3 (600)	6M/6F	3M/3F	3M/3F
T-DM1	10 (2000)	6M/6F	3M/3F	3M/3F

- 6-month study; q3w x 8 doses + 6 week recovery
- Timing driven by clinical development plan (support NDA filing)
- Results comparable to IND tox (same target organs); tolerated up to 10 mg/kg

T-DM1 Developmental and Reproductive Toxicity Strategy

- Did not conduct fertility, embryofetal, or peri- and post-natal toxicology studies on T-DM1 or its components
  - Herceptin pregnancy label D
  - Herceptin DART studies in monkeys not informative of post-marketing findings in pregnant women (i.e. monkey not good model for antigen-dependent effects)
  - DM1 cytotoxic; targets rapidly dividing cells
  - Maytansine is embryolethal, teratogenic, and clastogenic to mice (single doses at gestation days 7 or 8)
  - T-DM1 dose required to achieve clinically relevant exposure of DM1 not feasible in rats or monkeys
  - Approach consistent with ICH S9

T-DM1 Genotoxicity Testing Strategy

- To assess mutagenicity, conducted reverse bacterial mutation assay on DM1 – results negative
- DM1 expected to be aneugenic/clastogenic based on MOA
- Presence of increased mitotic figures seen in (all) in vivo toxicity studies evidence for T-DM1 and DM1 as putative genotoxicants
- For confirmation, conducting in vivo micronucleus rat study with DM1

Phase I Target Organs of Toxicity Identified in Toxicology Studies

- Total of 24 patients treated 0.3-4.8 mg/Kg in PhI
  - DLT at 4.8 mg/Kg, rapidly reversible Grade 4 thrombocytopenia
  - All other clinical findings ≤ Grade 2 (e.g.↑ LFTs, nausea, vomiting, alopecia, and neuropathy)
  - No cardiac-specific toxicity observed

Impressive Antitumor Activity of T-DM1 in a Phase II study in MBC patients

- **Multi-institutional, open label, single arm Phase II trial (N = 100)**
- **Substantial clinical benefit in a heavily pretreated Her2+ MBC population:**
  - Prior exposure to an anthracycline, a taxane, capecitabine, lapatinib and trastuzumab
  - Two HER2-directed regimens in the metastatic setting
  - Progressive disease on last regimen received
  - Number of agents for metastatic disease: Median: 7; Range: 1-15
- **In a prior proof-of-concept Phase II study, a confirmed objective response rate (ORR) of 26.9% by IRF and 38.9% by INV assessment, and median PFS of 4.6 months per IRF and INV assessment was observed.**

Tumor Response	IRF (N=110)	Investigator (N=110)
Objective Response Rate, % (95% CI)	32.7 (24.1–42.1)	30.0 (22.0–39.4)
CR	0	1.8
PR	32.7	28.2
SD*	46.4	52.7
PD	18.2	13.6
Clinical Benefit Rate, % (95% CI)	44.5 (35.1–54.3)	40.0 (31.1–49.3)

IRF - Independent Review Facility
Objective Response - CR or PR determined by two consecutive tumor assessments at least 28 days apart;
Clinical Benefit - objective response or SD maintained for at least 6 months.
**Including unconfirmed PRs.*

Baselga et. al, SABCS Dec 2009

Key Toxicity Messages for T-DM1

- Preclinical toxicities for T-DM1:
  - Mainly antigen-independent and consistent w/MOA of DM1
  - Concordant across species
  - Did not worsen with chronic dosing
  - Identified target organs translated to clinic
  - Clinically monitorable, manageable
- T-DM1 expected to be teratogenic and genotoxic based on mechanism of action of DM1

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