

# ***Predictive Teratology-*** **Lessons Learned and Current** **Applications**

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# Unexpected Teratogenicity as a Cause of Attrition

## ■ *Impact*

- Teratogenicity is related to:
  - ~10% of safety-related attrition
  - ~ 4.5% of historic attrition (based upon DPC/BMS history)
- Ranked third in safety-related attrition:
  - Cardiovascular (27.4%);
  - Liver (12.2%);
  - Teratogenicity (9.6%)

# Impact of Unexpected Teratogenicity Issues

## ■ “Last minute timing” of drug teratogenicity findings

- Results unknown until just prior to enrollment of women of child-bearing potential (WCBP) into clinical trials
  - Typically Phase IIb

## ■ Immediate questions to address related to drug teratogenicity findings

- Can clinical trials in WCBP be started?
- Will all back-up compounds do the same?
- Are the adverse effects related to the chemical structure?

## ■ Results of teratogenicity findings

- Can prohibit inclusion of WCBP into clinical trials
- May halt progression of a project
- Can adversely impact a drug’s labeling

# Origins of Teratogenicity

## ■ “On Target” Teratogenicity

- Related to intended pharmacology because many drug targets have essential roles in embryo-fetal development.

## ■ “Off Target” or “Near Off Target” Teratogenicity

- Related to structural chemistry
- Related to selectivity against the desired target
- Could be proactively identified using predictive screening methods.

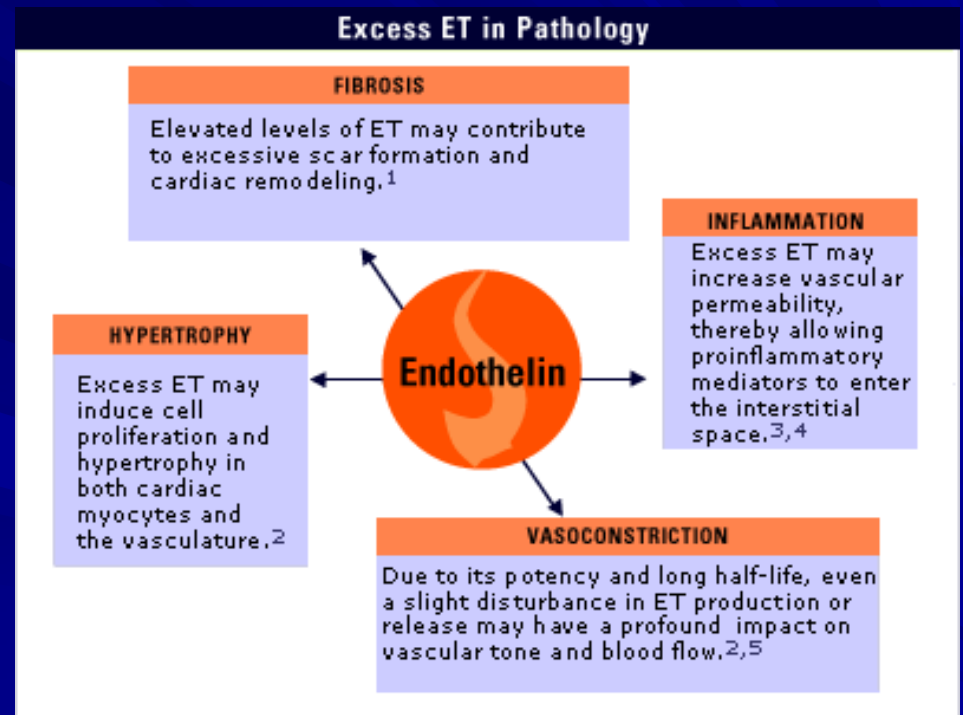
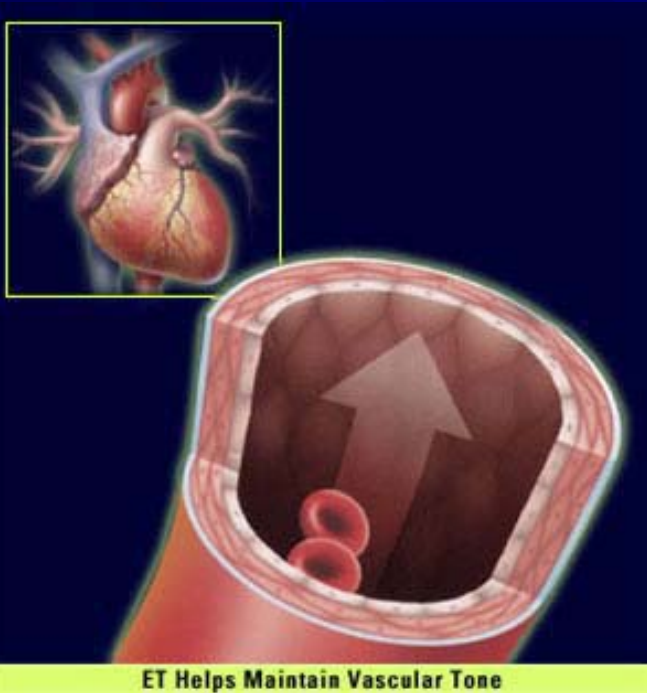
## ■ Opportunity exists to identify both types of liabilities early in the Discovery Phase

# “On Target” Teratogenicity

- Approximately half of teratogenic marketed and unmarketed compounds attributed to target-based mechanism
- Proactive assessment of the literature can identify potential teratogenic liabilities facilitating decisions that may reduce attrition risk
  - Helps define therapeutic/commercial potential
  - Facilitates appropriate matching of indication based upon risk
  - Helps prioritize Discovery targets
  - Helps define drug development plans

**Endothelin Receptor  
Antagonists:  
A Case Study of Target-Based  
Teratogenicity**

# Endothelin Pathway: Adult Function and Pathological Implications



<http://www.endothelinscience.com/endothelin.cfm>

Acetelion, Inc

# Pharmaceutical History with Endothelin Receptor Antagonists

## ■ Compounds

- SB-217242, L753,397 (ETA/ETB receptor antagonists)- published
- BMS ET-A selective receptor antagonist

## ■ Indications

- Potential therapy for pulmonary hypertension, congestive heart failure, renal diseases

## ■ Progression

- Toward phase IIb
- Segment II Embryo Fetal Development studies initiated

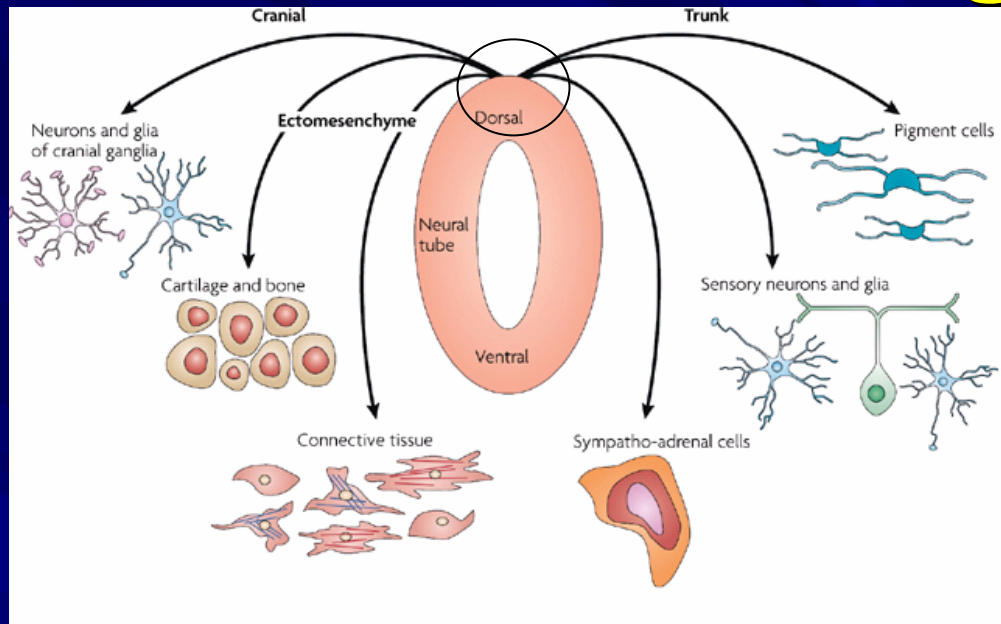
## ■ Issue

- “Unexpected” teratogenicity

## ■ Impact

- Eventual termination of project
- Teratogenicity finding significant contributor to attrition

# Neural Crest Lineages



## Cranial neural crest derivatives

- Cranial nerve ganglia (V, VII, IX and X)
- Schwann cells and meninges (pia and arachnoid)
- Melanocytes
- Craniofacial connective tissues and bones
- Cells of the truncocoelal cushions of the heart

## Trunk neural crest derivatives

- Spinal (sensory) and autonomic ganglia
- Schwann cells
- Melanocytes
- Medulla of the adrenal gland

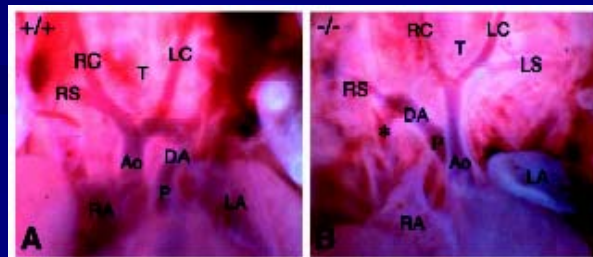
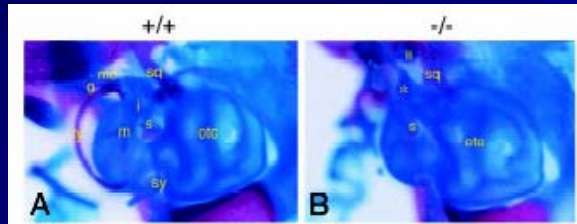
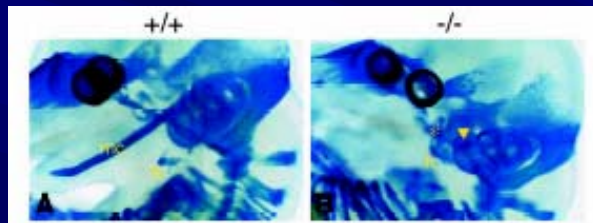
# Endothelin Expression in Embryogenesis

- High ET-A receptor expression in neural crest cell lineages that contribute to craniofacial structures, the pharyngeal arches and cardiac outflow track
- ET-1 expression identified in the endothelium of aortic arches, dorsal aorta and endocardium
- ET-3 and ET-B receptor associated with crest-derived enteric ganglions and pigmentation cells

# ET-A Receptor Knockout Mouse



- Associated malformations in knockout mice include pharyngeal arch, aortic arch and cardiac derived defects:



- Mandibular hypoplasia and clefting
- Microglossia
- Hypoplasia of thymus and thyroid
- Hypoplastic auricles
- Aortic arch and septation defects

# ET-3/ET-B Receptor Related Mutations

## Pigmentation and Enteric Neuron Depleted Effects



- **Human Mutations:** Hirschsprungs Disease (Megacolon); Waardenburg syndrome (pigmentation defects)
- **Murine spontaneous mutants:** *Piebald -lethal*, *Lethal spotting* (pigmentation and megacolon)
- **Murine knockouts:** ET-3 and ETB receptor (pigmentation and megacolon)



# **ET-A/B Receptor Antagonists Recapitulate ET-1/ET-A KO Phenotype**

■ Rats treated with SB-217242 (Treinen, et al Teratology 1999)

- Mandibular hypoplasia and clefting
- Microglossia and aglossia
- Hypoplastic auricles (microtia)
- Hypoplasia of thymus and thyroid
- Aortic arch and septation defects

■ Similar results with L-753,037 and BMS ET-A receptor antagonist (Spence, et al Reproductive Toxicology 1999)

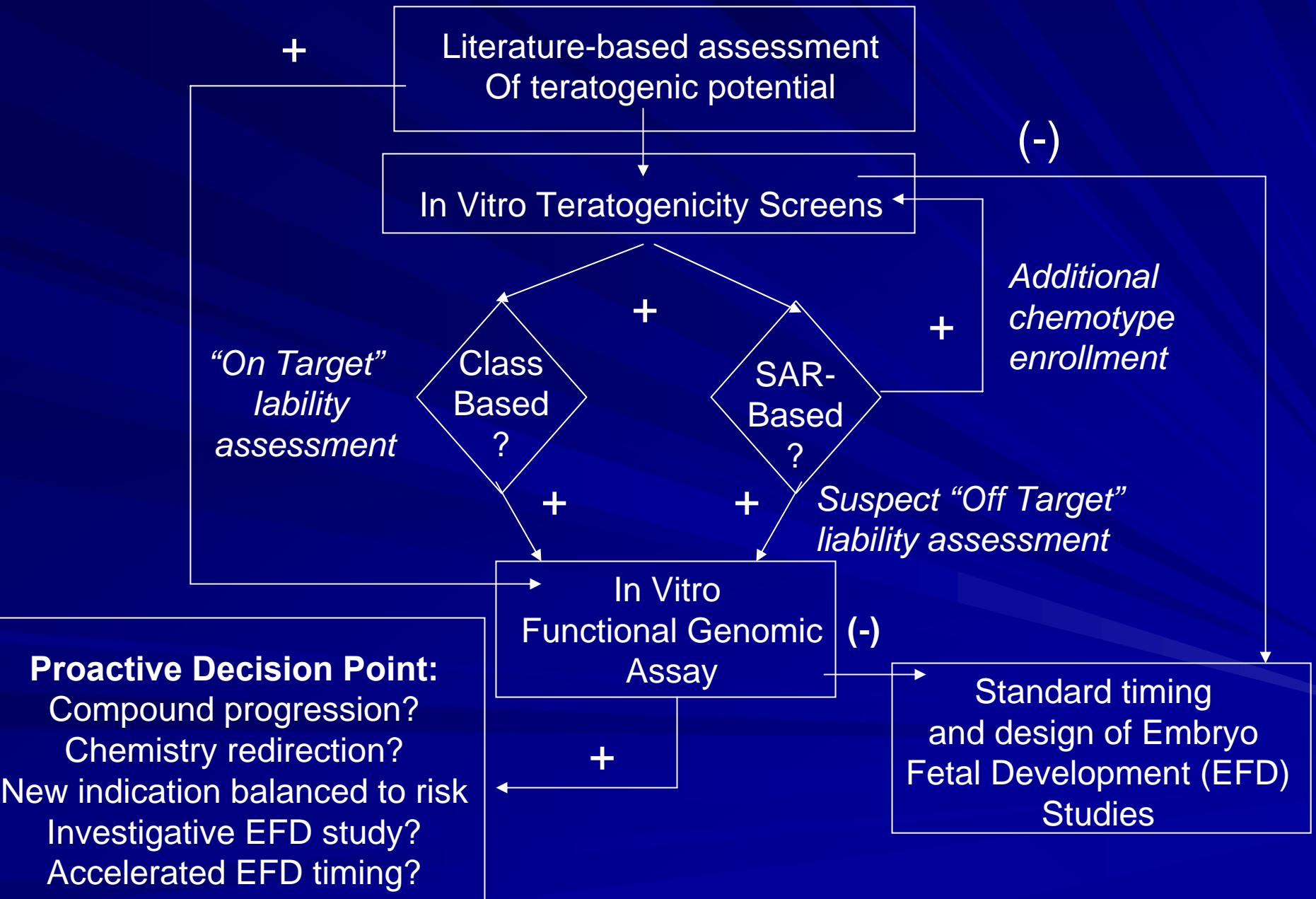
# Risk-Benefit of Teratogenicity Findings

Drug / Drug-target	Risk	Benefit	Balance
NNRTIs	Off Target Teratogenicity	HIV/AIDs	√
Presenilin	Target Based Teratogenicity	Alzheimers	√
<b>Endothelin-1</b>	<b>Target-based Teratogenicity</b>	<b>Hypertension</b>	<b>X</b>

# What can we do now?

- Proactively review published literature to make early assessments pertaining to teratogenic liability
- Apply developmental systems to characterize target expression and function in embryogenesis
- Apply in vitro teratogenicity screens

# Teratogenic Liability Process and Decision Tree



# Rodent Whole Embryo Culture Model

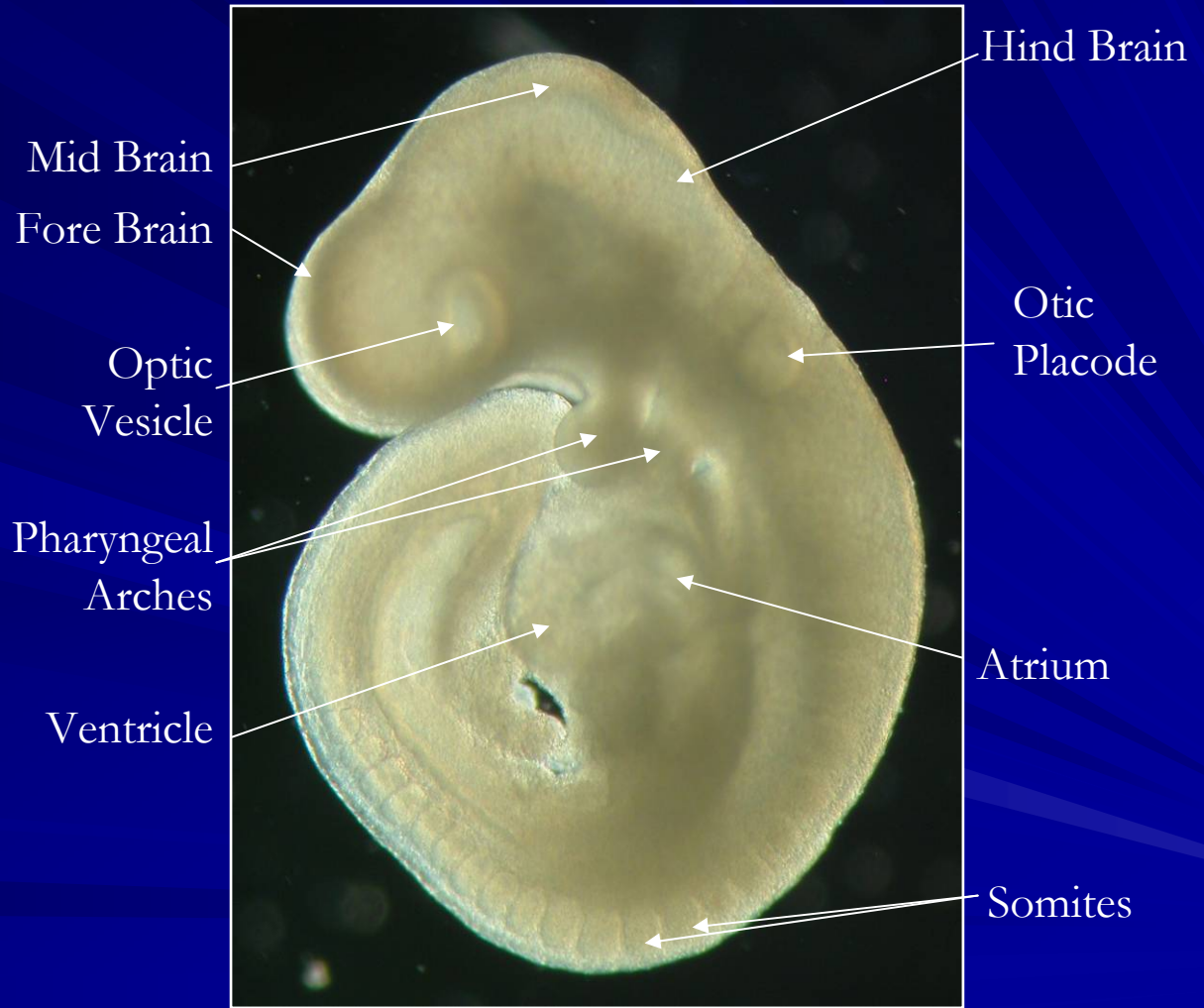


Embryo

Day 9 Embryo  
prior to culture



~ 24 hrs post-culture



~ 44 hrs post-culture

# Effect of BMS ET-A Receptor Treatment on Cultured Rat Embryos (Gestational Day 12)



Rostral View Isolated 1st Arch

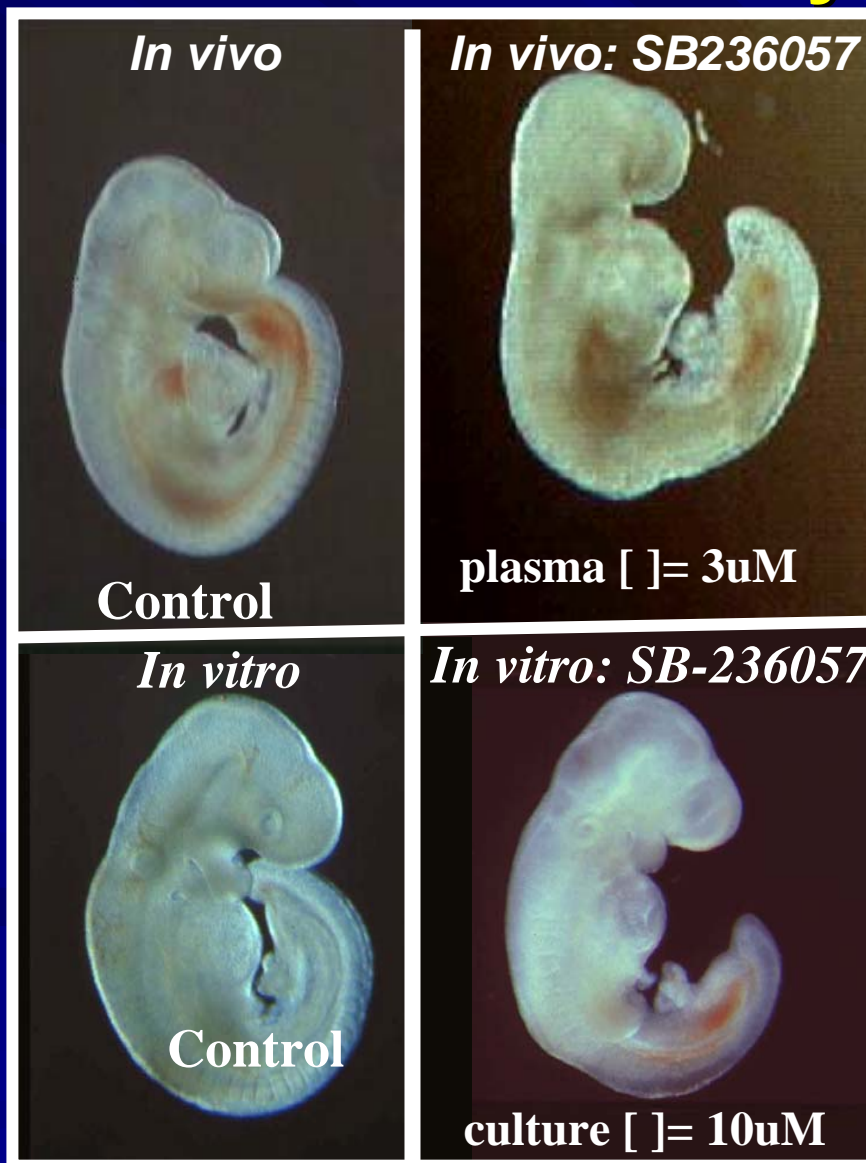


**Use of the Rodent Whole  
Embryo Model For  
Mechanistic Studies:  
*Delineation of On or Off Target  
Teratogenicity***



- SB-236057: 5-HT1B inverse agonist previously being developed for anxiety-depression indications.
- Identified to be a potent axial-skeletal teratogen in mice, rabbits and rats.
- Teratogenicity suspected to be caused by compound chemistry, not intended pharmacology.

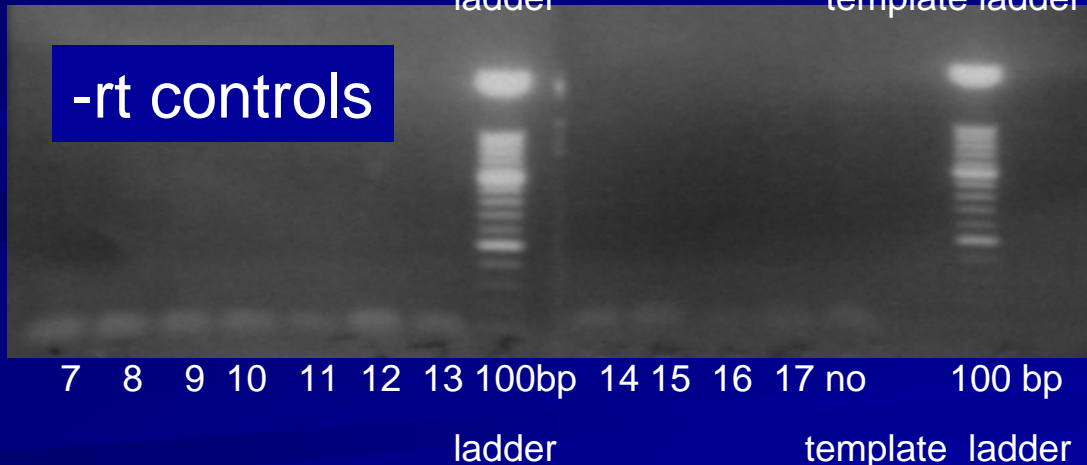
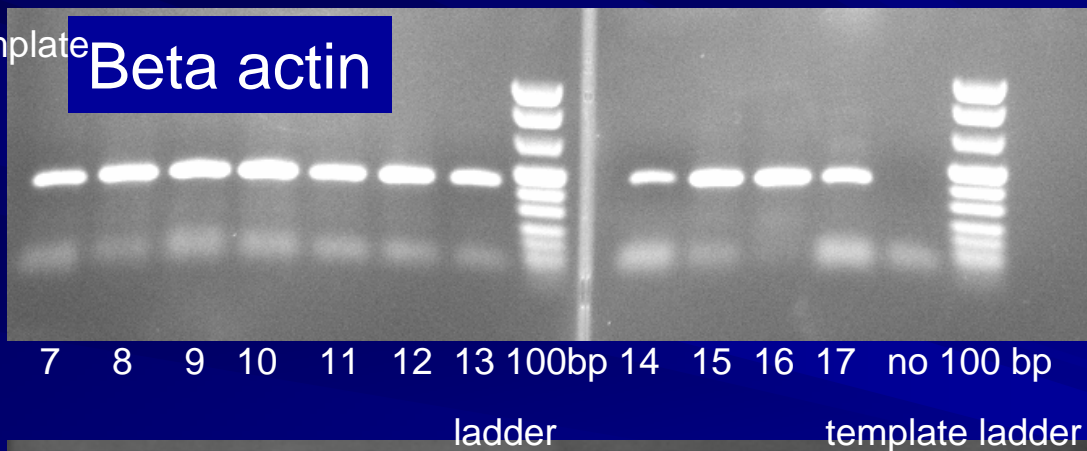
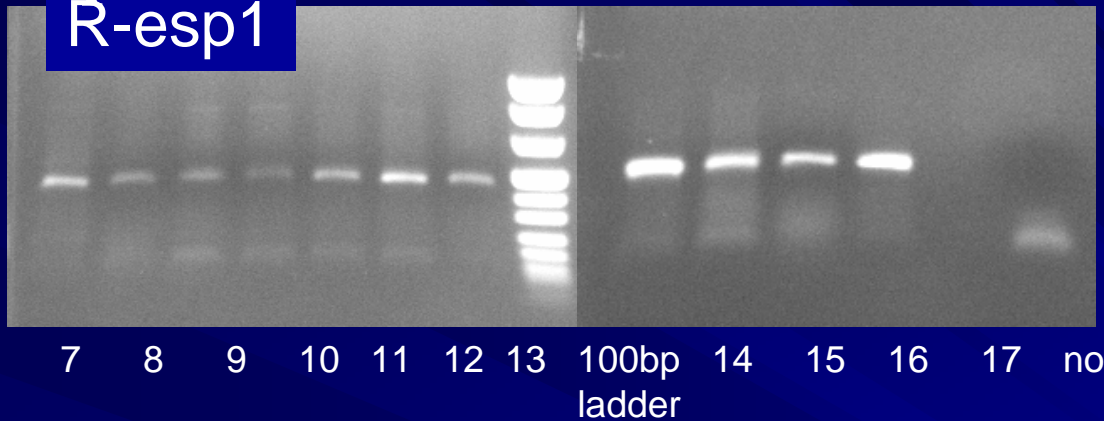
# ***In vivo* and *In vitro* Evaluation of SB-236057 Treated Rat Embryos (GD 11)**



# SB-236057: Mechanism Studies

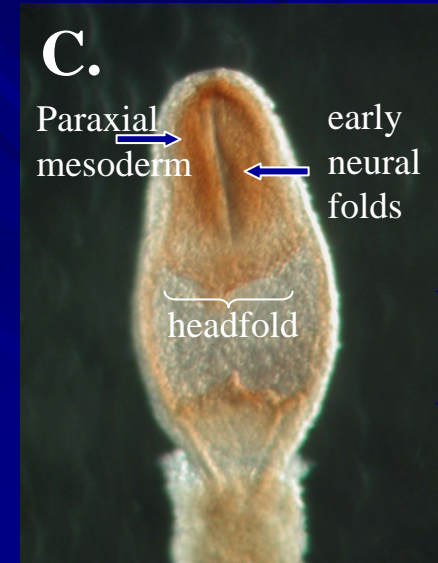
- Small molecule-protein binding studies using phage display identified r-esp1 as having high binding affinity to SB-236057
- R-esp1 suspected to be a putative molecular oscillator – developmental proteins essential for timing and patterning of axial formation
- R-esp1 expression and functional characterization studies were undertaken to determine whether this protein could be a potential SB-236057 “off-target”

# Expression Characterization of "Off Target": R-esp1



**R-esp1 Expression in Rat Embryo-Fetal Development determined by PCR**

# Expression of R-esp1 Determined by Wholemount In Situ Hybridization



# Morpholino Antisense Applications and Concepts



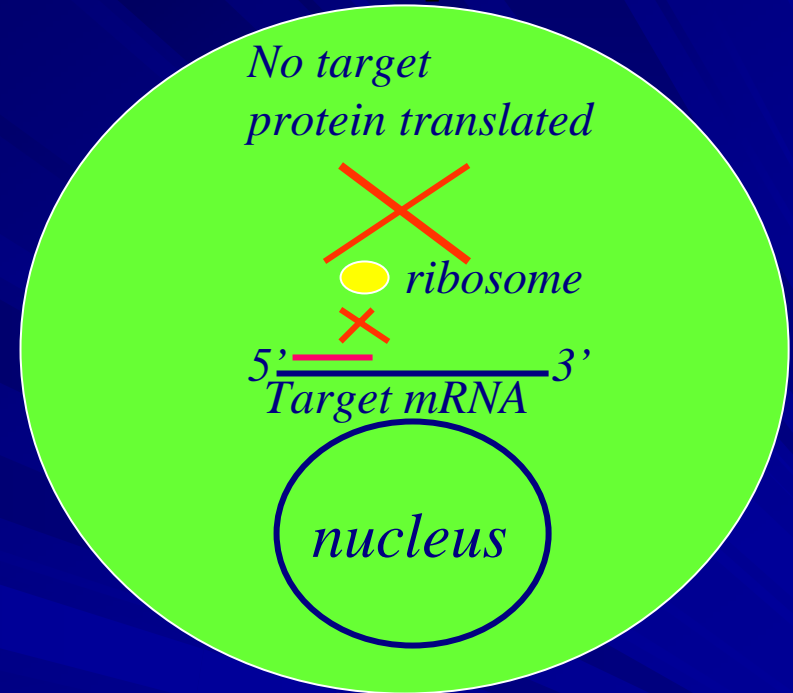
Amniotic injection with 50 nl morpholino oligonucleotide



Culture 48 hours



Examine day 11 pc embryo



Morpholino antisense are designed to bind to the 5' region of the target mRNA and block ribosomal binding by steric hinderance. This will lead to loss-of-function by reduction in protein levels but not by reduction in mRNA levels

# Validation of “Off-Target” Teratogenicity: Comparison of Affected Morphology of Embryos Treated with R-esp1 Antisense versus SB-236057



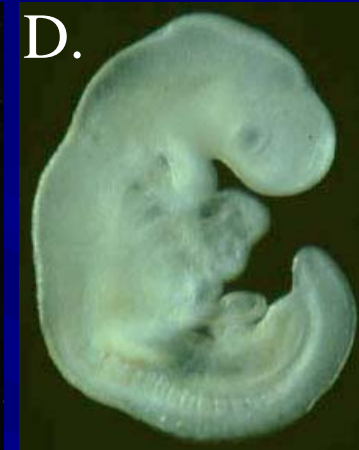
r-esp1 mismatch I



r-esp1 antisense I



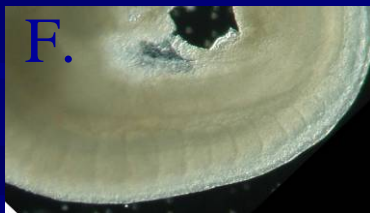
r-esp1 antisense II



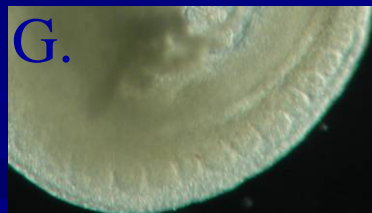
1uM SB236057



10uM SB236057



F.



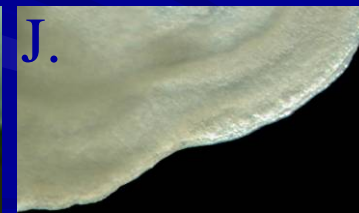
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H.

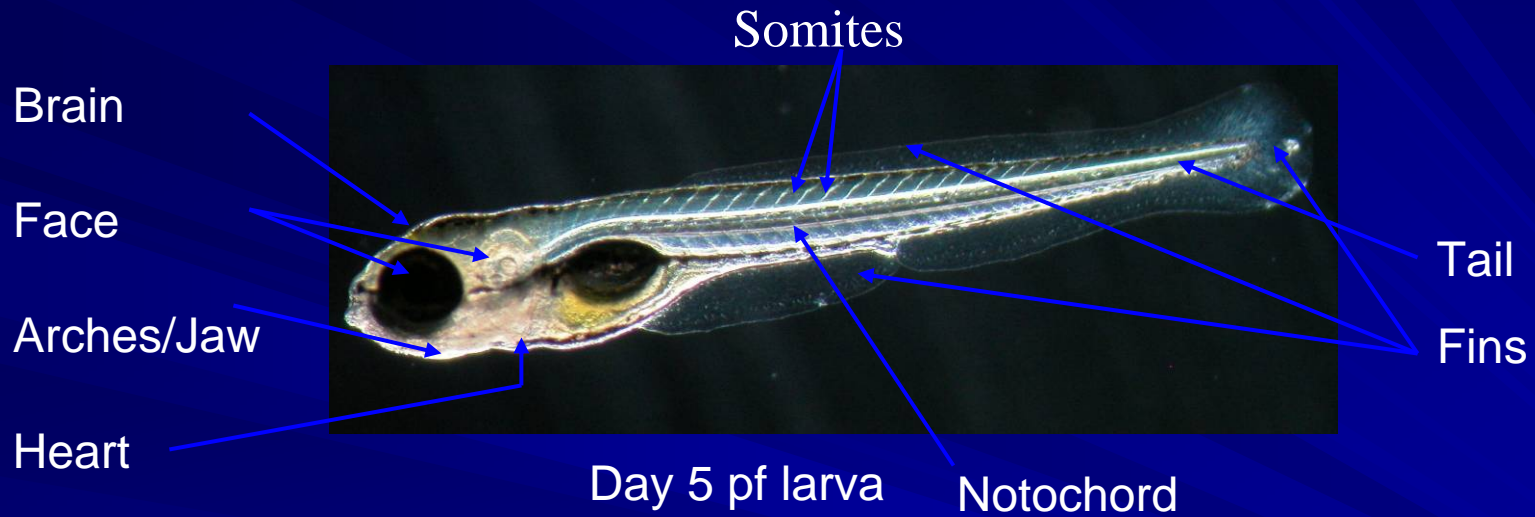


I.



J.

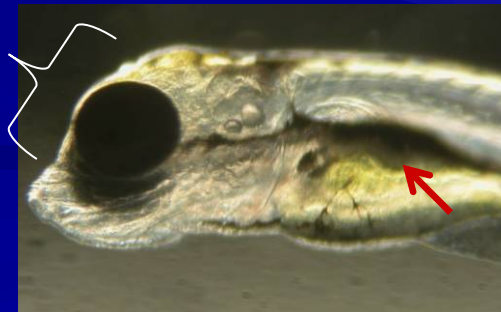
# Zebrafish Embryo-Larva Model



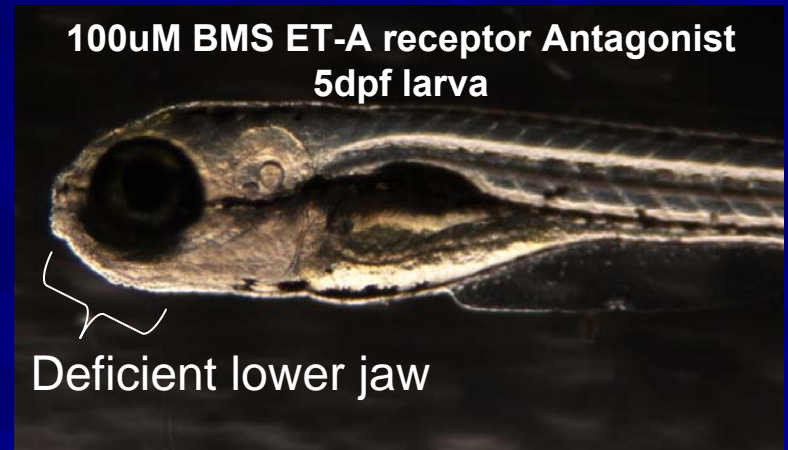
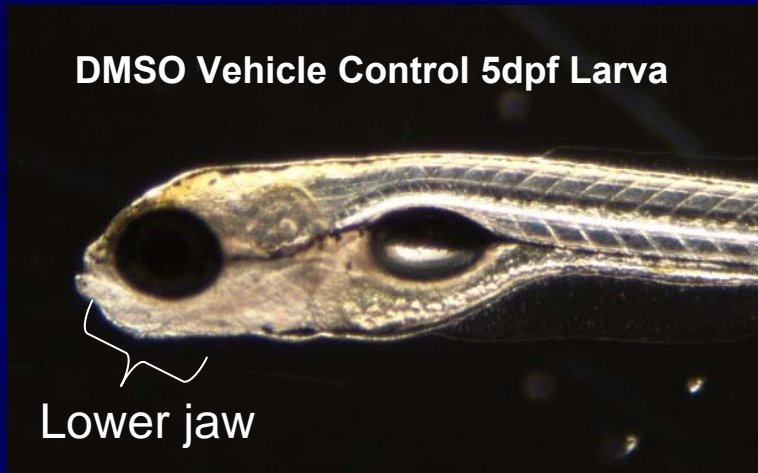
**Normal**



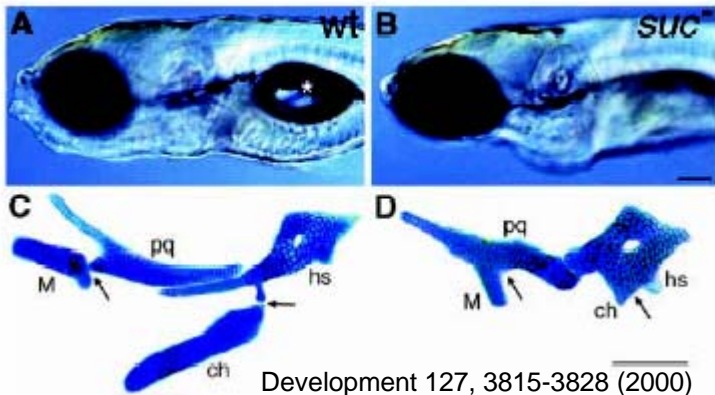
**Malformed**



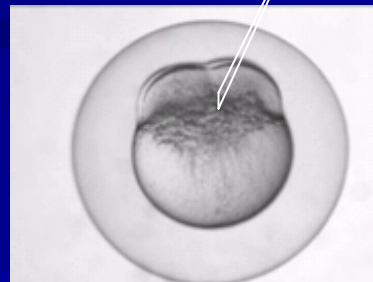
# BMS ET-A Receptor Antagonist Effect Compared to ET1 Null Mutant



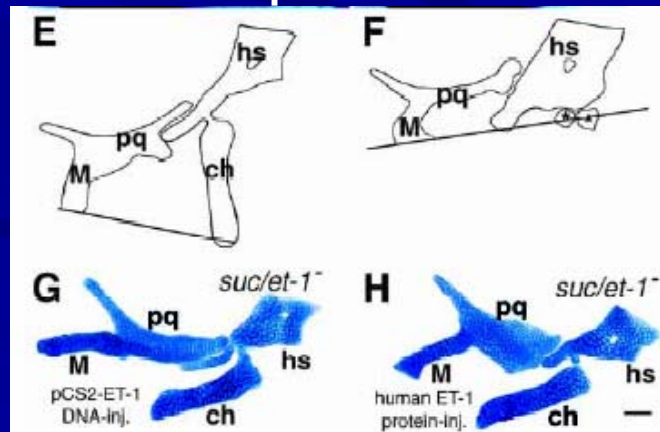
## ET-1 Spontaneous Mutant: *Sucker*



Ectopic expression recovery Experiment:  
ET-1 recombinant DNA microinjection



## Verification of Effected Target: Rescue Experiment Results



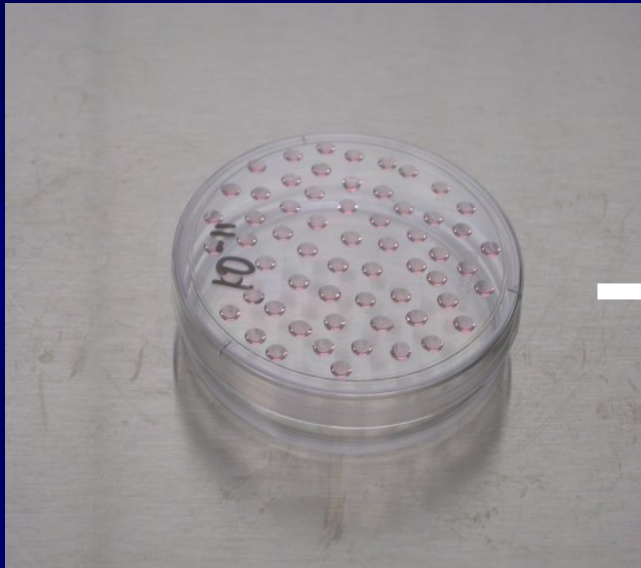
# Predictive Teratogenicity Assays

- Various assays under exploration by ECVAM and/or industry
  - Applying several developmental model systems derived from multiple species
  - Differential assay endpoints: molecular, protein-based or morphological
- Assays evaluated for predictivity, accuracy and precision with a battery of compounds with *in vivo* reproductive toxicology safety data.

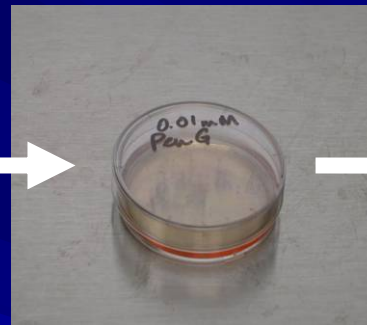
# ECVAM In Vitro Developmental Toxicology Assay Validation Efforts

- Rat micromass, rat whole embryo culture (WEC) and mouse embryonic stem cell test (EST) evaluated
- EC50 cytotoxicity evaluation of NIH3T3 fibroblast cells served as surrogate for adult/maternal toxicity and included in prediction model
- Linear discriminate statistical model generated to classify teratogenic potential of test agents
- Validation conducted across multiple laboratories
- Overall Concordance Results:
  - Micromass: 70%
  - Rat WEC: 80%
  - Mouse EST: 78%

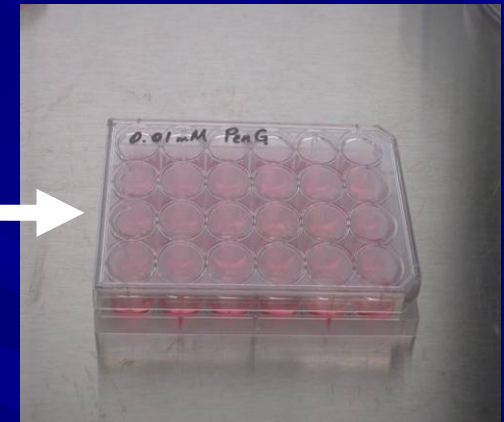
# Standard Embryonic Stem Cell Test



Day 0 - Day 3  
“Hanging Drops”



Day 3 - Day 5  
“Embryoid Body”  
Formation



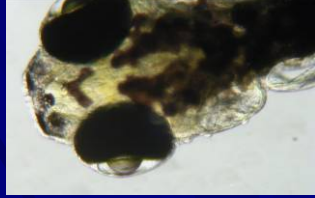
Day 5 - Day 10  
Cardiomyocyte  
Functional  
Differentiation  
**Day 10 Endpoint:**  
IC50 of reduced # of  
EBs with beating  
myocardial cells



# Molecular Embryonic Stem Cell Assay (MESCA)

- Multiple laboratories developing molecular-based ES assays
- Goals
  - Develop molecular endpoints
  - Shorten assay period
  - Enhance concordance
  - Increase assay throughput

# Zebrafish Teratogenicity Assay



- Multiple laboratories exploring model and running validation studies
- Assessment of viability rates, morphology scores and/or dysmorphology calls assessed along a dose range
- Selected lethality concentration (ie LC50) ratio-ed against highest concentration not producing dysmorphology (NOAEL)
- Validation results define threshold value for classifying compounds as potentially teratogenic

## Results:

– BMS results: 88% concordance

NOAEL based upon single calls

NOAEL based upon group  
Score average (4-5 considered  
Within range of normal)

### Example:

RAW DATA					
	0.5% DMSO	0.1 uM	1 uM	5 uM	10 uM
SCORE - Heart	5	5	5	5	2
	5	5	5	3	3
	5	5	5	3	5
	5	5	5	5	3
	5	5	3	3	2
	5	5	3	4	4
N	6	6	6	6	6
Mean	5.0	5.0	4.3	3.8	3.2
SD	0.0	0.0	1.0	1.0	1.2

# Where are we now?....

- Available literature and technological advancements have improved ability to proactively access teratogenic liability
  - Enhanced knowledge of the biological relevance of the target in organogenesis
  - Can directly access effects of the respective compound on organogenesis in multiple species
- Information will enhance
  - Proactive decision making regarding lead compound selection, assessment and risk-benefit analysis surrounding developmental toxicity liabilities

# Acknowledgements

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- Ruth Mulvey
- Lei (Grace) Gong