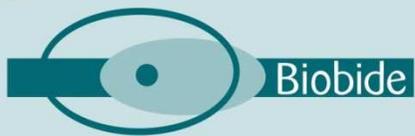


El modelo alternativo de pez cebra y su aplicación en el proceso de "Drug Discovery"



*V Jornada Rema
Pamplona 5 Abril 2011*



Introduction

Biobide is a biotech company that aims to help our customers worldwide developing tailor made solutions to maximize R+D productivity and minimize risks in the Drug Discovery process, integrating zebrafish animal model with innovative tools, adding value to their R+D+i mainly in the preclinical area (toxicology, safety and efficacy).

Private



Genetrix Group
www.genetrix.es



Mondragon corporation
www.mondragon-corporation.com

Public



Basque Government
www.euskadi.net



Guipuzcoa's County Council
www.gipuzkoa.net



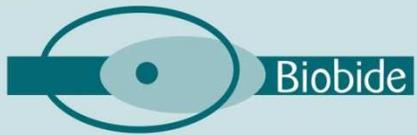
Biobide

Introduction

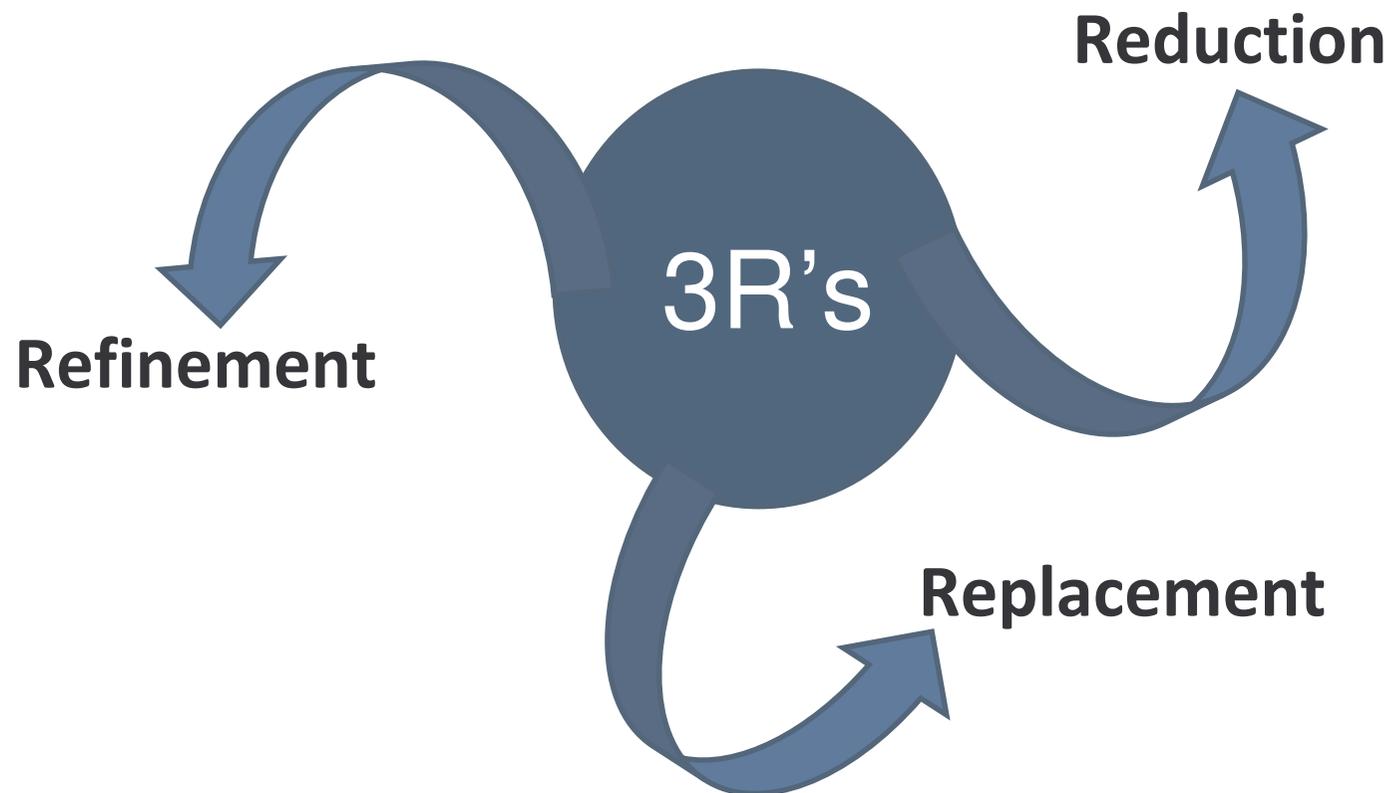


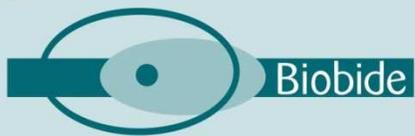
"You mean you're going to test
it on a guinea pig *now*?"

*"La utilización de animales vivos sigue siendo necesaria para proteger la
salud humana y animal" Directiva 2010/63/UE*



Introduction





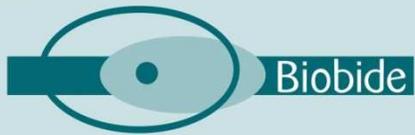
Introduction

DD&D and Approval Process is long and expensive

Discovery/ Preclinical Testing		Phase I	Phase II	Phase III	FDA, EMA,...		Phase IV
Years	6,5	1,5	2	3,5	1,5	15 total	Additional post marketing testing required by FDA
Test Population	Laboratory and animal studies	20 to 100 healthy volunteers	100 to 500 patient volunteers	1,000 to 5,000 patient volunteers	Review and approval process		
Purpose	Assess safety, biological activity and formulation	Determine safety and dosage	Evaluate effectiveness look for side effects	Confirm effectiveness and monitor adverse reactions from long- term use			
Screen Rate	5,000 compounds evaluated	5 enter trials			1 approved		

Source: Pharmaceutical Research and Manufacturers of America, www.phrma.org





Introduction

Characteristics of zebrafish

- High genetic homology with human > 85%

- Fast development / organogenesis
- Small size
- External fertilization and embryogenesis
- High productivity: 50-150 eggs/week
- Direct administration of compounds into the medium of embryos
- Transparent embryos

- Low cost
- Fewer ethical impediment

Appropriateness of the model for human assays:

- Suitable to screen for human drugs
- Models for efficacy and organ specific toxicity screens

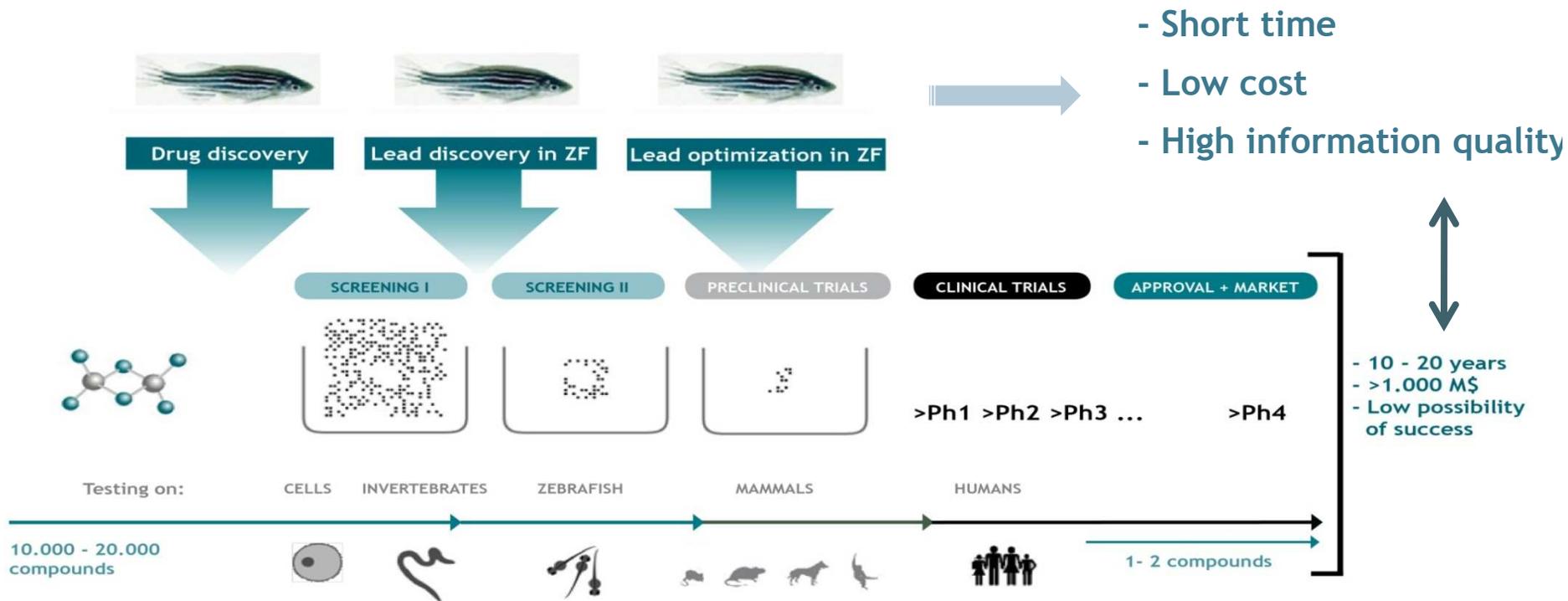
Easy manipulation for assay development:

- Easily sourced model
- Statistically significant result, with small quantity of drug
- Visualization of results by dyes (fluorescence, antibodies, etc.)
- Suitable for automated screenig (HTS)

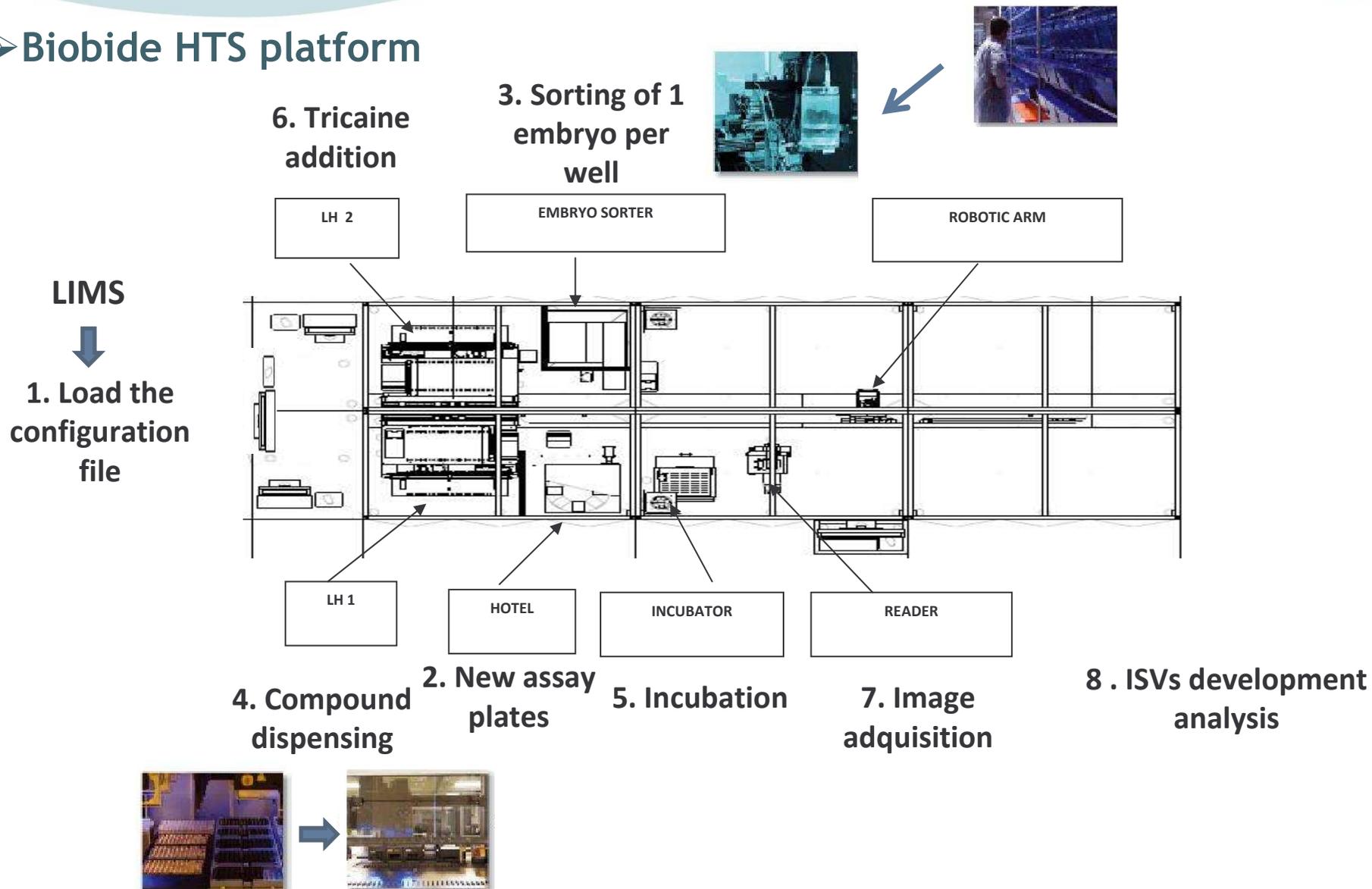
Wide utility:

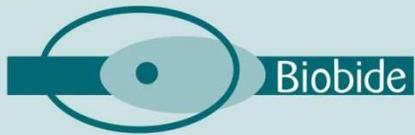
- Cost / time efficiency
- Highly informative results

Introduction



➤ Biobide HTS platform





Positioning

Exemplary model vs surrogate

Exemplary

Basic research

Elucidate fundamental physiological mechanisms

Understanding evolutionary processes

Can represent a higher taxon

Surrogate

Clinical/Applied research

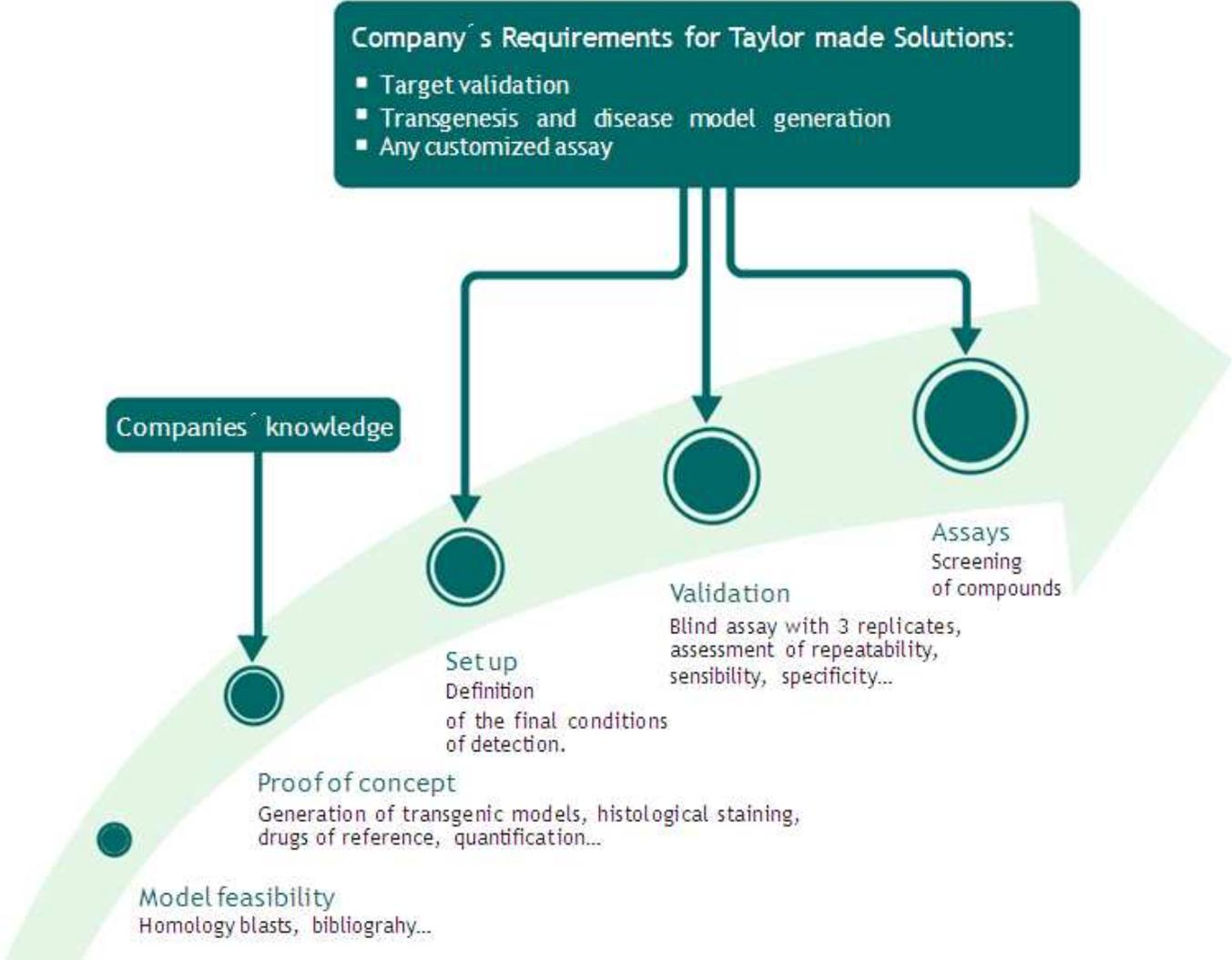
Understand disease etiology and mechanisms

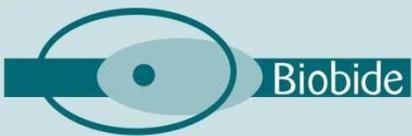
Develop and test possible therapies

Surrogate matches target in relevant features

Surrogate responds to manipulations as target would do

Adapted from Bolker 2009

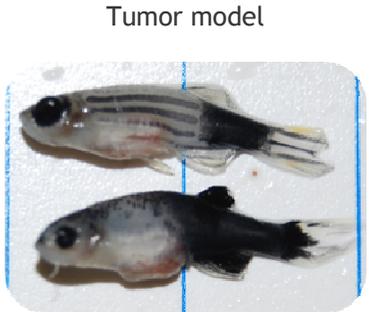
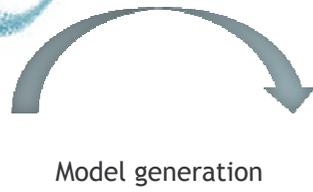
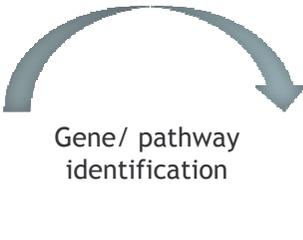




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Efficacy (Tailor-made solutions)

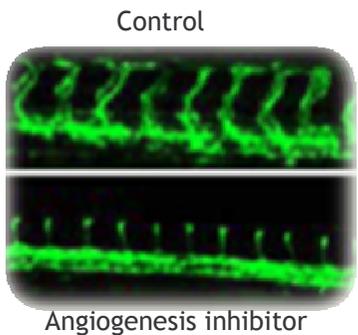
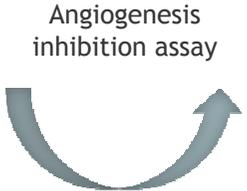
Disease model generation

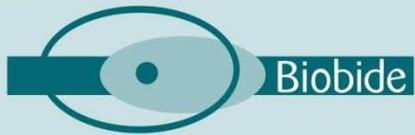


Target Validation

Analysis
(Histopathological, molecular,...)

Compound screening





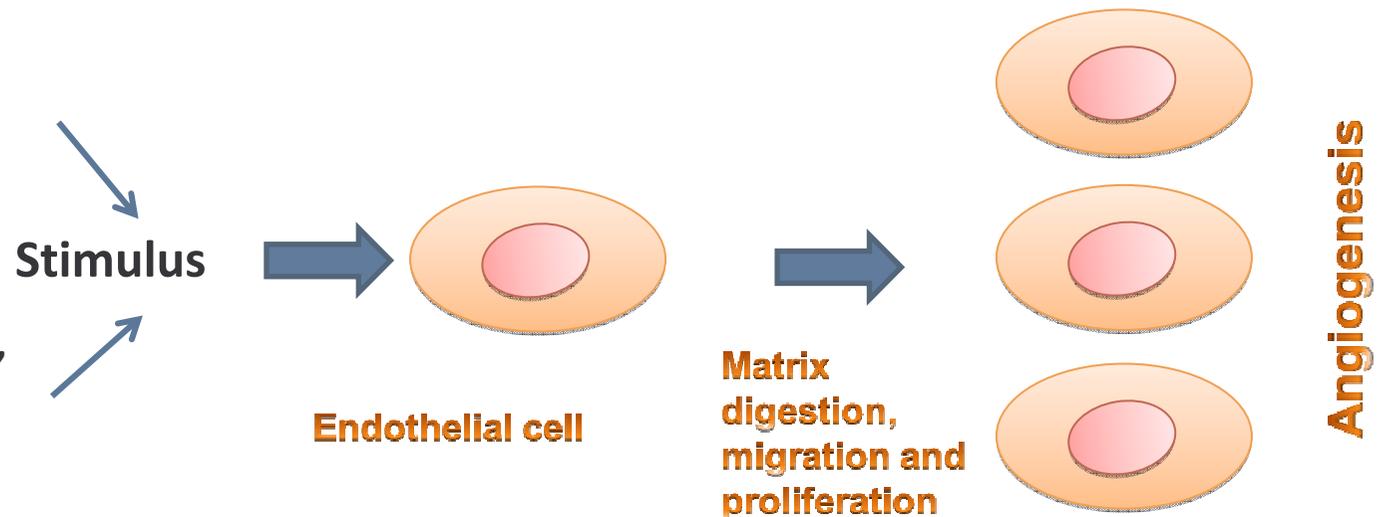
Development of an automated assay to detect angiogenesis inhibitors

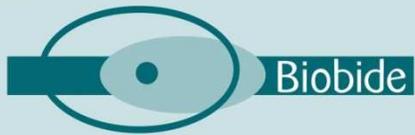
•Angiogenesis: development of new blood vessels from the existing vasculature

- Development
- Pathologies

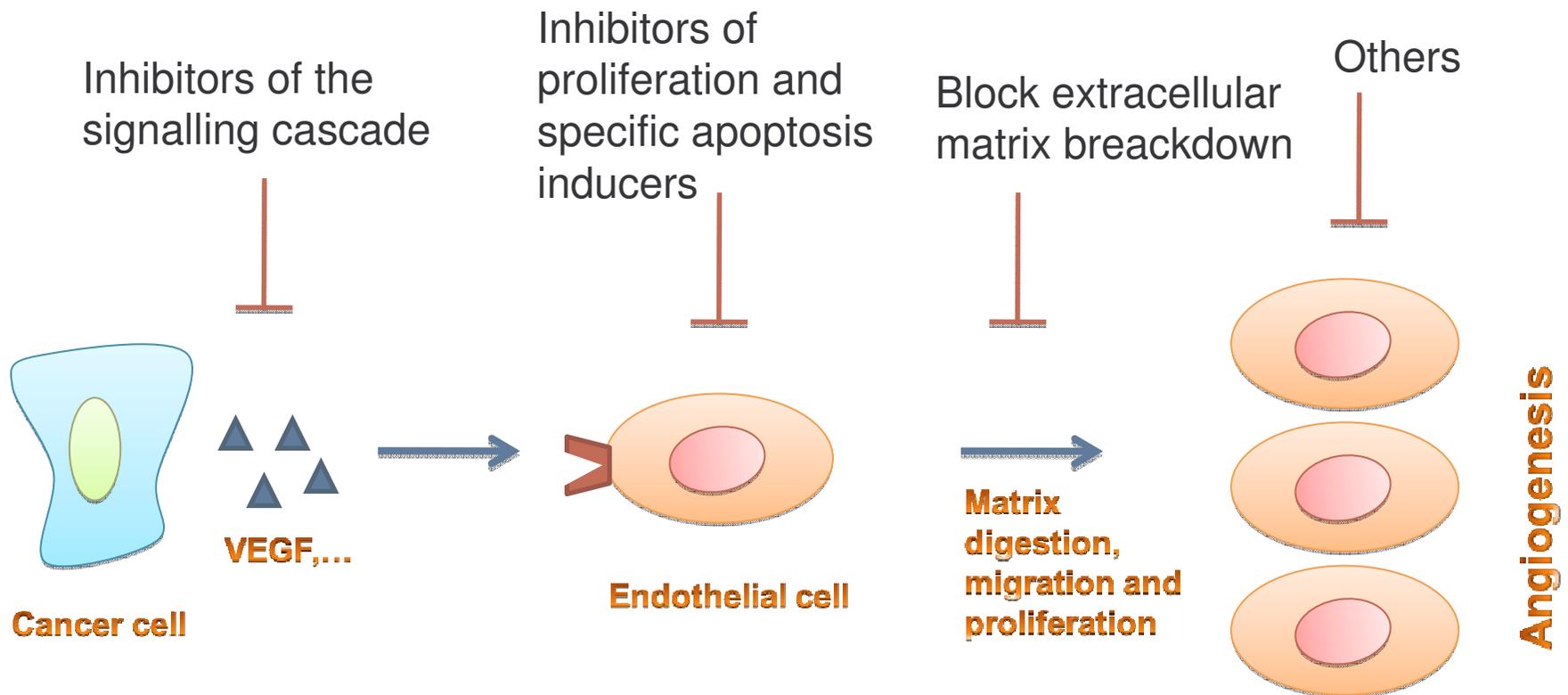
Wound repair,
regeneration, ischemia

Retinopathies, arthritis,
psoriasis and cancer
(tumoral growth and
metastasis)





Angiogenesis inhibition



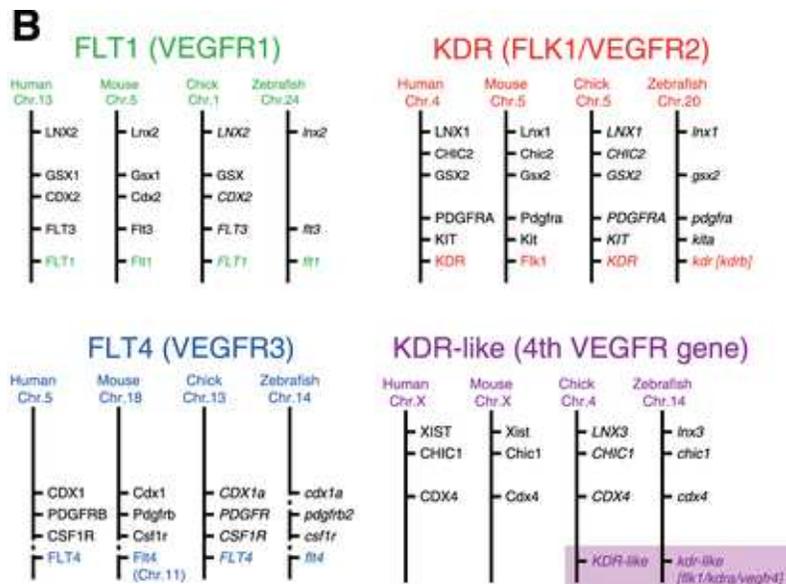
“In vivo” models are necessary to understand compounds properties



- Corneal micropocket
- Chick chorioallantoic membrane (CAM)
- Mesentery
- Sponge/Matrix implant
- The disc assay (DAS)
- Matrigel plug

- The main molecular pathways that regulate angiogenesis in mammals are conserved in zebrafish

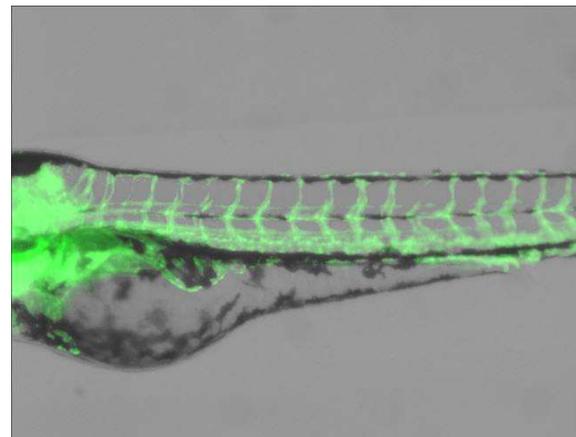
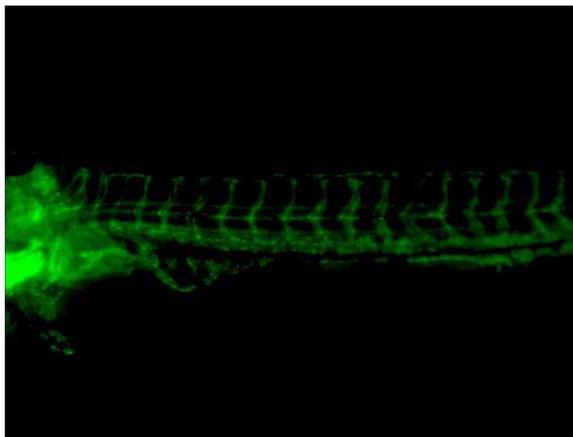
VEFG (vascular endothelial growth factor)



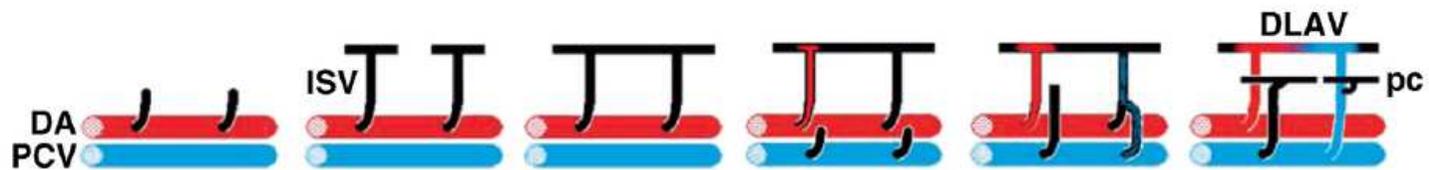
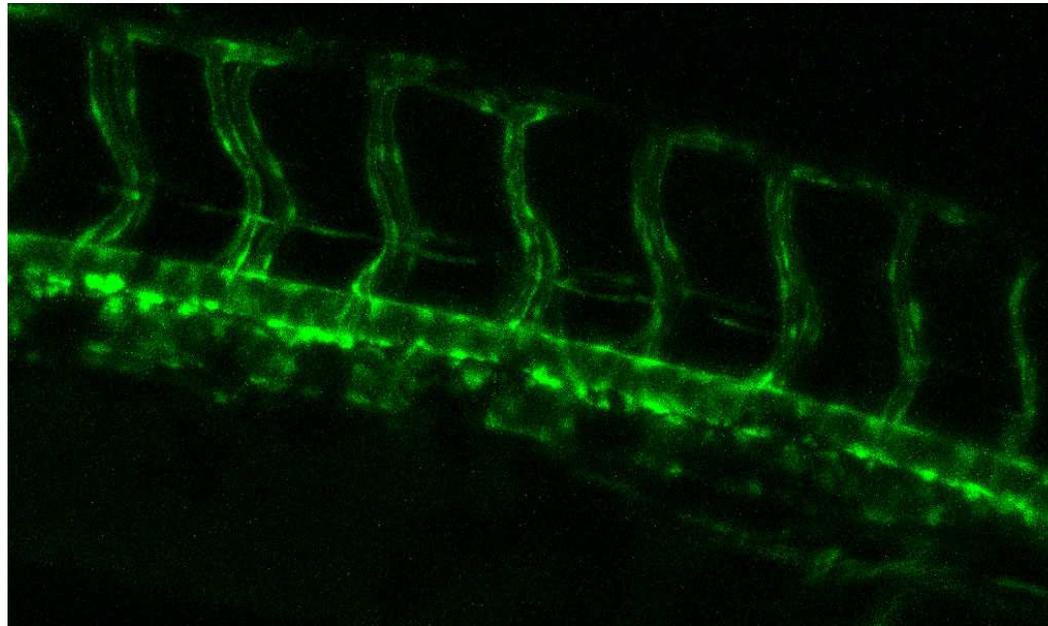
Other pathways

- Angiopoietin/ Tie2
- Notch
- Wnt
- TGFb/Alk1
- FGF
- PDGF
- S1P/Edg1
- Slit/robo
- Semaforin/Plexin
- Netrin/Unc5b
- Cellular matrix/integrins
- ...

Bussmann et al., 2007.



Sprouting angiogenesis



Isogai et al., 2003

DA: Dorsal Aorta
 PCV: Posterior Cardinal Vein
 DLAV: Dorsal Longitudinal Anastomotic Vessel

pc: parachordal vessel
 ISV: Intersengmental Vessel



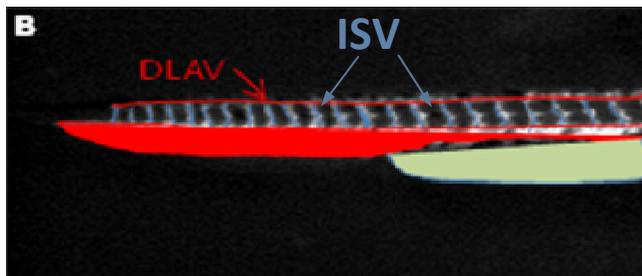
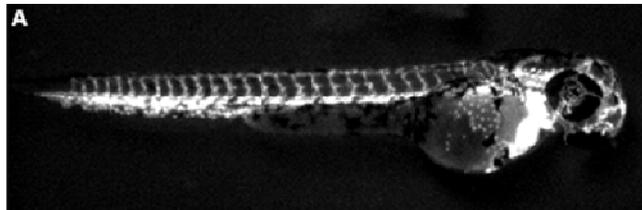
Validated Antiangiogenic assay



Image analysis

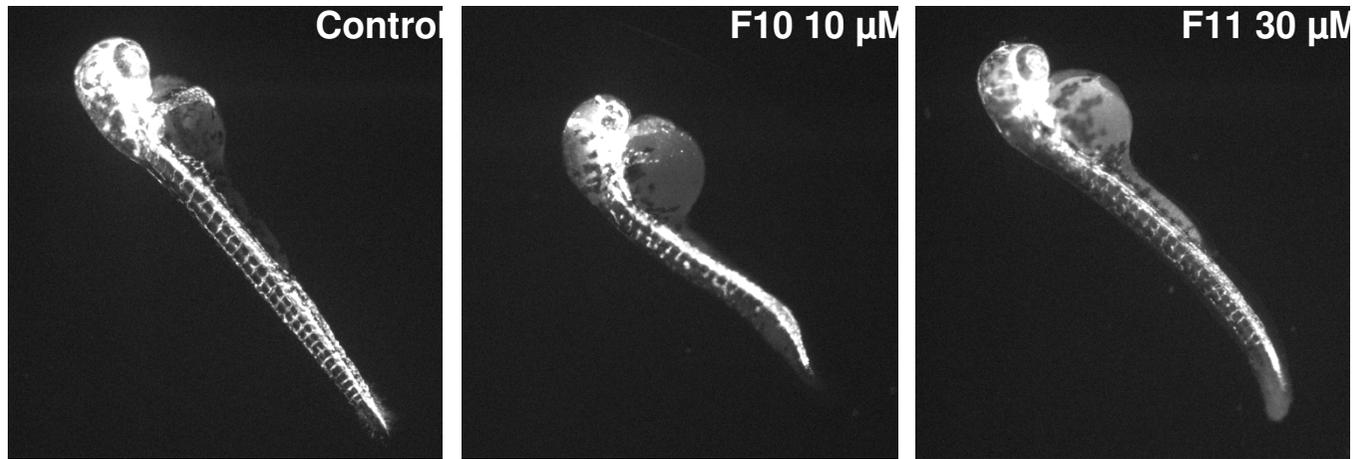
The pictures obtained are analyzed by counting two different parameters:

- Total number of intersegmental vessels present in the trunk
- Number of intersegmental vessels that are complete, getting to the DLAV

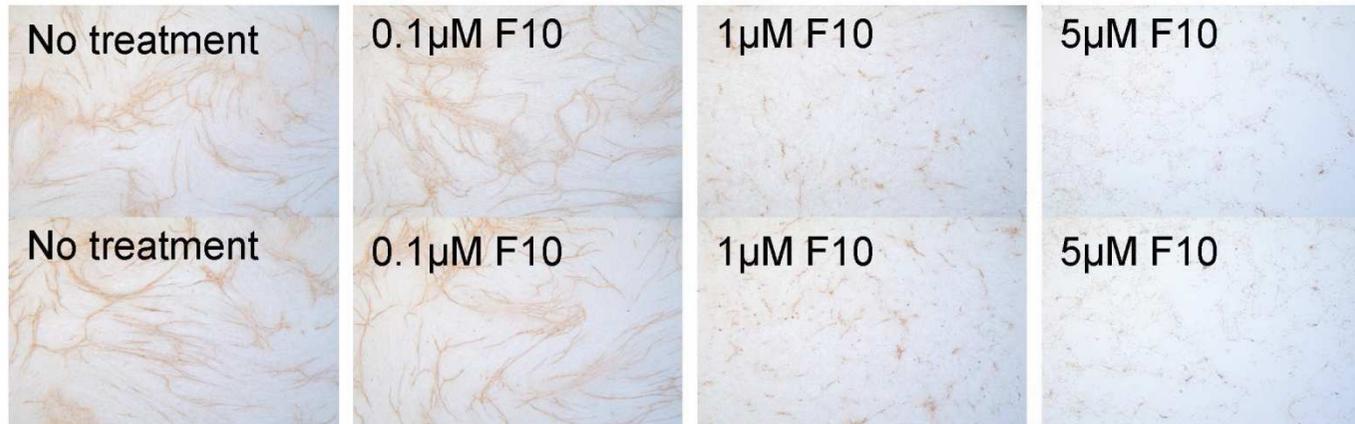


The figures show a whole embryo (A) and the part of the trunk in which vessels are quantified (B), which represents the same picture but without the head and the yolk (most bulging part).

DLAV: Dorsal Longitudinal Anastomotic Vessel
ISV: Inter-Segmental vessels



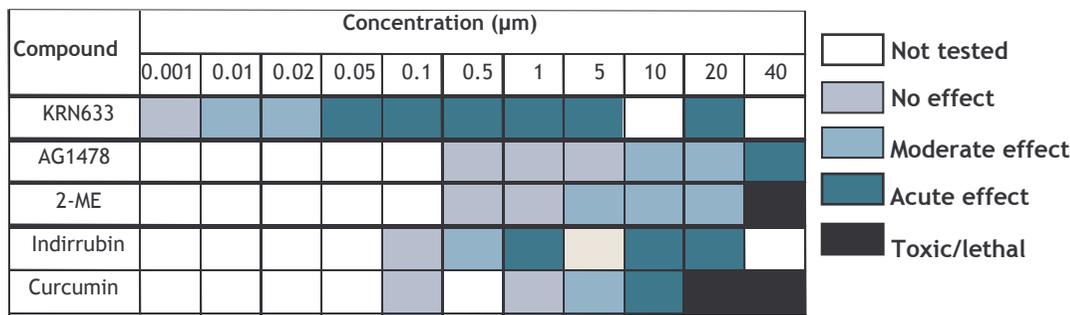
HUVEC cells



Camus et al (ready to submit)

Validated Antiangiogenic assay

Manual Validation



Automatic Validation

262 compounds Tyrosine kinase inhibitors library

12 positive at 30 uM (4.6%)

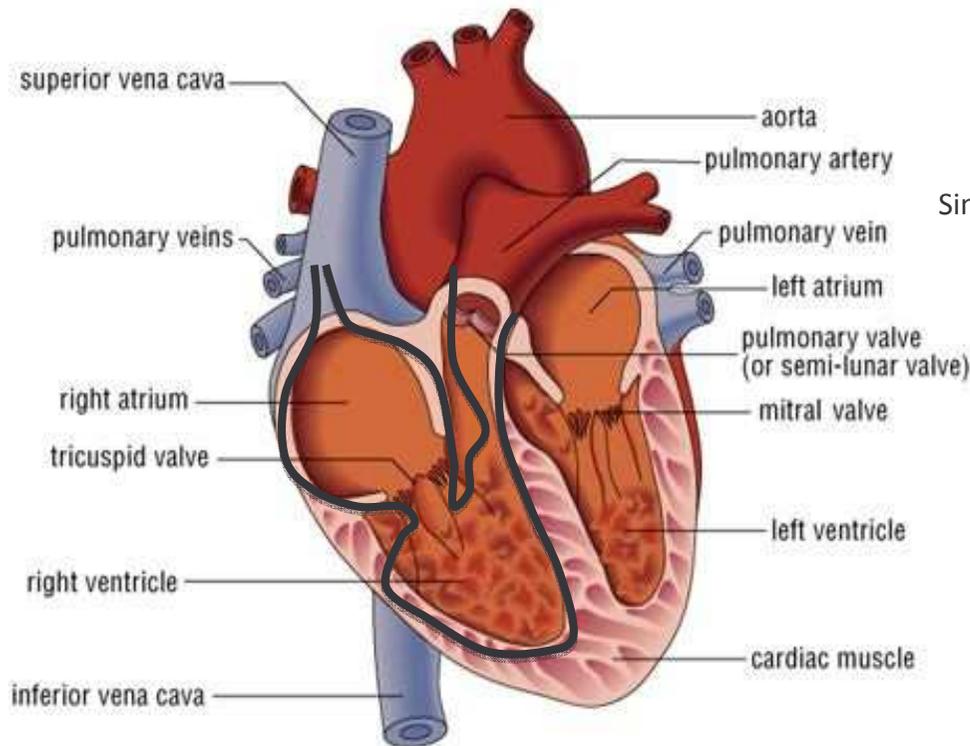
5 non-toxic (2%)



COMPOUND	ANGIOGENESIS INHIBITION
KRN633	Positive
ZD6474 (Vandetanib)	Positive
Sunitinib malate	Positive
Sorafenib Tosylate	Positive
PD173074	Positive
PD166866	Positive
AG-1296	Positive
PDGFR tyr kin inhibitor V	Positive
Tie2 Kinase inhibitor	*Negative
Bosutinib	Positive
AG1478	Positive
Indirubin-3'-oxime	Positive
Fumagillin	*Negative
NS-398	Positive
HIF-1 Inhibitor	*Negative
NVP-BEZ235	Positive
2-Methoxyestradiol	Positive
Paclitaxel	Positive
Tubulysin A	Positive
H-Gly-Pro-Arg-Pro-OH	*Negative
Tyrphostin AG490	Negative
Bestatin	Negative
Acetamide	Negative
E64	Negative
O6-benzylguanine	Negative
Cyclosporine A	Negative
4-Methylpyrazole hydrochloride	Negative
N-Acetyl-L-cysteine	Negative
Amiodarone hydrochloride	Negative
cis-Diammineplatinum(II) dichloride	Negative

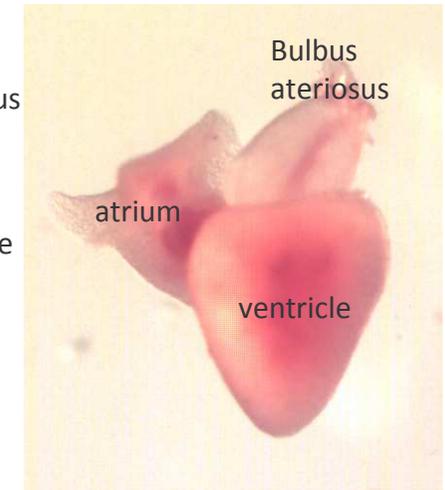
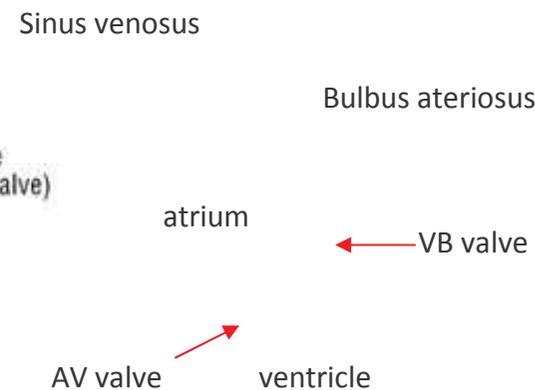
Validated Cardiotoxicity assay

Heart's Morphological similarities:

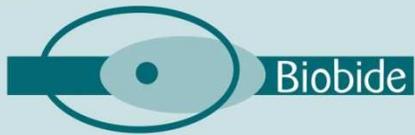


Helicon Publishing Ltd. 1999

Human heart



Zebrafish heart



Validated Cardiotoxicity assay

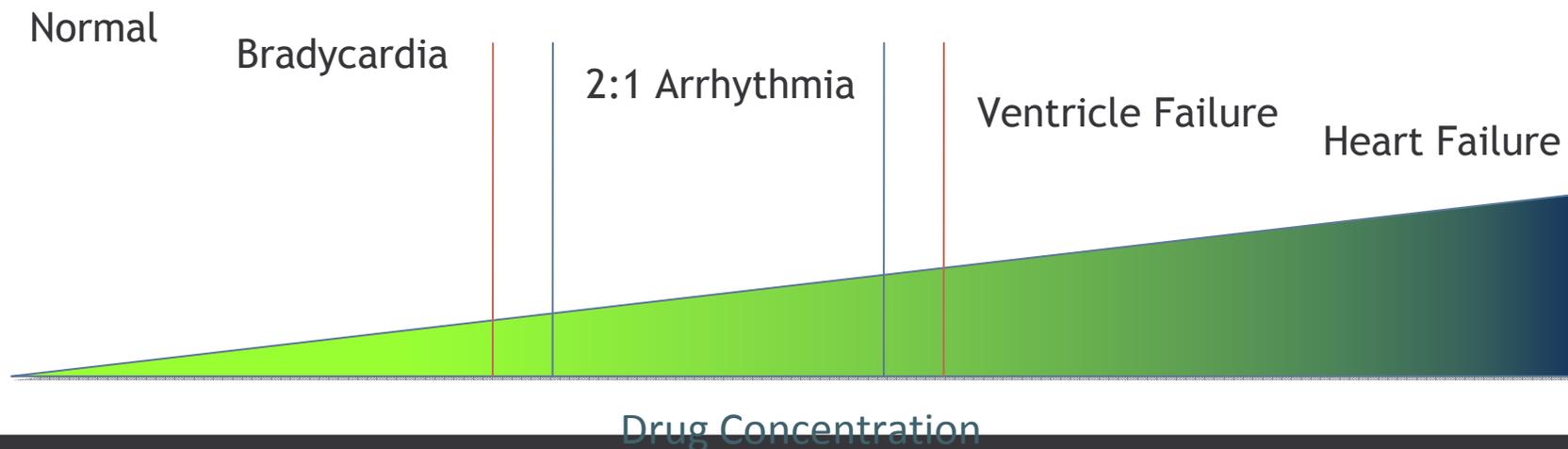
Automated method to detect cardiotoxicity by in vivo HTS in ZF embryos

Target: QT interval prolongation in humans

Rationale:

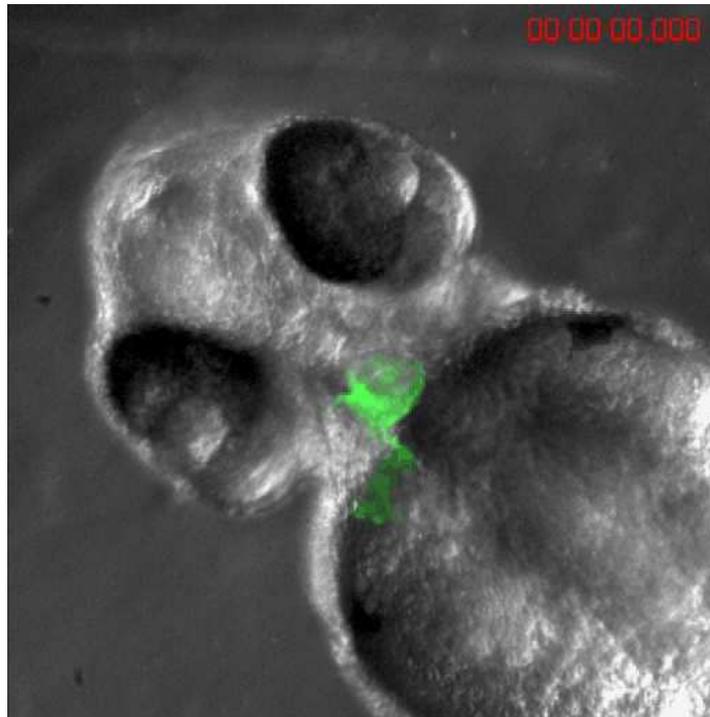
Zebrafish: zERG ↔ 2:1 arrhythmia

Humans: hERG ↔ Long QT Syndrome (LQTS)

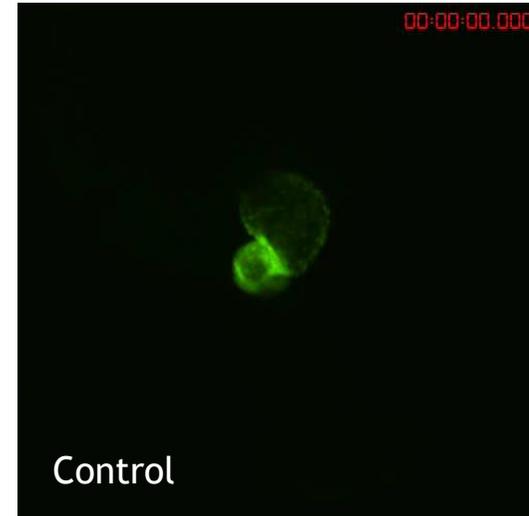


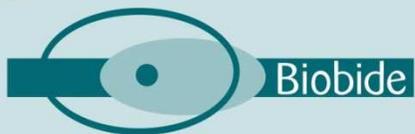
Validated Cardiotoxicity assay

Image analysis and transgenics



48 h zebrafish embryo-Biobide





Validated Cardiotoxicity assay

High Sensitivity, Specificity and Predictivity

	QT-Prolongation	No QT-Prolongation
Positive in Zebrafish	10 TP (90.9%)	2 FP (6.7%)
Negative in Zebrafish	1 FN (9.1%)	28 TN (93.3%)

Sensitivity: 93.3%
Specificity: 90.9%

False negative: 6.7%

False positive: 9.1%

Predictivity: 92.3%

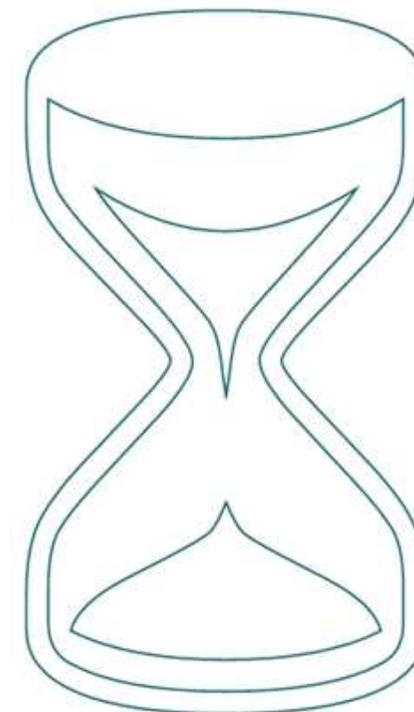
N= 6000 embryos



Conclusions

Advantages

- Fast and efficient assays in early stages of the Drug Discovery and Development process
- Toxicity, safety and efficacy assays
- Translational results: close similarity to human and other known models
- Increases selection arguments: a tool to prioritize drug candidates
- Certified under Good Laboratory Practices (GLP's) in August 2009 for *in vivo* toxicity, Pharmacodynamics and Safety Pharmacology
- Along with 3 R's theory (Reduction, Refinement and Replacement)





Acknowledgements

Biobide

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- Igor Lizardi
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- Itziar Irijalba

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- Celine Paloc, Vicomtech
- Conchi Rodriguez, Salk Institute
- Idoya Calvo, CIB Navarra
- Ilir Dubova, Salk Institute
- Marta Garcia-Granero, CIFA
- Roberto Gonzalez, Tekniker
- Valesca Martinez, Koniker
- Leopoldo Laricchia, CMRB



IN VIVO ZEBRAFISH HTS/HCS

Early Drug Discovery

Toxicity assays

Safety assays

Efficacy assays

www.biobide.com

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Spain. Tel: 943 309 360 Fax: 943 309 370. E-mail: info@biobide.es