Tufts UNIVERSITY | Cummings School of Veterinary Medicine

From salamanders to stem cells: key role for veterinary and medical doctors in advancing regenerative medicine

no conflicts of interest to declare

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Challenges for public health

Many incurable conditions that are managed but not cured

- Inherited diseases
- Physical ailments (infectious, non-infectious, acute, chronic)
- Mental illness
- Obesity-related illness
- Addiction and related illnesses

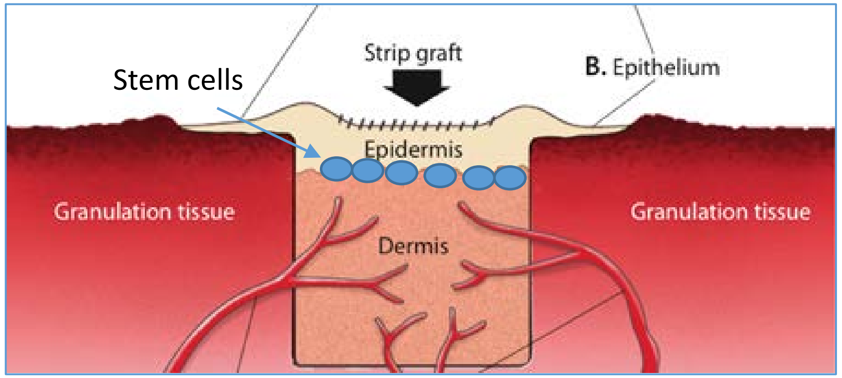
What is 'Regenerative Medicine'?

"process of replacing, engineering or regenerating cells, tissues or organs to **restore or establish normal function**"

Mason and Dunnil. *Regenerative Medicine*, 2008, 3(1), 1–5 [47]

What is 'Regenerative Medicine'?

Skin Graft



e.g.

- Bone marrow transplants
- Pure stem cell transplants
- Tissue grafts (skin, cornea)
- Bioengineered organs (ear, bladder)
- Gene therapy (DNA, RNA)

Regenerative Medicine

The guiding motto in the life of every natural philosopher should be, 'Seek simplicity and distrust it.” --Alfred North Whitehead

Regenerative Medicine

Potential solutions

One drug, <u>one</u> target

Regenerative Medicine

Potential solutions

One drug, one target

vs

Regen Med approach, multiple targets

Regenerative Medicine

Potential solutions

One drug, <u>one</u> target
vs
Regen Med approach, <u>multiple</u> targets

Complex disease problems

Autoimmune diseases, heart failure, neurodegenerative diseases (e.g., Alzheimer's)

Goals of this new discipline

- Address natural complexity
- Leverage natural healing ability
- Employ interdisciplinary approaches
- Improve efficacy, benefit : cost, and safety

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Veterinary medicine

Regenerative Medicine

'…potentially disruptive technology…' replacing major pharmaceuticals, devices, prostheses.

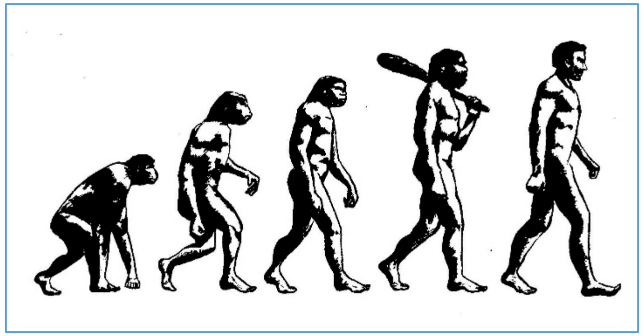
Mason and Dunnil. *Regenerative Medicine*, 2008, 3(1), 1–5 [47]

Guiding principles of regenerative medicine

"Study nature, love nature, stay close to nature. It will never fail you."
Frank Lloyd Wright

Guiding principles of regenerative medicine

•Evolution



http://elitetrack.com/evolution-strength-training-personal-perspective-51-years-experiences-part-three

•Developmental biology

•Regeneration biology in animals

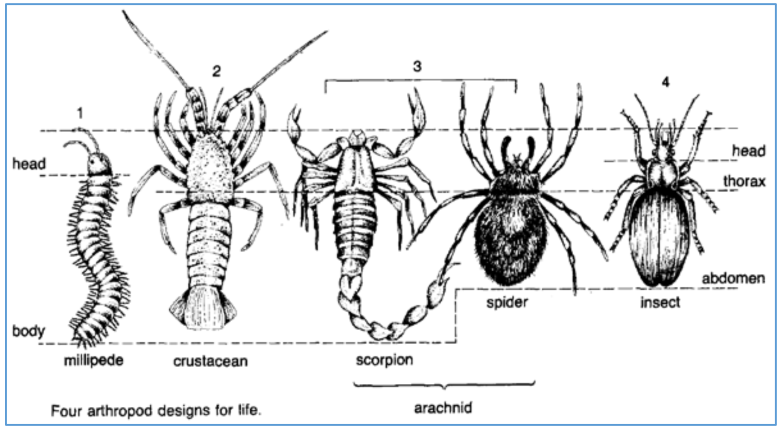
Guiding principles of regenerative medicine

Regeneration in the animal kingdom

Urodeles

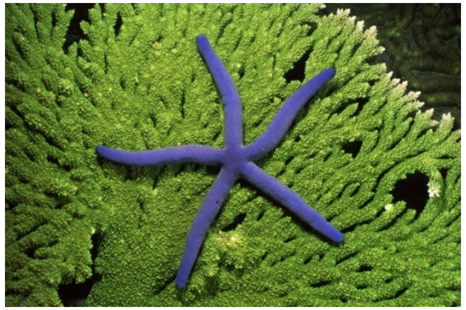
Lizards

Arthropods



Coelenterates

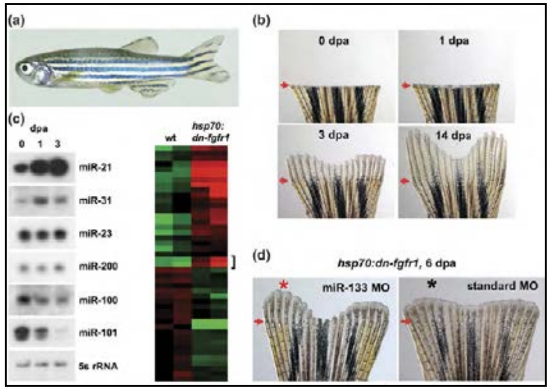
Echinoderms



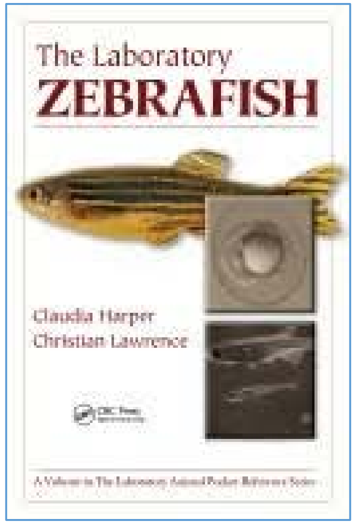
Guiding principles of regenerative medicine

- Fish

Tail, fins, organs

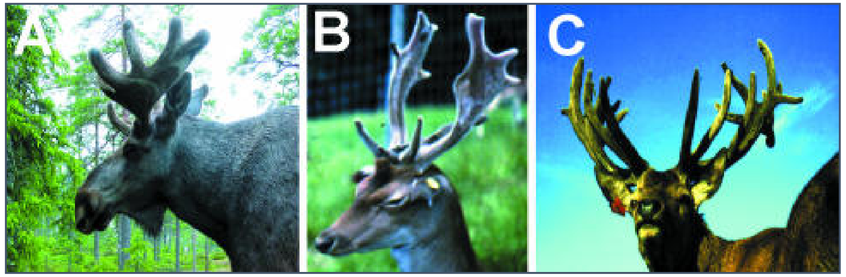


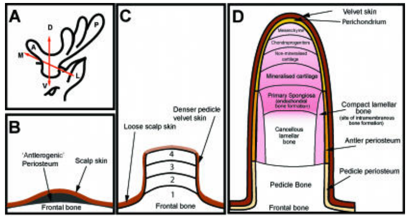
Yin *Current Opin Genetics and Develop* 2008



- Mammals

Cervid antlers





Gyurjan *Mol Genet Genomics* 2007

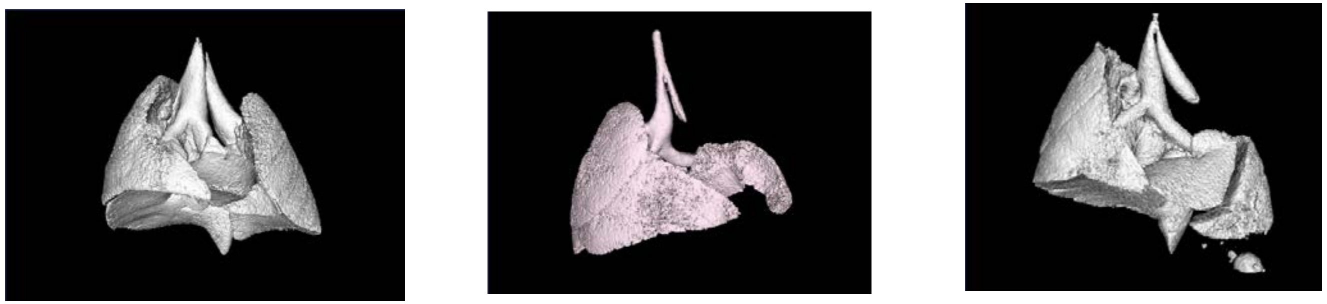
Humans and veterinary species: have evolved with limited regenerative capacity

Regeneration

- Tips of digits
- Blood system
- +/- Ear holes

Quasi-regeneration (compensatory growth)

- Liver, kidney, and lung in children only



Salamanders – ‘experts’ at regeneration

Mexican salamander

Order: Caudata (urodeles):

- Shed tail or leg to get away from predator (autotomy)
- Regenerate limbs, eye, spinal cord, tail, skin, etc.
- **Retain many juvenile traits (neoteny)**

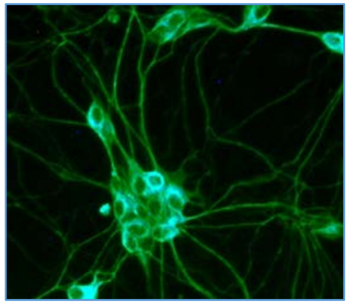
http://www.arkive.org/axolotl/ambystoma-mexicanum/image-G19629.html

Key events in re-growing a limb (in salamanders)

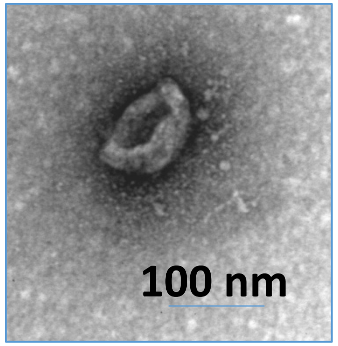
Regeneration in Mexican Salamander

1. Rapidly close skin defect (<1 d)
2. Quell inflammation, remodel
3. Stem cells arise, divide
4. Specialized growth factors pattern growth

'Next generation regeneration' – *which approach? how will these be tested?*

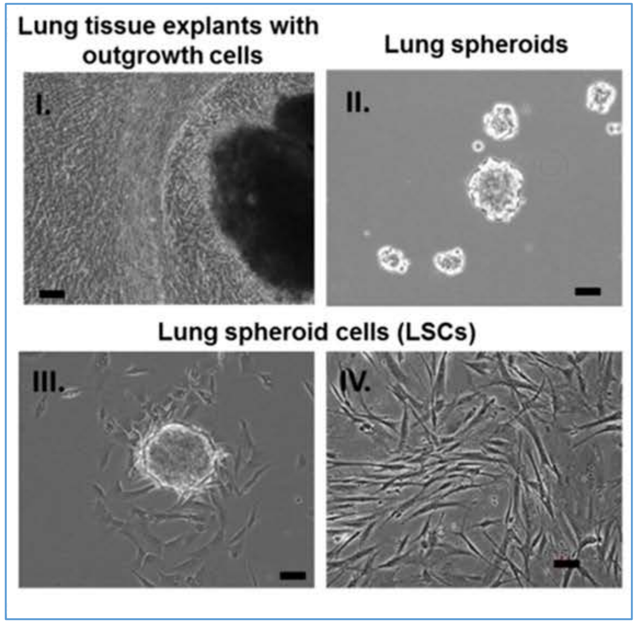


- Stem cells
- Biological chemicals
  (DNA, RNA, proteins)
- Bio-mimetic scaffolds
- Nanoparticles

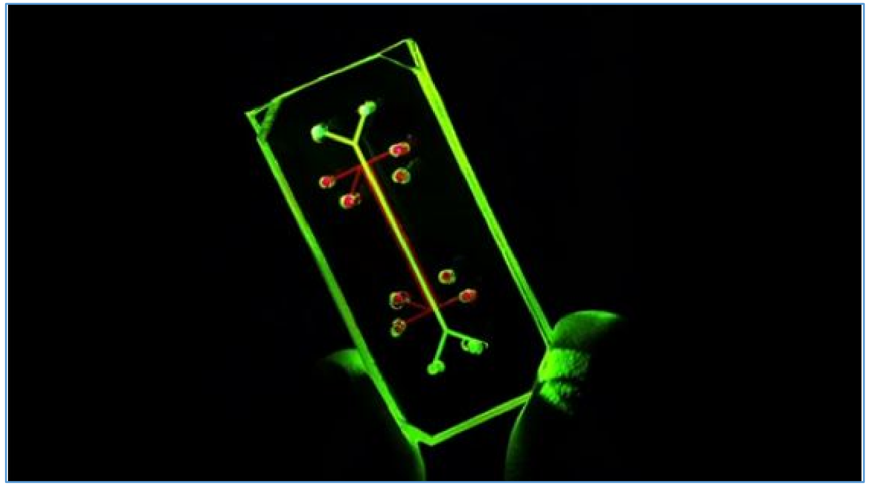


Initial testing in the dish

- Cells (including stem cells)
- 'Organs on a chip'
- Organoids



Henry et al. *Stem Cells Trans Med* 2015



http://www.ted.com/talks/geraldine_hamilton_body_parts_on_a_chip.html

Animal models

- Zebra fish
- Rodents
- Non-human primates
- Large animals

Naturally occurring disease, i.e. veterinary patients, are underutilized in biomedical research

- Dogs (78M), Cats (80M), Horses (6M), Birds (8M)
- Veterinarians: 67,000 (AVMA 2008)
- University Teaching Hospitals: >27

    e.g. Caseload @ Tufts University: >40,000/yr

- Specialists, referral centers, clinical trial consortium

Mouse vs. Dog vs. Human

'sister species' (Dog, humans)

	Mouse	Dog	humans
environment	-	+++	+++
life expectancy	2-3 yr	~15 yr	76-81
chromosomes	40	78	46
genes	23,000	20,000	19,000
#base pairs	~3.0B	~2.8B	~3.3B
shared DNA sequence	~65-75%	~80-90%	(100%)
size	0.02 kg	5-50 kg	70kg
anatomy	+	++	+++
physiology	+	+++	+++
genetic diversity	+	+++	+++
natural diseases	+/-	+++	+++
biomarkers	+	+++	+++
patient management	-	+++	+++
therapeutic responses	+	+++	+++

Mouse vs. Dog vs. Human

10-12,000 years ago

‘sister species’ (Dog, humans)

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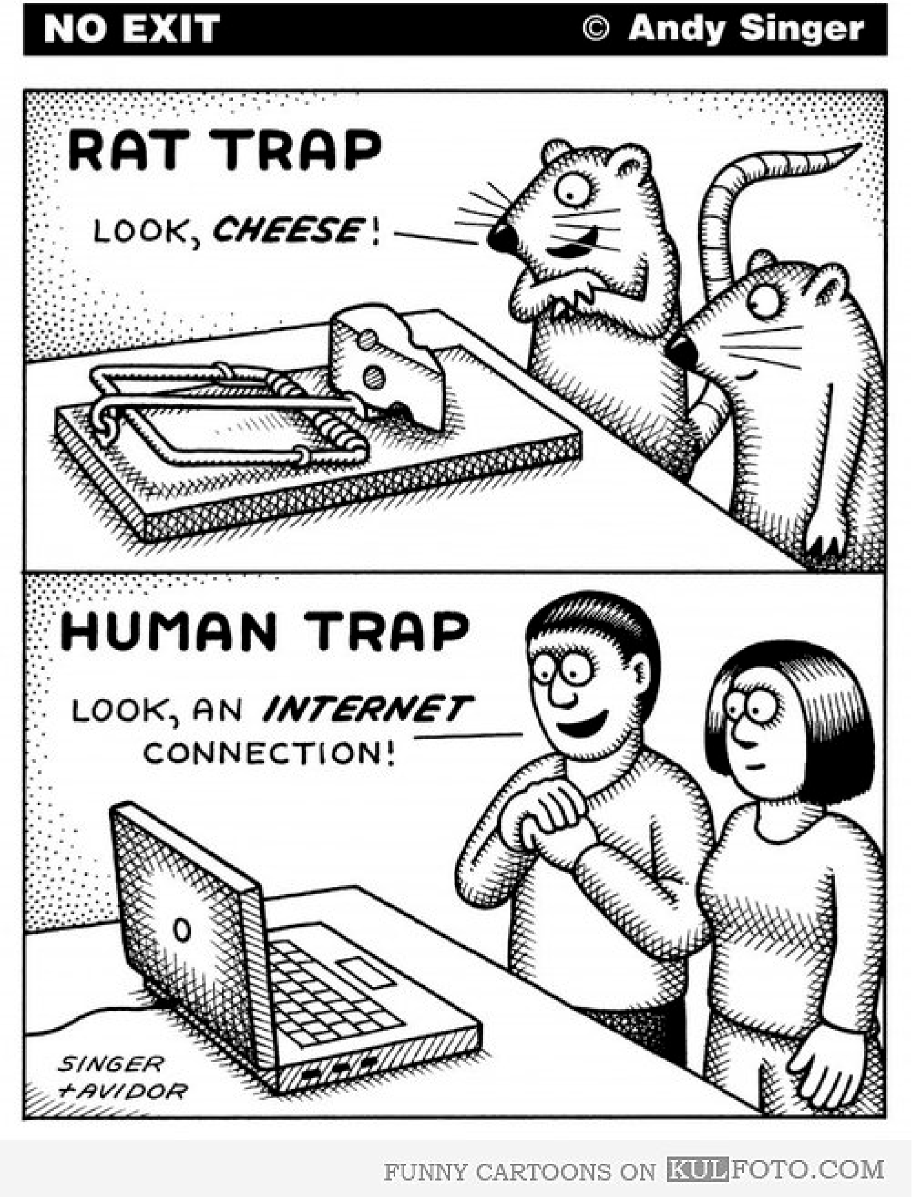
anatomy	+	++	+++
physiology	+	+++	+++
genetic diversity	+	+++	+++
natural diseases	+/-	+++	+++
biomarkers	+	+++	+++
patient management	-	+++	+++
therapeutic responses	+	+++	+++

OMIA - Online Mendelian Inheritance in Animals

	dog	cattle	cat	sheep	pig	horse	chicken	goat	rabbit	Japanese quail	golden hamster	Other	TOTAL
Total traits/disorders	631	444	318	229	226	221	208	74	62	43	40	517	3013
Mendelian trait/disorder	246	180	82	96	50	41	126	13	31	32	28	162	1087
Mendelian trait/disorder; key mutation known	173	87	46	42	23	29	38	8	7	9	3	73	538
Potential models for human disease	342	159	182	98	85	116	42	30	37	11	15	269	1386

http://omia.angis.org.au/home/

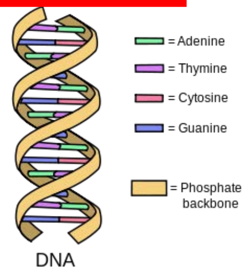
Are we different or just the same?



Genetic basis for disease

coding

DNA → **messenger RNA** → protein…cells, tissues



https://commons.wikimedia.org/wiki/File:DNA_simple2.svg

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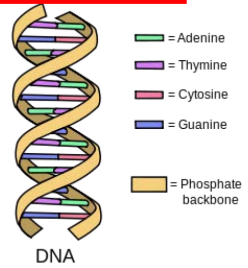


sequence variations or mutations → Abnormal proteins Diseased organs

https://commons.wikimedia.org/wiki/File:DNA_simple2.svg

Genetic basis for disease

- Human, canine genome projects
- **Genome wide association study (GWAS)**

↓

Genetic basis for disease → Risk, Cures

Genetic basis for disease

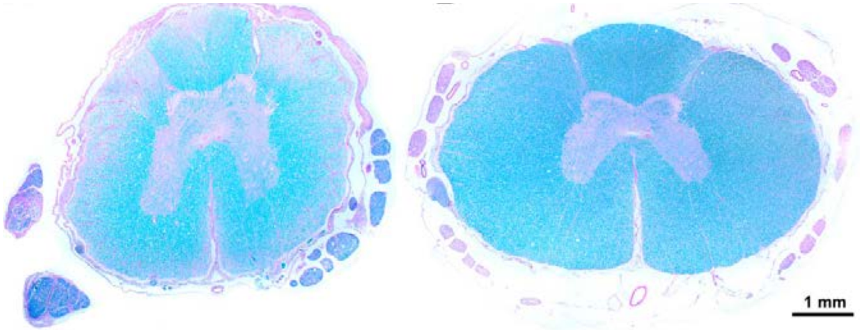
- Human, canine genome projects
- **Genome wide association study (GWAS)**

Genetic basis for disease → Risk, Cures

- Type II diabetes
- Parkinson's
- Heart disorders
- Obesity
- Crohn's disease
- Prostate cancer
- Response to anti-depressant medication

Natural model of human ALS form, 2% (SOD1 mutation)

Awano PNAS 2009



Spinal cord of Corgi with DM   Normal dog

Genetic basis for disease

- Human, canine genome projects
- **Genome wide association study (GWAS)**

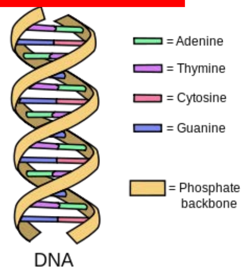
↓

However, majority of GWAS studies yield links between *disease* and *non-coding DNA* (previously referred to as 'junk DNA')

Genetic

coding

DNA → **messenger RNA** → protein…cells, tissues



https://commons.wikimedia.org/wiki/File:DNA_simple2.svg

Genetic vs. Epigenetic

coding

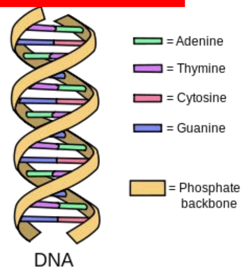
DNA → **messenger RNA** → protein…cells, tissues

non-coding

micro RNA

long non-coding RNA

piwi RNA

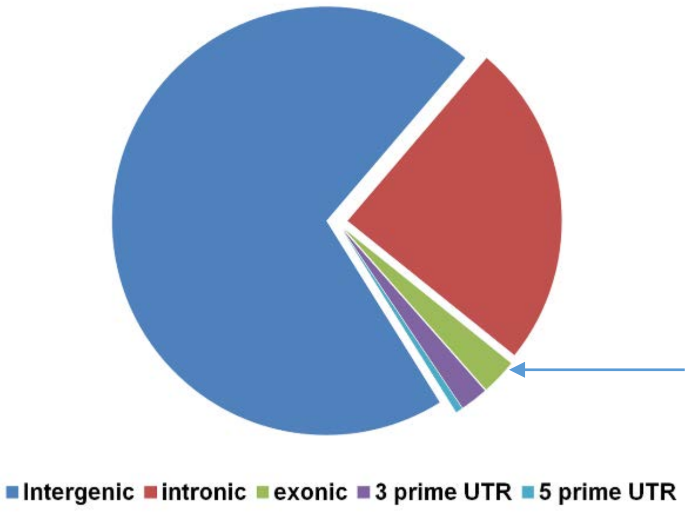


DNA Methylation
Histone modification

https://commons.wikimedia.org/wiki/File:DNA_simple2.svg

Our DNA is 98% non-coding, but not junk

- Human, canine genome projects
- Genome wide association study (GWAS)

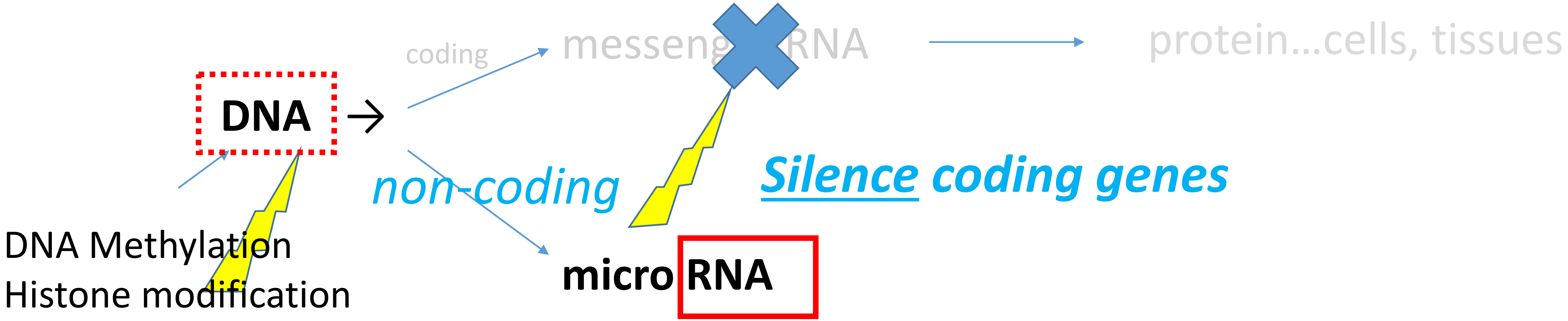


DNA coding
for proteins

98% 3%

% genome (DNA) non-coding

Epigenetics:
nature's feedback system



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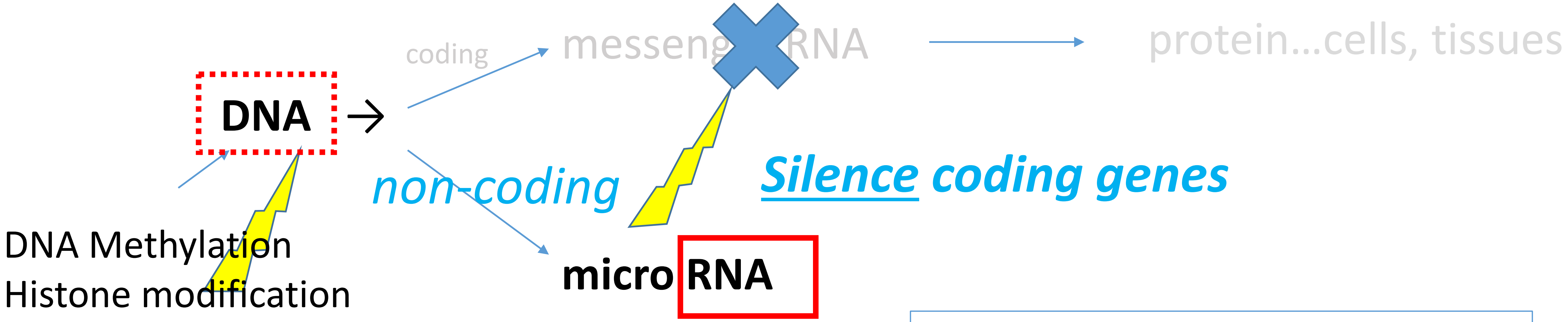
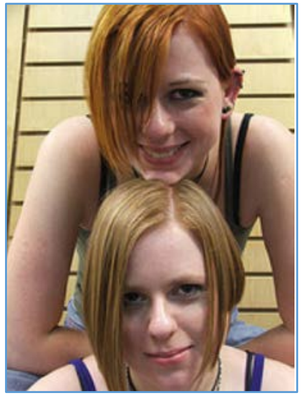


- **Evolution**
- **Anatomy, physiology**
- **Expression of traits (coat color)**
- **Natural Diseases**

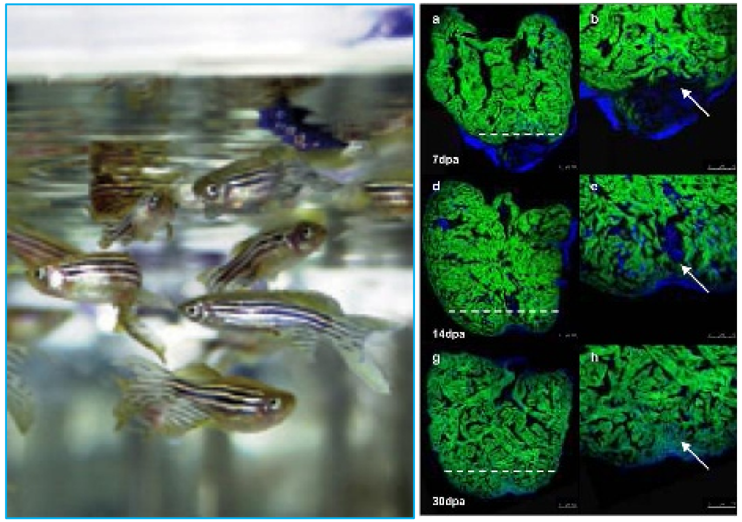
Epigenetics

Gene ↔ Environment



- Phenotype (size, shape, coat, color, etc)
- Many diseases of public health importance

Exploiting epigenetic tools to treat heart disease

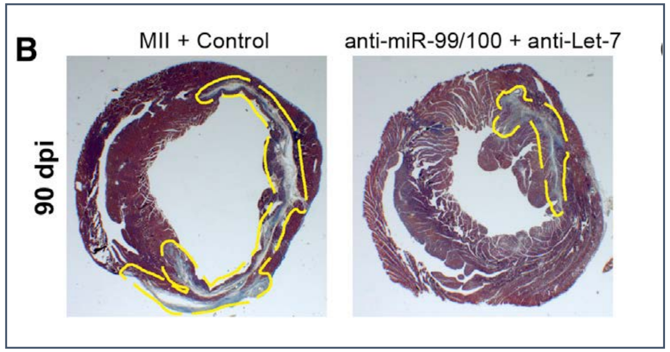


Zebrafish can regenerate 20% heart muscle, a mouse or human cannot…

http://www.devbio.biology.gatech.edu/?page_id=398

Adapt this Technology

…however, the mouse heart can be epigenetically reprogrammed to turn on heart regeneration.

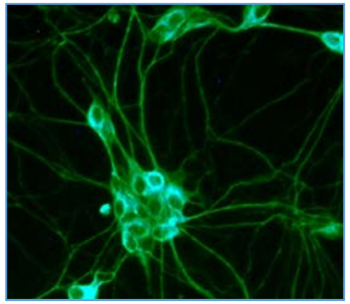


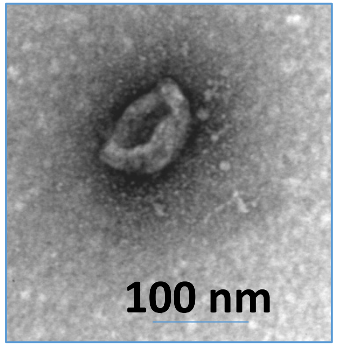
marked reduction in scar!!

Aguirre et al., 2014, Cell Stem Cell 15, 589–604

Which strategy is most effective? cost effective?

- Stem cells
- Biological chemicals
  (DNA, RNA, proteins)
- Bio-mimetic scaffolds
- Nanoparticles





5 day human embryo

Stem cells

- Fertilized embryo – **totipotent** – *whole body*
- Embryonic stem cells (ESC) – **pluripotent** – *all cell types*
- Induced pluripotent stem cells (iPSC) – **pluripotent** – *all cell types*
- Adult stem cells –**multipotent** – *limited range of cell types*

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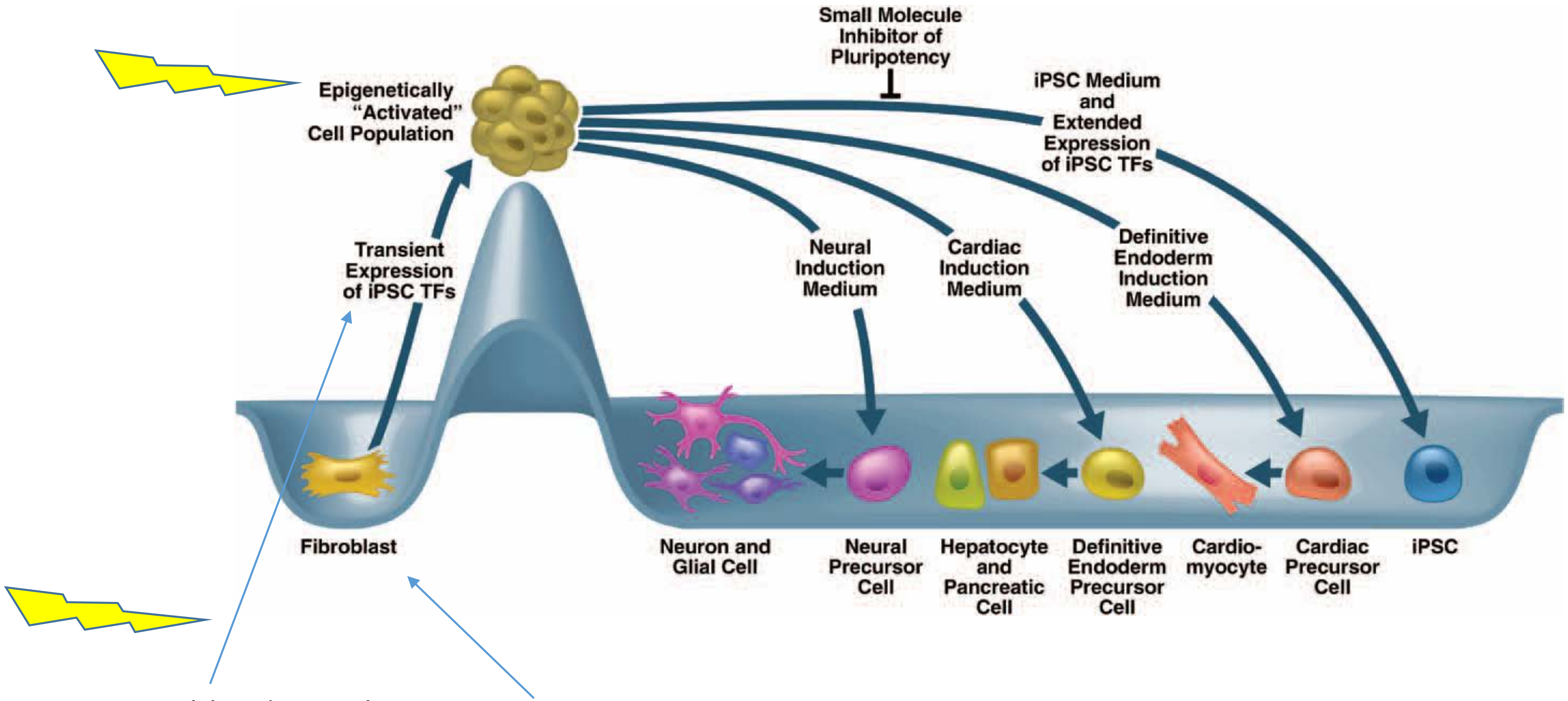
Unlocking pluripotency to make cell replacements



blood sample

skin sample

Ma et al. Circ Res. 2013;112:562-574

Advantages of reprogramming to pluripotency

- Requires only a blood or skin sample
- No immune response to your self (?)
- Basic studies of development, toxicology, etc

Induced pluripotent stem cells and reproduction…
e.g., saving endangered species

somatic cells

↓

iPSCs

↓

Spermatogonal SC
Oocytes

↓

IVF

Offspring (F1)

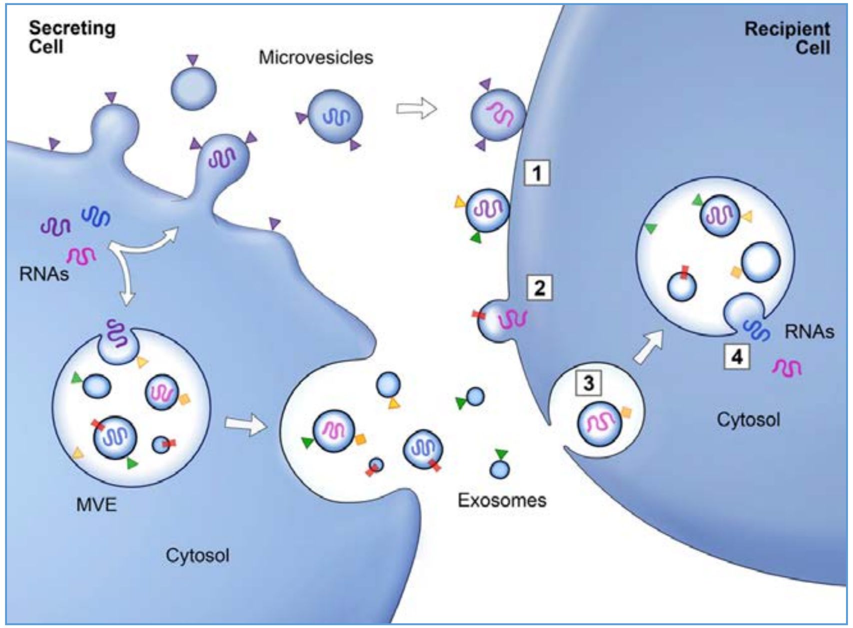
Inducing pluripotency in somatic cells from the snow leopard (Panthera uncia), an endangered felid
Verma et al. Theriogenology.
2012 Jan 1;77(1):220-8

Multipotent mesenchymal stem cells (MSC) are currently the most widely used stem cells for therapies

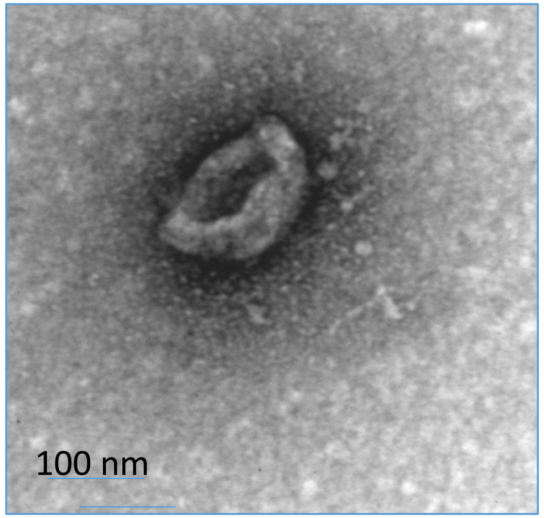
- Derived from uncontroversial sources
- No epigenetic reprogramming
- Naturally pro-regenerative
- Evidence they extend lifespan Kim et al STEM CELLS TRANSLATIONALMEDICINE 2015;4:1–11
- >289 clinical trials in humans vs. 1 for iPSC (Japan) and 2 for ESC (clinicaltrials.gov)

Mesenchymal stem cells (MSC) make many chemicals and nanoparticles with potent epigenetic signals

STEM CELLS



Nanoparticle from canine MSC

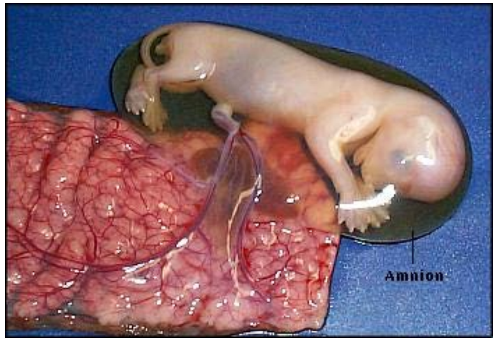


Canine chorionic MSCs EVs
(TEM by Vicky Yang)

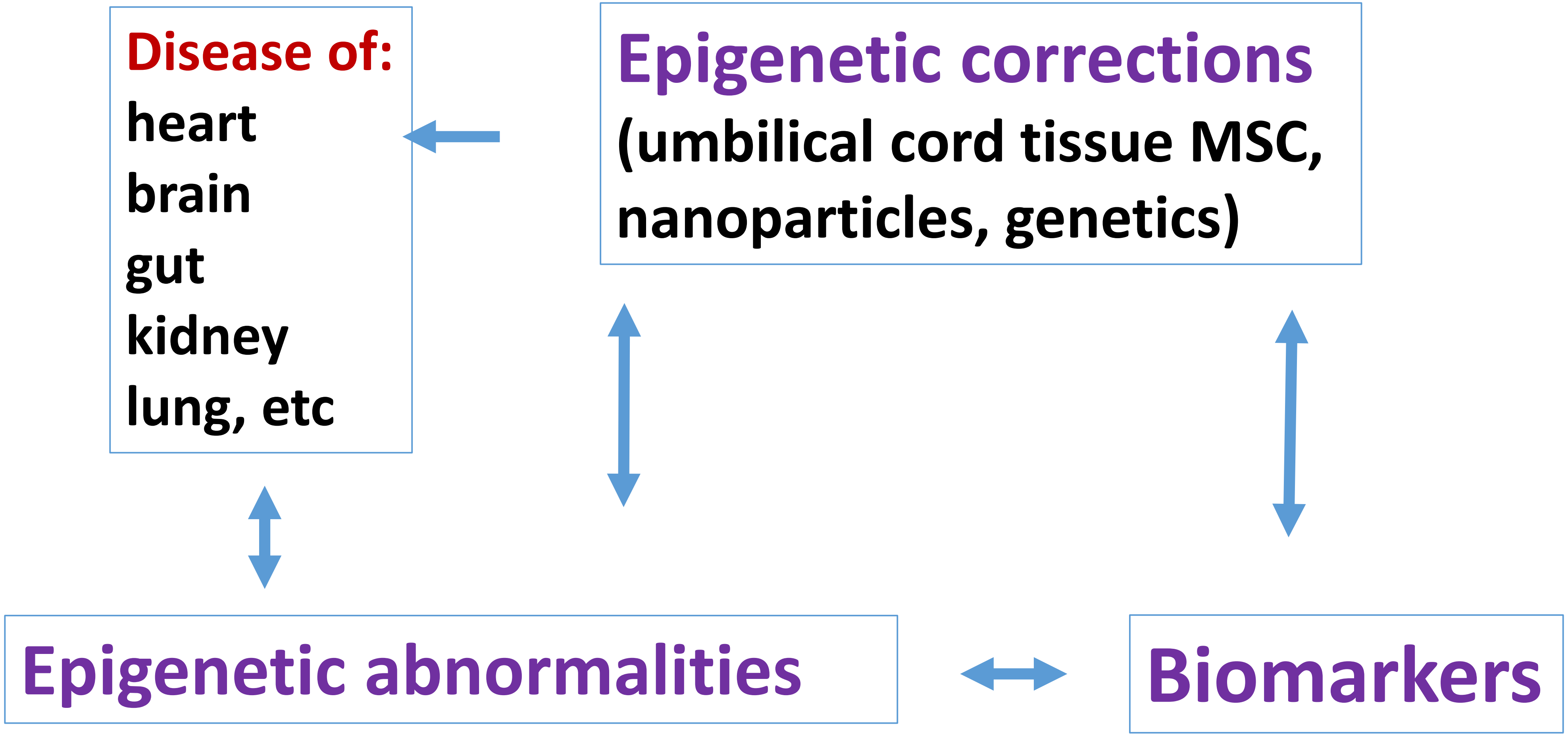
Raposo et al. *J. Cell Biol*. (2013) Vol. 200 No. 4 373–383

Umbilical cord and placental tissue as source of stem cells and exosomes (nanoparticles)

- Underutilized, discarded tissue source
- Pre-natal ('young') stem cell features (multipotency, pluripotency in many reports)
- Potent 'pro-regenerative signals'
- Animals: Identical siblings (2-10…)



General approach



General approach

Mitral valve disease

↑miRNA 29b, 30a derived from umbilical MSC, nanoparticles, genetic sources

Epigenetic abnormalities:
↓ miR-29b, -30a in valve cells

↓ ↑

Human and rodent model data (Beth Israel, Dr. Saumya Das)

Biomarkers
↓ blood levels of miR-29b, -30a

General approach – getting more personal…

Mitral valve disease

↑miRNA 29b, 30a derived from umbilical MSC, nanoparticles, genetic sources

"Theranostics"

Epigenetic abnormalities:
↓ miR-29b, -30a in valve cells

Biomarkers
↓ blood levels of miR-29b, -30a

Human and rodent model data (Beth Israel, Dr. Saumya Das)

Process of developing clinical trial in companion animals

- Identify problem
- Articulate vision
- Scientific rationale
- Design study
- Study review
- Funding
- Recruit patients
- Screen patients
- Test / Treat patients
- Monitor patients
- Review results

Process of developing clinical trial in companion animals

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Novel approach to treatment of patients after CPR

(Drs. Elizabeth Rozanski and Vicky Yang – Cummings School)

- Identify problem → CPR* success rate is very low in humans/animals
- Vision → CPR outcome can improve by protecting brain, heart
- Rationale → Stem cells shown to improve CPR outcome in rats
- Design study → Identify molecular disturbances thus potential targets in blood after CPR in dogs

*Cardiopulmonary Resuscitation

Biomarkers that appear in blood after CPR (in dogs)

Groups:

CPR dogs:	**6 dogs that survive CPR for at least 1 hr**
ICU Controls:	**6 dogs in ICU**
Healthy controls:	**6 healthy young Beagles**

Intervention:

Blood Sampling:	1 and 6 hrs after CPR (return to spontaneous circulation)
Owner consent:	*prior* to 1 hr blood sample (above)
Owner incentive:	Reimbursement for blood tests that would *normally be taken* during this period.
Endpoints:	**Survival, neurologic outcome, and biomarkers (microRNA)**

Naturally occurring diseases shared with humans (dogs, cats) - candidates for new approaches

- Heart
- Neurodegenerative
- Stroke
- Epilepsy
- Alzheimer's / dementia
- Auto-immune (skin, brain, etc)
- Diabetes
- Asthma
- Arthritis
- Cancer
- Disc degeneration
- Obsessive-compulsive disorder
- Age-related syndromes

Regenerative medicine / stem cell trials at Cummings

http://sites.tufts.edu/vetclinicaltrials/regenerative-medicine-stem-cell-trials/

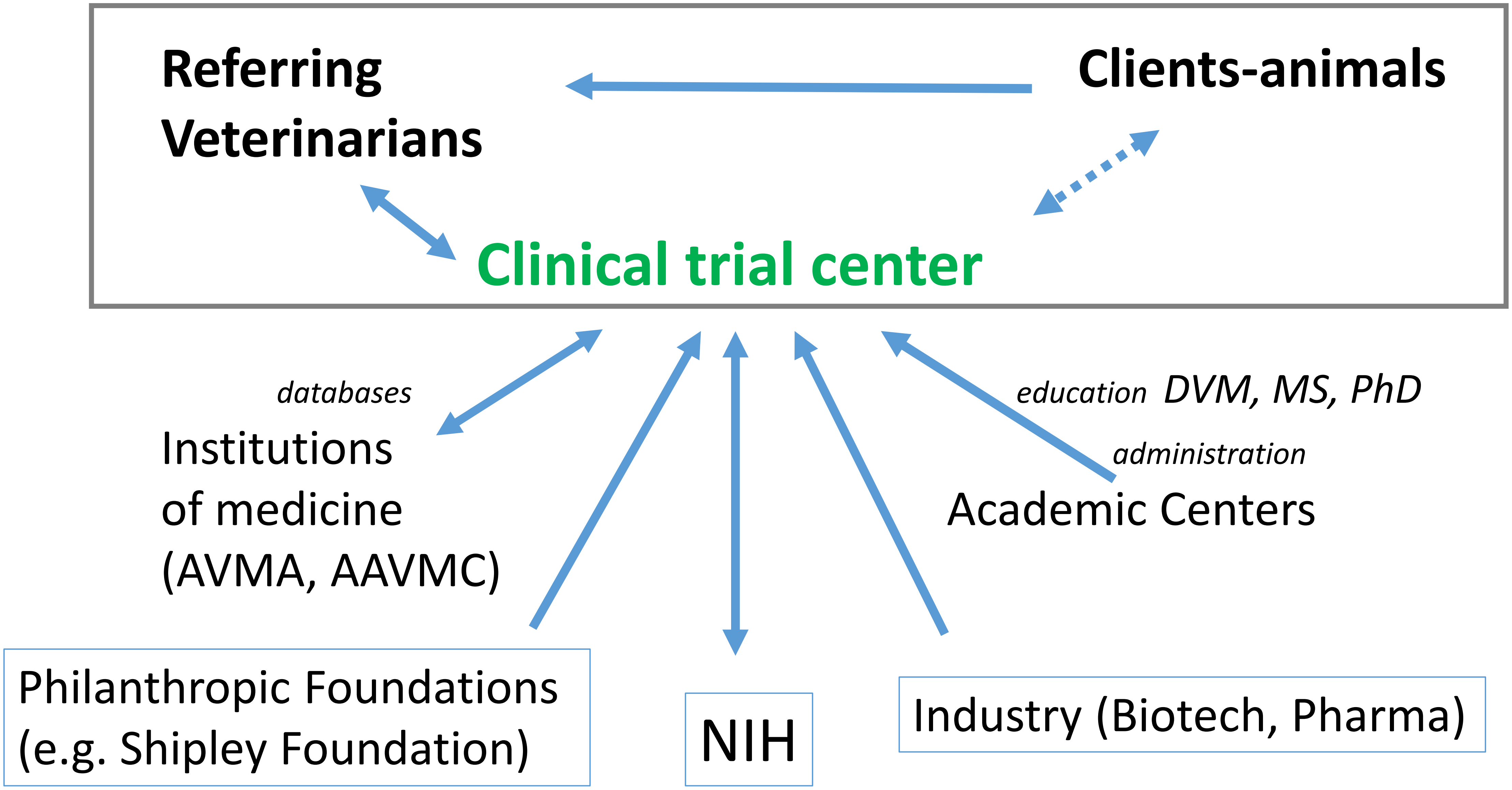
Stem cell trials in canine patients for:

- Mitral valve disease and associated heart failure – open
- ARVC – heart disease that causes sudden death - open
- Allergic (atopic) dermatitis - open
- Inflammatory bowel disease – open
- Perianal fistulas / fistulizing Crohn's Disease- open
- Auto-immune kidney disease - open
- Spinal cord compression due to disc degeneration – pending

Biomarker studies in canine patients:

- Biomarkers relating to CPR – open
- Biomarkers for mitral valve disease and ARVC - open

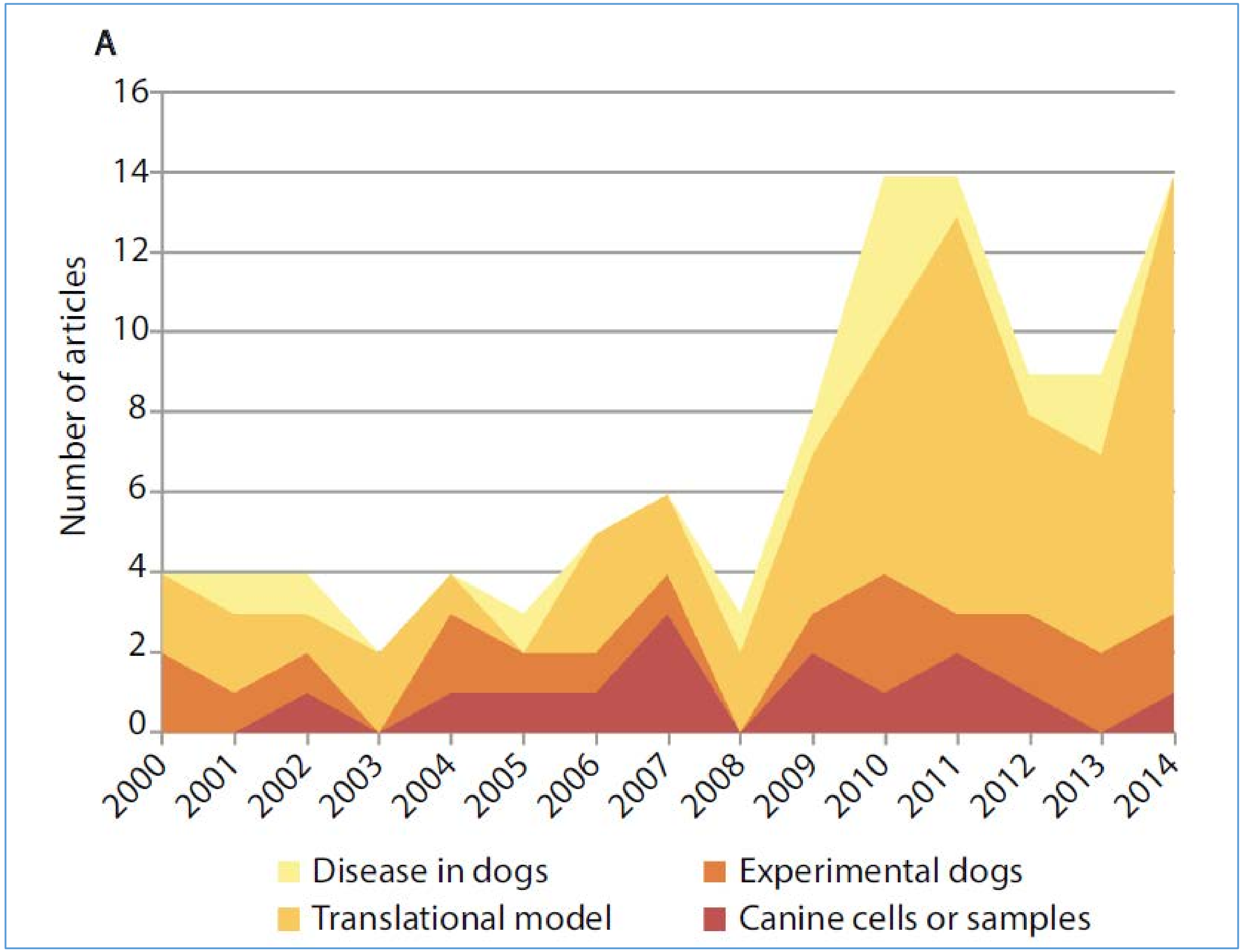
Evolving paradigm in regenerative medicine at Cummings



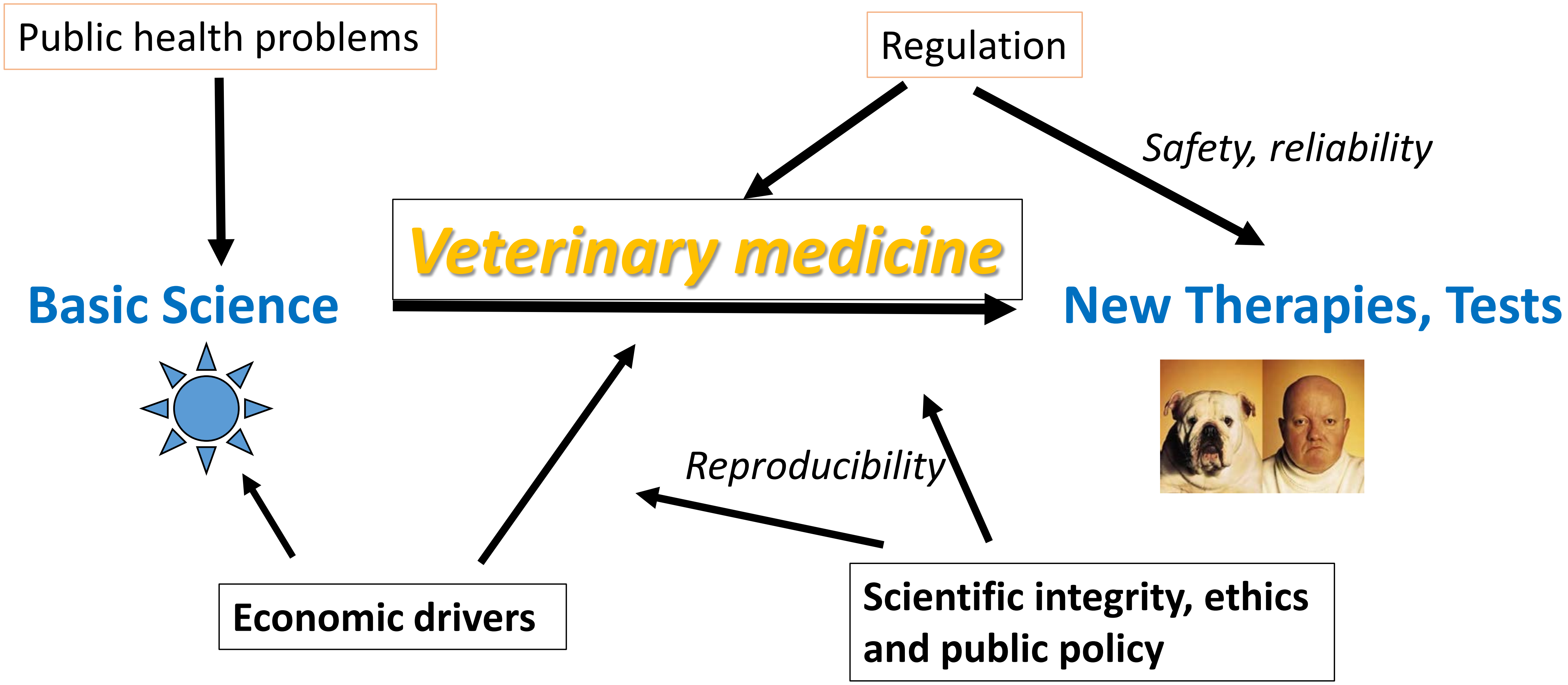
‘One Health’ : intersection: humans, animals, & environment

‘One Literature’

Mary Christopher, DVM, PhD. *Science*
2 September 2015 Vol 7 Issue 303 303fs36



Veterinary medicine – proof of principle, safety, reproducibility, translation to humans



Peter Doherty, BVSc, MVSc, PhD, Nobel Prize 1996

https://www.avma.org/News/JAVMANews/Pages/111201o.aspx

Quote

“**What could be more gratifying than to discover, describe, and explain some basic principle** that no human being has ever understood before? This is the stuff of true science. Those **societies that foster and harness that passion will be the prosperous, knowledge-based economies** of the future.”

The Beginners Guide to Winning the Nobel Prize,
by Peter Doherty

Mary Christopher, DVM, PhD. *Science*
2 September 2015 Vol 7 Issue 303 303fs36

Discussion

- Veterinary and human doctors working together
- Better understanding of natural disease models
- Employing natural disease models in biomedical research
- Better outcomes for human and animal patients

http://vetsites.tufts.edu/rml/

Regenerative Medicine Laboratory

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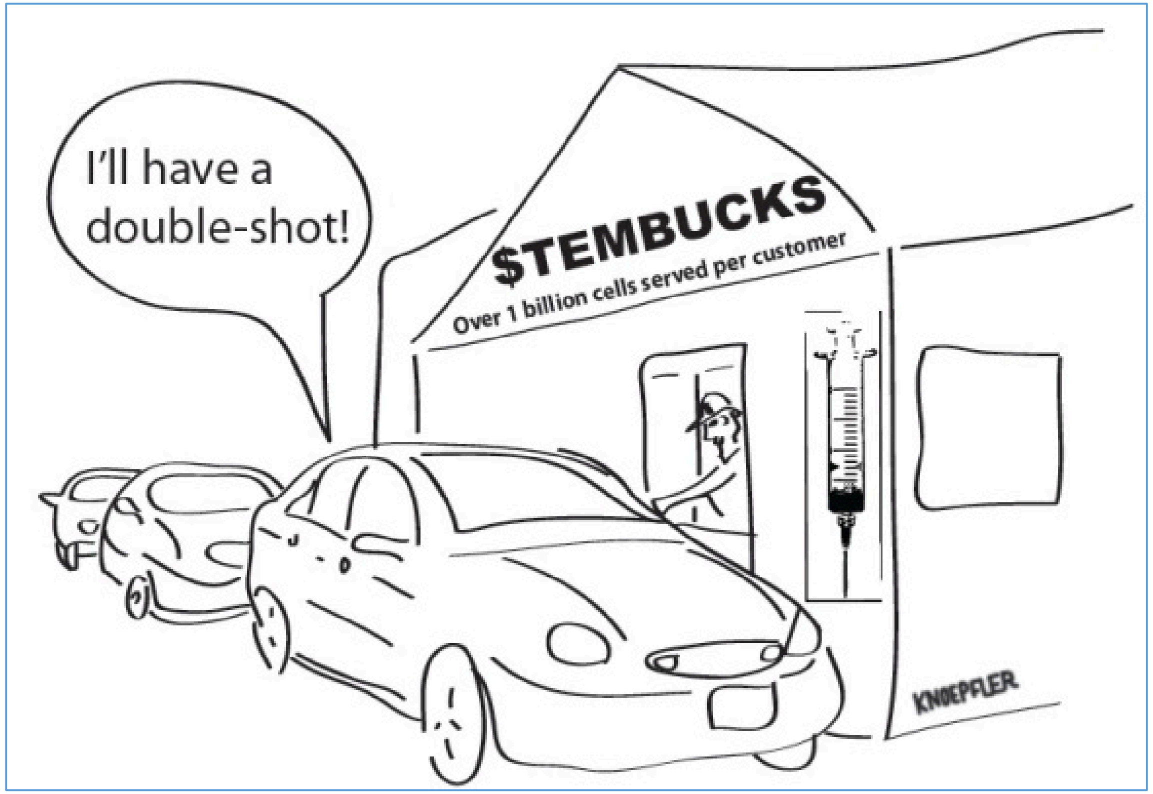
Cummings School Faculty: Drs. Elizabeth Rozanski (ECC), Lluis Ferrer (Derm), Andrea Lam (Derm), Cyndie Webster (GI), Mary Labato (Renal), Dominik Faissler (Neurology), Jennifer Graham (Exotics), Suzanne Cunningham and John Rush (Cardio), Nick Robinson and Arlin Rogers (Pathology)

Collaborators outside Cummings School:

Edward Ingenito (Brigham and Womens)
Dan Weiss (Univ VT, Pulmonary Center)
Alan Fine (BU Medical Center)
Julia Paxson (College of Holy Cross)
Alex Mitsialis (Children's Hospital)
Carla Kim (Childrens, Harvard Stem Cell Inst.)
Robert Lanza (Advanced Cell Technology)
Diana Bianchi (Tufts Medical Center)
Saumya Das, MD, PhD (Beth Israel)
Jason Aliotta (Brown Univ, Rhode Island Hospital)

Questions?



"Bring me a stem cell."