Challenges for Basic and Preclinical Research
or
"10% of The Time it Works Every Time"

With apologies to Ron Burgundy, "Anchorman"

BioCurate
www.biocurate.com.au

SUHF Conference, Karolinska Institutet
October 22, 2019
g.begley@biocurate.com

Agenda

1. Disclosure information
2. Stumbling into Research Reproducibility
3. How Poor Basic Research ("Sick Science") impacts Clinical Medicine
4. Why Do We Have This Problem?
5. What Should We Be Doing?

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1) Disclosure Information

C. Glenn Begley CEO, BioCurate Pty Ltd

Consultant & SAB member for biopharma companies, academic institutions

CSO: 2016 -2017 Akrivea Therapeutics, Thousand Oaks, CA
2012- 2016 TetraLogic Pharmaceuticals, Malvern, PA

Non-Executive Director: 2012-2017 Oxford BioTherapeutics

Consultant: 2012-2017 for 30 start-up biotech, pharma companies

VP and Global Head, Hem/Onc Research, Amgen, Thousand Oaks, CA: 2002- 2012
- Issue of scientific reproducibility highlighted
- Continue to hold stock in these companies

>20 years: Australian physician-scientist
- involved in first identification of human G-CSF,
- first clinical studies of growth factors including “Stem Cell mobilization” with G-CSF

I will not discuss off-label use and/or investigational use of drugs

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2) Stumbling into Research Reproducibility …

Between 2002-2012, Amgen was not able to reproduce the seminal findings from 47of 53 “top tier” publications.

- publications that reported something completely “new”
- 47 findings from 46 different labs: we have a **systemic problem**

The major finding was not reproduced!

In the majority, data was not reproduced by the original investigators with their reagents in their lab

Amgen’s experience is not unique….

Begley and Ellis. Nature (2012) 483: 531

Begley's Position Statement.

- These results do not challenge the validity or legitimacy of the scientific method
- Not talking about fraud: the subject is laziness, sloppiness, ignorance, exaggeration, desperation
- The vast majority of investigators want to do the right thing
- This debate, occurring in public, confirms the strength our scientific system

I am not

- "anti-experimental failure": we learn more from failure than success
- "anti-academia", "anti-University": this is human behavior

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We get what we incentivize

Begley's Position Statement.

- These results do not challenge the validity or legitimacy of the scientific method
- N
- T
- T ystem
-

The advances in medical treatment have been truly outstanding: we have every reason to remain optimistic that research will continue to deliver.

The issue is the "opportunity cost".

This could be solved immediately by Funding Agencies

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We get what we incentivize

Predicting Studies That Will Not Reproduce: High-Profile Studies Typically Fail at Multiple Levels

Begley's six criteria for judging scientific reports:

1) Were studies blinded?
   - Almost never
2) Were all results shown?
   - Typically not
     - "representative examples" & data selection bias
     - western blots that show only a slice; no size markers
3) Were experiments repeated?
   - Typically not
     - westerns/immuno-precipitation usually only performed once
     - use 1/2 RNAis and in 1/2 cell lines
     - confusion between replicates and independent experiments
4) Were positive and negative controls shown?
   - Typically not
5) Were reagents validated?
   - Typically not
     - Lack of specificity IHC with a polyclonal anti-peptide antibodies
     - siRNA and small molecule inhibitors
6) Was the analysis appropriate (e.g. cell growth/statistical tests)?
   - Typically not

Begley. Nature (2013) 497; 433

BE SKEPTICAL

The first principle of science: “that you must not fool yourself, and you are the easiest person to fool”
– Richard Feynman

3) How Poor Basic Research Impacts Clinical Medicine

"Cutting Edge" Stem Cell Therapy for Heart Disease -
a treatment that never was, and should never have been…

Preclinical Rationale 1.

Direct injection of BM stem cells into the myocardium adjacent to infarct

Bone marrow cells regenerate infarcted myocardium

Donald Orlic†, Jan Kajstura*, Stefano Chimenti*, Igor Jakoniuk*, Stacie M. Anderson†, Baosheng Li*, James Pickel‡, Ronald McKay‡, Bernardo Nadal-Ginard*, David M. Bodine†, Annarosa Leri* & Piero Anversa*

** Department of Medicine, New York Medical College, Valhalla, New York 10595, USA*

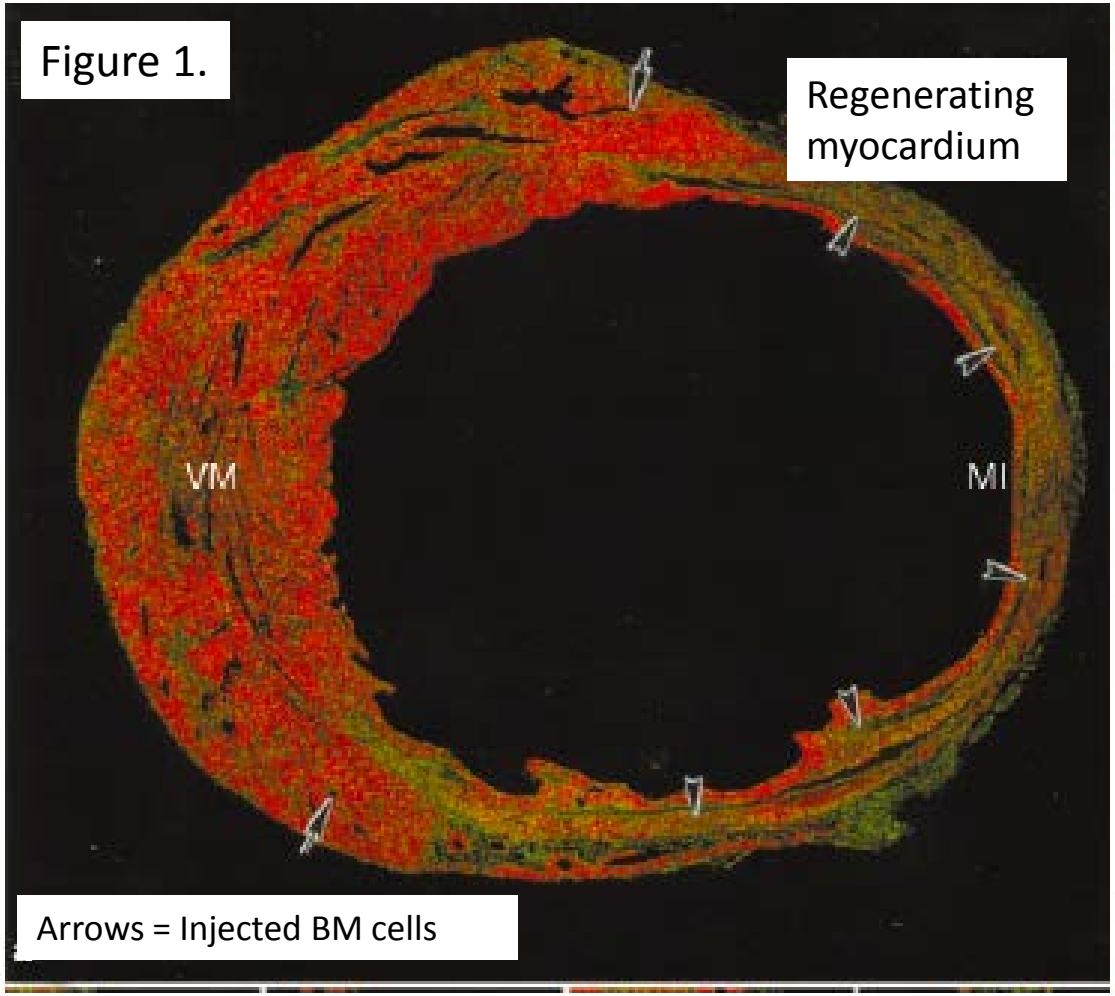
† Hematopoiesis Section, Genetics and Molecular Biology Branch, NHGRI, and ‡ Laboratory of Molecular Biology, NINDS, NIH, Bethesda, Maryland 20892, USA

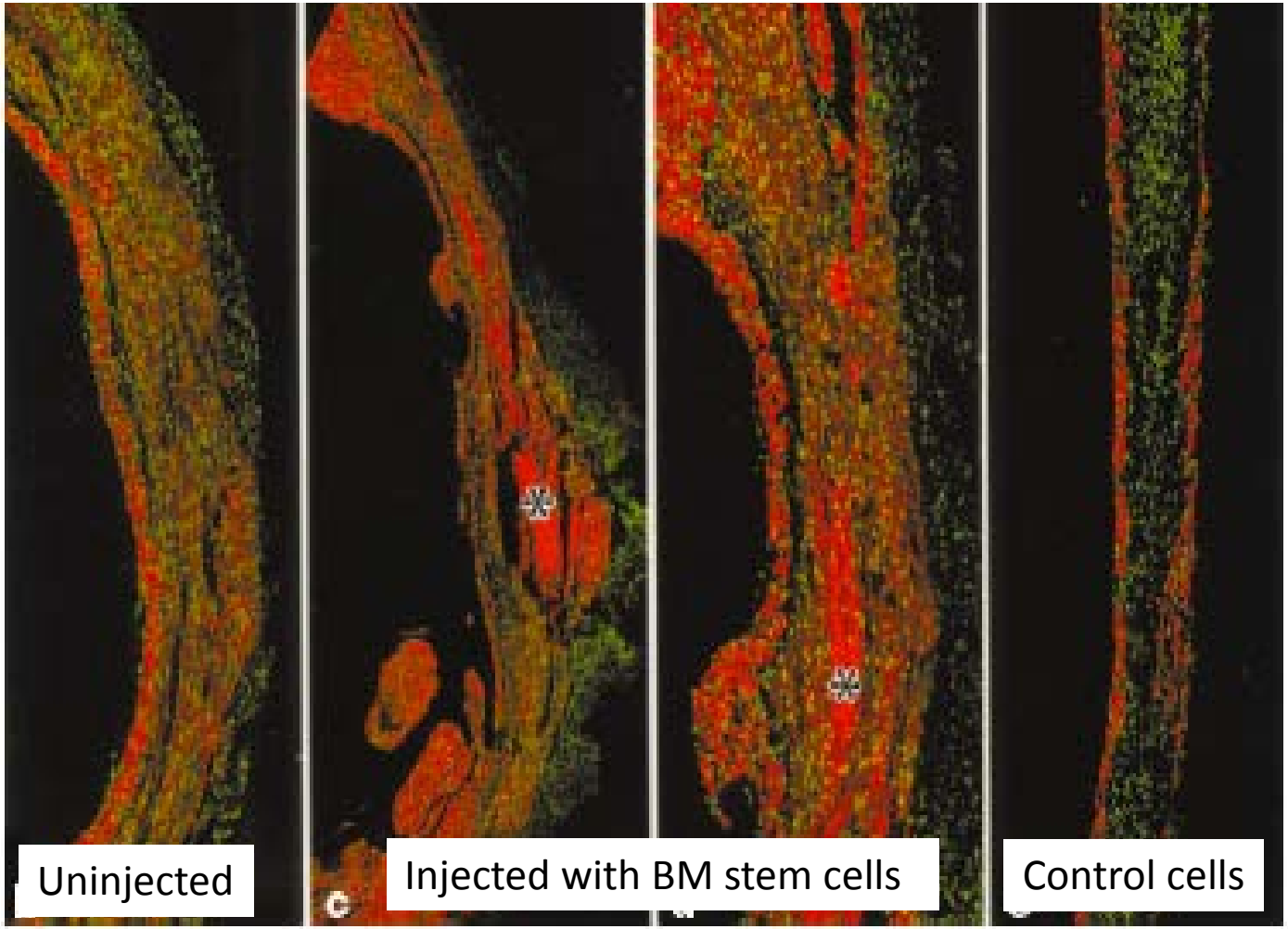
Cited >8,000

Nature 410; 701 (2001)

Preclinical Rationale 1.

Direct injection of BM stem cells into the myocardium adjacent to infarct - mouse model





Red – cardiac myosin. Green = propidium iodide labeled nuclei
Asterisk = necrotic muscle cell

Cited >8,000

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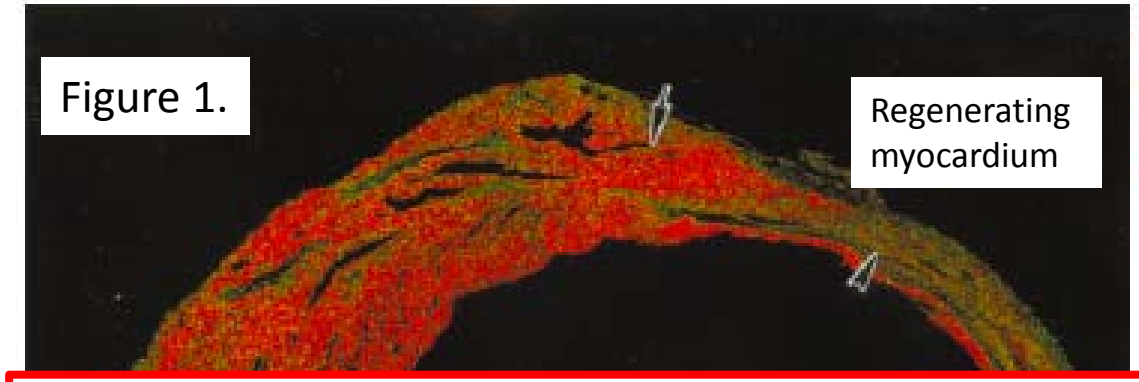
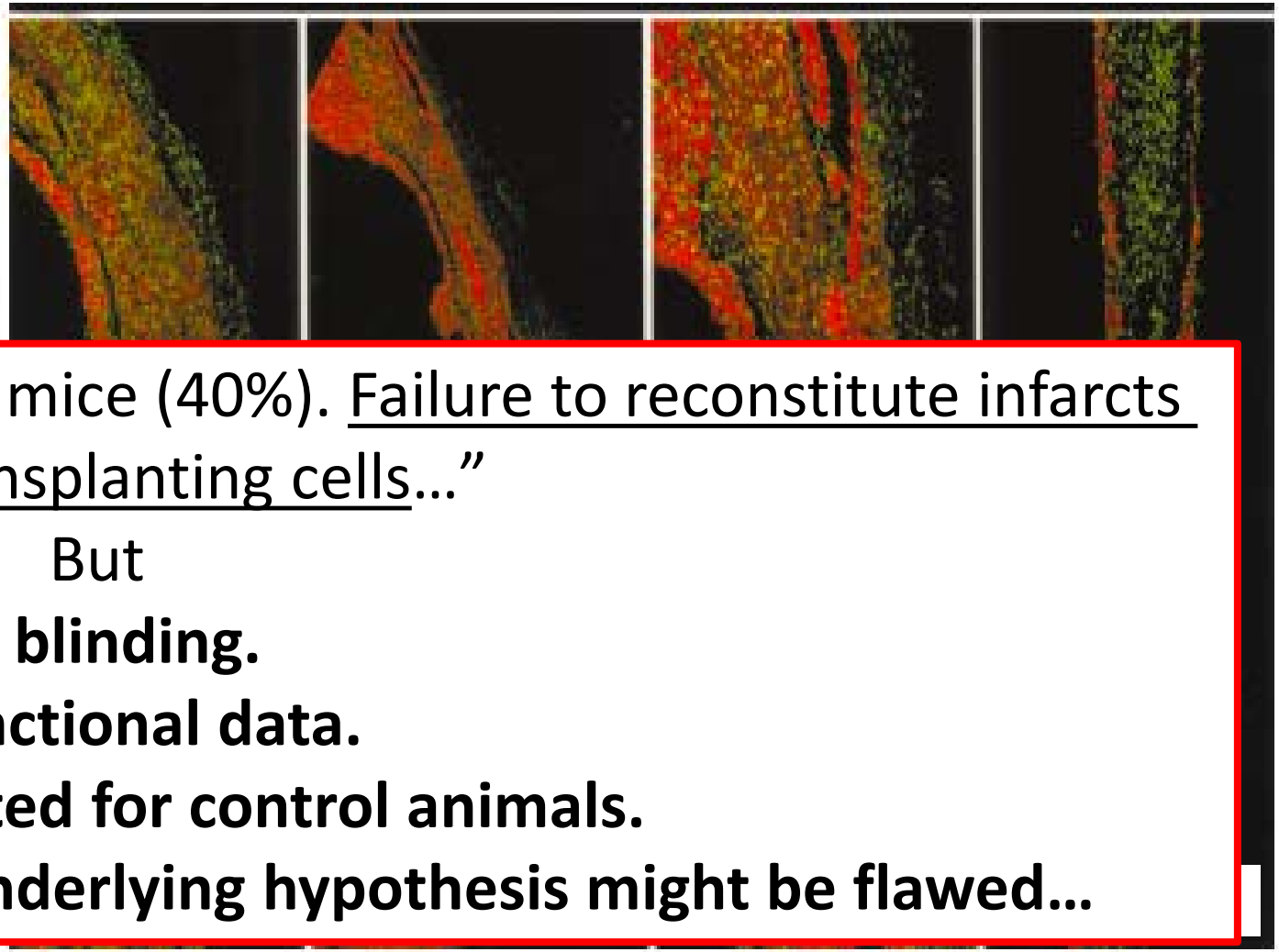




"Repair was obtained in 12 out of 30 mice (40%). Failure to reconstitute infarcts was attributed to the difficulty of transplanting cells…"

But

No blinding.

No functional data.

No data presented for control animals.

Did not contemplate that the underlying hypothesis might be flawed…

Red – cardiac myosin. Green = propidium iodide labeled nuclei
Asterisk = necrotic muscle cell

Cited >8,000

Nature 410; 701 (2001)

Preclinical Rationale 2.

Increased Survival with G-CSF alone - Sufficient to Mobilize & Direct BM cells to Repair the Heart

Mobilized bone marrow cells repair the infarcted heart, improving function and survival

Donald Orlic*, Jan Kajstura†, Stefano Chimenti†, Federica Limana†, Igor Jakoniuk†, Federico Quaini†, Bernardo Nadal-Ginard†, David M. Bodine*, Annarosa Leri†, and Piero Anversa†‡

†Cardiovascular Research Institute, Department of Medicine, New York Medical College, Valhalla, NY 10595; and *Hematopoiesis Section, Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institute of Health, Bethesda, MD 20892

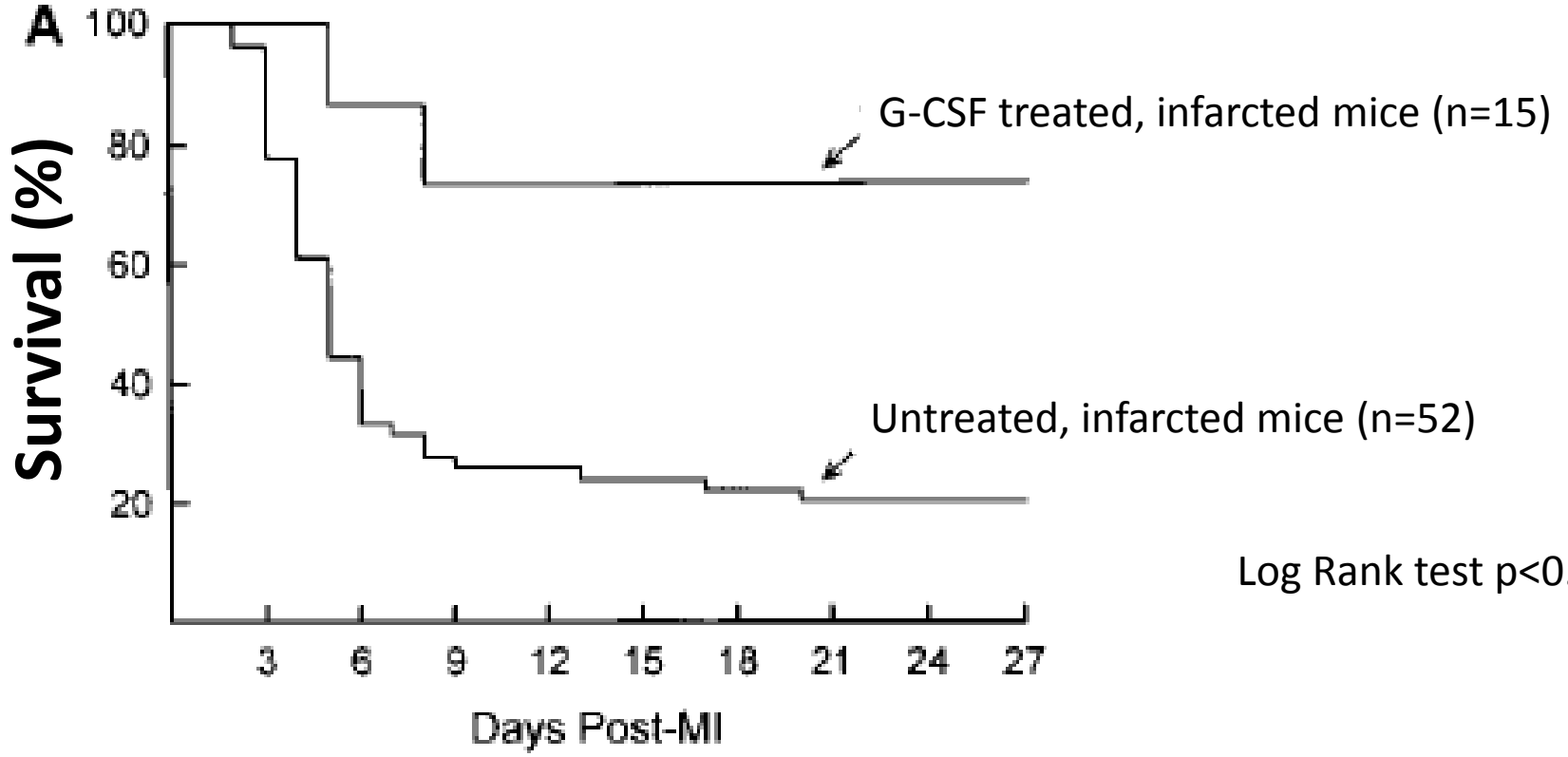
Edited by Eugene Braunwald, Partners HealthCare System, Inc., Boston, MA, and approved June 29, 2001 (received for review April 11, 2001)

Cited ~3,000 times

Preclinical Rationale 2.

Increased Survival with G-CSF alone - Sufficient to Mobilize & Direct BM cells to Repair the Heart

Figure 1A.



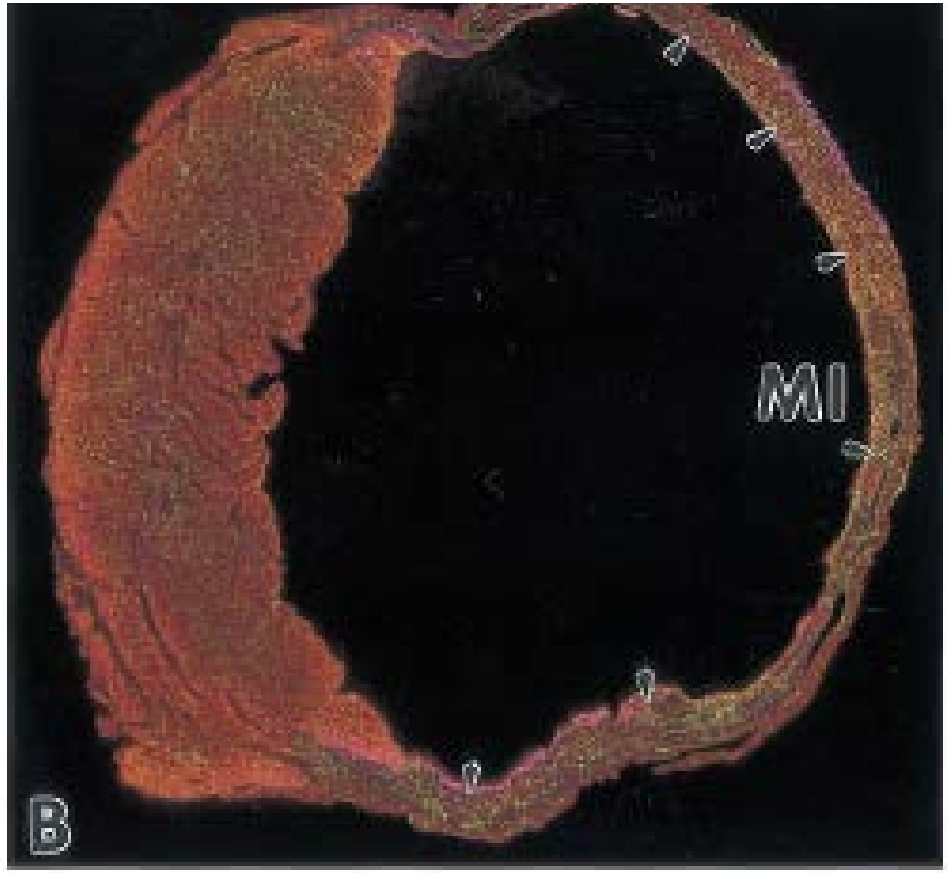
Log Rank test p<0.0001

Cited ~3,000 times

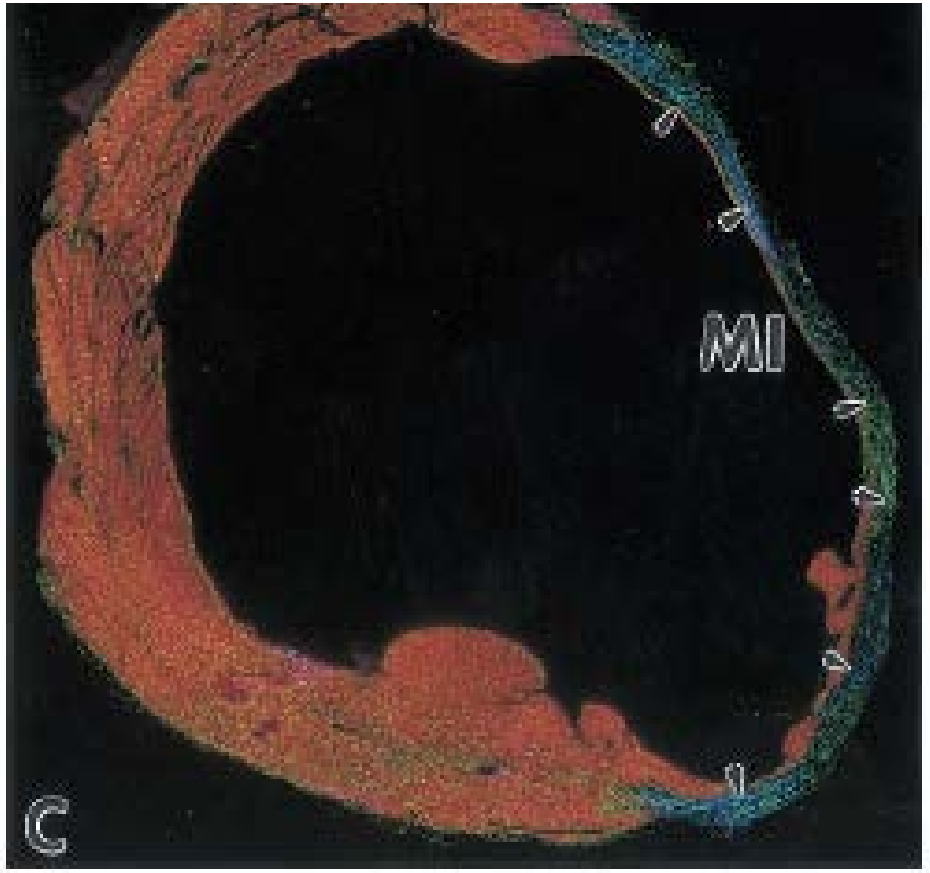
Preclinical Rationale 2.

Increased Survival with G-CSF alone - Sufficient to Mobilize & Direct BM cells to Repair the Heart

Infarct in G-CSF treated mouse shows healing.



Infarct in non-G-CSF treated mouse



Cited ~3,000 times

Forming myocardium shown by arrowheads.
Red = cardiac myosin. Yellow-green = propidium iodide labeling of nuclei. Blue = collagen

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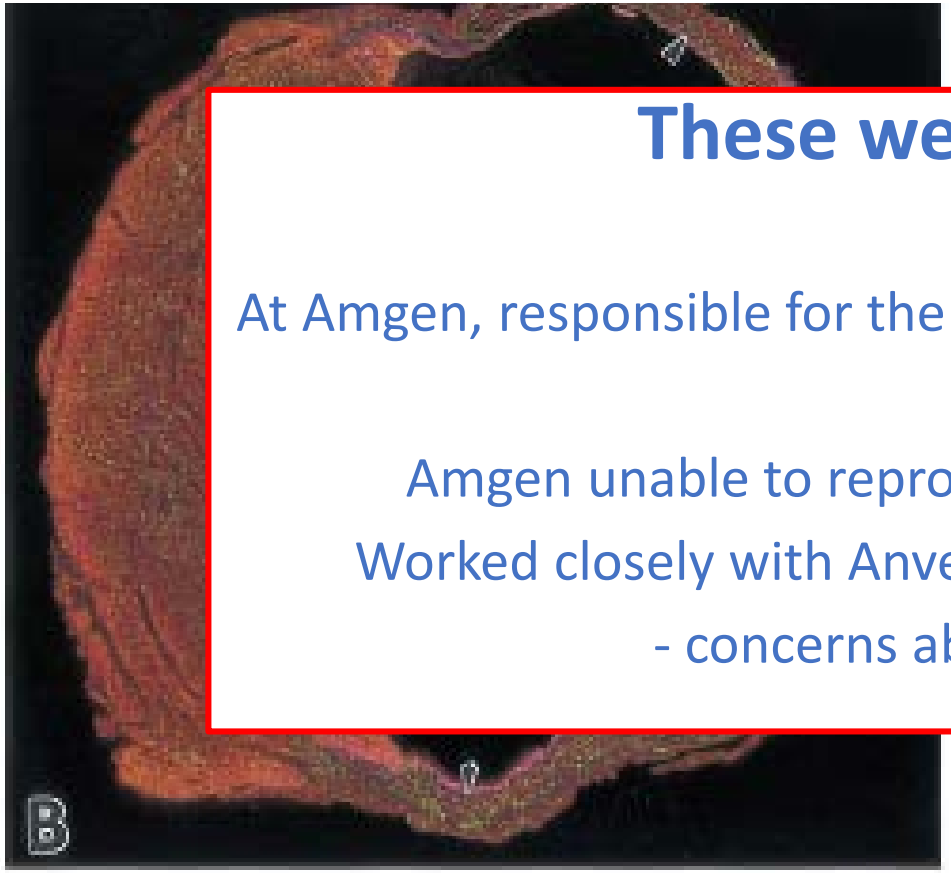

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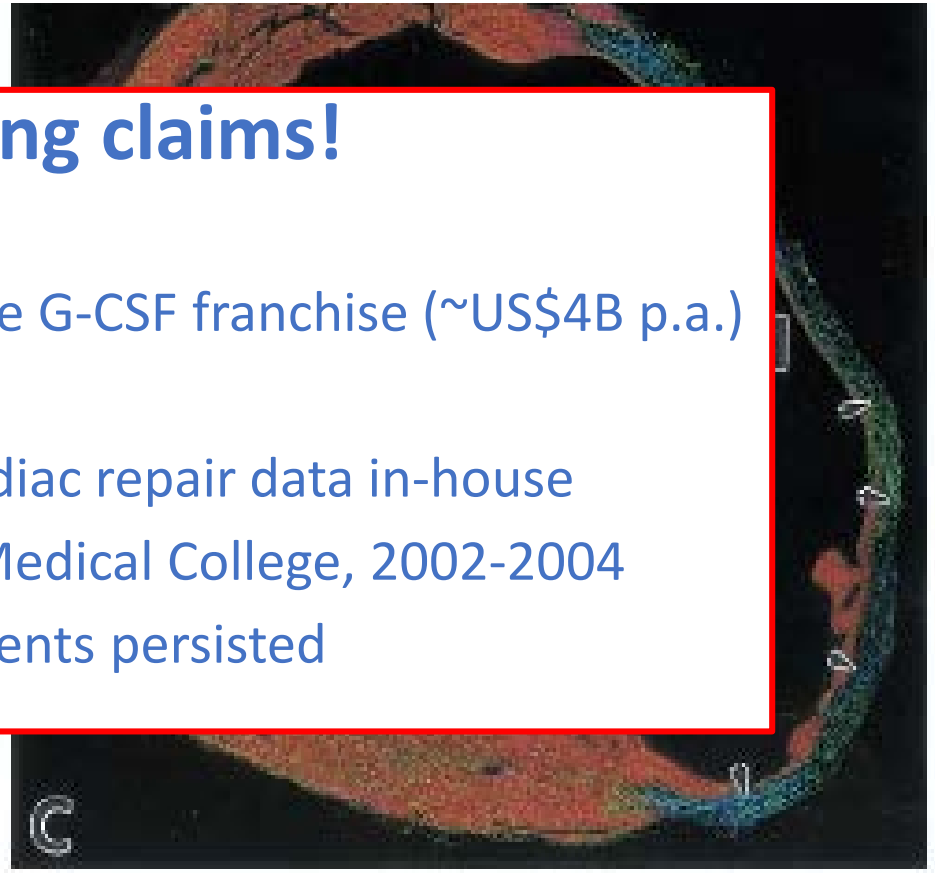


These were stunning claims!

At Amgen, responsible for the science of the G-CSF franchise (~US$4B p.a.)

BUT

Amgen unable to reproduce the cardiac repair data in-house

Worked closely with Anversa lab, NY Medical College, 2002-2004

- concerns about experiments persisted

Cited ~3,000 times

Forming myocardium shown by arrowheads.
Red = cardiac myosin. Yellow-green = propidium iodide labeling of nuclei. Blue = collagen

Preclinical Rationale 3.

Claim that G-CSF acts Directly on Cardiac Cells

G-CSF prevents cardiac remodeling after myocardial infarction by activating the Jak-Stat pathway in cardiomyocytes

Mutsuo Harada[1,4], Yingjie Qin[1,4], Hiroyuki Takano[1,4], Tohru Minamino[1,4], Yunzeng Zou[1], Haruhiro Toko[1], Masashi Ohtsuka[1], Katsuhisa Matsuura[1], Masanori Sano[1], Jun-ichiro Nishi[1], Koji Iwanaga[1], Hiroshi Akazawa[1], Takeshige Kunieda[1], Weidong Zhu[1], Hiroshi Hasegawa[1], Keita Kunisada[2], Toshio Nagai[1], Haruaki Nakaya[3], Keiko Yamauchi-Takihara[2] & Issei Komuro[1]

Key Claims: 1) Here we show that G-CSF acts directly on cardiomyocytes and promotes their survival after myocardial infarction. G-CSF receptor was expressed on cardiomyocytes and G-CSF activated the Jak/Stat pathway in cardiomyocytes.

2) These results suggest that G-CSF promotes survival of cardiac myocytes and prevents left ventricular remodeling after myocardial infarction through the functional communication between cardiomyocytes and noncardiomyocytes.

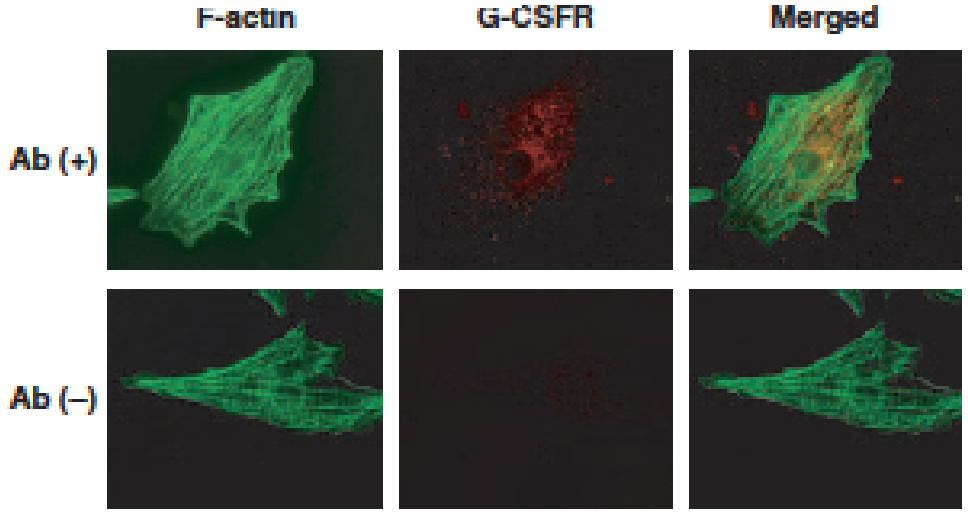
Cited >630

Nat Med 11:305 (2005)

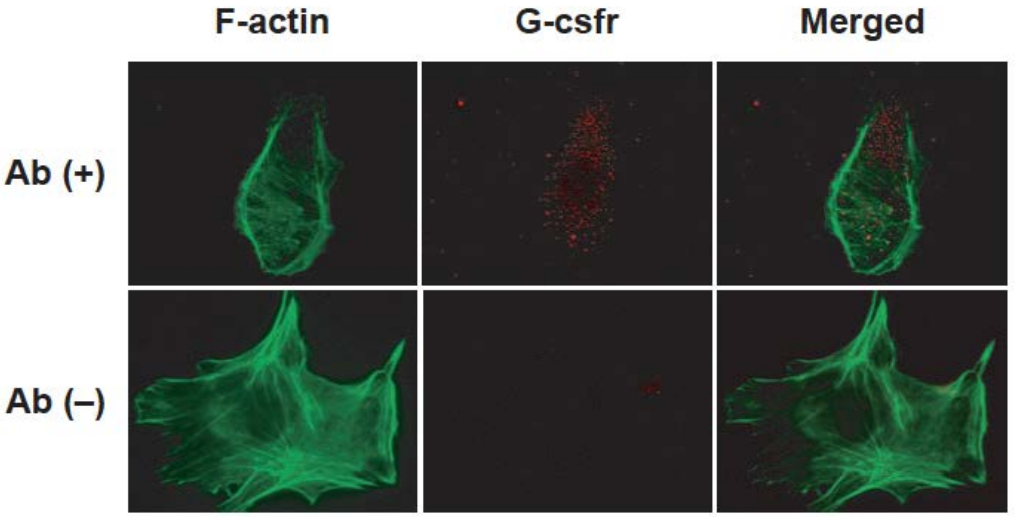
Preclinical Rationale 3.

Claim that G-CSF acts Directly on Cardiac Cells

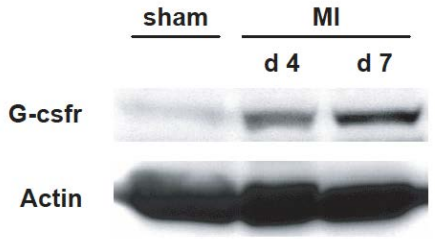
Cardiomyocytes



Cardiac fibroblasts



Harada et al. Used Santa Cruz (SC) "anti-G-CSF Receptor" Ab



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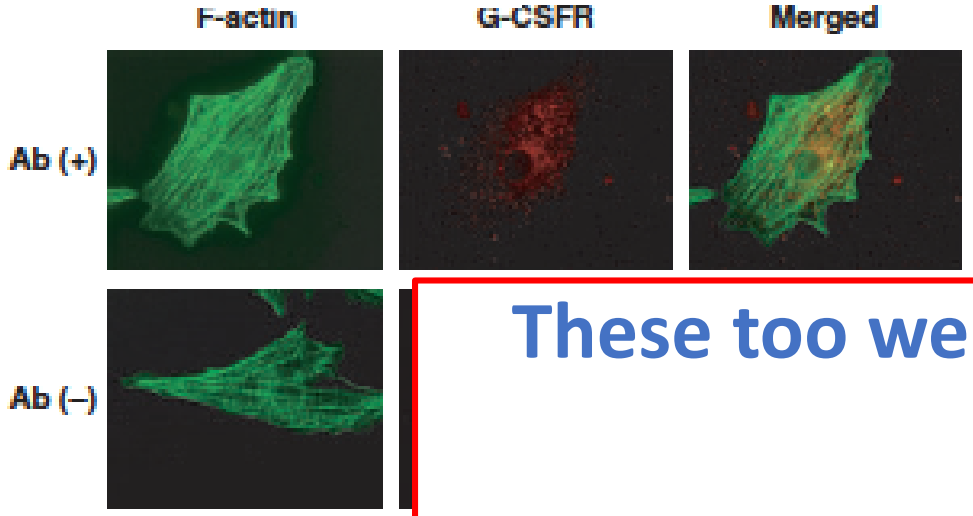

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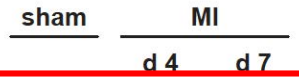
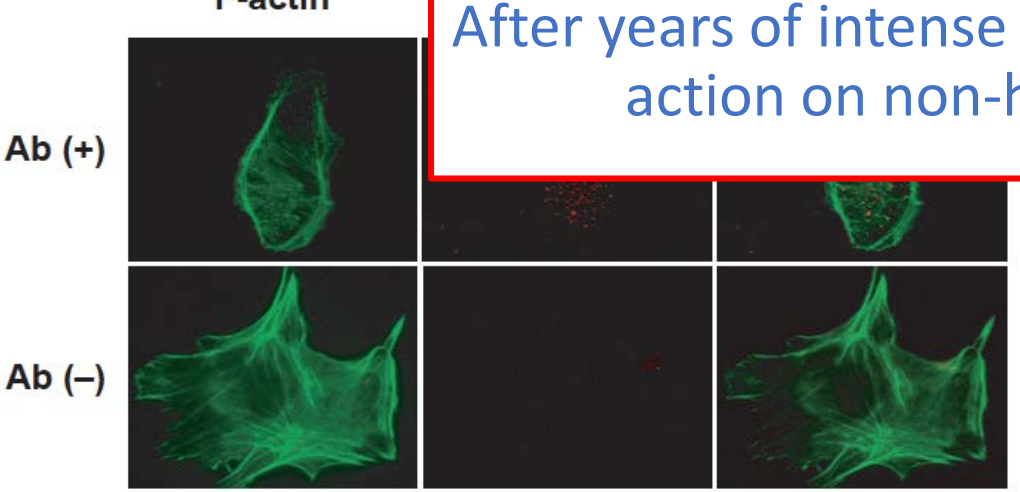


These too were unprecedented, spectacular claims!

After years of intense scientific interest in G-CSF, the claim of an action on non-haemopoietic cells was remarkable.

Cardiac fibroblasts



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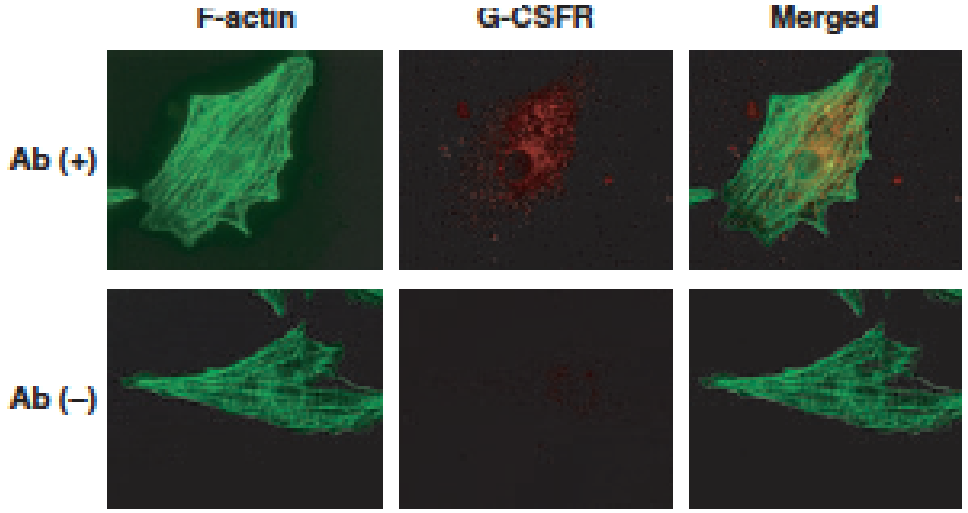

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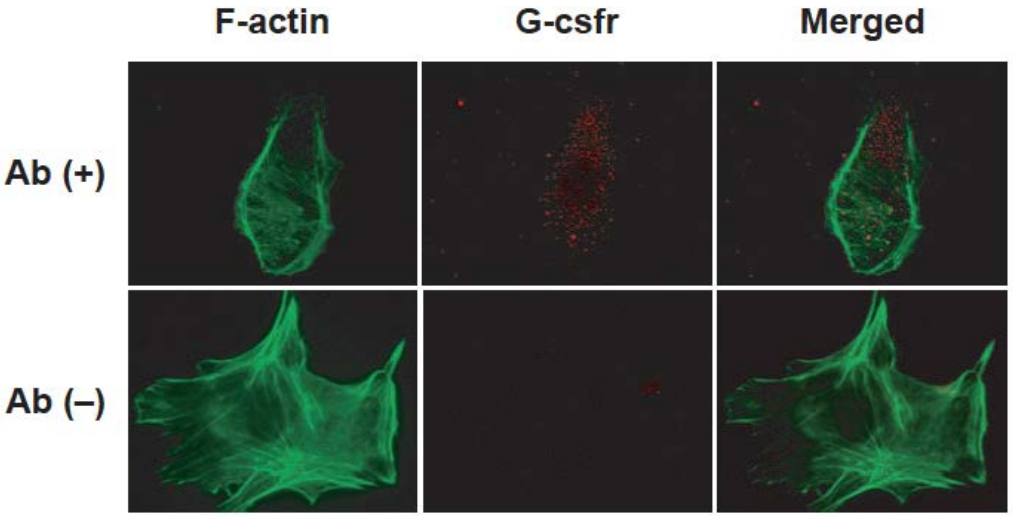

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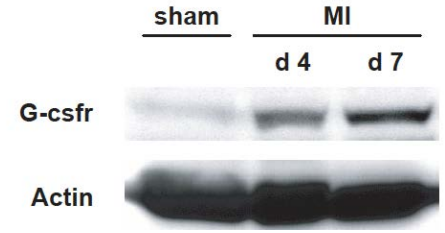
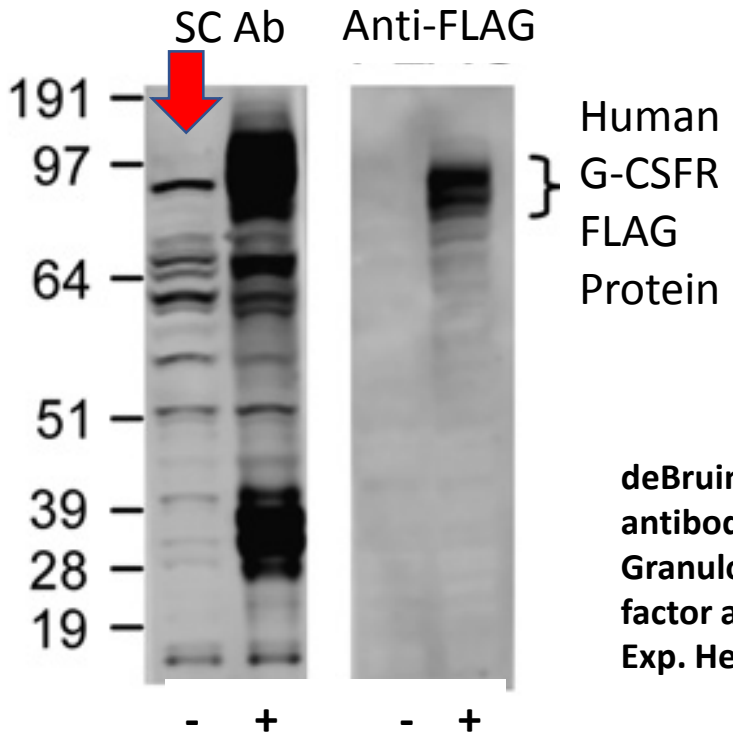


Which band is this?

Versus



deBruin et al. Most purported antibodies to the human Granulocyte colony stimulating factor are not specific. Exp. Hem. 38:1022 (2010)

Preclinical Rationale 4.

The Preclinical Reports Kept Flooding In…….

Approximately 1000 “confirmatory” publications from multiple independent laboratories,

with very few (n=6) papers refuting these findings.

Multiple Clinical Studies Examining Autologous Hematopoietic Stem Cells (ASCs) for Heart Disease.

Country	Pts	Indication	Approach	Status	Report	G-CSF Co.
UK	148	CHF	Intra-coronary vs intra-myocardial G-CSF BM	Completed 2013	No	No
Denmark	48	IHD	G-CSF vs VEGF plasmid	Completed 2015	No	No
Canada	86	AMI	G-CSF vs placebo	Unknown	No	No
Slovenia,USA	110	Cardiomyopathy	Intra-coronary ASCs vs G-CSF	Completed 2013	LVEF ↑	No
Brazil	182	Cardiomyopathy	Intra-coronary vs placebo	Terminated	No	No
Switzerland	50	IHD	G-CSF for 6 months	Completed 2015	No	No
USA	35	IHD	G-CSF	Completed 2005	No	No
USA	150	IHD	Intra-cardiac CD34+ vs placebo	Completed 2009	No	No
UK	20	Cardiomyopathy	Intra-coronary G-CSF BM cells	**Ongoing**	-	No
Slovenia,USA	110	Cardiomyopathy	Intra-coronary vs intra-myocardial ASCs	Completed 2014	No	No
Mexico	10	Paediatric c'pathy	Intra-coronary BM cells	Suspended	No	No

Source: Clinicaltrials.gov

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Source: Clinicaltrials.gov

How Did G-CSF Become a “Cutting-edge Therapy” for Cardiac Disease?

HEALTH

The New York Times

SUBSCRIBE LOG IN

He Promised to Restore Damaged Hearts. Harvard Says His Lab Fabricated Research.

Dr. Piero Anversa, in his home in Manhattan. "I have done nothing to deserve this," he said.

Annie Tritt for The New York Times

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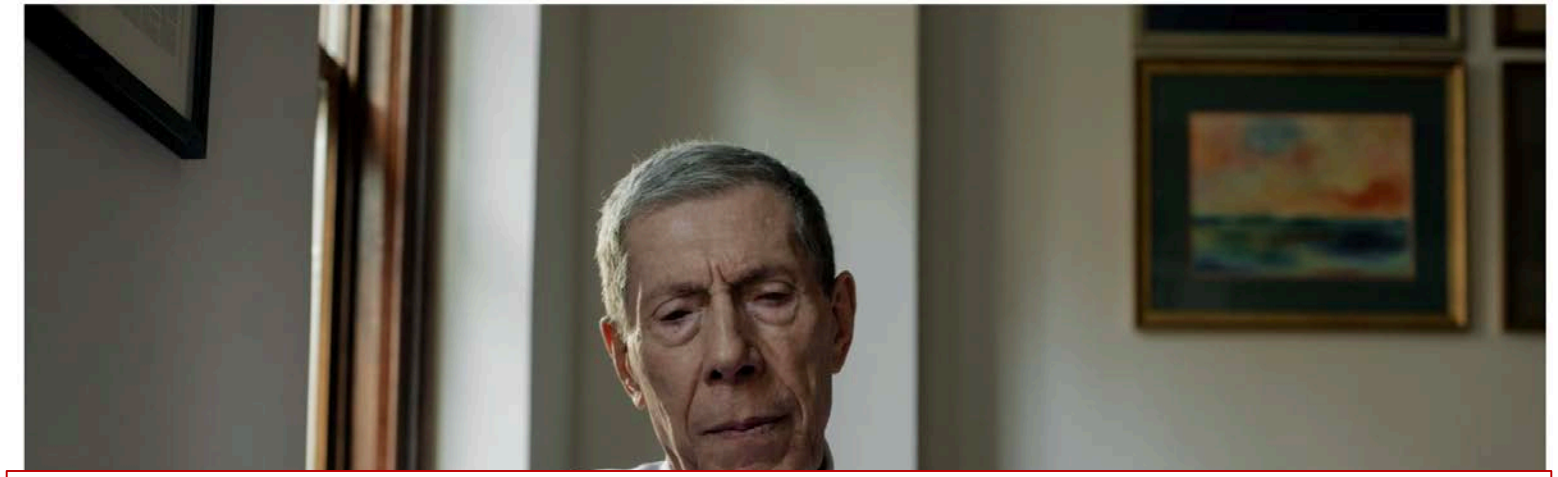

Harvard: Professor Anversa fired
recommends retraction of 31 papers
repays ~$10M to NIH
BUT the original papers still stand

Dr. Piero Anversa, in his home in Manhattan. "I have done nothing to deserve this," he said.

Annie Tritt for The New York Times

Three meta-analyses: no benefit, but did not contemplate that the underlying rationale may be flawed

BE SKEPTICAL

The first principle of science: “that you must not fool yourself, and you are the easiest person to fool”
– Richard Feynman

4) Why Do We Have This Problem? – Perverse Incentives

The Problem:

There is No Metric for Research Quality

Research is judged by (i) Stature of the Journal (Nature, Science, Cell)

(ii) Number of citations:

Medical Research Council (UK) report 2006-2013[1]; 94,000 publications

Average 2.8 citations; "Highly cited" > 4 ; "Very highly cited" > 8

Both based on false assumptions, that Journal & citation are surrogates for quality

In academia

There is no metric for quality

Versus industry

The clinic is unforgiving

Cost of academic research waste in the USA estimated at US$28B p.a.[2]

1. Chapman S, http://theconversation.com/are-citation-rates-the-best-way-to-assess-the-impact-of-research-5464)
2. Freedman et al. PLOS Biology June 2015

The Problem:

The Academic Research Cycle: no metric for quality

This is the metric of Academic success

Behavior is driven by perverse incentives, with few/no negative consequences.

Every scientific paper is undeclared self-promotion, self-advertising.

We get what we incentivize

5) What Should We Be Doing?

We Have a Systemic Problem With Preclinical Research:

Our System Tolerates (Encourages?) Poor Quality Science

The principal responsibility rests with the Investigator and their host Institution

This requires a multi-pronged approach:
Institutions, Funders, Journals, Advocates, Press

Patients expect, and certainly deserve, more

In Conclusion,

This is Not a “Reproducibility Crisis”, it is an “Innovation Opportunity”

Creating a Future For Young Scientists

Begley’s “Robin Hood” Philosophy of Funding Science - Fund Quality not Quantity

Solutions:

As scientists, we could

- Read papers before we cite them
- Refuse to cite papers of poor quality
- Refuse to accept the Journal as any surrogate for quality

  i.e., promotion/grants/hiring/post-doc ‘success’
- Focus on methods rather than the results
- Do things properly ourselves

Journals

- Blinding of reviews by Editors, Reviewers
- Pay reviewers
- Limit publications per scientist (e.g. maximum of 2 publications p.a.)

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Solutions:

Institutions – Demonstrate "Good Institutional Practice" *

- Review of published papers e.g. Scientific "M&M"
- Compulsory, annual methods training for PIs, trainees
- Random reviews of lab note books
- Require Guideline compliance/data sharing
- More realistic/accurate/honest public statements
- More long-term funding

With real consequences
- loss of lab space
- loss of trainees
- loss of grants

Governments & Funding Agencies

- Make funding quality the priority, rather than a "top-tier" Journal
- Demand Good Institutional Practice ("GIP") as a requirement for funding
- Require Licensing of Bio-medical Scientists
- Maximum of 2 publications p.a. – only review 2 most recent publications

Winters et al PLOS ONE June 2019

* Nature (2015) 525: 25-27

In Conclusion,

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Creating a Future For Young Scientists

Begley’s “Robin Hood” Philosophy of Funding Science - Fund Quality not Quantity

Solutions: Consumers, Patient advocates, Press

- Demand quality research

  i.e., experiments that are blinded,
  repeated,
  controlled,
  with validated reagents,
  show all the data;
  use appropriate data analysis

Winters et al PLOS ONE June 2019

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