SINEUPs: antisense long non-coding RNAs as tools to increase protein synthesis

...

Learning from experience

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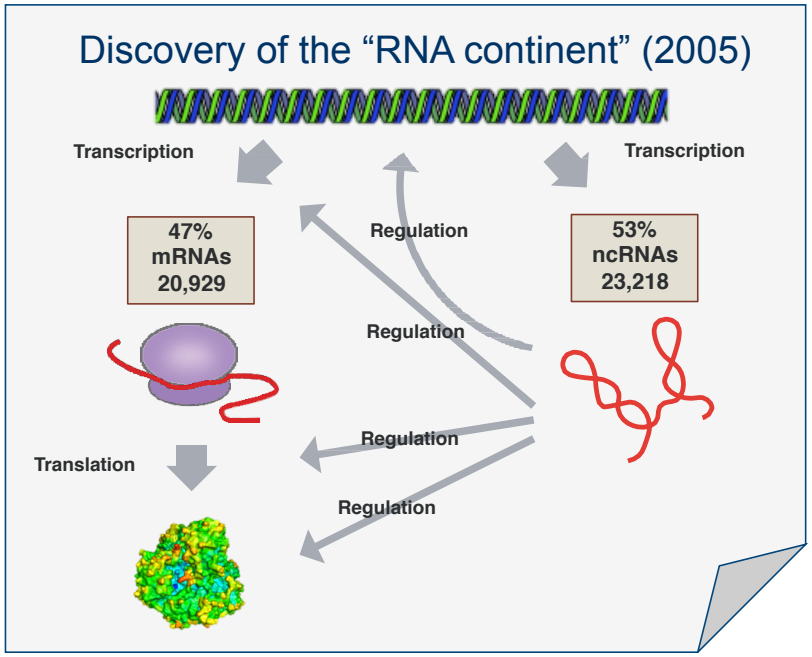
Starting point: basic science

- Exploring what the genome encodes
  - Identification of non-protein coding RNAs
    - Including “antisense” RNA

- (it will be a key point: basic science is needed for innovation
  - There is no true innovation without initial, often unexpected discovery)

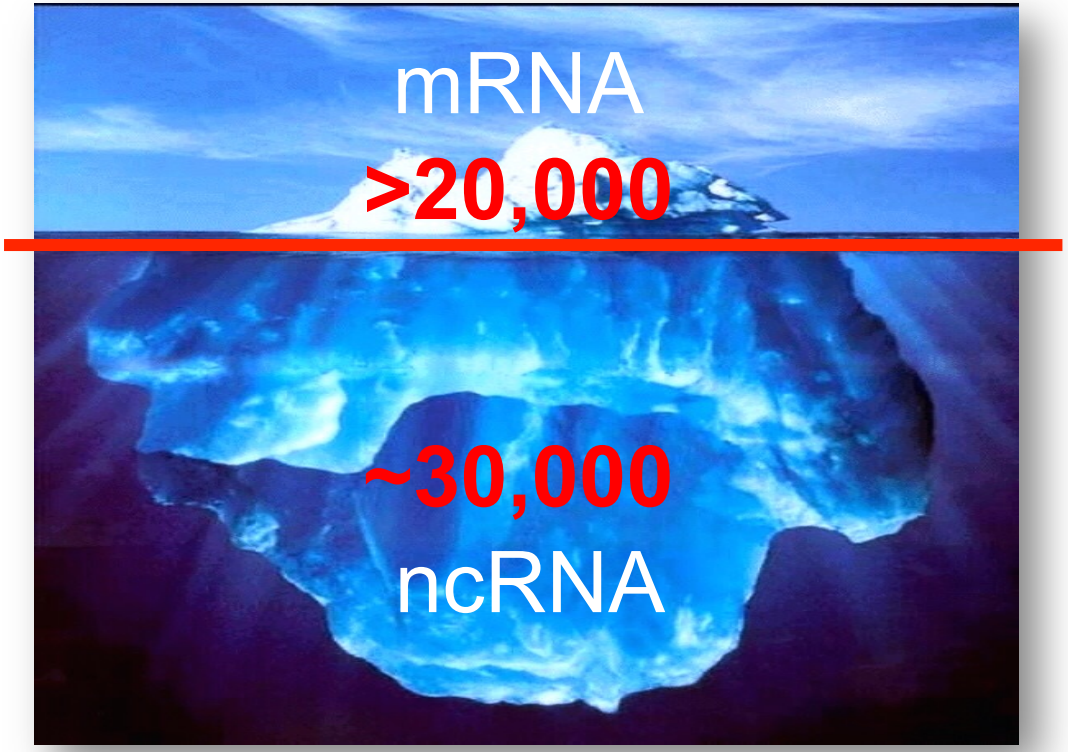
New frontier of ncRNA

Still we know very little



>72.7%

Multiple references in PubMed



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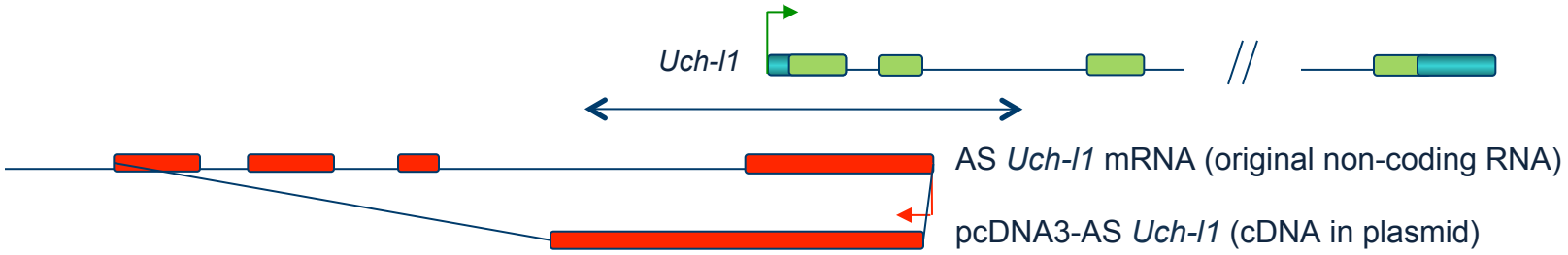
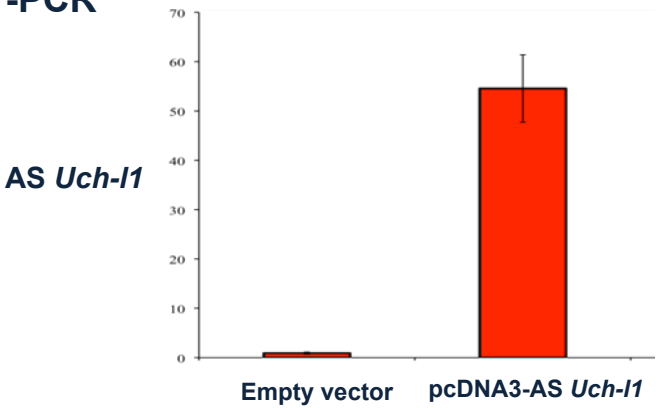
No reference in PubMed

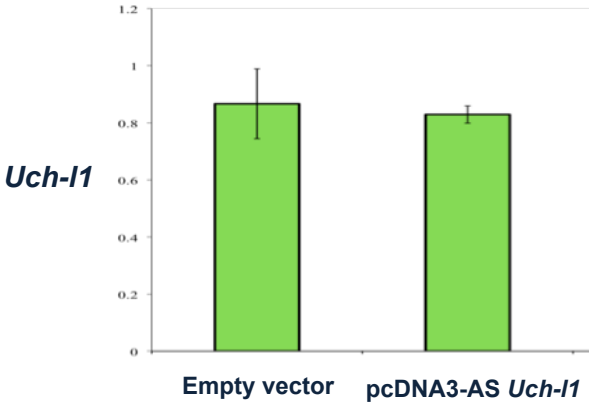
SINEUPs: an important example of antisense RNA function

AS Uch-l1 regulates endogenous UCH-L1 (=PARK5) protein expression

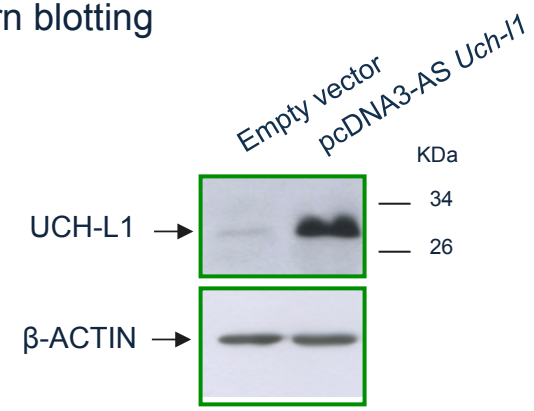


qRT-PCR





Western blotting



No changes at RNA level

Protein level:
dramatically enhanced!!

Carrieri *et al, Nature* 491, 454 (2012)

Definition of SINEUPs

SINE sequence that UP-regulate protein transilation

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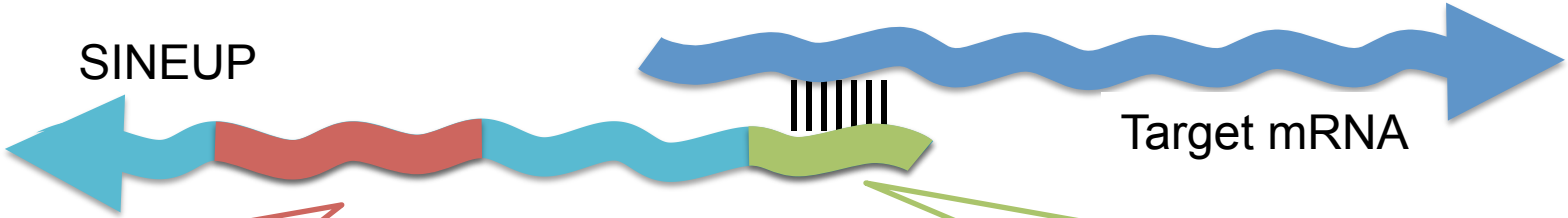
Key features



Effector Domain (ED)

- ✓ Having function to enhance protein translation

Biding Domain (BD)

- ✓ Complementary sequence to a region of target mRNA

- ➢ Customizable design for any proteins
- ➢ Up-regulates protein synthesis 2-5 folds
- ➢ Acts on endogenous mRNA and exogenous targets

Can we use this?

- When “more protein is needed”
- Are there profitable applications of ARTIFICIAL SINEUPS (SINEUPs designed for any mRNA)

Applications of SINEUPs

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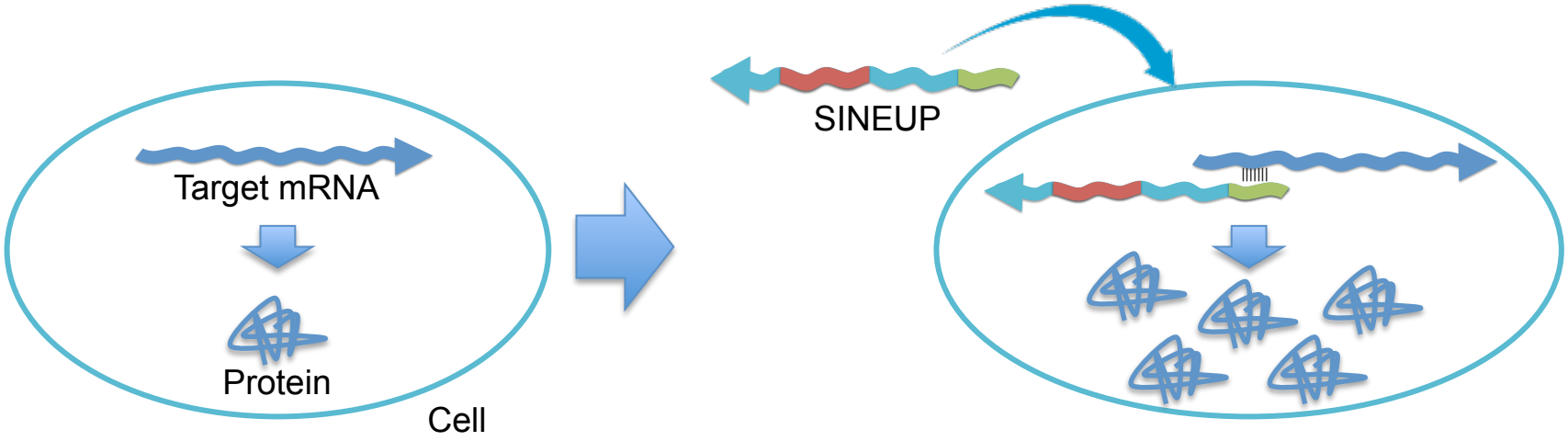
The first & only ncRNA to increase protein synthesis

(*Nature* 491, 454, 2012)

Mostly antisense are known for down-regulation



Biotechnology in Lab

- Counterpart of gene down-regulation technologies
- Boost protein synthesis for crystallography etc.

Bioproduction

- Efficient production of proteins (antibodies etc.)

Therapeutics

- Disorders with low-level proteins (Haploinsufficiency)
  - Neurodegenerative disorders
  - Liver diseases

Advantages: Safe & Site-specific control

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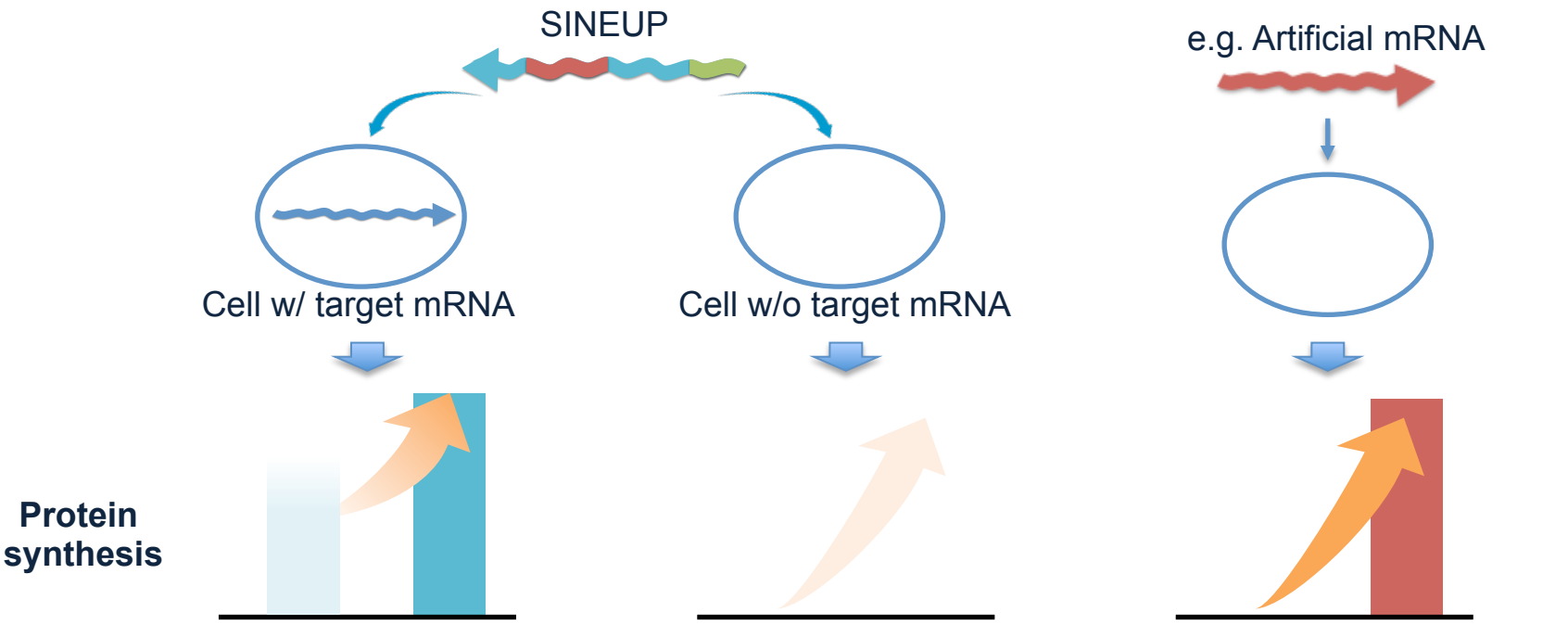
Endogenous mRNA-mediated up-regulation is natural and simply boosts pre-activated mechanisms.



✓ Natural up-regulation
✓ NO unexpected network perturbation

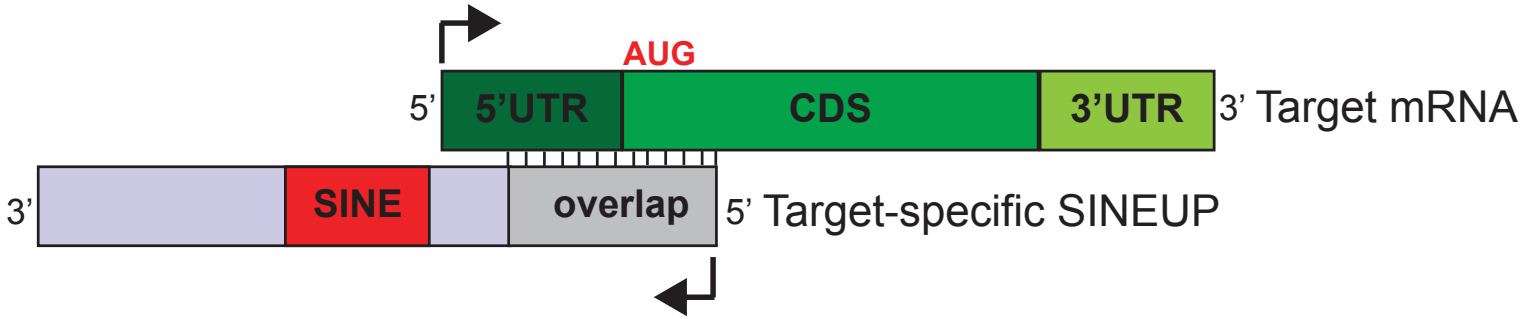
✓ NO effect

✓ Unexpected network perturbation potentially causes adverse reaction

Intellectual property?

- Search shows we are the first and only groups with this technology
- Patent
  - strategies

Intellectual Property part 1



FUNCTIONAL NUCLEIC ACID MOLECULE AND USE THEREOF.
(TransSINE TECHNOLOGIES 50% - SISSA 50%)

- Patent granted in the U.S.A (USPTO 9353370), very broad umbrella patent covering the whole class of SINE and similar molecules
- Patent application in evaluation process in EU (20120763652) and Japan (2014-514921)

Exclusive License to TransSINE TECHNOLOGIES of 50% owned by SISSA

- for the whole duration of the patent

When to start a company?

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- Be sure about technology
- Make some sort of plan
  - … it never works as you planned
- Define the missions
  - Develop the platform for making SINEUPs a strong technology
    - Much more than a “patent”
  - Prove this is useful

- Ready to go… GO!

TransSINE TECHNOLOGIES

Founded back in 2011; a RIKEN Venture company

Dr. Piero Carninci

Deputy Director of the RIKEN Center for Integrative Medical Sciences
Founder of TransSINE Technologies (2011) and Representative Director.
Consulting and Scientific Advisory board position of DNAform Inc.
Inventor of more than 30 patents (including cDNA cloning, CAGE Technologies, and SINEUPs)
Publication record: published more than 300 papers, *h*-index=99, (>70,000 citations).
Main awards: Biotec Award (2001), Yamazaki-Teichi Award (2007), Chen Award (2014), Shimadzu award (2016) and others.

Prof. Stefano Gustincich

Deputy Director for Technologies for Life Science, Director of the Department of Neuroscience and Brain Technologies, IIT, Genova, Italy
Full Professor, SISSA, Trieste, Italy
Founder, TransSINE Technologies Inc. (since 2011)
Founder, PARKscreen s.r.l. (since 2011)
Inventor of SINEUPs
Publication records: He is author of ~100 scientific publications in peer-reviewed journals, with an *h*-index of 38 (~12000 citations)
Main award: Italian Innovation Prize for Biotechnology (2011)

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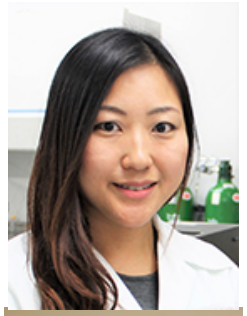
Constructing the team



Dr. Hazuki Takahashi
Reseach and development, patenting,
Research scientist @RIKEN, Yokohama

Dr. Michael Jones
Head Licensing, commercial, company dev. strategies
CellGS CEO, Cambridge (England)

Dr. Silvia Zucchelli
Head Research & Development
Assistant Professor, Universita' Piemonte Orientale, Novara (Italy)
Visiting Scientist, SISSA, Trieste, Italy

Prof. Claudio Santoro
Advisor in recombinant antibodies, general advisor
Full Professor, Universita' Piemonte Orientale, Novara (Italy)

SINEUPs technology

Syntetic, against any target

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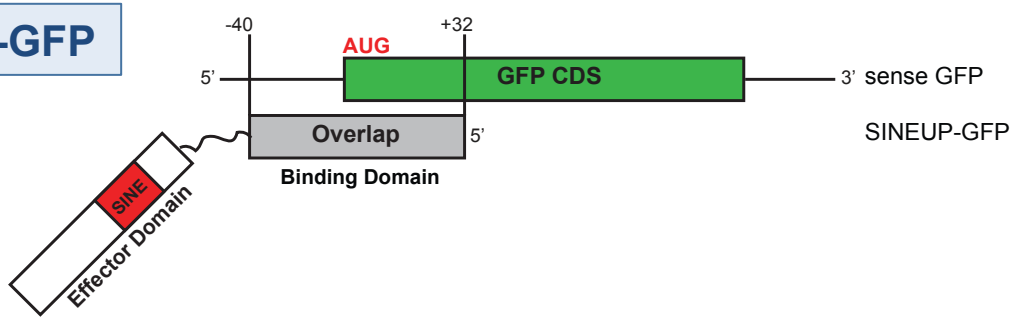
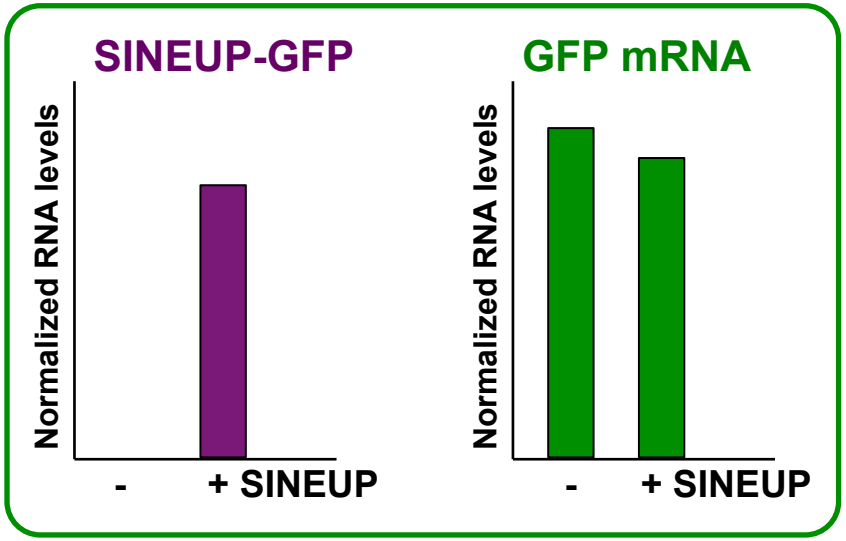
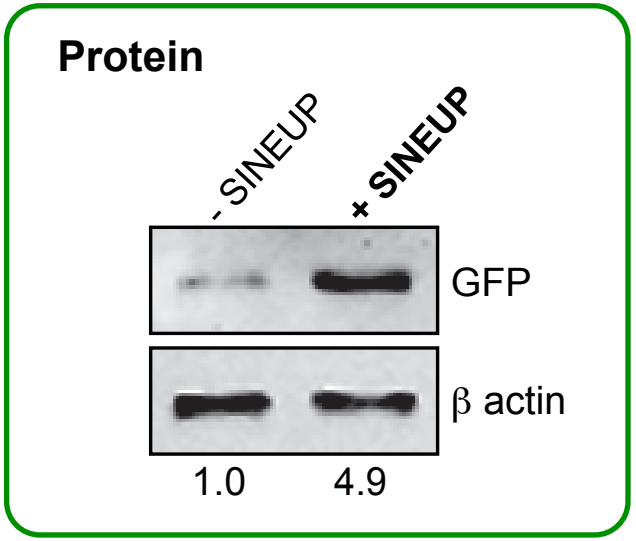
Synthetic SINEUPs: SINEUP-GFP



Unchanged GFP mRNA



Increased GFP PROTEIN



Carrieri C. *et al.*, Nature, 2012

Zucchelli S.,* Fasolo F.* *et al.*, Front Cell Neurosci 2015

Synthetic SINEUPs for RNA therapy for Haploinsufficiencies

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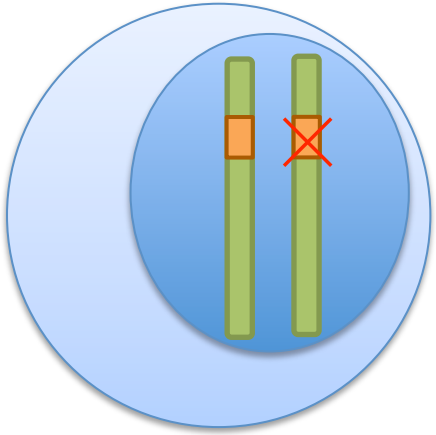
At least 300 Haploinsufficiency genes are known
(Dang *et al*., European Journal of Human Genetics, DOI: 10.1038/ejhg.2008.111, 2008)



Not enough protein → Disease/ Pathogenesis

+ SINEUP

Recover the protein level

Healthy/Remission

- ✓ SINEUP acts on a specific mRNA isoform
- ✓ No uncontrolled ectopic effect
- ✓ Increase at physiological levels

SINEUPs as Therapeutic Tools

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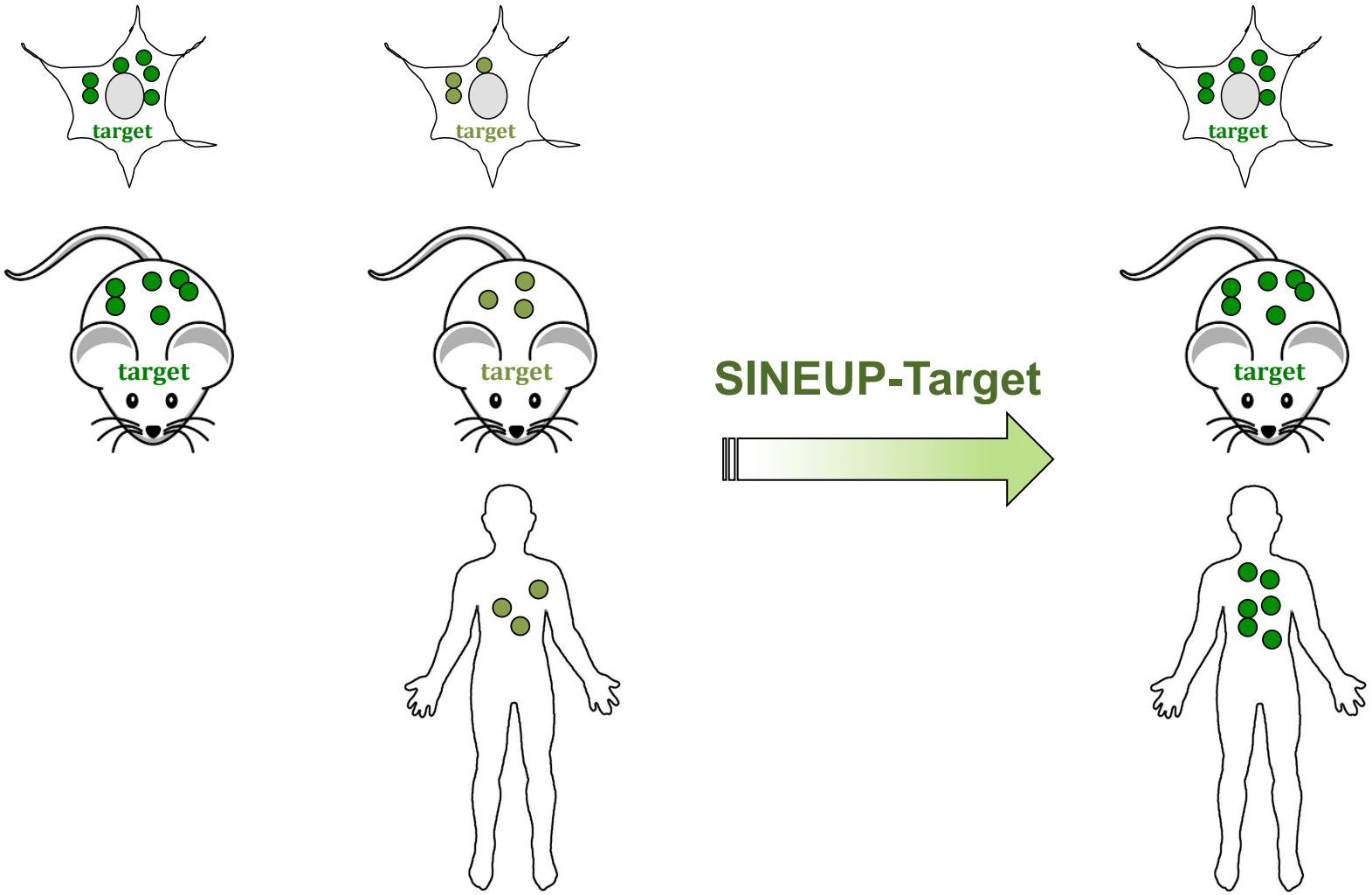

Healthy

Haploinsufficiencies
Or condition that require more protein

Therapeutic SINEUPs

SINEUPs: *in vivo* model of human diseases in Medaka

Microphtalmia with Linear Skin Lesions (MLS)

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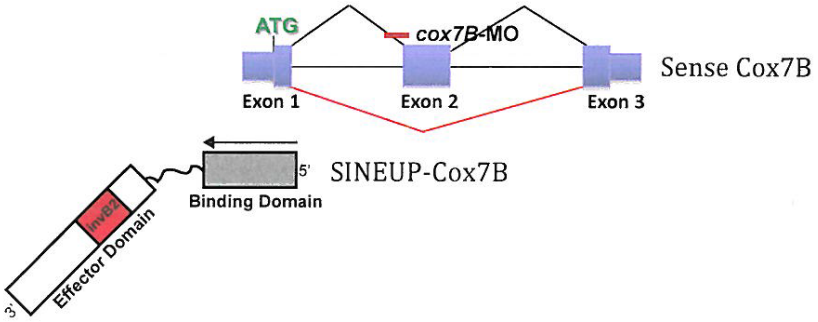
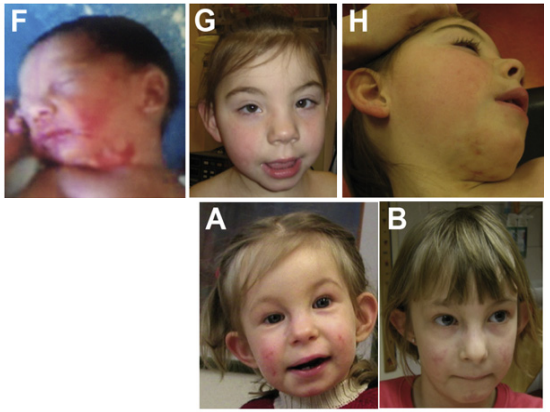
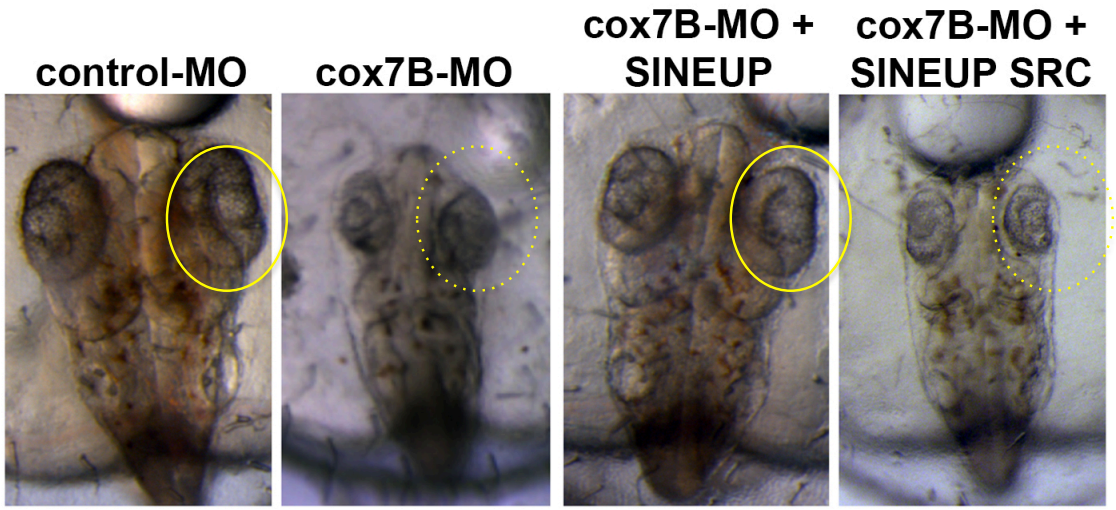
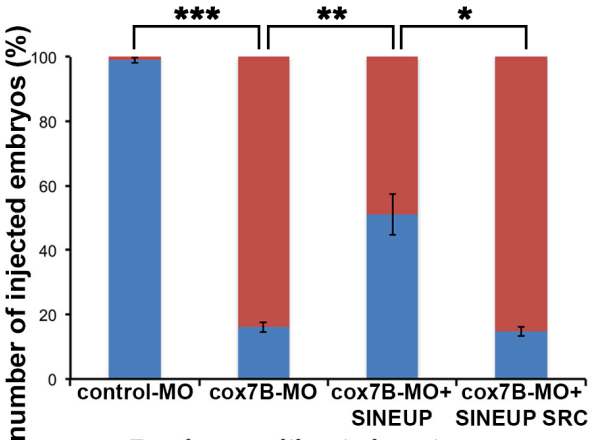
SINEUP-Cox7B







Embryo:	WT	MLS	MLS + SINEUP	MLS + ctrl
Eye:	Normal	Diseased	Normal	Diseased



Indrieri A. *et al.,* Scientific Reports, 2016

Yes it works, let's make a big company!!

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- Fishes are not humans
  - Make the POC with a mammalian (mouse)
- Let's raise money!
- **But… "it's too early!"**
  - Must provide **evidence** before we invest!

VC: Difference in attitudes between Japan and USA

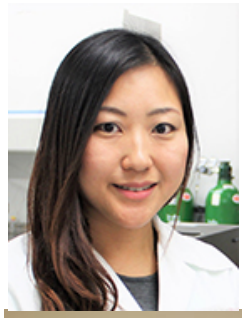
Japan is very hard for startups and new ideas

Japan	USA (and few others)
"It's impossible", especially without evidence	"It's interesting: let's invest to produce the evidence"
Few venture capitalists; people with salary-man experience but without experience as VC.	Many venture capitalists, specialized in high risk/high return investment. Losing it all acceptable, but aiming at few strong return of investments.
Hands-off / return (return required, of not risk taker fails, no next chance)	Hands-on / support
The system to grow corporate venture capital (CVC) is immature. Sometimes the company acquires IP from the external startup company in CVC in early phase, no growth outside as Biotech. Project stopped to early because treated as a corporation project	VC: evaluated also for the number of outstanding people they have raised and the experienced created. Failure acceptable, this is experience. Do what works, if not change. Biotech companies are independent.

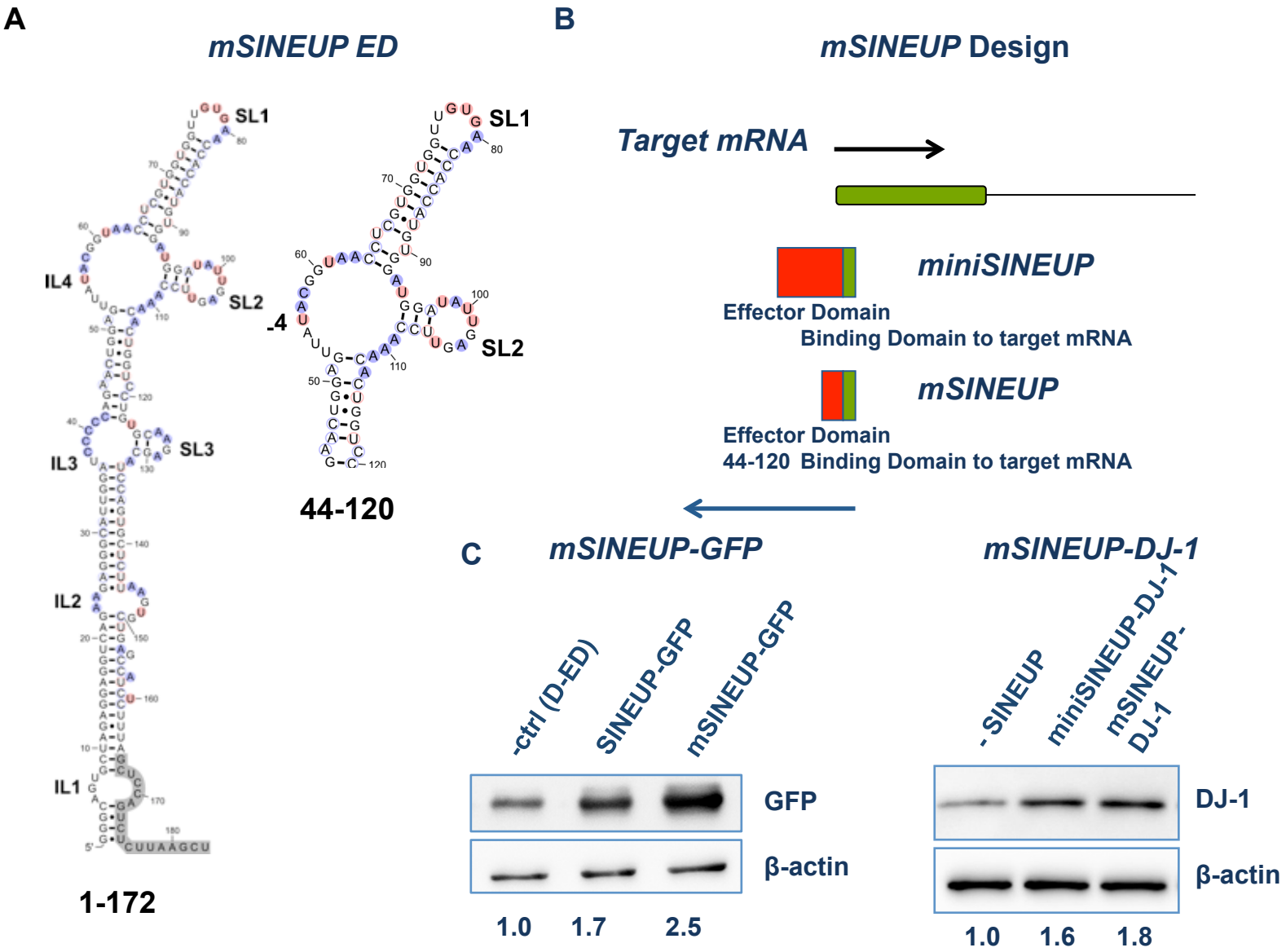
Summarized and adapted from the presentation by Kiichi Kubota, PeptiDream Inc. at BioPharma Expo 2018

Hmmmm…

- **Use our labs as much as possible**
  - Grants for innovations and anything
- Reinforce **international collaborations**
  - Work as a global team
  - Nights/early morning calls, trips, personnel exchange
- Try anything possible to **survive**
  - Sale reagents as well
  - No salaries but stock options

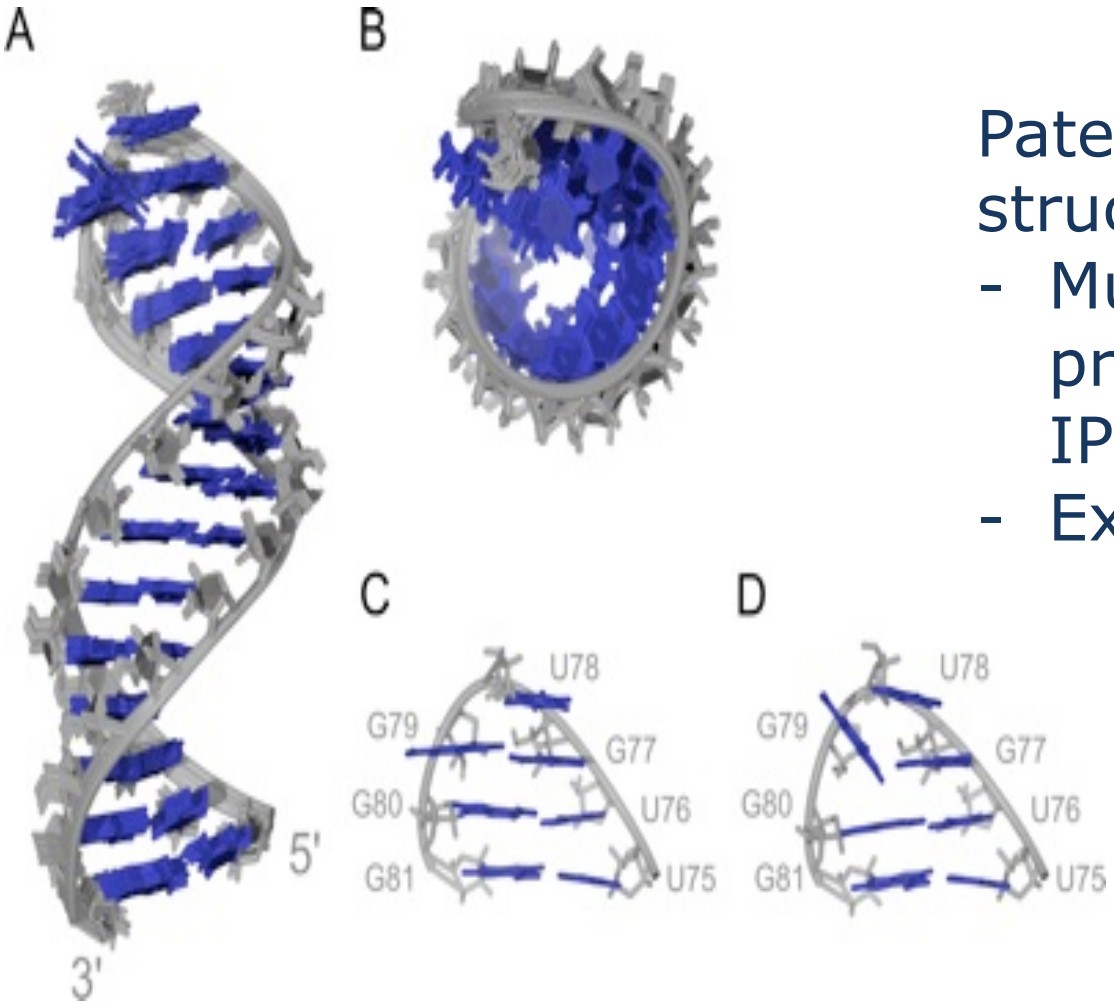



SINEUPs: development of more active motifs



Podbevšek *et al.*, Sci Rep 2018

High-resolution structure of SL1

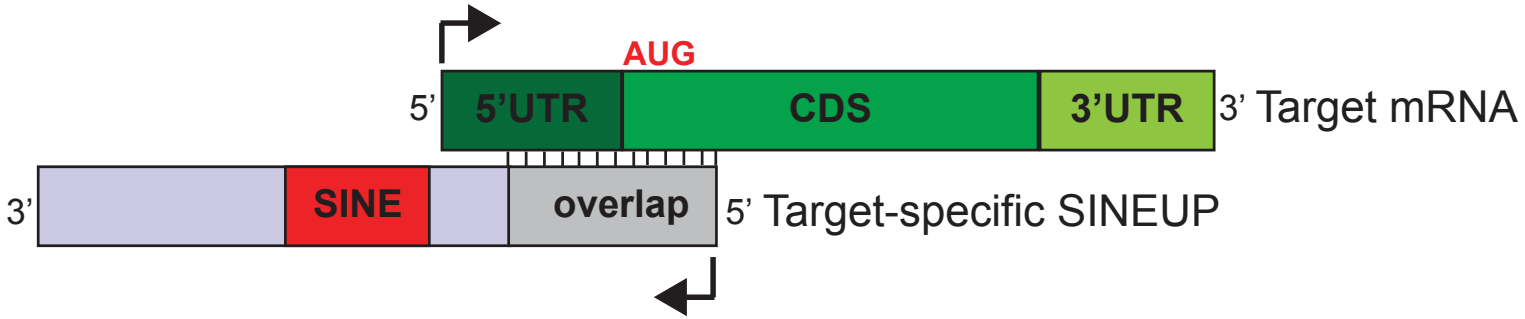


Patenting new structures.

- Multiple barriers to protect the dominant IP position
- Extend protection

Podbevšek, et al., Scientific Reports, 2018

Intellectual Property - 2



STRUCTURAL DOMAINS OF ANTISENSE RNA MOLECULES UP-REGULATING TRANSLATION.
SISSA & TransSINE Technologies

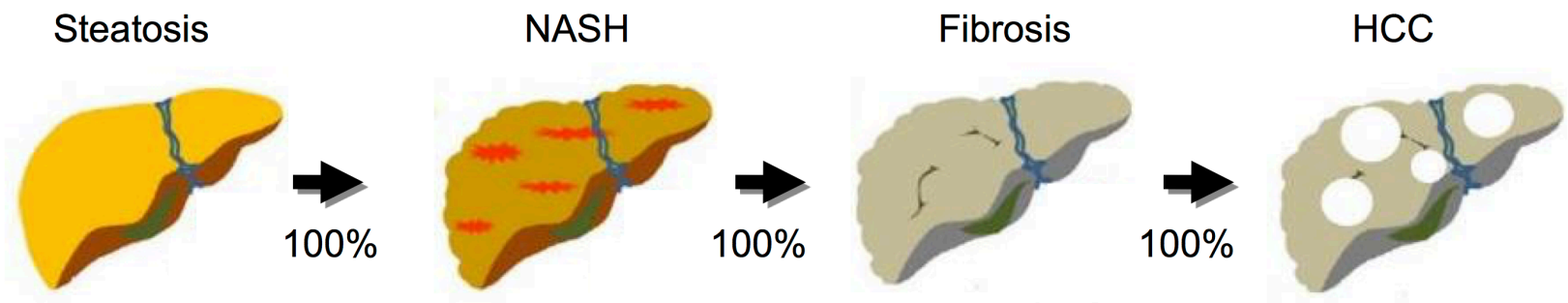
Patent Application in Italy - Serial No. 102018000002368. (05/02/2018).

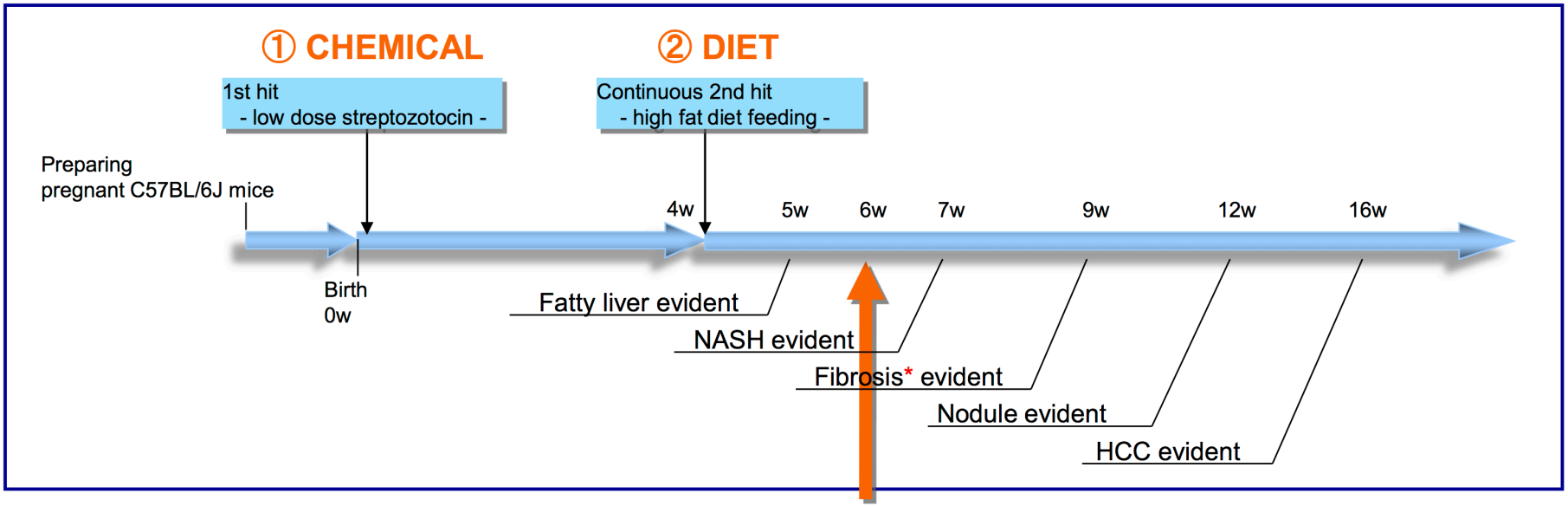
Exclusive License to TransSINE TECHNOLOGIES from co-owner (SISSA) for the whole duration of the patent

POC in an animal model

- Needed as POC
- Liver easy for delivery
- Some disease need action on transcription regulation
- Hnf4a is a strong gene target for SINEUPs
  - (Resetting the transcription factor network reverses terminal chronic hepatic failure; Nishikawa et al 2016; http://dx.doi.org/10.1172/JCI73137)

STAM™: In vivo predictive pharmacology model





All mice at 6 weeks of age meet "baseline" criteria as in the case of clinical trial in human

Fatty change (+)
ALT↑
NAFLD Activity score↑

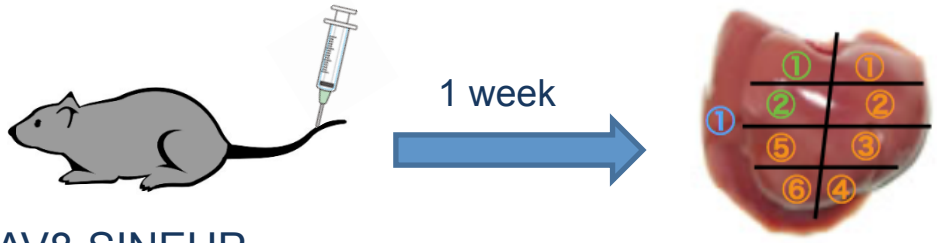
* *Perisinusoidal fibrosis rese*

AMED

SINEUPs: *in vivo* activity

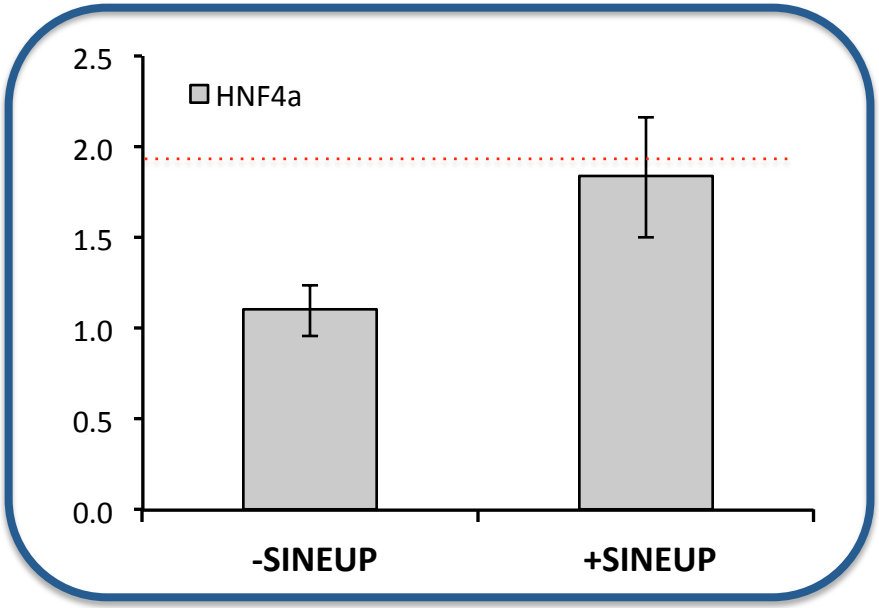
SINEUP-HNF4a

Liver therapy



AAV8-SINEUP-HNF4a injection

Liver collection/ protein analysis



PROTEIN

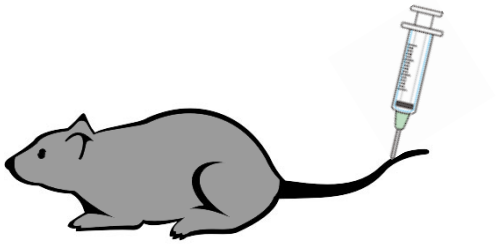
=

mRNA

SINEUP for HNF4a as therapeutic strategy for Non Alcoholic Fatty Liver Disease (NASH)

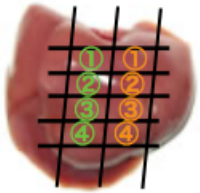
Nitta K., *et al*., RIKEN, unpublished

Flow of *in vivo* experiments



AAV8 virus infection
(Systemic injection)
C57BL/6J (8 weeks)
Titer: 4 x 10^{12} vg

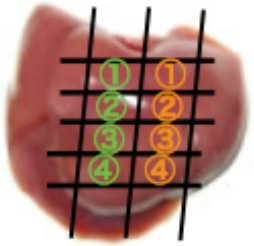
1 week

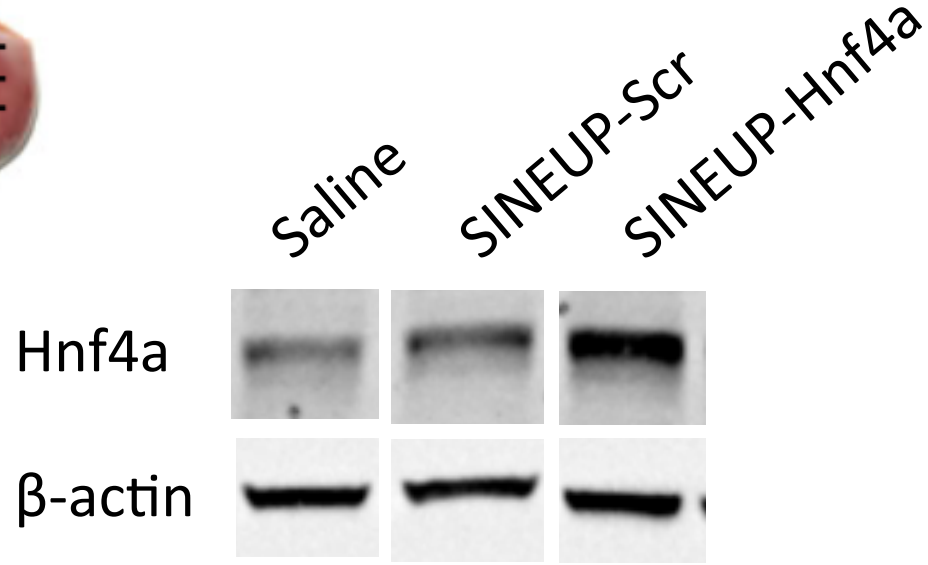


Liver sampling

Western blotting
qPCR (DNA, RNA)
Transcriptome analysis

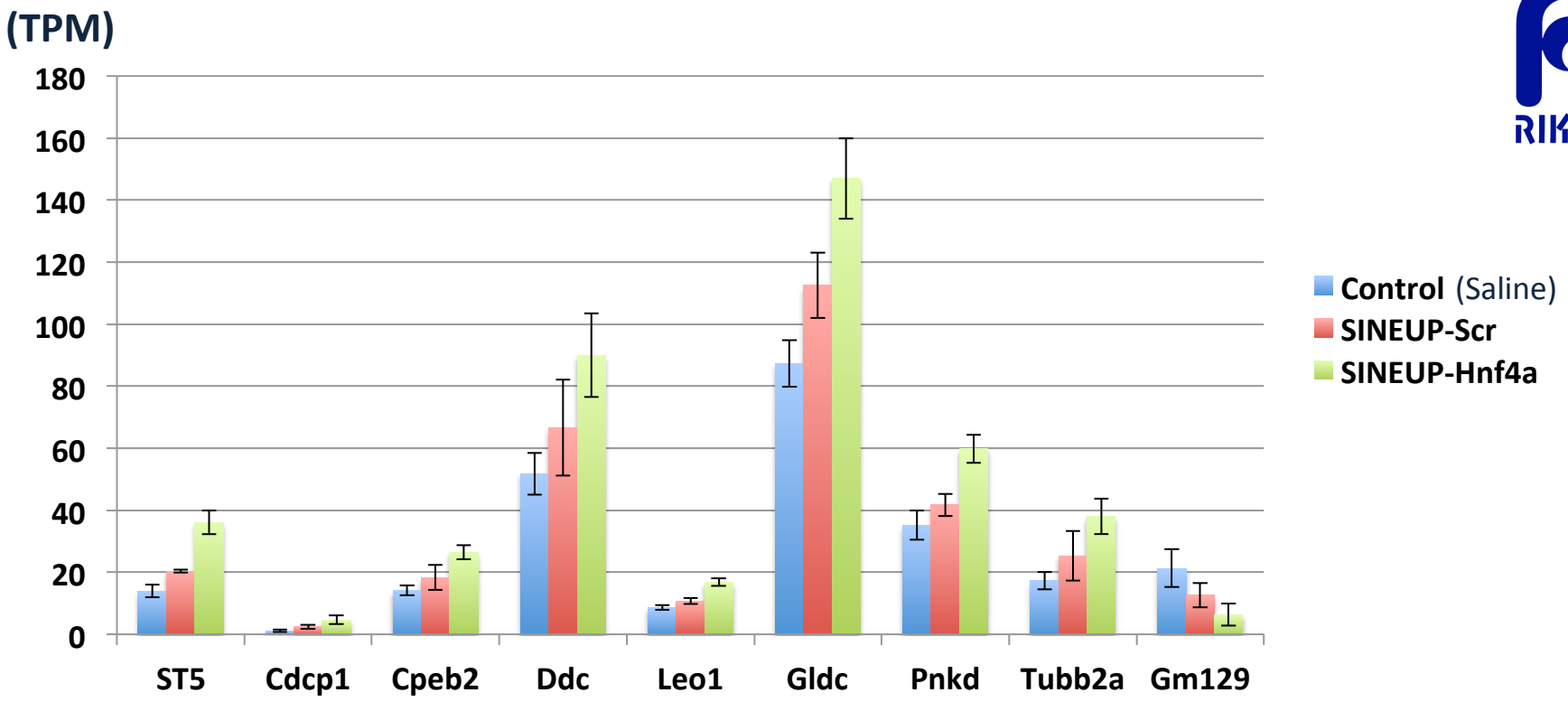
SINEUP-Hnf4a is functional in mouse liver





>1.7 folds up-regulation!

Genes downstream to HNF4a are positively regulated!



- ST5 is directly regulated by Hnf4a (Intron seq has TFBS)
- ST5 is ERK regulator in β-cell in pancreas (Gupta et al., 2007)

SINEUP-Hnf4a enhances Hnf4a translation *in vivo*

We have the POC!

- (much more data; other evidence by the collaborators at SISSA and IIT not shown)
- Who will talk with us?

TransSINE Technologies discussing investments from overseas VC

Getting VC investment on (seed money; 5-7M Pounds) based on results so far, including strong IP and collaborative work also coordinated by TT.

- TransSINE Technologies partnering with TT in UK.
- TV investment committee analysis successful (Boston, September 7 2018).
- Finalizing the seed investments on
  - Platform development
  - Preclinical development of 3 targets (still confidential)

Competitors

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- **Moderna**
  -> Technology: modified mRNA therapy (Refs)
  -> Deals: raised >1.4 billion $

- **Opko-CURNA**
  -> Technology: Antisense lncRNAs targeting ASOs (Nature Biotechnology, 2012)
  -> Deals: Opko acquisition of CuRNA (10 million $ upfront)

- **Ionis Pharmaceuticals**
  -> Technology: uORF targeting ASOs (Nature Biotechnology, 2016)
  -> Deals: GSK (others?)

- **MiNA Therapeutics**
  -> Technology: saRNAs (Hepathology, 2014)
  -> Deals: ?

Goals

Develop SINEUP potential and transfer applications to the society

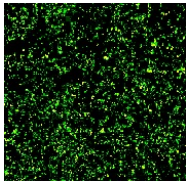
Patenting

JP/US/EU Patents

Patenting

RIKEN

Perform SINEUP experiments with animal model/HT screening



Showcase of SINEUP potentials

R&D Commissions

Licensing or IP acquisition

Buy out or Licensing

Customers

(Companies / Academia)

TransSINE TECHNOLOGIES

Develop SINEUPs pipeline for Pharma/Biotech targets

Acknowledgement

- Funding
  - Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan
  - Japan Agency for Medical Research and Development (AMED)

- Other key colleagues
  - Kazuhiro Nitta, RIKEN
  - Harshita Sharma, RIKEN