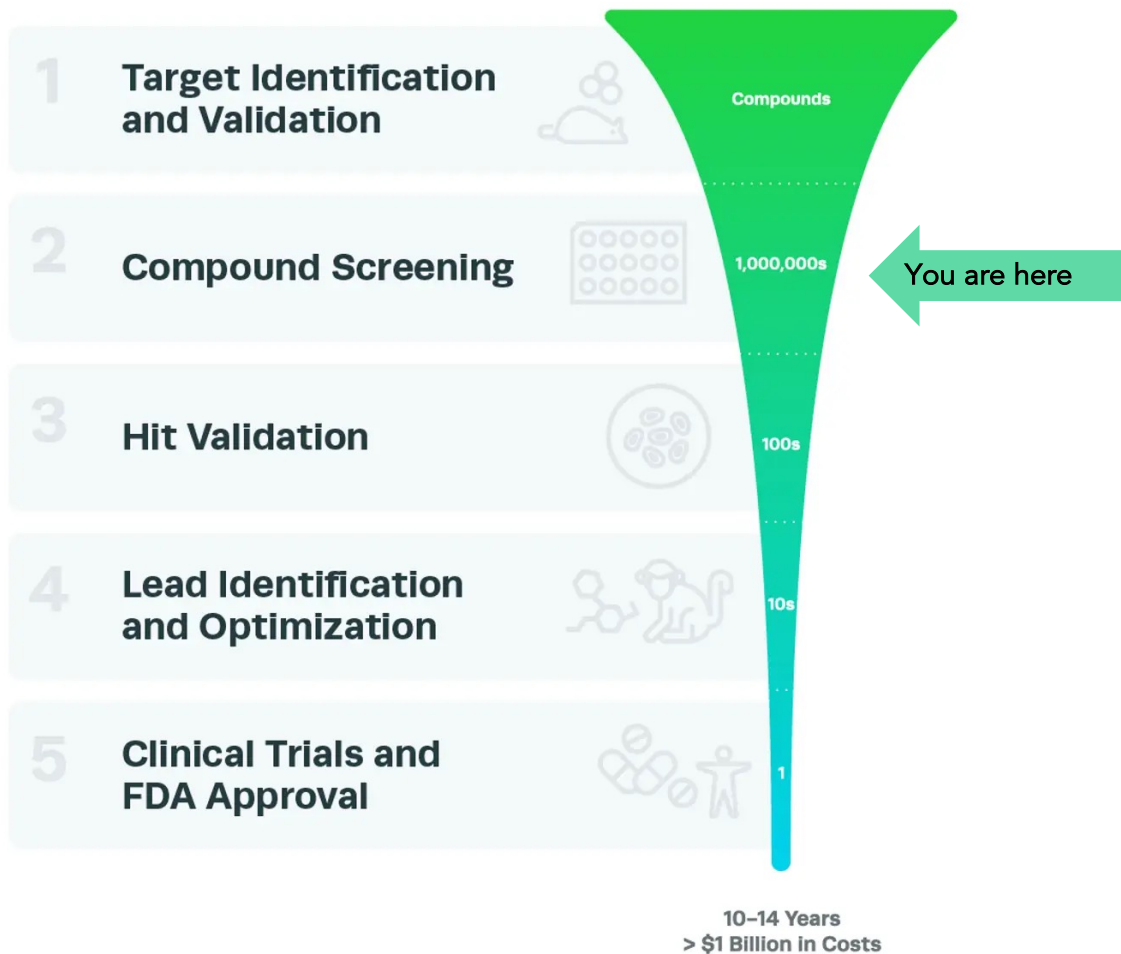


L5 – A Probe Discovery Vignette

February 28, 2023

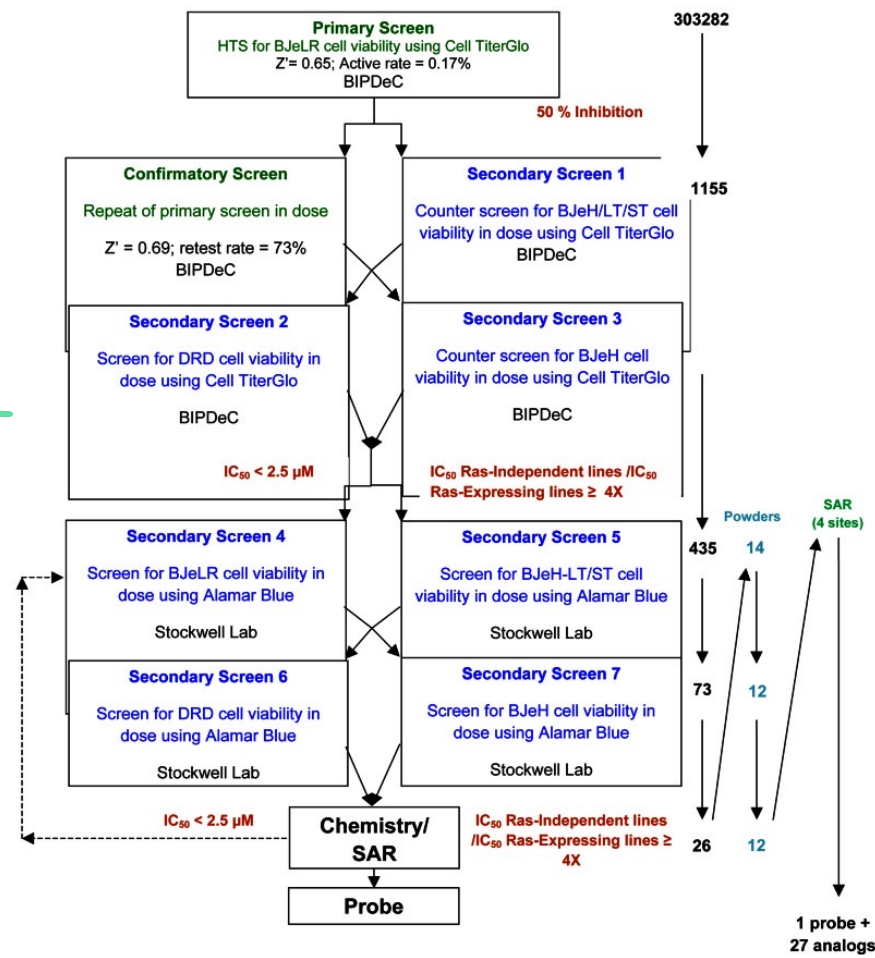
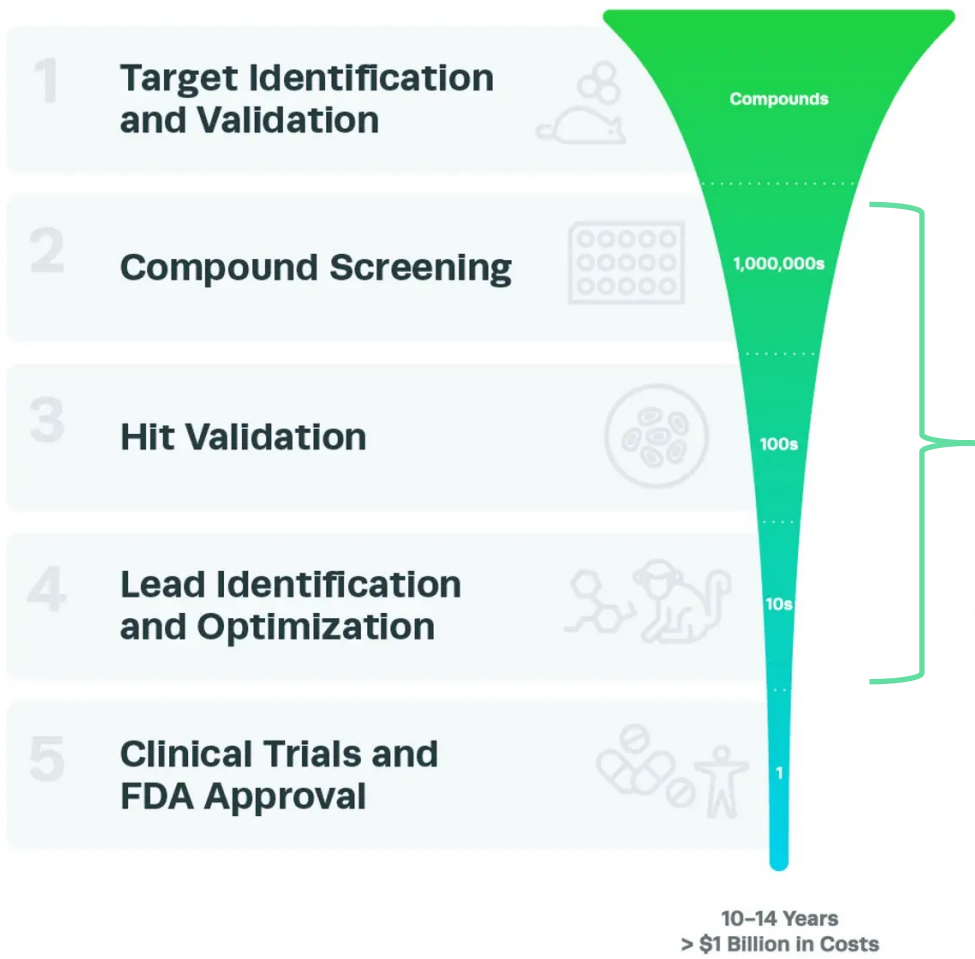
Question from L1 – What comes next?

Drug discovery funnel

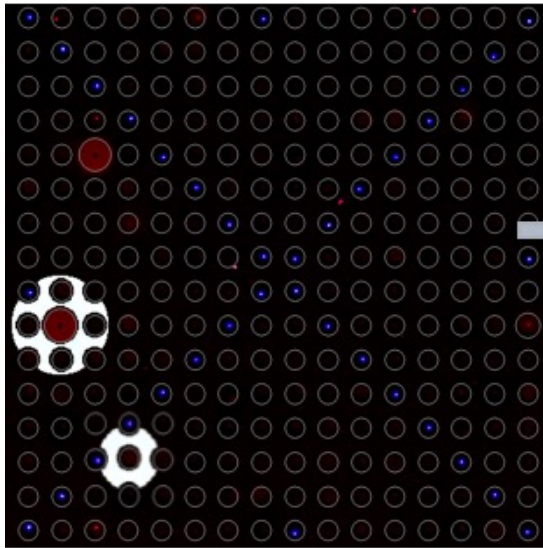


Heading down a 'critical path'

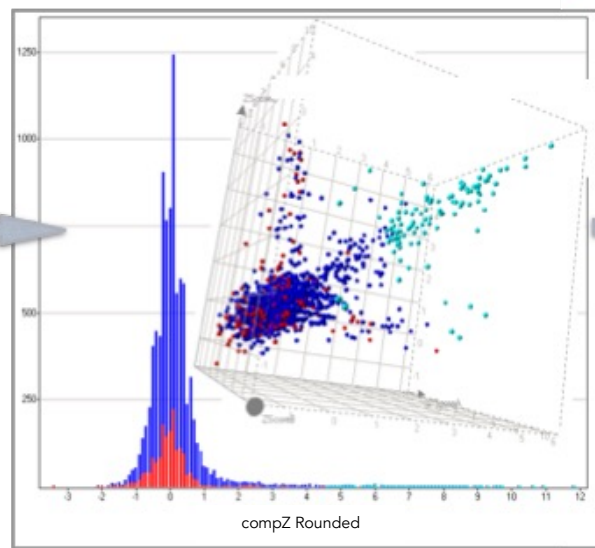
National Institute of Health (NIH)
Molecular Libraries Critical Path Scheme
Stockwell Lab Columbia + Broad Institute BIPDeC



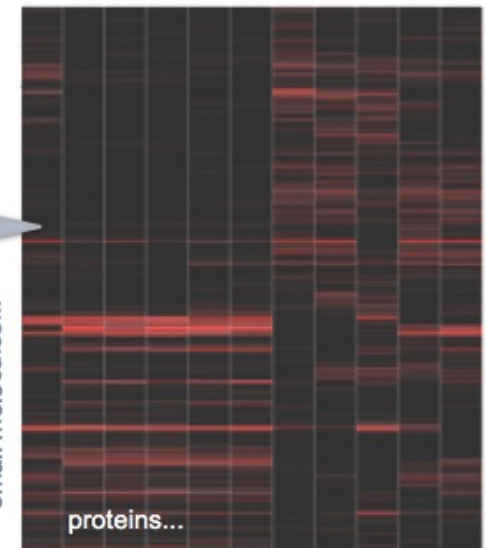
From L2: hits to probes → validation



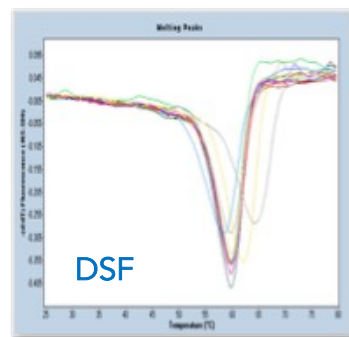
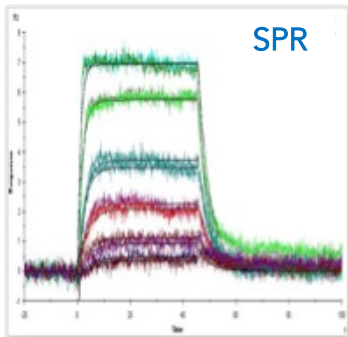
fluorescent features reveal putative **protein-ligand interactions**



compute composite Z-scores, **'hit' calls**

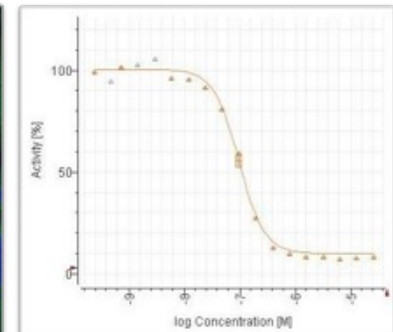
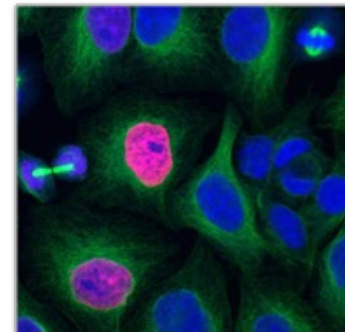


specificity analysis across proteins



secondary, quantitative **binding assays**

+



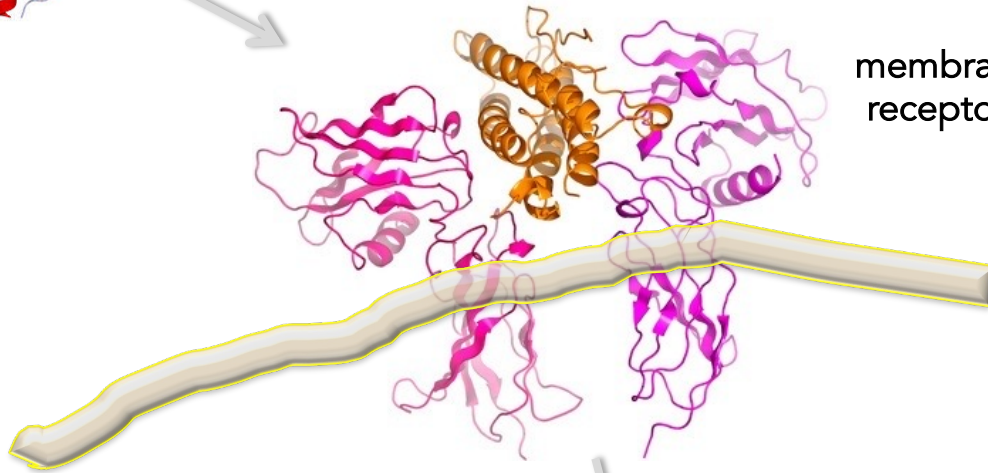
functional assays (e.g. cellular, biochemical)

the '20.320 version' of biology

extracellular factors



membrane receptors

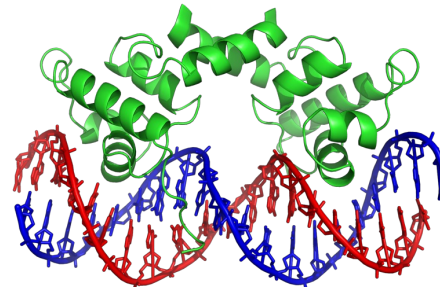


intracellular signaling proteins

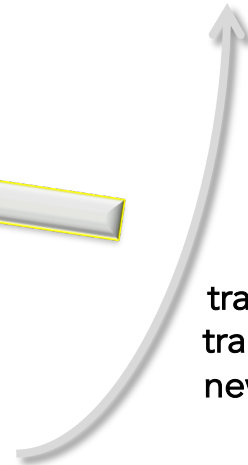


cellular response

transcriptional regulators



transcription translation of new proteins

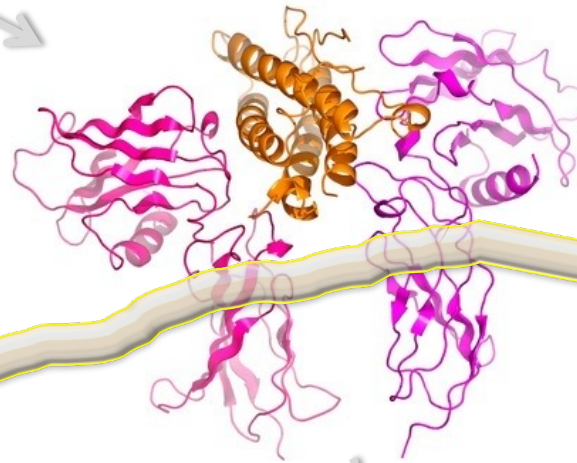


experimental methods
mathematical models
manipulate targets/systems

extracellular factors



membrane receptors



ion channels
receptor kinases
(oncology, neurology,
mood disorders,
inflammation)

cellular
response

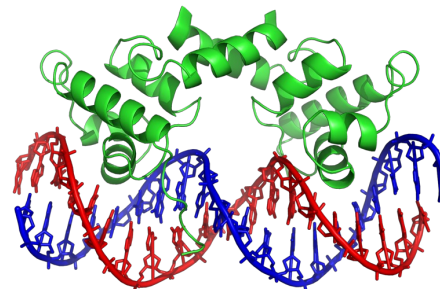
intracellular
signaling
proteins



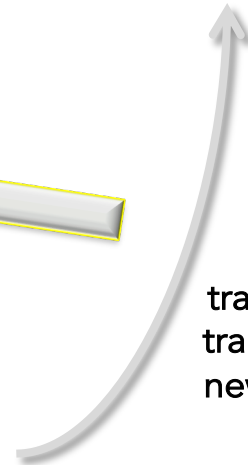
kinases
metabolic enzymes
(many diseases)



transcriptional
regulators



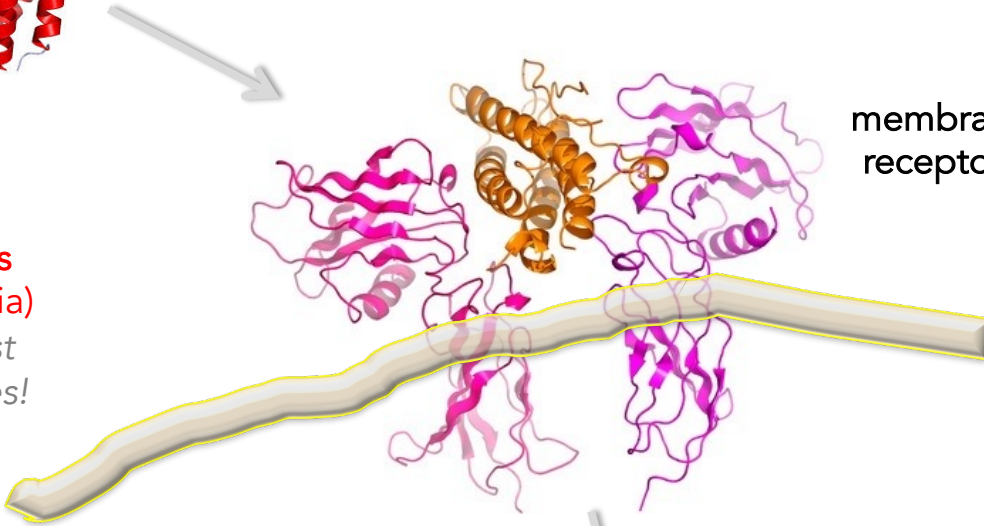
transcription
translation of
new proteins



extracellular factors



clotting factors
(DVT, hemophilia)
*one of the best
drugged classes!*



membrane receptors

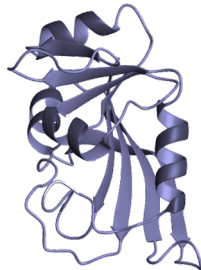


ion channels
receptor kinases
(oncology, neurology,
mood disorders,
inflammation)

**cellular
response**



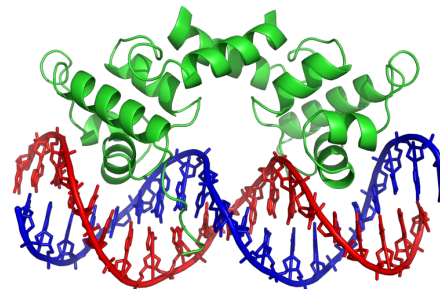
kinases
metabolic enzymes
(many diseases)



intracellular
signaling
proteins



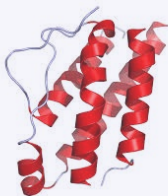
transcriptional
regulators



nuclear hormone receptors
(many diseases)

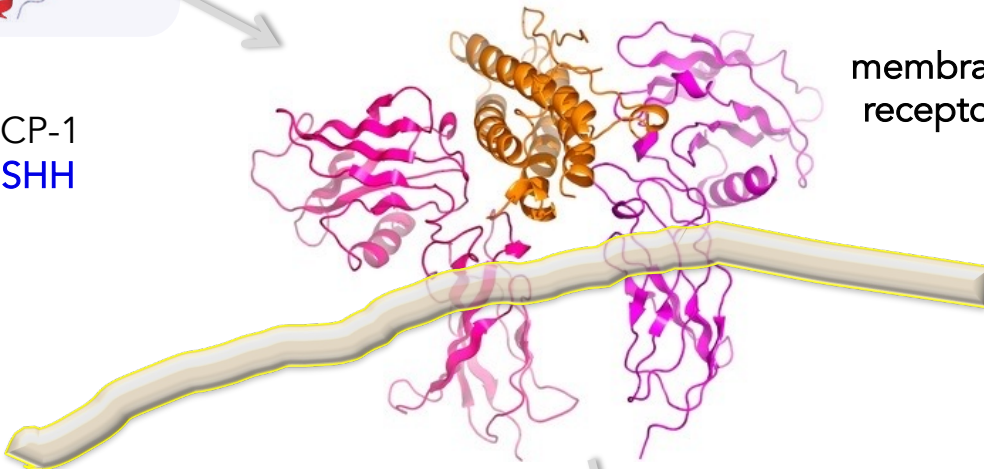


extracellular factors



cytokines – IL-4
chemokines – MCP-1
growth factors – SHH

membrane receptors



intracellular signaling proteins

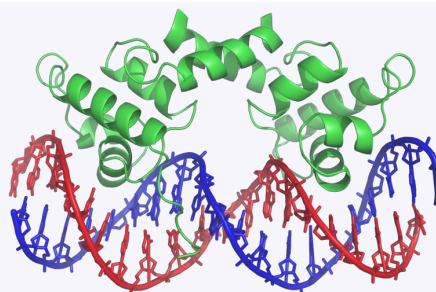


cellular response



epigenetic enzymes - HDACs
latent cytoplasmic TFs – NF-kappaB
nuclear hormone receptors – FOXA1
classic transcription factors – MYC, MAX

transcriptional regulators



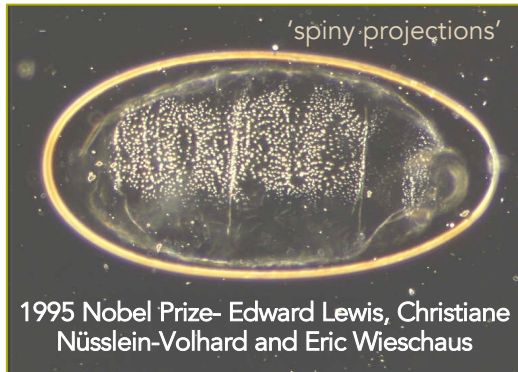
Sonic hedgehog protein

important role in development including limb and brain development

1978- Embryogenesis

Mutational Genetic Screen

mutant hedgehog drosophila larva



Desert and Indian
(Dhh and Ihh)

Hh genes

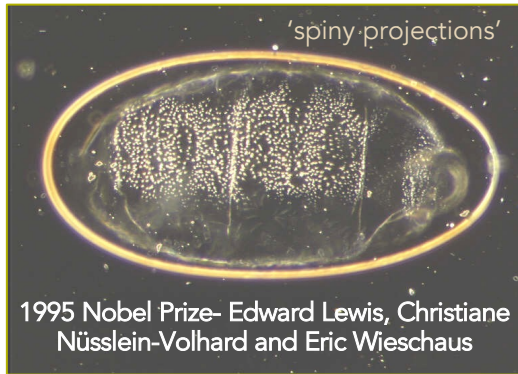
Sonic hedgehog protein

important role in development including limb and brain development

1978- Embryogenesis

Mutational Genetic Screen

mutant hedgehog drosophila larva



Hh genes



Desert and Indian
(Dhh and Ihh)



~1991
Sonic
(Shh)

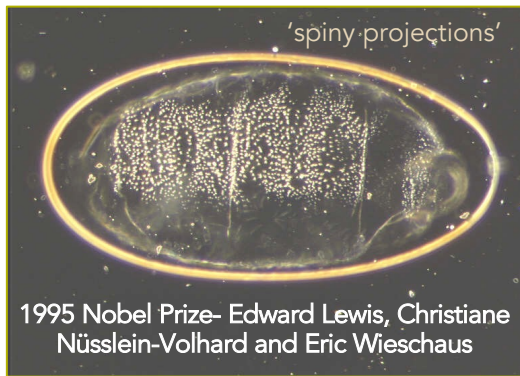
Sonic hedgehog protein

important role in development including limb and brain development

1978- Embryogenesis

Mutational Genetic Screen

mutant hedgehog drosophila larva



mutations in Shh are linked with *Holoprosencephaly (HPE)*



'cyclopia'

Hh genes



Desert and Indian (Dhh and Ihh)

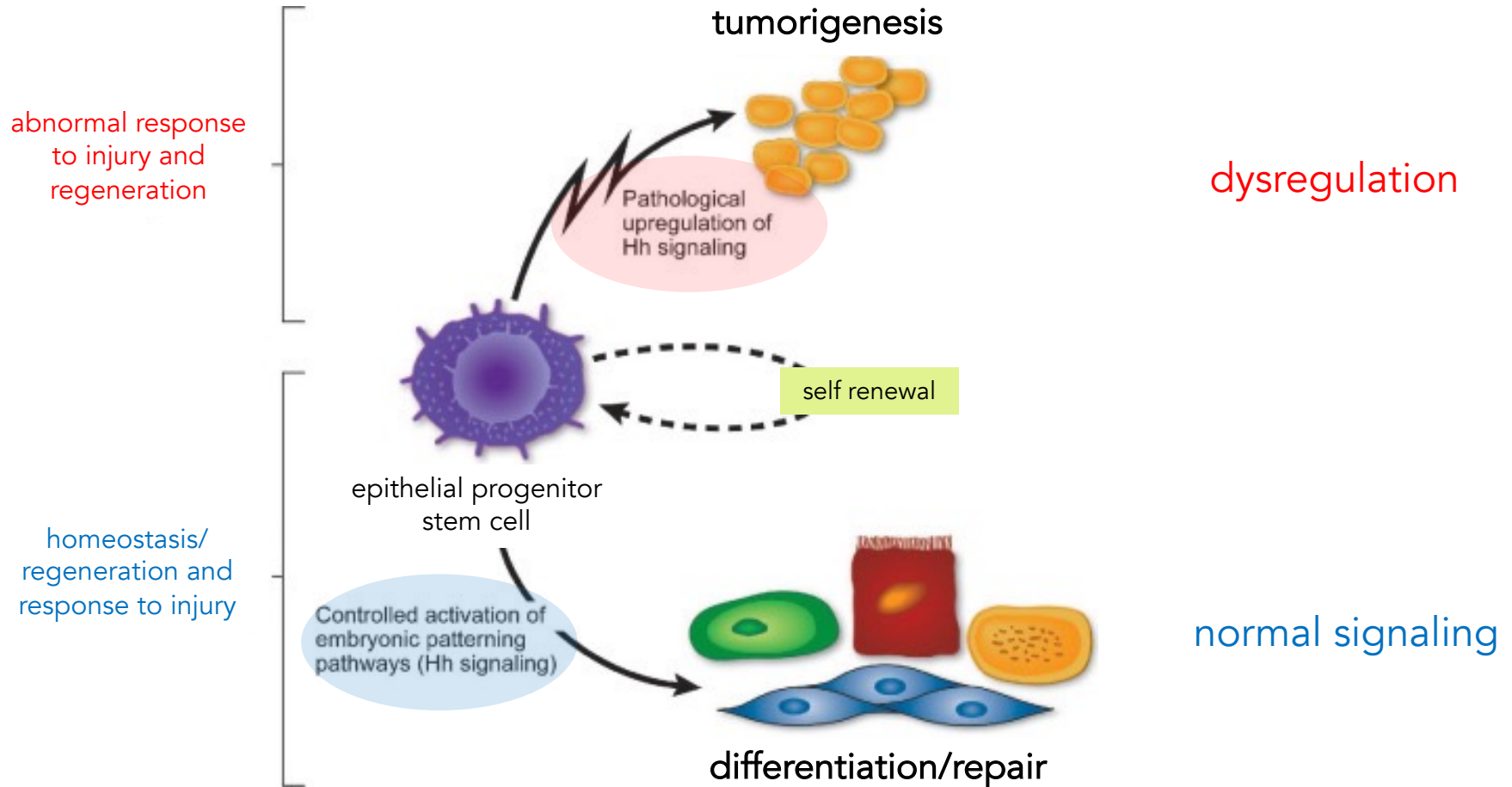


~1991 Sonic (Shh)

M. Muenke, *Seminars in Developmental Biology* Vol. 5, 293-301, 1994

Hedgehog signaling goes beyond embryogenesis

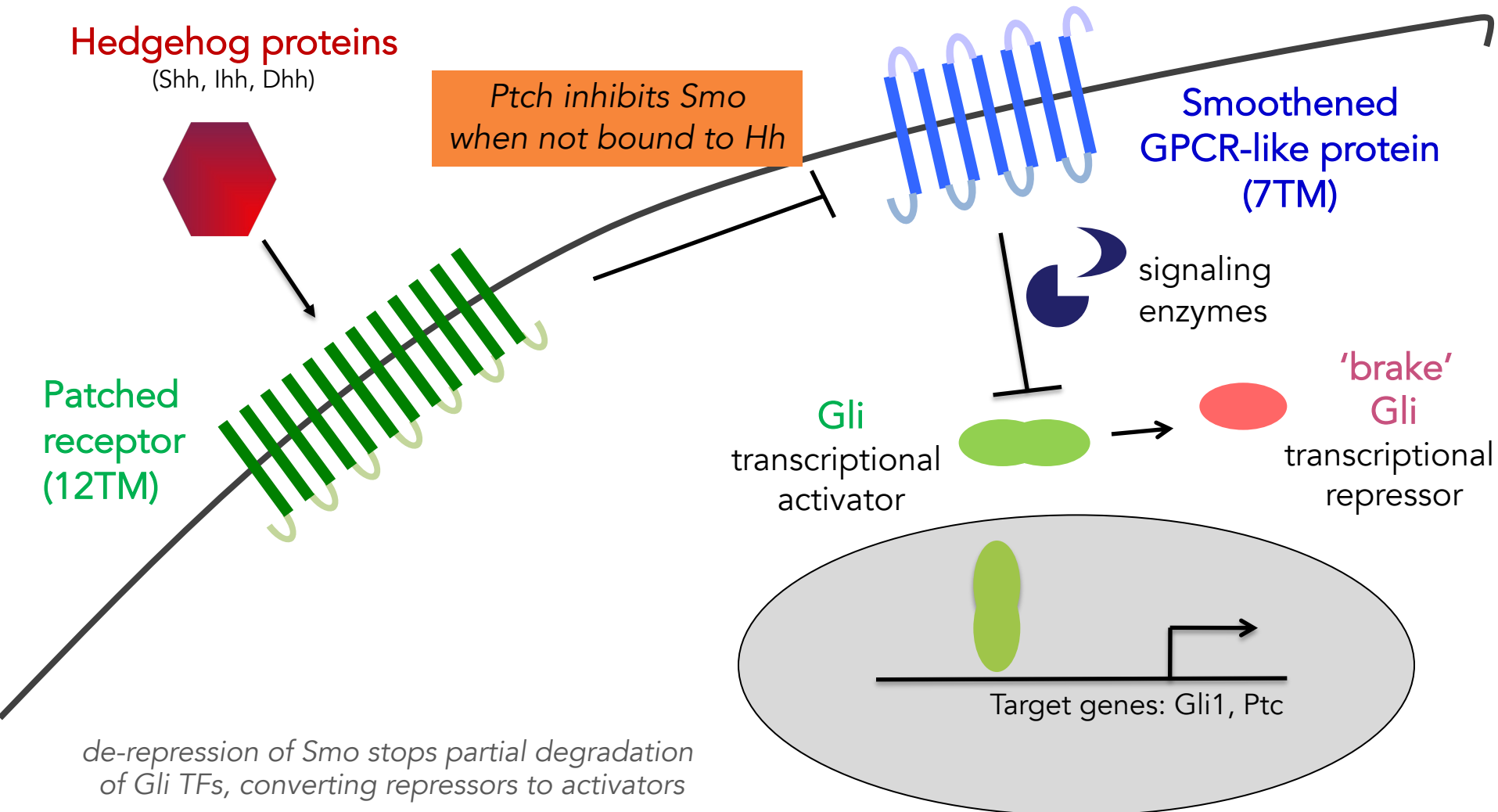
development, differentiation, and disease



Hh signaling pathway involved in embryogenesis plays a critical role in the maintenance of stem cells in adult life and cellular responses to injury

Hedgehog proteins 'de-repress' Smoothened

Hh-Ptch binding interaction activates Gli-driven transcription



overexpression of **SHH**

Pancreatic Cancer (70%) Prostate Cancer

Gastric Cancer

Lung Cancer

Medulloblastoma

Ovarian Cancer

activating mutations in **SMO**

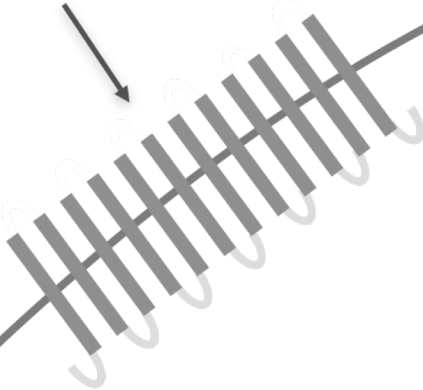
Basal Cell Carcinoma

Ovarian Cancer

Hedgehog proteins
(Shh, Ihh, Dhh)



Patched receptor



Smoothed
GPCR-like protein



signaling
enzymes

Gli
transcriptional
activator



Gli
transcriptional
repressor



loss or mutation of **PTCH1**

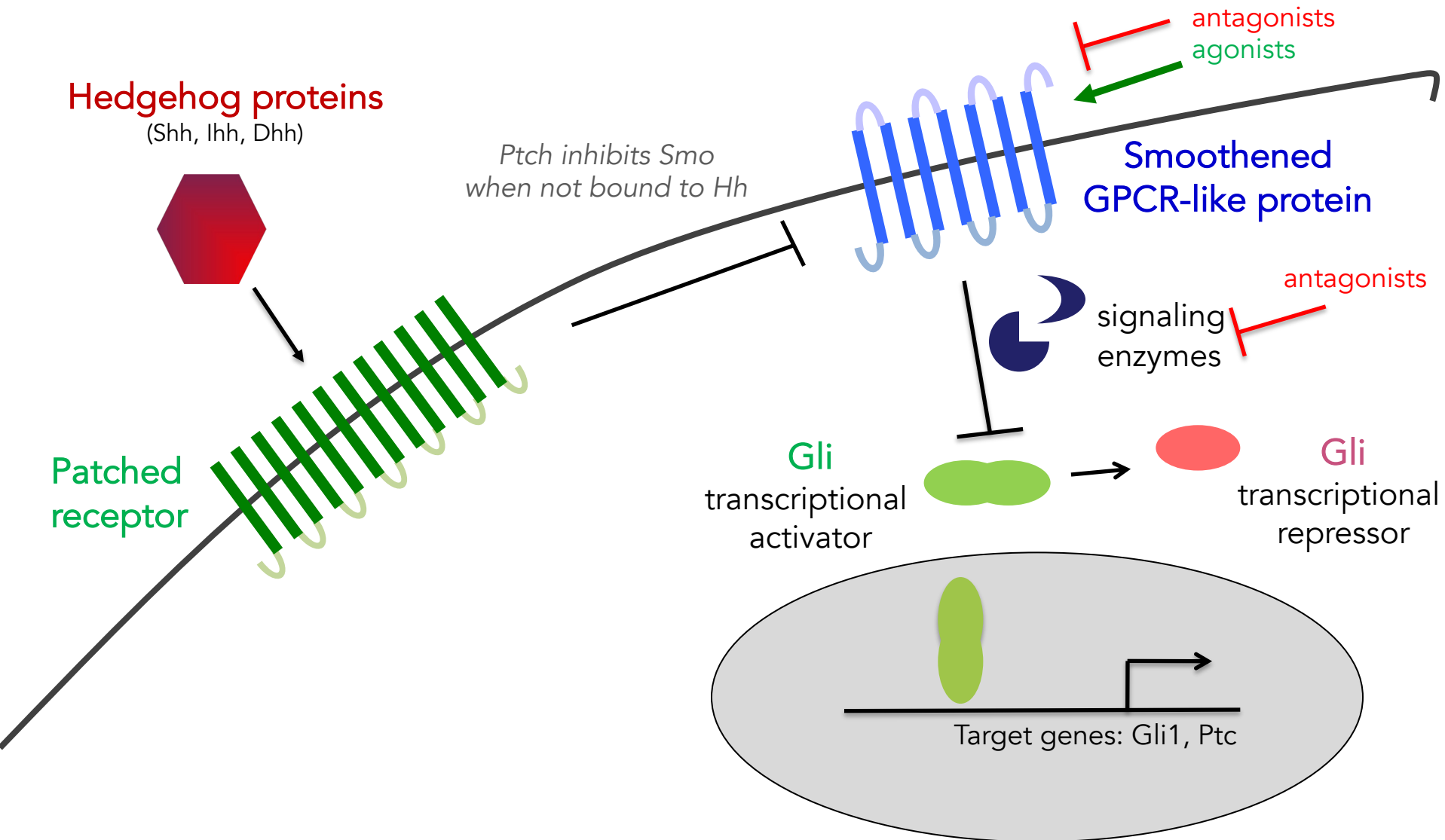
Basal Cell Carcinoma

Medulloblastoma

Rhabdomyosarcoma



Drugs targeting Hedgehog pathway

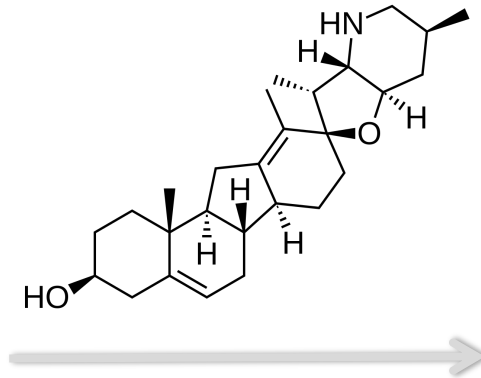


Cyclopamine

Smo antagonist and Hh pathway inhibitor



Veratrum californicum
wild corn lily



11-yr investigation
By US Dept of Agriculture



cyclopic lamb born of
a mother sheep that
ate corn lily

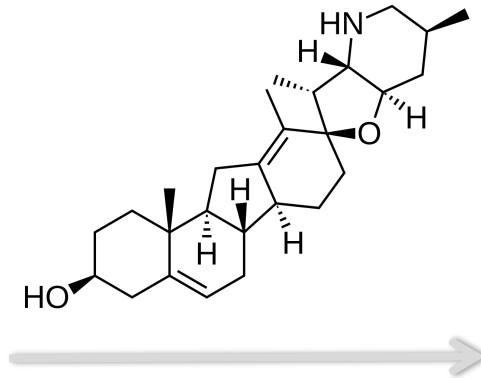
(Idaho farm, 1957)

Cyclopamine

Smo antagonist and Hh pathway inhibitor



Veratrum californicum
wild corn lily



11-yr investigation
By US Dept of Agriculture



cyclopic lamb born of
a mother sheep that
ate corn lily

(Idaho farm, 1957)

Beachy & Chen Labs (Stanford): Cyclopamine inhibits Hh signaling by influencing the balance of active and inactive Smoothed protein

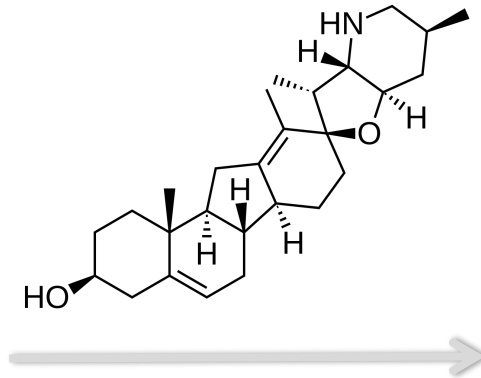
How did they arrive at this conclusion?

Cyclopamine

lead for development of anti-cancer agents

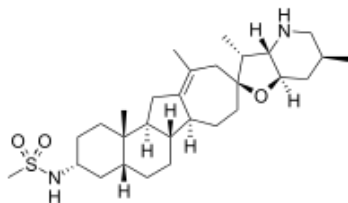


Veratrum californicum
wild corn lily

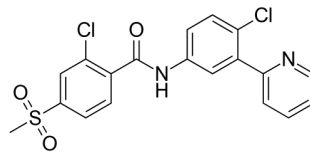


cyclopic lamb born of
a mother sheep that
ate corn lily

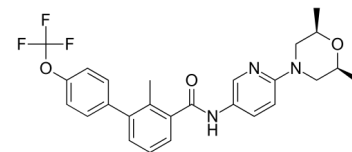
Adult cancers - basal cell carcinoma, medulloblastoma, prostate, breast, pancreas



saridegib



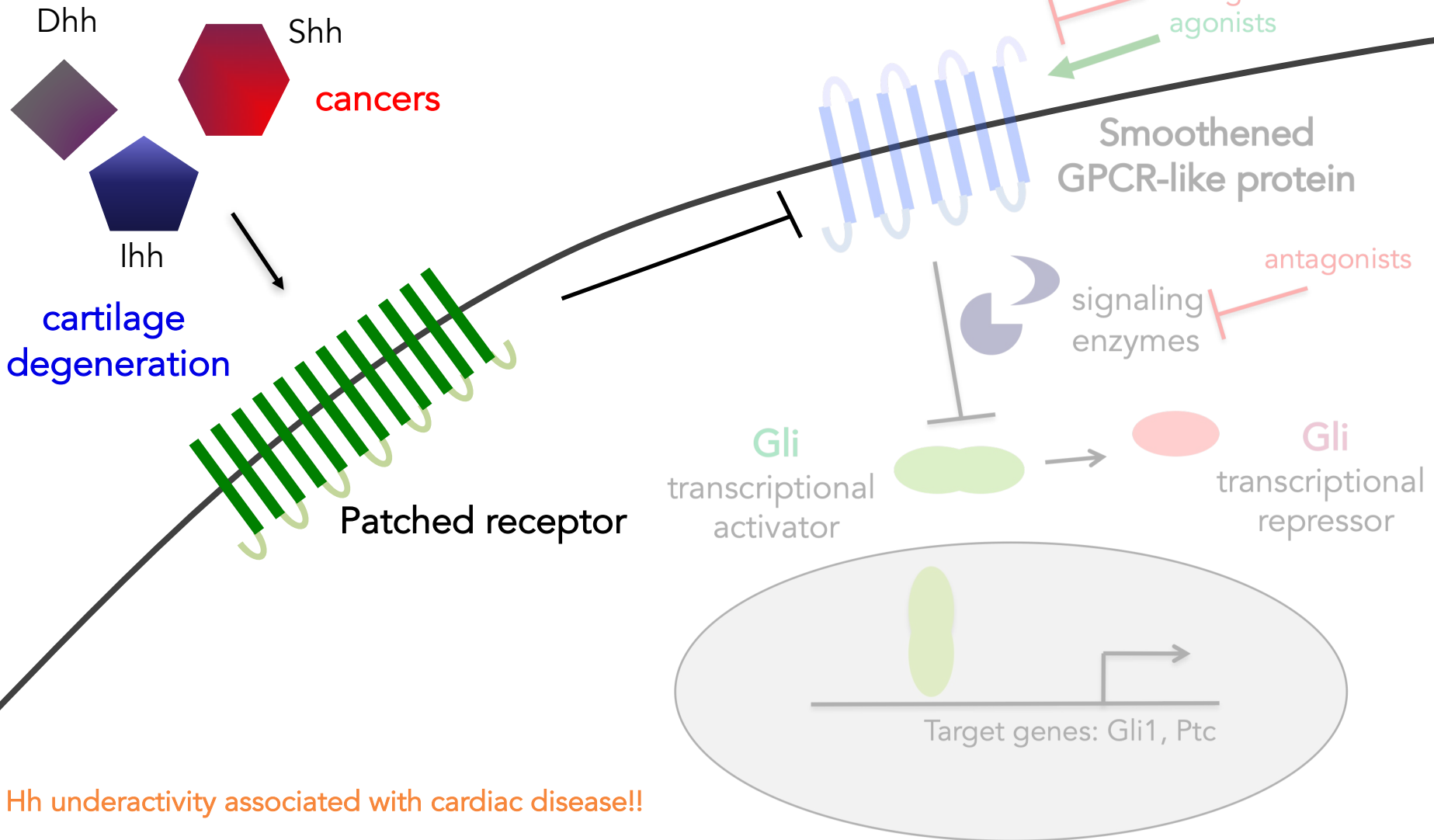
vismodegib



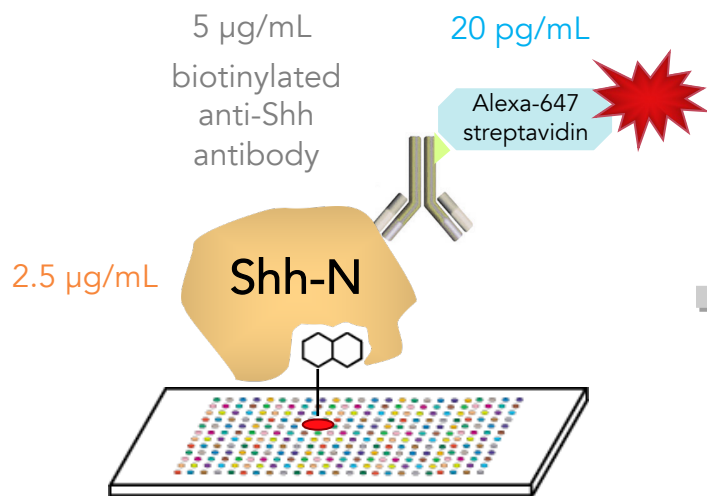
sonidegib

Selective targeting of Hh signaling upstream of Smo

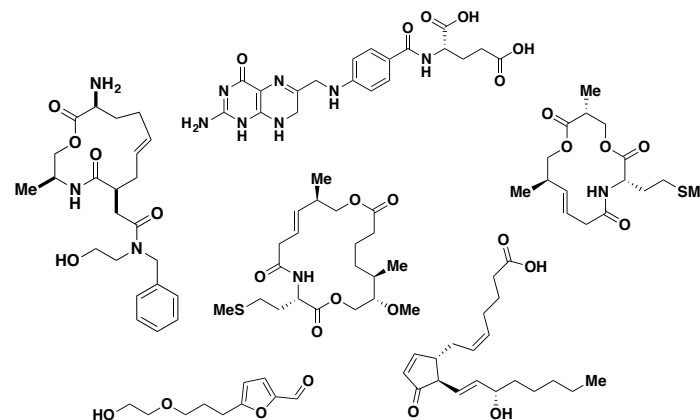
gonadal dysgenesis, neuropathies



SMM assay: 20 kDa Shh N-terminal fragment



19 SMM hits



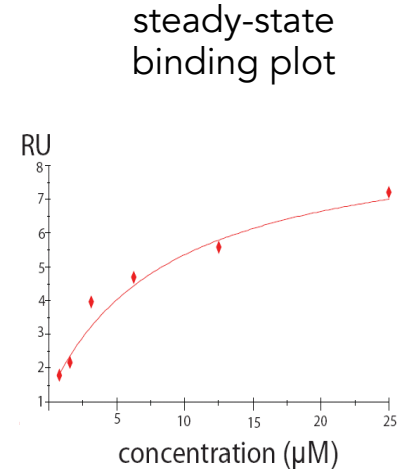
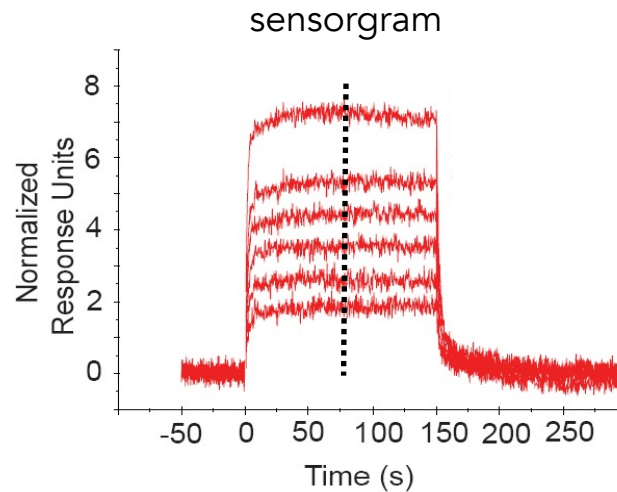
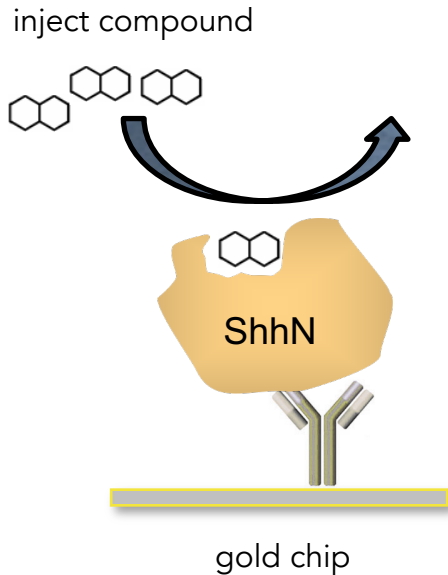
Angela, Broad Fellow

Lee Peng, MGH

Ben Stanton, Harvard



SPR experiments for Shh SMM hits

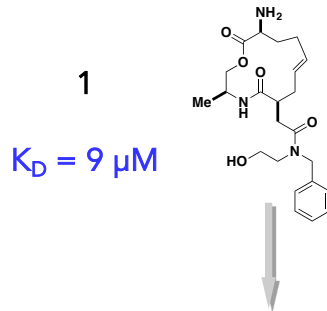


$$K_D = 9 \mu\text{M}$$

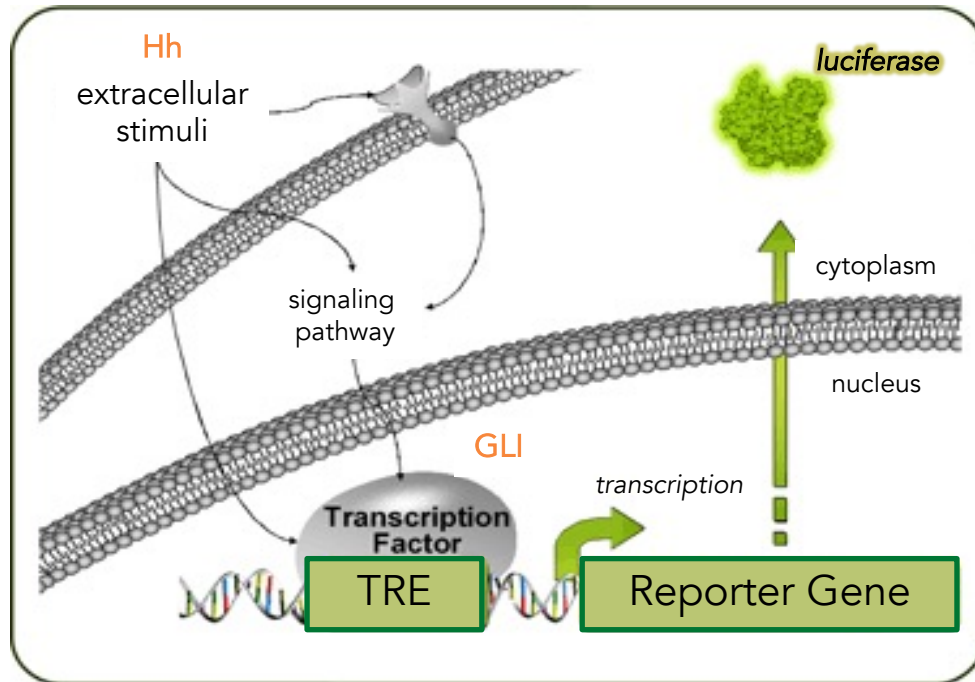
- reverses orientation from primary assay
- measures binding between immobilized protein and compounds injected in solution
- kinetic measurements
- ranking assays (k_{on} vs. k_{off} , % Ru_{max})
- compound affinity characterization

Measuring *GLI*-dependent transcriptional activity

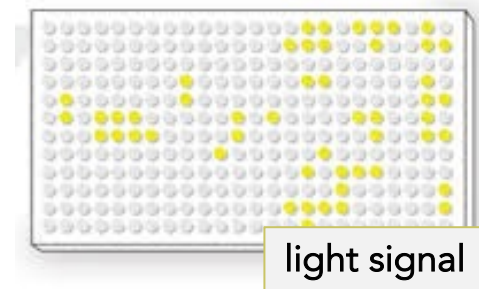
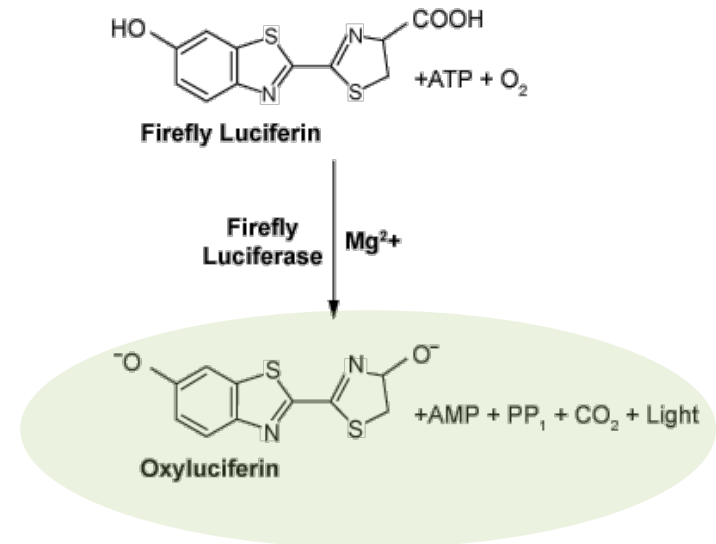
quantitative assay for hedgehog signaling



NIH/3T3 cell line transfected with *GLI*-responsive reporter assay vector

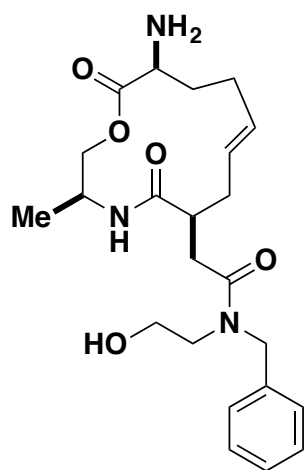


Luciferase reaction



Measuring *GLI*-dependent transcriptional activity

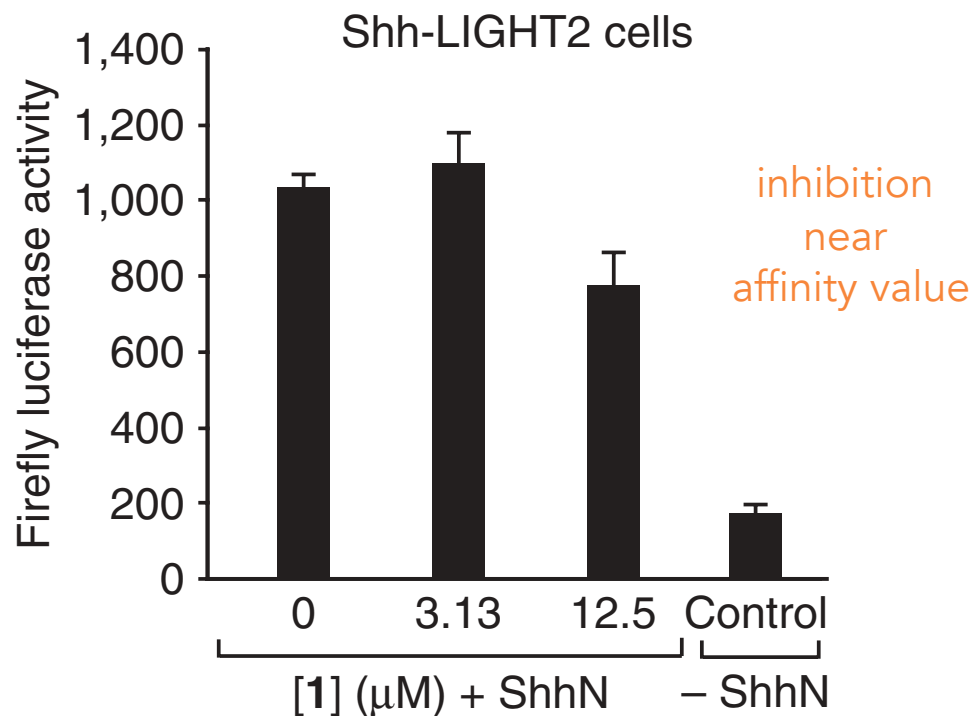
SMM hit modulates transcriptional output in preliminary experiment



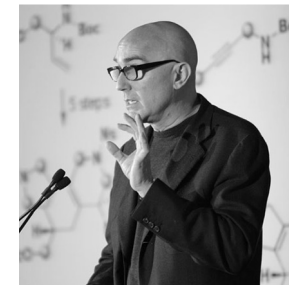
$K_D = 9 \mu\text{M}$



reporter assay



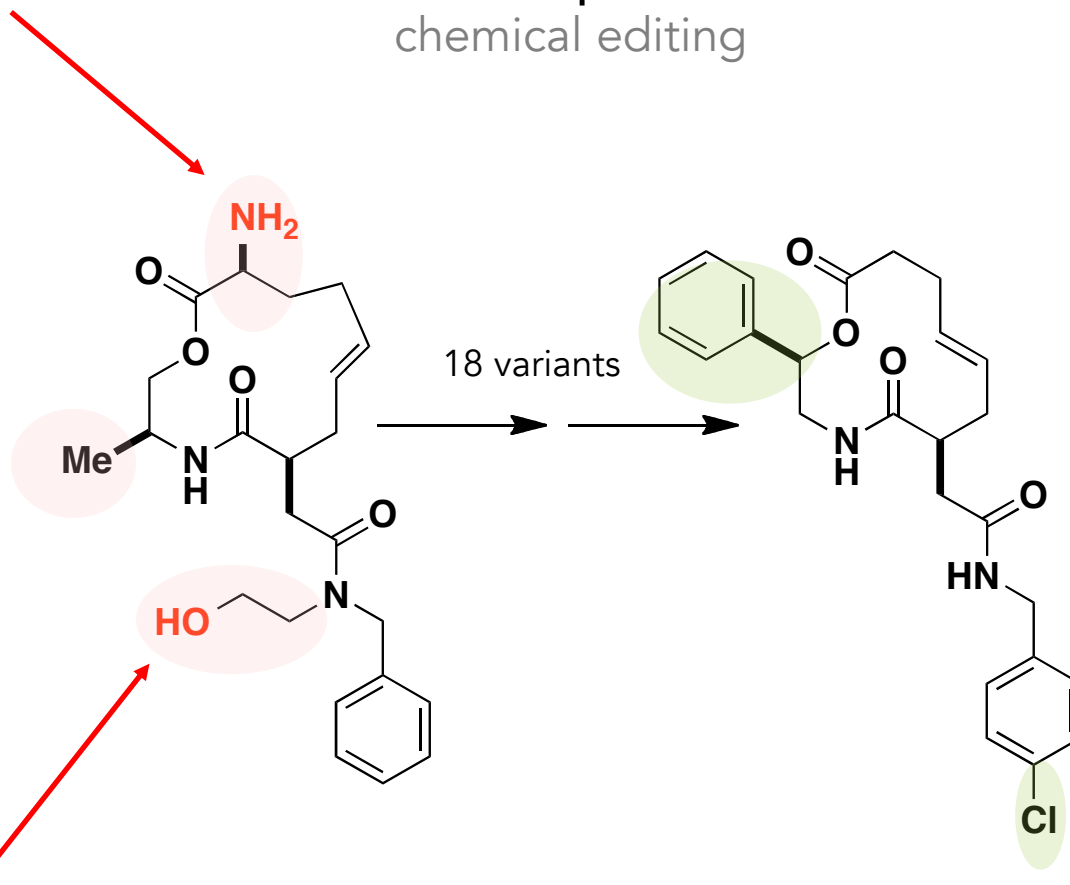
each value represents 5 technical replicates
error bars denote standard deviation



site of attachment to SMM

Hit to probe

chemical editing



site of attachment to SMM

$K_D = 9 \mu\text{M}$

$K_D = 3 \mu\text{M}$

improved binding affinity

Remove – ethanolamine, methyl, amine and carbon

Add – phenyl, chloro groups



Doctor Ivo "Eggman" Robotnik



Sonic the Hedgehog

Robotnikinin

Shh binder and antagonist

nature
chemical biology

A small molecule that binds Hedgehog and blocks its signaling in human cells

Benjamin Z Stanton^{1,2,7}, Lee F Peng^{1-3,7}, Nicole Maloof¹, Kazuo Nakai², Xiang Wang¹, Jay L Duffner¹, Kennedy M Taveras¹, Joel M Hyman⁴, Sam W Lee⁵, Angela N Koehler¹, James K Chen⁴, Julia L Fox⁶, Anna Mandinova⁵ & Stuart L Schreiber^{1,2}

Small-molecule inhibition of extracellular proteins that activate membrane receptors has proven to be extremely challenging. Diversity-oriented synthesis and small-molecule microarrays enabled the discovery of robotnikinin, a small molecule that binds the extracellular Sonic hedgehog (Shh) protein and blocks Shh signaling in cell lines, human primary keratinocytes and a synthetic model of human skin. Shh pathway activity is rescued by small-molecule agonists of Smoothed, which functions immediately downstream of the Shh receptor Patched.

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Guest Blog

In Silico et Vivo: When Life Science Draws Inspiration from Video Games

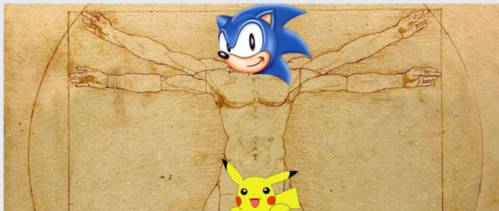
By Jon Chang on October 12, 2012

You Got Your Genetics In My Videogames

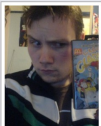
We here at GameCola's crack news team are dedicated to searching far and wide for videogame news, but today I'll be daring to traverse the very time-space continuum to bring you news from several year

Contributed by Christian Porter on April 8th, 2011 in News Posts

With content involving Christian Porter, Dr. Eggman, Dr. Robotnik, genetics, News, Pikachu, Pikachurin, Pokémon, retinitis pigmentosa, Robotnikinin, science, Sonic Hedgehog Homolog, Sonic the Hedgehog



We here at GameCola's crack news team are dedicated to searching far and wide for videogame news, but today I'll be daring to traverse the very time-space continuum to bring you news from several years in the past—just for you, valued reader!



Finally, my precious Mega Drive, we are one. My plans are complete.

Normally I expect to see references to videogames in *Game Informer*, not the science journal *Nature*, but it looks like gamer geneticists have been tasked with naming the mammalian gene responsible for the growth of fingers and toes, stem cell division, and the organization of the brain. It's name? Sonic Hedgehog Homolog. Yes, this means that Sonic the Hedgehog is inside of all of our genes, and, judging based on his love of the azure rodent, is now all over the inside of an overly-excited Matt Jonas's underwear upon hearing this information.

Additionally, it turns out that Harvard researchers created an equally hilarious inhibitor to counteract Sonic Hedgehog Homolo: Robotnikinin.

RETROIST
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Retroist Sonic the Hedgehog Podcast

By Retroist April 9, 2010 2 Comments

RETROIST PODCAST

SONIC THE HEDGEHOG

9 February 2013 / 1,237 notes / 6 comments

Share Trivia <https://tumblr.co/ZqM2Ivdiid7>

DID YOU KNOW GAMING?

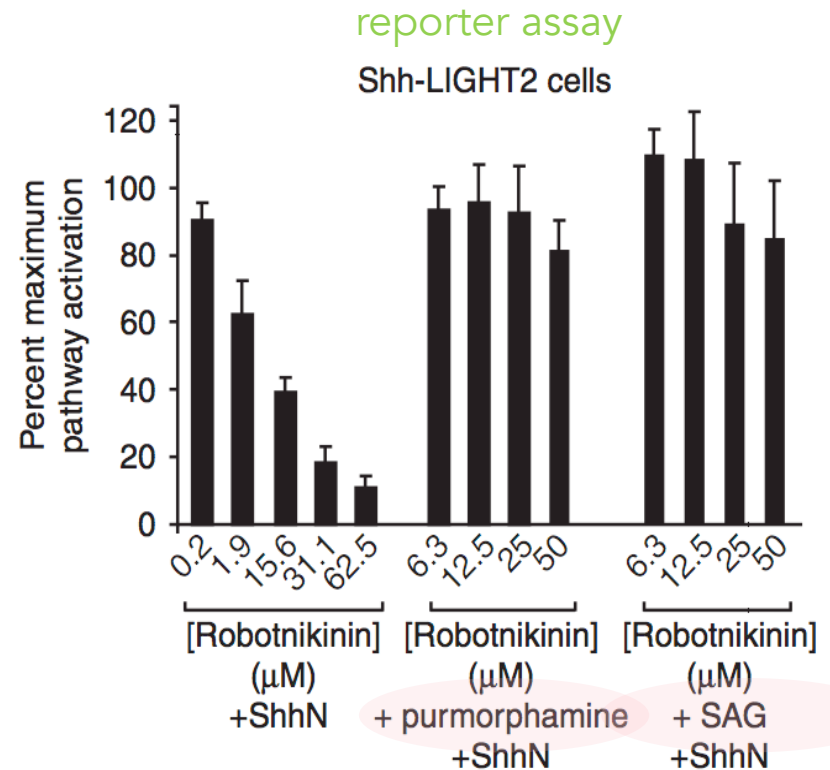
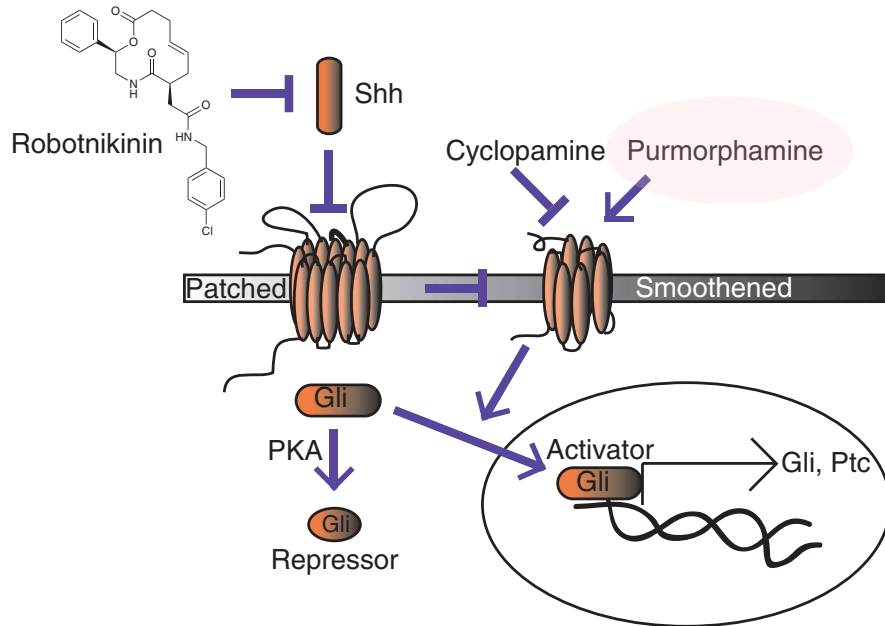
THE GENE NAMED 'SONIC HEDGEHOG' HAS AN INHIBITOR (SOMETHING THAT STOPS THE PROCESS OF A GENE) NAMED 'ROBOTNIKININ', NAMED AFTER SONIC'S NEMESIS, DR. IVO 'EGGMAN' ROBOTNIK.

SUBMITTED BY WTFISASONIC
DIDYOUKNOWGAMING.COM

Sonic the Hedgehog.

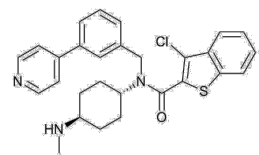
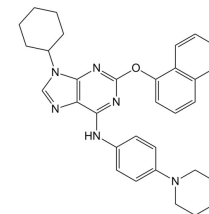
<http://www.tssznews.com/2009/02/21/sonic-hedgehog-vs-robotnikinin/>

Gli inhibition by Robotnikinin is rescued by a Smoothened agonist



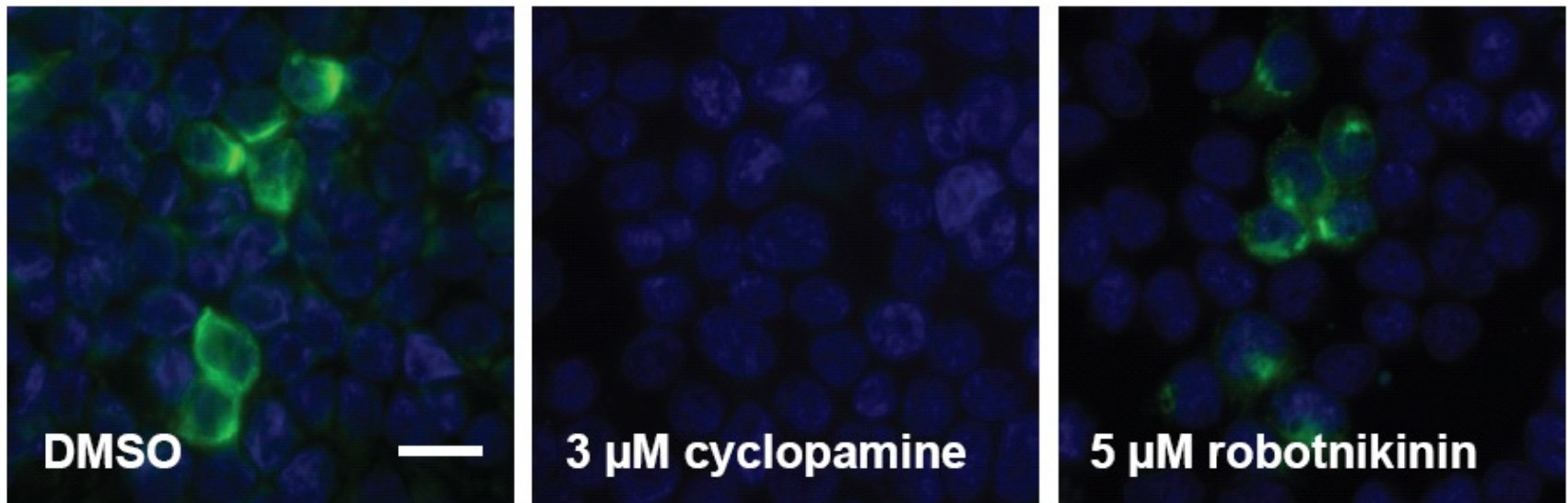
rescue experiments are common
in systems biology

validate mechanistic hypotheses



Ligand competition assays to assess specificity

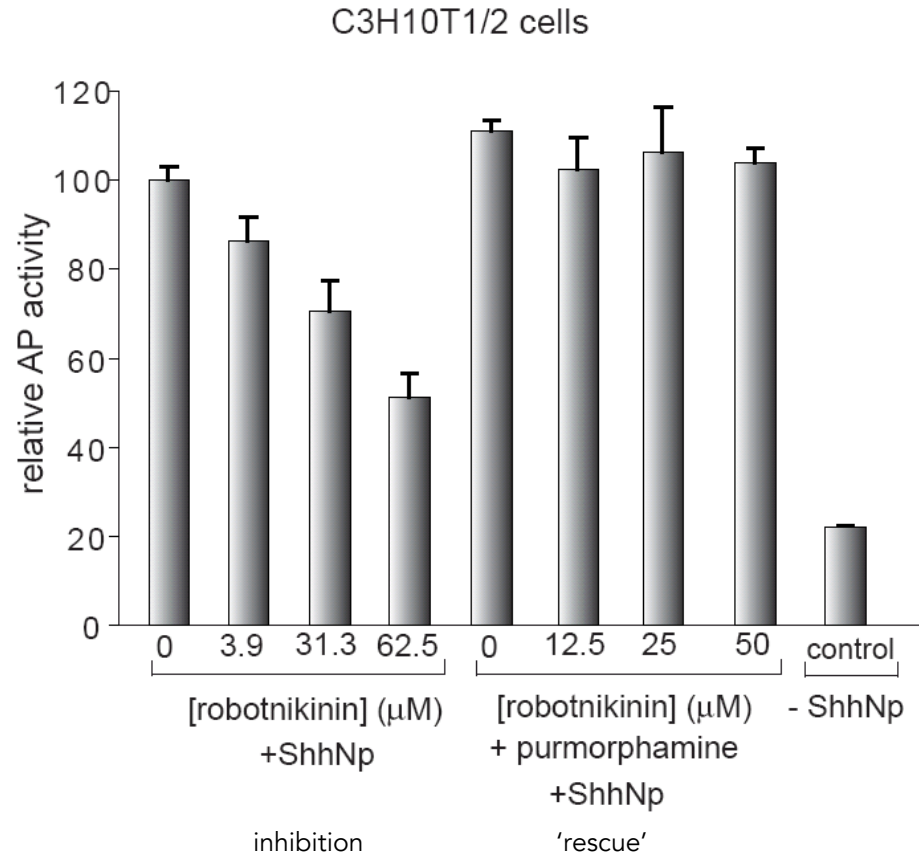
BODIPY-cyclopamine binds to Smoothed at cell surface



Smoothed-overexpressing human embryonic kidney cells

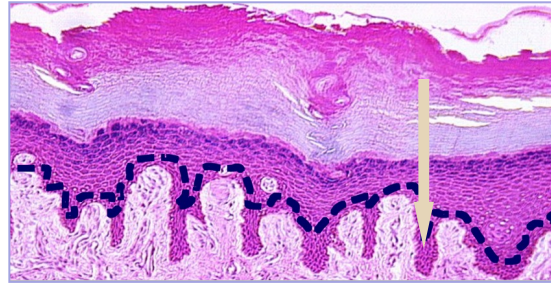
Conclusion: Robotnikinin does not compete with a labeled Smo ligand

Inhibition of stem cell differentiation



mouse mesenchymal stem cells differentiate into osteoblasts and upregulate alkaline phosphatase (AP) when stimulated with N-palmitoylated ShhN

Skin: Robotnikinin lowers levels of *GLI2* mRNA in primary human keratinocyte cells

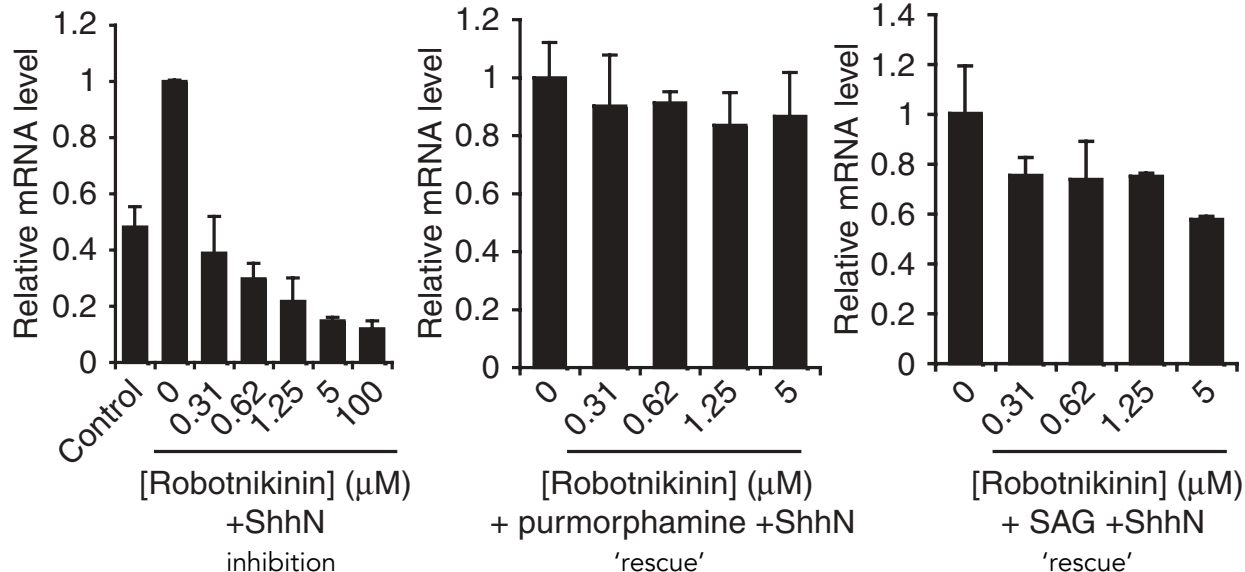


primary human keratinocytes
isolated from the basal cell layer

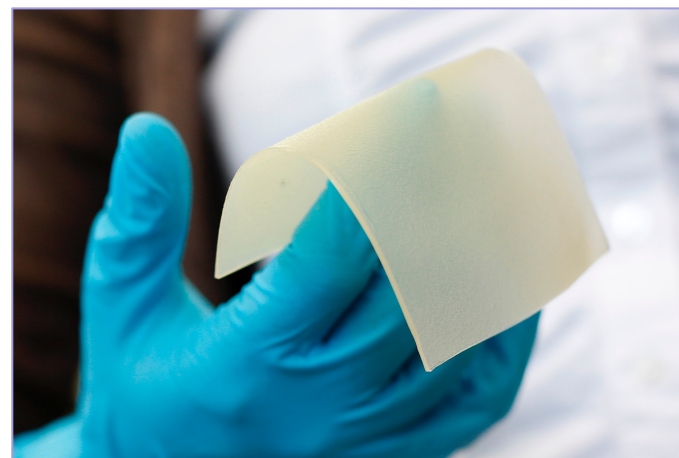
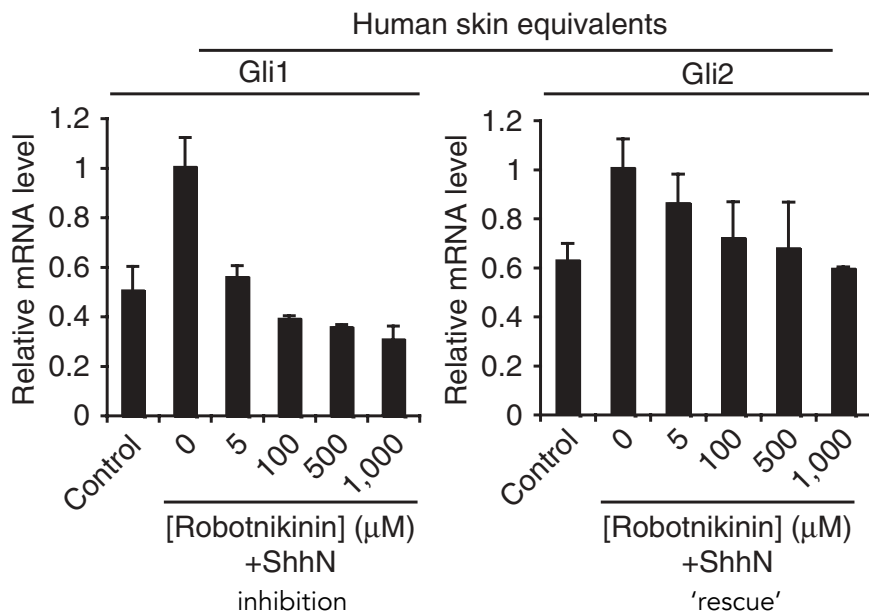


measure mRNA by quantitative
PCR after 30-hr treatments

Gli2



Robotnikinin blocks lowers levels of *GLI1* and *GLI2* mRNA in synthetic human skin



MGH synthetic human skin model:

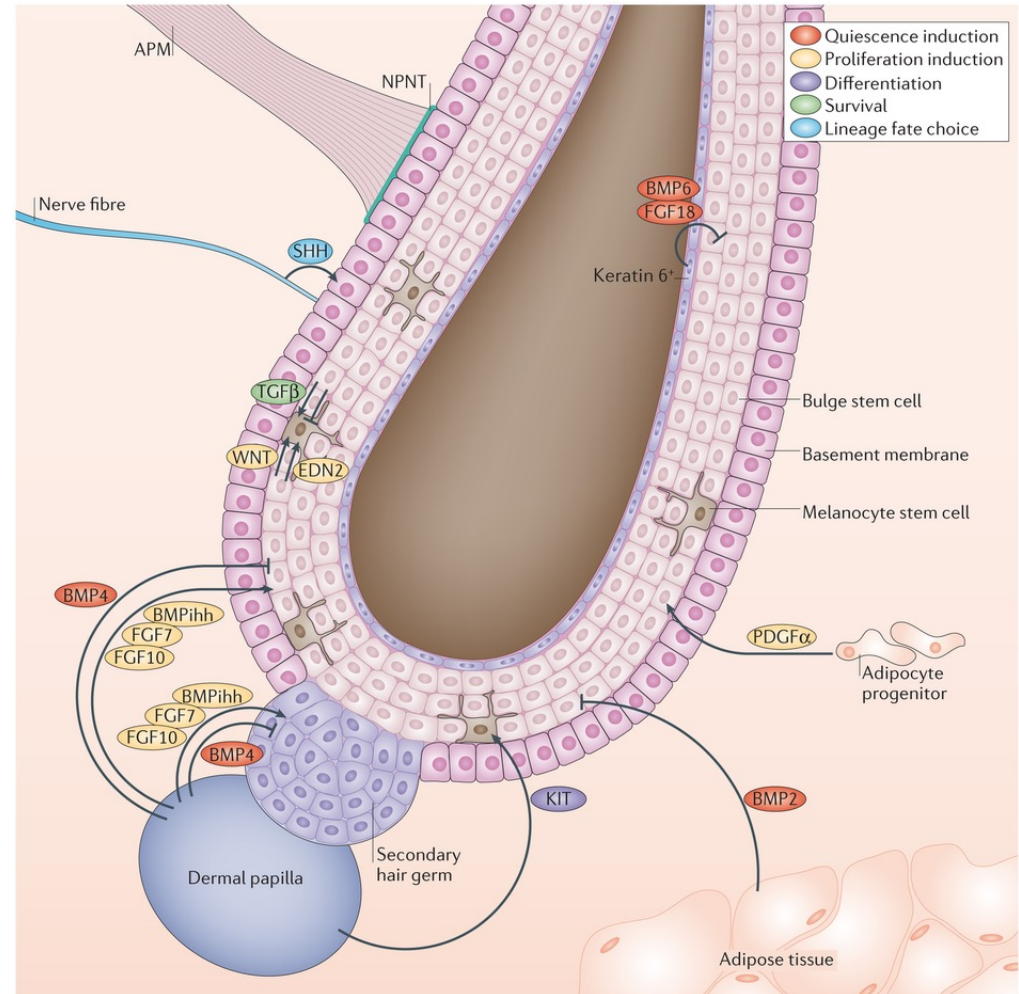
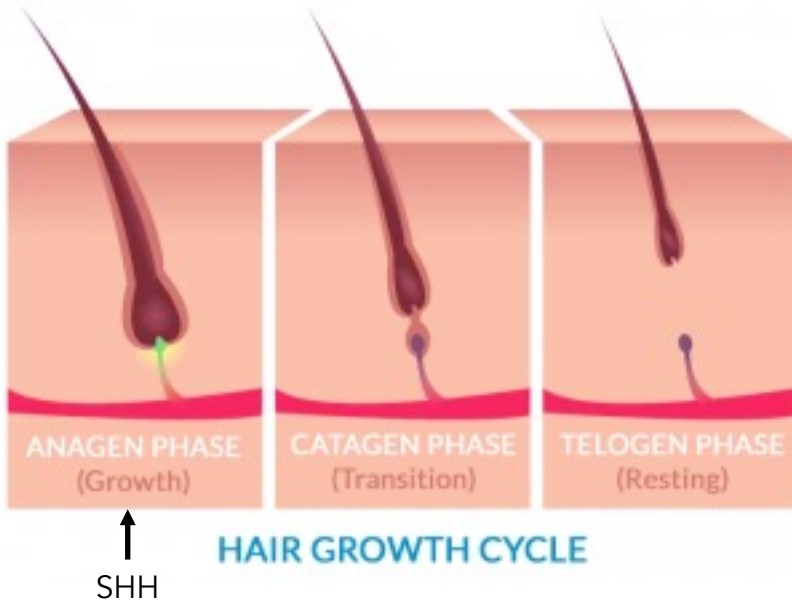
1. Extract dehydrated collagen matrix from skin grafts
2. Populate matrix with primary keratinocytes
3. Culture to form several dermal layers
4. Incubate with compound, analyze by qPCR and histology

Anna Mandinova, MGH



Structure-activity relationship
Syn-skin punches

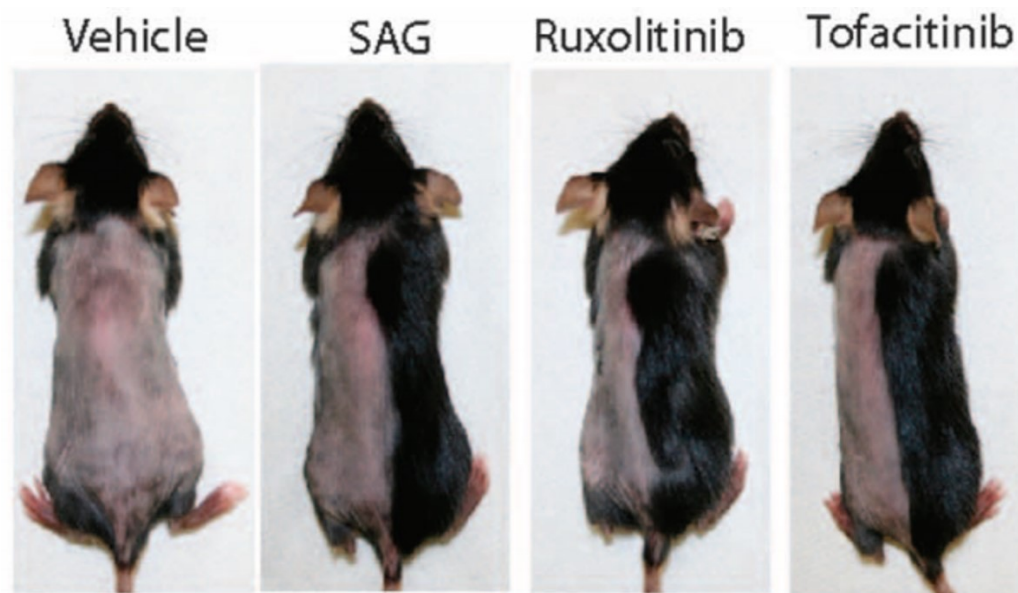
Shh and the hair follicle – a regulator of luscious locks



Nature Reviews | Molecular Cell Biology

lower levels of Hh expression or signaling is associated with baldness

Exploring stimulation of Shh pathway as a way to promote hair growth



Seven-week-old wild-type mice were shaved and treated daily with either a topical application of vehicle control, sonic hedgehog agonist (SAG), 3% ruxolitinib (JAK1/2 inhibitor), or tofacitinib (JAK3 inhibitor). Skin was harvested at the indicated time points and stained with hematoxylin and eosin (H&E). Images of mice were taken at D21 of treatment. Harel et al. *Sci. Adv.* 2015

Smoothened agonists used in our rescue experiments

Robotnikinin inhibits hair growth *in vitro*

8 days post depilation



10 uM robotnikinin

DMSO

12 days post depilation

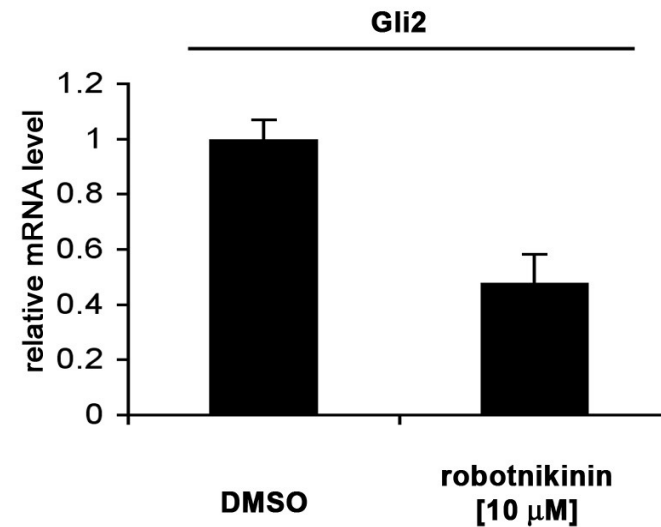
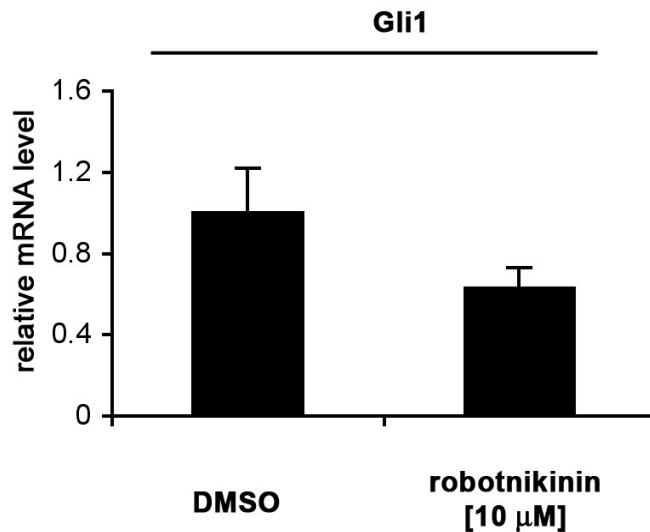
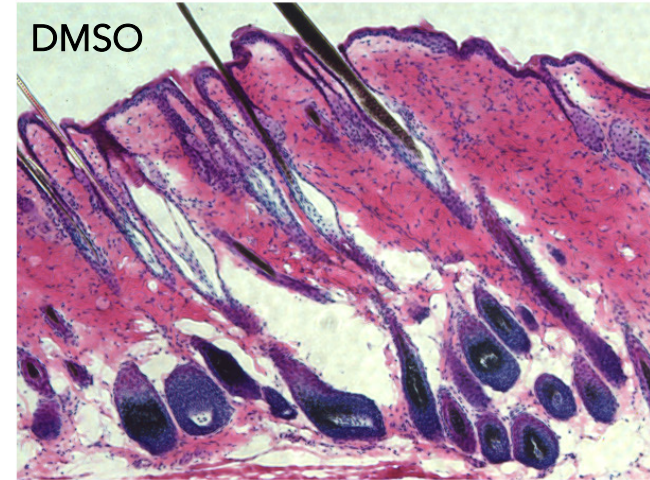


10 uM robotnikinin

DMSO

Robotnikinin causes hair follicles to fail anagen phase entry

12 days post depilation



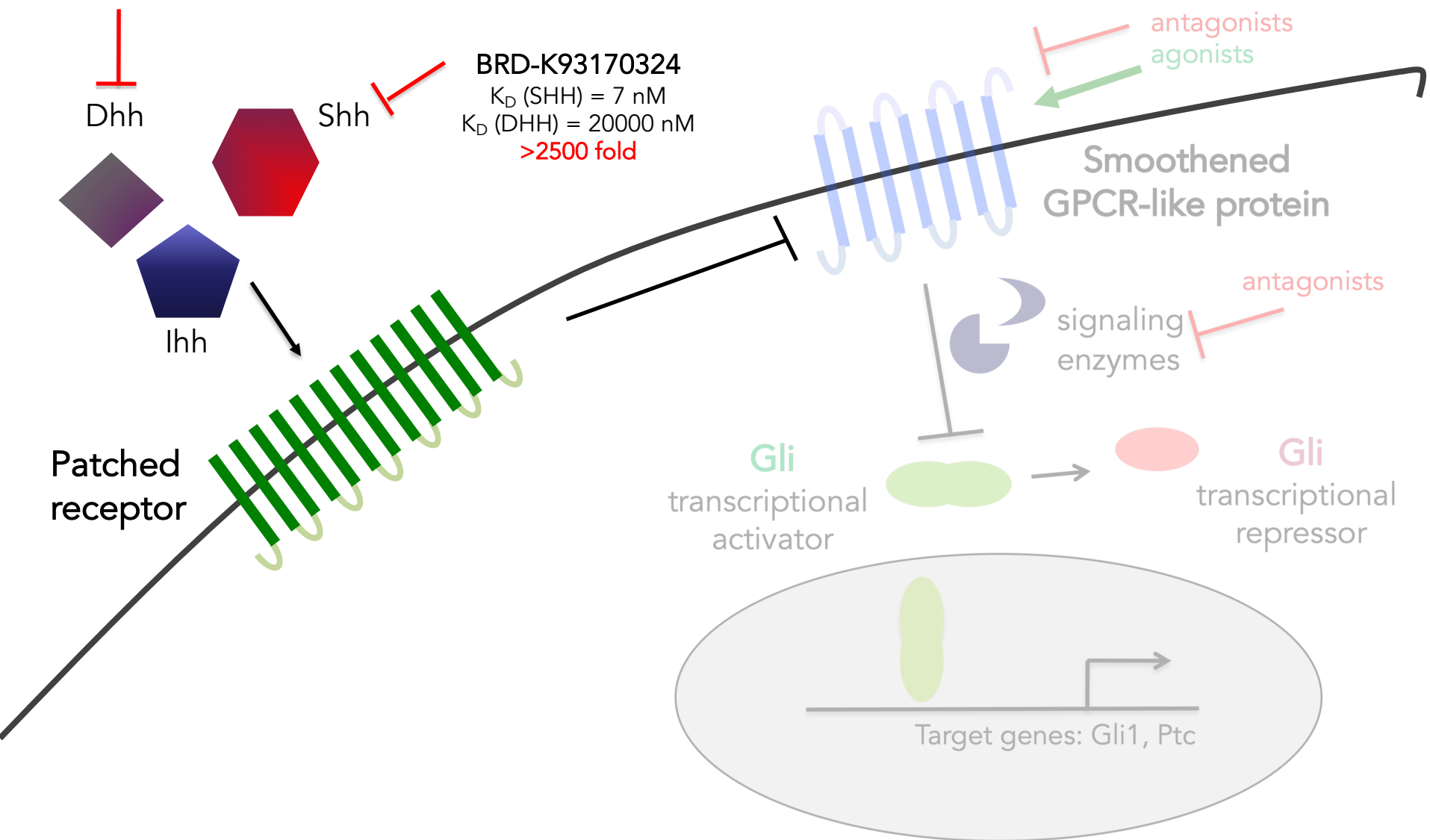
robotnikinin treatment shows no signs of inflammation or failed skin differentiation

Improving Hh homolog selectivity

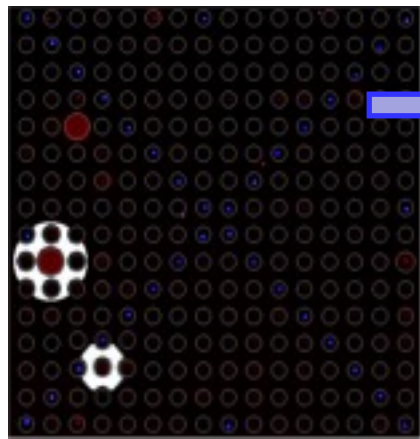
medicinal chemistry

BRD-K81967595
 K_D (SHH) = 9500 nM
 K_D (DHH) = 13 nM
>500 fold

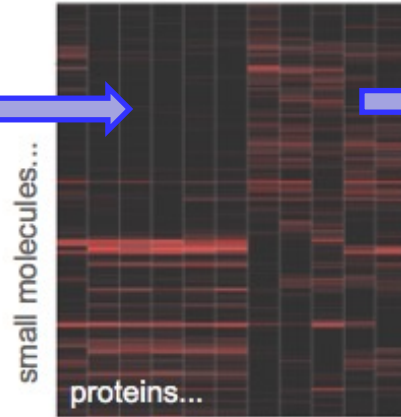
BRD-K93170324
 K_D (SHH) = 7 nM
 K_D (DHH) = 20000 nM
>2500 fold



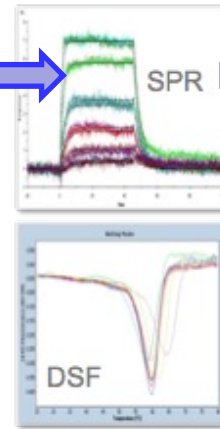
Path for probe discovery, validation, and development



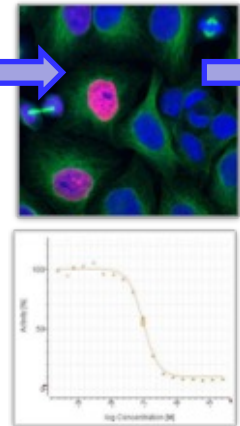
SMM



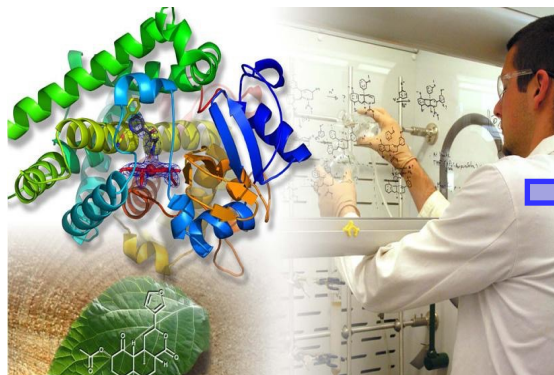
specificity analysis



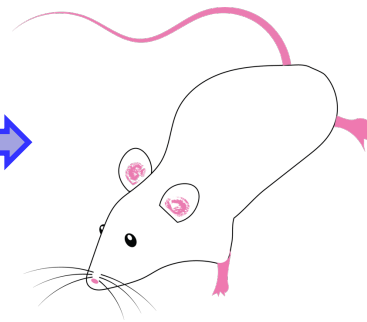
secondary binding



cellular or biochemical assays



optimize molecules using chemistry



additional cell biology animal models

Into the eye of the cytokine storm

New Direction - IL-4 and profiling pro-and anti-inflammatory cytokines



Arturo Vegas, Boston University



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Letters

A Small-Molecule Inhibitor to the Cytokine Interleukin-4

Sean P. Quinnell, Becky S. Leifer, Stephen T. Nestor, Kelly Tan, Daniel F. Sheehy, Luke Ceo, Shelby K. Doyle, Angela N. Koehler, and Arturo J. Vegas*

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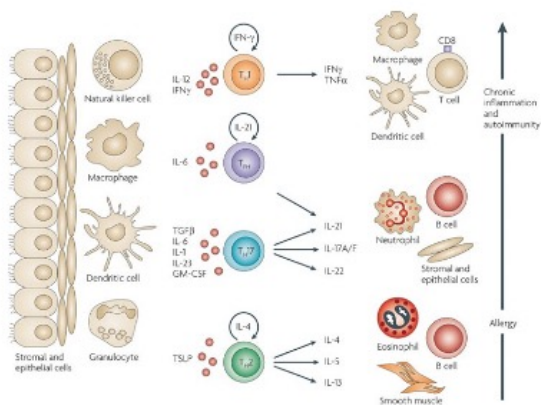
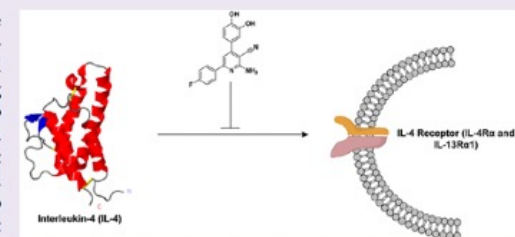
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Supporting Information

ABSTRACT: Interleukin-4 (IL-4) is a multifunctional cytokine and an important regulator of inflammation. When deregulated, IL-4 activity is associated with asthma, allergic inflammation, and multiple types of cancer. While antibody-based inhibitors targeting the soluble cytokine have been evaluated clinically, they failed to achieve their end points in trials. Small-molecule inhibitors are an attractive alternative, but identifying effective chemotypes that inhibit the protein–protein interactions between cytokines and their receptors remains an active area of research. As a result, no small-molecule inhibitors to the soluble IL-4 cytokine have yet been reported. Here, we describe the first IL-4 small-molecule inhibitor identified and characterized through a combination of binding-based approaches and cell-based activity assays. The compound features a nicotinonitrile scaffold with micromolar affinity and potency for the cytokine and disrupts type II IL-4 signaling in cells. Small-molecule inhibitors of these important cell-signaling proteins have implications for numerous immune-related disorders and inform future drug discovery and design efforts for these challenging protein targets.

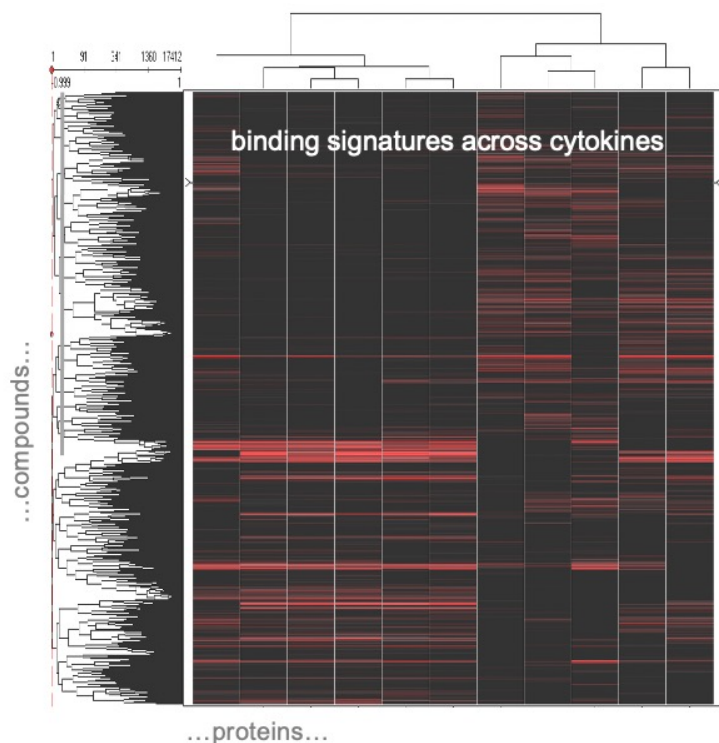


Nature Reviews | Drug Discovery

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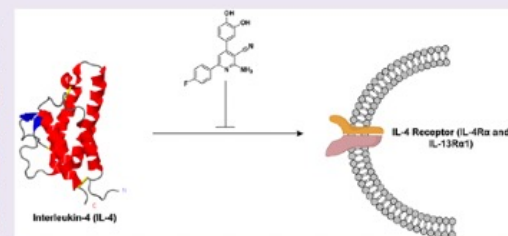
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Upcoming Lectures

2/9/23	Lecture 1	Intro to chemical biology: small molecules, probes, and screens
2/14/23	Lecture 2	Small Molecule Microarray (SMM) technique
2/16/23	Lecture 3	Our protein target – MAX
2/21/23	No Lecture	
2/23/23	Lecture 4	Quantitative evaluation of protein-ligand interactions
2/28/23	Lecture 5	An SMM ligand discovery vignette for sonic hedgehog
3/2/23	Lecture 6	KB-0742: A Phase 2 clinical candidate discovered by SMMs
3/7/23	Lecture 7	Wrap up discussion for Mod 1 experiments and report