Experimental Therapeutics Centre

D3

Bridging academic science and clinical research in the search for novel targeted anti-cancer agents

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Experimental Therapeutics Centre & D3, A*STAR

ESMO Conference, Singapore

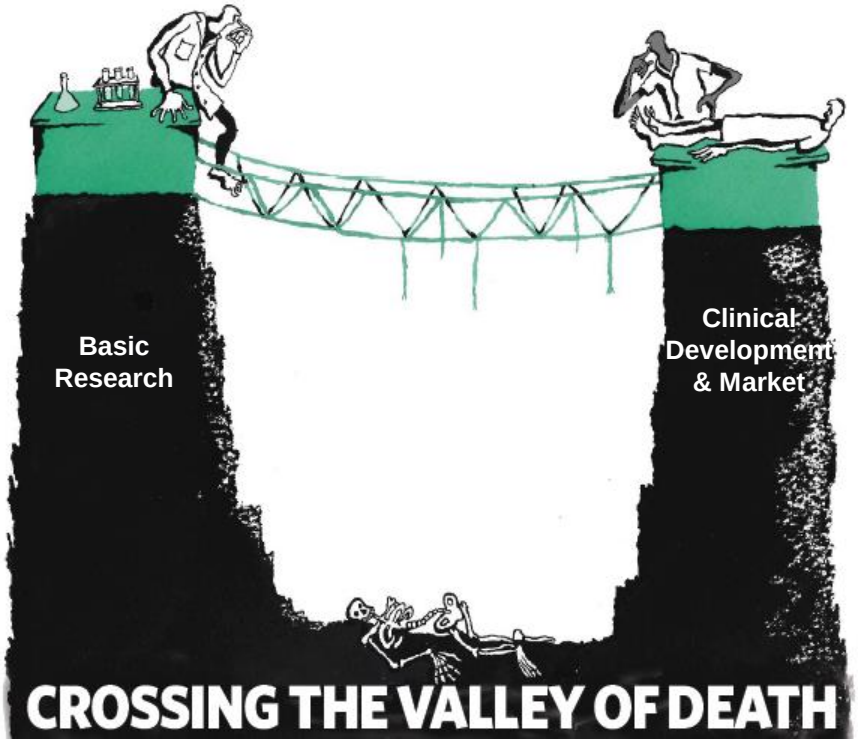
19th December 2015

Debating points

- Who we are – Mission & strategies
- Collaborative model between public sector and industry
- Two projects producing clinical candidates: Mnk/Abl, Wnt/porcupine
- Possible role of Public Sector R&D in drug discovery in Asia

Mission of ETC/D3 – Capturing the Opportunities!

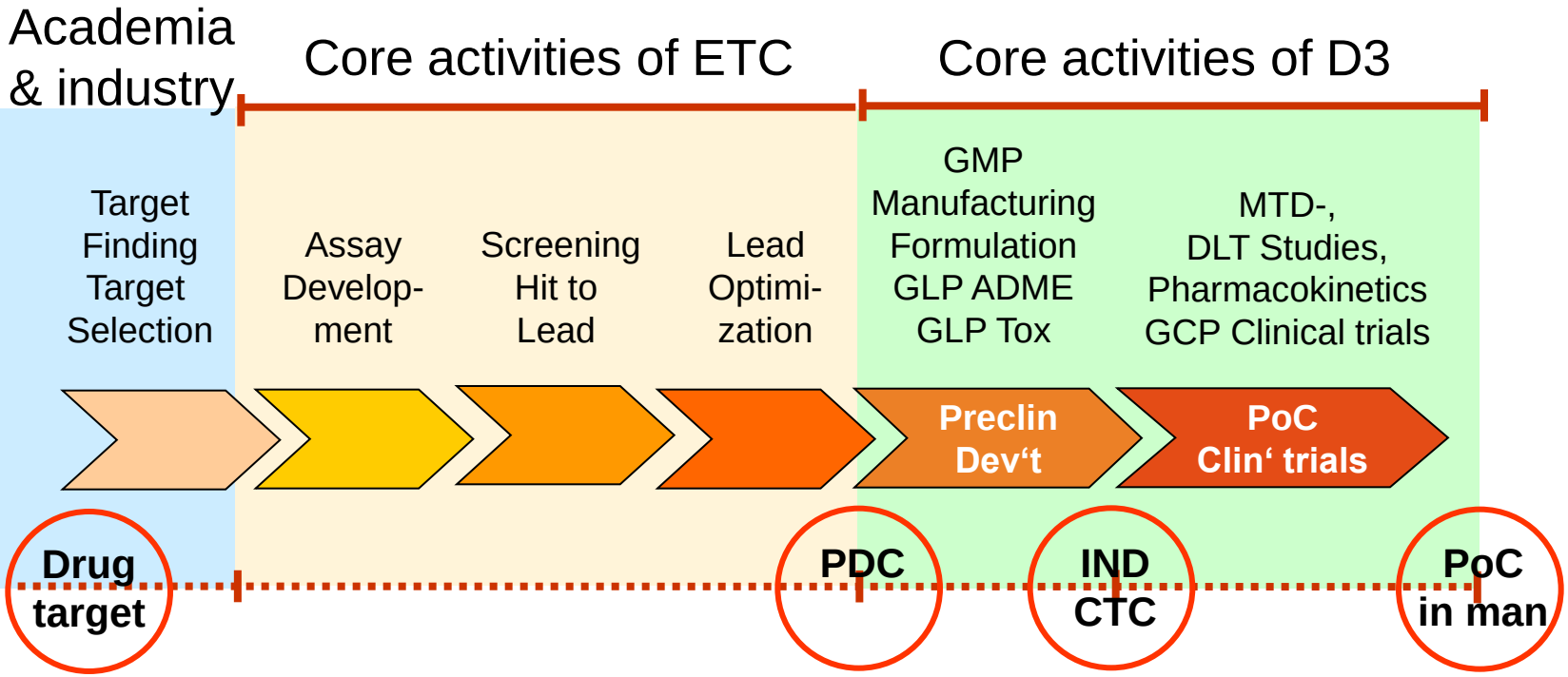
- To build bridges between basic science and the clinic – translational R&D
- To guide early-stage scientific discoveries towards Proof-of-Concept clinical trials in man
- To serve unmet medical needs in Singapore and the region through innovative product candidates
- To generate economic benefit



Picture : Translational Research, Nature Vol 453, 12 June 2008

From Drug Target to Proof-of-Concept in man!

Pathway for a small-molecular weight compound, as an example



Legend

GCP, Good Clinical Practice
GMP, Good Manufacturing Practice
GLP, Good Laboratory Practice
DLT, Dose-limiting toxicity
ADME, Absorption, Distribution, Metabolism, Excretion

IND/CTC, Permission to start clinical trials
MTD, Maximally tolerated dose
PDC, Preclinical Development Candidate
PoC, Proof-of-Concept

Comprehensive Capabilities and Resources

Primary focus on Oncology and Infectious Diseases

Skill bases & Technologies

- *Cell-based Assay Development*
- *Protein Biochemistry*
- *High Throughput Screening*
- *Medicinal Chemistry*
- *Antibody Technologies*
- *Molecular Diagnostics*
- *Analytics – Mass Spectrometry*
- *High end NMR*
- *Bioinformatics*
- *Preclinical Pharmacology*

Priority Therapeutic & Product Focus

- **Oncology**
- **Infectious Diseases**
- **Other Indications**

Products

- **Drug Candidates**
- **Vaccine Candidates**
- **Diagnostics & Biomarker Candidates**

Resources of ETC & D3

- ETC: 88 FTEs, 50% for biochemistry, cell biology, analytics, HTS – 50% for medicinal chemistry and computational chemistry; 24 FTEs outsourced
- D3: small team of experts plus range of specialized consultants

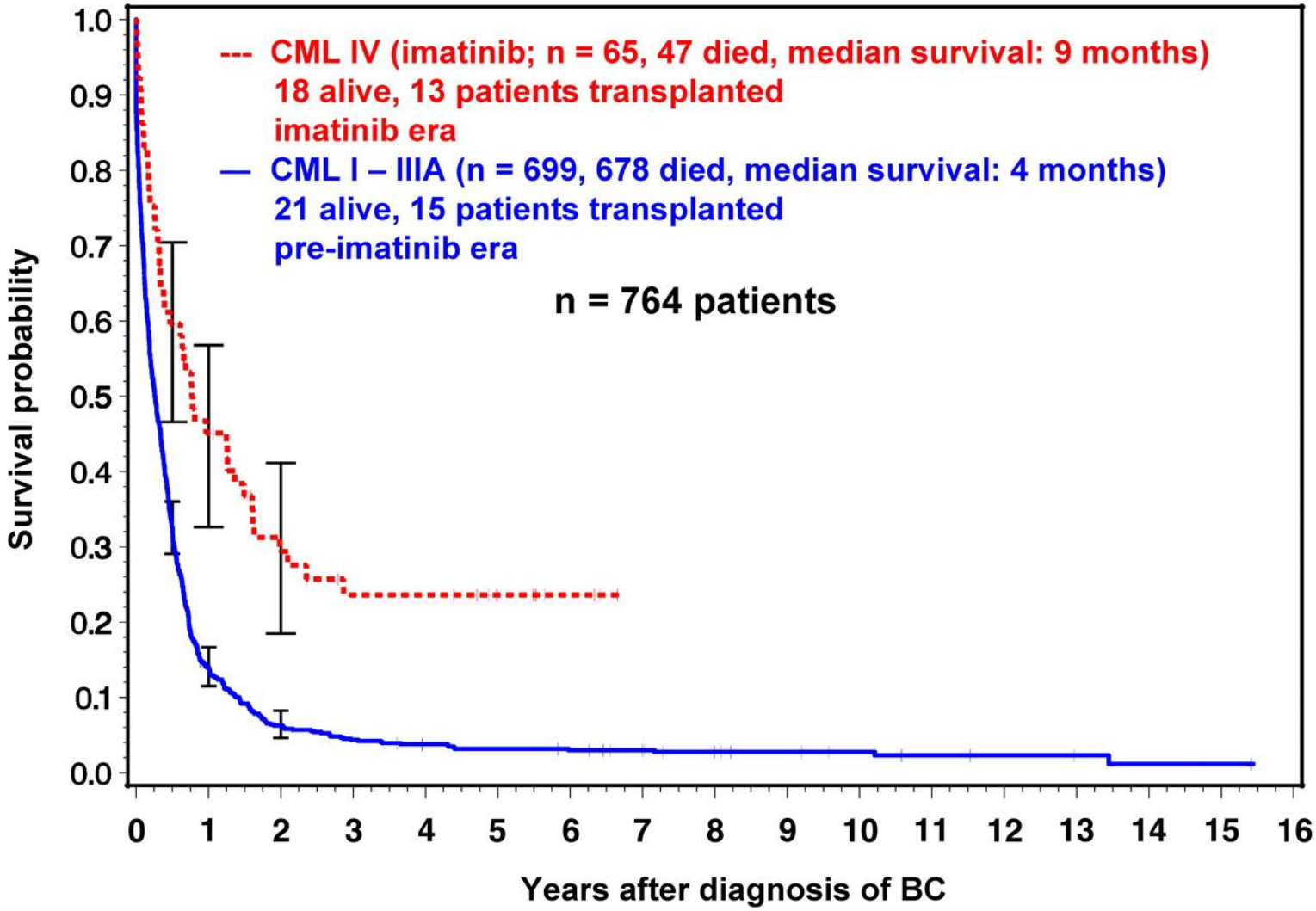
A first example of a drug candidate born in Singapore

A case of inhibiting simultaneously two targets

- one well known and hard to target: BCR-ABL/BCR-ABL^{T315I}
- one with a complex biology: MNK1/2
- Indication: drug-resistant Blast Phase of CML, imatinib-resistant PH1+ALL, DLBCL?

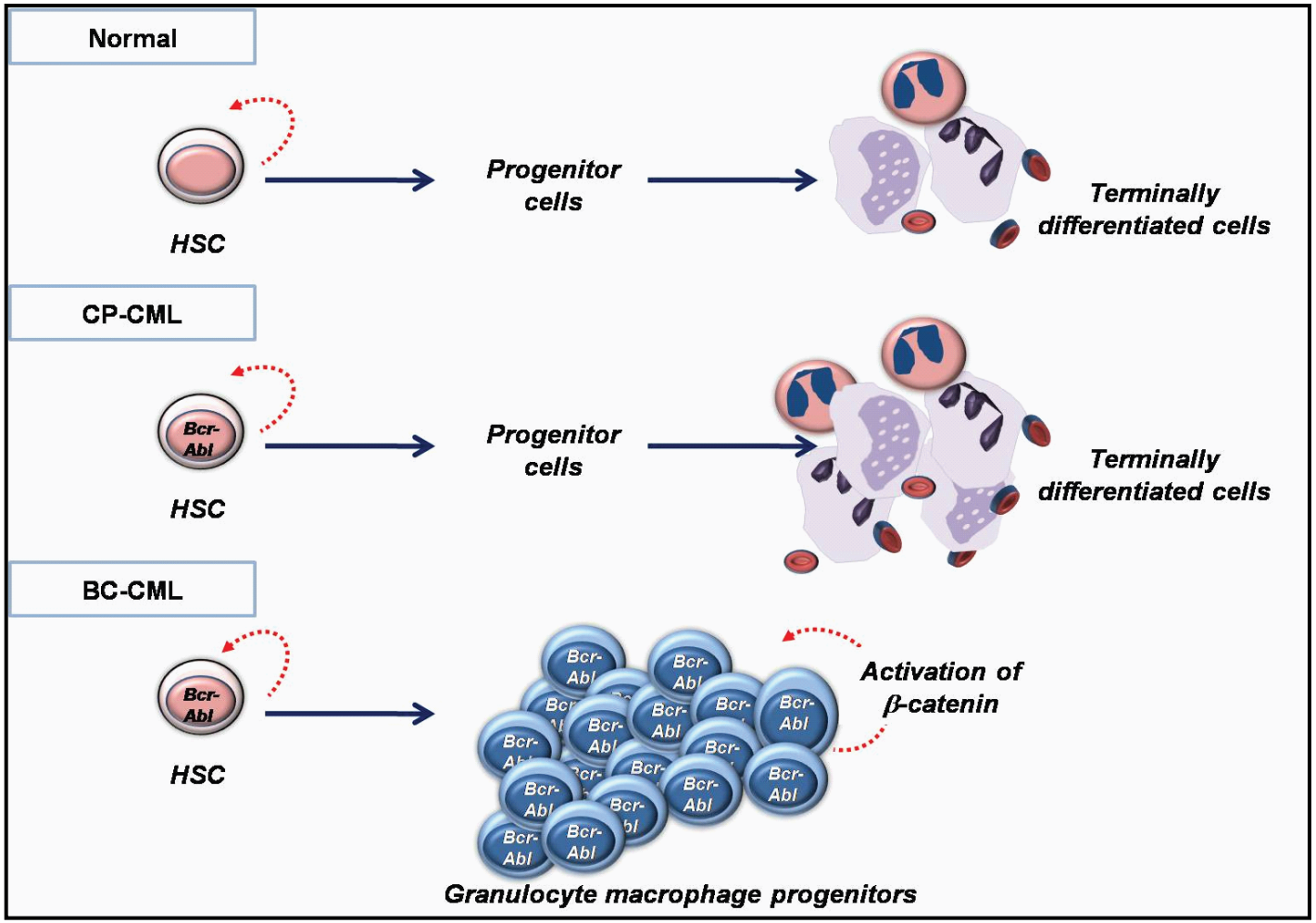
➢ *Collaboration between Prof Tiong S. Ong and Dr Sharon Lim, Duke-NUS and ETC/D3*

‘Blast crisis is the major remaining challenge in the management of Chronic Myelogenous Leukemia (CML)’



Hehlmann R, How I treat CML blast crisis, Blood 2013

CML – increased & unregulated growth of myeloid cells in the bone marrow & their accumulation in the blood



β-catenin-mediated self-renewal is an important feature of myeloid blast crisis granulocyte macrophage progenitors

eIF4E overexpression & phosphorylation activates β-catenin in Blast Crisis (BC) Leukemic Stem Cells (LSCs)

Lim et al. PNAS 2013

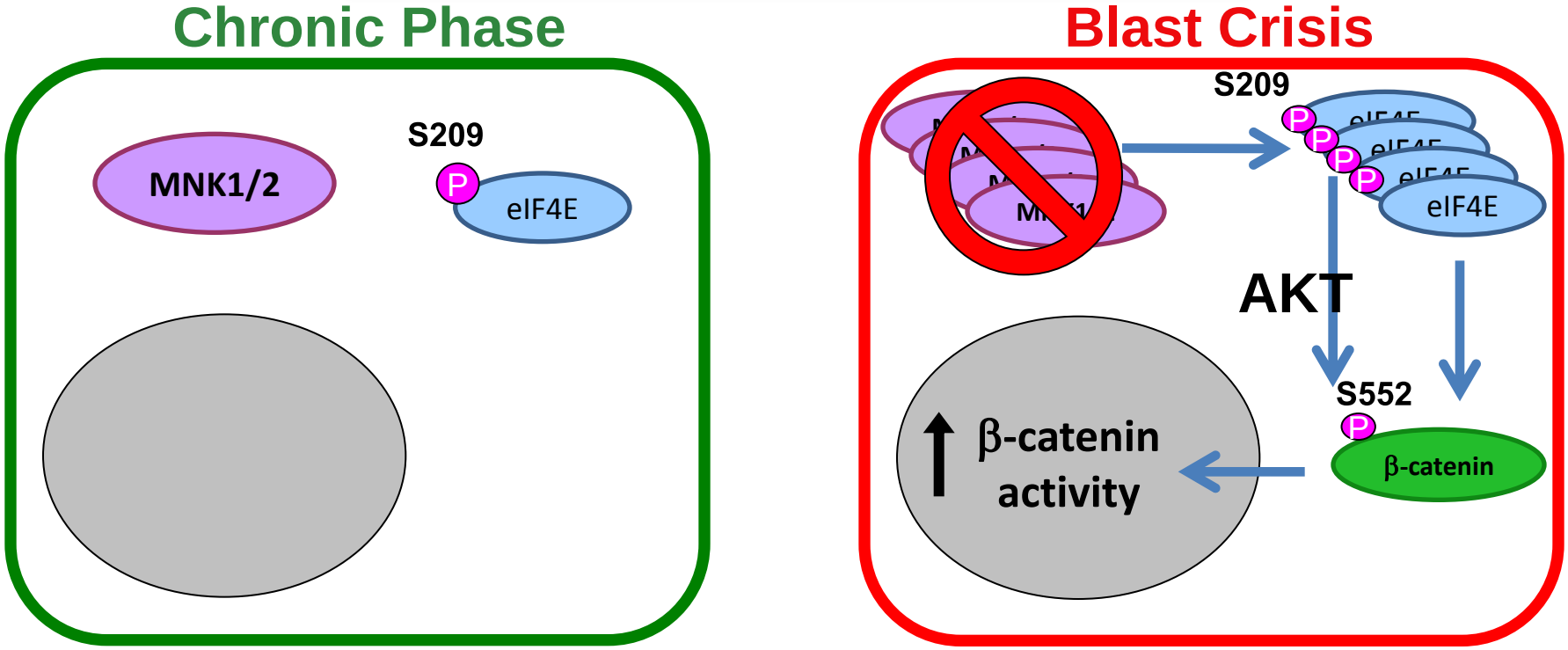
PNAS

Targeting of the MNK–eIF4E axis in blast crisis chronic myeloid leukemia inhibits leukemia stem cell function

Sharon Lim[a], Tzuen Yih Saw[a], Min Zhang[b], Matthew R. Janes[c], Kassoum Nacro[d], Jeffrey Hill[d], An Qi Lim[a], Chia-Tien Chang[a], David A. Fruman[c], David A. Rizzieri[e], Soo Yong Tan[f], Hung Fan[b,c], Charles T. H. Chuah[a,g], and S. Tiong Ong[a,g,h,i,1]

[a]Cancer and Stem Cell Biology Program, Duke–National University of Singapore Graduate Medical School, Singapore 169857; [b]Division of Hematology/Oncology, Department of Medicine, University of California, Irvine, CA 92868; [c]Institute for Immunology and Department of Molecular Biology & Biochemistry, University of California, Irvine, CA 92697; [d]Experimental Therapeutics Centre, Agency for Science, Technology and Research (A*Star), Singapore 138669; [e]Division of Cellular Therapy, Department of Medicine, Duke University Medical Center, Durham, NC 27710; Departments of [f]Pathology and [g]Hematology, Singapore General Hospital, Singapore 169856; [h]Department of Medical Oncology, National Cancer Centre, Singapore 169610; and [i]Division of Medical Oncology, Department of Medicine, Duke University Medical Center, Durham, NC 27710

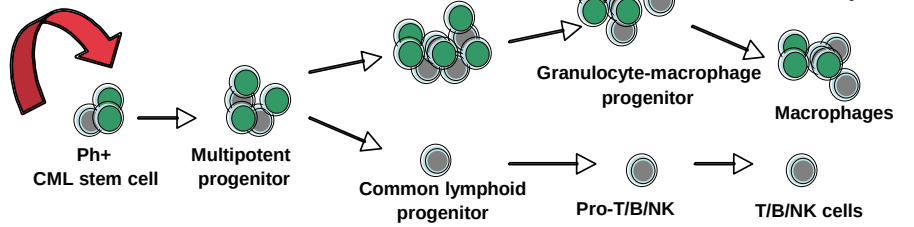
Edited by Dennis A. Carson, University of California, San Diego, La Jolla, CA, and approved May 10, 2013 (received for review February 8, 2013)



Clinical Hypothesis

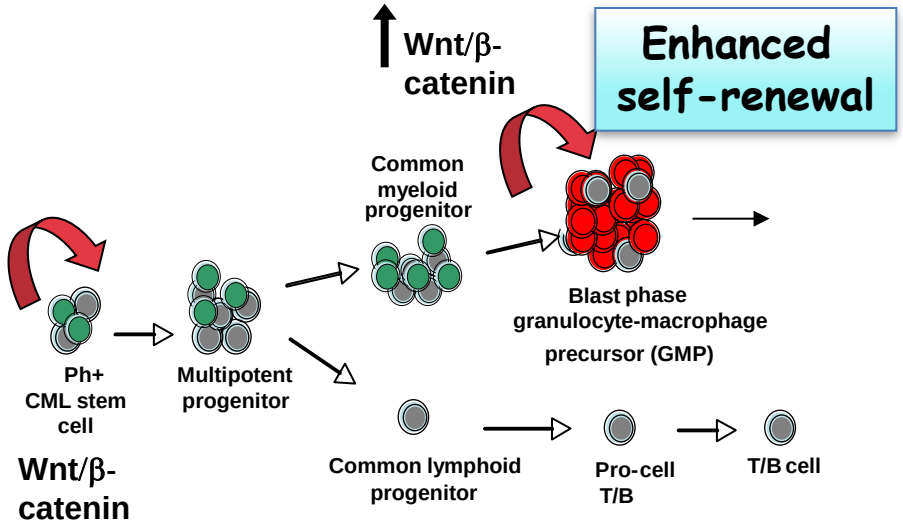
While BCR-ABL inhibitors control CP CML, MNK inhibitors by targeting BC LSCs, will control BC CML

CP CML



Controlled with BCR-ABL inhibitors

BC CML



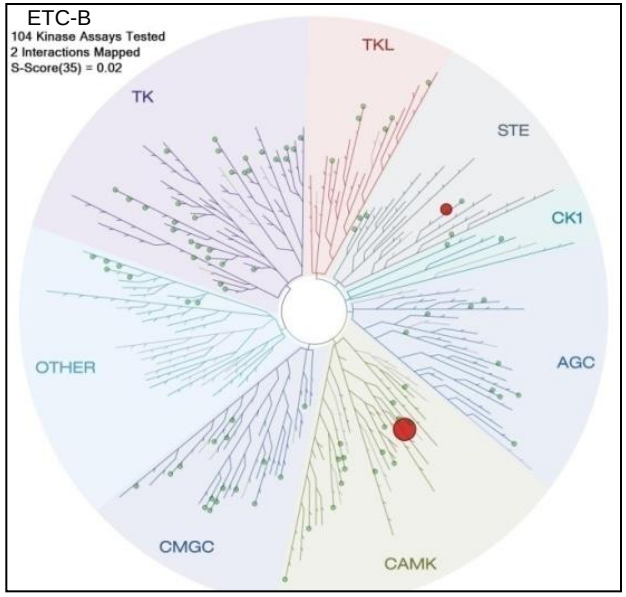
Controlled with MNK inhibitors *assayed by:*

In vitro **Serial Replating Assay (SRA)**	***In vivo*** **Serial Transplant-tion Assay**

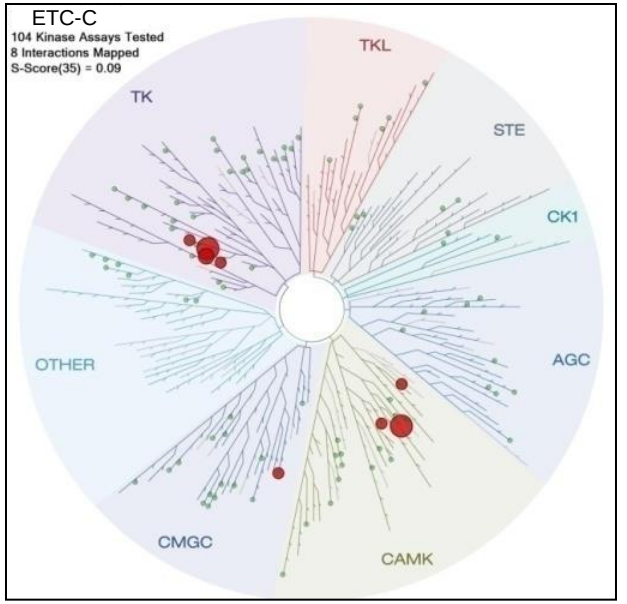
Selective Mnk Inhibitors

Profile of ETC-B and ETC-C: *in vitro* selectivity

ETC-B



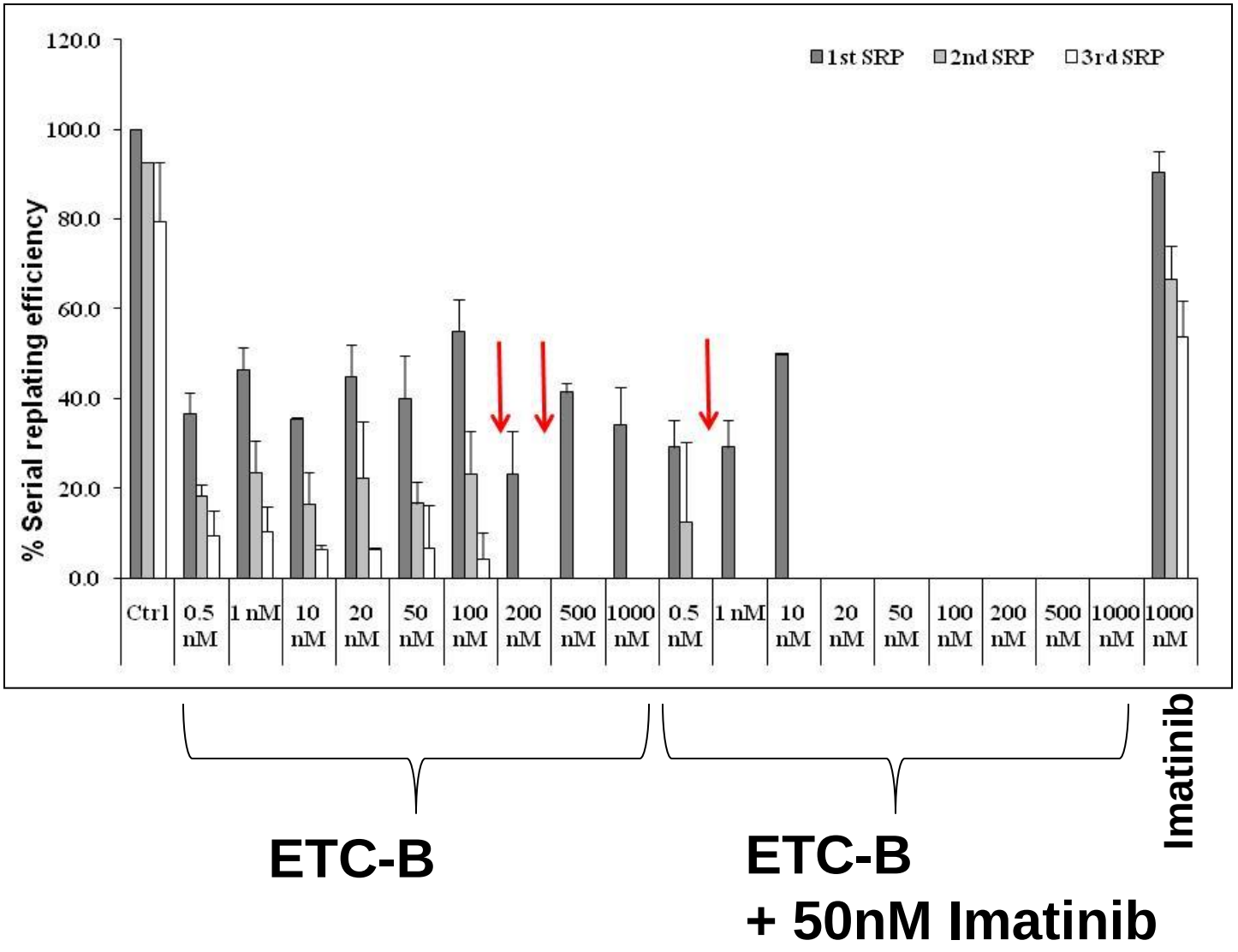
ETC-C



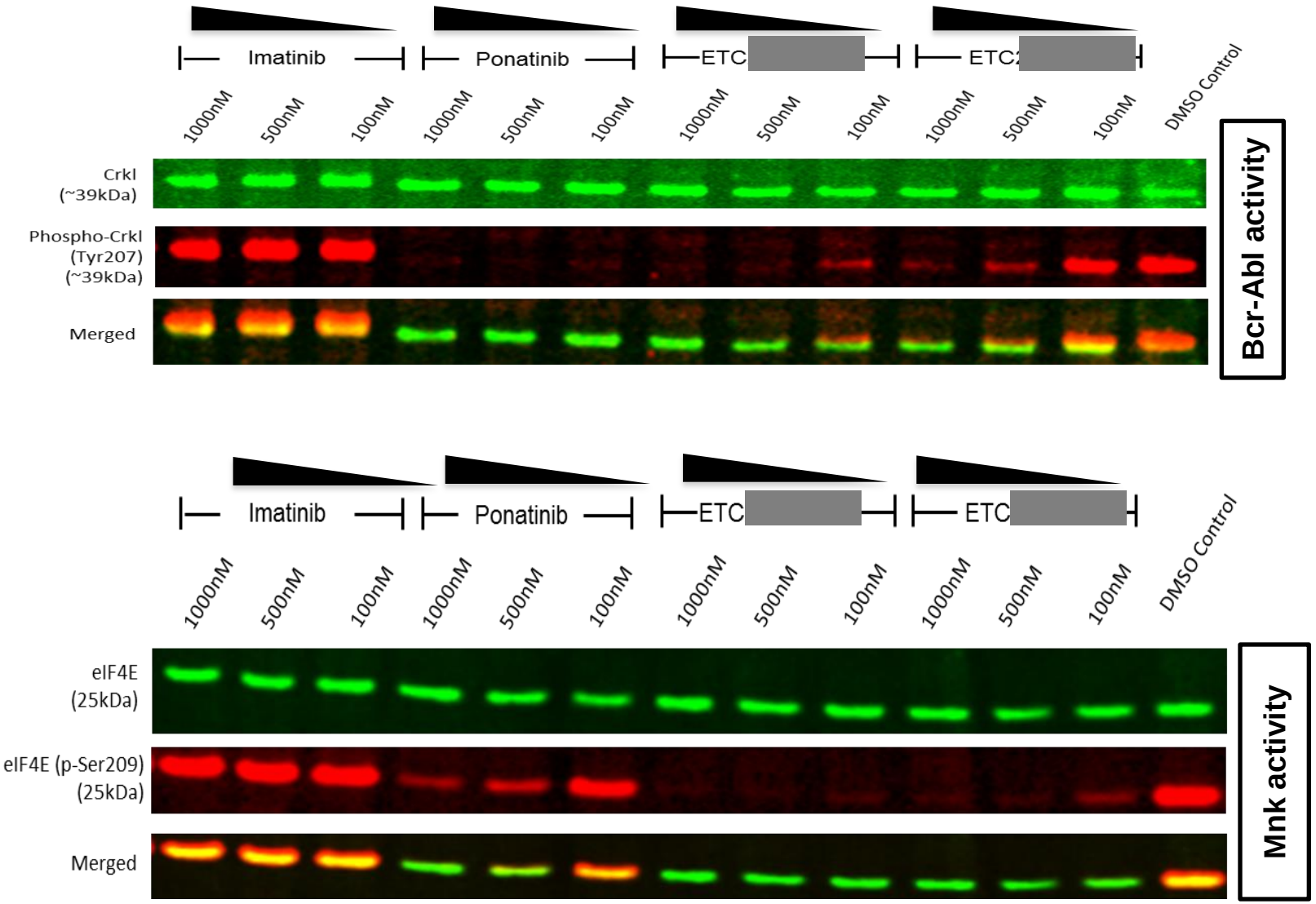
% Inhibition	Number of kinases inhibited	
	ETC-B	ETC-C
>90	1	3

Screening of 104 kinases @ 1 μM

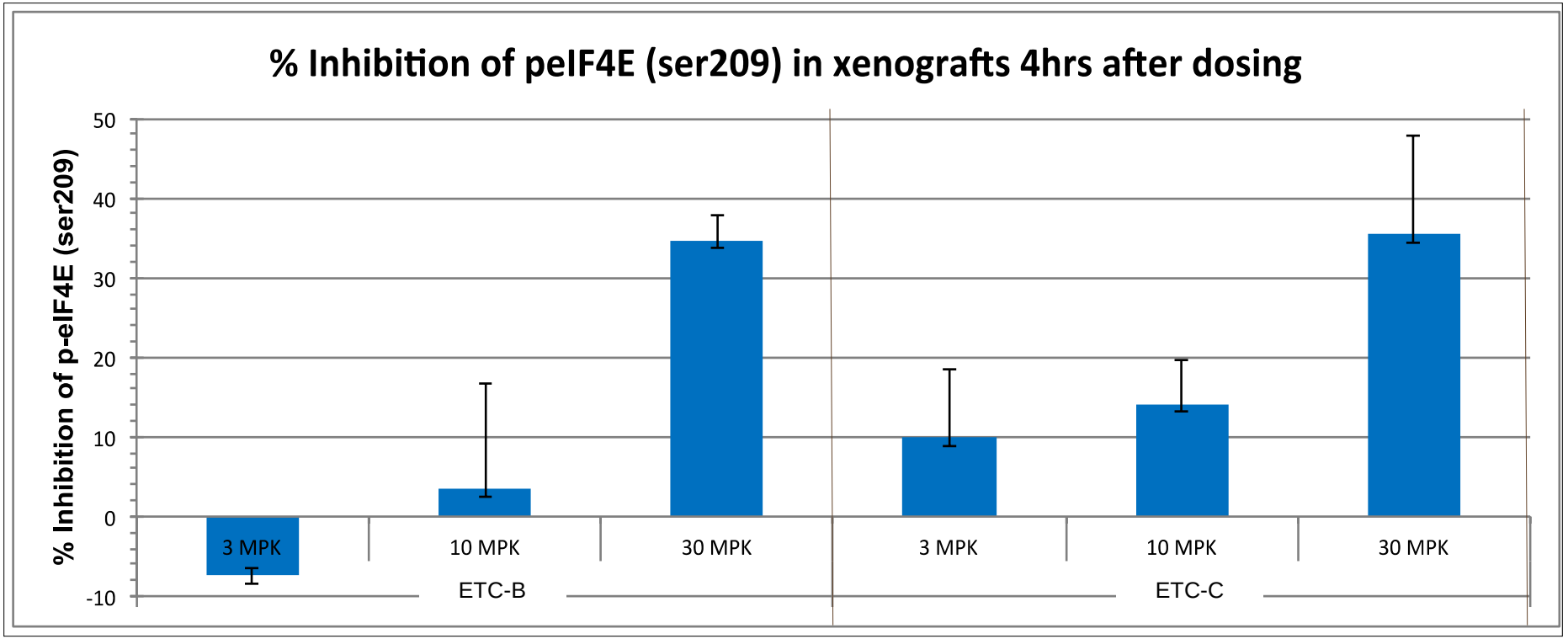
Combination of Mnk & Bcr-Abl Inhibitors on Primary BC CML LSCs



In Vitro Validation of Biomarkers in CML Cell Line K562 After Compound Treatment



In Vivo Validation of Biomarkers in K562 Xenograft Model



- **Dose dependent inhibition of p-eIF4E observed with both compounds**
- **~35% inhibition of p-eIF4E (ser209) is observed in tumor xenograft excised from mice treated with 30MPK of compound treatment**

A second example – Development of Wnt Signaling Inhibitors

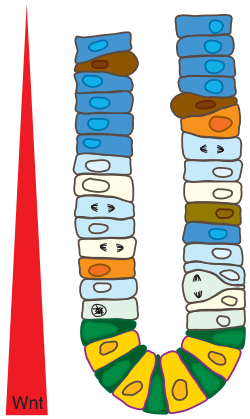
Collaboration between Prof David Virshup/Dr Babita Madan, Duke-NUS and ETC/D3

To identify compounds that block Wnt secretion with the potential to act as specific inhibitors of Wnt signaling for anti-cancer drug development

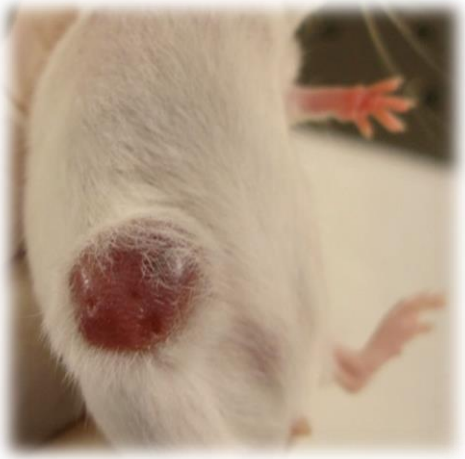
What Wnts do….

- Regulate cell fate, differentiation and morphogenesis during development
- Regulate stem cell proliferation and differentiation throughout life
- Implicated in diverse processes including bone metabolism, inflammation, wound healing, atherosclerosis, angiogenesis, pathologic fibrosis
- Dysregulated in multiple cancers by mutation, epigenetics, miRNAs

Xenopus axis determination

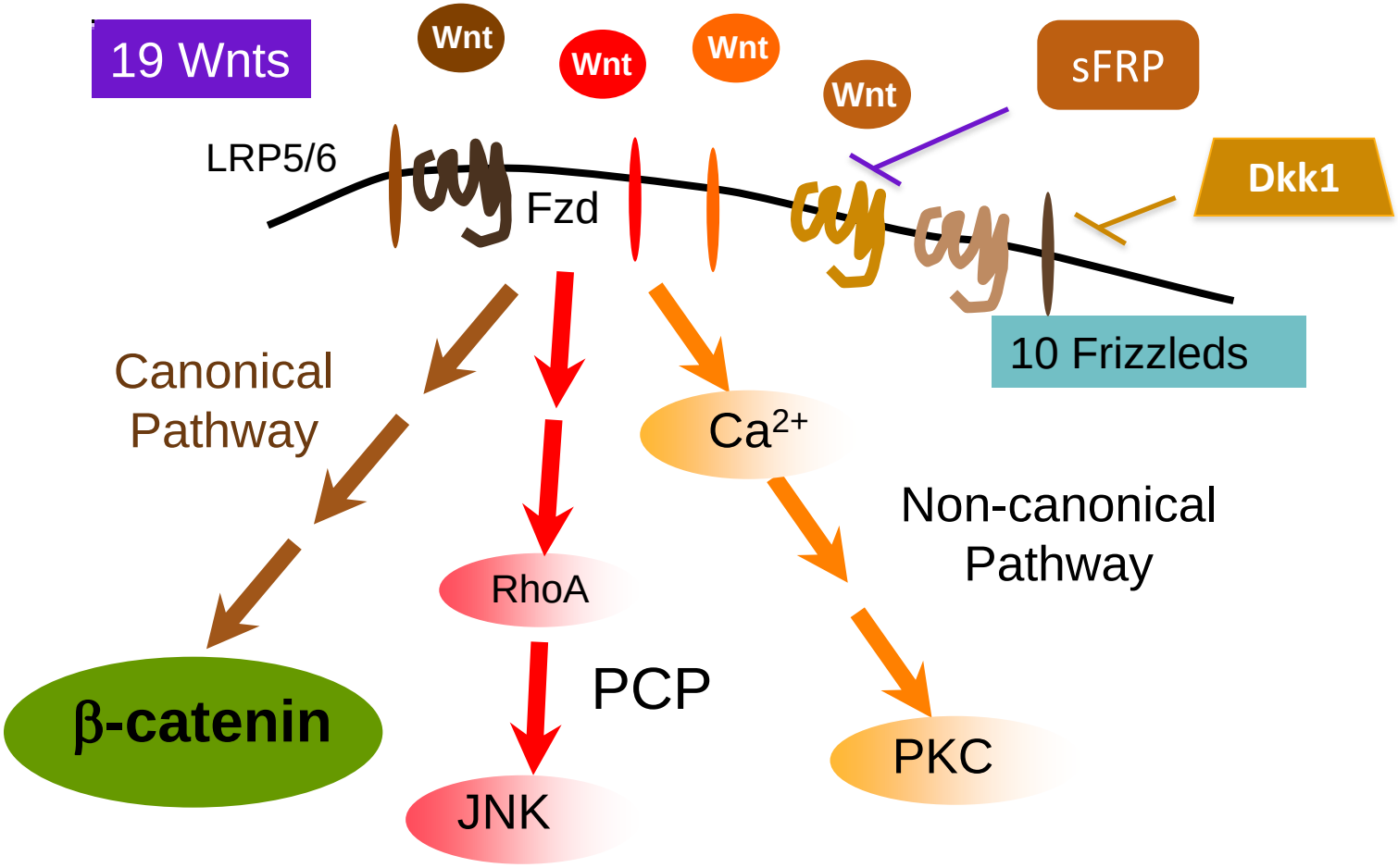


Intestinal crypt driver



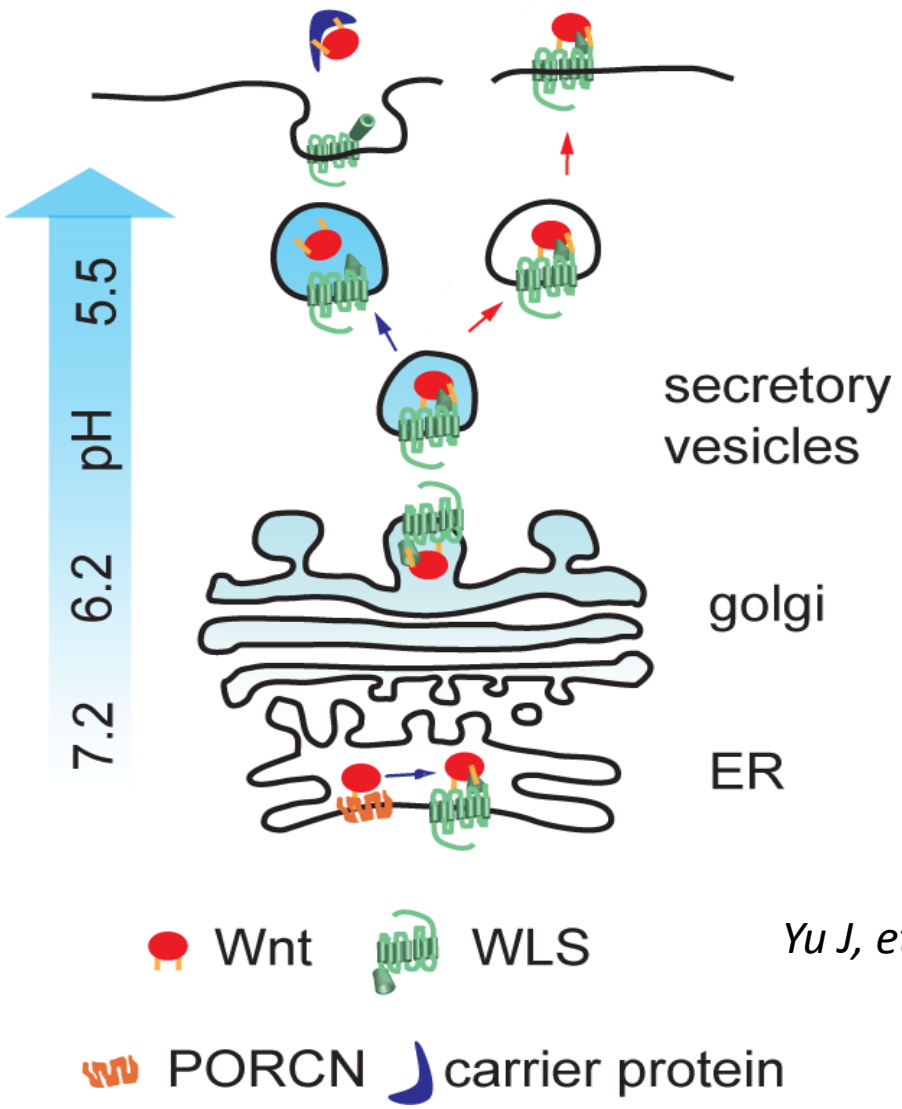
MMTV->Wnt1

Wnt Signaling is really complex



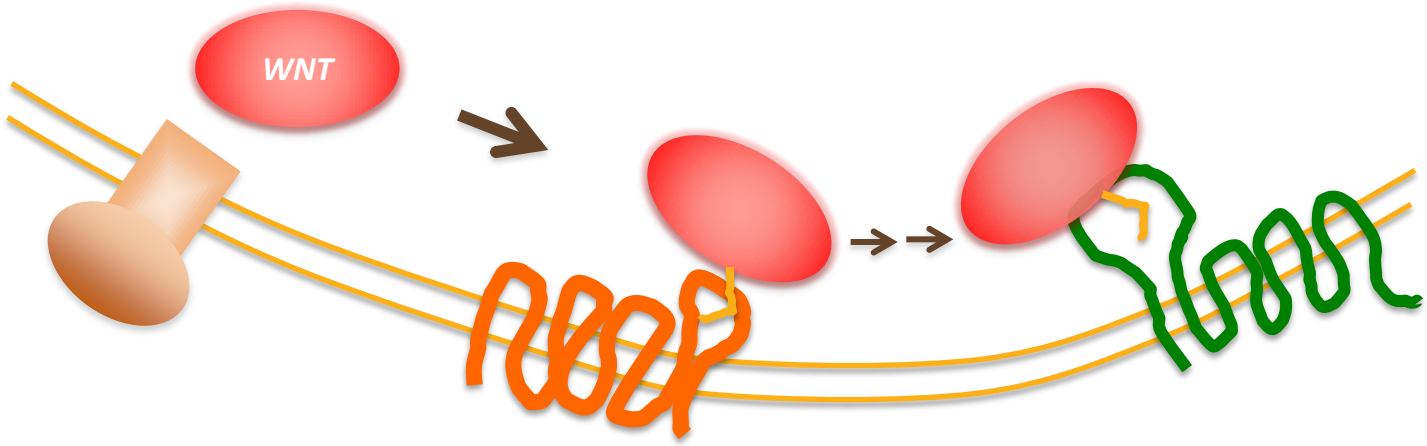
Other important things happen

The Wnt secretion pathway



Yu J, et al., Dev Cell. (2014) in press

PORCN and WLS are key regulators of global Wnt production



PORCN

Membrane **B**ound

O-**a**cyl **t**ransferase

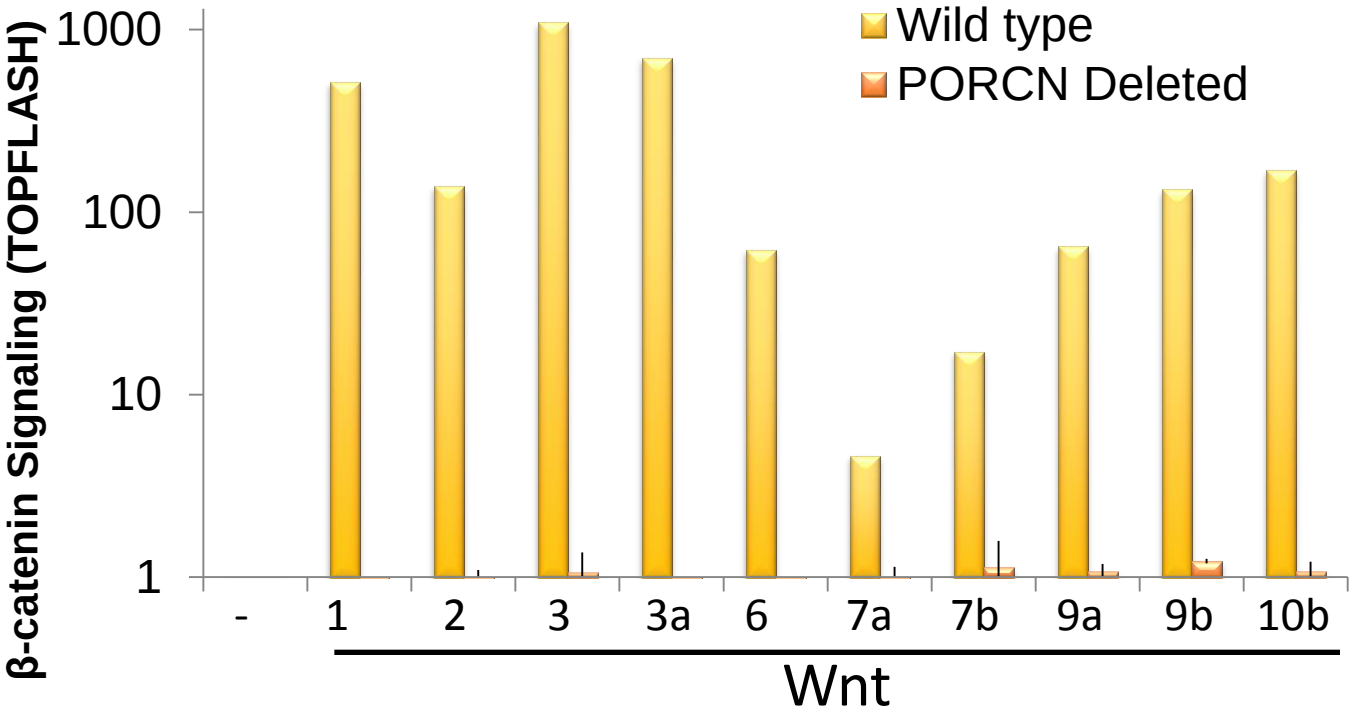
Transferase palmitate to conserved serine on Wnt

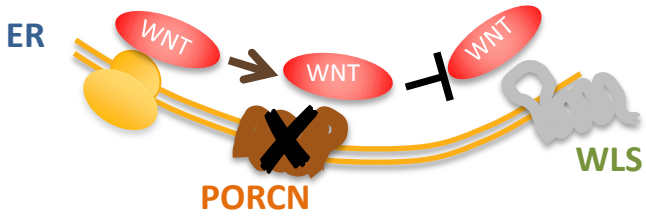
WLS

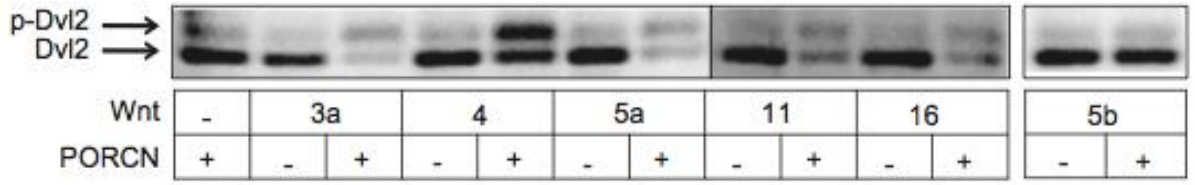
Transports palmitoleated Wnt to PM

Genetic loss of PORCN abrogates function of all human Wnts

PORCN Null HT1080 cells

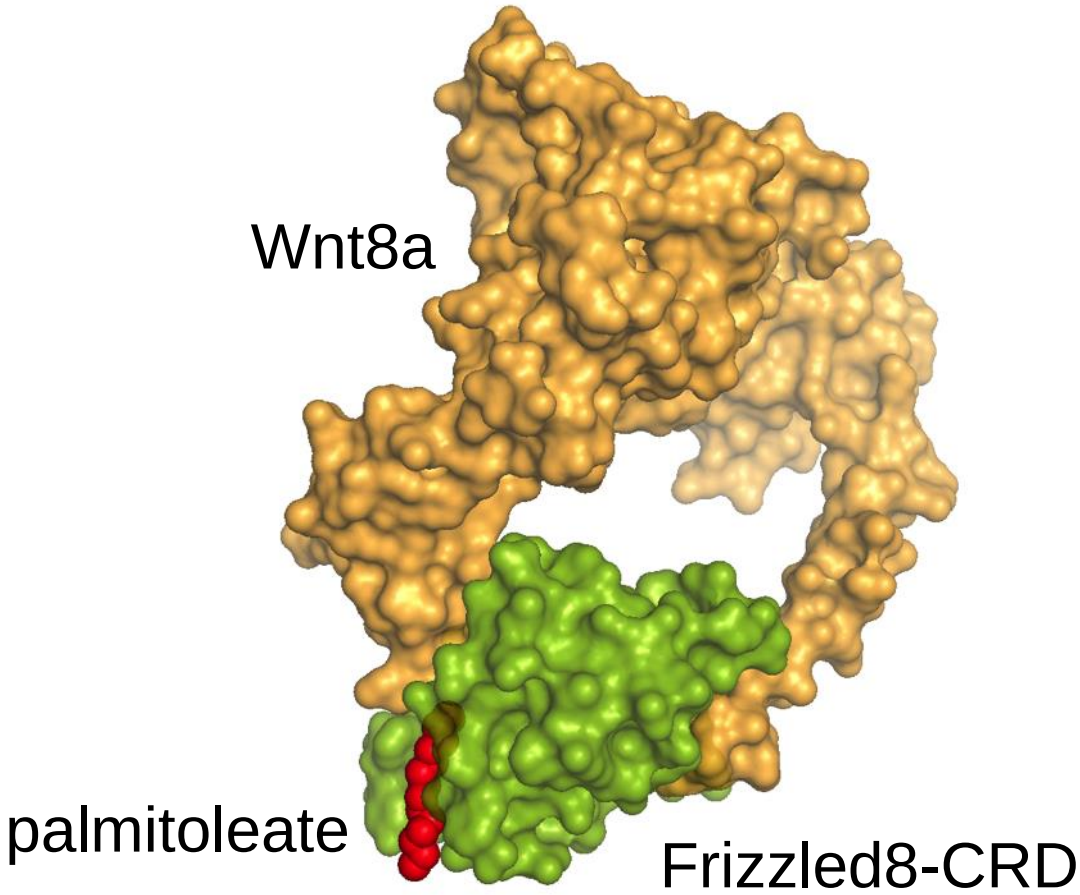






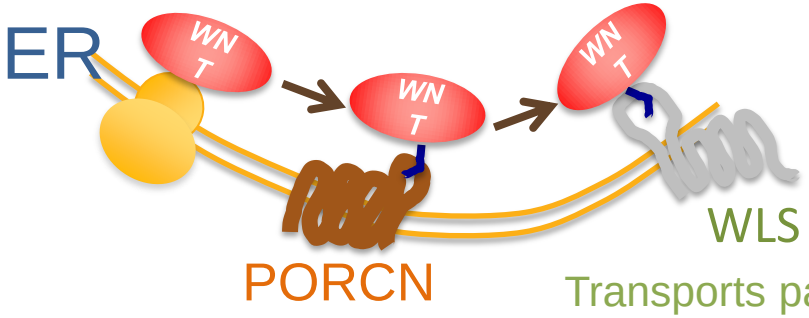
Najdi R, et al., Differentiation. 2012;84:203–13.

Essential role for palmitoleate in ligand-receptor interaction



Janda, C.Y., Waghray, D., Levin, A.M., Thomas, C., and Garcia, K.C. (2012). Structural Basis of Wnt Recognition by Frizzled. Science 337, 59–64.

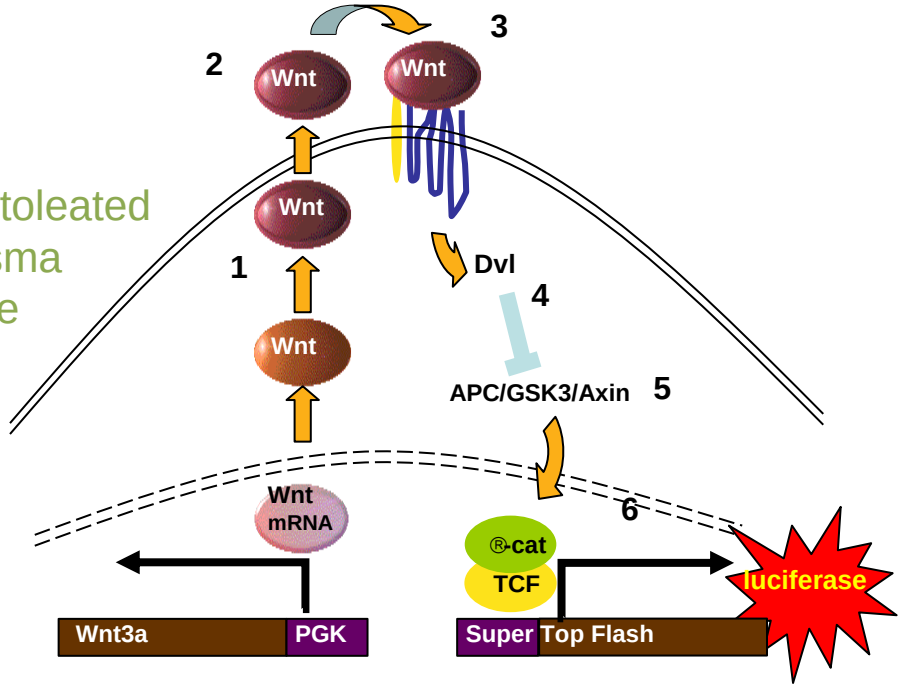
Can we interfere with Wnt Production using small molecules ?



O-acyl transferase, transfers palmiteolate to conserved serine on all Wnts

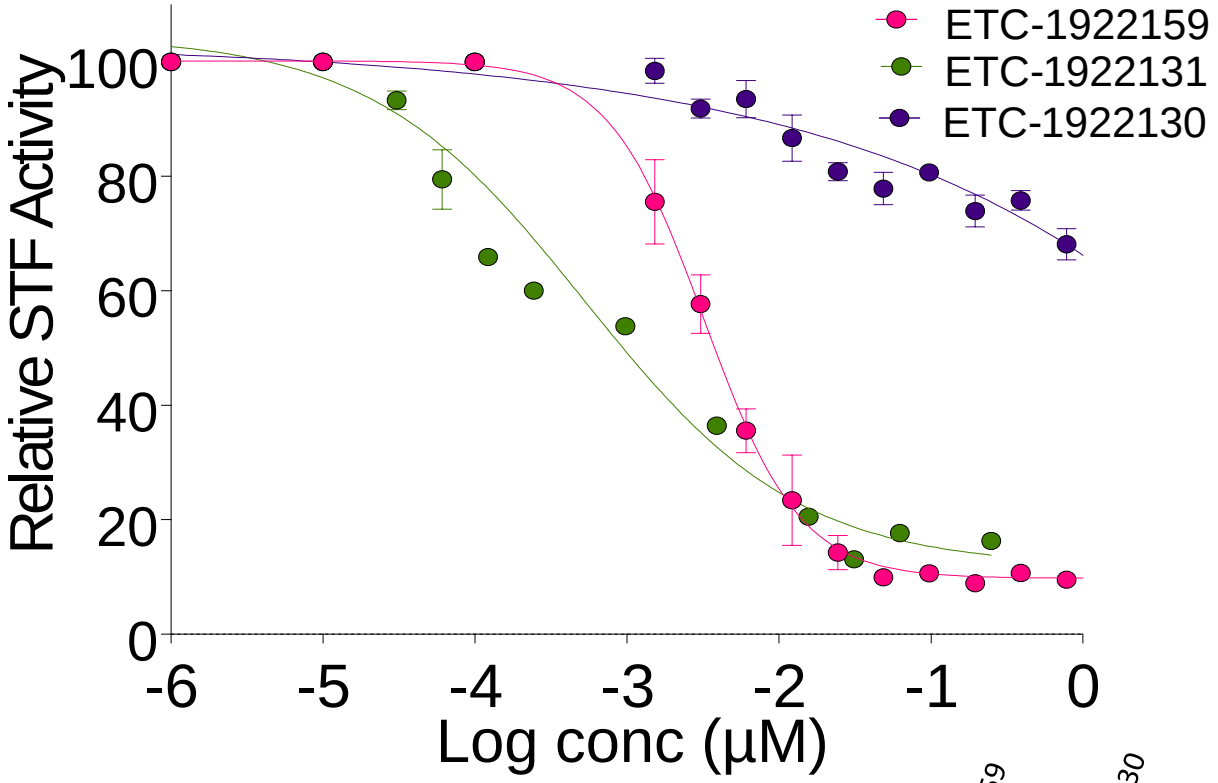
Transports palmitoleated Wnt to Plasma membrane

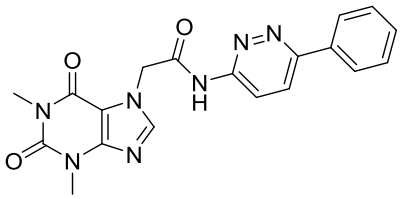
Wnt Pathway Multistep Drug Screen



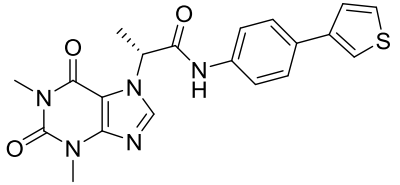
Gary Coombs; May Ann Lee and Horst Flotow at Experimental Therapeutics Centre

Inhibition of Wnt/β-catenin activity in STF3a Cells

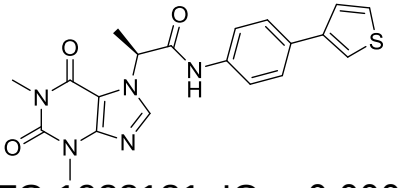




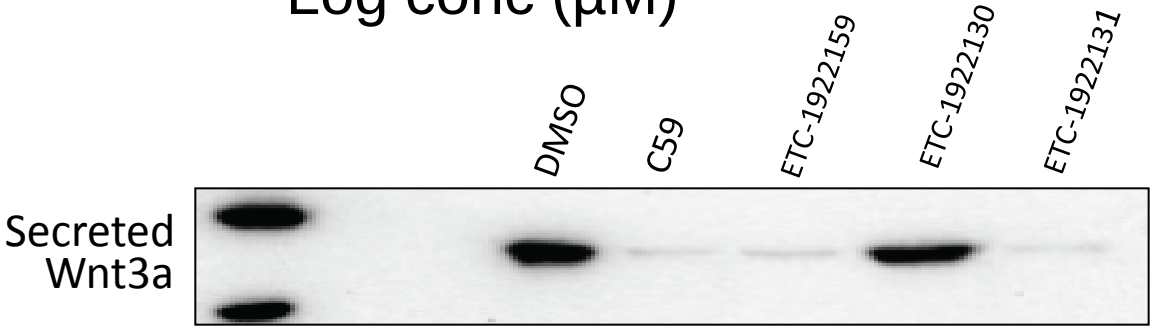
ETC-1922159 IC_{50}: 0.003μM



ETC-1922130 IC_{50}: NI



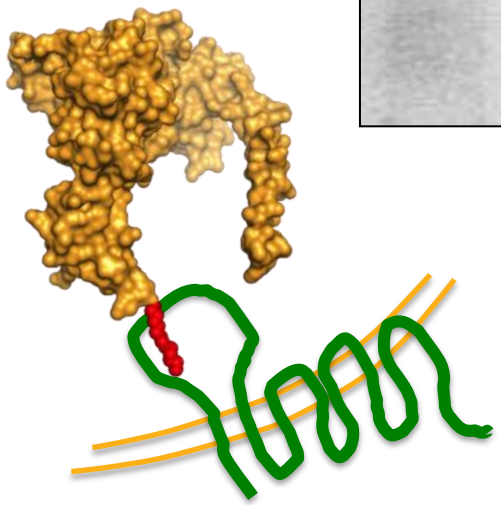
ETC-1922131 IC_{50}: 0.0004μM

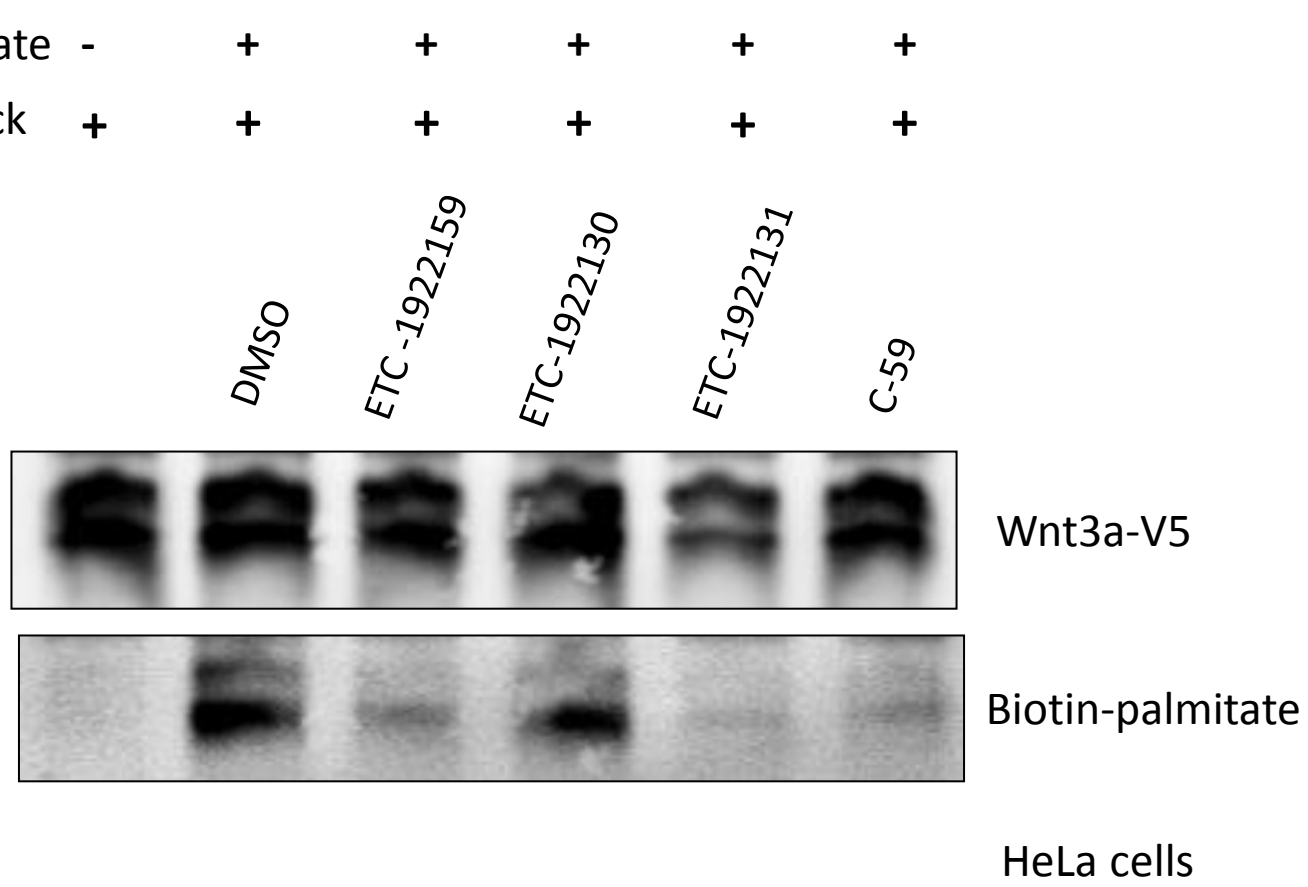


Ke Zhiyuan

Palmitoleation of Wnt3a is inhibited by PORCN inhibitors

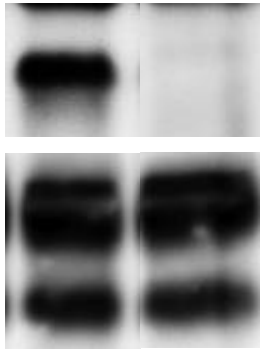
- Label cells with alkyne-palmitate
- IP Wnt via V5 tag
- Click with azido-Biotin
- SDS-PAGE
- Probe for Wnt3a-V5, Biotin-palmitate

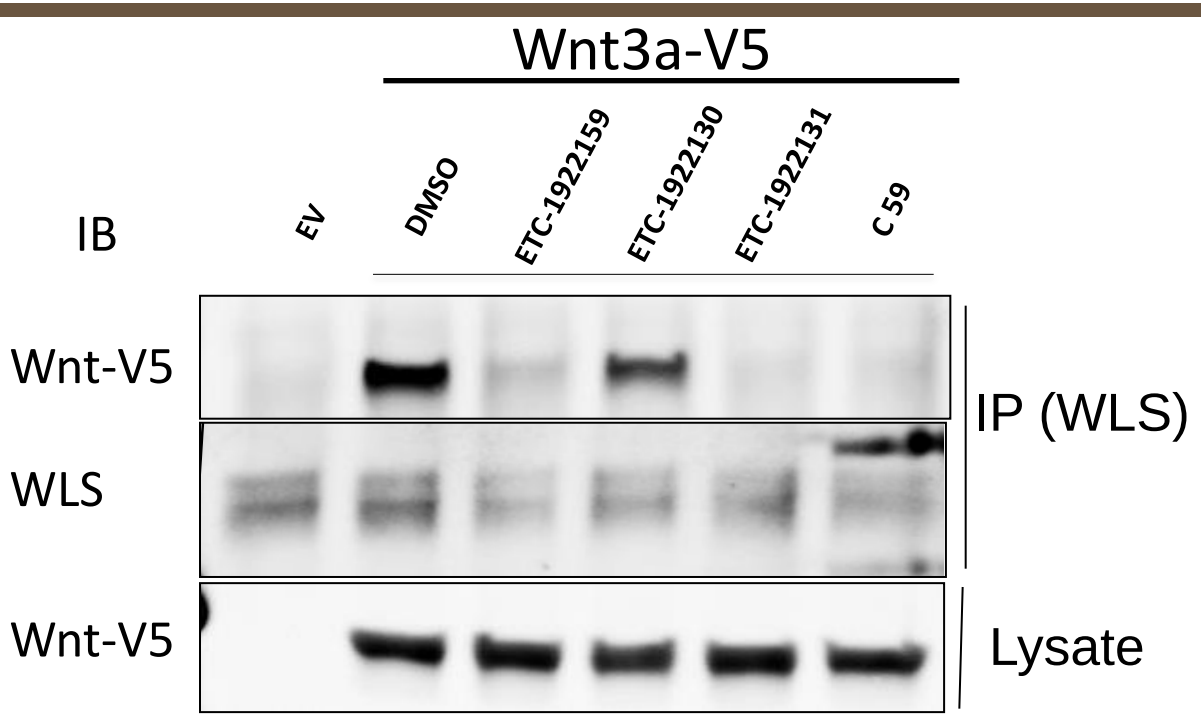


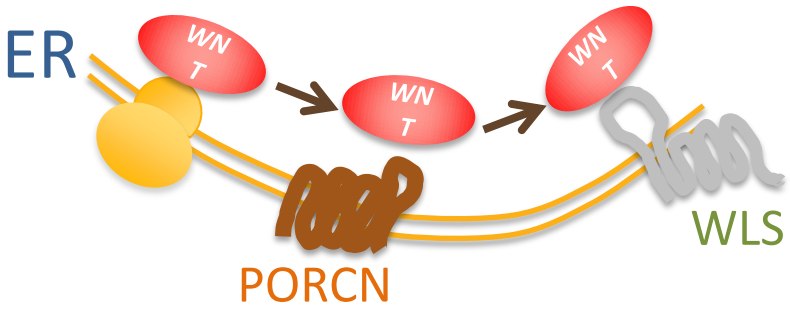


Interaction of WNTs with WLS is dependent on PORCN-mediated palmitoleation

mWnt3a	WT	S209A
hWls-V5	+	+

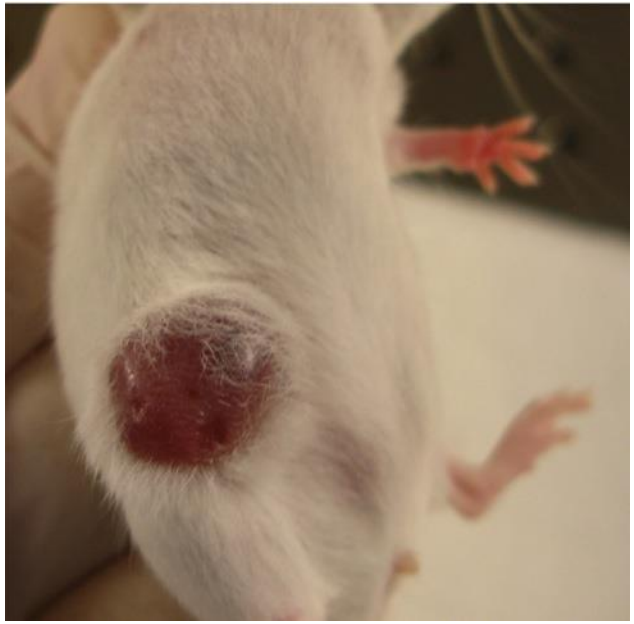






- IP WLS
- -/+ PORCN inhibitor
- Probe for Wnt-V5 and WLS

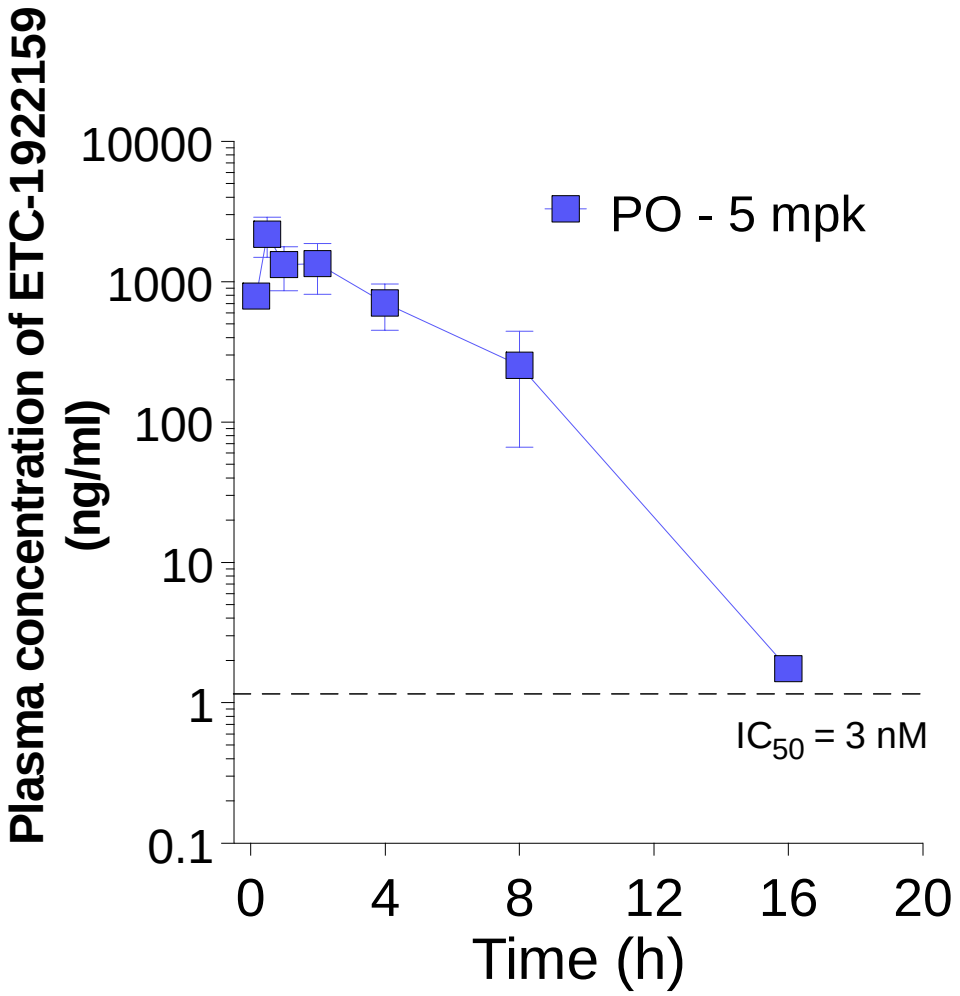
Do Porcn Inhibitors block the growth of cancers?



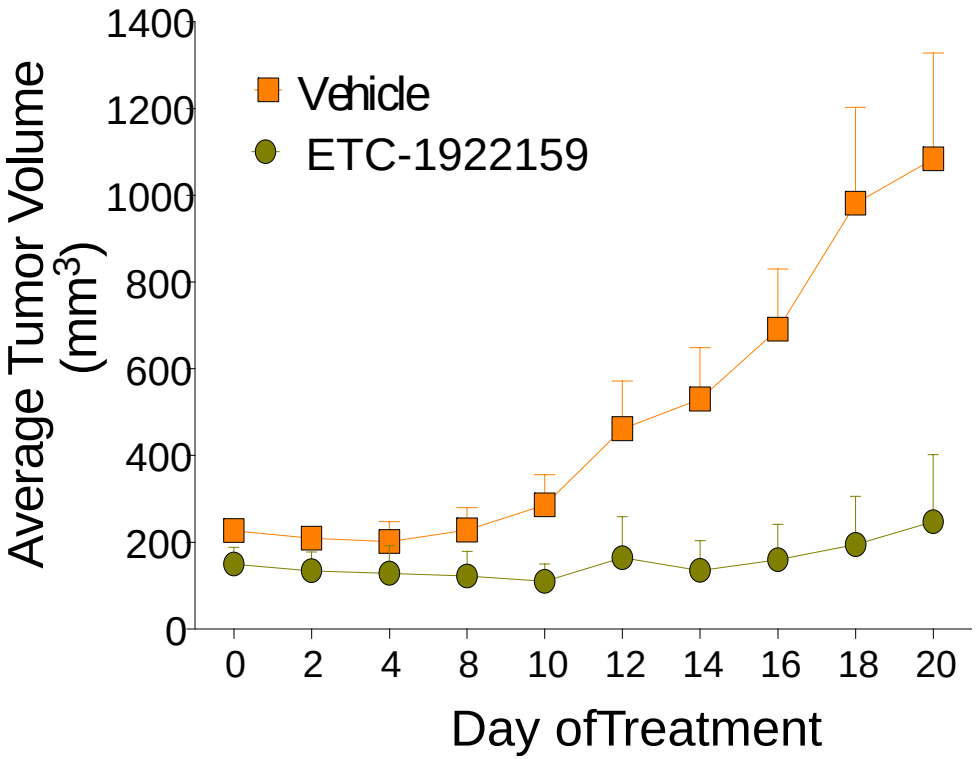
MMTV-Wnt1 mouse:
a genetic model of mammary cancer

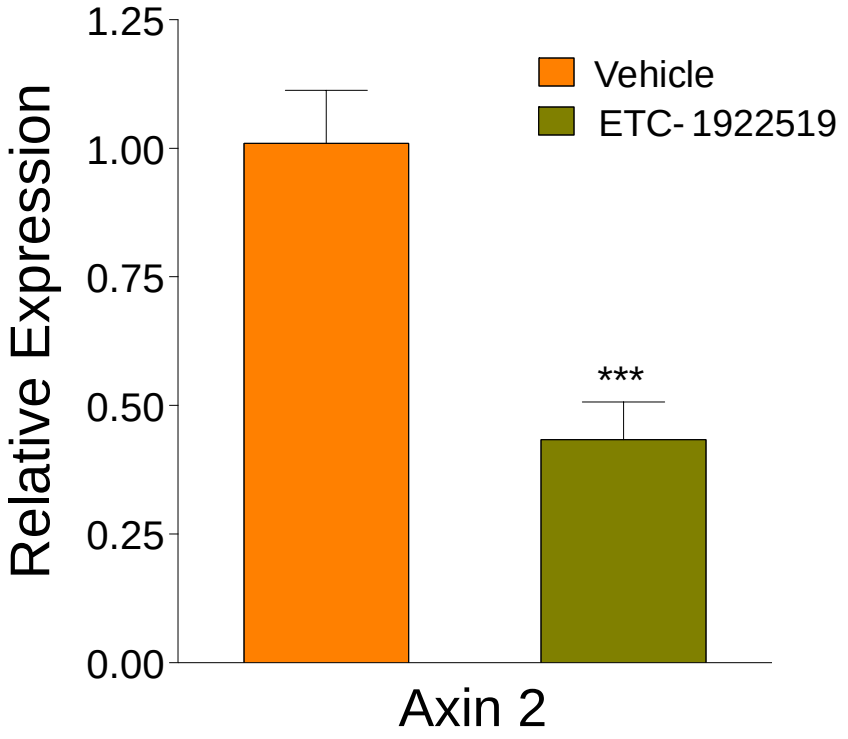
TgN(Wnt1)1Hev; Varmus and co-workers, Cell *55*, 619–625. (1988)

ETC-1922159 is orally bioavailable

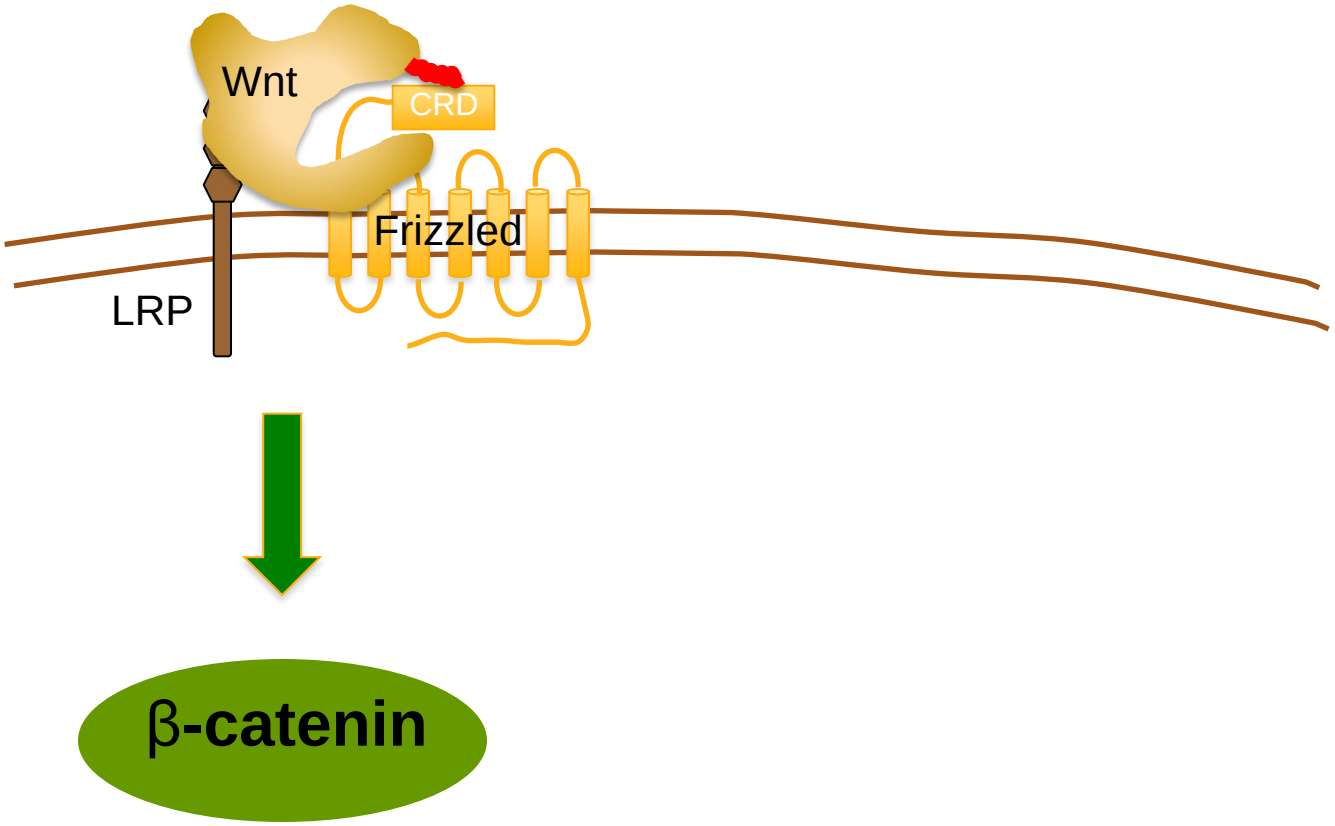


ETC-1922159 prevents growth of the teratocarcinoma PA-1 tumors in mice

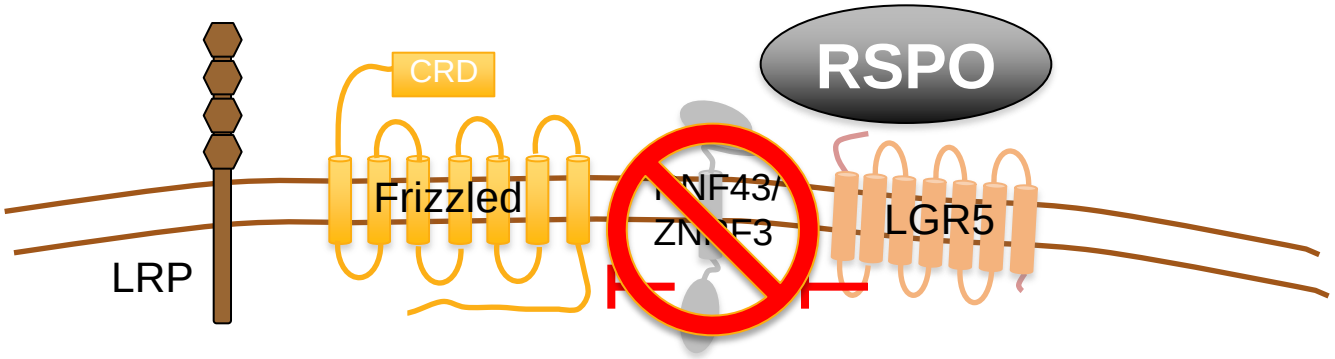




New findings in the Wnt pathway reveal predictive biomarkers



Wnt receptors are regulated, too



Loss of function of RNF43/ZNRF3, or Gain of Function of R-Spondins, make cancer cells much more sensitive to Wnts

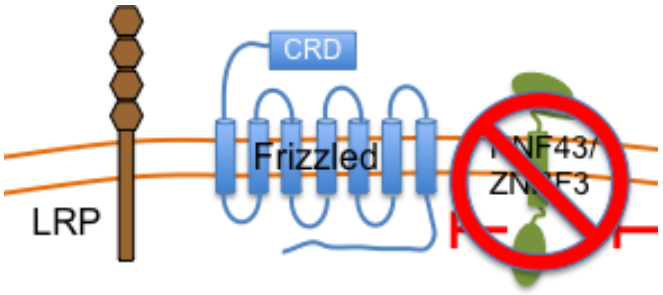
Frequency of these mutations in various cancers

Tumor Type	Frequency of mutation
Colorectal	~ 10 % RSPO translocations
Colorectal	~ 3-5% RNF43
Gastric	~ 4-8% RNF43
Pancreatic	~ 4 % RNF43
Head and Neck	~ 18% NOTCH1
Ovarian Mucinous	~ 10% RNF43
Endometrial	~ 22% RNF43

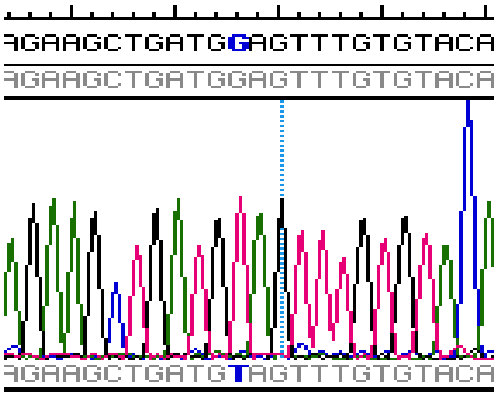
http://www.cbioportal.org/public-portal/
http://cancer.sanger.ac.uk/cancergenome/projects/cosmic/

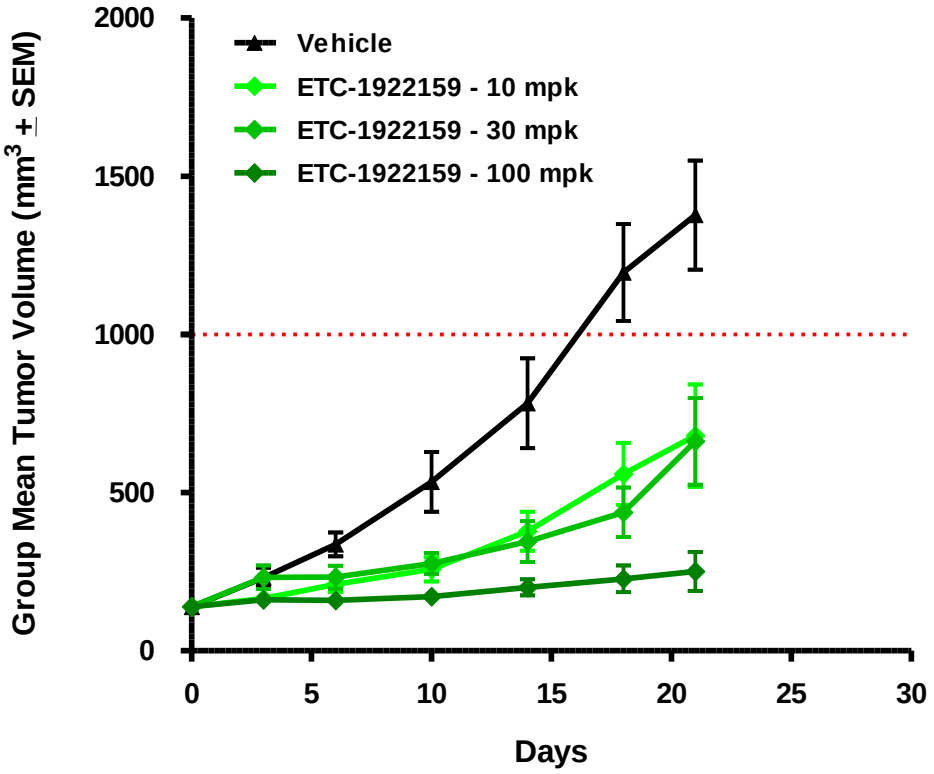
Kinde, et al. (2013). *Science Translational Medicine*, *5*(167), 167ra4–167ra4.
Ryland, et al. (2013) *The Journal of Pathology*, *229*(3), 469–476
Seshagiri, et al. (2012). *Nature*, *488*(7413), 660–664.

ETC-1922159 is effective therapy for HPAF-II RNF43 mutant, pancreatic cancer xenografts

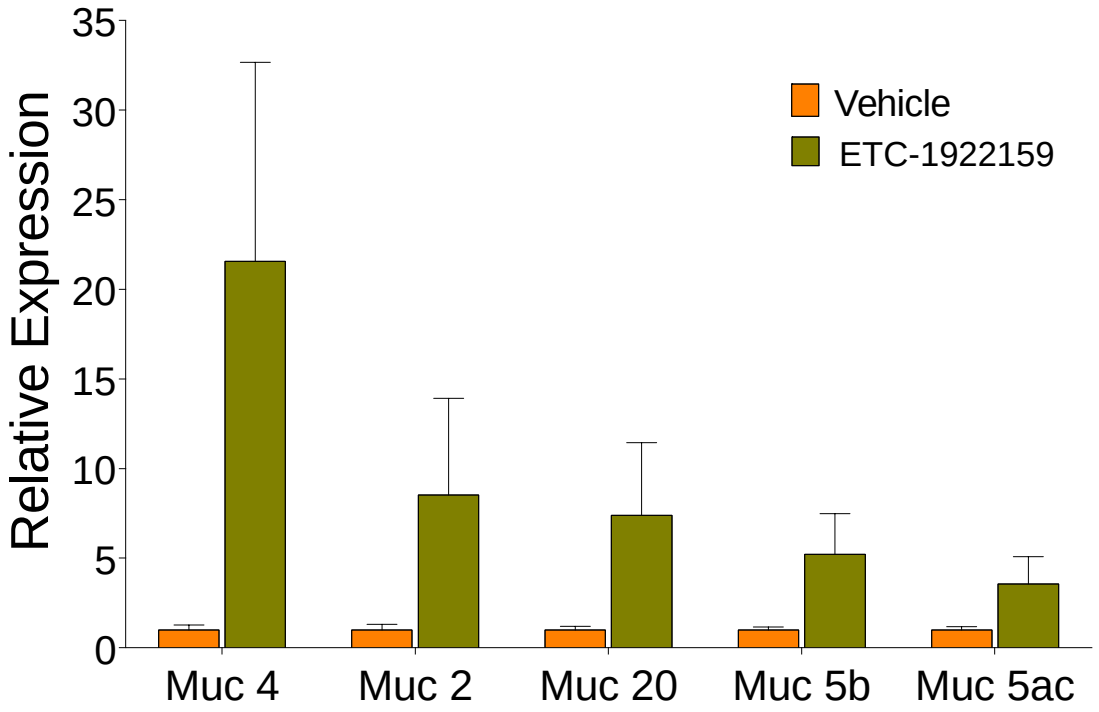


E174X in exon 3 of RNF43

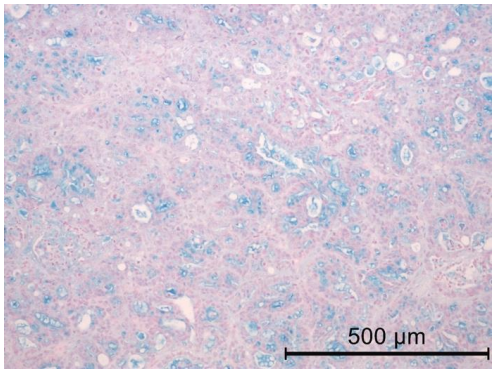




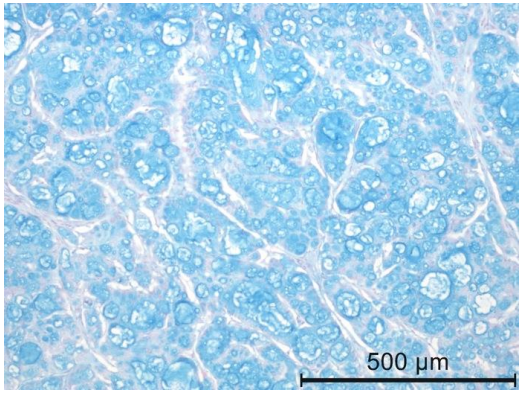
Treatment with ETC-1922159 promotes differentiation: HPAF-II, pancreatic cancer xenografts



Vehicle

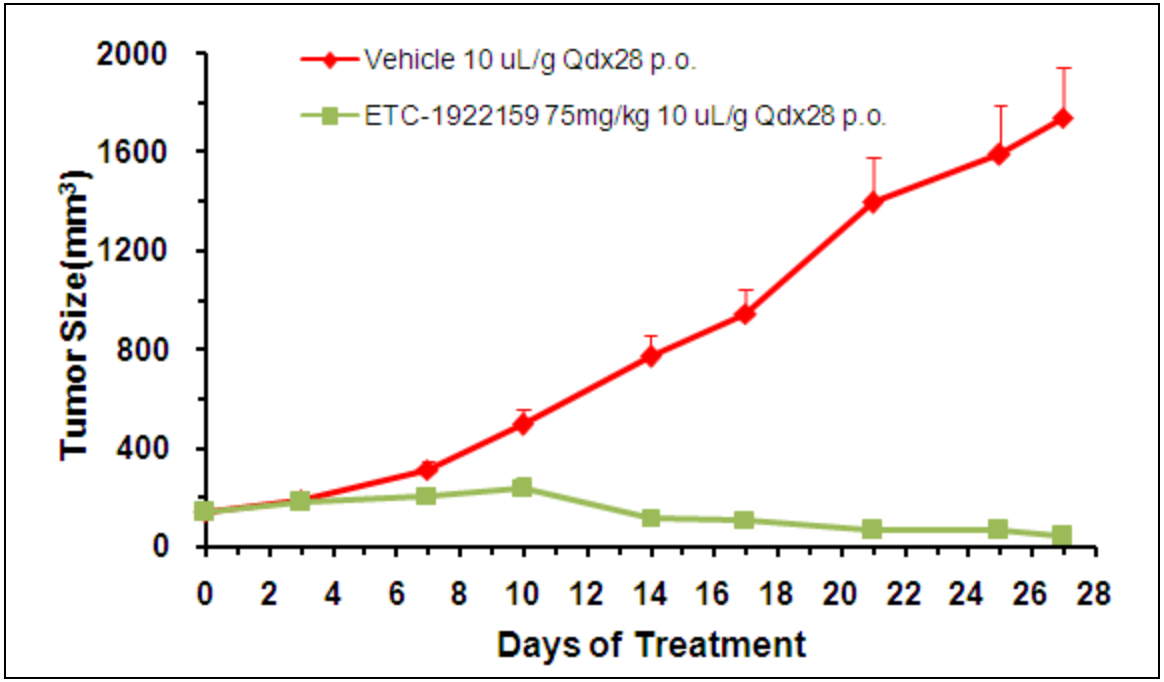


ETC-1922159



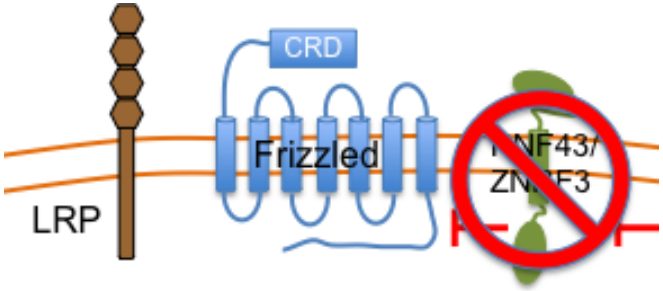
Mucin (Alcian Blue Staining)

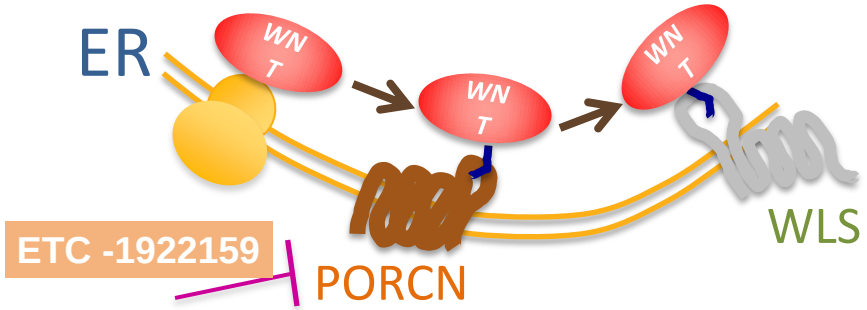
Colorectal Cancer PDX with a PTPRK-RSPO3 fusion gene: efficacy of ETC-1922159

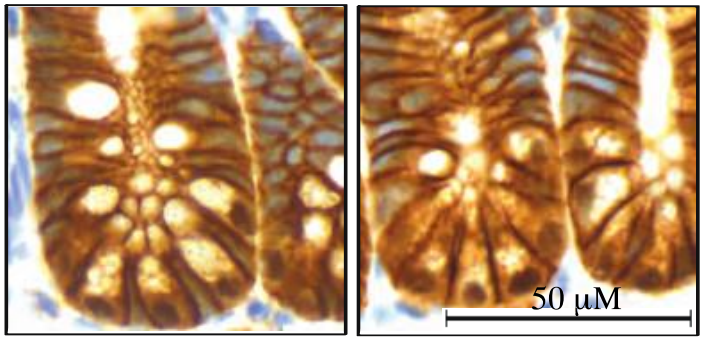


This compound has reached CTC (HSA) and IRB approval and shall enter clinical trials shortly (June 2015)
It is now renamed ETC-159

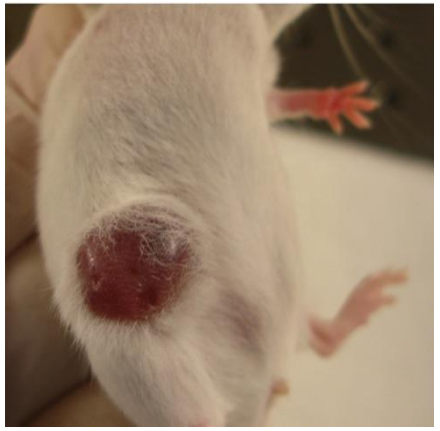
Wnt/Porcupine project – Conclusions





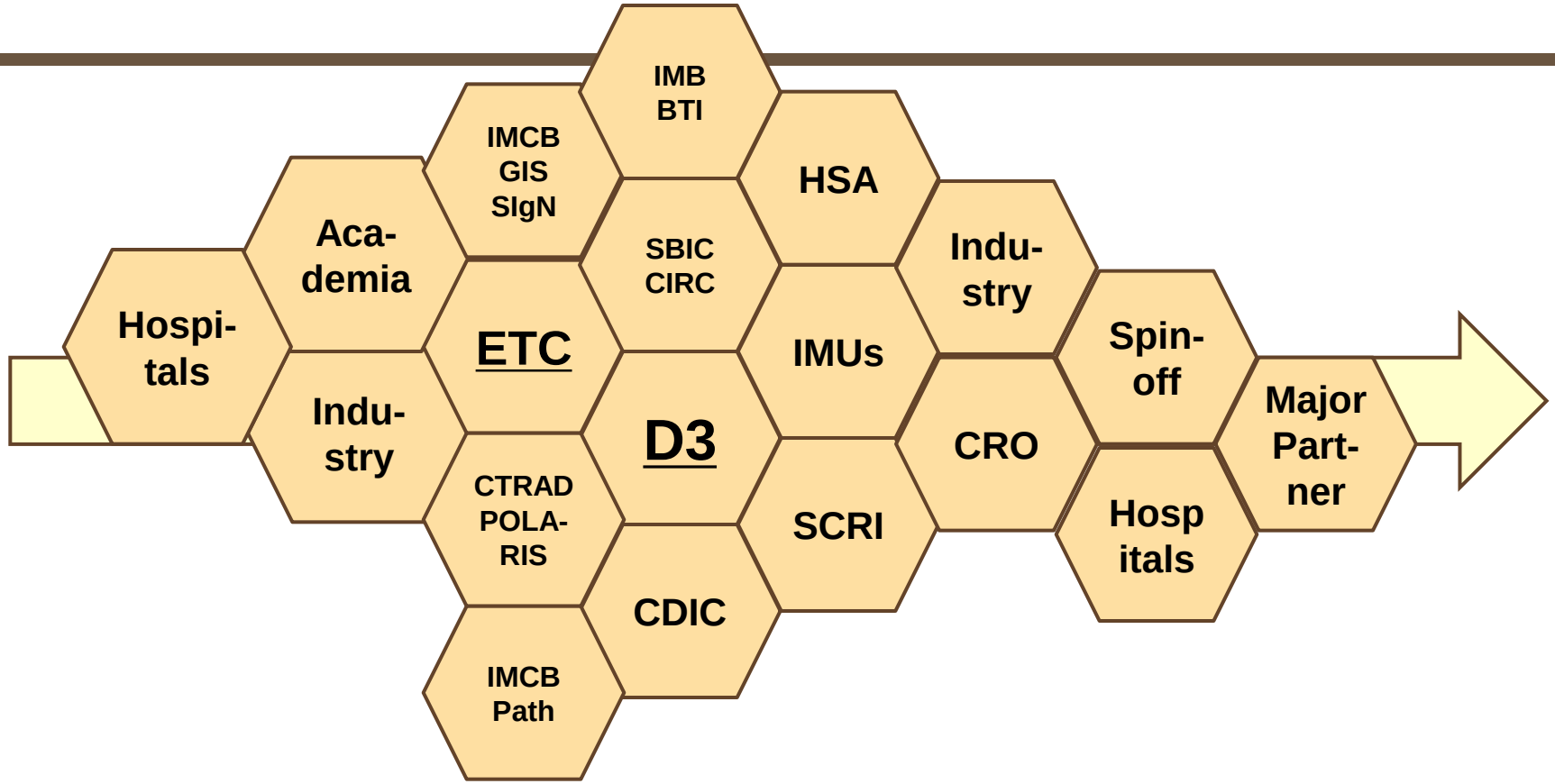


Intestinal homeostasis is not affected by therapeutic doses of PORCN inhibitors



Small molecule inhibitors of PORCN inhibit secretion of all Wnts and block proliferation of Wnt dependent tumors

Singapore is a role model for collaborative R&D and ETC/D3 can be a catalyst



ETC/D3 creates value through networking with our public research institutions and hospitals, and is a core interface with the pharma & biotech industry

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Thank you!