Development of chemical probes for CLK kinases

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Eshelman School of Pharmacy

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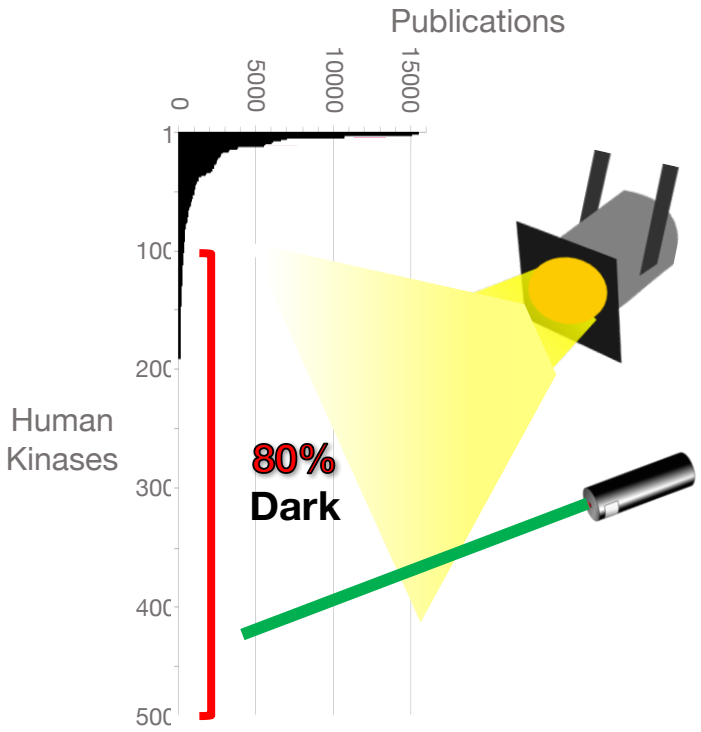
Extreme Open Science Meeting January 2018

Let me introduce myself…

- Born in Barcelona, Spain.
- BSc in Chemistry at University of Barcelona (2006-2011).
- Masters in Organic Chemistry at University of Barcelona (2011-2013).
- PhD in Organic Chemistry at University of Strathclyde (2013-2017).
  - 3-Month industrial placement at GSK in Stevenage (09/2016-12/2016).
- Postdoctoral Research Associate at UNC-SGC (08/2017-present).

SGC-UNC Kinase projects



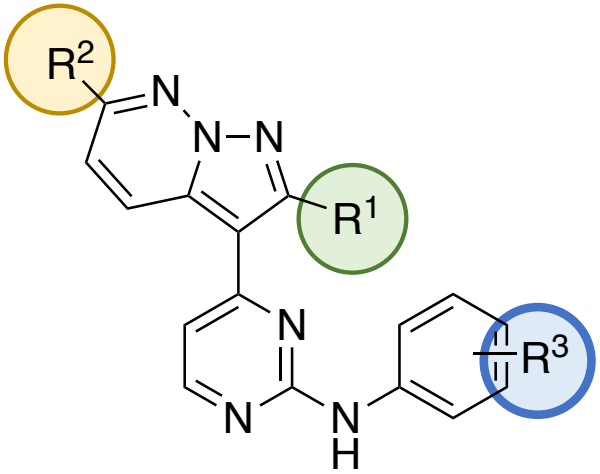
Kinase Chemical Probes

- ✓ Potent
- ✓ Selective
- ✓ Cell active
- ✓ Negative control

(Collaborations with other SGC sites and partners)

CLK1/2/4 inhibitor

GW807982X R^1 EtO
R^2 H
R^3 *m*-OMe/CF_3



Compound progression path

1. Compounds tested at Luceome.
    - %Inhibition at 1 µM
    - IC_{50} for compounds with >75% inhibition
2. NanoBret cell assays
3. Broad selectivity screening of potent, cell active compounds

CLK2 inhibitors may be useful in cancer

- CLK2 plays a role in controlling cell cycle and survival of glioblastoma
  - “Cdc2-like kinase 2 is a key regulator of the cell cycle via FOXO3a/p27 in glioblastoma” https://www.ncbi.nlm.nih.gov/pubmed/25670169
- Inhibtion of CLK2 modulates EMT splicing patterns and inhibits breast tumor growth
  - “CLK2 Is an Oncogenic Kinase and Splicing Regulator in Breast Cancer”https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5042015/

Acknowledgments

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Dr Chris Asquith
Dr Yi Liang

Luceome Biotechnologies
Reena Zutshi

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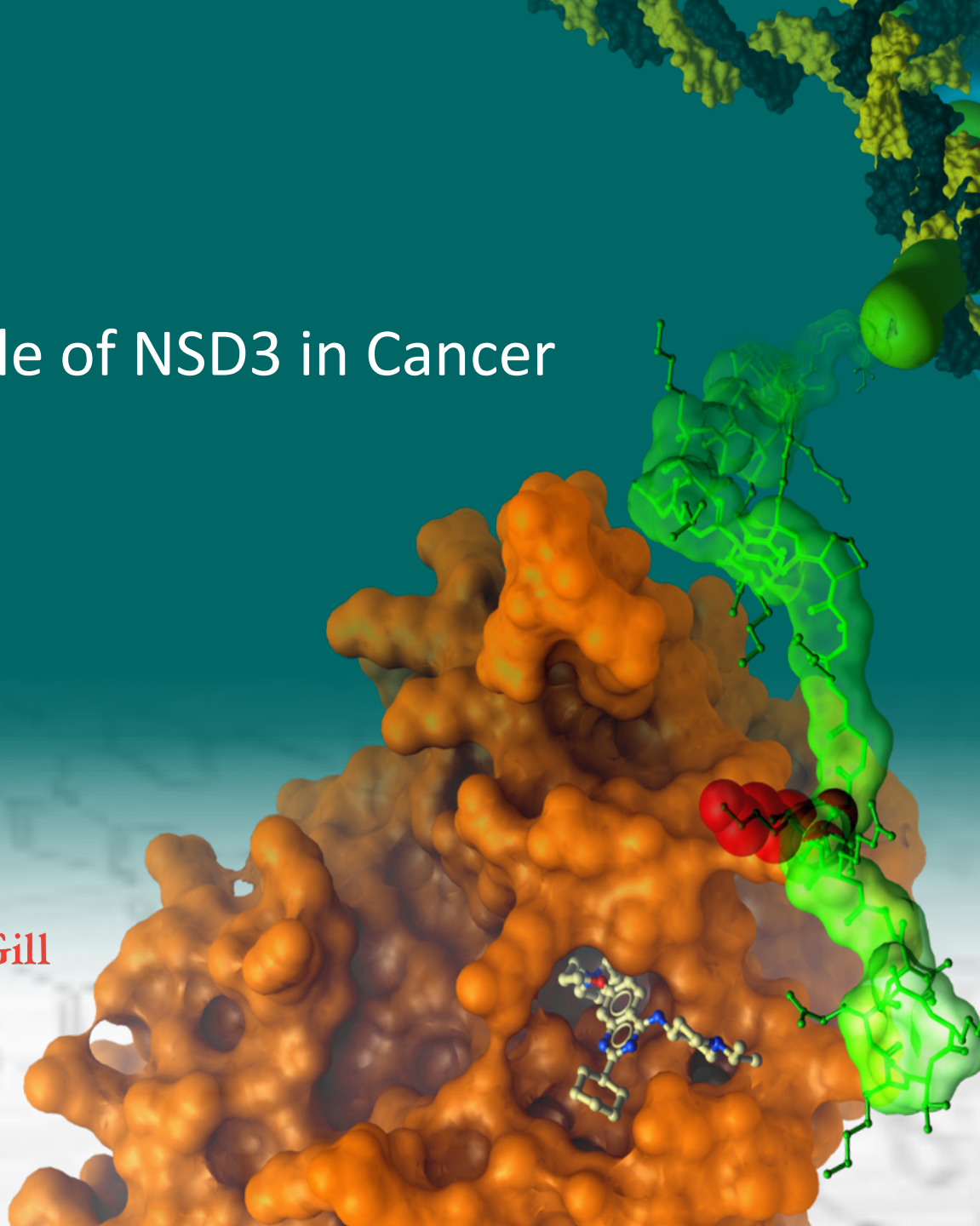
at CHAPEL HILL

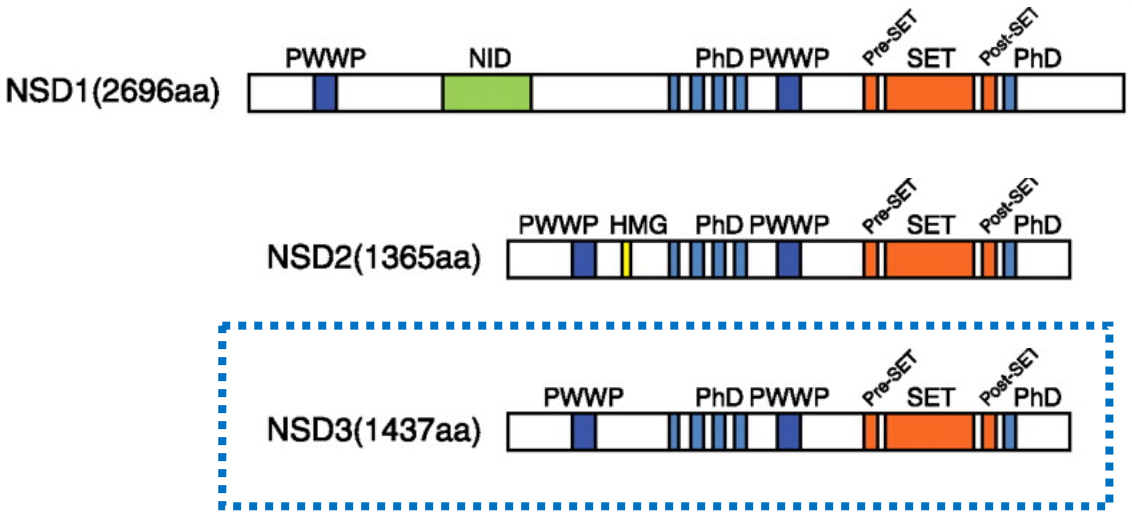
Understanding the Role of NSD3 in Cancer

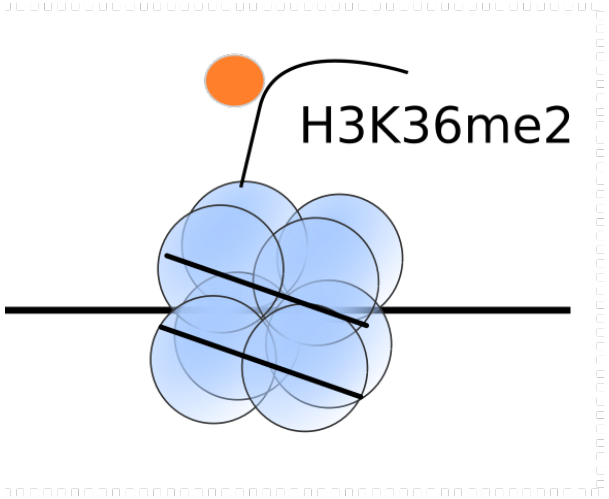
David Dilworth
Postdoctoral Fellow
SGC Toronto

19-01-2018



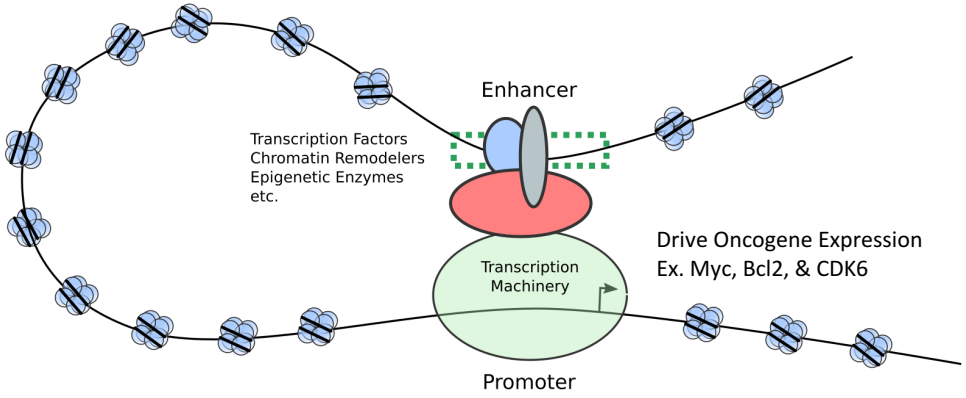
The Nuclear SET Domain containing protein (NSD) family of Histone Methyltransferases

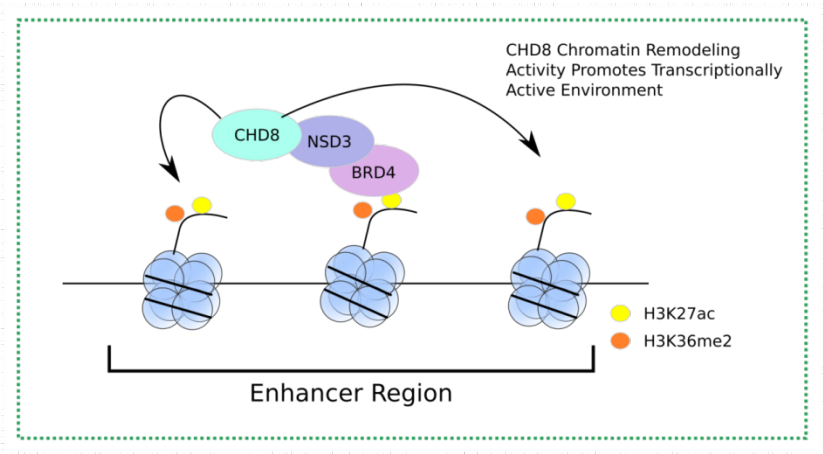




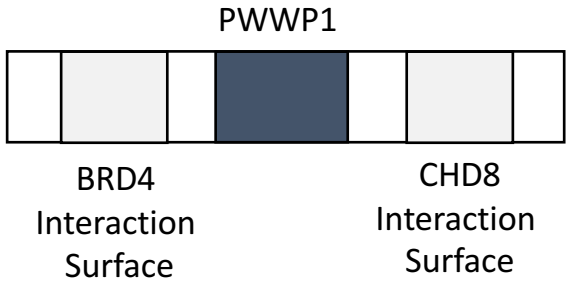
Yan Li et al. J. Biol. Chem. 2009;284:34283-34295

NSD3-Short is Required for BRD4-Mediated Maintenance of Acute Myeloid Leukemia (AML)





NSD3-Short as an Adapter Protein



- Chen et al (PMID: 26626481) discovered that NSD3-Short is sufficient and the PWWP1 reader module is required.
- PWWP1 domain may represent a good target in the treatment of AML and potentially other cancers with similar oncogene dependencies.

Central Questions

1. How does NSD3 influence the epigenetic landscape of oncogene enhancers in AML?

Central Questions

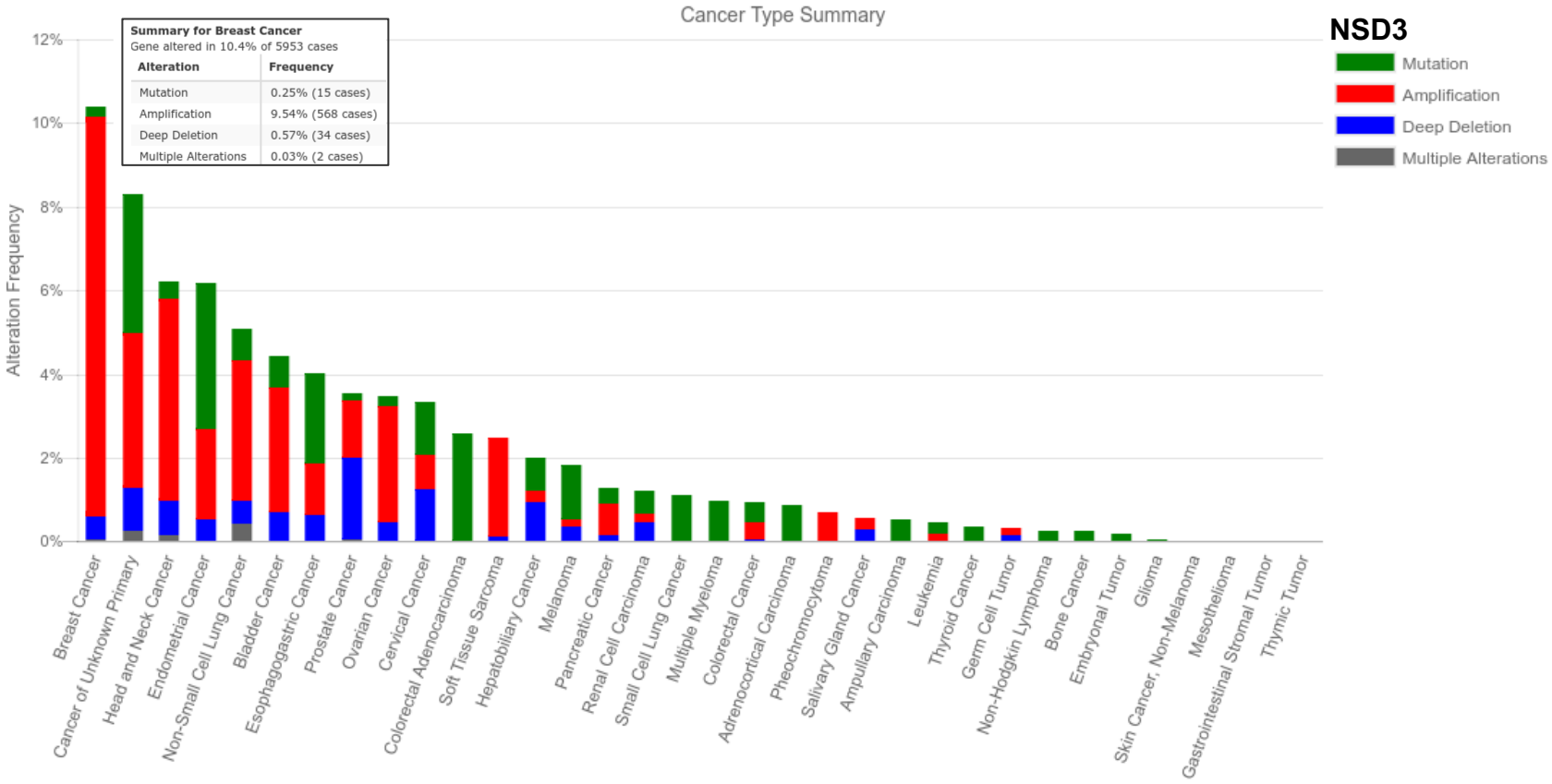
2. What is the role of NSD3's PWWP1 reader domain?

Central Questions

3. Does NSD3's function at enhancers also promote oncogene expression in other forms of cancer?

NSD3 is Amplified in ~10% of cBioPortal Breast Cancer Cases:

However, almost nothing is known about how it contributes to the disease.



cBioPortal: Cerami et al., Cancer Discov. 2012 and Gao et al. Sci. Signal. 2013

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FUNDING PARTNERS
The SGC is a registered charity (number 1097737) that receives funds from AbbVie, Bayer Pharma AG, Boehringer Ingelheim, Canada Foundation for Innovation, Eshelman Institute for Innovation, Genome Canada through Ontario Genomics Institute [OGI-055], Innovative Medicines Initiative (EU/EFPIA) [ULTRA-DD grant no. 115766], Janssen, Merck KGaA, Darmstadt, Germany, MSD, Novartis Pharma AG, Ontario Ministry of Research, Innovation and Science (MRIS), Pfizer, São Paulo Research Foundation-FAPESP, Takeda, and Wellcome [106169/ZZ14/Z].

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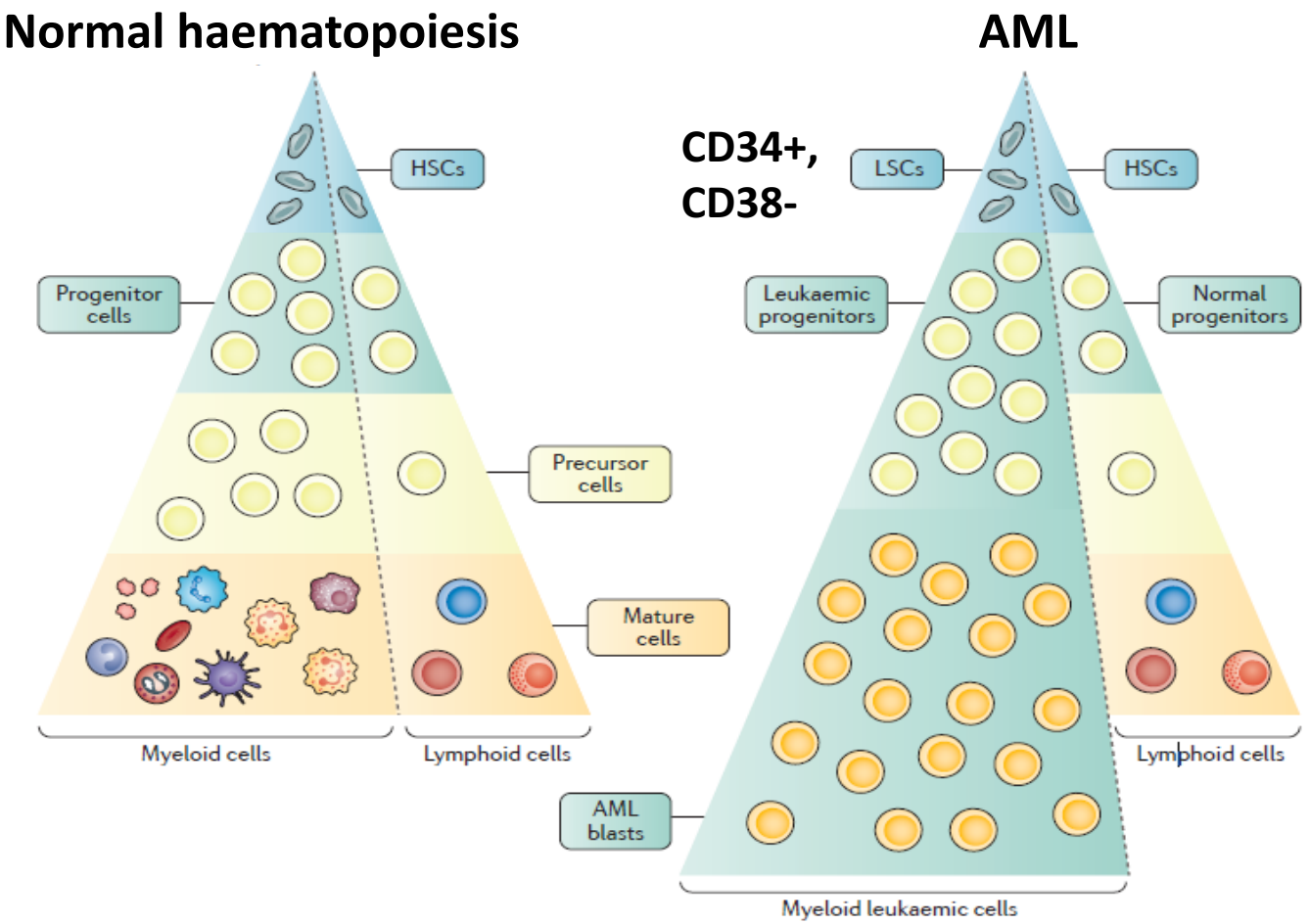
Testing Polycomb Repressive Complex 2 (PRC2) Inhibitors in Acute Myeloid Leukemia

Genna Luciani
SGC Toronto
January 19, 2018

AML: Acute myeloid leukemia



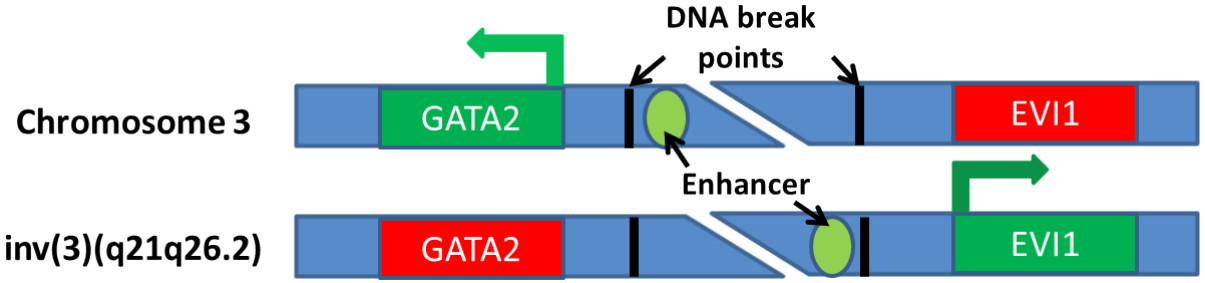
- Abnormal growth and differentiation of HSCs
- Most common acute leukaemia in adults: 3-4 in 100 000 each year worldwide
- The overall outcome of standard cytotoxic chemotherapy is poor:
  - 5-year overall survival below 50%
  - below 20% for patients older than 60 years

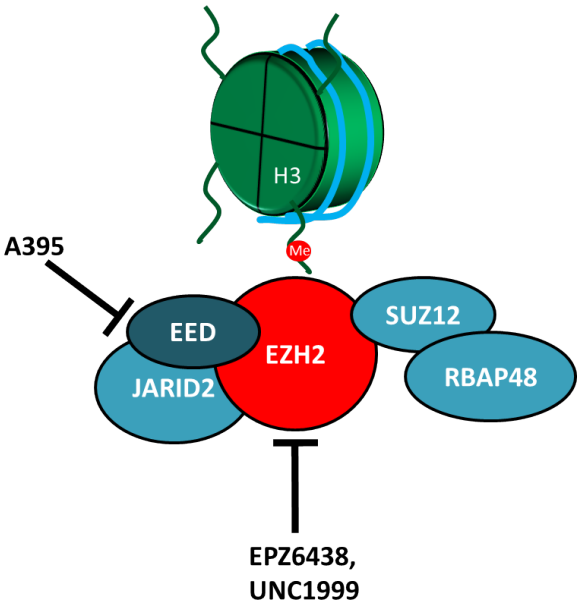
Gregory *et al.*, 2009

Figure from Khwaja *et al.*, *Nat. Rev. Dis. Primers*

Testing polycomb repressive complex 2 (PRC2) inhibitors in inv(3) acute myeloid leukemia

- AML with inv(3) has very poor prognosis driven by epigenetic regulator
- Responds poorly to treatment
- Often times co-occurs with deletion of chromosome 7





- PRC2 repressive complex methylates lysine 27 on histone 3
- Inhibitors exist to both EZH2 and EED

- **Investigate how EVI1 interacts with the PRC2 complex components**
- **Determine how modulating PRC2 with chemical probes affects leukemia in inv(3) samples with and without chromosome 7**

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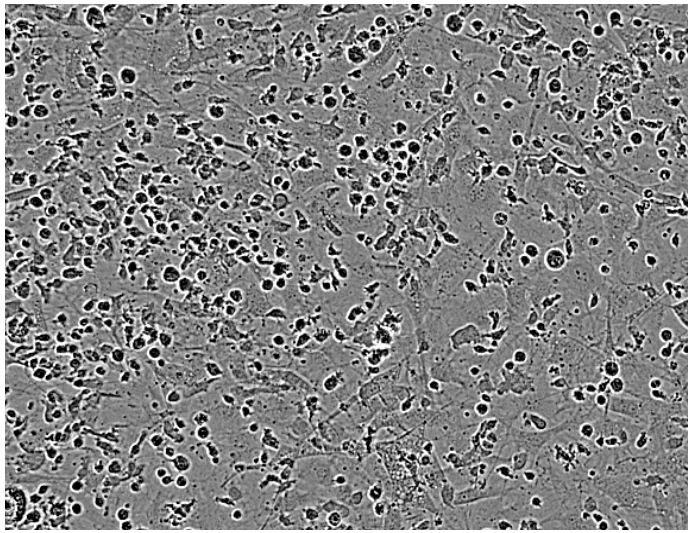
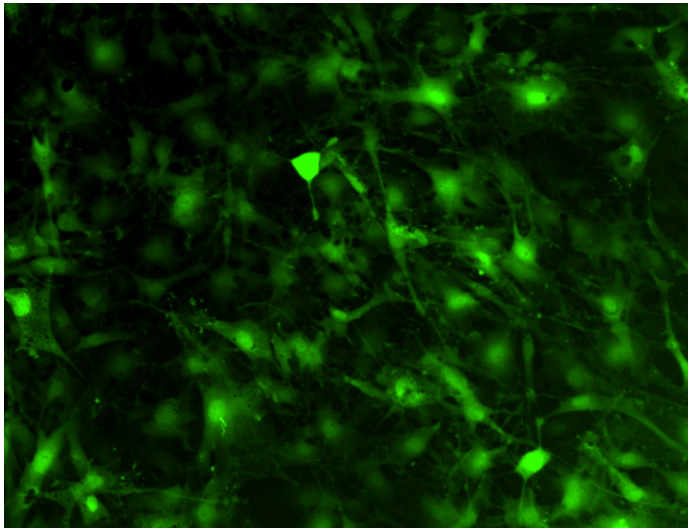
Co-culture patient leukemic cells on fluorescent stroma cells





Cell collection

MACSQuant flow cytometer

- Assess viability of non fluorescent cells (blasts) with and without PRC2 inhibitor treatment

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Lily Xie
RJ He

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Eric Lechman

MSSM
Jian Jin

LEUKEMIA & LYMPHOMA SOCIETY OF CANADA
fighting blood cancers

Mitacs

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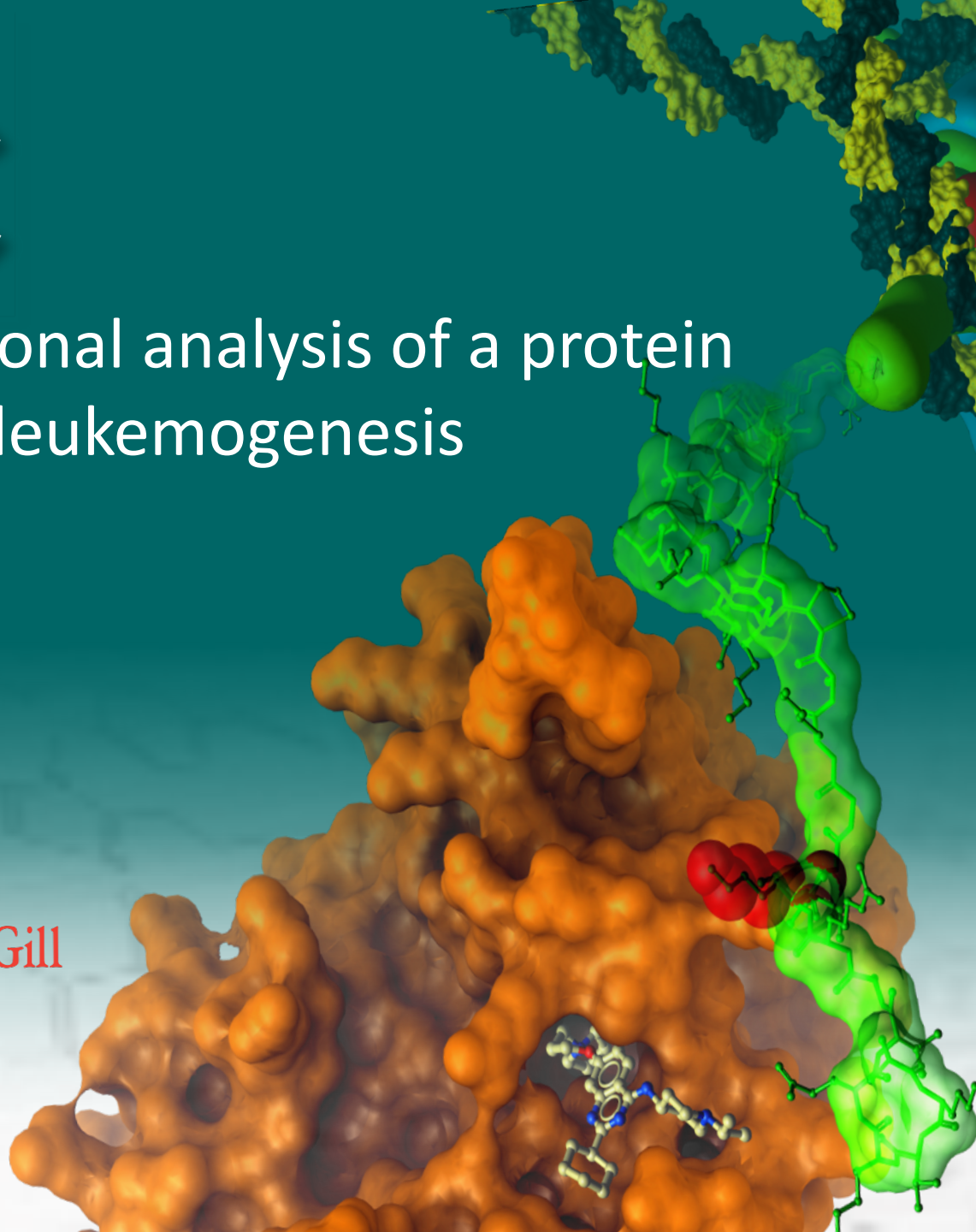
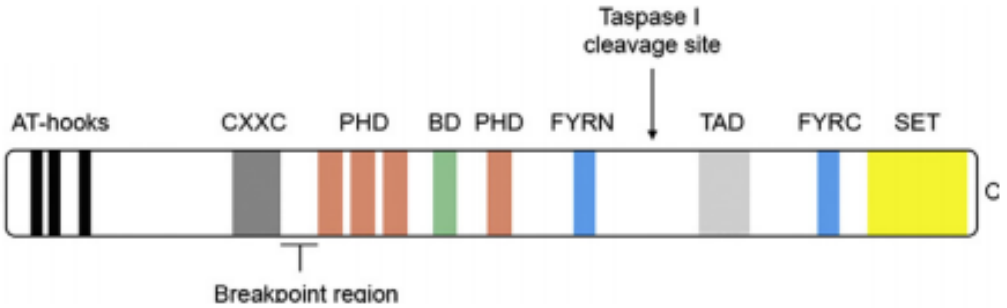
Structural and functional analysis of a protein complex involved in leukemogenesis

Heng Zhang
University of Toronto

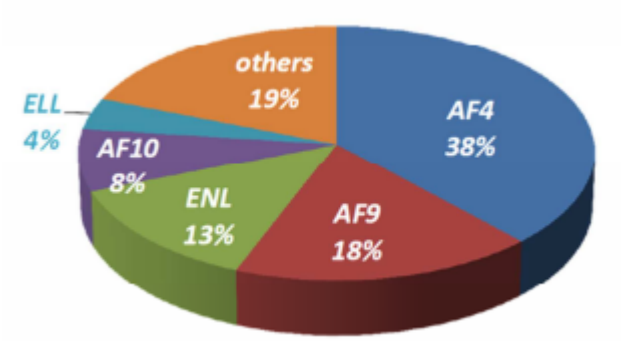


MLL rearrangements trigger leukemias

MLL1 protein



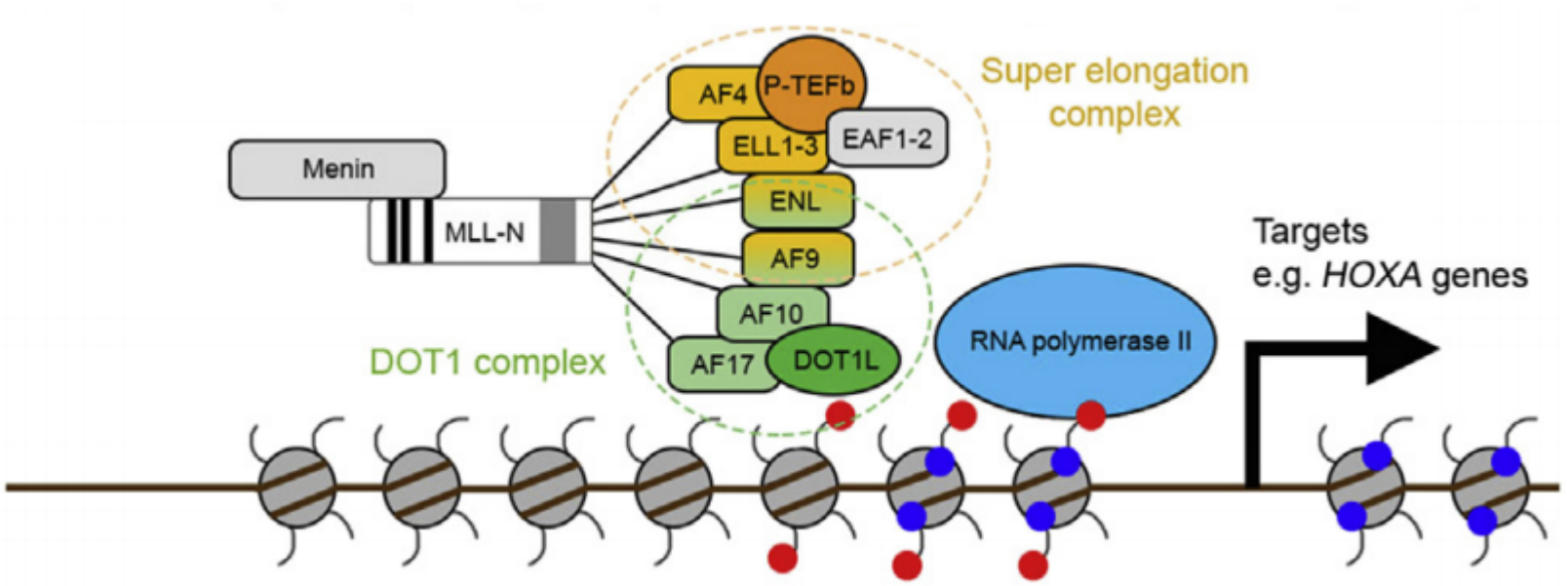
MLL1-fusion proteins



Frequency of the most common MLL translocation partner genes

Chen et al 2015, Chopra et al 2015

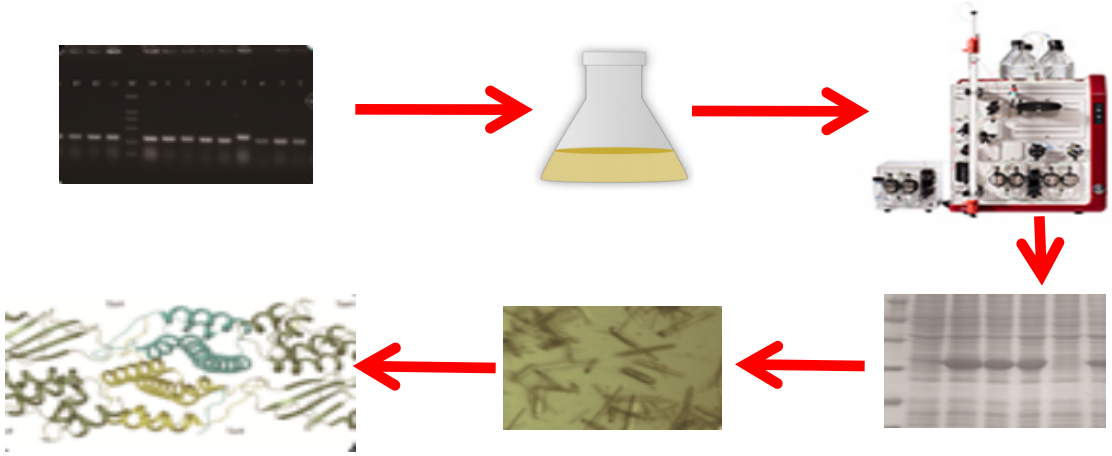
DOT1L is involved in MLL-fusion-associated leukemogenesis



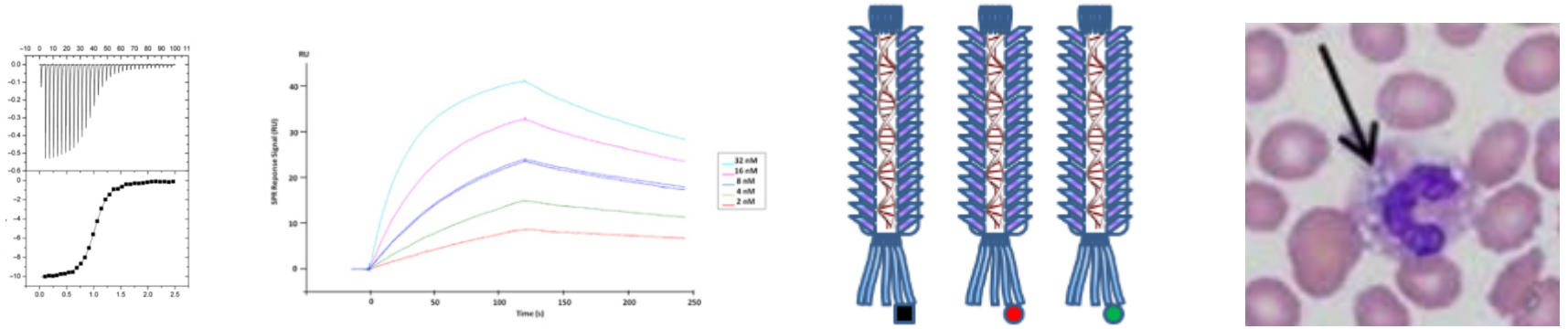
Proposed mechanism of deregulated gene expression by common MLL1 fusions proteins

Chopra et al 2015

Characterization of the DOT1L–MLL complex



Protein expression, purification, crystallography and structure determination.



Functional and biochemical analysis of the DOT1L-MLL complex

ACKNOWLEDGEMENTS

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Dr. Su Qin
Yanjun Li

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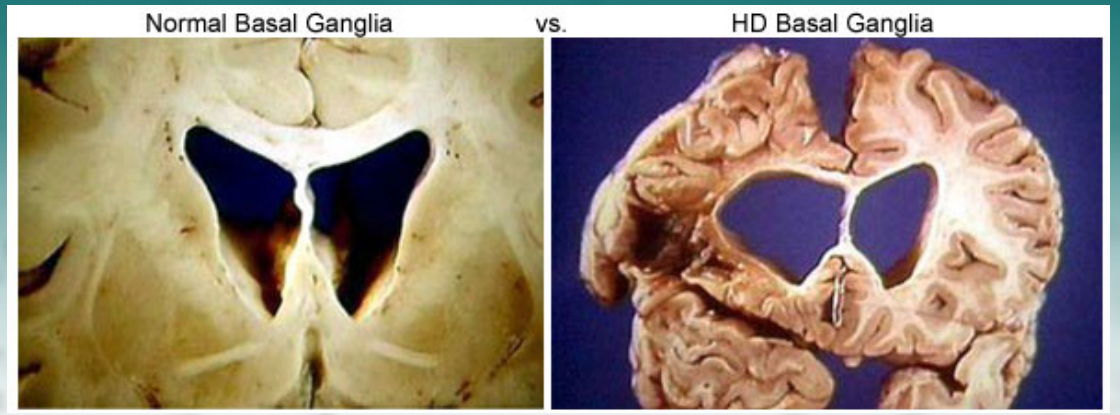
Function of Huntingtin protein

Jolene Ho (Takis Prinos Group, SGC Toronto)

- Huntingtin is the protein that is mutated in Huntington's disease (HD), and is coded for by the gene *HTT*
- Mutation: CAG repeat expansions in *HTT* lead to an abnormally long polyglutamine (polyQ) region in the protein
- Age at onset and age at death are correlated to length of polyQ region



Source: Singer, Jonathan. Huntington's Disease. Online. Available at: http://ist-socrates.berkeley.edu/~jmp/HD.html

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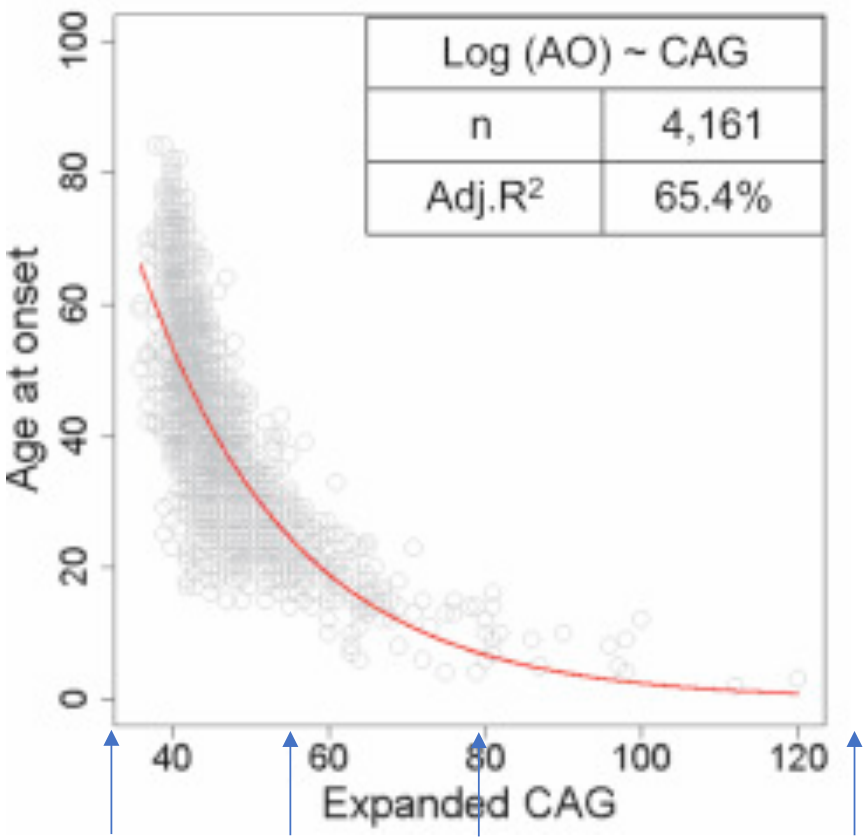
Karolinska Institutet Anno 1810 · University of North Carolina at Chapel Hill · Goethe Universität Frankfurt am Main · McGill · Unicamp · University of Oxford

Aims

- Overexpression of HTT with different lengths of polyQ regions in mammalian cells
  - Function of WT/mutant HTT
- Knockdown of HTT in mammalian cells
  - Normal function of HTT
- Treatment of cells with WT/mut HTT with epigenetics chemical probes → effect on HTT expression/localization
  - Possible therapy
- Observe biological and functional effects in these cells

HTT Constructs

A **Figure 1, Keum *et al.*, Am J Hum Genet 2016**



Length of polyQ region	Q23	Q54	Q79	Q145
Construct name	D01	D02	D04	D06

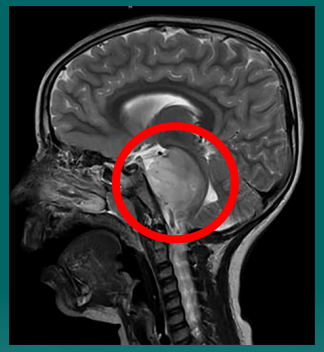
Alma Seitova
Peter Loppnau
Rachel Harding

The Hunt for DIPG Drug on the Shoulder of FOP

Jong Fu Wong
Cell Biologist, Alex Bullock's team, SGC Oxford

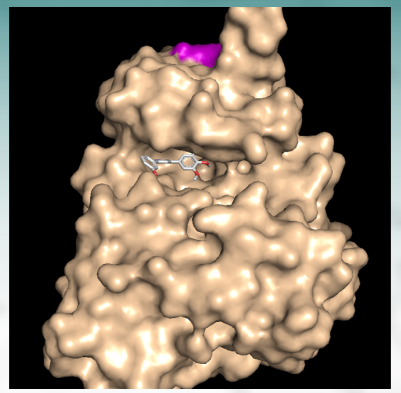
The BRAIN TUMOUR CHARITY

- The Brain Tumour Charity
- Diffuse Intrinsic Pontine Glioma
- ~6-7 years old children
- Only radiotherapy, median survival 9-12 months
- Mutations: H3K27M in 78% ALK2 in 24%
- ALK2 had been studied extensively in Fibrodysplasia Ossificans Progressive (FOP)



cancer.gov

R206



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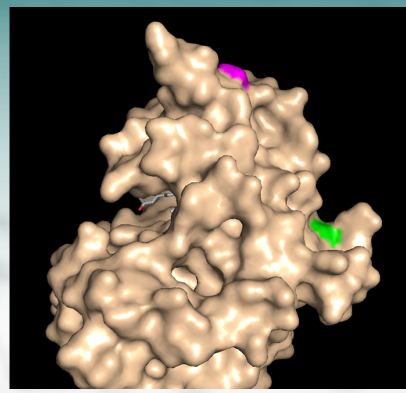

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GOETHE UNIVERSITÄT FRANKFURT AM MAIN

McGill

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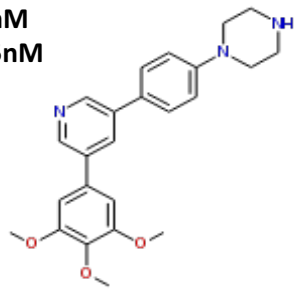
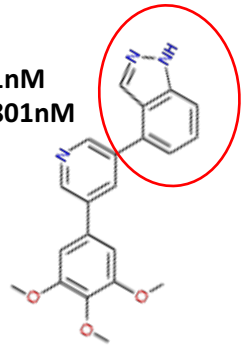
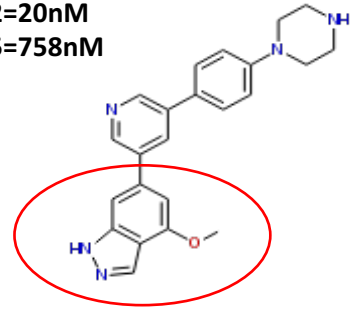
Cellular Screening for ALK2 Inhibitors

- ❖ Improve selectivity
- ❖ Less metabolically active
- ❖ Better central nervous system penetrance

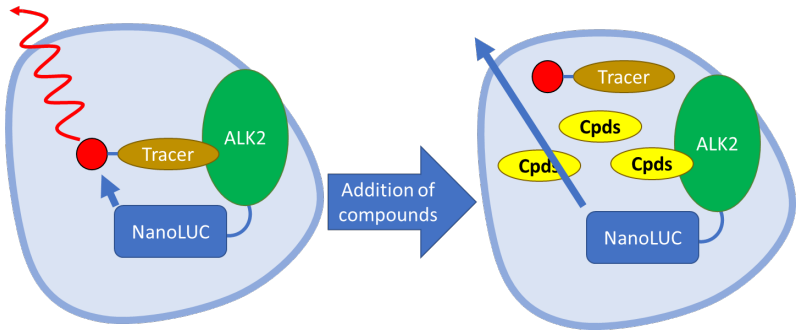
	K03841 OICR0015167A01	K62821 OICR0015105A01	K63024 OICR0015149A01
In vitro IC50	ALK2=14nM ALK5=175nM	ALK2=21nM ALK5=1801nM	ALK2=20nM ALK5=758nM

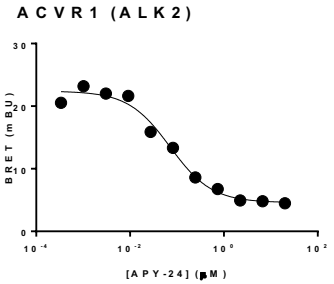




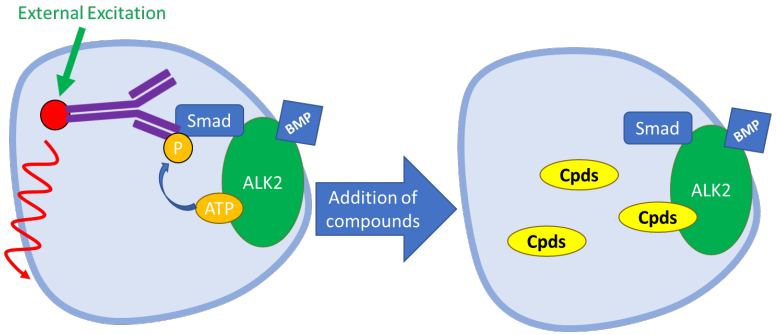


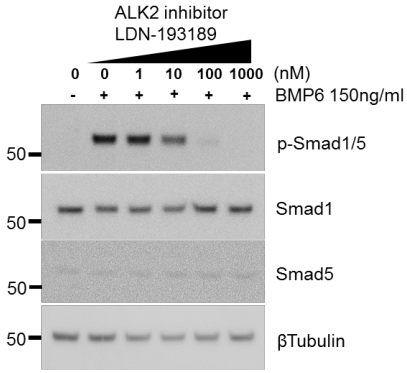
NanoBRET Target Engagement Assay (Promega kit developed with SGC)

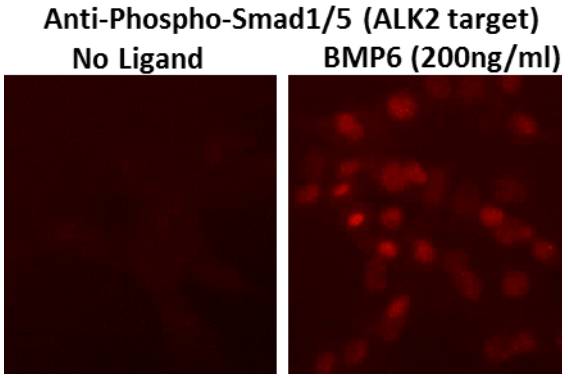




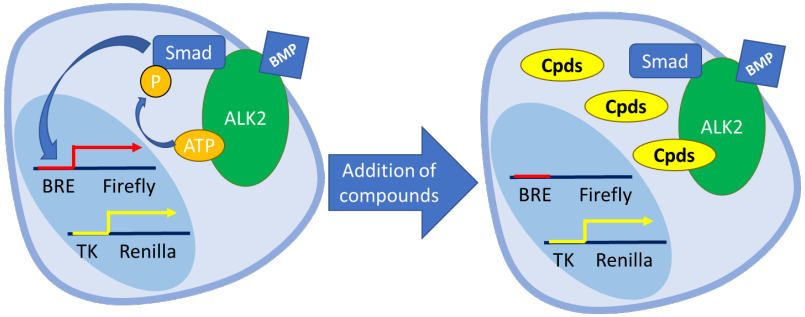
Cellular SMAD phosphorylation





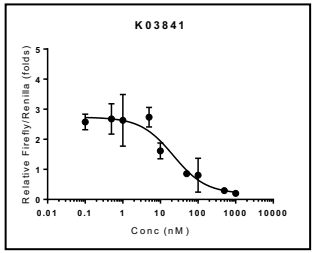


Dual Luciferase Assay (DLA)



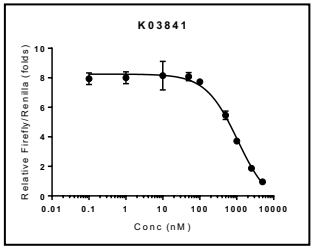
In vitro IC50=14nM
Cellular IC50=22nM

ALK2



ALK5

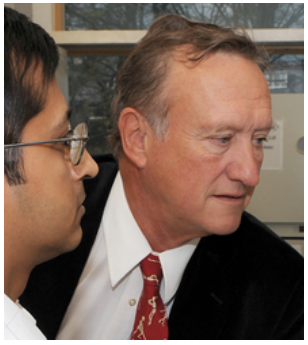
In vitro IC50=175nM
Cellular IC50=1063nM



Understanding the pathogenic mechanism of ACVR1/ALK2 mutations

Liz Brown
DPhil student – supported by the Simcox Family Scholarship
Supervisors: Alex Bullock, Gillian Farnie – SGC Oxford

2018.01.19



The Simcox Family

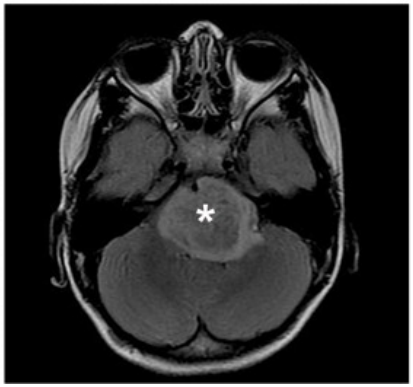
Background – DIPG, FOP and ACVR1

- **Fibrodysplasia ossificans progressiva –**

  A genetic disorder in which soft tissue progressively mis-differentiates into bone (heterotopic ossification)
- **Diffuse Intrinsic Pontine Glioma –**

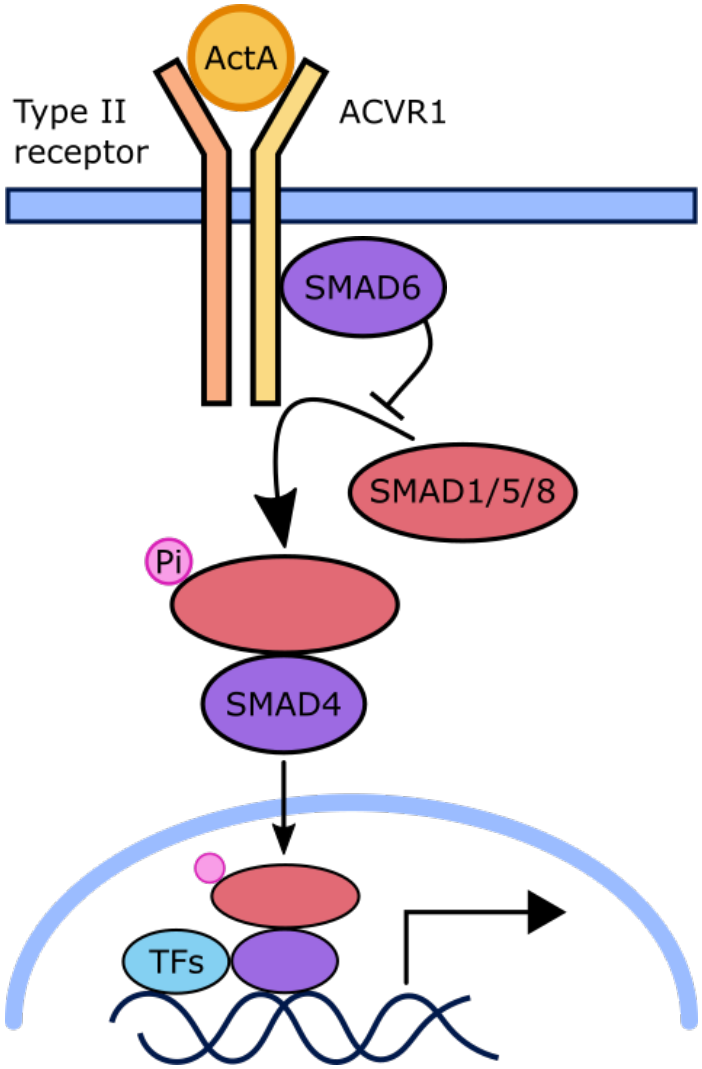
  A paediatric brainstem tumour that is characteristically diffuse and infiltrative
- Both diseases carry **mutations in ACVR1/ALK2**, a serine/threonine kinase that activates BMP signaling (along with histone and PI3K mutations in DIPG)
- Mutant **ALK2 signals in response to the binding of activin** in addition to its natural ligand BMP6

Joh-co [CC BY-SA 3.0], via Wikimedia Commons



Monje (2011) 10.1073/pnas.1101657108

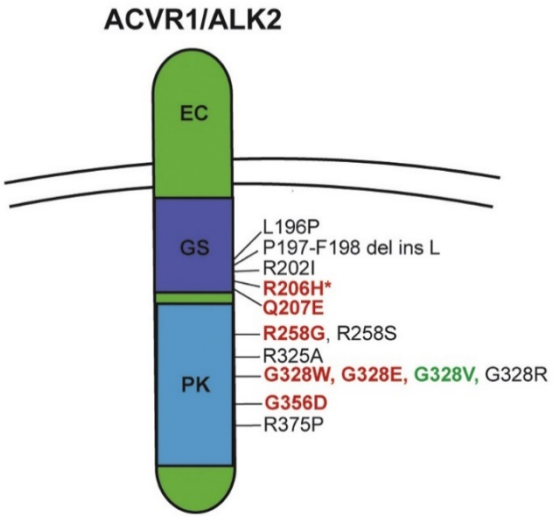
How do ACVR1 mutations alter BMP signalling?

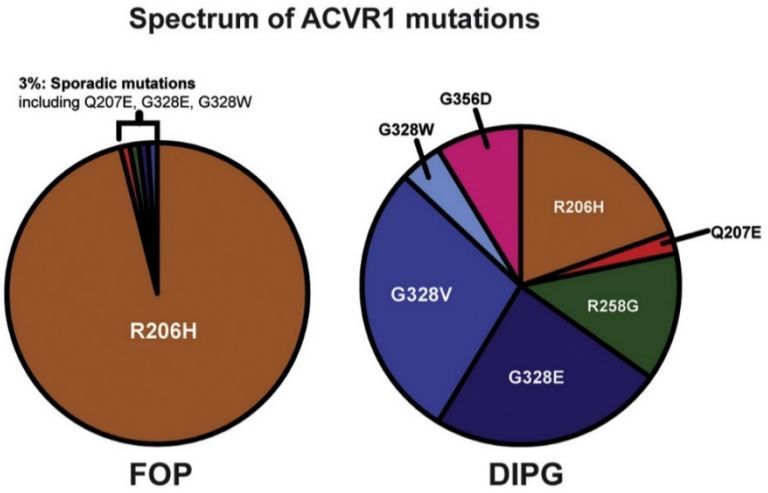


- Recorded ACVR1 mutations are all found in the intracellular domains
- We hypothesize that the mutations:
  - Reduce interactions with negative regulators such as FKBP12 or Smad6/7 and promote signaling
  - And potentially increase interaction with positive regulators such as Smad1/5/8 or its type II receptor and promote signaling

Do all mutations affect the cell similarly? Are they equally susceptible to inhibition?

- Most *in vitro* drug screening carried out on WT ACVR1 or only the R206H mutant – the major mutation found in FOP
- I will screen drug efficacy, study signaling changes and assay phenotypic changes in cells with the wider range of mutations found in DIPG





Han, H. J., Jain, P., & Resnick, A. C. (2017). *Bone*. https://doi.org/10.1016/j.bone.2017.08.001

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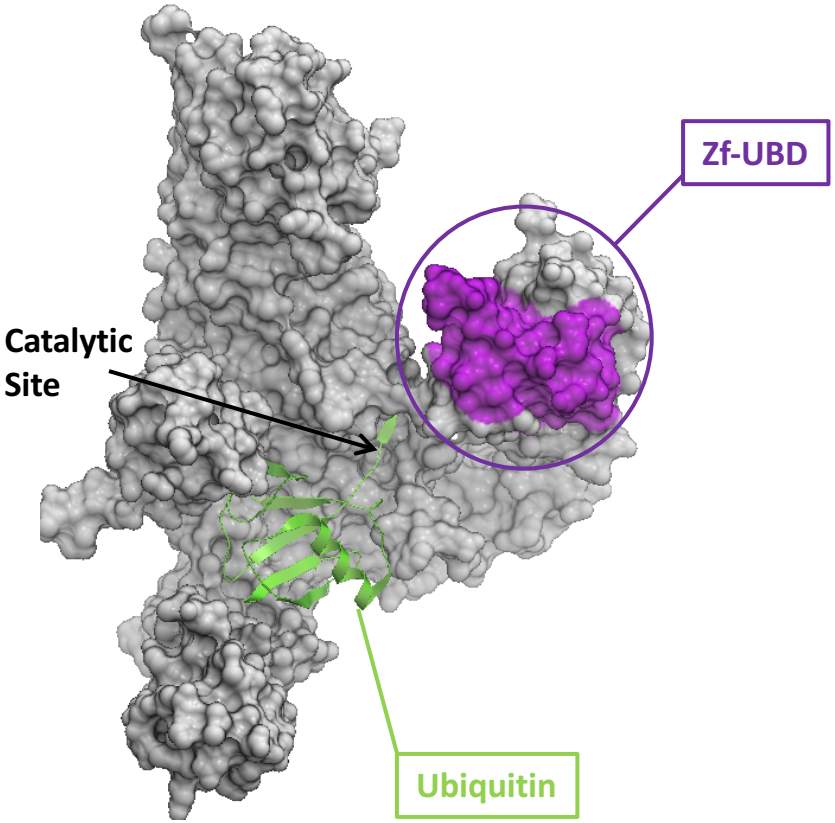
Development of Chemical Probes Against USP5 Zf-UBD

Mandeep Mann, MSc Candidate

Structural Genomics Consortium, University of Toronto

Extreme Open Science Initiative
Ottawa, January 19 2018

USP5 Background



- Ubiquitination can mark proteins for degradation, alter their cellular location, affect their activity, and promote or prevent protein interactions
- USP5 is responsible for the recycling of free ubiquitin from unattached ubiquitin chains (Komander *Nature Rev Mol Cell Bio* 2009)
- The Zinc Finger Ubiquitin Binding Domain (Zf-UBD) of USP5 recognizes the free C-terminal Gly-Gly motif of ubiquitin
- Binding of ubiquitin to Zf-UBD allosterically activates the catalytic activity of USP5 (Reyes-Turcu *Cell* 2006)
- Small molecule catalytic inhibitors of USP7 and USP1 were recently reported (Turnbull *Nature* 2017, Kategaya *Nature* 2017, Liang *Nat Chem Biol* 2014)
- Is the USP5 Zf-UBD a valid therapeutic target?

USP5 Disease Association

- Cancer therapy
  - Depletion of USP5 increases p53 levels (Dayal *JBC* 2008)
  - USP5 knockdown leads to increased DNA damage and apoptosis in pancreatic cancer cells (Kaistha *Oncotarget* 2017)
- Neurological disorders
  - USP5 Zf-UBD interacts with a voltage-dependent calcium channel leading to inflammatory and neuropathic pain (Garcia-Caballero *Neuron* 2014)

Project Outline

- Expression and purification of USP5 Zf-UBD
- Development of biophysical assays for screening compounds
  - Fluorescence Polarization
  - Differential Scanning Fluorimetry
  - Surface Plasmon Resonance
  - Isothermal Titration Calorimetry
- High resolution, 3D crystal structures
- Computational approaches
- Collaborate with chemists and cell biologists

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Matthieu Schapira

Rachel Harding

Mani Ravichandran

Cheryl Arrowsmith

Aled Edwards

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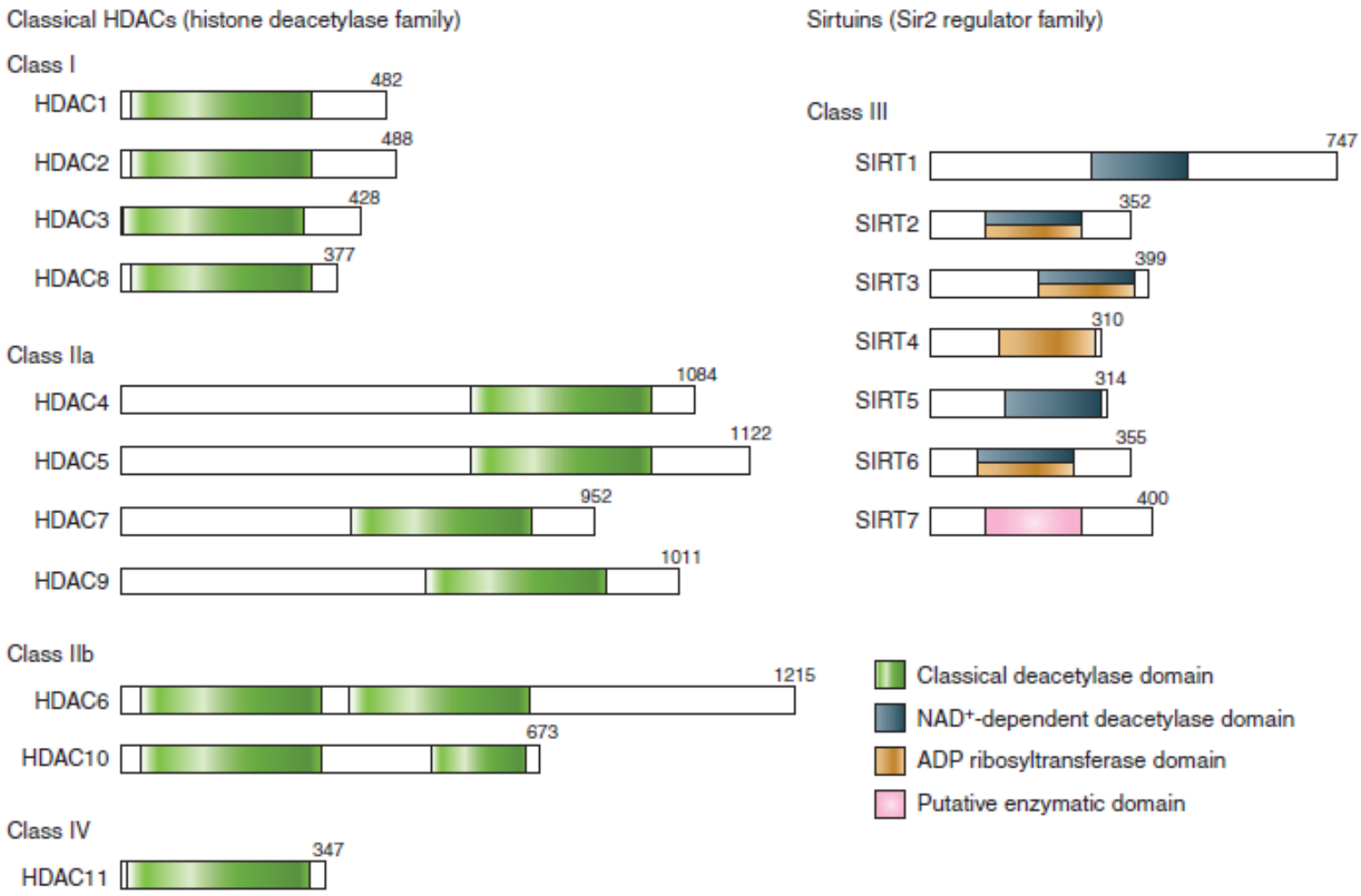
Developing assays and screening methods for discovery of chemical probes for histone deacetylases (HDACs)

Megha Abbey (PDF)
Molecular biophysics,
Masoud Vedadi's team,
SGC Toronto

19th January 2018

Histone deacetylase (HDAC) family



Modified from Seto *et al.*, *Cold Spring Harb Perspect Biol.*, 2014; 6(4):a018713

HDACs and disease association

HDAC	Disease
HDAC1	Breast, colorectal, liver, gastric, lung cancer, myeloma, Atrichia with papular lesions, retinoblastoma, Rett syndrome
HDAC2	Breast, colorectal, pancreatic, liver, gastric, lung cancer, medulloblastoma, CTCL, endometrial stromal sarcoma, Rett syndrome
HDAC3	Breast, colorectal, liver, gastric, lung cancer, Friedreich's ataxia
HDAC4	Gastric cancer, Brachydactylic mental retardation syndrome, developmental coordination disorder
HDAC5	Colorectal, liver, lung cancer, medulloblastoma, AML
HDAC6	Breast, pancreatic, liver cancer, CTCL, AML, X-linked dominant chondrodysplasia
HDAC7	Colorectal, pancreatic cancer
HDAC8	Neuroblastoma, Cornelia de Lange syndrome, Wilson Turner X-linked mental retardation syndrome
HDAC9	Medulloblastoma, malignant gastrointestinal neuroendocrine tumour, corneal staphyloma, Jaw-Winking syndrome, Peters anomaly
HDAC10	Cervical, gastric, lung cancer, Neuroblastoma
HDAC11	Colon, prostrate, ovarian, breast cancer, pituitary tumors, pancreatic neuroendocrine tumor, gliomas, renal I/R injury

Dali-Youcef *et al.*, *Sci Rep.,* 2015, 5:9087; Klieser *et al.*, *Human Pathol.,* 2017, 65:41-52; Li *et al.*, *Cold Spring Harb Perspect Biol.,* 2016, 6(10); Mrug *et al.*, *Am J Physiol Renal Physiol.,* 2013, 305(7):F973-4; Roche *et al.*, *Eur J Med Chem.,* 2016, 121:451-483; Wang *et al.*, *Cell Biol Int.,* 2017, 41(12):1290-1295; Zhang *et al.*, *Hepatol Res.,* 2017, 47(2):149-159.

Development of HDAC inhibitors

Screening of compound libraries using high-throughput assays

Five approved HDAC inhibitors

Inhibitor	HDAC Class specificity	Disease
Vorinostat	I, II, IV	Cutaneous T-cell lymphoma
Belinostat	I, II, IV	Peripheral T-cell lymphoma
Panabiostat	I, II, IV	Multiple myeloma
Valproic acid	I, IIa	Epilepsia, biopolar disorder, migraine
Romidepsin	I	Cutaneous T-cell lymphoma

Side-effects include gastrointestinal disturbances (nausea, vomiting), fatigue, liver toxicity, hematologic problems (thrombocytopenia, neutropenia, anaemia), QT prolongation with risk of fatal arrhythmia

Need for Selective inhibitors/drugs

Designing and optimize new high-throughput assays for screening

- Substrate (Target) specificity
- Specificity for binding partner

HDAC11	Colon, prostrate, ovarian, breast cancer, pituitary tumors, pancreatic neuroendocrine tumor, gliomas, renal I/R injury

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All members of the team

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Extreme Open Science Initiative, SGC

Aled Edwards
Matthieu Schapira
Rachel Harding

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Design and Synthesis of Chemical Probe for AAK1/BIKE

High Priority Probe Project
(SGC-UNC, Campinas, Oxford)

SGC-UNC funding: Eshelman Institute for Innovation, UNC

Nirav R. Kapadia

Post Doctoral Research Associate, SGC-UNC,UNC-Chapel Hill, NC : March 2016-present
PhD, City University of New York, New York, NY : August 2010-Jan 2016
[Advisor: Wayne Harding, "Synthesis of Novel Aporphine-Inspired Neuroreceptor Ligands"]
MS, Fairleigh Dickinson University, Teaneck, NJ: Jan 2008-Dec 2009
BPharm., Sardar Patel University, Gujarat, India: August 2003-April 2007

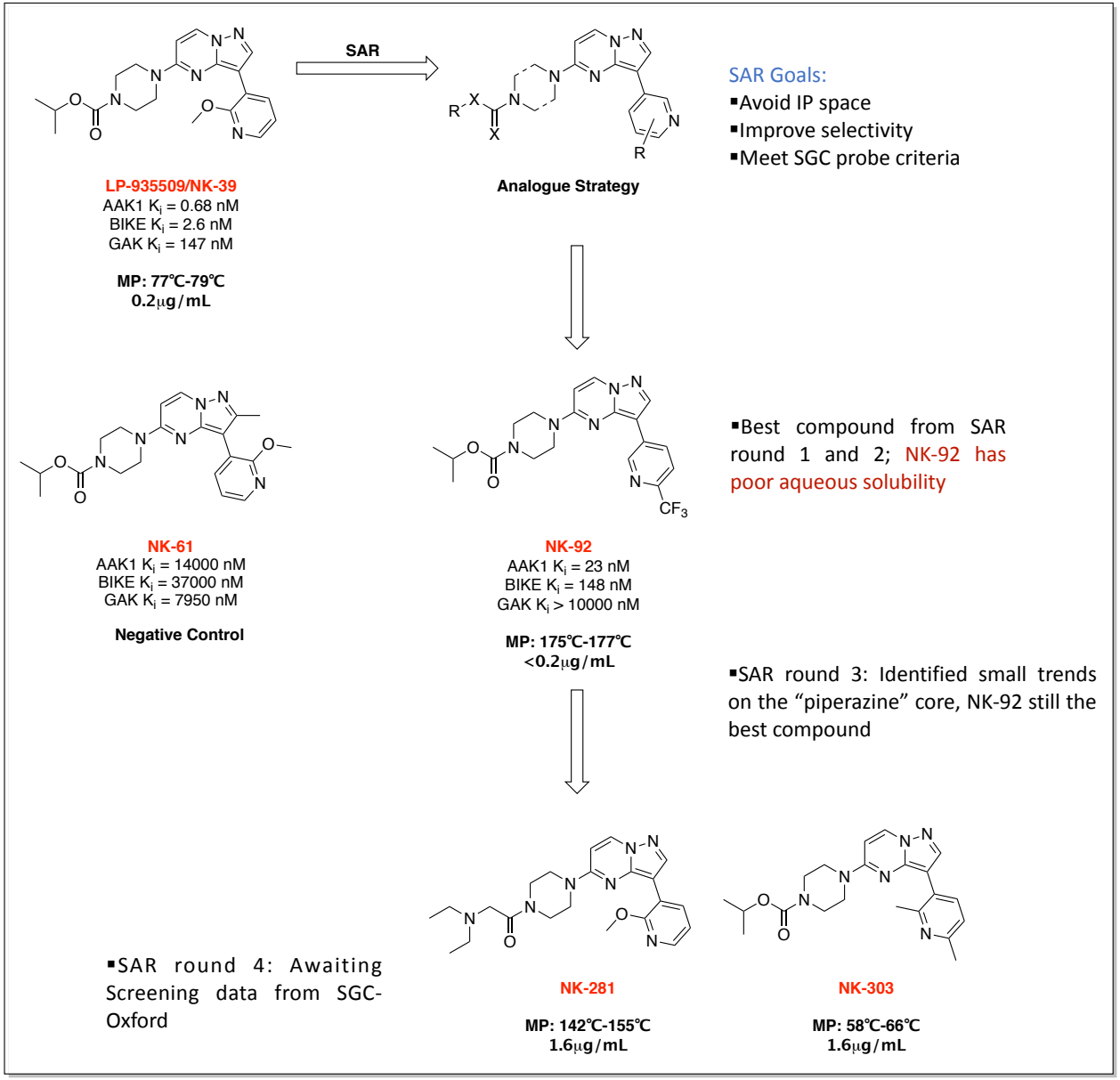
Project Overview

- **AAK1: A**daptor protein 2-**A**ssociated **K**inase **1**

- **Understudied kinase:** 17 citations in 2010 bibliometric analysis

- **Function:** involvement in clathrin-mediated endocytosis

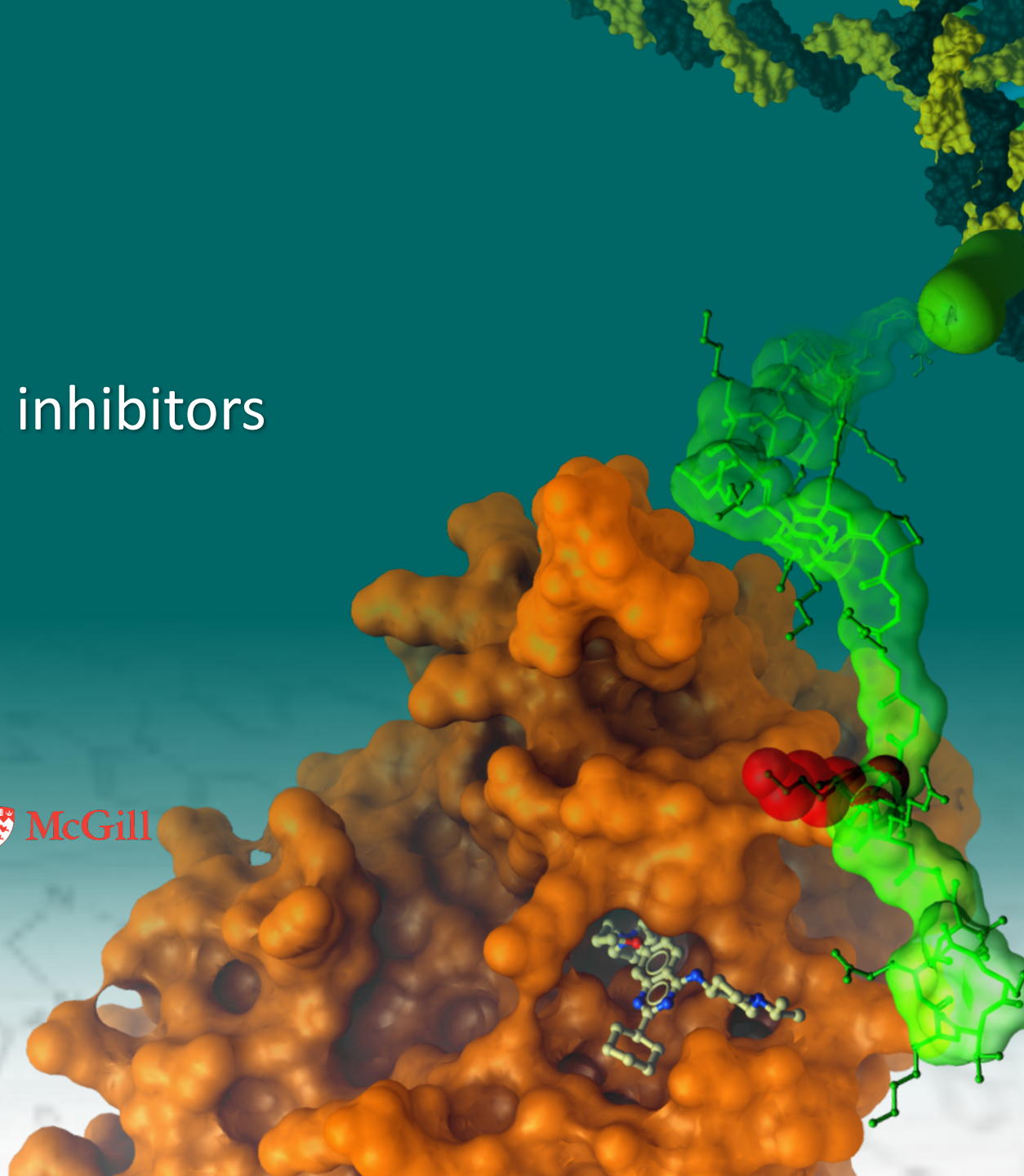
- **Disease link:** ALS, Alzheimer's disease, bipolar disorder, pain, Parkinson's disease, schizophrenia



Hunting for ACVR1 inhibitors

Roslin Adamson
Growth Factor Signalling group
University of Oxford

Ottawa 19 Jan 2018

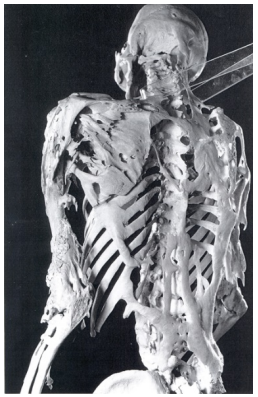


Same mutations, different diseases

ACVR1/ALK2

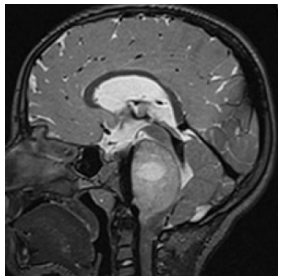
Fibrodysplasia ossificans progressiva (FOP)

- Rare genetic disease characterised by progressive conversion of soft tissues into bone (heterotopic ossification)
- Mutations in bone morphogenetic protein (BMP) receptor ACVR1 alter signalling
- R206H most common of about 12 (in 97% of cases)
- Current treatment purely symptomatic

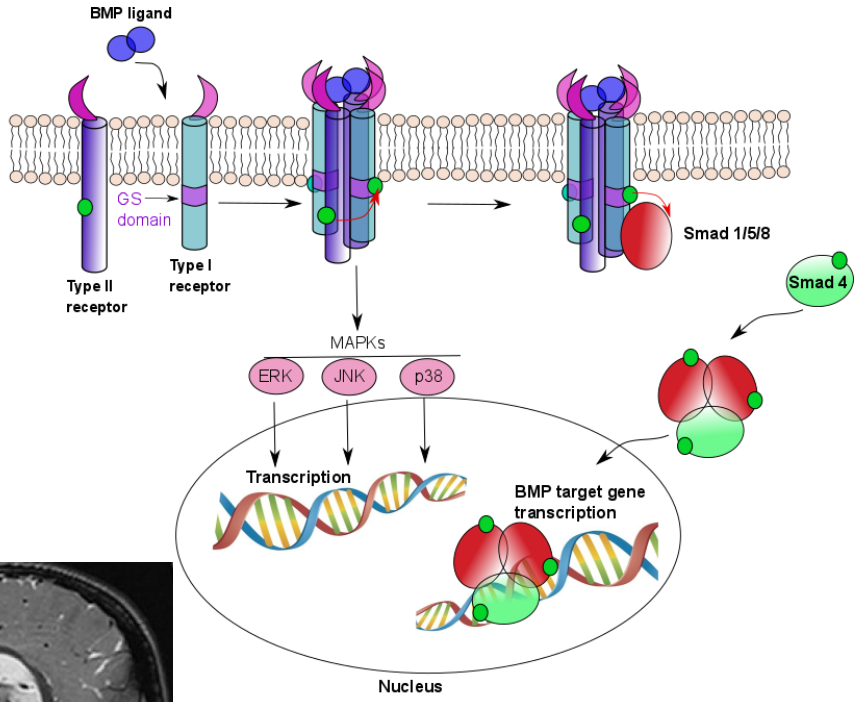


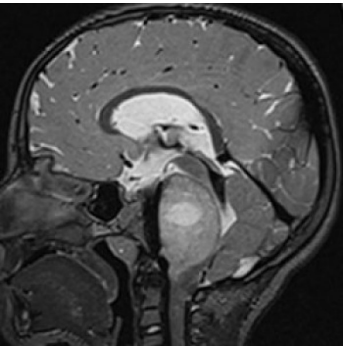
Diffuse intrinsic pontine glioma (DIPG)

- Aggressive childhood brain cancer in pons in brainstem
- Lifespan after diagnosis 9-12 months
- Surgery impossible, radiotherapy ineffective, no chemotherapy available
- Many of the same FOP mutations in ACVR1 are found in DIPG (~25%) + H3 histone mutations

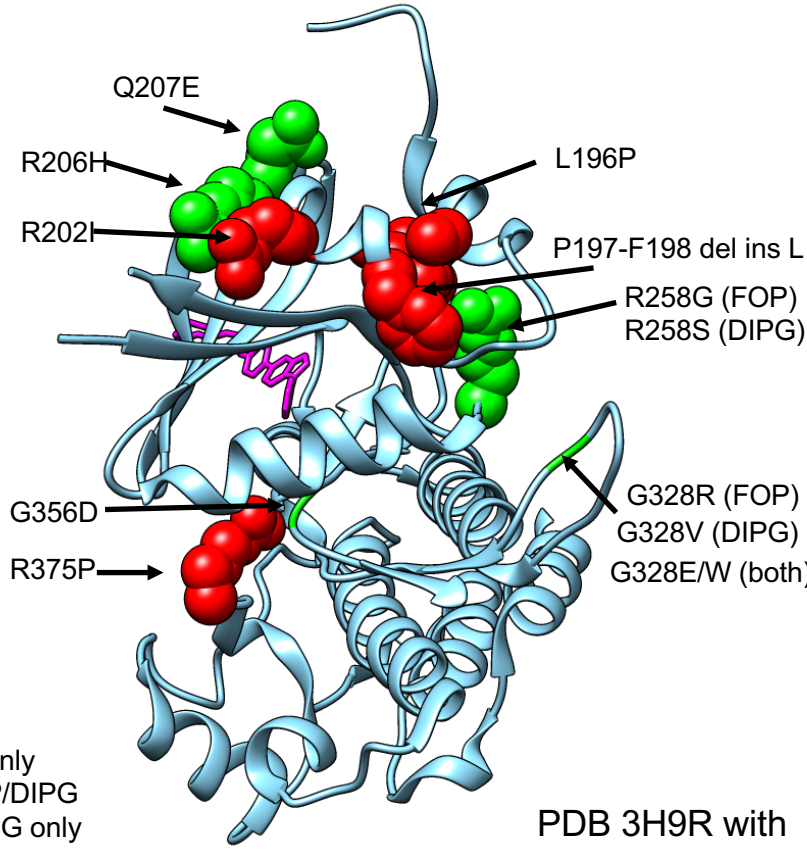


ACVR1 signalling and structure





https://www.sciencedaily.com/releases/2014/04/140407101737.htm



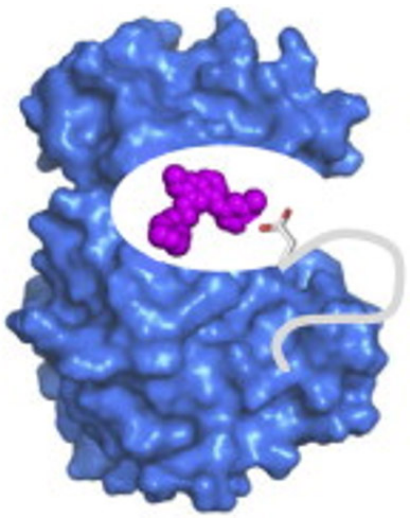
Red – FOP only
Green – FOP/DIPG
G328V – DIPG only

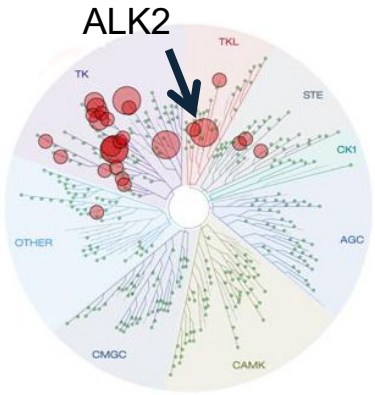
PDB 3H9R with dorsomorphin

Allosteric inhibitors offer exquisite selectivity

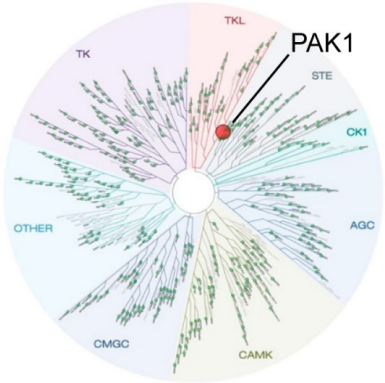
Use structural biology to look for:

- potentially more selective ATP competitive inhibitors
- fragments that bind allosteric sites and can be built into drug-like molecules

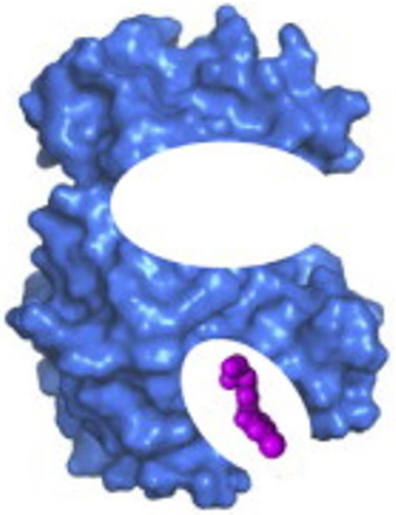




e.g. saracatinib



e.g. NVS-PAK1-1



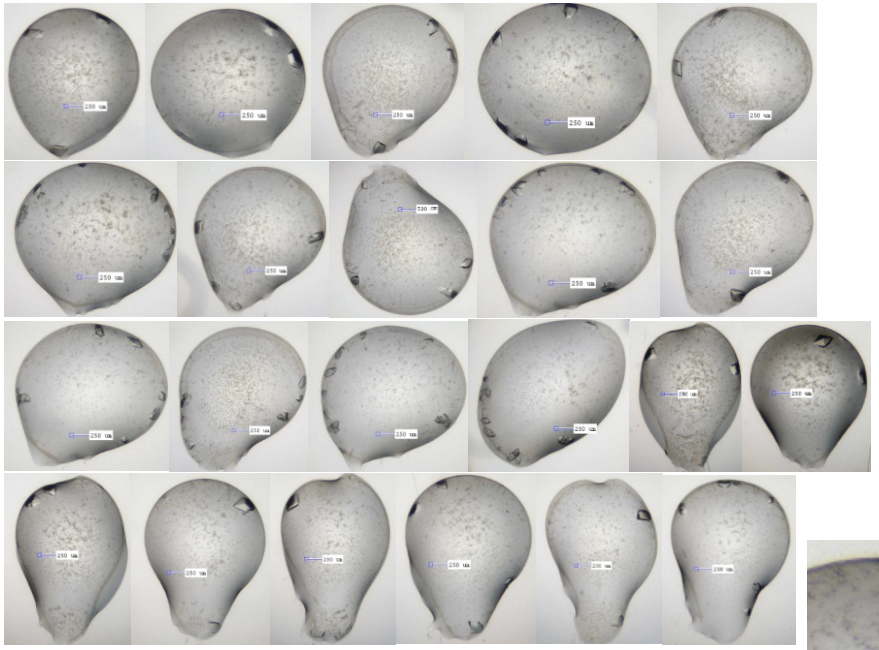
ATP site inhibitor

Allosteric inhibitor

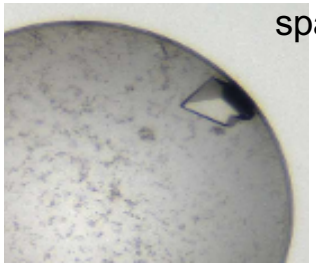
TRENDS in Pharmacological Sciences

Crystallography

Fragment screening for allosteric binding site



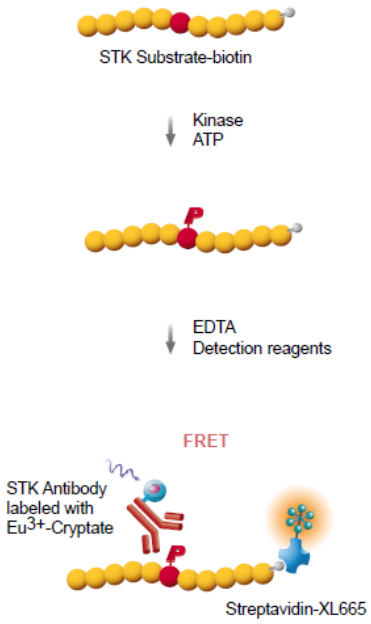
ACVR1 co-crystallised with LDN 193189



- Original structure (3Q4U) conditions (20 % PEG 3350, 0.2 M ammonium citrate dibasic) fine screened and crystals used for initial solvent testing and limited fragment screening
- 92 crystals mounted: 29 soaked in 5-30 % DMSO with or without 25 % ethylene glycol.
- 63 soaked in various compounds
- 52 total datasets below 3.4 Å
- 43 datasets ranging in resolution from 1.7-2.5 Å were collected (83 %)
- Currently preparing models in different space groups to refine the data

HTRF KinEASE assay

Aim: to develop a robust, highly sensitive assay for inhibitor screening



Kinase phosphorylates substrate in the presence of ATP

Biotin-labelled substrate binds labelled strepavidin

Labelled antibody binds phosphorylated peptide

FRET takes place between labelled substrate and SA within the Forster range

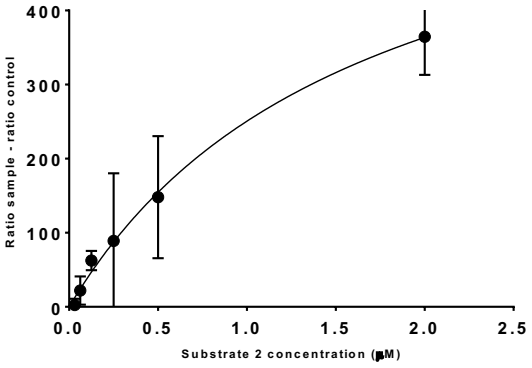
8. Optimization of the kinase assay

A typical development for a HTRF® KinEASE™-STK assay consists of the following steps:

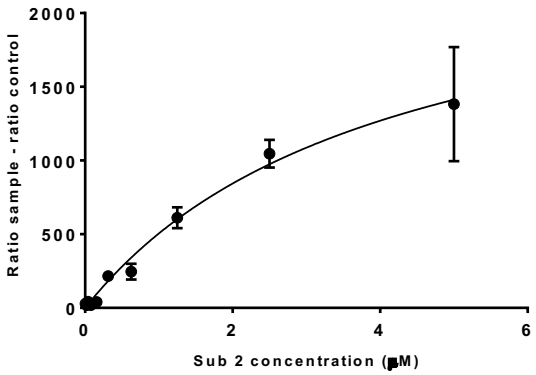
1. Substrate selection (only possible with HTRF® KinEASE™-STK discovery kit)
2. Enzyme titration
3. Kinetic study
4. Substrate titration
5. ATP titration
6. Biotin/streptavidin ratio optimization
7. Inhibitor IC50 determination

HTRF KinEASE assay data

Requirements: to be able to assay ~1 nM enzyme



[ACVR1A-c096] = 5 nM
[ATP] = 100 uM



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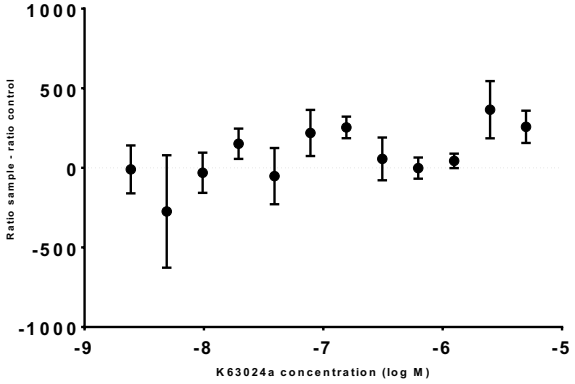

5 nM ACVR1A-c096
5 mM MgCl2
2.5 µM substrate 2
100 µM ATP
4:1 biotin:SA ratio
Compound 5 µM to 2.4 nM

Everything fine until inhibitor added

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Dan Pinkas
Ellie Williams
Jong Fu Wong
Zhou Chen
Liz Brown
Alice Fox

Growth Factor Signalling Group

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