# Set de inhibidores de proteínas quinasas KCGS y su aplicación en el estudio y modulación de estas proteínas

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**Investigator Postdoctoral (2017-2020) SGC-UNC**

**PROSIC, Enero 2022**

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# Alfredo Picado

sgc-unc.org

## OPEN SCIENCE

## Our Beginning

The SGC (Structural Genomics Consortium) is a not-for-profit, public-private partnership that performs basic science of relevance to drug discovery. Our research is conducted at several sites around the world to produce reagents, proteins, antibodies, assays, and data that support exploration of the human genome. All material and intellectual output of the

# Resumen

- Ciencia abierta para todos y “chemical probes”
- Proteínas quinasas.
- Construcción del set selectivo de inhibidores de quinasas (KCGS) .
- Aplicaciones del set KCGS en el inicio del desarrollo de medicamentos.

SGC

www.thesgc.org


wellcome trust INNOVATION.CA GenomeCanada Ontario FAPESP UNC ESHELMAN INSTITUTE FOR INNOVATION imi

abbvie Pfizer BAYER Boehringer Ingelheim Janssen Takeda NOVARTIS MERCK

**FAQ**

Fundada en 2003

Publicaciones científicas- open science

No patentes o PI

>250 científicos

**Resultados**

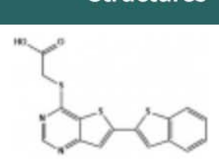
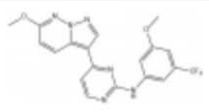
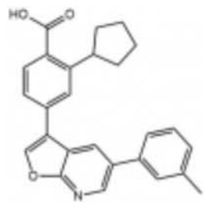
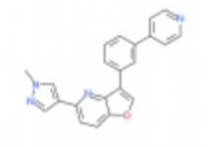
Estructuras de Proteínas

Anticuerpos

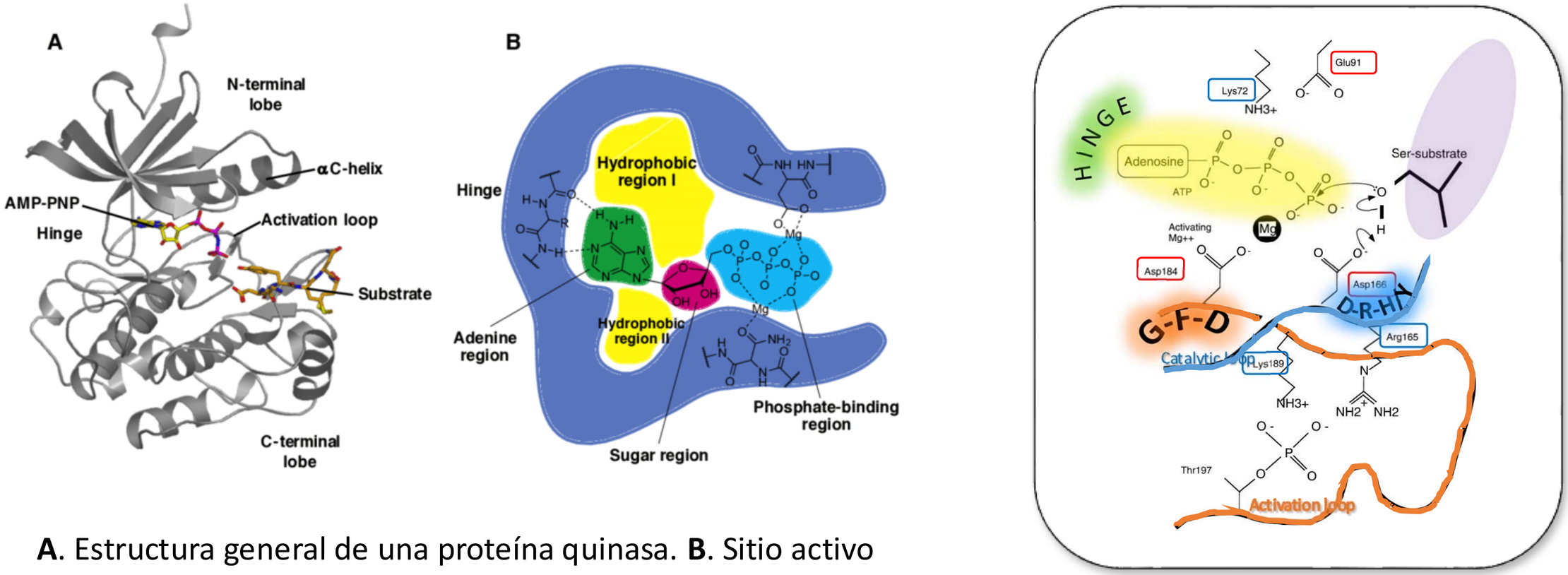
Chemical probes

Ensayos en tejidos humanos

# Chemical Probes

| Name | Structures | Protein Family | Specific Targets | Control | In Vivo Activity* | Added |
|---|---|---|---|---|---|---|
| SGC-STK17B-1 |  | Kinase | STK17B/DRAK2 | SGC-STK17B-1N | | 02/20 |
| SGC-CLK-1 |  | Kinase | CLK1, CLK2, and CLK4 | SGC-CLK-1N | no | 02/20 |
| SGC-CAMKK2-1 |  | Serine-threonine protein kinase | CAMKK1, CAMKK2 | SGC-CAMKK2-1N | no | 01/20 |
| MU1210 |  | Kinase | CLK1, CLK2, CLK4, DYRK1A, DYRK1B, DYRK2 | MU140 | no | 05/19 |

# Proteínas Quinasas




**A**. Estructura general de una proteína quinasa. **B**. Sitio activo


Lorente-Macías, Álvaro. Design synthesis and biological evaluation of 6-alkoxypurine derivatives as kinase inhibitors. DO - 10.13140/RG.2.2.21386.62404

**1950**
Estudio en quinasas, transferencia catalizada de fosfato de ATP

**1986**
Staurosporine

**1995**
Programas de drogas en quinasas

**2001**
Gleevec, aprobado por el FDA

**2002**
500+ quinasas el genoma humano

**2005**
Estudio del “kinome”

**2007**
Kinobeads – Selectividad celular

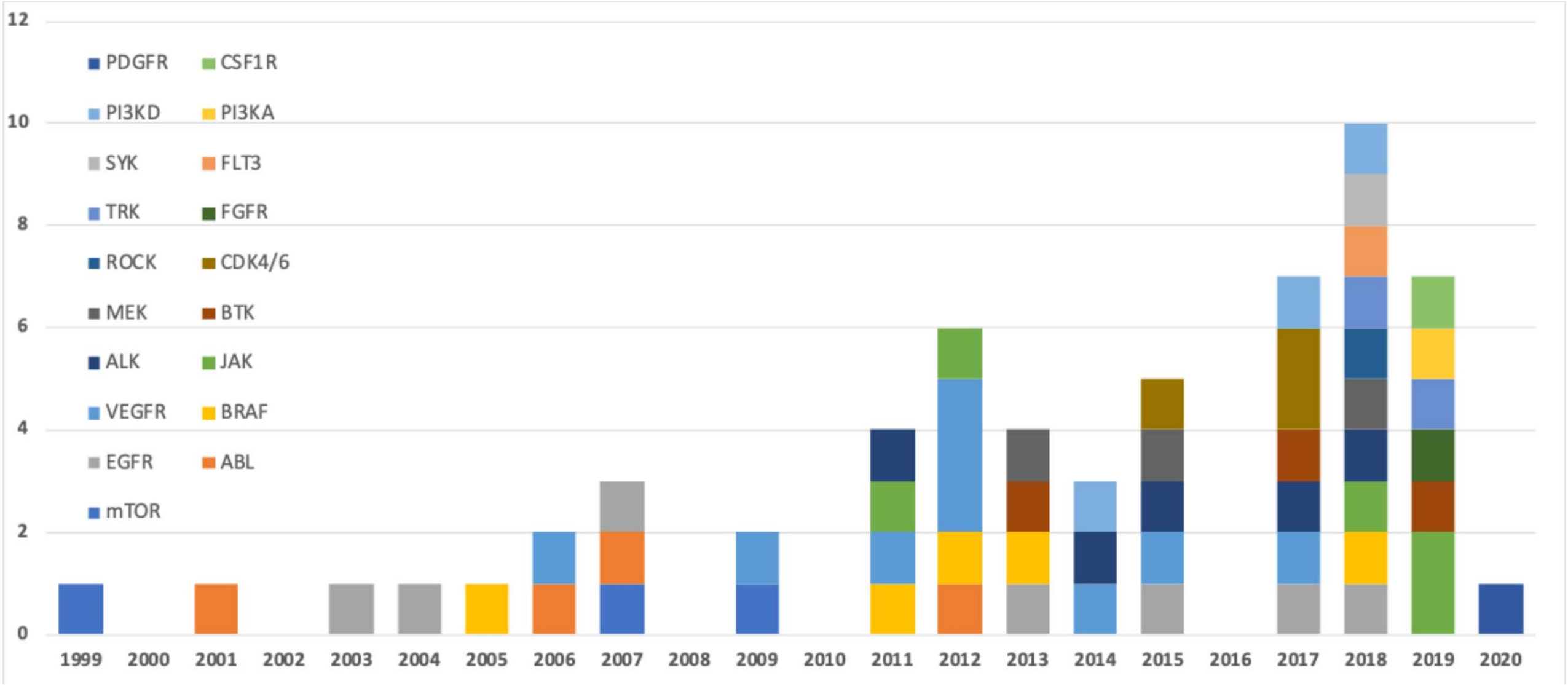
**2017**
Cientos de estructuras cristalinas

**2020**
59 medicamentos aprobados FDA

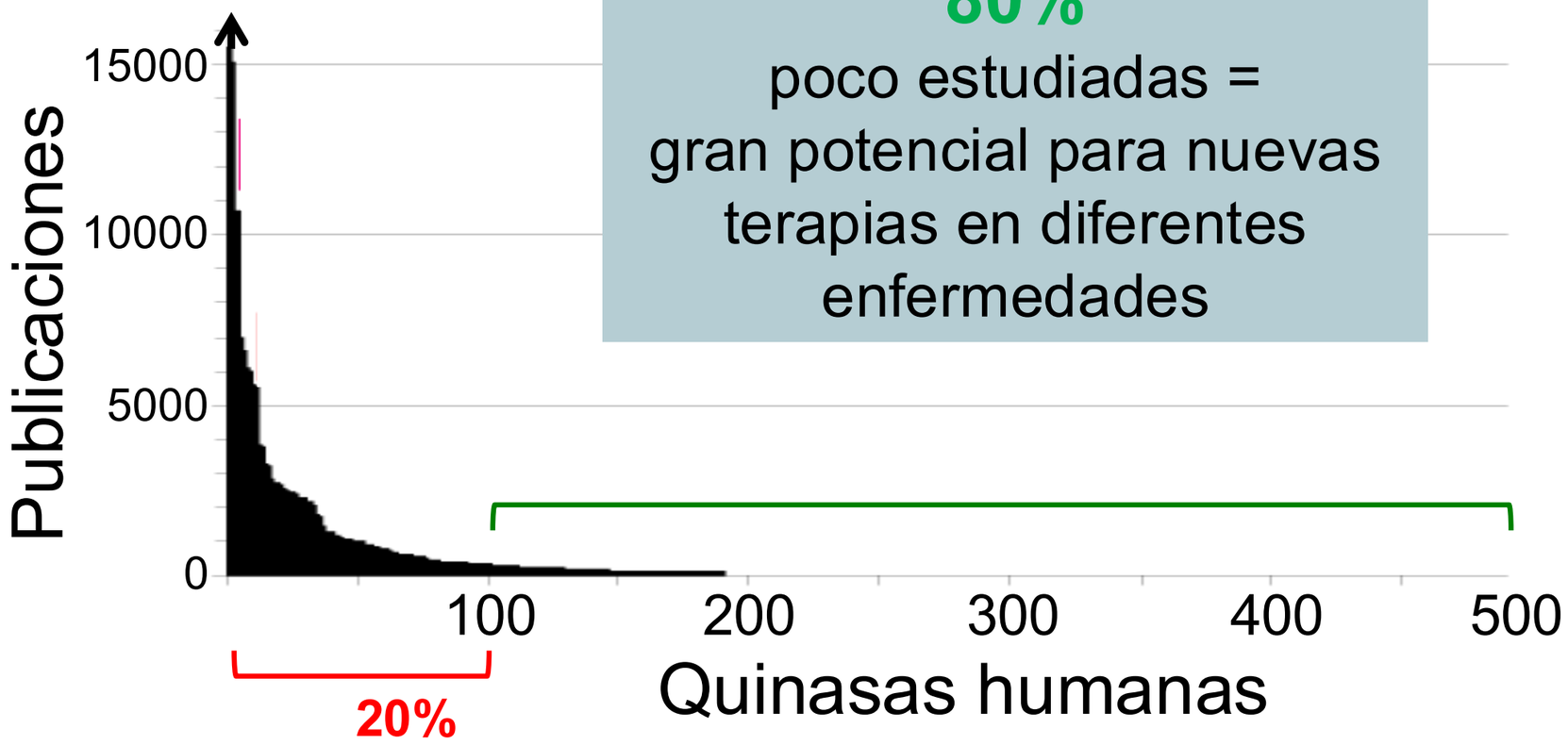
# Quinasas: gran potencial

Kinase Chemogenomic Set

## FDA Kinase Inhibitor Approvals



# Ignoramos mucho del "kinome"




Kinase Chemogenomic Set

- 90% de la investigación en 20% de las quinasas.
- Las quinasas poco estudiadas, relevantes para entender la biología.
- Estrategias para atender el 80% poco estudiado no enseñara su rol en enfermedades, impactando pacientes.

*Nat. Chem. Biol.* (2010) 6 166-9

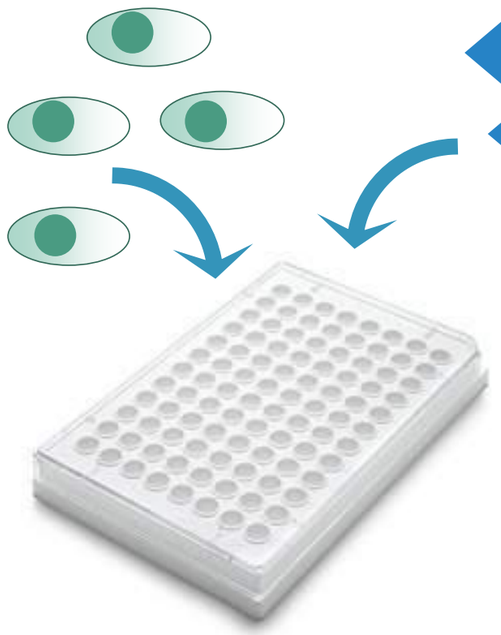
# Kinase Chemogenomics Set

Kinase Chemogenomic Set

Determinar la función de todas las quinasas usando un set pequeño de inhibidores

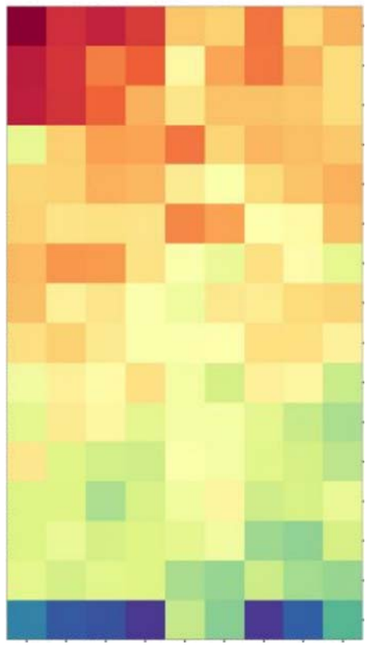
Ensayos fenotípicos relevantes a la enfermedad

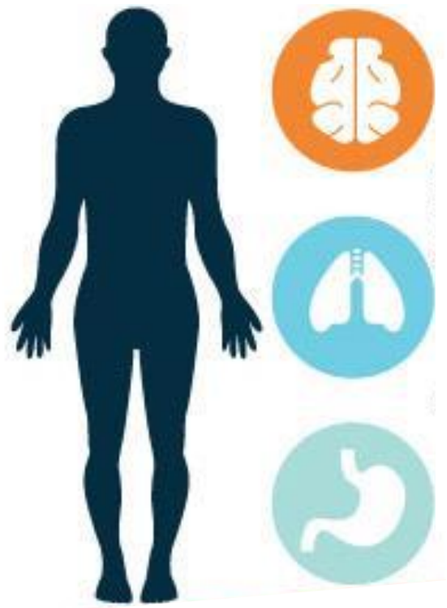
Inhibidores de quinasas



KCGS: Chemogenomic Set

Estudiar todas las 500 quinasas



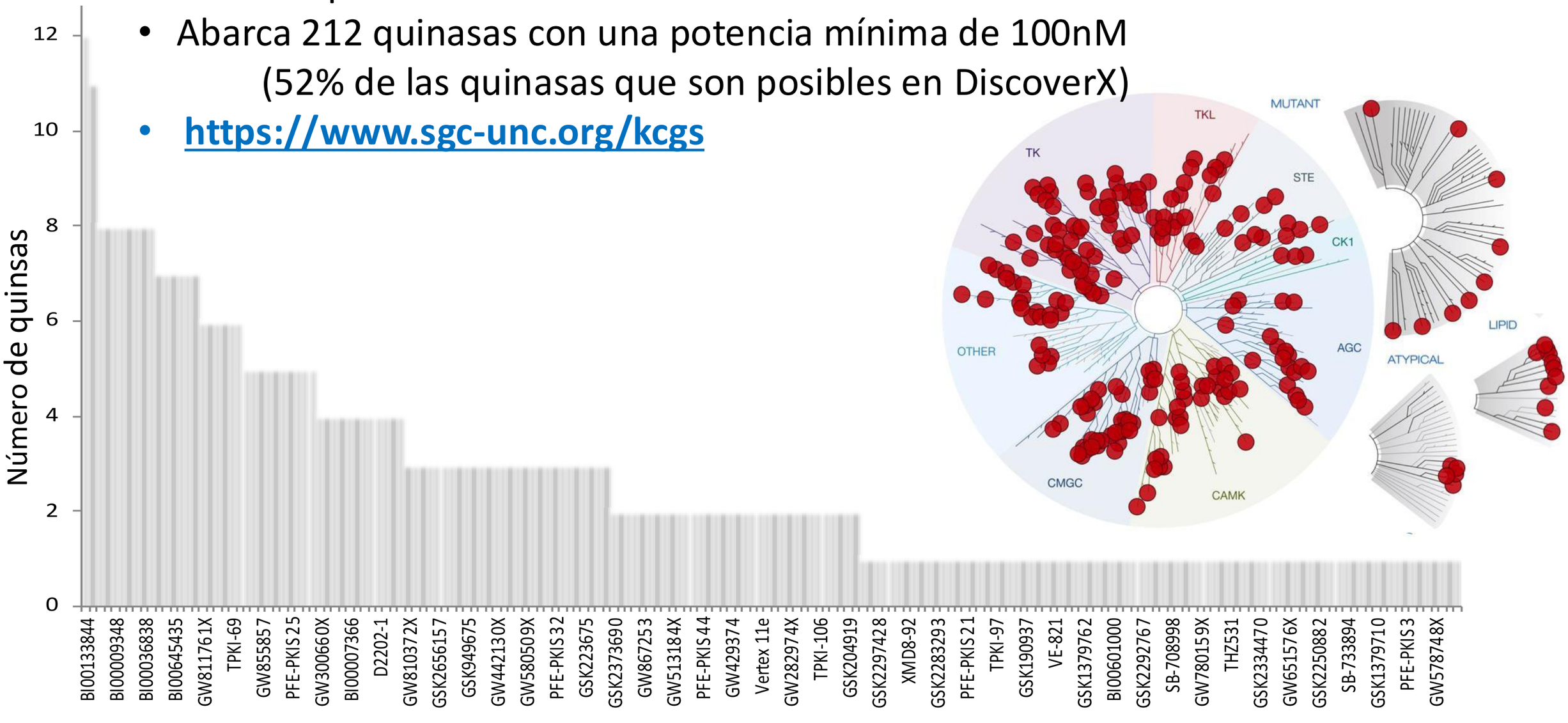


Indentificar la quinasa correspondiente con la respectiva enfermedad

*Nat Rev Drug Disc* (2017) **6** 285–296

# KCGS v 1.0

- 187 compuestos
- Abarca 212 quinasas con una potencia mínima de 100nM
  (52% de las quinasas que son posibles en DiscoverX)
- **https://www.sgc-unc.org/kcgs**

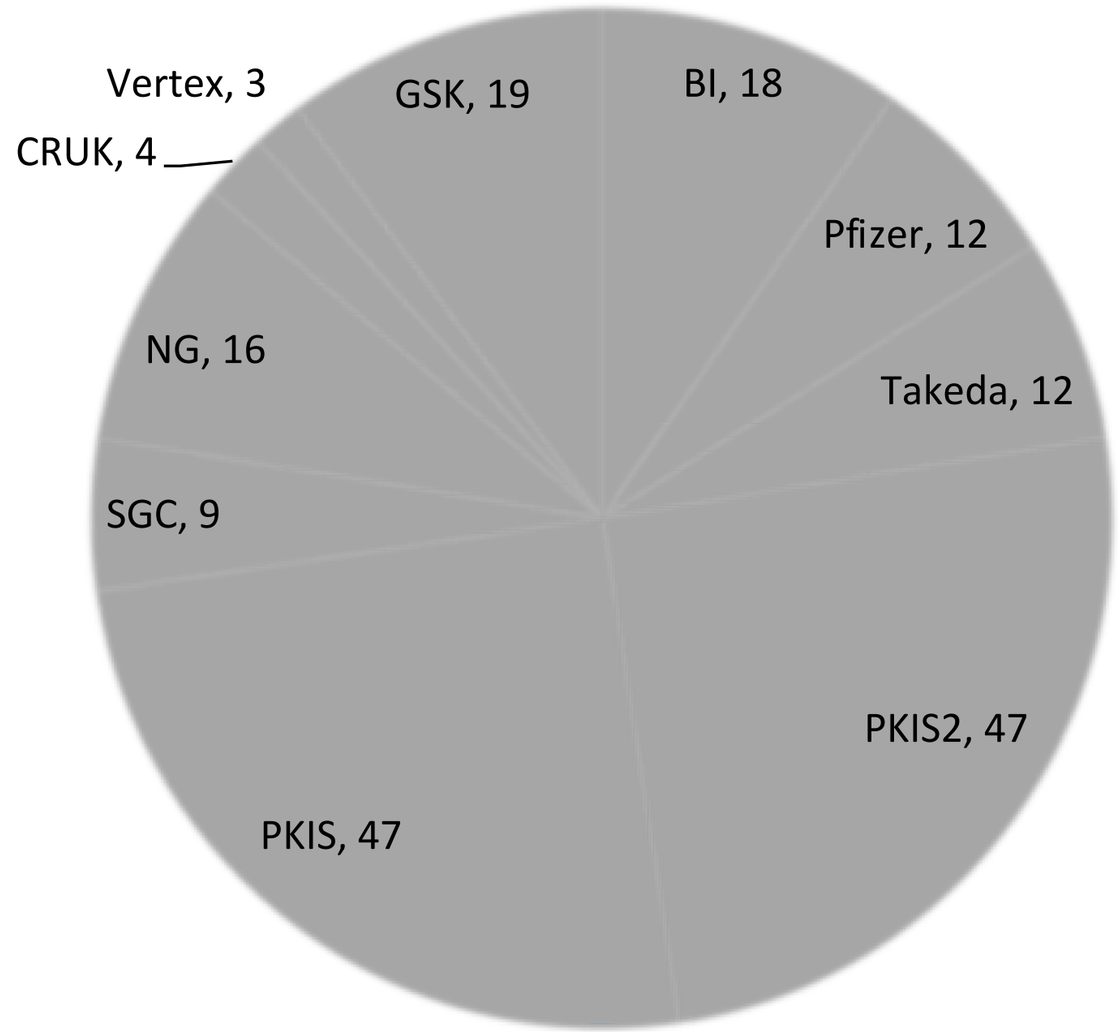


# KCGS donaciones por origen

Versión 1.0 tiene compuestos de:

5 compañías farmacéuticas:
BI, GSK, Pfizer, Takeda, and Vertex
3 Laboratorios/instituciones:
Nathanael Gray, CRUK, SGC

Versión 1.1 será un set suplemental para la v.1.0 e incluirá nuevos compuestos de Abbvie, Bayer, AstraZeneca, Merck, and BI



# Herramienta para la comunidad

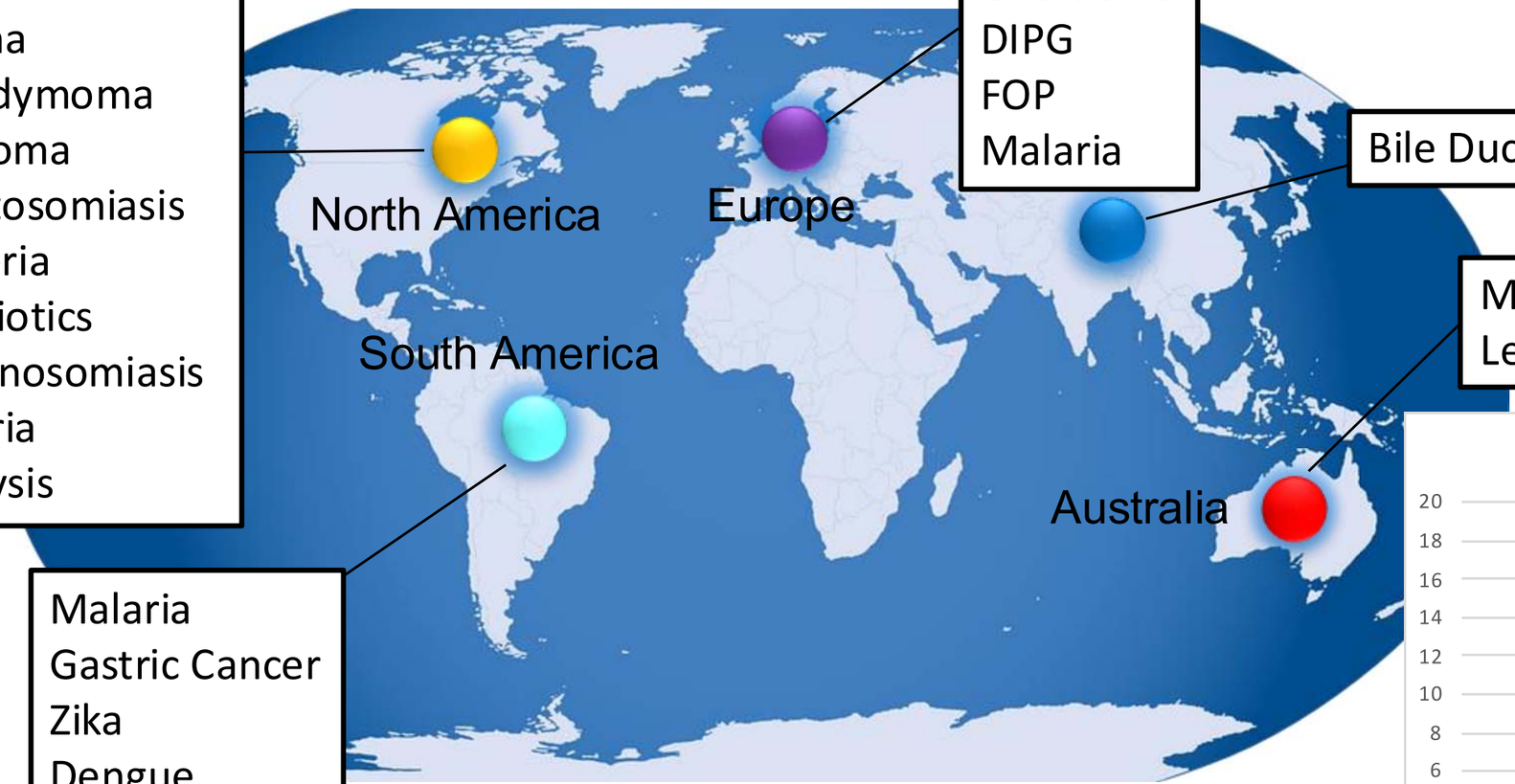
Kinase Chemogenomic Set

TNBC
Glioma
Ependymoma
Myeloma
Schistosomiasis
Progeria
Antibiotics
Trypanosomiasis
Malaria
Paralysis

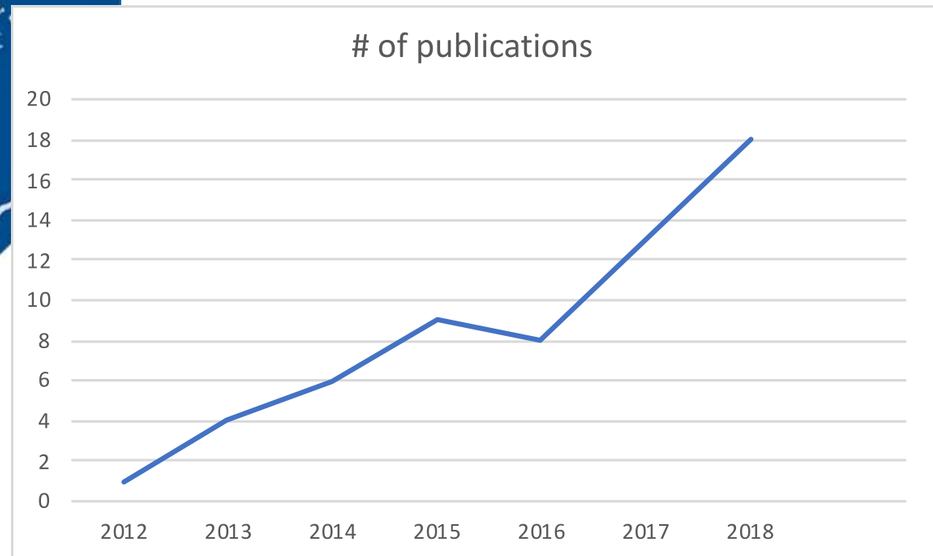
Chordoma
DIPG
FOP
Malaria

Bile Duct cancer

Malaria
Leukemia

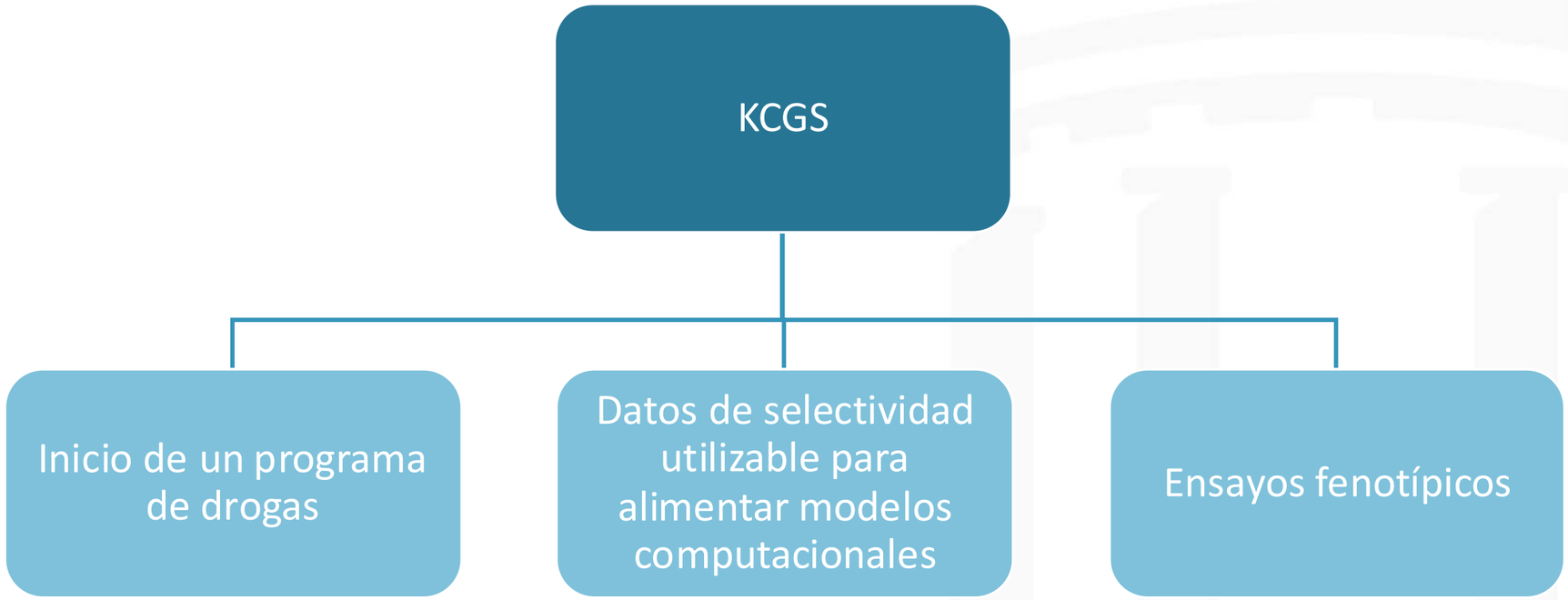



Malaria
Gastric Cancer
Zika
Dengue




"Comprehensive Characterization of the Published Kinase Inhibitor Set" (PMID: 26501955)
"Progress towards a public chemogenomic set for protein kinases and a call for contributions" (PMID: 2876711)

# Aplicaciones del set KCGS




International Journal of *Molecular Sciences*

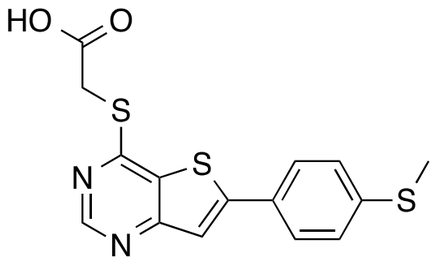
MDPI

*Article*

**The Kinase Chemogenomic Set (KCGS): An Open Science Resource for Kinase Vulnerability Identification**

*Int. J. Mol. Sci.* **2021**, *22*(2), 566; https://doi.org/10.3390/ijms22020566

# Punto de partida para un “STK17B chemical probe”

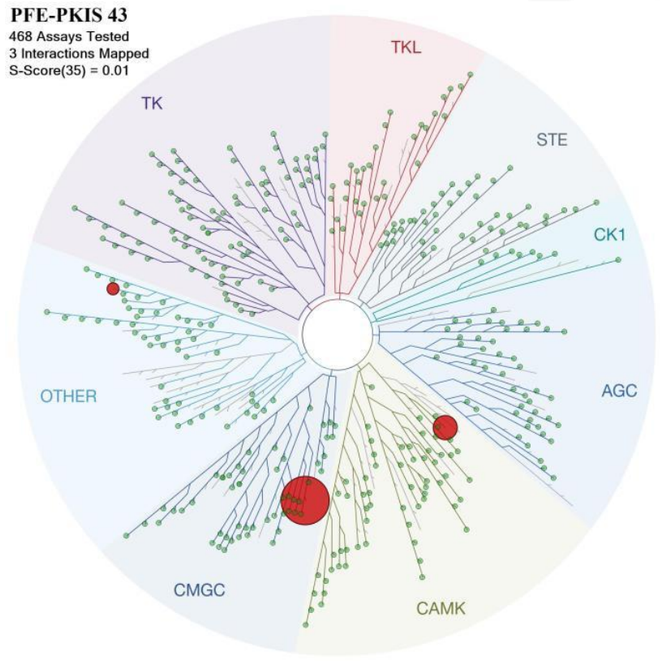



**PFE-PKIS 43**

**Pfizer**

BMCL (2011) 21, p.5952

TPL2 IC50 = 34 μM




| Quinasa | % Inhibición@ 1uM | $K_d$ |
|---|---|---|
| SRPK2 | 100 | >10,000 |
| DRAK2 | 97.7 | 3.8nM |
| AURKB | 78 | 39nM |
| TNK1 | 57 | -- |
| HIPK1 | 55 | -- |
| HIPK2 | 52 | -- |
| KIT | 51 | -- |
| DRAK1 | 37 | 220nM |

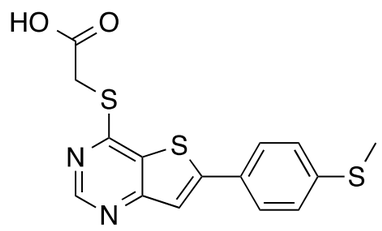
- **STK17B** esta sobre expresada en carcinoma hepatocelular y cáncer de pecho.
- **STK17B** es abundante en células T-maduras.
- **“STK17B** knockout” en ratones resultó en resistencia a enfermedades inmunes (ej. Diabetes tipo 1)

# Primeros resultados-Hits

**% Inhibition**

| Compuesto | DRAK1 | DRAK2 |
|---|---|---|
| PFE-PKIS 14 | 98 | 100 |
| PFE-PKIS 31 | 98 | 100 |
| PFE-PKIS 43 | 37 | 98 |
| PFE-PKIS 9 | 83 | 99 |

KINOMEscan data @ 1µM

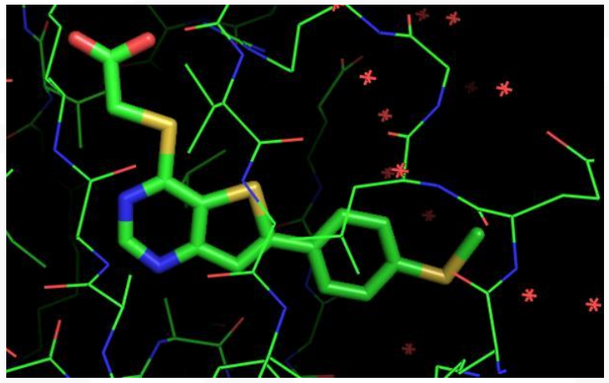



**PFE-PKIS 43**

<u>Selectivad en el Kinome (DiscoverX)</u>

**STK17B = 3.8 nM**
STK17A = 220 nM
AURKB = 39 nM
*No otras quinasas @ 1 µM*

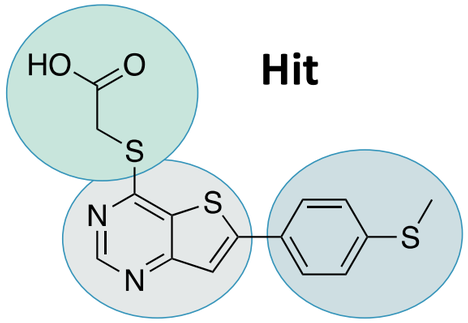
**Estructura co-cristalina PKIS-43/DRAK2**



<u>Solubilidad</u>– 42 *µg*/mL
<u>Estabilidad en Microsomas</u>
(% remanente despues de 30min) – 0%

<u>Siguientes pasos</u>:
1. Explorar la SAR alrededor de **PKIS 43**
2. Mejorar la estabilidad metabólica del compuesto(s) en estudio

# Resultados




**Hit**

100+
Productos Finales

**% inhibición**,
KINOMEscan at
DiscoverX

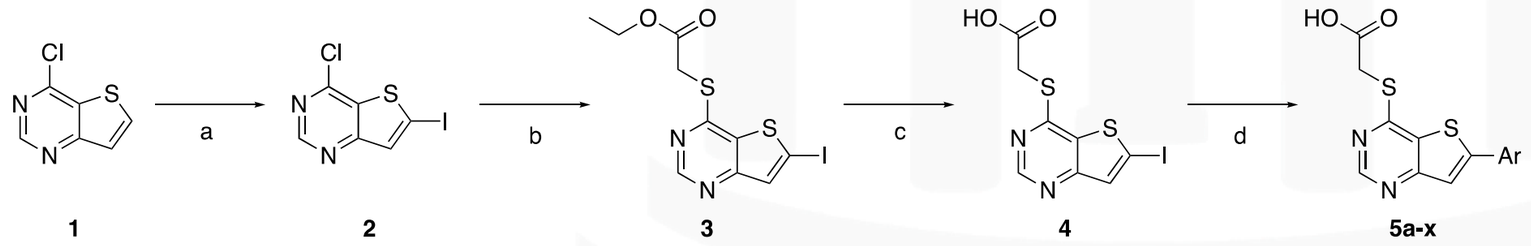
**Ensayo de Afinidad**
% inhibición DRAK2 & DRAK1
DRAK2 $IC_{50s}$
Split-Luciferace (Luceome)

**DRAK2 $IC_{50s}$**
nanoBRET
Ensayo celular (HEK293)

**Ensayo Enzimático**
$IC_{50s}$
Eurofins

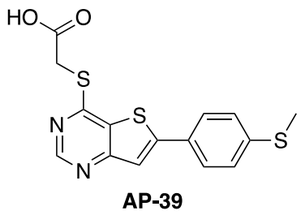
**Otras determinaciones**:
Solubilidad, estabilidad microsomal, permeabilidad

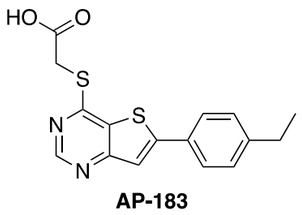
**Síntesis de las thieno[3,2-*d*]pyrimidines.**

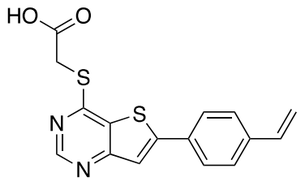


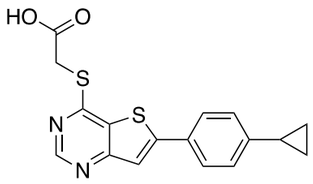
# Selección del "chemical probe"

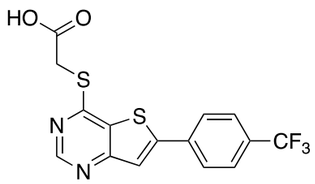
**Candidatos**

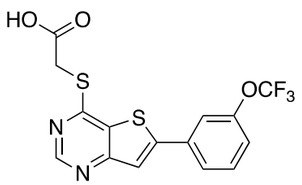

**AP-39**

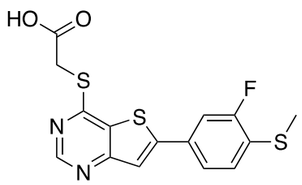

**AP-183**

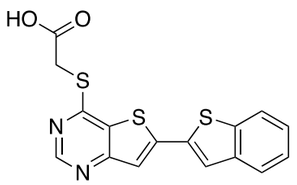

**AP-186**


**AP-229**

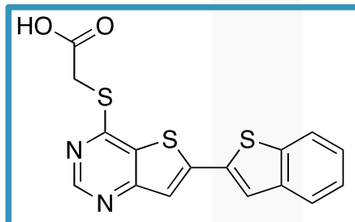

**AP-203**

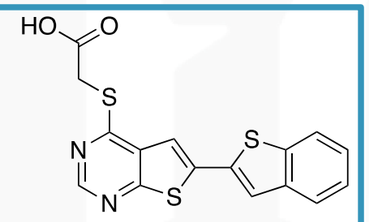

**AP-187**


**AP-123**


**AP-194**

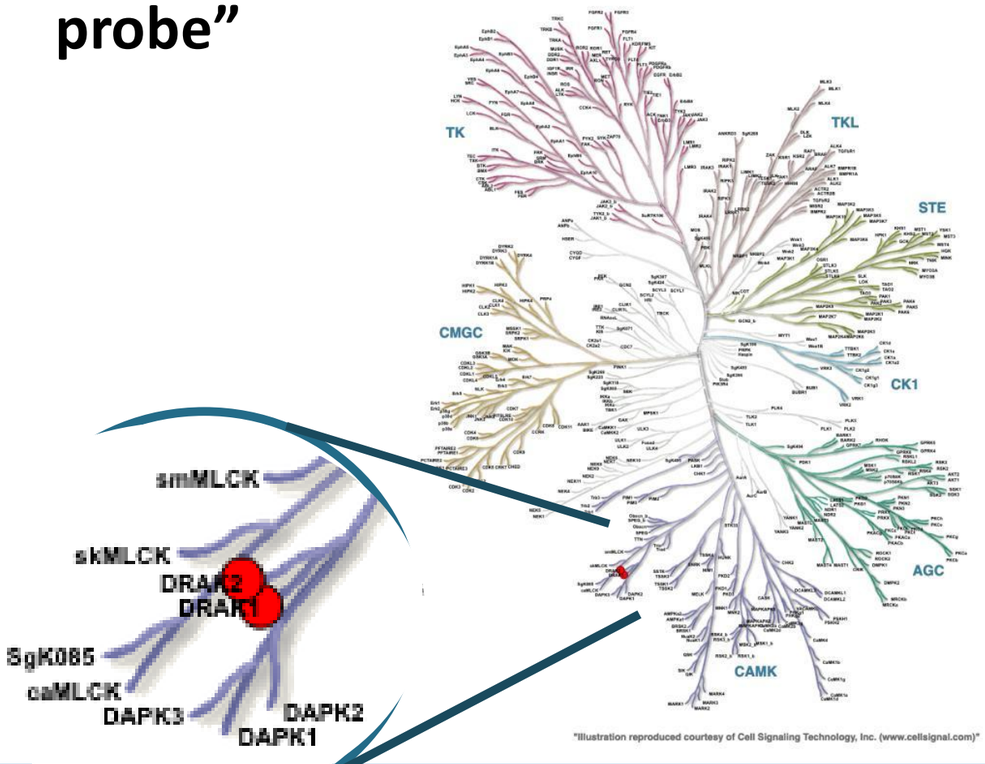
- **DRAK2 $IC_{50}$ <600 nM (celular)**
- % Inhibición de DRAK1 y DRAK2
- $IC_{50s}$ enzimáticos DRAK1 y DRAK2
- Estabilidad en microsomas >50% después 30 min

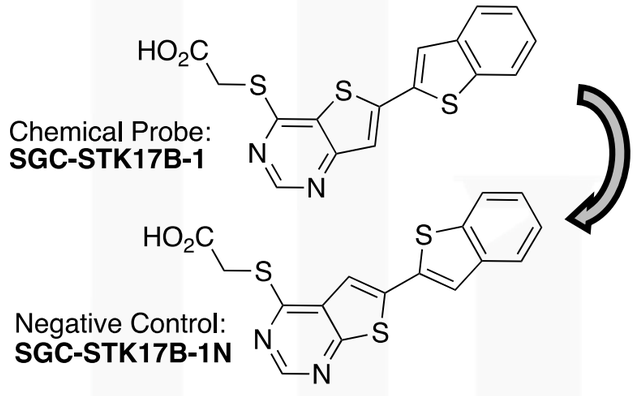

**AP-194**
**SGC-STK17B-1**


**AP-301**
**SGC-STK17B-1N**
**(Negative Conrol)**

# Dos interrogantes

1. **Porque el “chemical probe” >100-veces mas selectivo de STK17B sobre STK17A?**
   - ATP-sitio activo es idéntico
2. **Porque el “control negativo ” > 100-veces menos activos que el “chemical probe”**



# Estructura Cristalina

1. **Porque el “chemical probe” >100-veces mas selectivo de STK17B sobre STK17A?**
   - ATP-sitio activo es idéntico

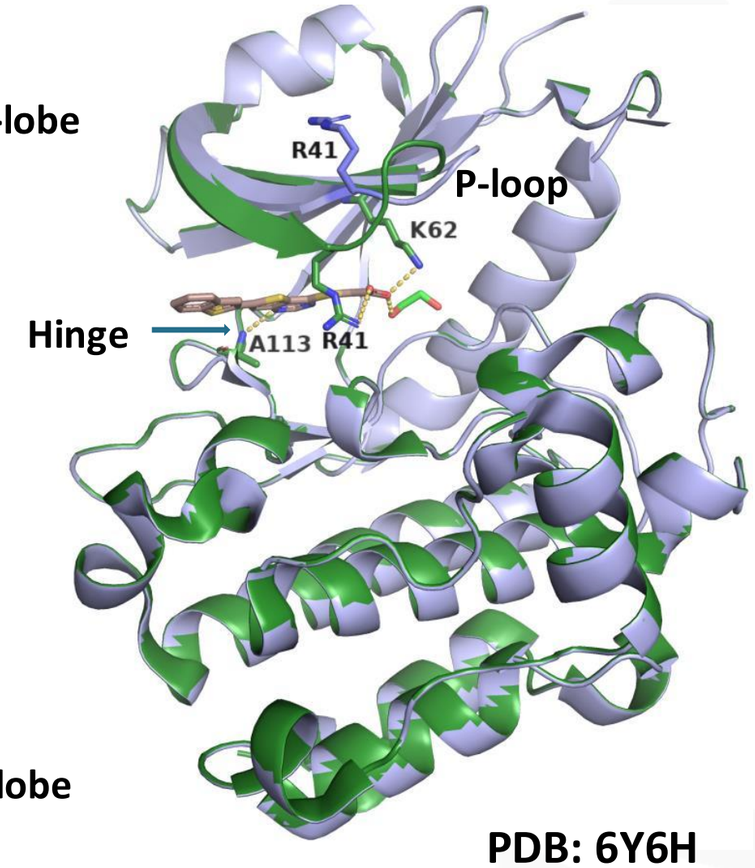
**SGC-Frankfurt**

Stefan Knapp, PhD

Apirat Chaikuad, PhD

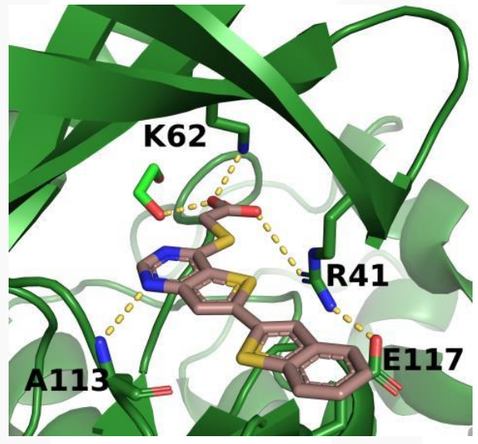
A

**N-lobe**

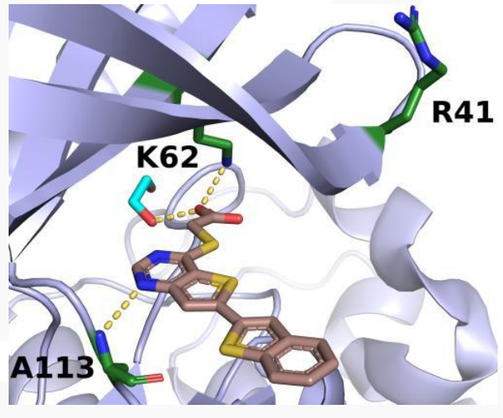



**PDB: 6Y6H**

B




C



# Dinámica Molecular (MD)

- **Kannan lab (University of Georgia) Evolutionary Systems Biology Lab, University of Georgia**

Safal Shrestha
(Estudiante)

Natarajan Kannan, PhD
(Profesor)

# Cálculos de acidez-pKa

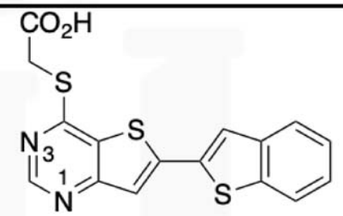
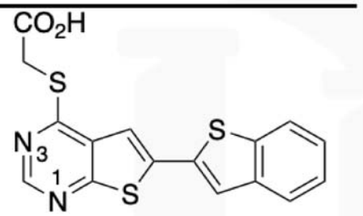
**2. Porque el "control negativo" es >100-veces menos activo?**

- Isómeros de posición de thienopyrimidine tienen la misma forma

**Dr. Shubin Liu**, Research Computing Center-UNC

- Desarrollo un método computacional usando Ab initio y DFT para predecir pKas basado en estructuras químicas

**Cálculos de pKa para el Probe and Control Negativo**

| |  AP-194 |  AP-301 |
|---|---|---|
| $pK_a$ (N1) [a] | 2.8 | 0.1 |
| $pK_a$ (N3) [a] | 1.1 | 0.5 |

[a] pKa was obtained from density functional theory (DTF) calculations using the molecular electrostatic potential as the descriptor

- **AP-194**. N1 es mas básico que N3
- **AP-301**. N3 es mas básico que N1
- N1 en **AP-194** es 500x mas básico que N1 en **AP-301**.

Chunying Rong, Bin Wang, Dongbo Zhao, Shubin Liu. (2020) Information-theoretic approach in density functional theory and its recent applications to chemical problems. WIREs Computational Molecular Science 10:4.

# “Chemical Probe” para modular la proteína STK17B


**A Chemical Probe for Dark Kinase STK17B Derives Its Potency and High Selectivity through a Unique P-Loop Conformation**

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# Trabajo actual: KCGS v.1.1

KCGS Availability

The current set is available to any investigator at a non-for-profit organization for an access fee of $3100 that off-sets the resynthesis cost of the small molecule inhibitors. Requestors receive:

- A copy of KCGS in 96-well plates as 1 µL of 10 mM DMSO solution
- A spreadsheet that lists chemical structure, target kinase, and literature references for each inhibitor
- 5 x 1 µL of 10 mM DMSO cherry picks upon request
- Access to the full kinase selectivity data for each inhibitor

For UNC Investigators KCGS is available through Infoporte CORES. For all other investigators KCGS is available from our distribution partner Ximbio.

A list of investigators that have received a copy of KCGS can be found here.

KCGS Resynthesis

To ensure the continued availability of KCGS, we have completed a project to resupply all of the inhibitors in the set.

https://www.sgc-unc.org/kcgs

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# Muchas Gracias!