Decision Tree for Illumination of Dark Kinases

Development of Dark Kinase Knowledgebase
Collaboration with KMC

Prioritize DKs for study

Expression Mutation Phenotype

CRISPRi/a

Small Molecule Inhibitors

Perturbation Based Phenotypic Imaging

Generate, validate, organize and distribute tools and reagents for study of DKs

Collaborations with IDG groups

Generation, systematization and dissemination of knowledge about DKs, networks, cellular phenotypes and human disease.

Characterization

Phosphoproteomics

Protein-protein Interaction

Reporter Signaling

Metabolomics

PRM Analysis of KO lines

DK Study in Primary Cells

Tangible Products

Completion of Dark Kinase Knowledgebase

Dark Kinase Pages

Chemical Tools

KO Cell Lines

Reagents

New Therapeutic Targets

Collaborations

Quantitative PRM/RNAseq

CRISPR Screens

CRISPR Screens

Yes Stop

Yes Stop

Yes Stop

Yes Stop

Stop

Stop

Stop

Stop

Stop

Stop
Initial DKK Establishment Path

Establish key data and interface with Pharos

Prototype DKK (interface and microsite testing)

DKK embedded as microsite in Pharos

Standalone DKK site

DKK Public (Ver 1.0)

Continued refinement of DKK prioritization based on data and community input

Continued engagement with KMC

Preliminary prioritization of Dark Kinases

Coordinated with KMC

Integration of public data sets (e.g., mutation freq.)

Standards development for DKK integration of additional data (images, etc.)

Year 1

Year 2
Dark Kinase Validated Multiplex PRM Assay – Decision Tree

Proteotypic DK Peptide Identification by LC-MS

PRM Survey Assay

DK Peptide Candidates

+ Target Tissue/Cell
LLOQ < 1 fmol

Qualified DK Peptides

Concurrency
Testing

Configured DK Multiplex PRM Assay

Repeatability (CV≤20%)
Matrix Selectivity
Peptide Stability
Reproducible DK detectability in target tissue/cell

Validated DK Multiplex PRM Assay
Dark Kinase CRISPR/Cas9 Knockouts– Decision Tree

Expression/Mutation cell type suitable for CRISPR/Cas9 targeting

PRM/RNAseq expression analysis

Prioritization of DKs for knockout

Characterize knockout phenotypes growth/apoptosis/migration/invasion perturbation-based phenotypic imaging

- No phenotype-stop
  - consider additional cell lines based on expression/mutation

Chemical tool inhibitor phenotype

Comparison to KO phenotype

Inhibitor-induced phenotype in primary cells

KO vs small molecule inhibitor phenotypic differences

Screen for off-targets

- other kinases/GPCRs/ion channels

Metabolic changes -KO vs inhibitor

Selection of kinases for CRISPRa/i

Assay for expression/activity change/phenotype relative to KO

Phosphoproteomics/PRM analysis of kinome dynamics +/- perturbation

DK KO & CRISPRa/i cell lines for distribution to research community

Collaborate with KMC and DKK informatics team for analysis of kinome dynamics with DK perturbation

DKK/KMC website download
Dark Kinase Chemical Tools – Decision Tree

Fluorescent tracers → optimize tracer → DK-Nluc fusions → DK-Nluc assay → No assay → Stop

Focused Screen → Literature cmpds, SGC Kinase sets, Kinase libraries → Active @ 10 μM → Medicinal Chemistry → IC50 < 1 μM, Aq. Sol. > 10 μM → Kinase Selectivity → S < 0.05 @ 1 μM → Non-Kinase Selectivity → ia @ 10 μM → DK Small Molecule Tool