Organizational meeting on RC4 grant

RFA-OD-10-005

Preamble

Some new data

Control Screens

Validation Screen:

Orthoganol Screen:

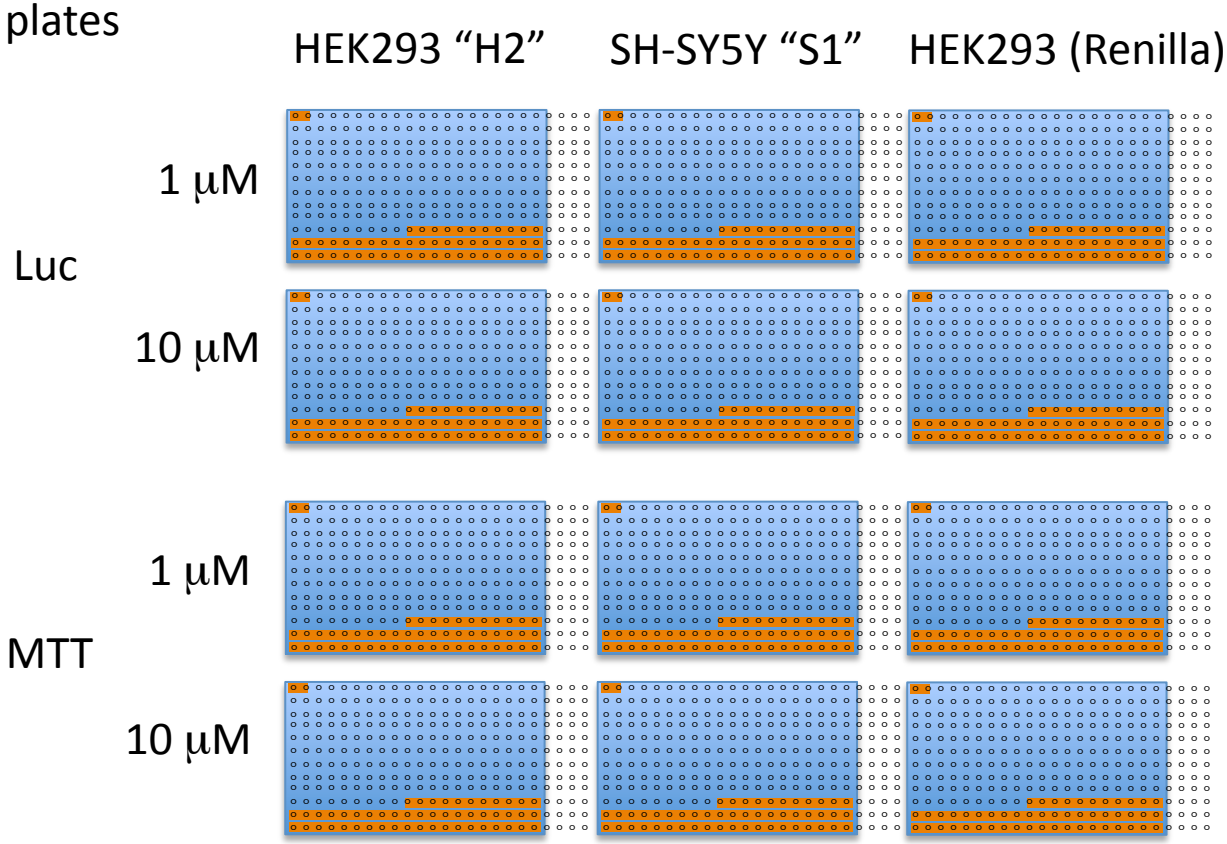
Counter Screen:

Orthogonal Screen

HEK293 or SH-SY5Y cells stably expressing ATXN2-Luc or ATXN2-RLuc

Conducted paired MTT and Luciferase assays for 1 μM and 10 μM compounds

384 well plates



Control DMSO only wells in orange

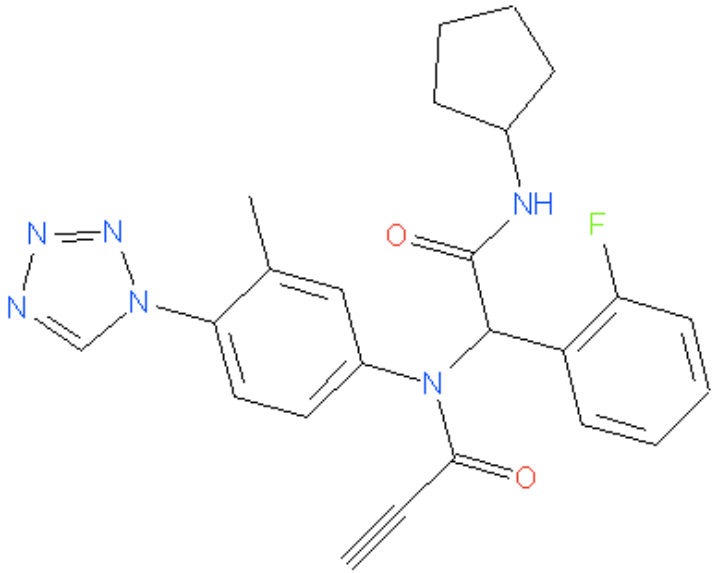
Data on four compounds that appear to inhibit both FFLuc and RLuc

		Luciferase data					MTT data				
Cell Line	Compound Conc.	Average control RLU	DL31-O10	DL34-O18	DL35-D3	UCLA48-A14	Ave MTT of Controls (Min value)	DL31-O10	DL34-O18	DL35-D3	UCLA48-A14
S1	10 μM	963±66	86	52	59	306	0.819±0.68 (0.511)	0.524	0.526	0.545	0.620
S1	1 μM	797±64	803	671 (-16%)	748	371 (-53%)	0.875±0.68 (0.418)	0.808	0.72	0.882	0.683 (-22%)
H2	10 μM	3766±197	358	127	212	726 (-81%)	0.873±0.90 (0.485)	0.538	0.506	0.495	0.660
H2	1 μM	3509±291	3265	3163 (-10%)	3002 (-14.5%)	1545 (-55%)	0.891±0.07 (0.470)	0.795	0.898	0.912	0.636 (-28%)
Ren	10 μM	359±132	26	60	31	75	1.18±0.31 (0.260)	0.342	0.331	0.338	0.532
Ren	1 μM	483±48	176 -(63%)	258 (-46%)	53 (-89%)	256 (-47%)	1.38±0.18 (0.380)	1.43	1.50	1.40	1.17 (-15%)
Result in HTS	10 μM		-5SD	-7SD	-6SD	-4SD					

Master Plate Well	Library & position	Vender
E2	DL31-O10	Asinex
E6	DL34-O18	Asinex
E9	DL35-D3	Asinex
H18	UCLA48-A14	Chembridge

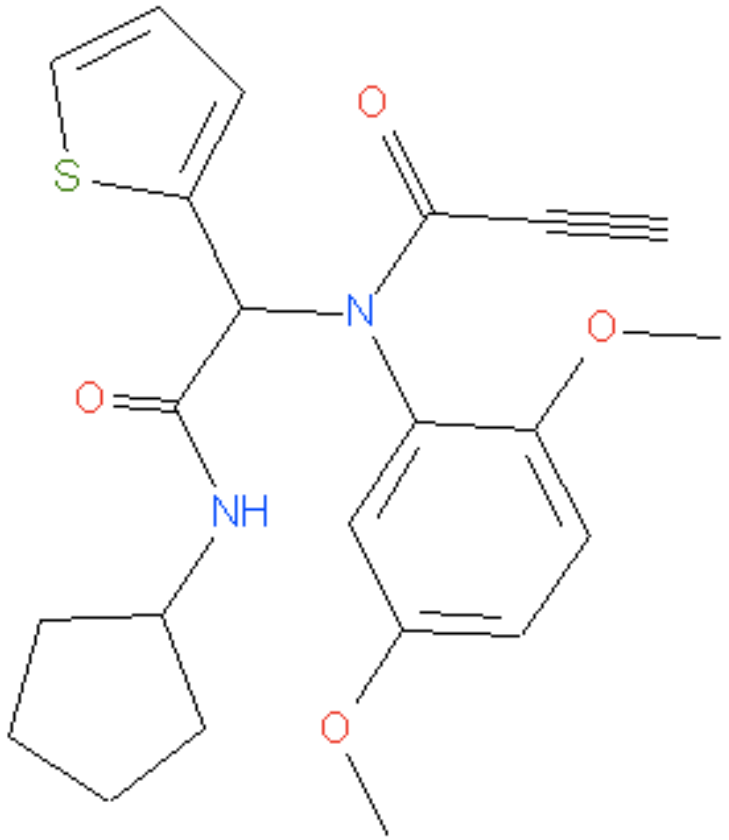
DL31-O10 ASN 06088274

Name	Value
STRUCTURE NAME	
Molweight	446.4768232
TPSA	93.01
HBa	7
HBd	1
IDNUMBER	ASN 06088274
PLATE	31
CELL	O010
WEIGHT	0.2
MOL_WEIGHT	446.48
STRUCT_FORMULA	C24H23FN6O2
VOLUME_DMSO	50_MICRO_L
CONCENTRATION	10_MICROMOL
VENDOR_LOT	433359



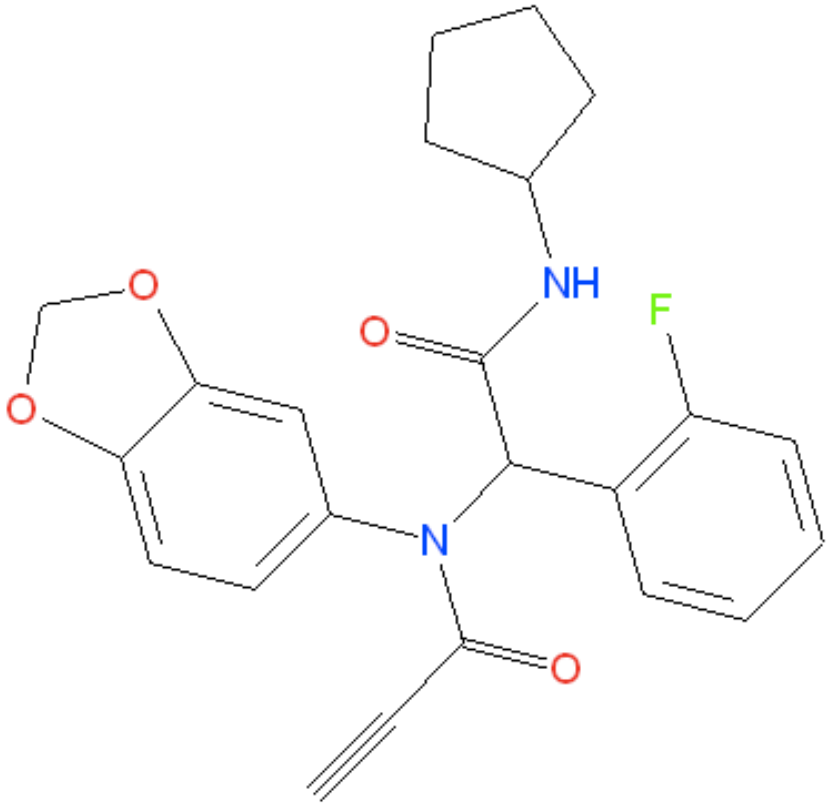
DL35-D3 ASN 06087944

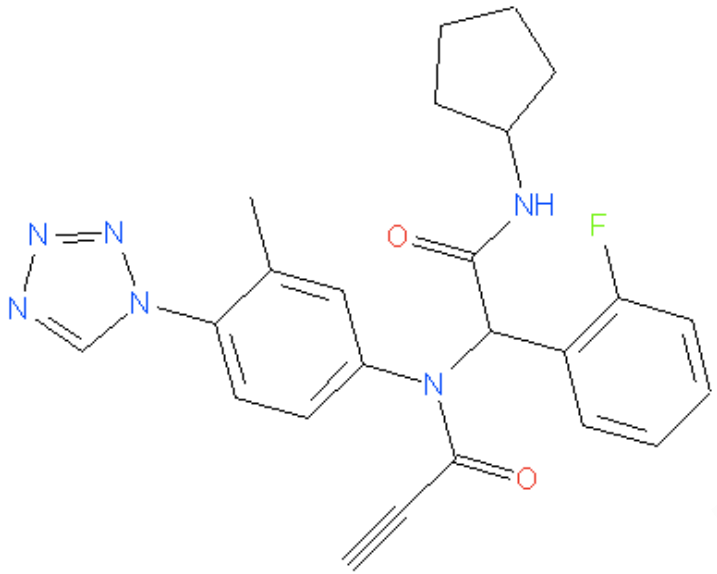
Name	Value
STRUCTURE NAME	
Molweight	412.50196
TPSA	67.87
HBa	8
HBd	1
IDNUMBER	ASN 06087944
PLATE	35
CELL	D003
WEIGHT	0.2
MOL_WEIGHT	412.51
STRUCT_FORMULA	C22H24N2O4S
VOLUME_DMSO	50_MICRO_L
CONCENTRATION	10_MICROMOL
VENDOR_LOT	433358

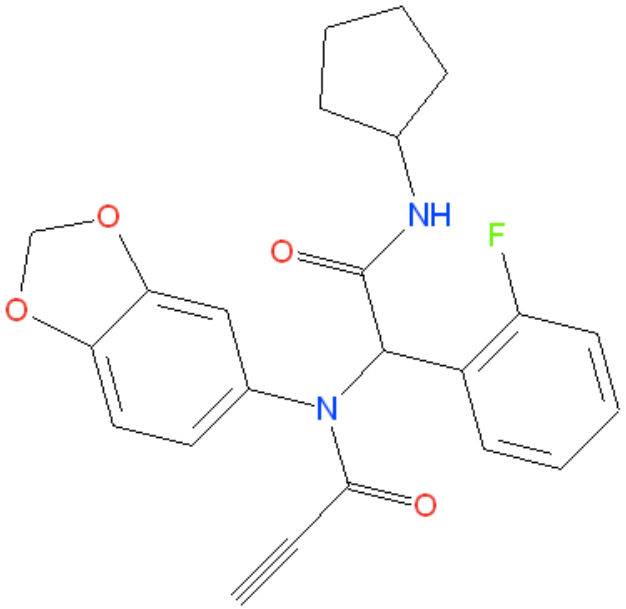


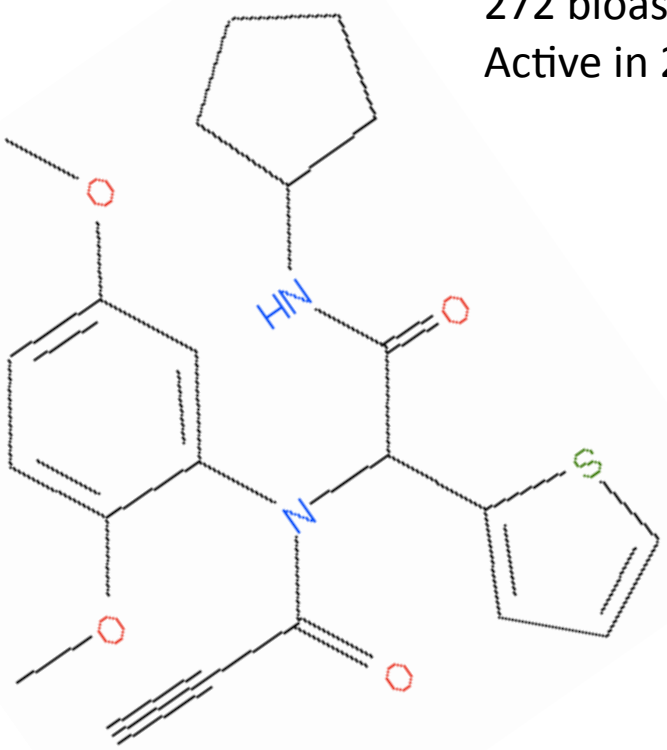
DL34-O18 ASN 06088278

Name	Value
STRUCTURE NAME	
Molweight	408.4222432
TPSA	67.87
HBa	8
HBd	1
IDNUMBER	ASN 06088278
PLATE	34
CELL	O018
WEIGHT	0.2
MOL_WEIGHT	408.43
STRUCT_FORMULA	C23H21FN2O4
VOLUME_DMSO	50_MICRO_L
CONCENTRATION	10_MICROMOL
VENDOR_LOT	430039





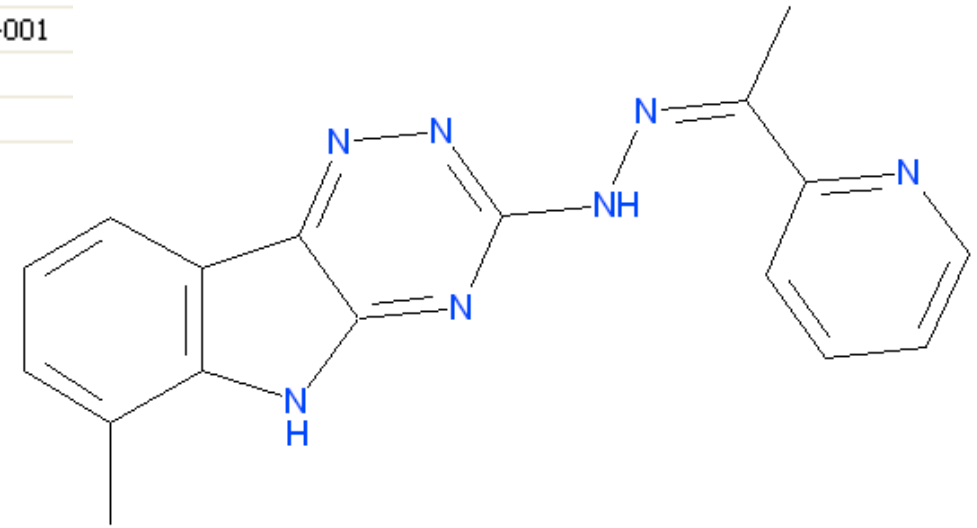




272 bioassays
Active in 25

UCLA48-A14 Chembridge 5625138

Name	Value
STRUCTURE NAME	
Molweight	317.3479
TPSA	87.98
HBa	5
HBd	2
ID	5625138
Plate	10189
Col	07
Row	A
Supplier	ChemBridge
Coordinates	A07
clogP	3.040000000000000e+000
RB	3
tPSA	9.174000000000000e+001
Hacc	5
Hdon	2



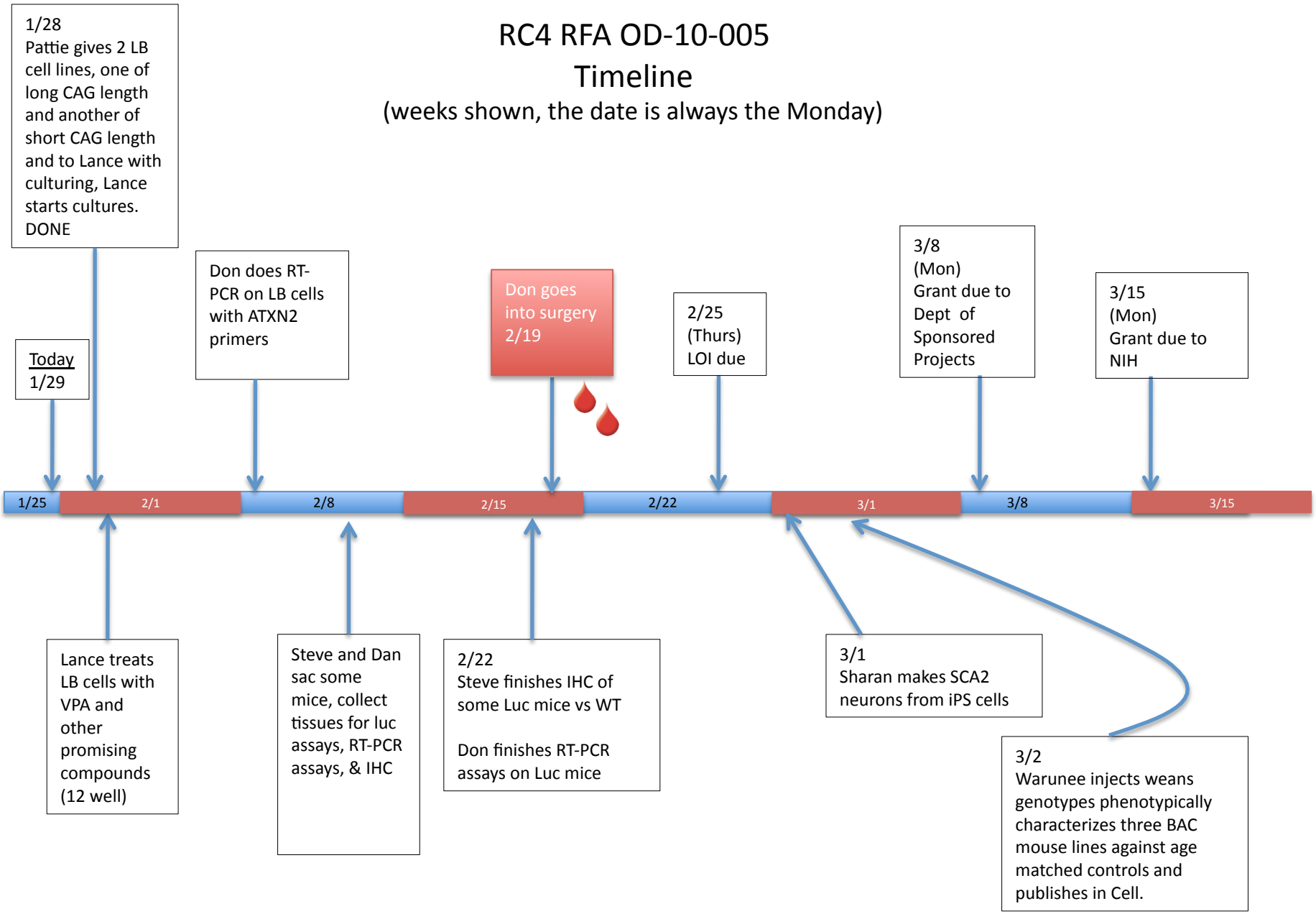
RC4
RFA-OD-04-005 (Recovery Act Limited Competition: NIH Director's Opportunity for Research in Five Thematic Areas (RC4))

Important Dates:

LOI: 2/15/10
Grant due: 3/15/2010
Review: June/July
Council meeting: Aug
Start Date: 9/1/2010

Budget:
Minimum $500,000 / yr for 3 yrs, or $1.5 million + $750,000 IDC
80 million is available, this is enough for about 35 grants (including IDC).

RC4 RFA OD-10-005
Timeline
(weeks shown, the date is always the Monday)



RFA

From "Scope and Specific Requirements”
Armed with a wealth of basic science discoveries and an understanding of the pathophysiology of various diseases, we are embarking on the next frontier in designing new diagnostic and therapeutic strategies. Molecular and cellular insights into a disease can be developed into screening assays on hundreds of thousands of compounds, and tested in disease models to identify the most promising leads that can sustain the drug development pipeline and attract public-private partnerships for further pursuits. Additional pathways to therapeutics from gene therapy, biologics, and stem cells (including iPS cells) are also showing great promise. The opportunity is here for translational science to develop small molecule-based, gene-based, protein/peptide-based and cell-based therapies for common as well as rare diseases.

From "Requirements”
The project or generated results and resources can be expected to become integrated with other NIH and privately funded research within a reasonable timeframe.

http://grants.nih.gov/grants/guide/rfa-files/RFA-OD-10-005.html

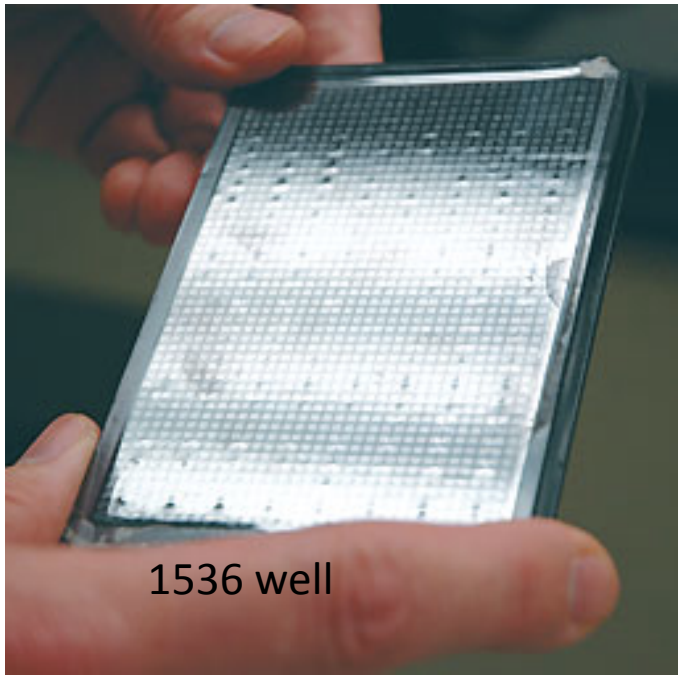
NCGC (National Chemical Genomics Center)

Chris Austin
Director

James Inglese
Deputy Director

"Anthropomorphic" Robot

Capabilities:
400,000
Compounds
screened with
seven doses



Specific Aims

Evaluation of active compounds identified in a screen of 65000 compounds.

a) Purchase of all compounds reducing expression by 2 SD (~200 compounds x ~$40 = $8000)
b) Orthagonal and counter screens

Identification of new compounds inhibiting ATXN2 by screening 400K compounds

a) Screening at National Chemical Genomics Center (NCGC)
b) Orthagonal and counter screens

Evalutaion of compounds' biological relevance

a) Compound inhibition of endogenous ATXN2 in SH-SY5Y cells and of polyglutamine expanded ATXN2 in lymphoblast cells
b) Evaluating compound ability to inhibit ATXN2-luciferase in an animal model
    I. Characterization of the mouse and ATXN2-Luc expression
        - IHC, RT-PCR compared to native atxn2
    II. Ability for compounds to inhibit ATXN2-Luc in Purkinje cells, and passage of the blood brain barrier
    III. Inhibition of ATXN2 in SCA2 neurons…
    IV. Ability for compounds to reverse ATXN2 rotorod phenotype in BAC transgenic mice.