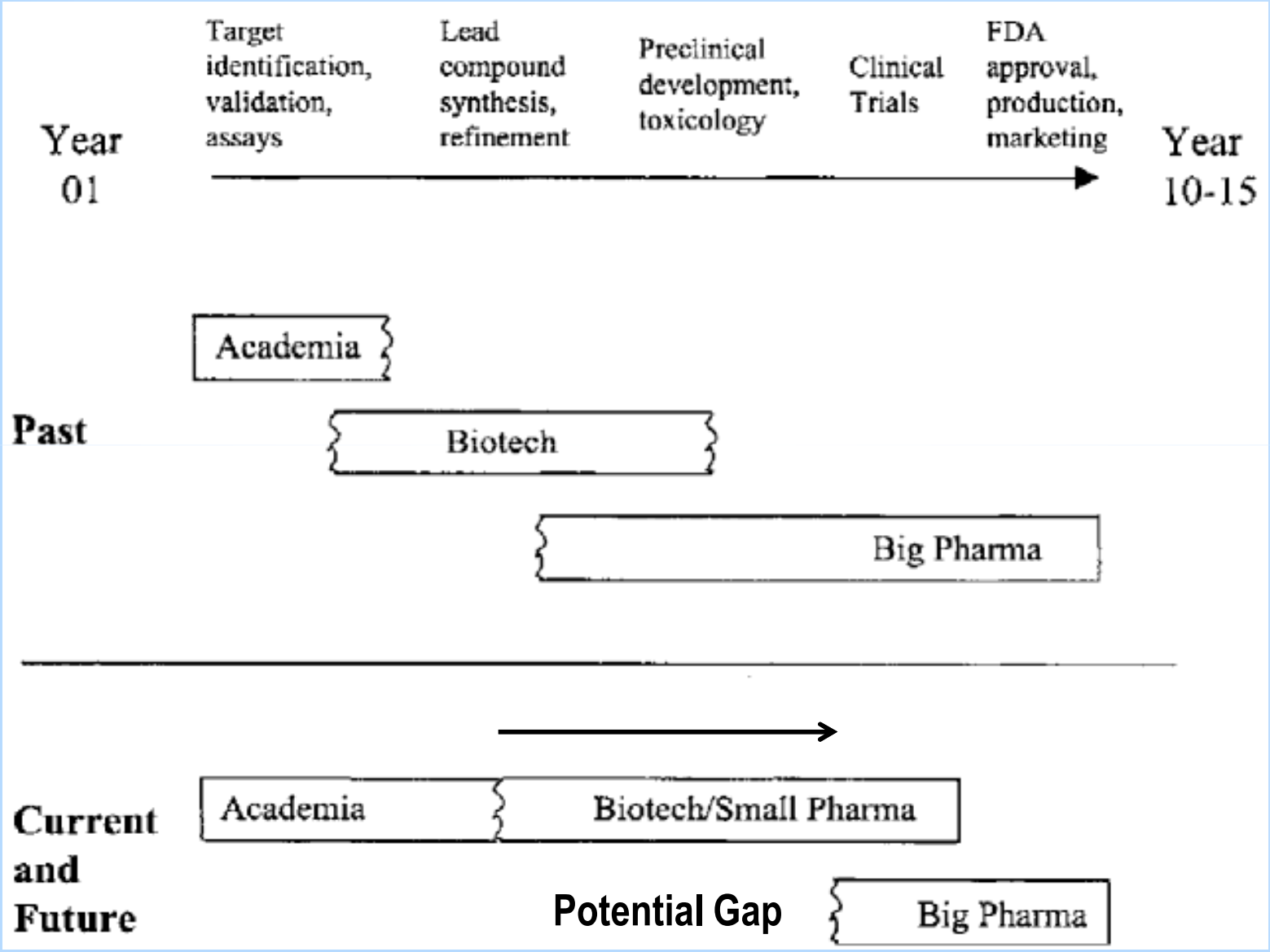
Etiology-based drug discovery for Amyotrophic Lateral Sclerosis

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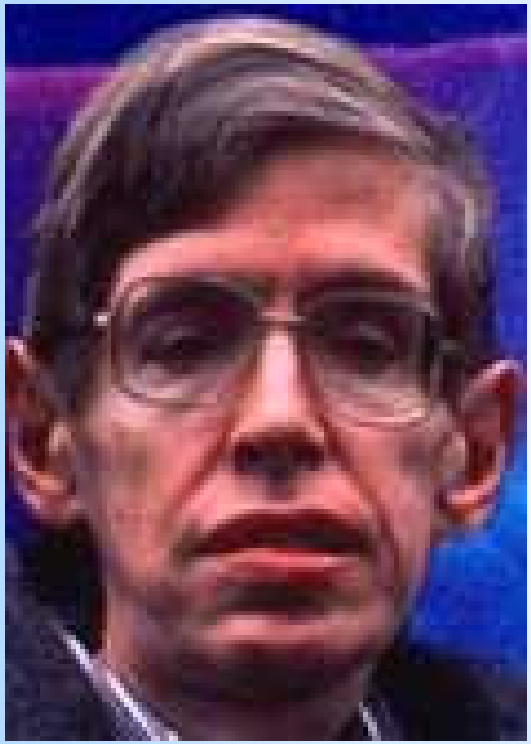
Increasing role of academic drug discovery research in the drug pipeline



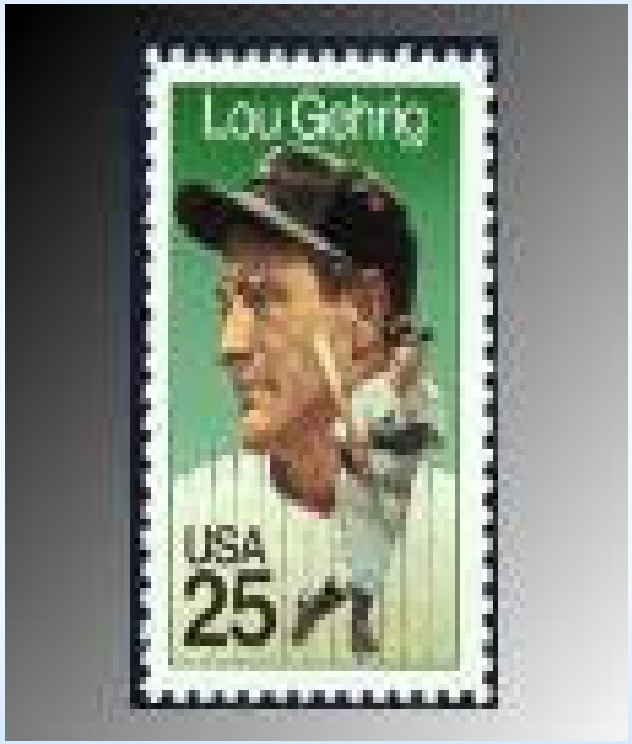
How do you accelerate Drug Discovery in an Academic Environment?

1. Early target identification– Genetics, DNA microarrays, Proteomics
2. Validation of relevant assays for compound screening (preferably cell-based). Direct effects on gene expression (Protein and/or mRNA measures)
3. Focused High-Throughput Screening (Spending constraints).
4. Screening of Smart Compound Libraries (Harvard, Scripps, etc).
5. Medicinal Chemistry optimization of lead structures (Have backups)
6. Feedback from assays to develop SAR and validate mechanism of action.
7. Perform preclinical pharmacokinetics and toxicity testing.
8. Demonstrate efficacy in preclinical trials (Animal models).

Amyotrophic lateral sclerosis (Lou Gehrig's Disease)



Stephen Hawking





Chairman Mao

ALS is an age-related, fatal, paralytic neurodegenerative disorder resulting from degeneration of large motor neurons in the brain and spinal cord. Lethality 2-5 years after onset. Riluzole is the only FDA approved drug that is currently used but does not significantly alter mortality.

❖ ALS occurs as sporadic disease (SALS) in 90% of cases, however, 10% of ALS cases are familial (FALS). Mutations in Cu, Zn superoxide dismutase (*SOD1*) are responsible for 20% while the newly discovered hexanucleotide repeat expansion in the *C9ORF72* gene is responsible for at least 60% of FALS cases.

❖ Transgenic mice over-expressing mutant SOD1, such as G93A develop paralysis and pathology reminiscent of SOD1-linked ALS in humans.

❖ Experiments in these model animals indicate that damage to motor neurons occurs due to the acquisition of a toxic function by mutant SOD1 and the onset of symptoms and pathology are dependent on the level of expression of the mutant SOD1.

❖ We reasoned that if the expression of SOD1 could be reduced, onset of disease could be postponed.

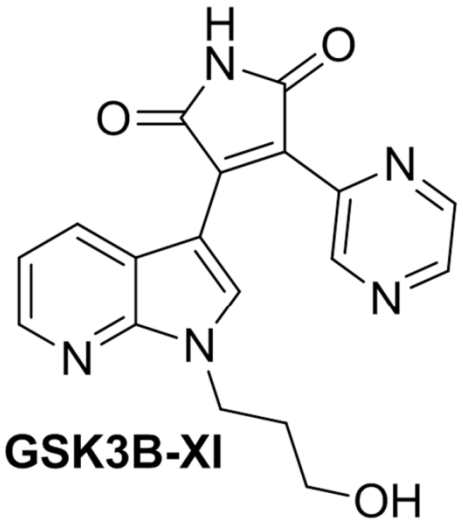
Part I. High throughput screening for inhibitors of SOD1 expression.

1. G93A mutant SOD1 (G93A) mouse fibroblasts were quantitatively plated in a 96 well format and incubated overnight.
2. The test compounds were applied in DMSO solution (range 0.1 to 50 µM final concentration) in triplicate wells to fresh media and incubated with the fibroblasts for 48 hr.
3. Aliquots of extracts were used for protein measurements and a specific human SOD1 ELISA assay. ELISA data was normalized to either total protein or to GAPDH activity.
4. Multiple libraries of compounds were screened: FDA-approved drugs, known protein kinase inhibitors; and natural products. ~2000 compounds

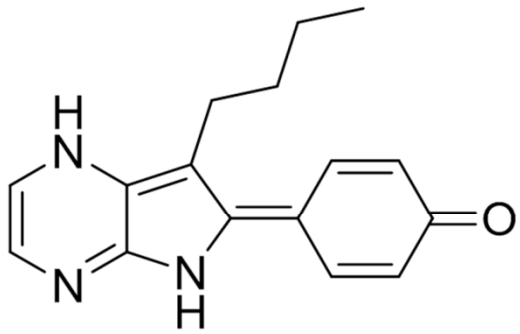
Results:

Hits were curated based upon low toxicity and potential neuroprotective activity. Many were protein kinase inhibitors with activity against GSK3-Beta and/or members of the cyclin-dependent protein kinase family.

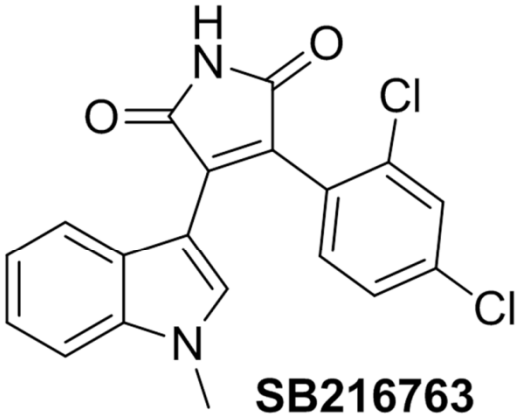
No Robotics used -- only a devoted medical student



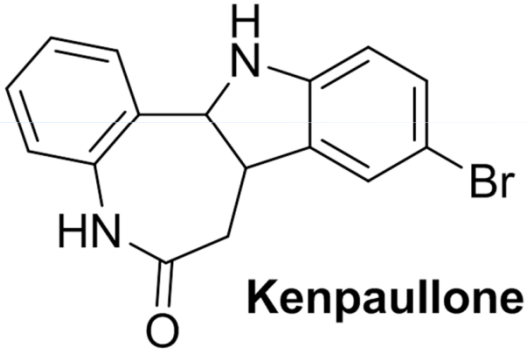
GSK3B-XI



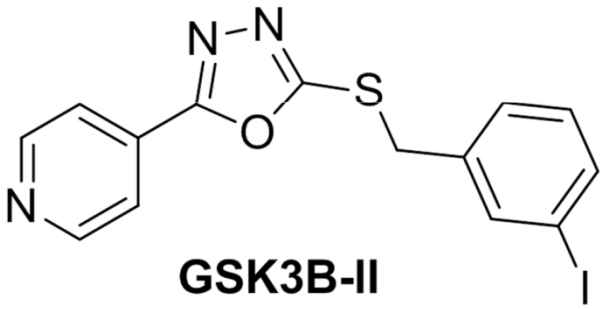
AloisineA



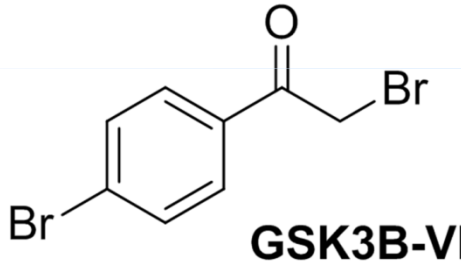
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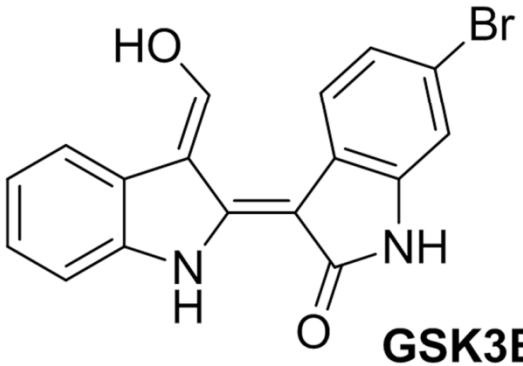
Kenpaullone



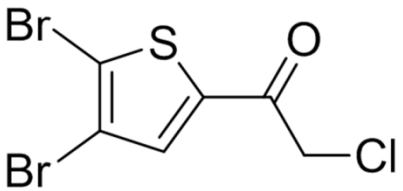
GSK3B-II



GSK3B-VII

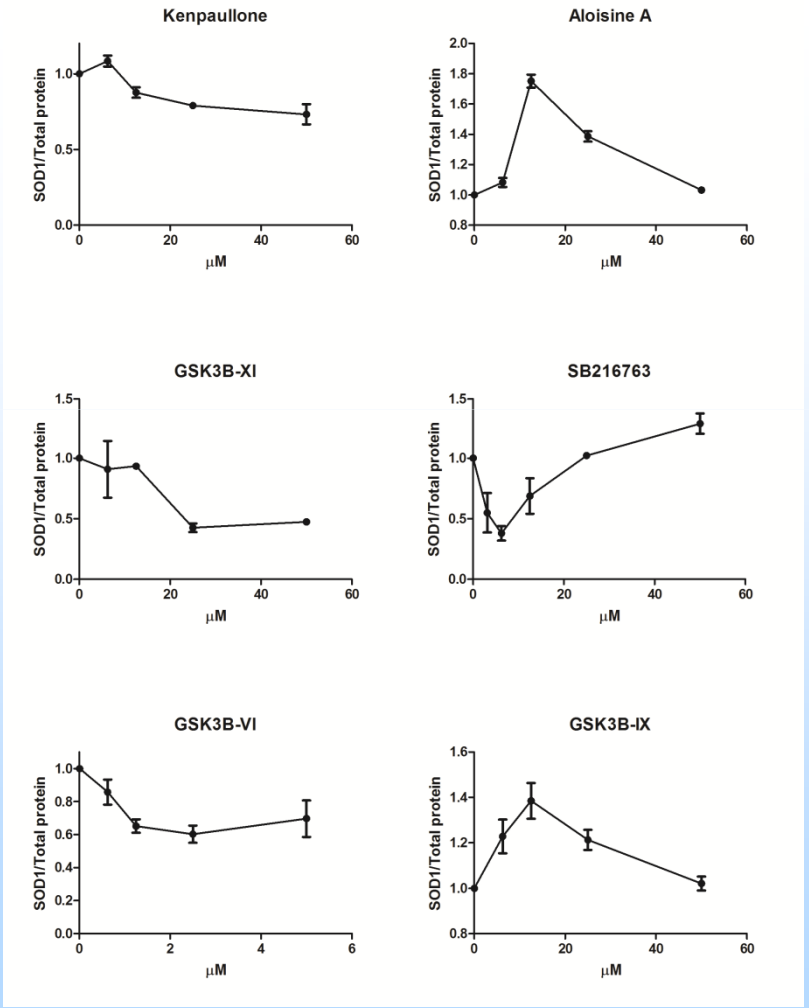


GSK3B-IX

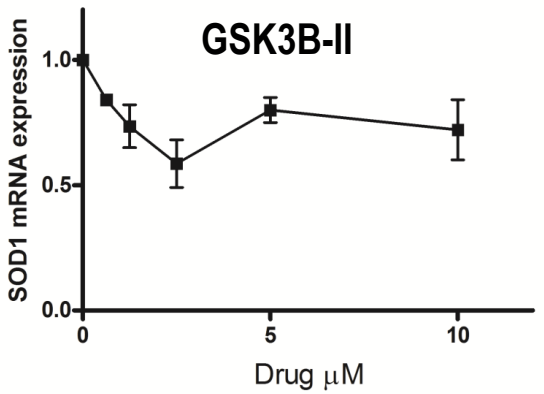


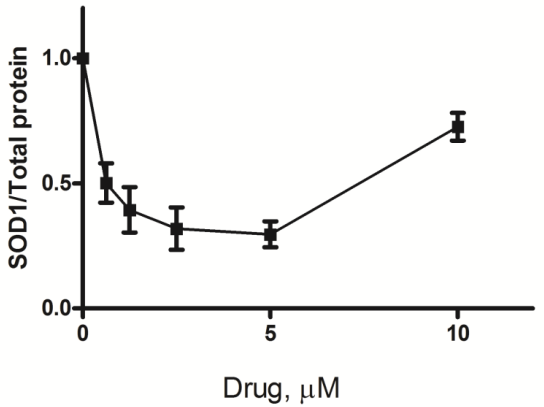
GSK3B-VI

Example inhibition of SOD1 expression curves from the cellular assay.



Many compounds exhibit biphasic curves





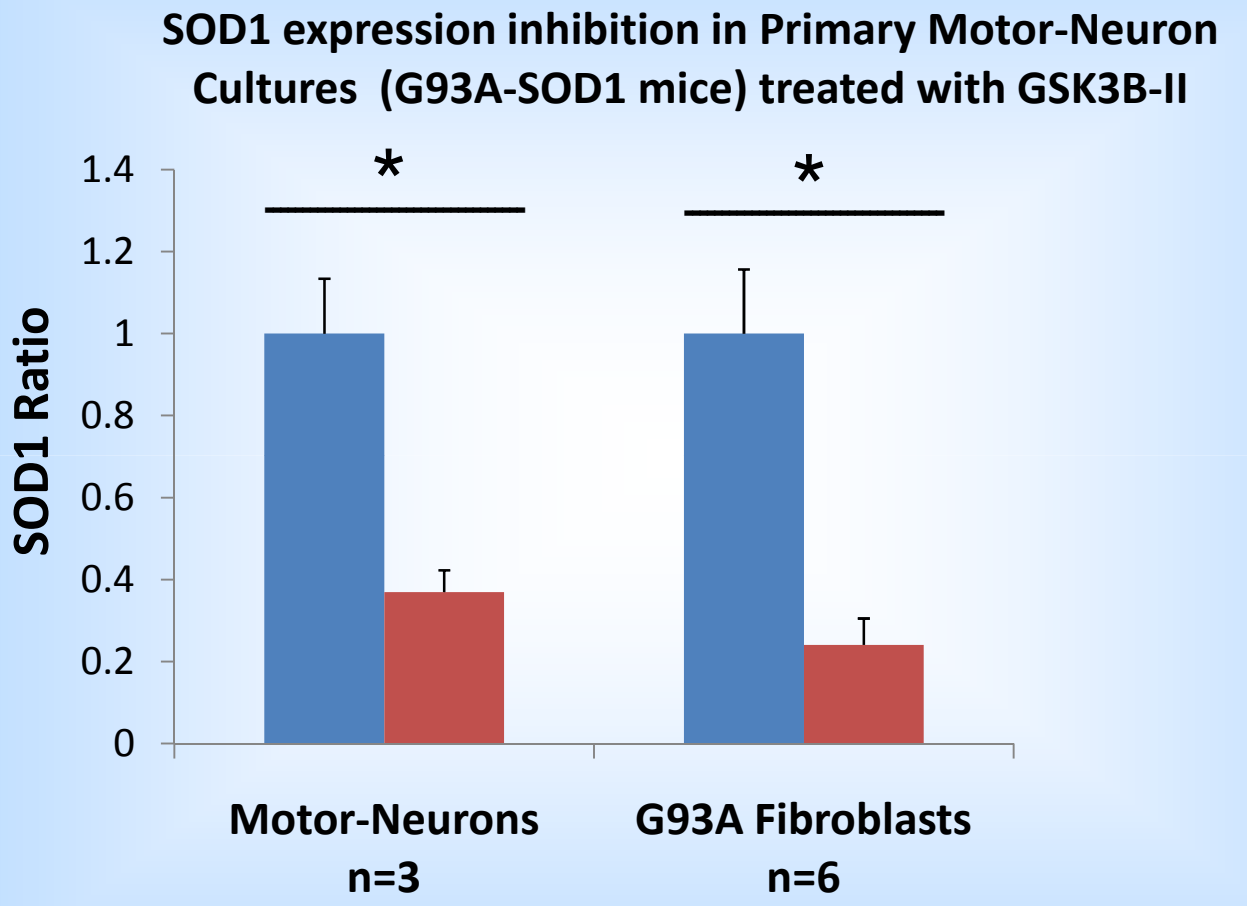
GSK3B-II displays upward Curvature in inhibiting both SOD1 mRNA and protein SOD1 expression.

Table I. GSK3β inhibitors and mutant SOD1 protein expression in mouse fibroblasts

ID	Name	GSK3β IC50 (nM)	SOD1 IC50 (nM)	Comments
1	GSK3B-II	390	625	Lead compound
2	GSK3B-IX	5	Activates	Also inhibits CDK5,CDK2, CDK1
3	GSK3B-XI	25	10000	Also inhibits CDK4
4	SB216763	32	3000	*In vivo* imaging of GSK3β

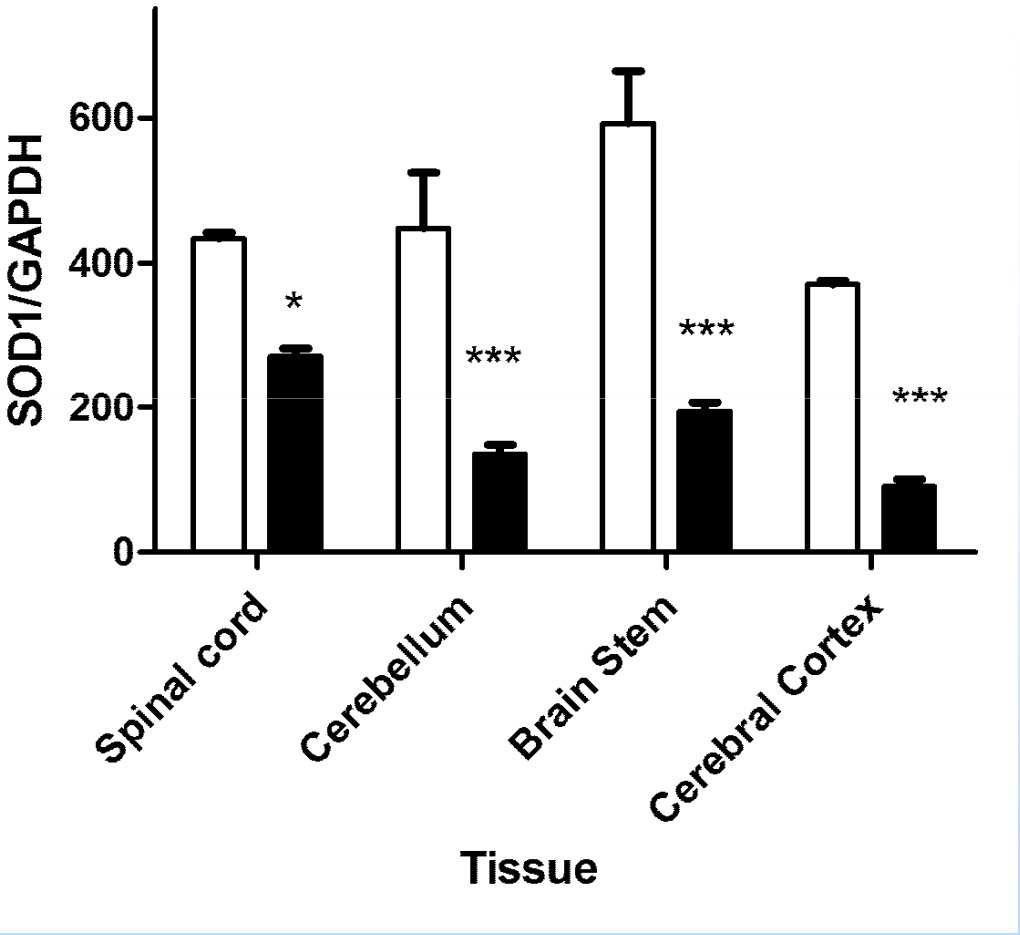
Inhibition of SOD1 expression is not correlated with the IC50 of the tested compounds towards GSK3β. It is not a class effect.

Assumed mechanism of action is on the regulation of the SOD1 Promoter.



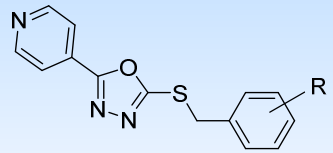
Primary motor neuron response is comparable to fibroblasts

GSK3B-II (20 mg/kg, IP 26 days) decreases mutant SOD1 expression in the G93A-SOD1 mouse

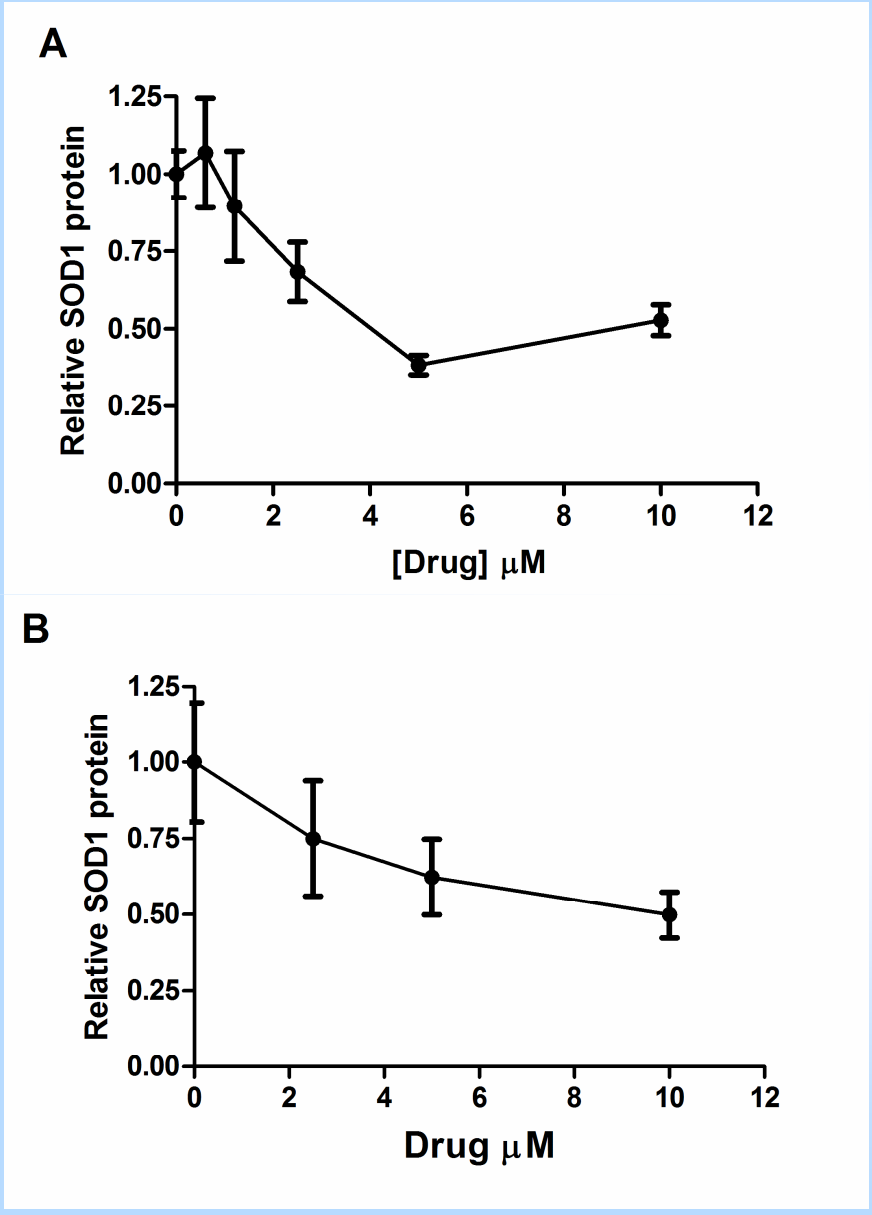


But.... Extended (beyond 1 month) and/or high dosage leads to toxicity

Medicinal Chemistry optimization of the lead compound

ID		ClogP[a]	Relative SOD1 expression 10 µM compound (p-value)[c]
7 (GSK3B-II)	R= 3-Iodo	2.91	0.73 (0.022)
NUCC-433	**R= 3-Methoxy**	**1.71**	**0.50 (0.005)**
NUCC-319	R= 3-Fluoro	1.93	1.43 (0.001)
NUCC-435	R= 4-Trifluoromethyl	2.82	1.57 (0.003)
NUCC-439	R= 3-Chloro	2.51	0.96
NUCC-432	R= 3-Nitro	1.53	1.17
NUCC-318	R=3-Bromo	2.65	0.91
NUCC-434	**R=4-Methoxy**	**1.71**	**0.47 (0.001)**
NUCC-440	R=4-Chloro	2.51	1.23 (0.09)
NUCC-320	R=4-Bromo	2.65	1.03
NUCC-321	R=4-Iodo	2.91	0.69 (0.026)
NUCC-324	R=4-Fluoro	1.93	0.86
NUCC-436	R=4-Methyl	2.29	1.26
NUCC-431	R=4-Nitro	1.53	1.02
NUCC-441	R=H	2.29	1.33 (0.07)

Lukas, T. J., Schiltz, G. E., Arrat, H., Scheidt, K., and Siddique, T. (2014) Bioorg.Med.Chem.Lett. 24, 1532-1537



NUCC-434 (A) and NUCC-433 (B) Inhibit SOD1 expression with less upward curvature in their dose response curves.

Distribution of GSK3B-II in mouse tissues after a 20 mg/kg injection (n=2)

Tissue	Time	Concentration (μM)
Brain	1 hr	0.944
Spinal Cord	1 hr	4.00
Serum	1hr	0.672
Brain	24 hr	0.077
Spinal cord	24 hr	0.855
Serum	24 hr	0.073

Distribution of CMIDD-434 in mouse tissues after a 10 mg/kg injection (n=2)

Time hr	Serum (μM)	Spinal Cord (μM)	Brain (μM)
2	0.153	0.057	0.029
4	0.334	0.162	0.047
24	0.123	0.113	ND*

The CMIDD drugs are currently undergoing testing in G93A-SOD1 mice for efficacy in reducing SOD1 expression and clinical improvements (onset, progression).

Part II. Pilot study for ACTH as a treatment for ALS in the G93A SOD1 mouse

1. There are reciprocal actions between pituitary neuropeptides and proinflammatory cytokines.

2. An ACTH analog influences neuromuscular function by electrophysiological enhancement of motoneurons and exhibits both neurotrophic and myotrophic properties.

3. ACTHar gel (Questcor) is used to treat infantile spasms and relapsing multiple sclerosis.

Experimental treatment arms for the study.
IM: intramuscular injection, SC: subcutaneous injection U/kg

Male G93A mice					Female G93A mice				
Control	120 IM	120 SC	60 SC	60 SC/w	Control	120 IM	120 SC	60 SC	60 SC/w
8	7	9	6	5	8	7	9	8	5

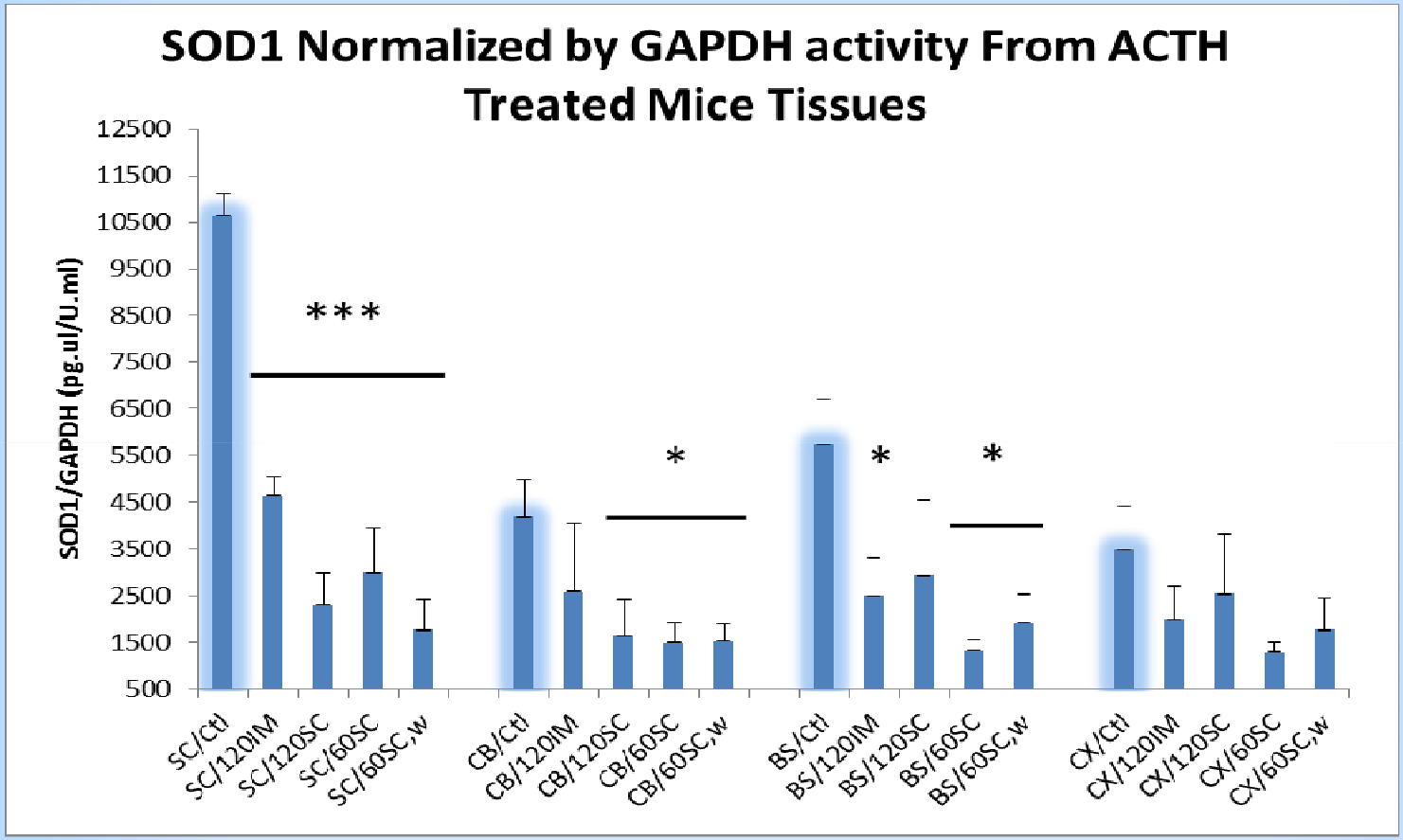
Clinical statistics of the timing of ALS-like symptoms with corresponding treatment doses.

Animal Group	Onset/Tremor Median Age	Paralysis Median Age	Endstage Median Age
Control	103	122	129
IM120	109	120	127
SC120	116	126	130
SC60	121	127	134
SC60W	115	130	138
Log-rank significance p=	0.0001	0.0509	0.318
Log-rank trend p=	0.0001	0.0023	0.355

Rotarod performances are significant for the treated groups (except for IM120) before the age of onset.

Animal Group	Males p-value (wks)	Females p-value (wks)
IM120	> 0.05 (all)	>0.05 (all)
SC120	< 0.01 (11-13)	<0.001 (11-14)
SC60	<0.01 (10-12)	<0.001 (10-16)
SC60W	<0.001 (9-11)	<0.01 (13)

Does ACTH have an effect on SOD1 levels in the SOD1 transgenic mouse?



Summary

Etiology-based treatments for SOD1-linked ALS are feasible with specific classes of small molecules (1,3,4-oxadiazoles).

ACTHar gel shows promise as a treatment for ALS and may specifically Benetit patients with SOD1-lunked disease.

Acknowledgements

Faculty Collaborators
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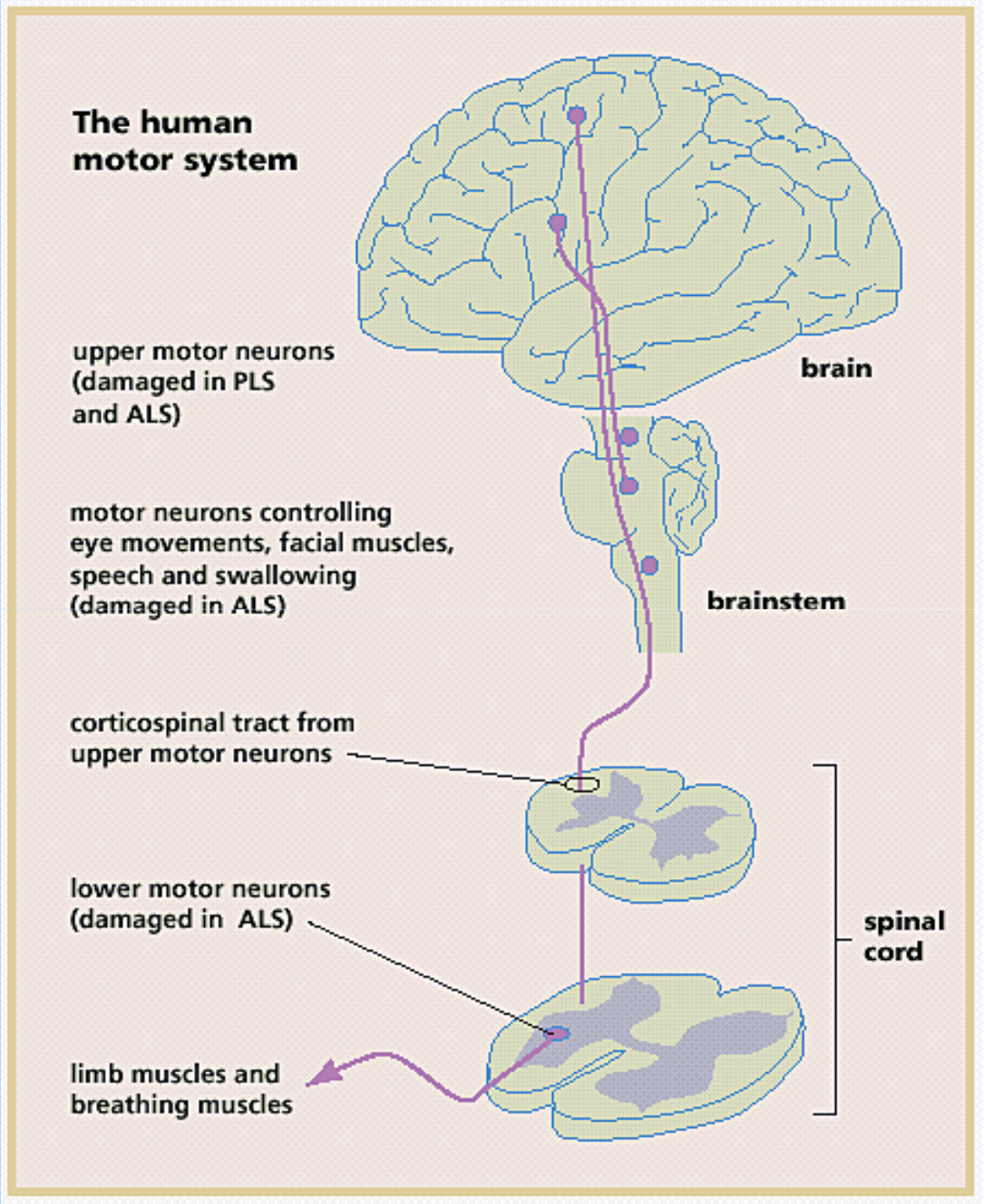
Animals
Rongen Fu
Erdong Liu

CMIDD
Center for Molecular Innovation and Drug Discovery

Karl Scheidt Gary Schiltz

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Pathology in ALS



Dysfunction of upper motor neurons in cortex
→ spastic paralysis

Dysfunction of lower motor neurons in brain stem and spinal cord
→ muscle weakness and atrophy
→ respiration failure and death in three years (50%) to five years (90%). No effective treatment.