A new approach to treating CNS, inflammatory, autoimmune and oncology diseases

POTENT AND SELECTIVE INHIBITORS OF HISTONE DEACETYLASE 6 (HDAC6)

BEYOND TUBASTATIN A: NEXTURASTAT, MERCAPTOACETAMIDES AND THQ'S SMALL MOLECULE INHIBITORS OF HDAC6

Inventors: Alan P Kozikowski,

UIC Ref #DF132,

Executive Summary

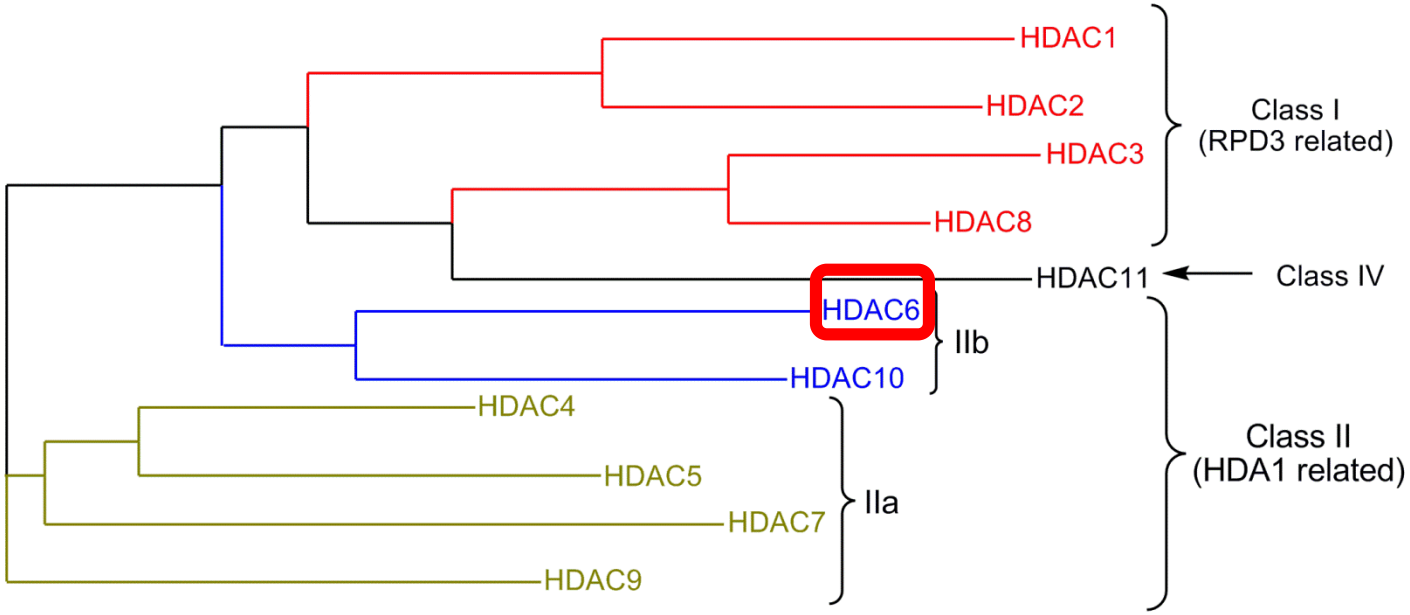
The Problem:

- There are numerous diseases that are related to dysregulated HDAC enzymatic function
  - Many of these diseases impact the immune system or are related to carcinogenic cellular pathways controlled by HDAC function.
  - There are numerous clinical candidates that are designed to inhibit HDAC activity.
  - The majority of these compounds are nonselective and lack of ability to target specific HDAC enzymes and can contribute to unwanted side effects.

The Solution:

- UIC medicinal chemists have developed several HDAC6 selective inhibitors that can be developed as promising therapies for autoimmune, oncology and inflammation indications.

HDACs belong to a large family of enzymes inhibitor selectivity is critical



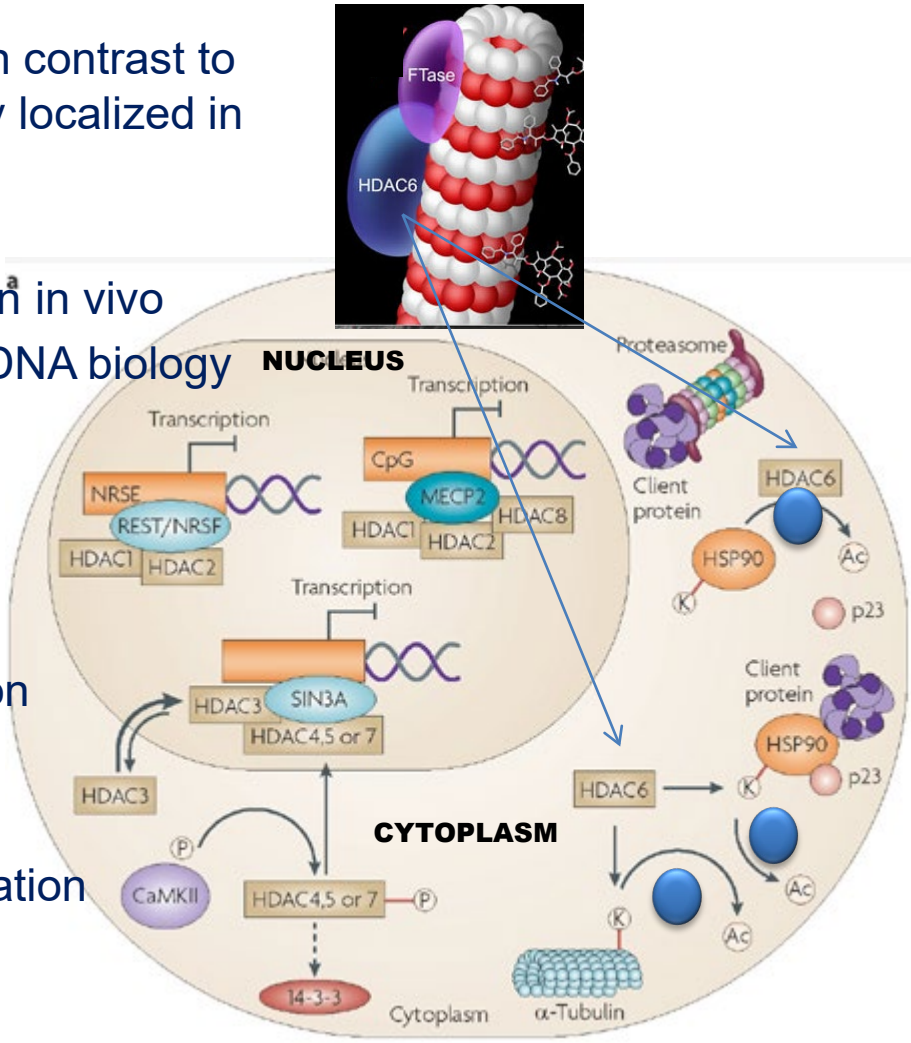
Three classes comprise the 11 known zinc dependent HDACs

- Class I: HDAC1, 2, 3, and 8
- Class II: HDAC4, 5, 6, 7, 9, and 10 Zn^{2+} dependent
- Class IV: HDAC11
- Class III: Sirtuins (7 known isoforms) – NAD^{+} dependent

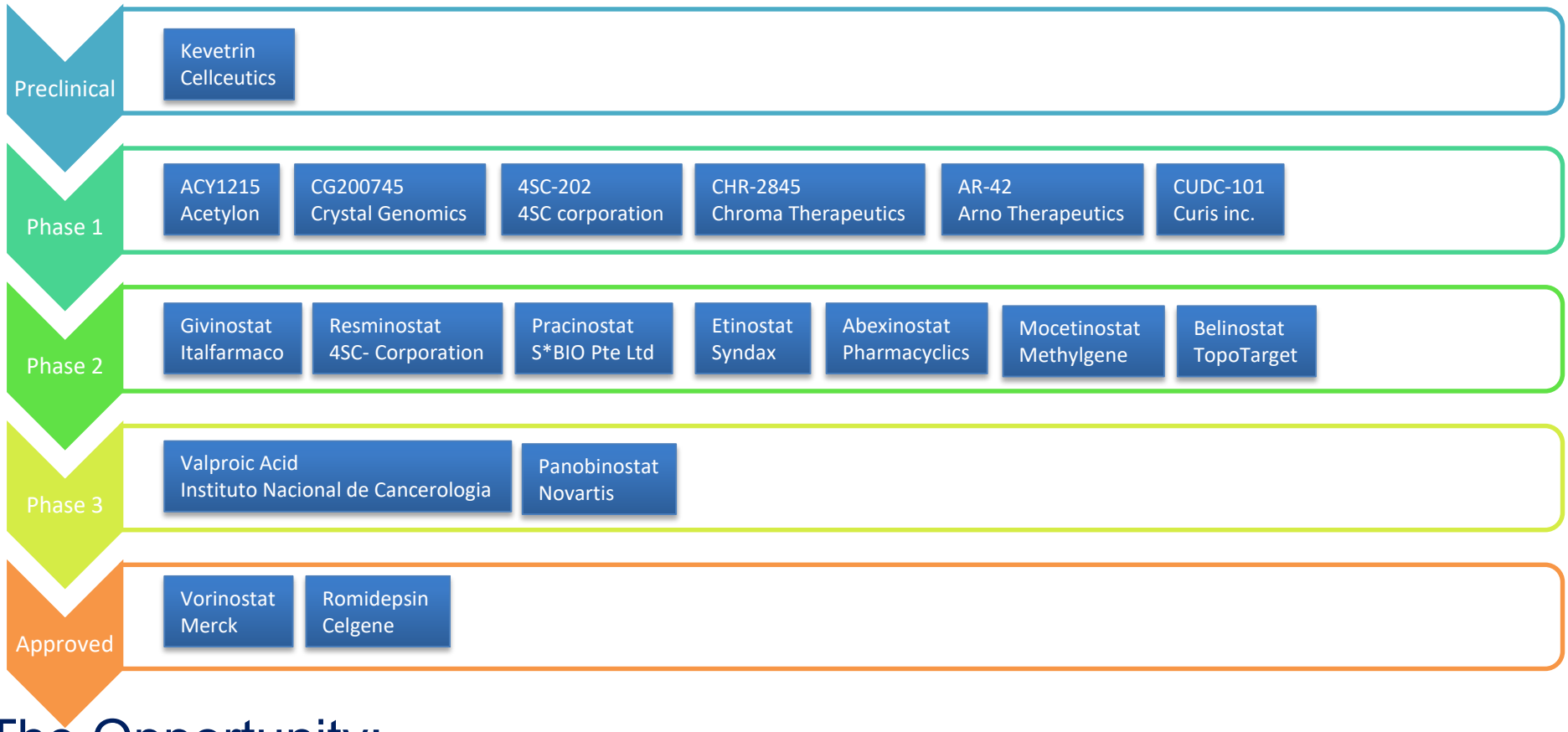
HDAC 6 differs from other HDACs and does not catalyze histones directly

HDAC6 is localized exclusively in the cytoplasm in contrast to most HDACs which are transiently or permanently localized in the nucleus.

- HDAC6 does not catalyze histone deacetylation[a] in vivo
- Better drug target since there is no impact on DNA biology
- HDAC6 has two main in vivo substrates:
  - Alpha tubulin is involved in cytoskeletal structural integrity and cellular motility
  - Hsp90 (heat shock protein) helps client proteins fold properly and maintain function
- HDACi's would impact both of these pathways and provide a novel interventional strategy in treating disease without affecting DNA modification pathways



Pipeline diagram of HDAC inhibitors in clinical development validating the target

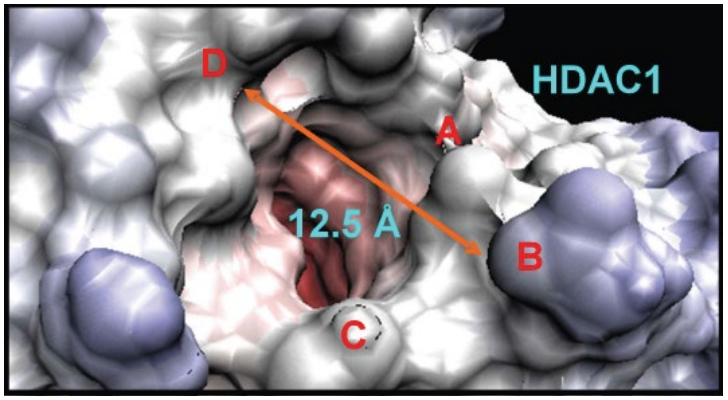


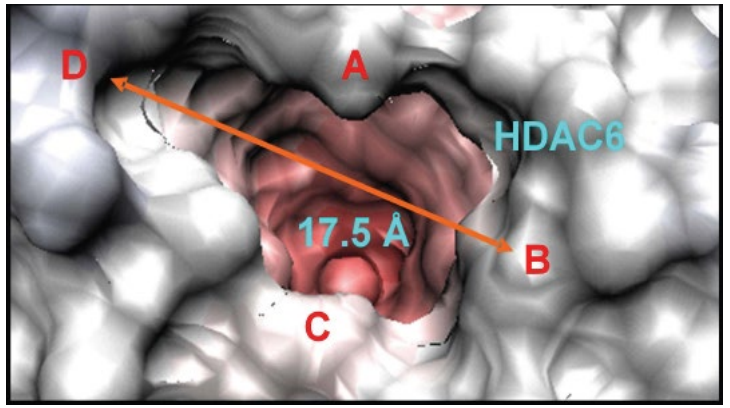
The Opportunity:

- The vast majority of HDAC inhibitors in clinical development have either poor selectivity or pan-specific properties which can lead to unwanted side effects. UIC compounds are highly selective for HDAC6.

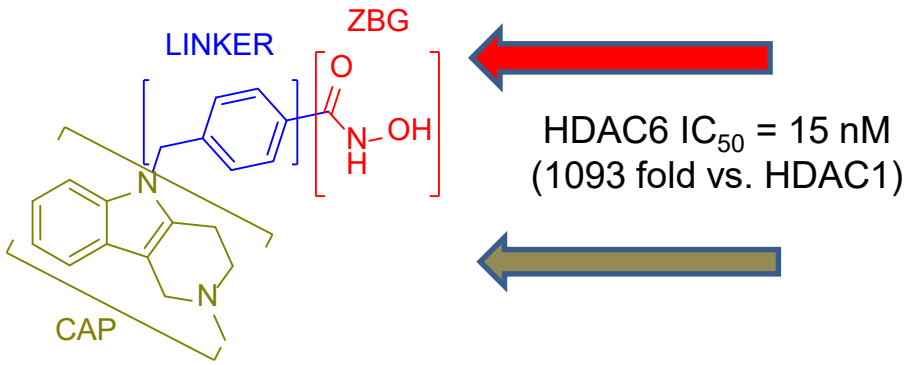
How Tubastatin A was designed

- Rational drug design:
  - Homology modeling revealed subtle differences in the region around the catalytic channel rim between HDAC1 and HDAC6





  - Bulky cap group was chosen to exploit this difference



HDAC6 IC_{50} = 15 nM
(1093 fold vs. HDAC1)

Zinc Binding Group utilizes a hydroxamate residue.

Carbazole Cap provides a bulky domain that fits HDAC6 not HDAC1

J. Am. Chem. Soc. **2010**, *132 (31)*, 10842-6

Significant improvements of UIC HDAC compounds over predecessors

Tubastatin A has superior selectivity for HDAC6 over other HDACi's

Compound:	• Tubacin
Inventor:	• Stuart Schreiber lab
Institution:	• Broad Institute Harvard-MIT

	Tubacin IC_{50} (μM)	Tubastatin A IC_{50} (μM)
HDAC1	1.40	16.4
HDAC2	6.27	>30
HDAC3	1.27	>30
HDAC4	17.3	>30
HDAC5	3.35	>30
HDAC6	0.004	0.015
HDAC7	9.7	>30
HDAC8	1.27	0.854
HDAC9	4.31	>30
HDAC10	3.71	>30
HDAC11	3.79	>30

Compound:	• Tubastatin A
Inventor:	• Alan Kozikowski lab
Institution:	• UIC Pharmacy

More improvements of UIC HDAC inhibitor compounds beyond tubastatin: the next generation

	Cmp 6 IC_{50} (μM)	Cmp 7 IC_{50} (μM)	Cmp 10 IC_{50} (μM)	Cmp 11 IC_{50} (μM)	Tubastatin A IC_{50} (μM)
HDAC1	16.9	>30	>30	NI[a]	16.4
HDAC2	>30	NI[a]	NI[a]	NI[a]	>30
HDAC3	22.8	>30	NI[a]	NI[a]	>30
HDAC4					>30
HDAC5					>30
HDAC6	0.0060	0.0077	0.0212	0.0067	0.015
HDAC7					>30
HDAC8					0.854
HDAC9					>30
HDAC10	NI[a]	NI[a]	>30	NI[a]	>30
HDAC11					>30

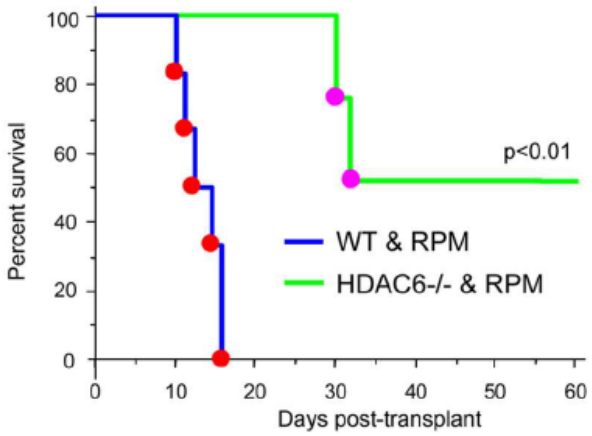
a. Non-Inhibitory (NI) as defined as no inhibition at a concentration of 50 μM.

Immunomodulation indication: Organ transplant validation model

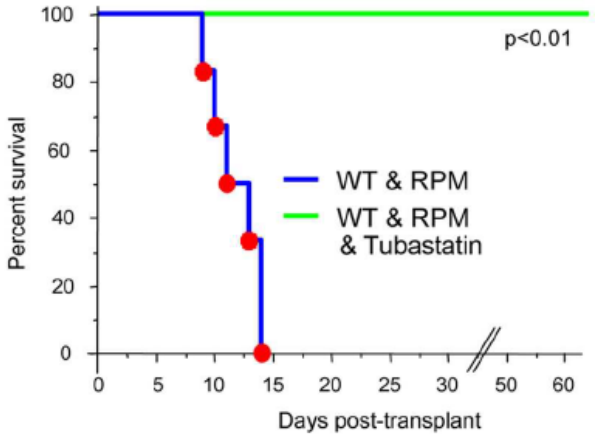
Tubastatin A combination with Rapamycin confers improved retention of organ transplants

Compounds:	• Rapamycin
Protocol:	• WT (Wild type) and HDAC6 -/- mice with cardiac allografts treated with Rapamycin
Conclusion:	• HDAC6-/- mice treated with Rapa retained transplanted organs longer than WT

Compounds:	• Rapamycin + Tubastatin A
Protocol:	• WT mice with cardiac allografts treated with Rapamycin + Tuba A
Conclusion:	• Combination of Rapa and Tubastatin A preserved organ transplants in 100% of mice



Tubastatin A used in conjunction with anti-rejection drug Rapamycin confers superior organ transplant retention times compared to HDAC6 KO animals treated with Rapa alone, suggesting Tubastatin A has additional benefits over HDAC6 inhibition alone.



Mol Cell Biol. 2011 May;31(10):2066-78

Neuroinflammation indication: Charcott-Marie-Tooth (CMT) validation model

Tubastatin A restores neuromuscular junctions and mitochondrial function to CMT model mice

Disease Information:

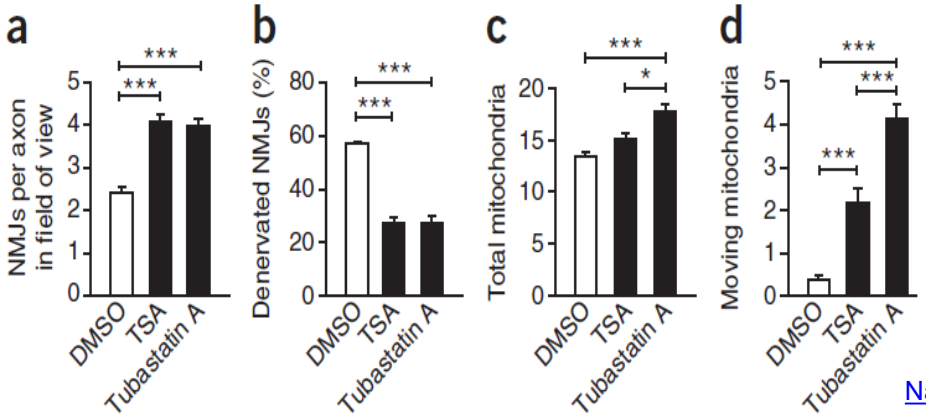
- CMT is the most common inherited disorder of the peripheral nervous system characterized by muscle atrophy, weakness and denervation. No treatment exists

Animal Model:

- Heat shock protein B1 (HSPB1 mutation) mimics axonal defects and muscular denervation in transgenic animals

Conclusion:

- Treatment with Tuba A leads to reversal of disease pathology.



CMT model mice treated with Tuba A or a nonselective HDAC inhibitor(TSA) are both able to rescue axonal transport defects and promote muscle reinnervation (a,b) Amount of visible Neuro Muscular Junctions per axon increased in contrast with dennervated NMJs. Total and fraction of moving mitochondria (c,d) increased in nerves cultured from TG mice model of CMT post HDACi treatment, suggesting CMT pathology can be treated using Tubastatin A.

Nat Med. 2011 Jul 24;17(8):968-74

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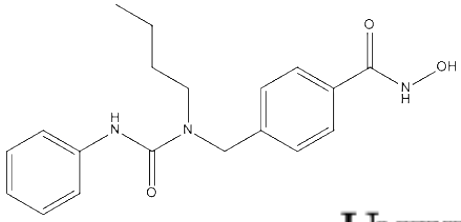
Nexturastat: HDAC6 inhibitor for the treatment of cancer

HDAC Isoform	Nexturastat IC_{50} (μM)	Tubastatin A IC_{50} (μM)
HDAC1	3.02	16.4
HDAC2	6.92	>30
HDAC3	6.68	>30
HDAC4	9.39	>30
HDAC5	11.7	>30
HDAC6	0.00502	0.015
HDAC7	4.46	>30
HDAC8	0.954	0.854
HDAC9	6.72	>30
HDAC10	7.57	>30
HDAC11	5.14	>30

J Med Chem. 2012 Nov 26;55(22):9891-9. doi: 10.1021/jm301098e. Epub 2012 Oct 23. Access article

In collaboration with the H Lee Moffitt Cancer Center, UIC has developed a potent and selective HDAC6 inhibitor called Nexturastat A which is highly active against B16 melonoma cells and more active compared to Tubastatin A. This activity against B16 cells is selective which improves safety.

compound	GI_{50} melanoma cells (μM)
Nexturastat A	14.3 ± 1.15
Tubastatin A	40.5 ± 1.21



IP Protection on the inventions:

- **2012-132 Urea-core hydroxamates, Nexturastat (Moffitt Cancer Center)**
  - National phase applications
  - US 9,409,858
- **2016-007 Mercaptoacetamides**
  - PCT Application
- **2017-076 Isoxazole Core HDAC-I (George Washington University)**
  - Provisional application

About The Investigators

Dr. Alan P. Kozikowski

- One of UIC's leading faculty member in Medicinal Chemistry with over 500 publications in drug discovery and design.
- KOL/Authority on epigenetics MedChem
- Alan's lab has collaborations with UNC, Cornell, Mayo Clinic, Moffit Cancer Center and the University of Chicago.

Dr. Wayne Hancock

- Chief of the Division of Transplantation Immunology
- Professor of Pathology and Laboratory Medicine, University of Pennsylvania School of Medicine

Dr. Eduardo Sotomayor

- FMR-Susan and John Sykes Endowed Chair in Hematologic Malignancies at Moffitt
- Director of George Washington Cancer Center

Dr. Alejandro Villagra

- Assistant professor Moffit Cancer Center and now at George Washington University

About The Inventors

Dr. Irina Gaisina

- Research Professor at University of Illinois at Chicago.

Dr. Joel Bergman

- Former postdoctoral fellowship at UIC, now at Pfizer Inc.

Dr. Jay Kalin

- Postdoctoral fellowship at Johns Hopkins University

Dr. Kyle Butler

- Postdoctoral fellowship at the University of North Carolina

Dr. Sida Shen

- Postdoctoral fellowship at the UIC.

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