A NOVEL VIRUS-INSPIRED APPROACH TO DISCOVER FIRST-IN-CLASS PRECLINICAL ASSETS FOR A RANGE OF THERAPEUTIC AREAS

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ENYO Pharma has developed an innovative systems biology approach to identify patentable chemistries directed at new human disease targets. Viruses are obligate intracellular pathogens that must modulate host cell pathways involved in countless cellular processes to complete their replication cycle. ENYO Pharma's approach identifies the host targets of a virus, and develops therapeutics mimicking the viral molecular mechanisms. As the target is a human pathway, ENYO Pharma's molecules have a therapeutic application beyond infectious diseases.

Figure 1 – ENYO Pharma's Approach to Discovery Research

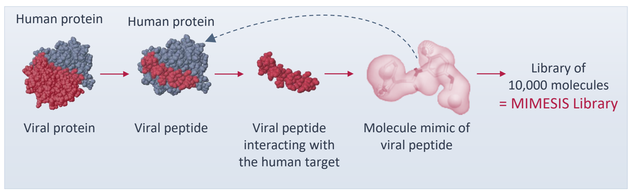
To meet the continuing need to improve drug discovery success metrics, ENYO Pharma has defined a new class of drug targets, selected by millions of years of viral evolution. These are the human targets engaged by viruses in order to seize control of host cell biology.
ENYO has constructed a curated database of all reported viral:human protein-protein interactions (PPIs) containing more than 40,000 PPIs. However, physical interaction between viral & human proteins alone does not identify a tractable drug target.
The success of ENYO Pharma's approach is based on 3 strategic decisions:

1. Attention is given only to those PPIs that have been mapped to short necessary sequences of 5 – 15 amino acids.
2. The approach is only applied to those short peptides that are biologically active in phenotypic assays.
3. Having identified a short bioactive peptide sequence, ENYO Pharma uses structure based design to identify small molecules that mimic pharmacophores on the peptide.

Intracellular proteins targeted by viruses = A novel class of drug development targets

Proprietary database & expertise

- Virus and human protein interactions
- 40,000 protein-protein interactions of virus-host in database
- Many interactions mapped to short peptide regions
- Peptides are bioactive in phenotypic assays



By this approach, a library of small molecules has been designed to bind the same pocket on human proteins as viruses do.

Figure 2 – Structure Based Design of Molecule Mimics of Bioactive Peptides

The 3D structure of each peptide is used as a template on which pharmacophoric queries are built and then used to search a database of small molecules. Poses obtained by fitting a 3D conformation onto a pharmacophore are then filtered, clustered and visually checked, forming a hit list for a given pharmacophore. Hit lists from all pharmacophores are then pooled together to form the MIMESIS library.

*Baell, J; et al. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. J. Med. Chem. 2010

$Bruns, R. F. et al. Rules for Identifying Potentially Reactive or Promiscuous Compounds. J. Med. Chem. 2012

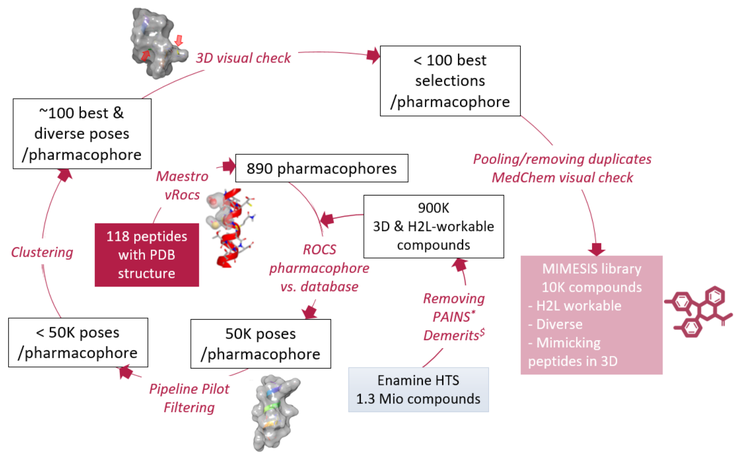
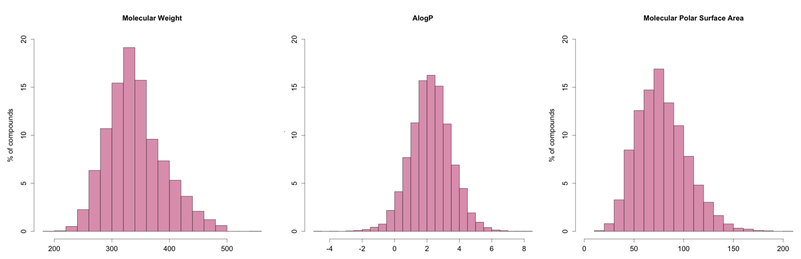


Figure 3 – Molecular Properties of the MIMESIS 10K Compound Collection

A high-level overview of the structural features of the MIMESIS collection demonstrates distribution around a mean considered favorable for drug-like substances.

*Bemis, G.; Murcko, M., The properties of known drugs. 1. Molecular frameworks. J. Med. Chem. 1996



Chemical Diversity

6300 different Bernis-Murcko* scaffolds

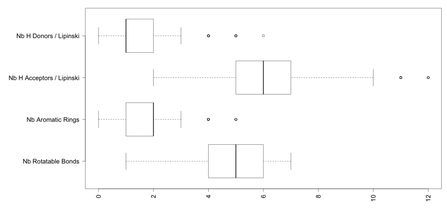


Figure 4 – Excellent Performance of the Mimesis Collection in Seven Phenotypic Screens

6 diseases with unmet clinical need were addressed by MIMESIS:

- **Infectious diseases**
  - Influenza virus
  - Human rhinovirus
  - Respiratory syncytial virus
  - Zika virus
  - *Mycobacterium tuberculosis*
- **Non infectious disease**
  - Induction of tumor Immunogenic Cell Death

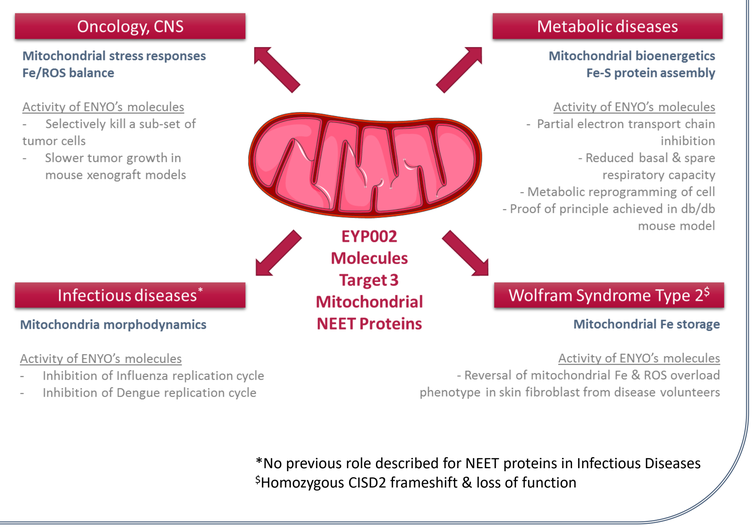
Screen	Cell line	Strain	Endpoint	% Hits at 25 µM	Validated Hits (with IC50)
Respiratory Syncytial Virus	Hep-2	A2	Inhibition of viral cytopathic effect (CPE)	0.24 % (25)	48 % (12)
Human Rhinovirus	HeLa Ohio	HRV-14	Inhibition of viral CPE	0.21 % (22)	63.6 % (14)
Immunogenic Cell Death (ICD)	MDA-MB-231, U2OS and Hepa1-6	/	Induction of key ICD hallmarks	0.93 % (96)	16.7 % (16)
Zika Virus	Huh7	PRVABC59	Inhibition of viral CPE	0.96 % (99)	23.2 % (23)
Mycobacterium tuberculosis	MRC-5	H37Ra	Inhibition of Mycobacterial CPE	0.16 % (19)	15.8 % (3)
Cytotoxicity	HepG2 and THP1	/	Cell viability (intracellular ATP)	2.6 % and 6.2 %	not relevant

Figure 5 – Modulators of NEET Protein with Broad Therapeutic Application

Molecules designed to mimic the pharmacophores on an anti-viral KSHV viral peptide were optimised using inhibition of Influenza replication as the primary assay. A novel lead series has been optimised with low nM potency. The target for these molecules is the NEET family of mitochondrial proteins:

- Regulate mitochondrial Fe homeostasis, the biogenesis of Fe-S proteins and stress responses from mitochondria.
- Until now, the only known small molecule modulators of the NEET proteins were molecules of the glitazone class.

ENYO Pharma's molecules mirror the cell biology uncovered during the last ten years of molecular cell biology studying the NEET proteins. This and positive *in vivo* data are strong validation that ENYO Pharma's molecules will have therapeutic applications beyond infectious diseases.



*No previous role described for NEET proteins in Infectious Diseases
$Homozygous CISD2 frameshift & loss of function

Figure 6 – Hit to Lead Optimization Has Yielded Additional Priority Series

Following screening of the MIMESIS compound collection, top priority chemical series have been optimized:

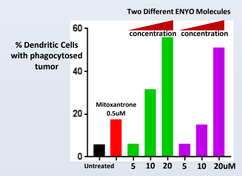
- One induces Immunogenic Cell Death in tumor cell lines,
- And another that inhibits RSV viral replication.

Initial validation of these series suggests they are promising starting points for drug discovery in Oncology and Infectious Diseases.

Immunogenic Cell Death (ICD)

- Programmed cell death that boosts the adaptive arm of immunity
- Stimulating the immune system in novel ways will address difficult-to-treat cancers unresponsive to current immunotherapies

Milestone: Treatment of tumor cells with ENYO molecules induces tumor cell phagocytosis by primary human DCs

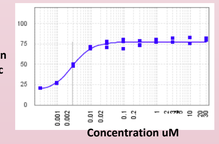


- Progressing to *in vivo* validation
- MedChem optimisation is ongoing

Respiratory Syncytial Virus (RSV)

- RSV is the most frequent cause of pediatric bronchiolitis and pneumonia and is an important pathogen in the elderly

Milestone: One series identified with potent, low nM anti-viral activity



- Molecule action is upon binding & fusion of virus at cell surface
- Progressing to *in vivo* validation via intra-nasal delivery

Conclusions:

- ENYO Pharma has defined a new class of drug targets, selected by millions of years of viral evolution.
- A collection of 10,000 molecules, designed to bind the same pocket in host proteins as viruses, has been successfully used in phenotypic screens to generate three starting points for drug discovery:
  1. NEET Protein modulators validated in Oncology, Metabolic and Infectious diseases
  2. Inducers of Immunogenic cell death validated in primary human dendritic cell systems
  3. Inhibitors of RSV replication

ENYO Pharma is dedicated to the development of these scaffolds for clinical evaluation. It is considering shared risk, "build to buy" partnerships in order to rapidly progress the chemical series to value inflexion points that may trigger licensing discussions.

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