Translational Investigations of Fenretinide in Childhood Cancer
Novel Ceramide-mediated Chemotherapies

Fenretinide, 4-HPR

Barry J. Maurer, MD PhD
Min H. Kang, PharmD
C. Patrick Reynolds, MD PhD

Cell Biology and Biochemistry, Pharmacology, Pediatrics and Medicine
TTUHSC SOM Cancer Center
The Texas Tech University System (TTUS) and Children’s Hospital of Los Angeles (CHLA) holds patents and patent applications (pending) on the use of fenretinide, fenretinide-containing drug combinations, and various formulations of fenretinide and ceramide-modulating co-agents for use in cancer therapy. As a co-inventor, BJM may potentially benefit financially from the development and future use of this IP through institutional revenue-sharing agreements.

BJM is a co-founder, equity holder, and officer of CerRx, Inc., Lubbock TX, a CPRIT-supported, clinical-stage drug development start-up company that licenses certain IP from CHLA and TTUS and may potentially benefit financially in these capacity.

A TTUHSC Conflict of Interest (COI) Management Plan is in place.
Desirable Properties for a New Chemotherapy

- Novel mechanism
- p53-independent
- Active in physiological hypoxia
- Complement existing therapy
Fenretinide
A novel increase of cytotoxic dihydroceramides

Fenretinide, 4-HPR

- Increase ROS in certain cancer cells
- Increase dihydroceramides in susceptible cancer cells in a concentration and time dependent manner
- p53-independent
- Activity in hypoxia

**Fenretinide** - increased production of **D-erythro-dihydroceramides** in cancer cells and blocks their conversion to ceramides.

**Ceramide Pathways**

Serine + palmitoyl-CoA $\xrightarrow{SPT}$ D-erythro-sphinganine $\xrightarrow{Fenretinide}$ D-erythro-dihydroceramides

D-erythro-Dihydroceramides $\xrightarrow{\text{Sphingomyelin (membranes)}}$ Ceramides $\xrightarrow{\text{Glucosylceramides}}$


Fenretinide Increases Dihydroceramides in Tumor Cell Lines and Xenografts

Human Neuroblastoma Tumor Xenograft

Control 4-HPR + ketoconazole

CHLA-90

pmole DHCer/nmole PO₄

DHCer Species

14:0 16:0 18:0 18:1 20:0 20:1 22:0 22:1 24:0 24:1

10⁻³ 10⁻² 10⁻¹ 10⁰ 10¹ 10²

Ceramide Pathways

**Fenretinide mimic** - increased production of **D-erythro-dihydroceramides** in cancer cells and blocks their conversion to ceramides.

Certain - But Not All - Dihydroceramides are Cytotoxic to Cancer Cells
Ceramide Pathways

**D-threo-PPMP** – blocks conversion of dihydroceramides and ceramides to non-toxic glucosylceramides; partially inhibits conversion to sphingomyelins.

Serine + palmitoyl-CoA ➔ SPT ➔ D-erythro-sphinganine ➔ D-erythro-Dihydroceramides ➔ Sphingomyelin (membranes)

Fenretinide

Ceramides ➔ GCS ➔ Glucosylceramides

D-threo-PPMP Increased Responses in Cell Line and Xenograft Models

**Graph 1:**
- **X-axis:** Survival Fraction
- **Y-axis:** 4-HPR
- **Legend:**
  - Control
  - PPMP
  - 4-HPR
  - H+P

**Graph 2:**
- **X-axis:** 4-HPR, µM
- **Y-axis:** D-, or L-threo-PPMP, µM
- **Legend:**
  - L-threo
  - D-threo
  - H
  - H + L-threo
  - H + D-threo

**Graph 3:**
- **Title:** 4-HPR + D-threo-PPMP improved survival in KCNR neuroblastoma murine xenograft
- **X-axis:** Day
- **Y-axis:** Tumor Size mm³
- **Legend:**
  - C1
  - C2
  - C3
  - C4
  - C5
  - C6
  - HP1
  - HP2
  - HP3
  - HP4
  - HP5
  - HP6
Ceramide Pathways

**Safingol** (L-threo-sphinganine) converted into L-threo-dihydroceramides

Safingol is catabolized to L-threo-DHCers

MOLT-4 Treated for 16 hrs
(Normalized with protein amount)
Fenretinide + Safingol

Synergistic (Multi-log) Increase in Cell Killing.
Minimally Toxic to Normal Cells

H-fenretinide; S-safingol; H+S=fenretinide + safingol
What Is the Molecular Mechanism of Fenretinide (and Dihydroceramide-mediated) Cell Death?

CPRIT RP150416 Individual Investigator Research Award for Cancer in Children and Adolescents

WHAT IT IS NOT

• Apoptosis
• Necrosis
• Paraptosis
• Necroptosis
• AIF-mediated
• Lysosomal cell death
• Anoikis

Features of some, Hallmarks of none
**What Does Happen?**

1. **Increased Unfolded Protein Response (UPR)/ ER stress**

   - Control
   - +3h
   - +6h
   - +12h

2. **Early golgi dispersal (TGN-46)**

   - Control
   - 4-HPR (H)
   - Saf
   - H+S
   - +6h
What Does Happen?

3) Accumulation of ubiquitinated proteins

4. Increased autophagy (LC3B II)
What *Does* Happen

- **Fenretinide** and **Safingol** lead to the formation of misfolded proteins.
- Misfolded proteins can be associated with **K48-linked Poly-Ub** and **K63-linked Poly-Ub**.
- **Hsp90i** and **IXAZOMIB** play roles in protein folding and aggregation.
- **UPS** (Ubiquitin-Proteasome System) is involved in the degradation of misfolded proteins.
- **HDAC6** and the **Lysosome** are involved in the degradation of aggresomes.
- **Autophagosome** formation is regulated by **siRNA ATG7 BECN1**.
- **MQ** can associate with **autophagosome** and **autolysosome**.

**Autophagosome staining**

- **Con**
- **H+S**
- **H+S + MQ**

**+12h** images show the progression of autophagy and aggresome dynamics.
Early inhibition of p97/VCP phosphorylation

[Diagram showing inhibition patterns with IgG controls and time points (+4h, +8h) for A-172 and T98G cells, using antibodies anti-pY and anti-p97/VCP.]
Current Working Hypothesis

Testing effects on p97-dependent proteins vs. canonical UPS-dependent proteins
What *Does* Happen
Enabled Identification and Testing of New co-Drugs

Xenograft testing in progress
Can High-Dose Fenretinide Be Delivered Clinically?

Retinoids are minimally water soluble

Previous McNeil/J&J/NCI oral capsule formulation

6 - 10 µM

NCI RAID-Supported Formulations

15 - 25 µM

40 - 70 µM
NCI RAID-Supported fenretinide/LXS oral powder


**Fenretinide Plasma Concentration (µM)**

Day 7 Steady-State Peak

- **Course 1** (No ketoconazole)
  - n = 15, courses = 28

- **Course 2** (Plus ketoconazole)
  - n = 22, courses = 40

\[ P = 0.0004 \]

T-test, 2-sided, Unequal variance
Fenretinide/LXS oral powder + ketoconazole
Increased MRD responses and PFS in Relapsed Neuroblastoma

Overall and Progression-Free Survival (n=34)
Excludes Patients with Baseline CR or BM Disease Only

Overall Survival
Median (95% CI): 30.2 (17.2, 35.6) Months

Progression Free Survival
Median (95% CI): 4.1 (1.3, 18.8) Months

Historical PFS - single agents

IV Fenretinide well-tolerated in Phase 1 trials*
Sustained Responses in T-cell Lymphomas
Signals of activity in adenocarcinomas of the GI tract


<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Dose (mg/m²/d)</th>
<th>Best Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>PTCL (ATL)</td>
<td>1810</td>
<td>PRu - (PFS 6 mo)</td>
</tr>
<tr>
<td>CTCL (Sezary)</td>
<td>1280</td>
<td>CR - (PFS 36+ mo)</td>
</tr>
<tr>
<td>CTCL</td>
<td>1280</td>
<td>unevaluable - withdrew</td>
</tr>
<tr>
<td>CTCL</td>
<td>1280/905</td>
<td>SD - much improved skin/prurititis</td>
</tr>
<tr>
<td>CTCL</td>
<td>905/640</td>
<td>SD - (PFS 3+ mo)</td>
</tr>
<tr>
<td>PTCL (ATL)</td>
<td>905</td>
<td>CRu - (PFS 30+ mo) - withdrew</td>
</tr>
<tr>
<td>CTCL</td>
<td>905/640</td>
<td>SD - (2 months)</td>
</tr>
<tr>
<td>CTCL</td>
<td>905</td>
<td>PR - (PFS 5 mo)</td>
</tr>
<tr>
<td>CTCL</td>
<td>640</td>
<td>SD - (PFS 8 mo)</td>
</tr>
<tr>
<td>PTCL (NOS)</td>
<td>640</td>
<td>PD</td>
</tr>
<tr>
<td>PTCL (ATL)</td>
<td>640</td>
<td>PD</td>
</tr>
</tbody>
</table>

29 year old female – Refractory Cutaneous T-cell Lymphoma (CTCL-Sezary)*

40 - 70 µM

*Currently in FDA accelerated-approval Phase 2b trial (FEN-T14) - Relapsed PTCL by CerRx, Inc.
Can Safingol Be Delivered Clinically?

A Phase I Clinical Trial of Safingol in Combination with Cisplatin in Advanced Solid Tumors

Conclusion: single agent safingol tolerated to at least 930 mg/m²; >20 µM plasma.

Can Fenretinide + Safingol Be Delivered Clinically?

- Well-tolerated in canines
- IND #110,514
- South Plains Oncology Consortium (SPOC)

SPOC 2010-002 - Phase I of IV Fenretinide + IV safingol in Adult solid tumors
ClinicalTrials.gov Identifier: NCT01553071; David Gerber, UTSW, Chair
Accruing to Dose Level 2
Can D-threo-PPMP Be Delivered Clinically?
CPRIT Bridging the Gap: Early Translational Research Award RP121060

PPMP and PPMP + 4HPR, well tolerated in rats by bolus or CIV*.

PPMP = 30 - 120 mg/kg/day CIV x 3 days
Plasma Cmax = 2.7 – 42 micromolar

PPMP = 60 mg/kg/day + 4-HPR 60 mg/kg/day CIV x 3 days
PPMP Plasma Cmax = ~16 micromolar
4-HPR Plasma Cmax = ~80 micromolar

*Kang laboratory, manuscript in prep
Grant Support (excluding private/foundational)

FENRETINIDE + SAFINGOL

- NIH NCI DTP Rapid Access to Intervention Development (RAID) (PI: C. Reynolds)
  "Formulation, IND-directed Toxicology, of intravenous Fenretinide and Safingol for Phase I trials"
- NIH NCI DTP Rapid Access to Intervention Development (RAID) (PI: B Maurer)
  "Formulation and IND-directed Toxicology of a Novel Oral Formulation of Fenretinide with Increased Ease of Administration and Increased Oral Bioavailability"
- NIH NCI 1-R43-CA92797-01A1 SBIR (PI: E. Grant, Molecular Express, Inc)
  "Development of Liposomal Fenretinide and Safingol"
- NIH NCI 1-R01-CA100895-01 (PI: B. Maurer)
  "Novel Ceramide-based Chemotherapy for Acute Leukemias"
- NIH NCI R21 CA161889-01 QuickTrials (PI's: B. Maurer and C. Reynolds)
  "A Phase I trial combining intravenous fenretinide and safingol to target overproduction of cytotoxic dihydroceramides in malignant cells"
- Cancer Prevention and Research Institute of Texas (CPRIT) RP100762 (PI: C. Reynolds)
  "Enhancing the anti-neuroblastoma activity of fenretinide by identifying and targeting sphingolipid pathways that confer resistance."
- NIH NCI R15 CA159298-01 NIH/NCI AREA (PI: B. Maurer)
  "Novel L-threo-sphinanines for ceramides-based cancer therapy."
- Cancer Prevention and Research Institute of Texas (CPRIT) CP120090 Company Formation Award (PI: W. Simpson, CerRx)
  A Phase IIa trial of IV fenretinide in Relapsed Peripheral T-cell Lymphoma (PTCL).
- NIH NCI R44 CA183316 SBIR (PI: W. Simpson, CerRx, Inc.)
  A Phase I trial combining IV fenretinide and IV safingol to target overproduction of cytotoxic dihydroceramides in malignant cell.
- Cancer Prevention and Research Institute of Texas (CPRIT) RP150416 Individual Investigator Research Award Cancer in Children and Adolescents (PI: B. Maurer)

PPMP

- NIH NCI 1-R41-CA102842-01 Phase I FLAIR/STTR (PI: B. Maurer)
  "PPMP as a Ceramide Catabolism Inhibitor for Chemotherapy"
- Cancer Prevention and Research Institute of Texas (CPRIT) RP121060 (PI: B. Maurer)
  Manufacture, Formulation, and IND-directed Toxicology of the Multifunctional Ceramide Catabolism Inhibitor D-threo-PPMP to enable Phase I Clinical Trials.
Acknowledgements

Maurer Laboratory - TTUHSC
• Nikhil Vad, PhD, Fellow
• Dong Wang, MD MS
• Michael Holliday, MD PhD
• Seyed Mohammad Abedi, MD PhD
• Shelby Ray Kenney, PhD, Fellow

Kang Laboratory - TTUHSC
• Hwangeui Cho, PhD, Fellow
• Hardeep Singh, PhD, Fellow
• Dattesh Verlekar, PhD Candidate

Reynolds Laboratory - TTUHSC
• Charlie Linch

Cancer Prevention and Research Institute of Texas (CPRIT)

Avanti Polar Lipids, Alabaster AL
Stephen W. Burgess, PhD
Dennis Graves
Dale C. Smith, PhD

Particle Sciences, Bethlehem PA
Bruce L. Frank, PhD
Matthew Bigert
Gary Gwozdz
Robert Lee, PhD

Bioanalytical Sciences Inc. (BASi), Evansville IN
L. David Hopper, DVM, PhD, DABT, RQAP-GLP
Philip A. Downing

CerRx, Inc., Lubbock TX
William Simpson
Kerry M. Barnhart, PhD
# Acknowledgements

**Pre-clinical**
Peter O’Donnell, MD PhD  
Sandeep Batra, MD  
Hongtao Wang, PhD  
Guoying Kong, MD  
Jun Wu, MS

**Animal models**
Bee-Chun Sun  
Ondrej Kalous, MD  
Vanessa Maldonado  
Vazgan Khankaldyyan

**PK/Mass Spec**
Kyung Hwa Hwang  
Tomas Frgala, MD  
Jitka Janeba, PhD  
Iva Chvilickova, PhD  
Shiny Xiaquin Wu

**Mass Spec-MUSC**
Alicja Bielawska, PhD  
Jacek Bielawski, PhD

**DTP/NCI/NIH**
B. Rao Vishnuvajjala, PhD  
Shanker Gupta, PhD  
Joseph Tomaszewski, PhD

**MOB/CCR/NCI/NIH**
Martin Gutierrez, MD  
Anthony Murgo, MD

**USC/Norris**
Allen Yang, MD PhD  
Jacek Pinski, MD  
Anthony El-Khoueiry, MD  
Ann Morhbacher, MD

**TTUHSC**
Sanjay Aswasti, MD  
Everardo Cobos, MD

**Joe Arrington Cancer Center - Covenant Medical**
Don Quick, MD  
Isaac Tafur, MD

- California Cancer Consortium (CCC)  
- Developmental Therapeutics Clinic Center for Cancer Research, NCI, NIH  
- New Approaches to Neuroblastoma Therapy (NANT) Consortium  
- Texas Tech University Health Sciences Center (TTUHSC)  
- South Plains Oncology Consortium (SPOC)
**Fenretinide** - increased production of **D-erythro-dihydroceramides** in cancer cells and blocks their conversion to ceramides.