Opportunities for Preclinical Evaluation of Novel Therapies

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Conflict of Interest Disclosure

- Paid Consultant
- Sponsored Research
- Shareholder
Can more sophisticated preclinical models improve success?
Companion Dogs - A unique resource

- 70 million dogs in US
  - 25% will be diagnosed with cancer
- Advantages for some cancer histologies
  - Spontaneous and competent immunity
  - Similar genetic and biologic behaviors
  - Represents genetic heterogeneity (host and tumor)
  - Rapidly translational (metabolism and physiology)
  - Similar allometric scaling and PK
  - Repeat or serial endpoint documentation
- Areas of potential limitation
  - Multiple variables to be considered, too heterogeneous?
  - Uniform and broad scientific awareness & acceptance
Cancer researchers usher in dog days of medicine

The dog as a cancer model

To the editor:
The dog has long been used as a model in drug discovery and development research because of its similarities to human anatomy and physiology, particularly with respect to the cardiovascular, urogenital, cancer research, scientific and clinical leaders from both human and veterinary oncology have come together to form a multidisciplinary consortium, the Canine Comparative Oncology and Genomics Consortium (CCOGC).

Translation of new cancer treatments from pet dogs to humans

Melissa Paoloni and Chand Khanna

Abstract | Naturally occurring cancers in pet dogs and humans share many features, including histological appearance, tumour genetics, molecular targets, biological behaviour and response to conventional therapies. Studying dogs with cancer is likely to provide a valuable perspective that is distinct from that generated by the study of human or rodent cancers alone. The value of this opportunity has been increasingly recognized in the field of cancer research for the identification of cancer-associated genes, the study of environmental risk factors, understanding tumour biology and progression, and, perhaps most importantly, the evaluation and development of novel cancer therapeutics.
The University of Illinois Experience
Hallmarks of cancer

1. Produce growth factors
2. Evade inhibitory signals
3. Invade neighboring tissues and metastasize to other parts or organs
4. Uncontrolled replication
5. Form new blood vessels
6. Evade apoptosis

Each categorical Hallmark can serve as a drugable target

Hanahan & Weinberg Cell 2000
Conventional Agents and Apoptosis

Vast majority of anticancer drugs perturb
- DNA synthesis/replication (S)
- Cell division (M)
- Directly damage DNA

1) *General* cytotoxins that induce death in all rapidly dividing cells
2) Requires *competent* apoptotic pathway
3) Further intensification unlikely to improve therapeutic outcomes

Underscores the need for personalized approaches to cancer therapy
The apoptotic cascade

Intrinsic Pathway

- Procaspase-9
- cyt-c
- Apaf1

Extrinsic Pathway

- Death Ligand
- Death Receptor

DNA Damage

- p53
- Bax

Procaspase-8/10

Apoptosome

- Procaspase-3
- Caspase-8/10
- Caspase-3

Apoptotic Death
Cancer cells evade apoptosis through the Intrinsic Pathway and the Extrinsic Pathway.

**Intrinsic Pathway**
- DNA Damage activates p53.
- p53 mutant leads to increased Bcl-2 expression.
- Bcl-2 prevents apoptosis.
- Procaspase-8/10 are not activated.
- Apaf1 is not activated.
- Apoptosome formation is inhibited.
- Caspase-3 and Caspase-8/10 are not activated.
- Apoptotic Death is not induced.

**Extrinsic Pathway**
- Death Ligand binds to Death Receptor.
- Procaspase-8/10 are activated.
- Caspase-3 is activated.
- Apoptotic Death is induced.
Direct reactivation of apoptosis in cancer

**Intrinsic Pathway:**
- Bcl-2
- DNA Damage
- p53
- Bax
- Apaf1
- Pro-caspase-3
- Apoptosome

**Extrinsic Pathway:**
- Death Receptor
- Death Ligand
- Pro-caspase-8/10

**Small Molecule:**
- Caspase-8/10
- Caspase-3
- Apoptotic Death
Benefits of direct procaspase-3 activation

- Apoptotic pathways funnel to procaspase-3
- Mutations in cancer are typically upstream of procaspase-3

Procaspase-3 levels are **elevated** certain cancers providing a preferential target

- PC-3 elevated in lung cancer
- PC-3 elevated in breast cancer
  - *Clin. Cancer Res.* 2003, 9, 738
- PC-3 elevated in hepatocellular carcinomas
  - *Mod. Path.* 2004, 17, 861
- PC-3 elevated in certain neuroblastomas
  - *Cancer Res.* 1997, 57, 4578
- PC-3 elevated in certain lymphomas
- PC-3 elevated in melanoma


Procaspase-3 elevated 6-fold in colon cancer
Biochemistry of procaspase-3

Procaspase-3 itself is active, ~200-fold less than caspase-3
Clark, C. and co-workers Biochemistry 2003, 42, 12298

Procaspase-3 can cleave procaspase-3 to active caspase-3

Thus, if we can enhance procaspase-3 activity and commit cells to die...could serve as a novel anticancer strategy platform

Rationale to screen a library of molecules with pro-apoptotic activity
PAC-1- 1st Procaspadase-3 Activating Compound

Paul Hergenrother Laboratory

A) PAC-1: Zn²⁺

“Sweet Spot”
Zinc Binding Affinity
Kd = 42 nM

Data generated by Diana West and collaborator Amy Palmer

Cell Death
2 Pivotal and Key Findings for PAC-1

Inverse relationship between procaspase-3 concentrations and sensitivity to PAC-1

Preferential overexpression of procaspase-3 by malignant relative to normal cells

Potential opportunity to target the most malignant cancer cells with PAC-1
Identification of PAC-1

22,000 compounds

Hergenrother Laboratory

In vitro

Enzymatic activity
SDS-PAGE/Western blot

Cell culture

Cancer cell lines
- Efficacy
- Apoptosis

Primary tumor samples
- Efficacy
- Pro-caspase-3 levels

Fan Laboratory

In vivo

PK data/toxicology
Mouse studies
Dog studies - research and pet dogs
Combination studies - pet dogs

PAC-1 Derivatives
SAR PK/Biodistribution (BBB vs. Non-BBB)
Marginal single-agent activity, cumbersome delivery, and toxicity—**No Go Decision**
Synergistic potential of low dose, pulsatile PAC-1

Virtually all cancer treatments are now “cocktails” of chemotherapeutics

Can the chelation of labile zinc from procaspase-3 synergize with:

1) Conventional anticancer drugs
2) Ionizing radiation therapy
3) Small molecule strategies

\[ K_d = 350 \text{ nM} \]

\[ K_d = 42 \text{ nM} \]

**APOPTOSIS**
Oral PAC-1 and Chemotherapy

- PAC-1 PK: Mice & dogs

Anticancer Activity in OS

![Graph showing PAC-1 50 mg/kg in different tissues.](image)

![Graph showing percent survival over time for different groups.](image)

Day 53 PAC-1/TMZ  
Day 18 Control
PC3 Expression in K9 OS

6 dogs with metastatic sarcoma treated with combination oral PAC-1 & intravenous doxorubicin.

Pulsatilie therapy well tolerated - Go Decision

Primary 18/20 (90%)
Metastases 6/7 (86%)
Exploiting BBB:PAC-1 synergizes w/ TMZ

**Cell death:**

- **U87**
  - 0 µM PAC-1
  - 3 µM PAC-1
  - 5 µM PAC-1

- **D54**
  - 0 µM PAC-1
  - 1 µM PAC-1
  - 1.5 µM PAC-1
  - 2.5 µM PAC-1

- **9L**
  - 0 µM PAC-1
  - 3 µM PAC-1
  - 5 µM PAC-1

**Apoptosis:**

- 0 µM PAC-1
- 20 µM PAC-1

- 0 µM TMZ
- 150 µM TMZ

- FITC
- PI

**Data provided by Rachel Botham**
PAC-1 synergizes with TMZ to extend survival in a mouse model of glioblastoma

Human oncosphere cells intracranially implanted
PAC-1 and TMZ given orally at 50 mg/kg each:
Early findings with combination oral PAC-1 and temozolomide

• 8 yr old, FS Labrador
• Acute onset cluster seizures
• Symptomatic management
  – Anticonvulsants
  – Oral prednisone
• MRI consistent w/meningioma
• Treatment with investigational combination
  – Oral PAC-1 (12.5 mg/kg), days 1-21
  – Oral Temozolomide (100 mg/m^2), days 8-12
  – Repeat cycle 28 days
Combination pulsatile therapy well tolerated with activity- Go decision
2 Pivotal Canine Feasibility Studies to be conducted in Chicago, IL

1) Oral TMZ + oral PAC-1
2) Definitive RTH + oral PAC-1
Conclusions

**PAC-1** directly activates procaspase-3 *in vitro* through the sequestration of inhibitory zinc ions

**PAC-1** readily penetrates the BBB and has potential in CNS cancers

**PAC-1** shows synergy with a *wide variety* of standard-of-care cancer drugs in *various* cancer models

**PAC-1** is orally available and can be given with conventional cytotoxins

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**Use of pet dogs with cancer in drug development and dose scheduling**

1) **Critical for the realization of toxicity and limited single agent activity**
2) **Necessary for development of tolerable dosing regimens to inform human trials**
3) **Provides preliminary evidence for combination studies to inform human trials**
Phase I human clinical trials of PAC-1

**Component 1:**
Late stage cancer patients

UI Cancer Center, Chicago

Once-a-day for 21 days, escalation to MTD

**Component 2:**
Glioblastoma patients

Johns Hopkins

Once-a-day for 21 days, + TMZ

ClinicalTrials.gov

Procaspace Activating Compound-1 (PAC-1) in the Treatment of Advanced Malignancies

This study is currently recruiting participants. (see Contacts and Locations)

Verified February 2015 by University of Illinois at Chicago

Sponsor:
University of Illinois at Chicago

Collaborator:
Vanquish Oncology, Inc.

Information provided by (Responsible Party):
Oana Danciu, University of Illinois at Chicago

ClinicalTrials.gov Identifier:
NCT02355535

First received: January 30, 2015
Last updated: February 23, 2015
Last verified: February 2015
History of Changes
Acknowledgements

Hergenrother Laboratory
Paul Hergenrother, PhD
Rachel Botham, BS
Howard Roth, BA
Isak Im, PhD

UIUC Pathobiology and VDL
Anne Barger, DVM, MS
Stephane Lezmi, DVM, PhD

UIUC Cancer Care Clinic
Laura D. Garrett, DVM
Jacqueline Wypij, DVM, MS
Zachary Neumann, DVM
Alycen Lundberg, DVM
Corrine Camero, DVM
Jenny Byrd, LVT
Rebecca Kamerer, LVT
Tara Bailey, LVT

Comparative Oncology Research Laboratory
Holly Pondenis, MS
Katie Wycislo, DVM
Lisa Schlein, DVM
Mark Byrum

UIUC Comparative Biosciences
Levent Dirikolu, DVM, PhD

North Carolina State University
Luke Borst, DVM, PhD