Measuring What Matters: Proliferation and Cell Death Biomarkers for Oncology Research

Thymidine Kinase 1 and Keratin 18

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Qualities of a Good Biomarker

1. Specific / Sensitive / Relevant to Disease
2. Accurate quantitation
3. Rapid and Robust
4. Non-invasive sample collection
5. High-throughput

Note: The Arocell TK210 Assay, M30 Apoptosense®, and M65® ELISA assays are for RESEARCH USE ONLY in the US.
Cancer: Fundamentally a disease of regulation of growth
Cell Proliferation
Proliferation as an Indicator and Target
BIOMARKERS FOR PROLIFERATING CELLS

• **DNA Replication Indicators**
  – Mitosis Counting
  – BrdU, $^3$H Thymidine, etc.

• **Protein Biomarkers**
  – **Ki-67**: Nuclear protein expressed in G1, S, G2 and M phases of cell cycle, but absent in G0.
  – **PCNA**: Nuclear protein elevated during the G1/S phase of the cell cycle.
  – **PTTG**: Securin protein degraded at anaphase onset.
  – **TK1**: Enzyme involved in DNA synthesis that are elevated during S phase.
IMMUNOHISTOLOGICAL BIOMARKERS

- Require biopsy – invasive
- Labor intensive
- Sampling issues
- Low reproducibility / subjective
TK1 (Thymidine Kinase 1)

- TK1 has a key function in the synthesis of DNA
- TK1 is synthesized during S phase and is degraded after cell division
- In cancer events, TK1’s cell cycle regulation is lost and TK1 levels are upregulated.

The relationship between fluorescence intensity of TK1 positive cells (Texas Red) and DNA content (DAPI) in 152 PC-3 cells. Mitotic cells are indicated by open circles.

(Wang, 2001)
Elevated TK1 enzymatic activity has long been used as a blood cancer biomarker, but the radioactive nature of the activity assay is not ideal.

Activity levels ≠ better measurement
In the case of solid tumors, serum TK1 protein levels are also elevated. The problem is that TK1 activity is low in fractions where protein levels are still high. Thus, for solid tumors, important information might be missed with an activity assay and there is a need of an antigen based assay.

(Jagarlamudi, 2015)
Figure 2: Gold-Standard $^3$H Labelled Thymidine Assay vs. TK1 ELISA

$r_s = 0.32$

$P = 0.12$
Arocell TK210 ELISA

• Developed components that cleared the hurdle of the complexes
  – Established a buffer to dissociate complexes increases detection capacity
  – Monclonal antibody against aa 210 increases sensitivity

• Results:
  – More robust ELISA that correlates better with the gold standard of enzymatic activity
  – Sensitive and specific research tool for solid tumors
Benefits TK210 ELISA

BENEFITS TO CELL CULTURE:
• Efficacy, tolerance, and dose response

BENEFITS OF TK 210 ELISA FOR XENOGRAFT STUDIES:
• Specific for human TK1 protein, thus can discriminate between human and mouse TK1 and TK2
• Measure drug effects over time, without the need to sacrifice the animal

BENEFITS OF TK 210 ELISA FOR CLINICAL RESEARCH STUDIES:
• Non-invasive serum based marker

New tools for clinical researchers (hematology and solid tumors):
• **Hematology - Old dog / New trick:** Reintroduction of TK1 with a new look… TK1 protein levels are just as good as activity measurements for blood tumor research but without the hassles of activity assays.
• **Solid tumor- New Kid on the Block:** New marker to look at for solid tumor research that is just now being explored.
Serum TK1 as a Breast Cancer Research Marker

After Kumar et al. Tumor Biology 2016
Cell Death
NORMAL

Enzymatic digestion and leakage of cellular contents

NECROSIS

Apoptotic body

Phagocytosis of apoptotic cells and fragments

APOPTOSIS
APOPTOSIS

Extrinsic apoptotic pathway
(Death receptor mediated)

Intrinsic apoptotic pathway
(mitochondrial mediated)

FASL/TRAIL

FADD

FLIP

FAS/DR

Bax

Bak

BH3 only proteins

Bcl-2 like proteins

mitochondria

Cyt-c

Bid

Caspase 8

Caspase 3

Apaf-1

Pro-Caspase 9

Caspase 9

Caspase 3

Smac

IAPs

(Hector et al., 2009)
Desired Qualities:
- Apoptosis specific
- Quantitative
- High-throughput
- Endpoint marker
- In the blood

(Cummings, 2008)
Keratin 18 (K18) (Cytokeratin 18, CK18)

Keratin 18 is found in almost all **epithelial** cells in the body, e.g.:
- liver
- lung
- intestines
- breast
- prostate

and **tumors** of these organs.

K18 is **not** expressed by neurons, muscle and connective tissues, skin and cells of the immune system.
The dead cells still count!

Cell death

Necrosis
- K18
- Necrotic cell
- Nucleus
- Leakage of full-length K18

Apoptosis
- Apoptotic cell
- Caspase cleavage of K18
- Caspase
- Disintegration of apoptotic bodies and release of K18 fragments

Only intact K18

Caspase-cleaved K18
Keratin 18

- Monoclonal Abs: M5, M6, M30
- CASPASE
- Additionally: K18-Asp396 or CK18-Asp396 ccCK18 ccK18

Olofsson et al., 2009
# M65® and M30 Apoptosense® ELISAs

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<thead>
<tr>
<th>Only intact K18</th>
<th>Caspase-cleaved K18</th>
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<tr>
<td><img src="image1" alt="M65® ELISA" /> M65 EpiDeath® ELISA</td>
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- **Measurement of intact and cleaved K18**
- **The M65 ELISAs measure total cell death** *(necrosis and apoptosis)*
- **Measurement of cleaved K18 only**
- **The M30 ELISAs measure** *(only apoptosis)*
Necrosis or Apoptosis?

**Apoptosis**
- M30 level: ccK18
- M65 level: total K18

High levels of caspase-cleaved K18 (ccK18) compared to total K18 (high M30:M65 ratio)

**Necrosis**
- M30 level: ccK18
- M65 level: total K18

Low levels of caspase-cleaved K18 (ccK18) compared to total K18 (low M30:M65 ratio)

*Krammer et al., 2004*
M30® mAb Specificity and Cross-reactivity

Specific for K18 and Apoptosis

Cross-reactive:
- Cow
- Macaque

Not reactive:
- Mouse
- Rat

Olofsson et al., 2007

Olofsson et al., 2009
Keratin 18 Use as a Biomarker in Xenograft Modeling

SCID FaDU model

Rat SW620 model

Olofsson et al., 2009
Efficacy of a new cancer drug can be shown by peaks of cell death upon successful dosing.

Kramer et al., 2006
Take-Home Messages:

1- TK1 protein (via TK210 ELISA) as a proliferation marker, and caspase-cleaved Keratin 18 (M30 Apoptosense® ELISA and the M65® ELISA) as an apoptosis marker, have strong support as relevant biomarkers for oncology studies.

2- Both of these biomarkers are valuable for the whole drug development process because of their uses in vitro studies and xenograft models to clinical studies.
Product Overview
All Products are for RESEARCH USE ONLY in the US

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ELISA Products
- TK210 ELISA

www.diapharma.com
Customer Support: 1.800.526.5224
Technical Support: 1.800.447.3846
Email: info@diapharma.com

VLVbio

ELISA Products
- M30 Apoptosense® ELISA
- M30 CytoDeath™ELISA
- M65® ELISA
- M65 EpiDeath® ELISA

Antibody Products
- M30 CytoDEATH ™mAb
- M6 & M5 Keratin 18 mAb

These products, as well as other unique oncology markers, can be found at:

www.diapharma.com