

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

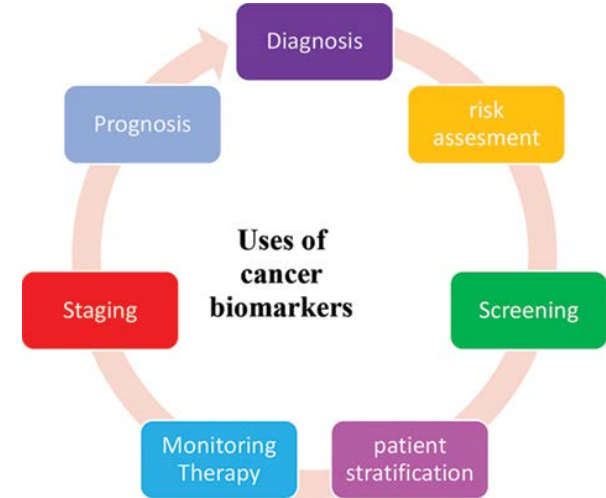
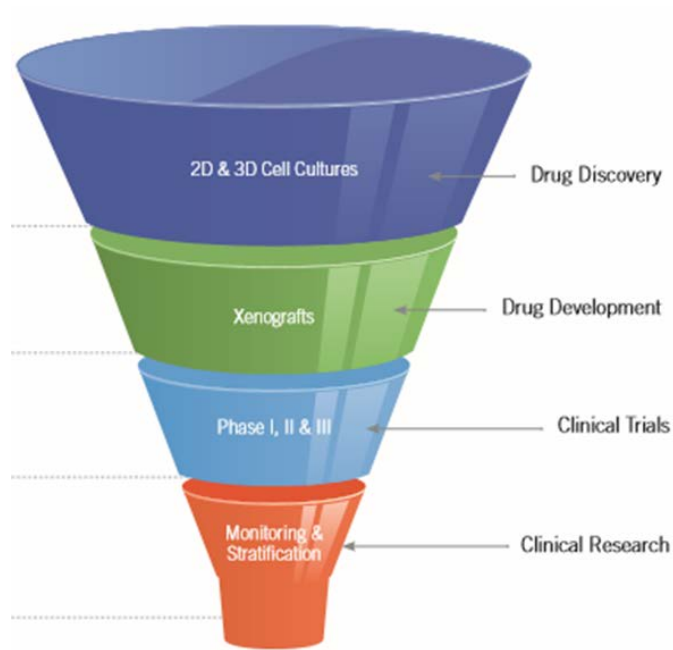
Thymidine Kinase 1 and Keratin 18



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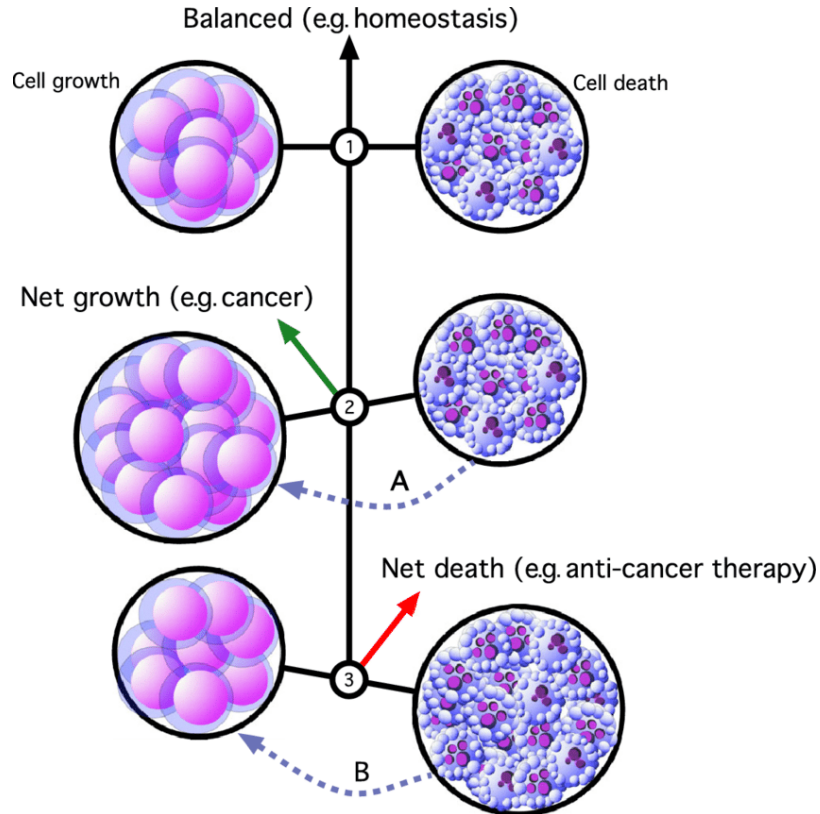
# Qualities of a Good Biomarker



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

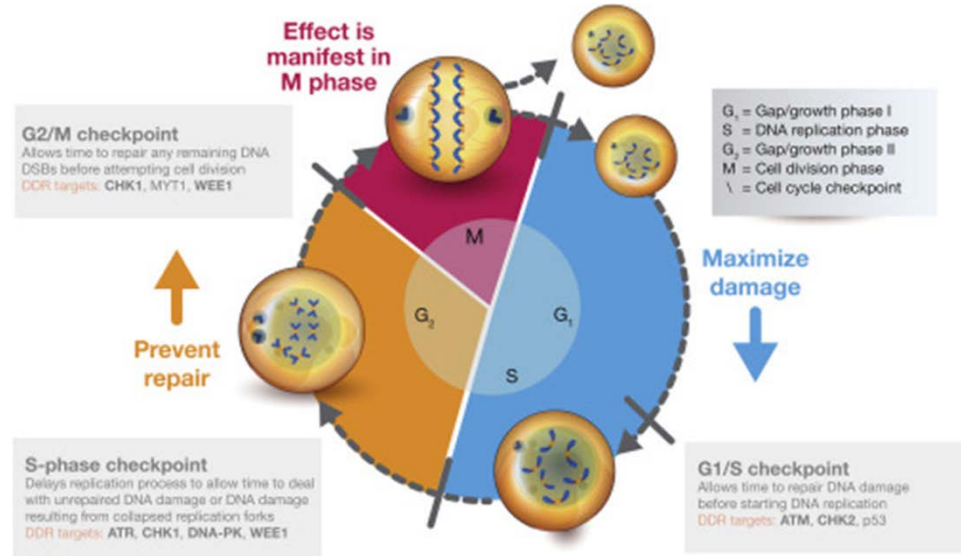
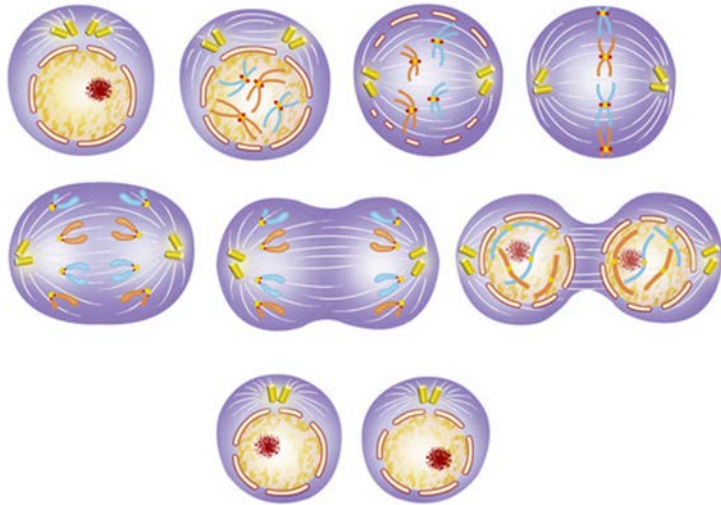
Note: The Arocell TK210 Assay, M30 Apoptosense®, and M65® ELISA assays are for RESEARCH USE ONLY in the US.

# Cancer: Fundamentally a disease of regulation of growth



# Cell Proliferation

# Proliferation as an Indicator and Target



Combinations with immunotherapy may achieve immune-mediated cell death at lower DNA damage thresholds

# BIOMARKERS FOR PROLIFERATING CELLS

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- *DNA Replication Indicators*

- Mitosis Counting
- BrdU, <sup>3</sup>H Thymidine, etc.

- *Protein Biomarkers*

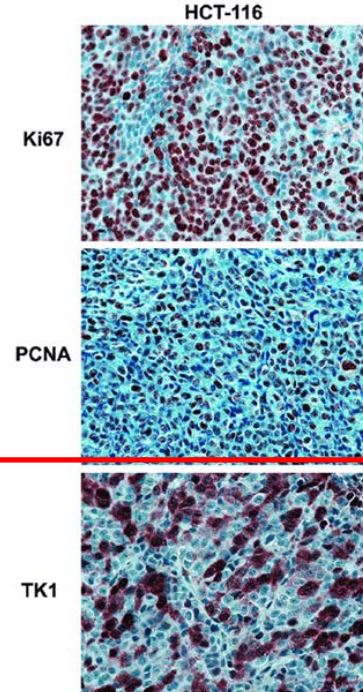
- *Ki-67:* Nuclear protein expressed in G1, S, G2 and M phases of cell cycle, but absent in G0.
- *PCNA:* Nuclear protein elevated during the G1/S phase of the cell cycle.
- *PTTG:* Securin protein degraded at anaphase onset.
- *TK1:* Enzyme involved in DNA synthesis that are elevated during S phase.

# BIOMARKERS FOR PROLIFERATING CELLS

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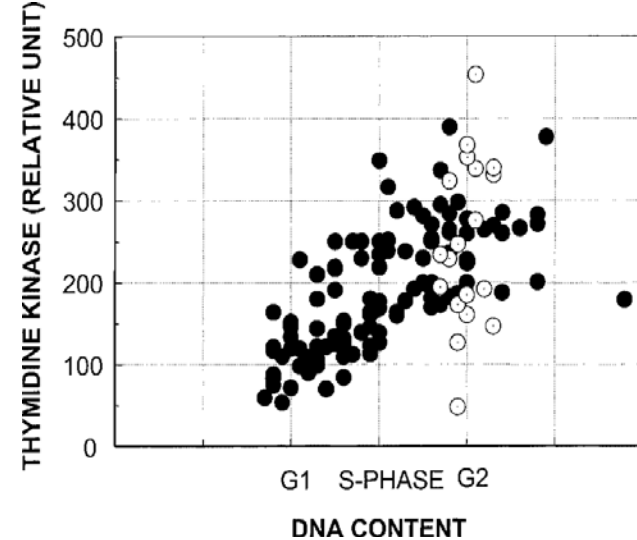
## IMMUNOHISTOLOGICAL BIOMARKERS

- *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)



# TK1 (Thymidine Kinase 1)

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.***

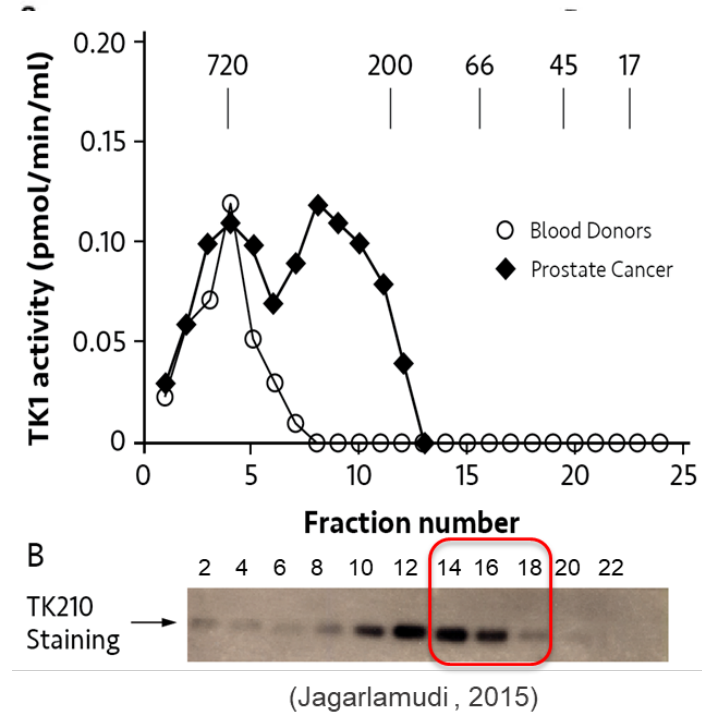
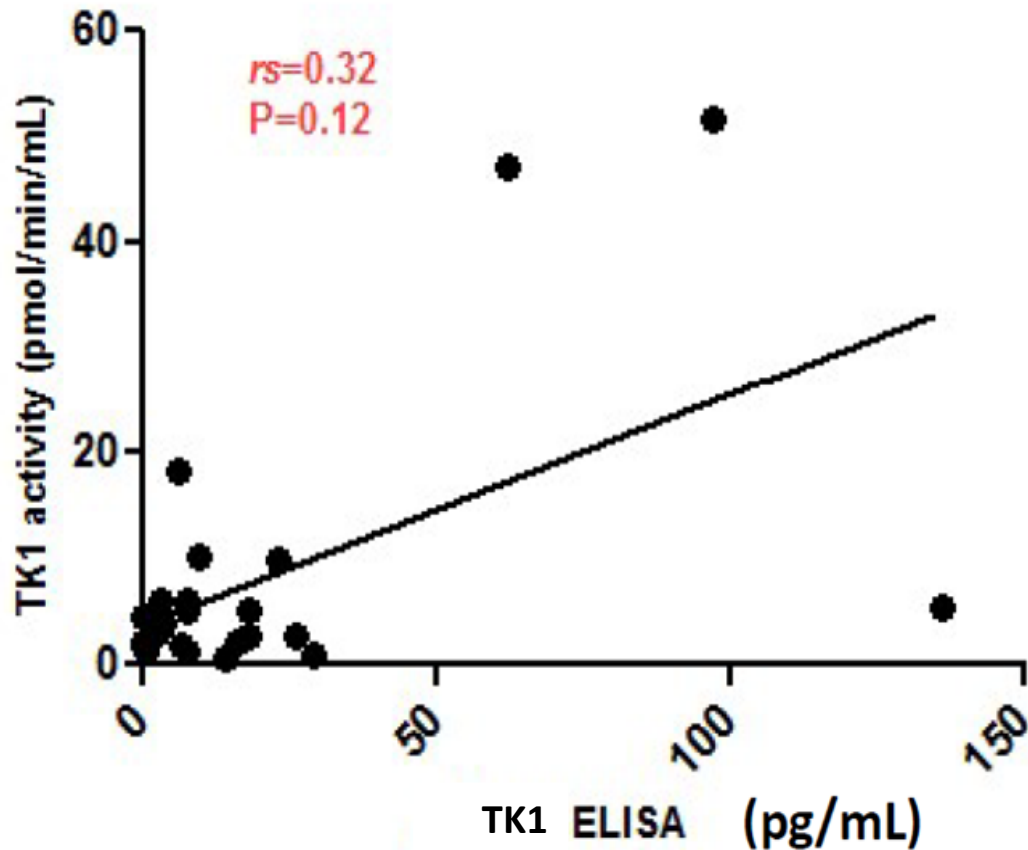


Figure 2: Gold-Standard  $^3\text{H}$  Labelled Thymidine Assay vs. TK1 ELISA



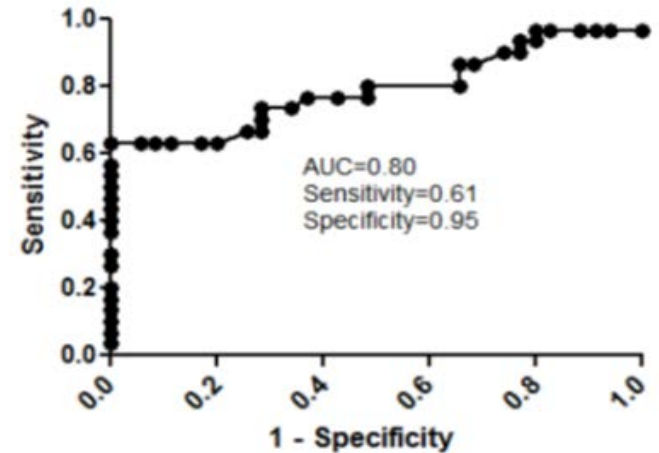
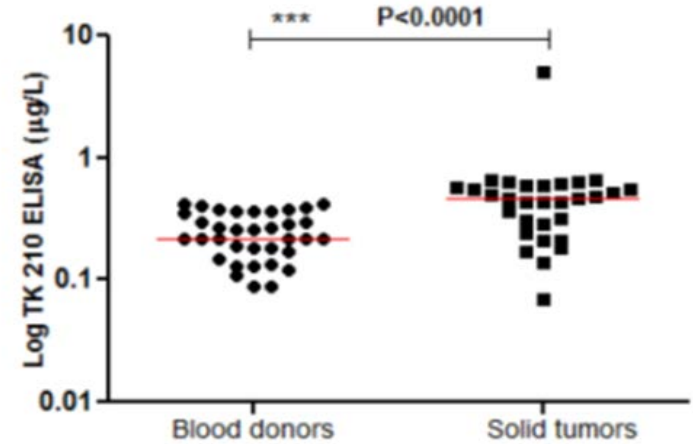
$^{125}\text{I}$ -  
iododeoxyuridin

based  
assays

TK1 ELISAs

# Arocell TK210 ELISA

- *Developed components that cleared the hurdle of the complexes*
  - *Established a buffer to dissociate complexes increases detection capacity*
  - *Monoclonal 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

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## 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

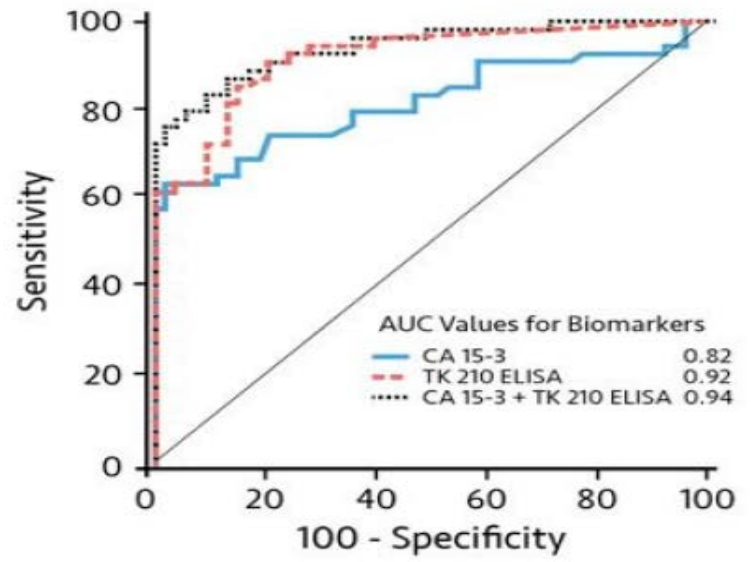
## BENEFITS OF TK 210 ELISA FOR CLINICAL RESEARCH STUDIES:

- Non-invasive serum based marker

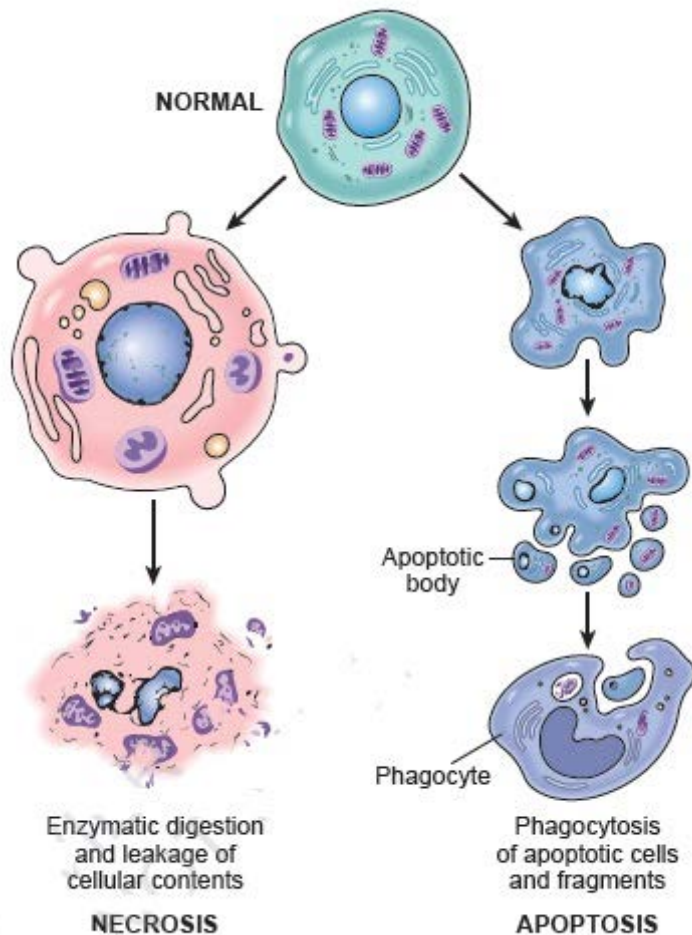
New tools for clinical researchers (hematology and solid tumors):

- **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



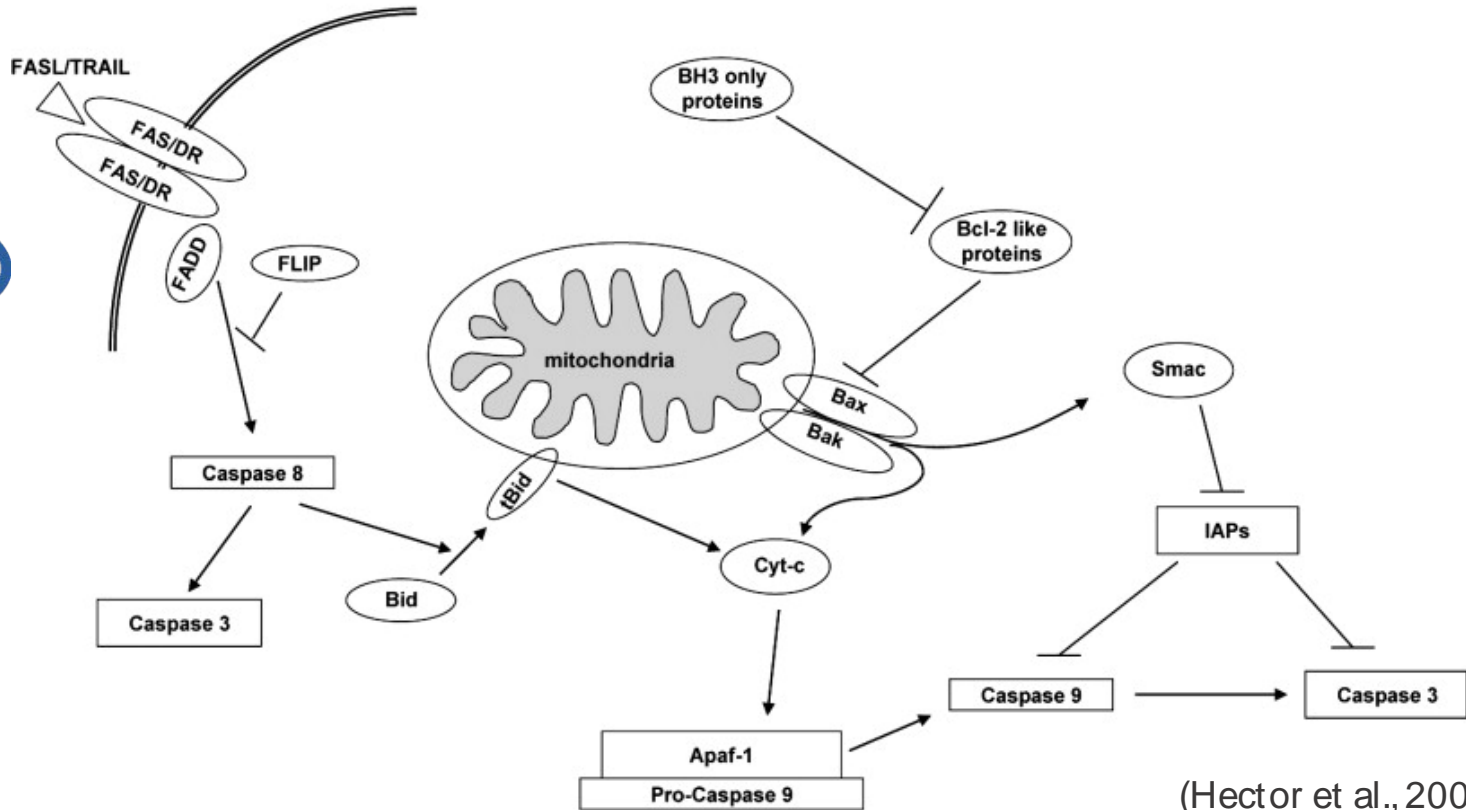
# Cell Death



# APOPTOSIS

Extrinsic apoptotic pathway  
(Death receptor mediated)

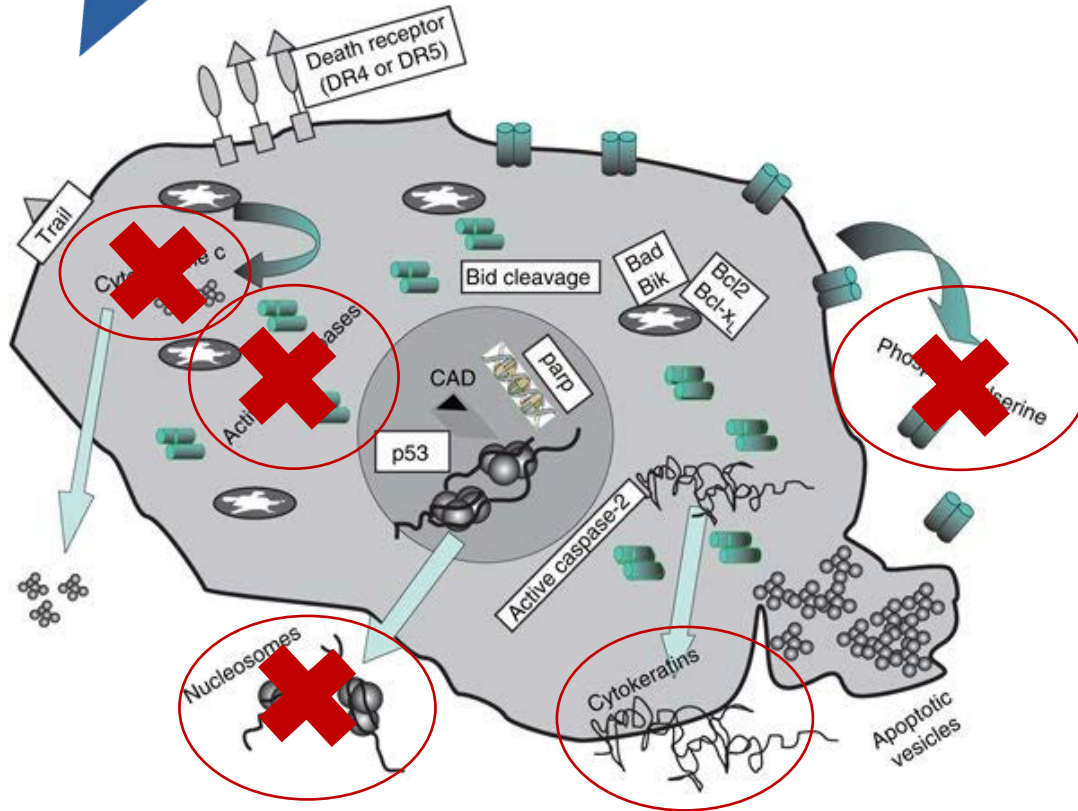
Intrinsic apoptotic pathway  
(mitochondrial mediated)



(Hector et al., 2009)



# APOPTOSIS BIOMARKERS



## Desired Qualities:

- ✓ Apoptosis specific
- ✓ Quantitative
- ✓ High-throughput
- ✓ Endpoint marker
- ✓ In the blood

# Keratin 18 (K18)

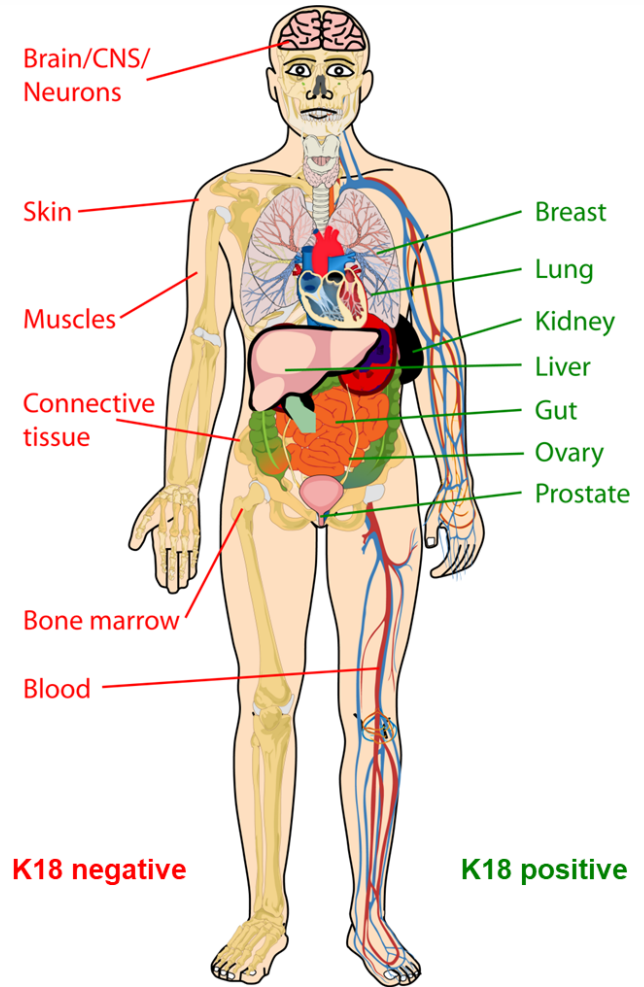
(Cytokeratin 18, CK18)

Keratin 18 is found in almost all epithelial cells in the body, e.g.:

- liver
- lung
- intestines
- breast
- prostate

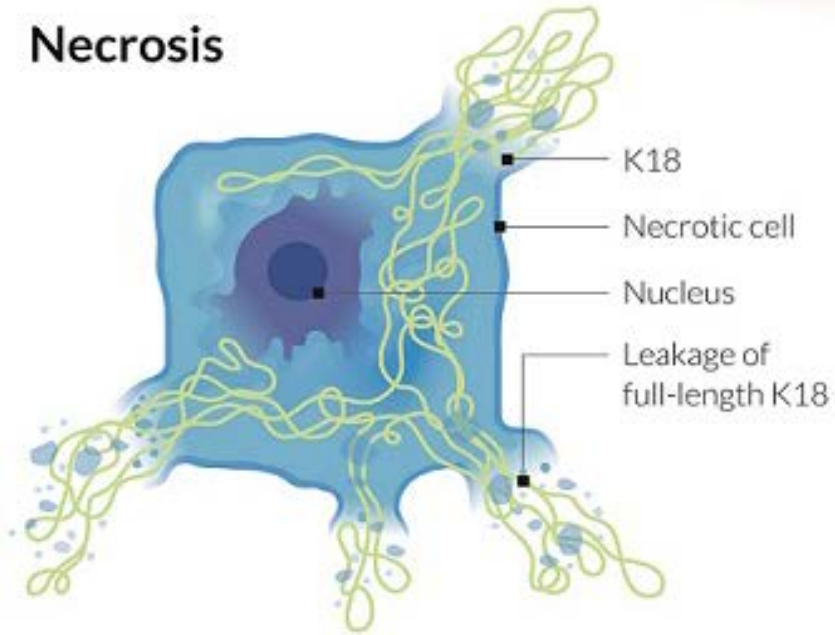
and **tumors** of these organs.

K18 is **not** expressed by neurons, muscle and connective tissues, skin and cells of the immune system.



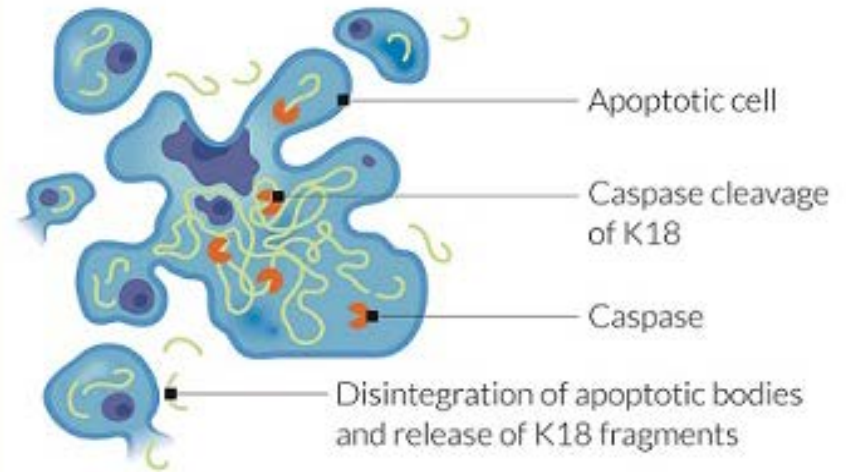
# Cell death

## Necrosis



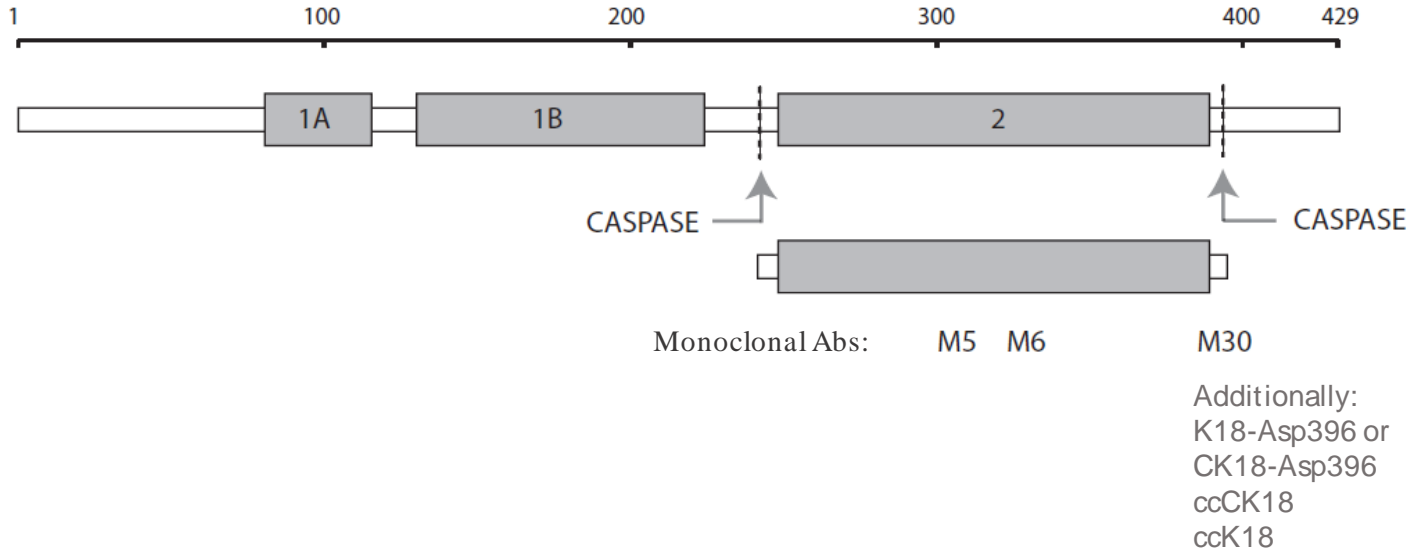
Only intact K18

## Apoptosis


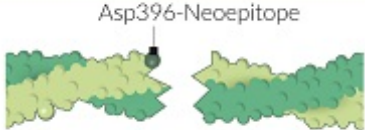
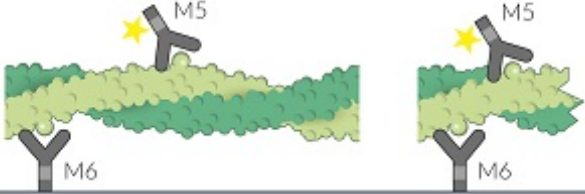
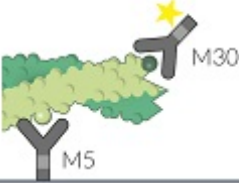


Caspase-cleaved K18

# Keratin 18



# M65® and M30 Apoptosense® ELISAs

Only intact K18	Caspase-cleaved K18
	
<p data-bbox="336 558 691 642"><b>M65® ELISA</b> <b>M65 EpiDeath® ELISA</b></p> 	<p data-bbox="973 558 1392 642"><b>M30 Apoptosense® ELISA</b> <b>M30 CytoDeath™ ELISA</b></p> 
<p data-bbox="336 887 765 912">Measurement of intact and cleaved K18</p> <p data-bbox="336 936 799 992"><b>The M65 ELISAs measure total cell death</b> <b>(necrosis and apoptosis)</b></p>	<p data-bbox="973 887 1344 912">Measurement of cleaved K18 only</p> <p data-bbox="973 936 1263 992"><b>The M30 ELISAs measure</b> <b>only apoptosis</b></p>

# 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

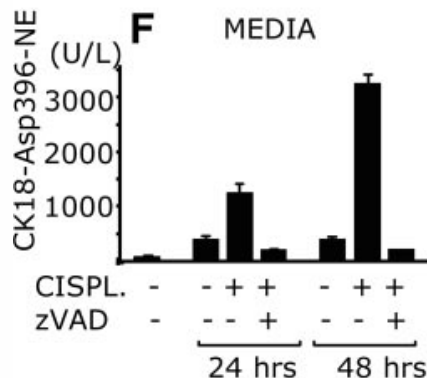
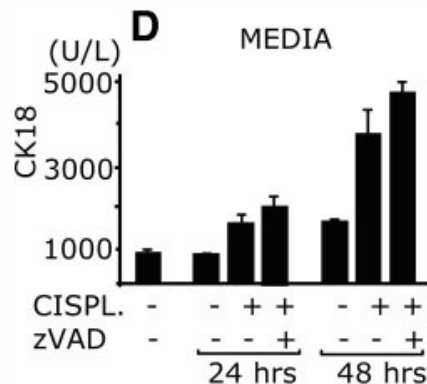
ccK18

M65 level

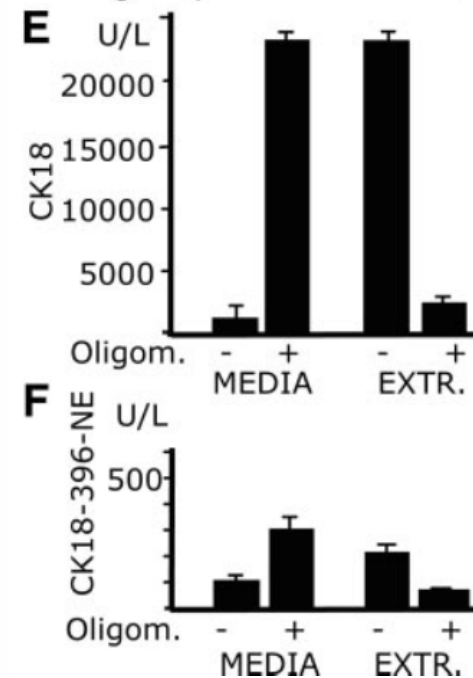
total K18

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

## Apoptosis

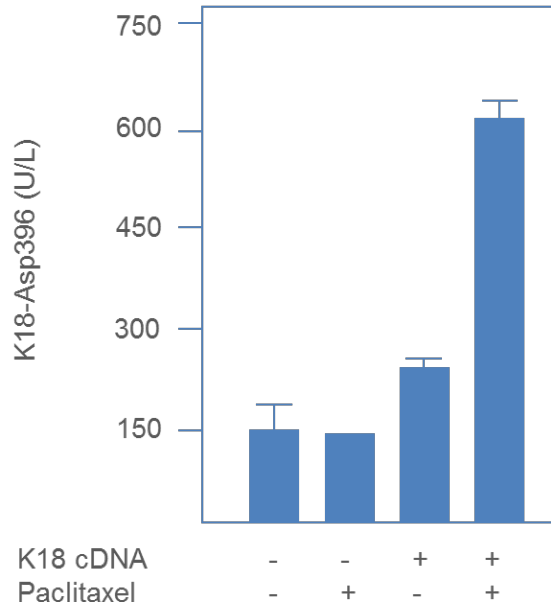


## Necrosis

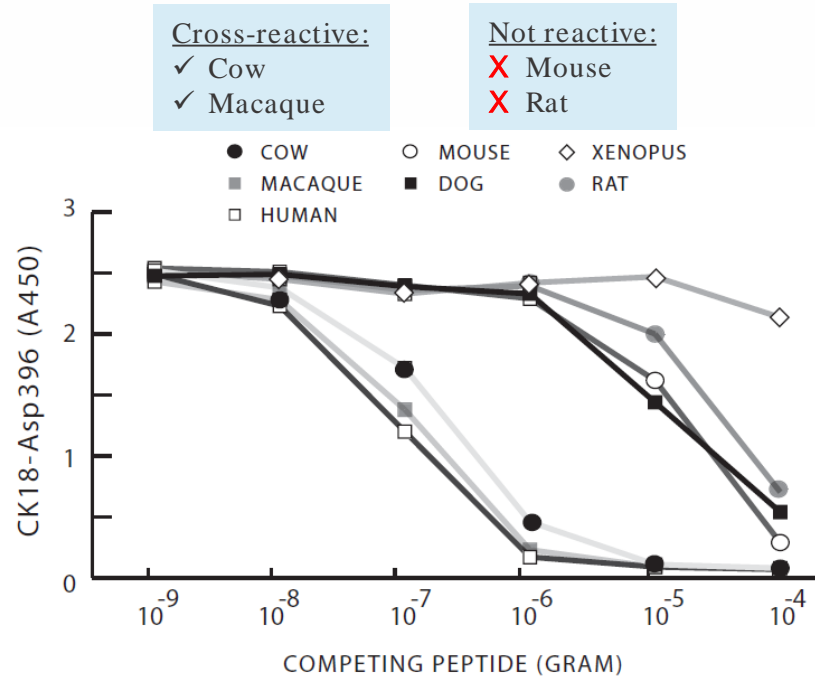


# M30® mAb Specificity and Cross-reactivity

Specific for K18 and Apoptosis



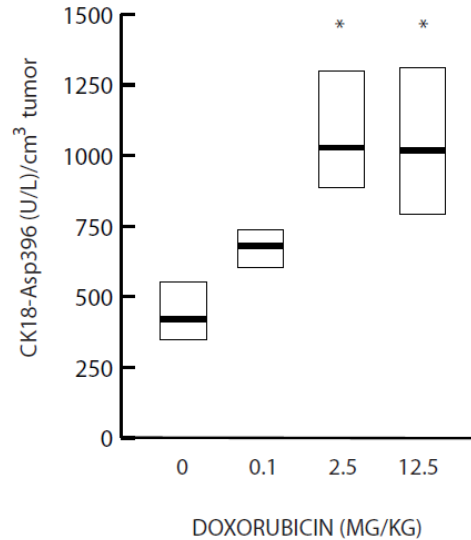
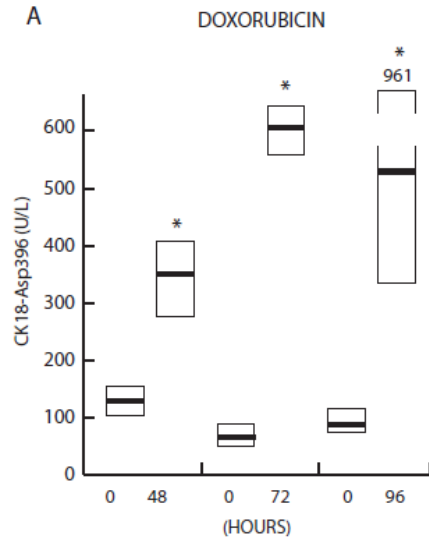
Olofsson *et al.*, 2007



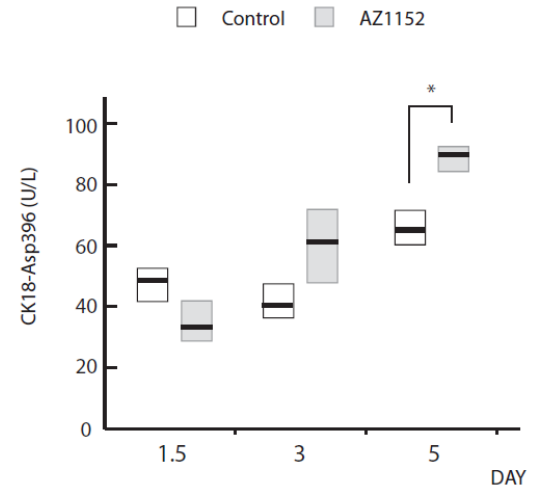
Olofsson *et al.*, 2009

# Keratin 18 Use as a Biomarker in Xenograft Modeling

SCID FaDU model

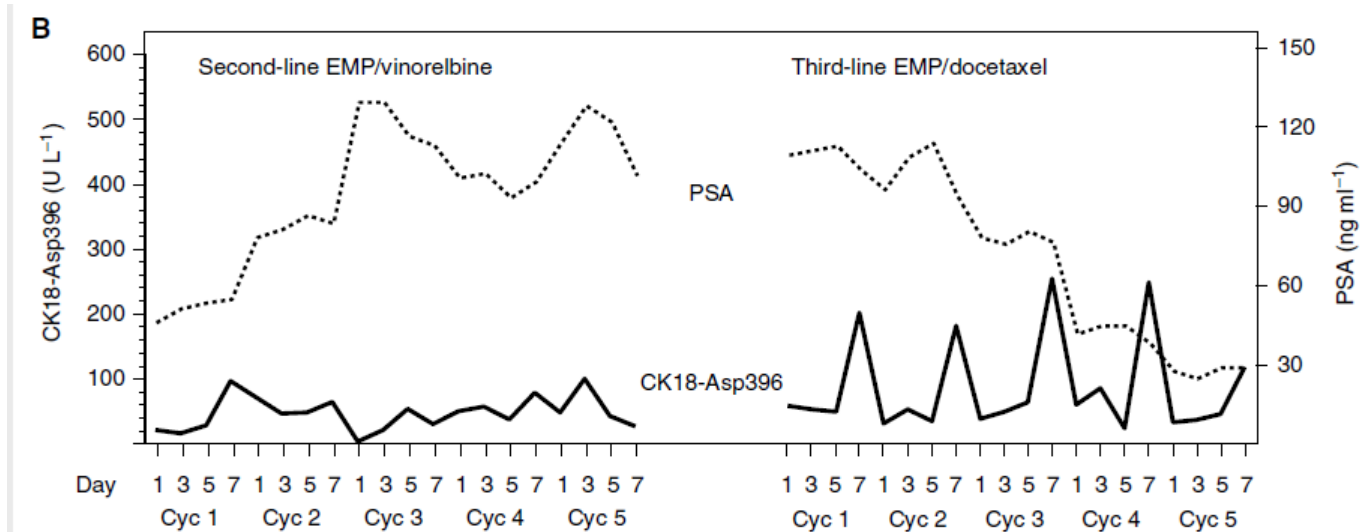


Rat SW620 model





# Keratin 18 Apoptotic Fragment for Clinical Research



Efficacy of a new cancer drug can be shown by peaks of cell death upon successful dosing.



## 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

## arCell

### ELISA Products

- TK210 ELISA



## 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](http://www.diapharma.com)

Customer Support: 1.800.526.5224

Technical Support: 1.800.447.3846

Email: [info@diapharma.com](mailto:info@diapharma.com)