Oncology Cell Death Products

from basic research to disease modelling to clinical trials

Peviva Cell Death Products

Monoclonal Antibody Products

- **M5 and M6 Keratin 18 mAb**
- **M30 CytoDeath™ caspase-cleaved K18 mAb** (unlabelled, biotin, fluorescein, orange)

Sample Type & Suitable Experiments

Product	Cell cultures	Spheroids	Xenografts	Blood samples
M30 Apoptosense ELISA	(✓)	(✓)	✓	✓
M30 CytoDeath ELISA	✓	✓	—	—
M65 ELISA	✓	✓	✓	✓
M65 EpiDeath ELISA	✓	✓	✓	✓

Peviva Cell Death ELISAs

Measurement of Cell Death Modes

Product	Apoptosis	Necrosis	Total cell death
M30 Apoptosense ELISA	✓	—	—
M30 CytoDeath ELISA	✓	—	—
M65 ELISA	✓	✓	✓
M65 EpiDeath ELISA	✓	✓	✓

The Peviva ELISAs are

- a tool for determination of cell death mode
- for cell culture/spheroids OR *in vitro* serum/plasma samples

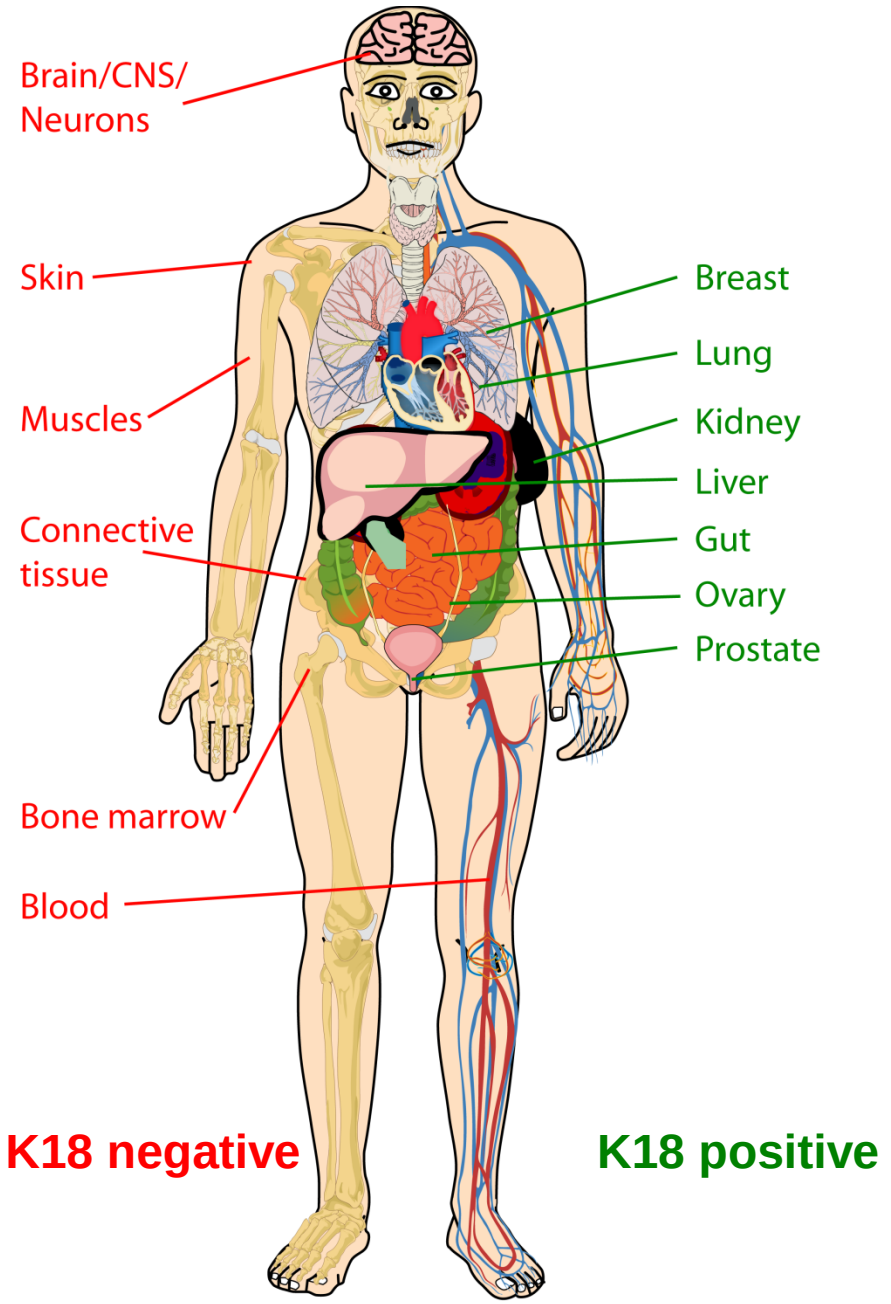
Keratin 18 in the Human Body

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

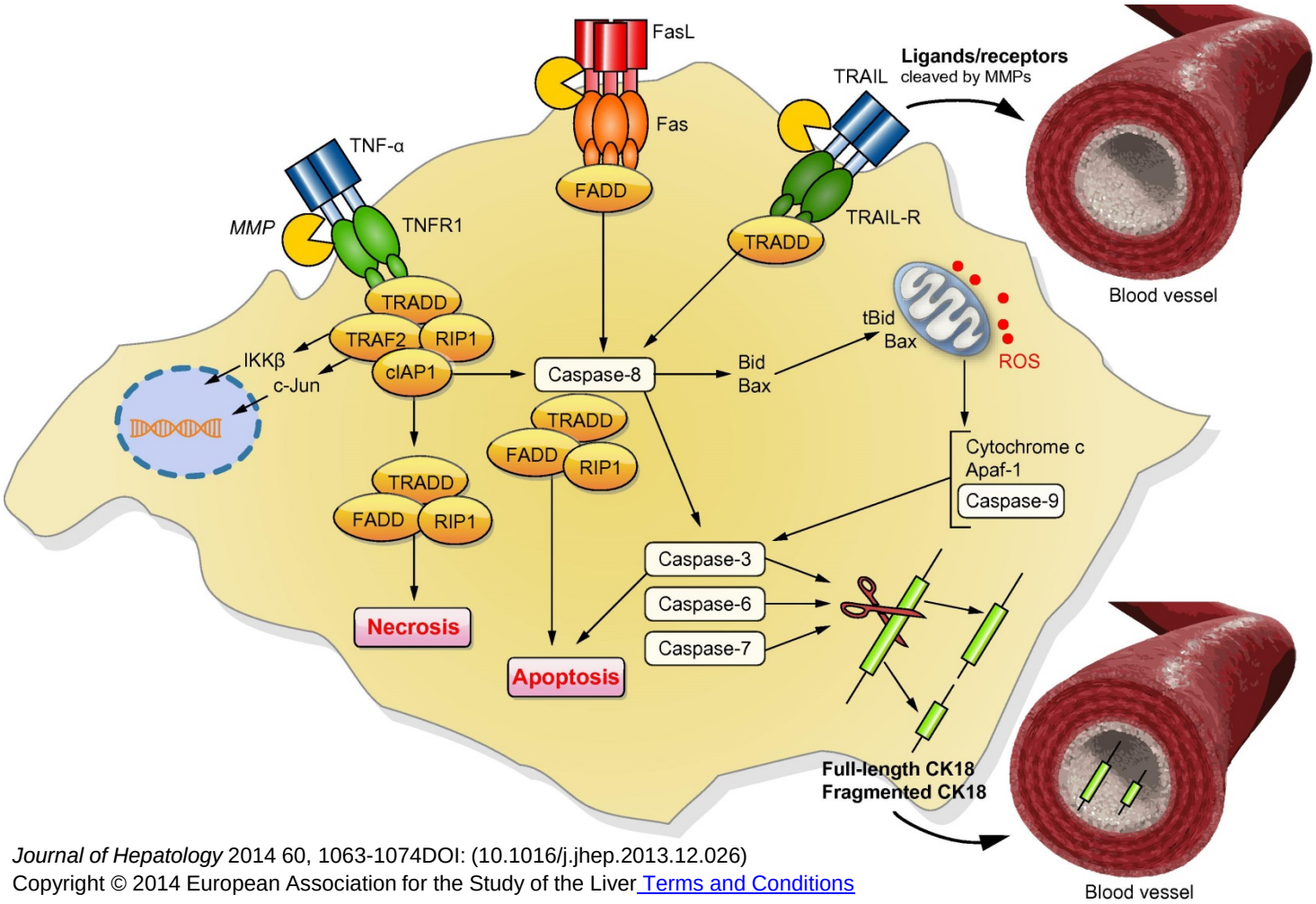
- liver
- lung
- intestines
- breast
- prostate

and **tumors** of these organs.

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

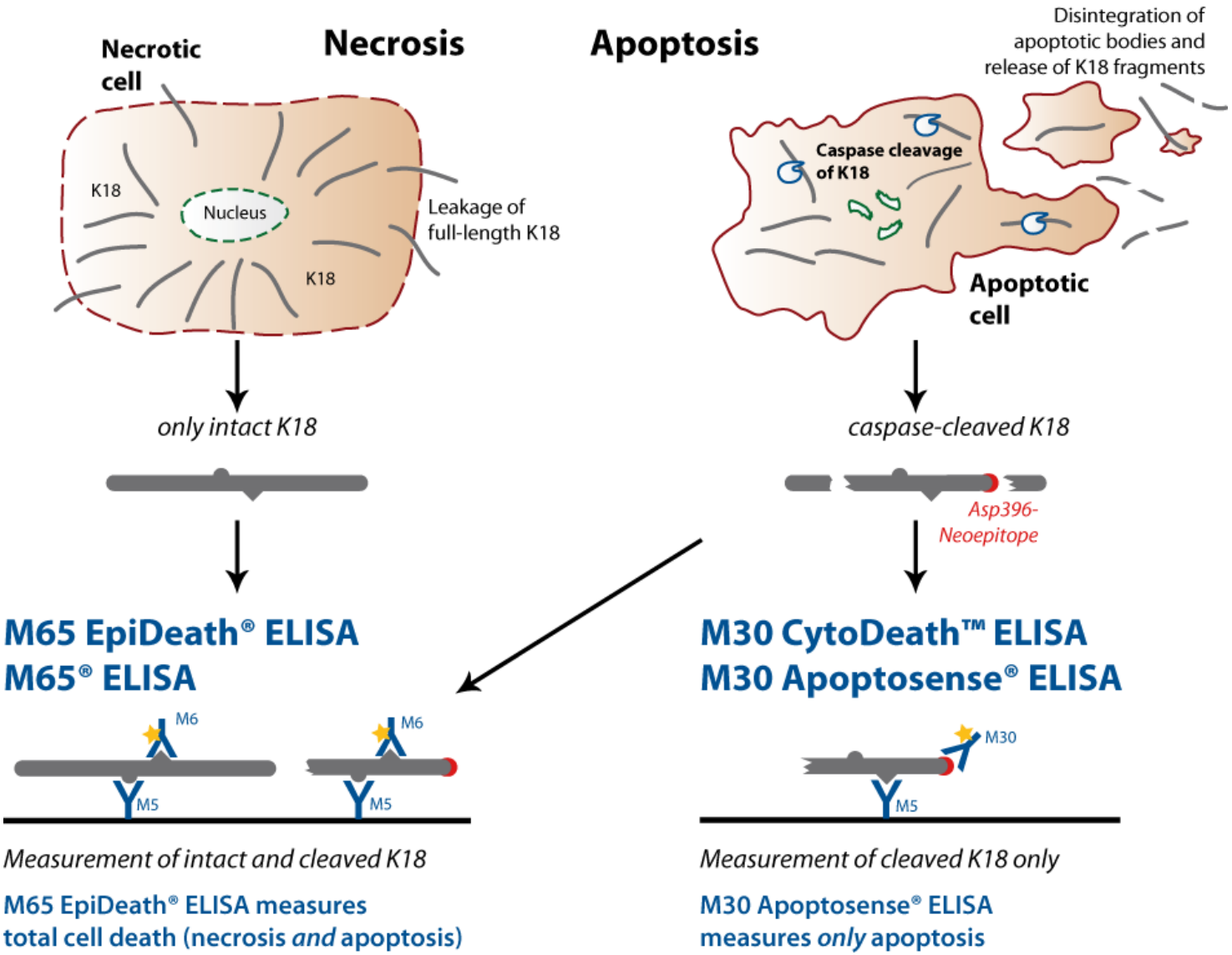


Biology and Mechanisms of Epithelial Cell Death: Necrosis vs. Apoptosis



Journal of Hepatology 2014 60, 1063-1074DOI: (10.1016/j.jhep.2013.12.026)

Epithelial Cell Death: Necrosis or Apoptosis



Oncology Drug Development Applications

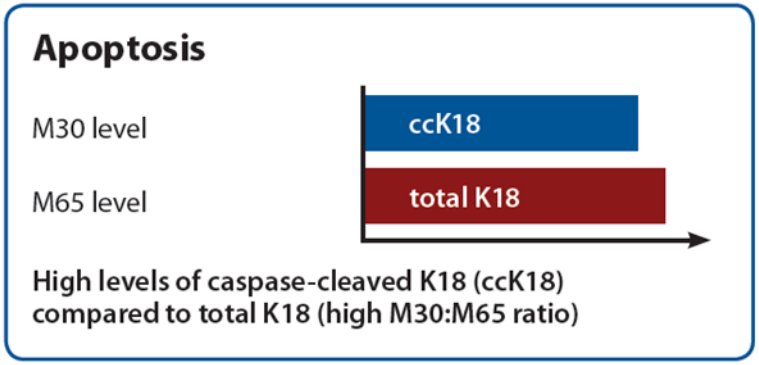
1) Determine Drug Mechanism of Action

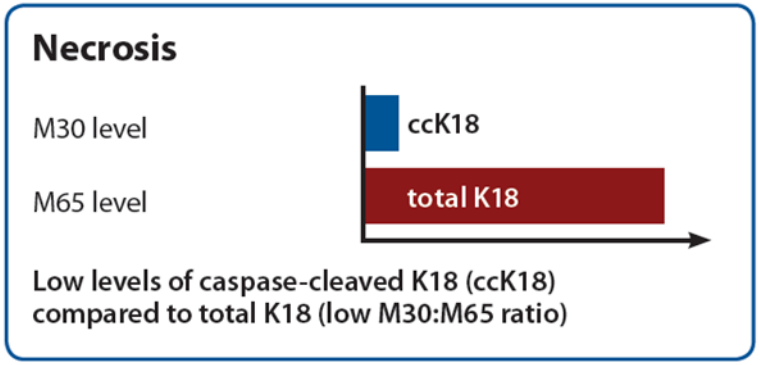
M30 Apoptosense® ELISA + M65®ELISA:

Identify Mechanism of Cell Death in Antitumor Drugs

- **M30:** detects *caspase-cleaved* cytokeratin 18 = **APOPTOSIS**
- **M65:** detects *intact* cytokeratin 18 = **NECROSIS**

M30:M65 ratios are used to determine cell death mode (apoptosis vs. necrosis):



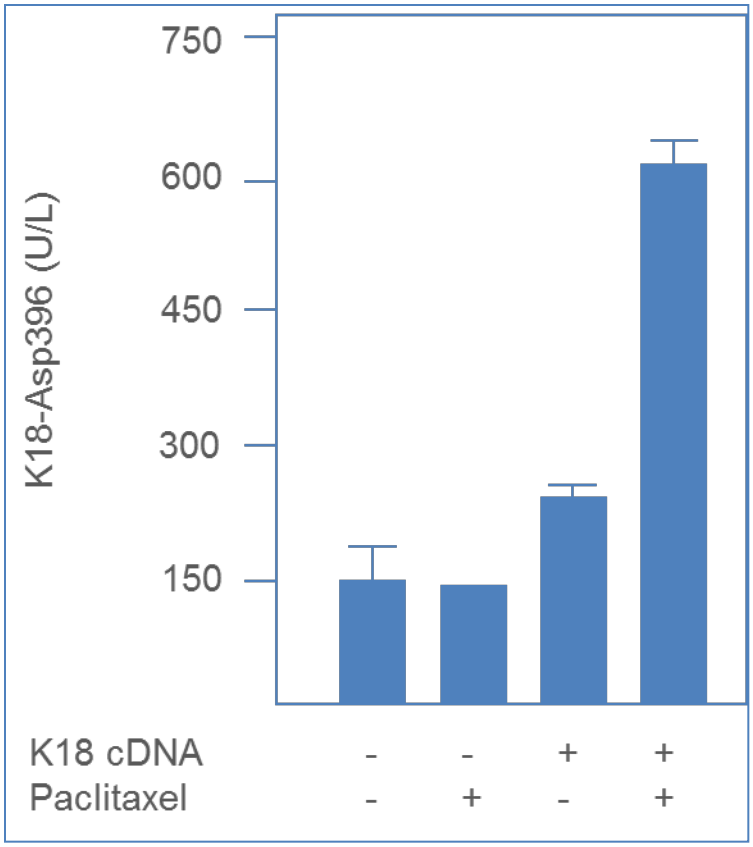


Kramer et al., Cancer Research 64 (2004) 1751-1756.

2) Determine Drug Mechanism of Action

Cancer Therapeutics Often Induce Apoptosis

- M30 antibody detects apoptosis with high specificity.
- Apoptosis from only K18 positive cells, such as epithelial tumour cells, is detected.

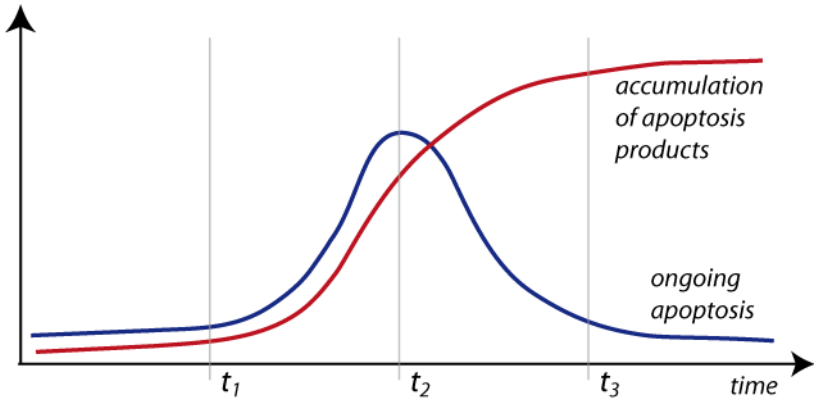


(K18 negative) fibroblasts treated with paclitaxel only induce M30 signal increase when transfected with K18 cDNA.

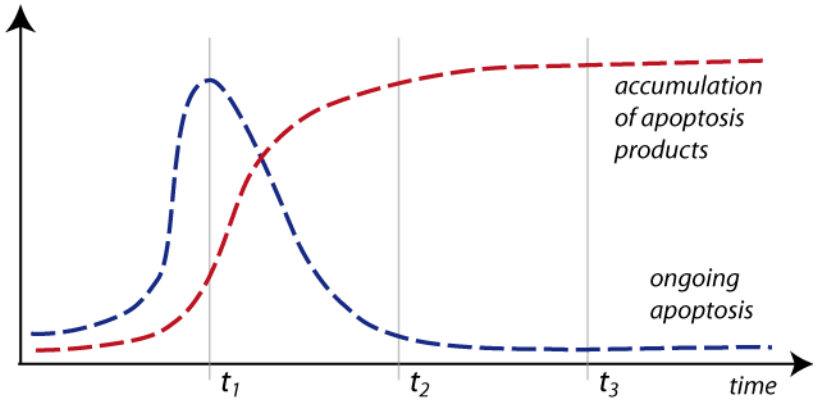
Measure Apoptosis Independently from Time

The accumulated apoptosis product measured by the M30 CytoDeath™ ELISA:

Slow induction of apoptosis



Rapid induction of apoptosis

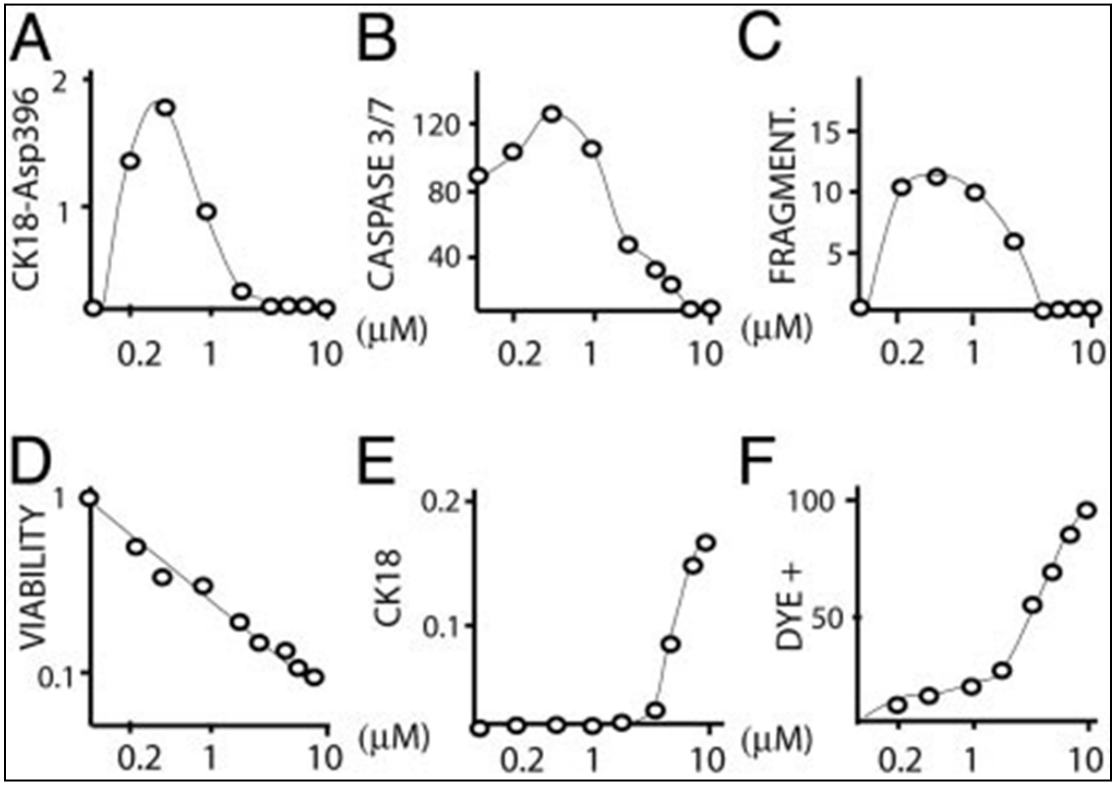


Approach combines *high-throughput* & *end-point measurements*. Assay quantifies *accumulation* of an apoptosis-generated product, which is semi-stable in cells and culture media. The signal from cells that have undergone apoptosis remains in the culture medium (or blood) after all apoptotic processes are completed and all cells are dead. A late time point (t_3 in the chart below) is sufficient to quantify the intensity of the apoptotic stimulus, regardless of its kinetics.

Methods that measure the number of apoptotic cells at t_1 or t_2 will often return incorrect results.

3) Determine Drug Mechanism of Action

Dose Response Assessment



Data in the graphs above indicate screening compound NSC567461 triggers an apoptotic response in tumor cells at low concentrations and a necrotic response at higher concentrations.

Erdal et al., PNAS (2005) 192-197.

4) Drug Screening: High Through-Put Applications

M30 CytoDeath™ ELISA:

Identify Pro-Apoptotic Antitumor Drugs via High-Through-Put-Screening

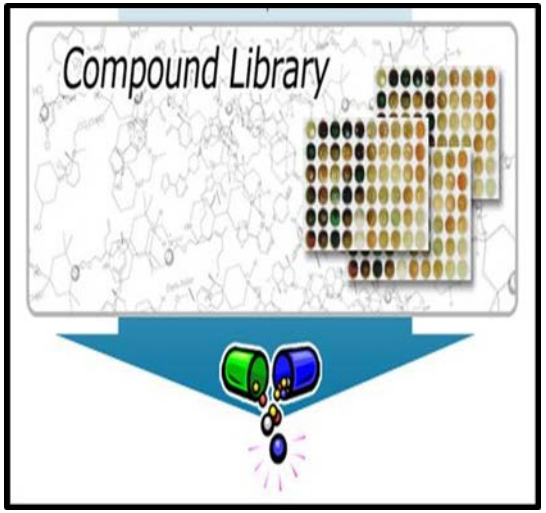
PLATE	SUFFIX	WELLID	WELLNBR	NSC	SAMPLE	STORCONC	CONCUNIT	VOLUME	MW	UHAZ	SOLIND	RESIDUE
4827	12	A02	2	32065	11	0.001	M	20	76.05	RPT	1	
4827	12	B02	14	3970	8	0.001	M	20	112		1	
4827	12	C02	26	4728	9	0.001	M	20	101		1	
4827	12	D02	38	4960	2	0.001	M	20	124		1	
4827	12	E02	50	5354	5	0.001	M	20	108	TOX	1	
4836	12	H10	94	3053	21	0.001	M	20	1255.43	HTX IV P R	1	
4836	12	A11	11	18268	6	0.001	M	20	3808	ALK CRC I	1	
4836	12	B11	23	71948	1	0.001	M	20	1349		1	
4836	12	C11	35	125066	32	0.001	M	20	1512.61	CRC IV P R	1	
4836	12	D11	47	125176	1	0.001	M	20	1393		1	
4836	12	E11	59	285116	5	0.001	M	20	1648.84	PSN	1	
4836	12	F11	71	333856	8	0.001	M	20	1336.48		1	
4836	12	G11	83	615993	1	0.001	M	20	1365		1	
4836	12	H11	95	622116	1	0.001	M	20	1869		1	
4837	12	A02	2	622124	1	0.001	M	20	3015		1	

National Cancer Institute (NCI)/Division of Cancer Treatment and Diagnosis (DCTD)/Developmental Therapeutics Program (DTP) : http://dtp.cancer.gov

Tumor Cell Lines (ex. NCI_{60})

1. Grow **human epithelial** tumor cell lines in culture.
2. Test drug candidates/compound library on cell lines to determine effective **pro-apoptotic** compound(s).

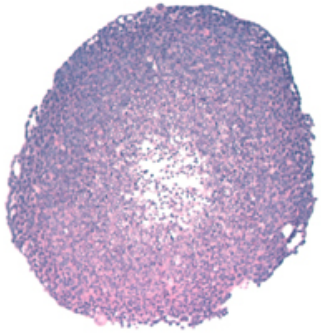
+



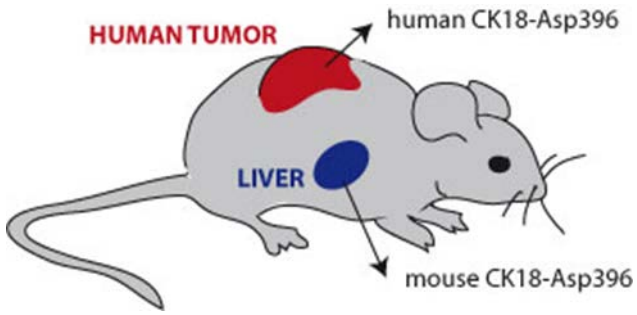
Compound Screening Library

5) Preclinical Drug Testing in Disease Models

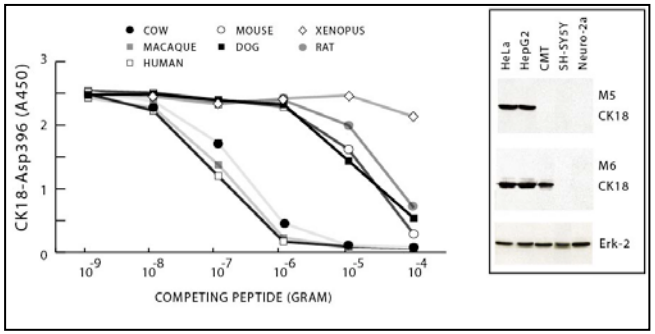
M30 CytoDeath™/M30 Apoptosense® ELISAs:
Evaluate Efficacy of Pro-Apoptotic Antitumor Drugs



2D Cell Culture *or* 3D Spheroids
M30 CytoDeath™ ELISA
M65® ELISA (necrosis assessment)



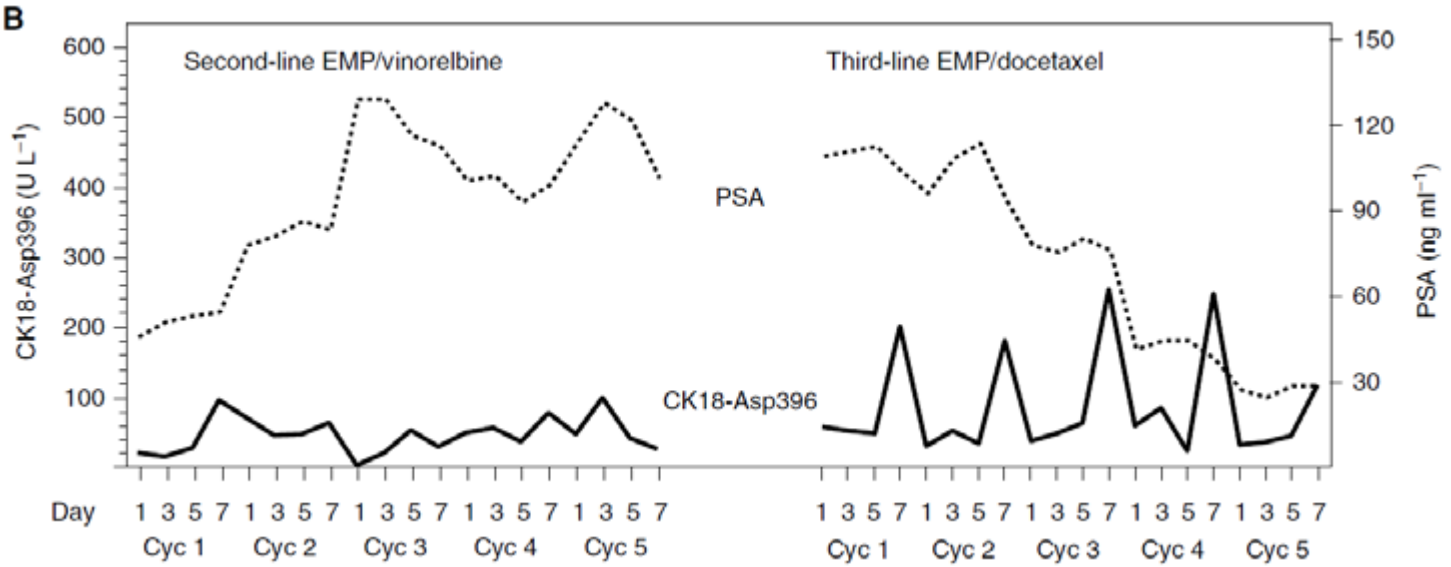
Xenograft Models
M30 Apoptosense® ELISA
M65® ELISA (necrosis assessment)



Olofsson et al., Cancer Biomarkers 5 (2009) 117-125.

- *Minimal/no* antibody cross-reactivity with mouse & rat species
- Apoptosis in tumor xenograft leads to increase in *human* ccK18 in mouse plasma

6) Drug Testing in Oncology Research Studies

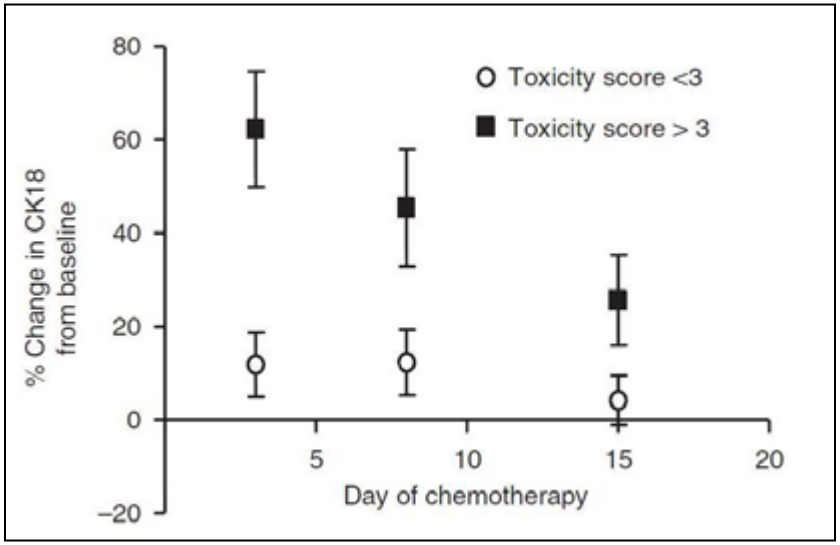


- Data from a research study where ccCK18 was induced during each cycle of chemotherapy is shown above (right). In the graphs, estramustine phosphate (EMP) in combination with docetaxel did not show a treatment effect (shown by stable ccCK18 serum levels and increasing PSA concentrations (left)). Switching to EMP-docetaxel therapy led to increases of ccCK18 levels in serum which correlated with decreases of PSA.

- Typically, samples should be tested 2-4 days after drug administration, depending on pharmacokinetics and mechanism of action.

Kramer et al., BJC 94 (2006) 1592-1598.

7) Drug Toxicity Evaluation in Oncology Research Studies



Changes in circulating CK18 in research participants with lymphoma following chemotherapy, according to CTCAE epithelial toxicity score.

- Use in drug safety research for *non-epithelial* tumors to assess treatment toxicity.
- CK18 levels as measured by the M65 ELISA® measure host toxicity; rises in CK18 levels provide early warning of epithelial toxicity.