Double Targeting as an Effective Anti-Cancer Strategy

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ALPHA-FETOPROTEIN: THE PROTEIN THAT NEVER GREW UP

Throughout the fetal proteins I have sifted,
Alpha-fetoprotein is surely gifted;
Of all the fetal proteins that I see,
The dominating force is AFP.
Its blood circulation is quite uncanny
Through every embryonic nook and cranny.
In one of the assays that man may devise
Its presence may indicate fetal demise.
The amount of AFP reflects
The presence of neural tube defects.
Workers at a famous Institute
Claim it's an albumin substitute;
Yet others have stated formulation
Pointing toward immuno-regulation.
Some rave of functions much more merrier,
For example, a blood transport carrier.
Functions which first may appear insipid
Are transports of steroid and of lipid;
It transports metals such as copper and zinc
And possibly serves as an estrogen sink.
It seems to peak during fetal duration,
But fails to attain adult maturation.
At birth when proteins go into rehirement,
AFP is just approaching retirement.
One could say "AFP over-runneth its cup",
It's a case of a protein that never grew up.

G.J. Mizejewski
November, 1979

Human AFP

- Natural delivery protein (70 kDa), $T_{1/2}$ = 3-5 days *in vivo*
- Substituted by albumin (67 kDa) after the birth
- Elevated in the blood during pregnancy and cancer:

  Healthy adults <10 ng/мл

  Pregnant: 15-100 ng/мл

  Cancer marker >200 ng/мл

  Abelev GI., Adv Cancer Res. 14:295–357, 1971; Gerald J. Mizejewski, Experimental Biology and Medicine, vol.226(5):377-408, 2001.
- Was registered as injectable drug in Russia

AFP Receptor:

- Embryo cells
- Cancer cells: Type I = 2,000/cell

  Type II = 135,000/cell

  Moro R., et al,Tumor Biol., 1993, v.14, p.116-130.

  Normal human cells do not have the AFP receptor with the only exception being small population monocytes.

  Gerald J. Mizejewski, Experimental Biology and Medicine 229:439-463, 2004.

AFP>Albumin Ligand Binding = Significant of its Fetal Uptake*

Over 70% of estrone (which bind strongly to rodent AFP) injected into the maternal circulation was found to be associated with AFP in the fetus.

Synthetic estrogens with lower AFP-binding affinity were not concentrated in the fetus.

LeGuern et al., Dev. Pharm. Ther.,4(Supple.1), 79, 1982.

Ka DHA-AFP: *Ka* DHA-albumin = 97:1,8

Anel A, et al, Febs Letters, v. 250, n. 1, 22-24, 1989.

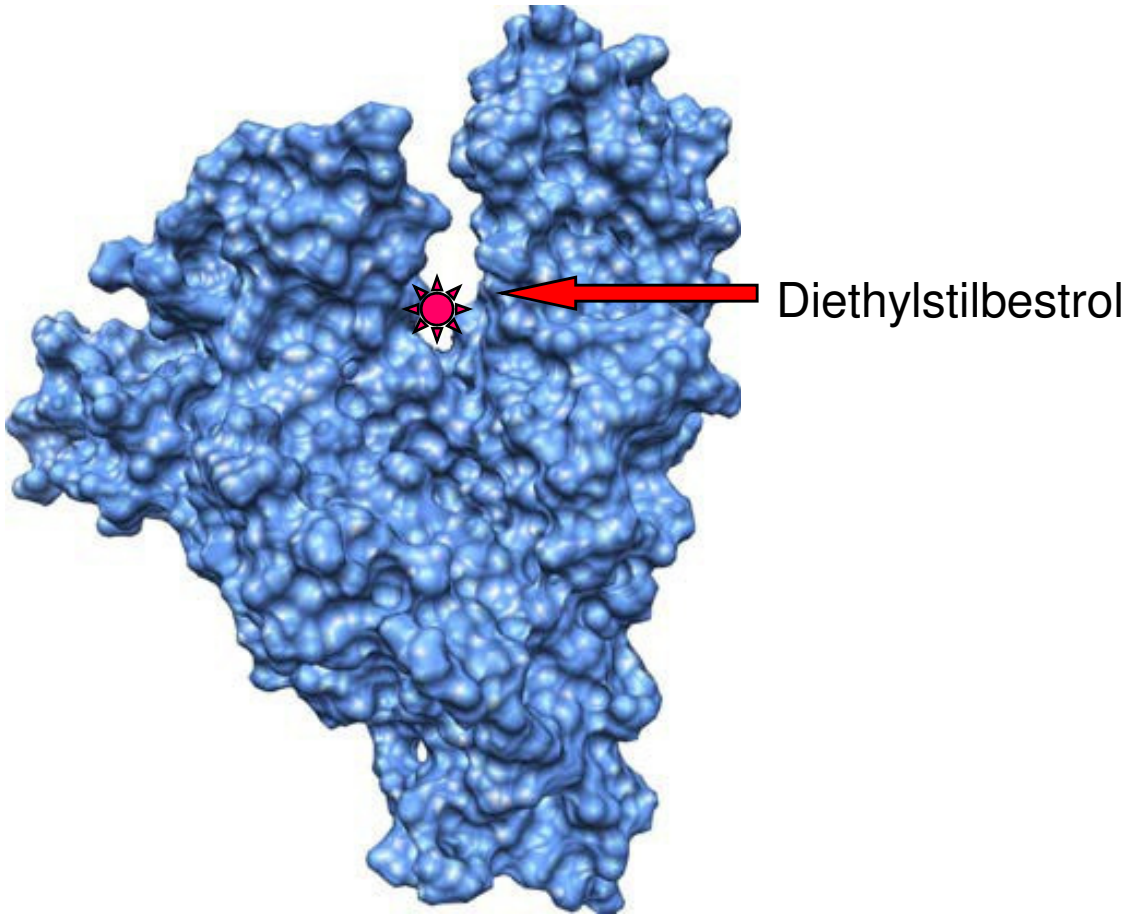
*Hsia JC, Deutsch HF, et al, An in vitro model of placental transfer of polyunsaturated fatty acids: the albumin-alpha-fetoprotein exchange system, Biological activities of alpha-fetoprotein, CRC Press, Inc., v.1, 205-211, 1987.

Chemotherapy Agents in Pregnancy

Agent	Generally Acceptable	Generally Unacceptable at any time	Not Enough Study to Recommend
methotrexate		X	
cytarabine		X	
5-FU	X		
cyclophosphamide	X		
doxorubicin	X		
bleomycin	X		
vincristine	X		
etoposide	X		
platinums			X
vinorelbine			X
taxanes			X

Mayer E.L. An Oncologist's Perspective on the clinical use of teratogenic products. Dana-Farber Cancer Institute, Boston, MA, Dec. 13, 2012.

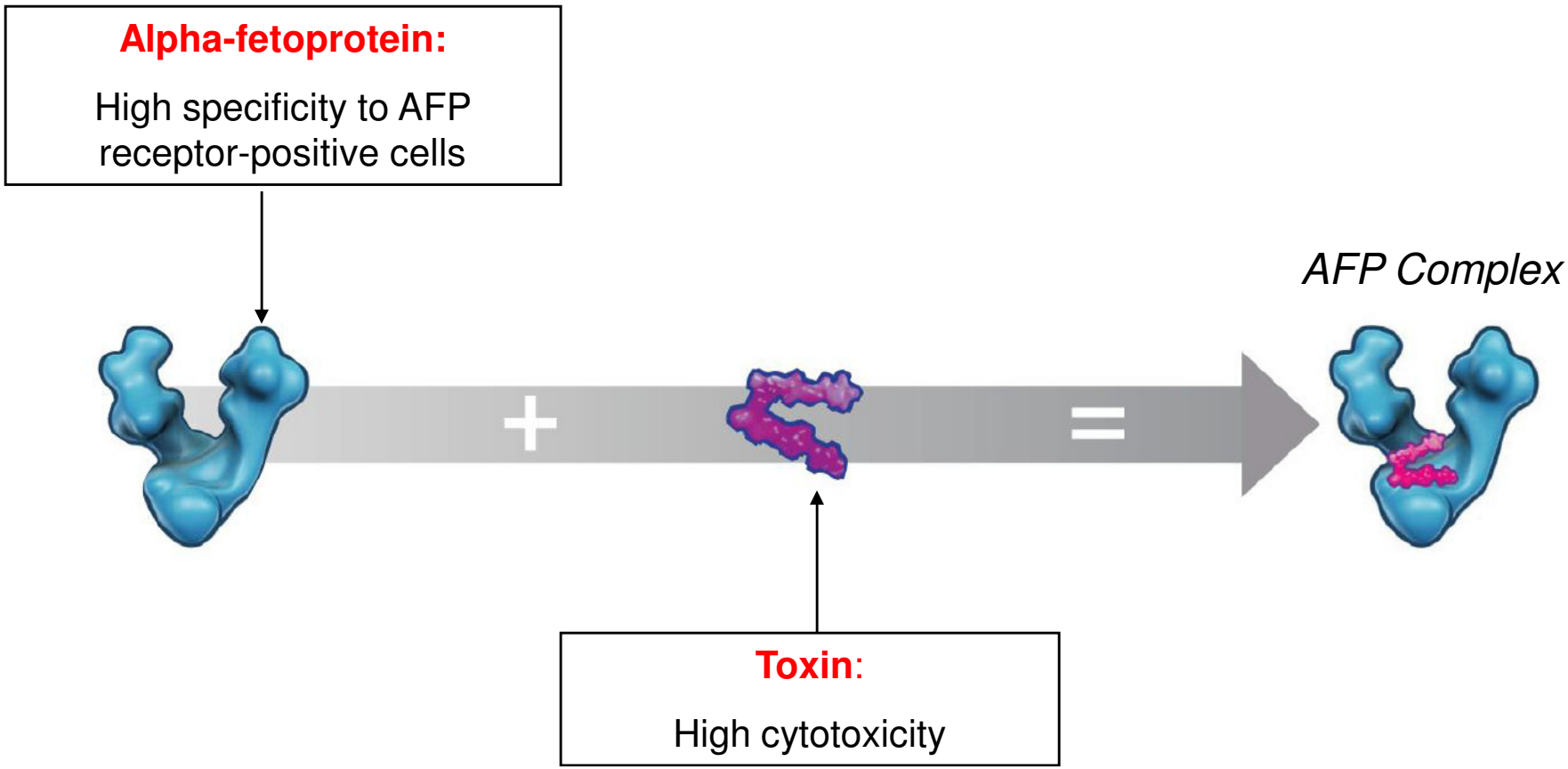
Diethylstilbestrol in the AFP Hydrophobic Pocket



Terentiev AA, Moldogazieva NT, Levtsova OV *et al*. Modeling of three-dimensional structure of human alpha-fetoprotein complexed with diethylstilbestrol: docking and molecular dynamics simulation study. *J Bioinform Comput Biol.* 10, 1241012 (2012).

"The whole is greater than the sum of its parts."

Aristotle



AFP receptor in Cancer Patients Serum

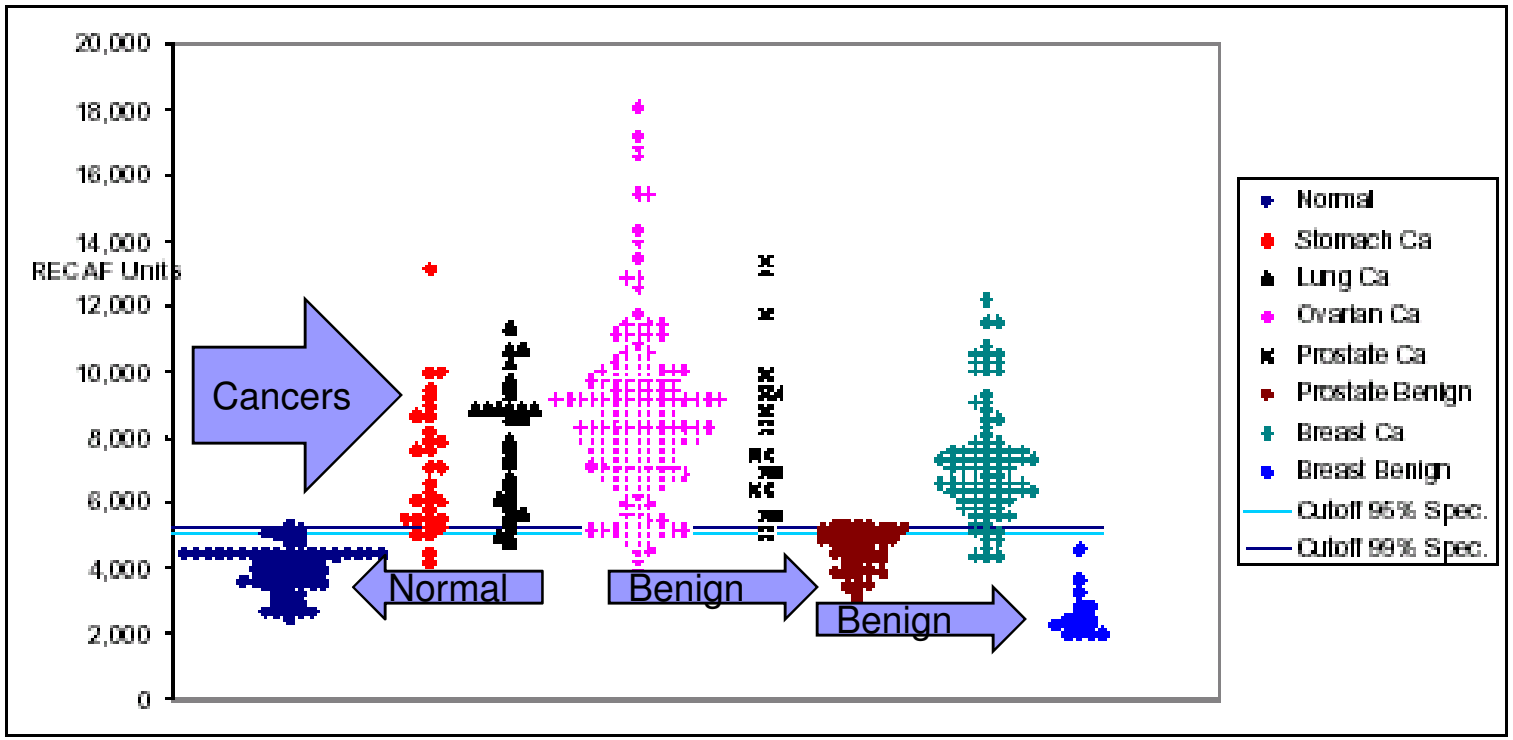


Figure 5. Distribution of RECAF values for normal, cancer and benign tumor samples. The horizontal lines mark the 95% and 99% specificity cutoff values.

Moro Ricardo et al., 2007, BioCurex Inc. and Pacific Biosciences Research Centre

Cancer cells toxin delivery by AFP

- AFP+toxin conjugates: internalization x50-1000 times ↑ by AFP receptor-positive cancer cells.
  Severin S.E., et al, Tumor Target, 2:299-306, 1996.

- AFP+toxin conjugate: overcomes multiple drug resistance (MDR)
  Moskaleva E.Yu., et al, Cell Biol. Int., 21(12):793-9, 1997.

- AFP-dioxin complex: toxicity x200-1400 times ↑ on cancer cells compared to dioxin alone.
  Sotnichenko AI, et al, FEBS Lett., 1999, v.450, p.49-51.

- AFP-Amphotericin B complex: clinical response 6 out of 8 cancer patients.
  Pak V.N., et al, US Patent # 6,878,688, 2005.

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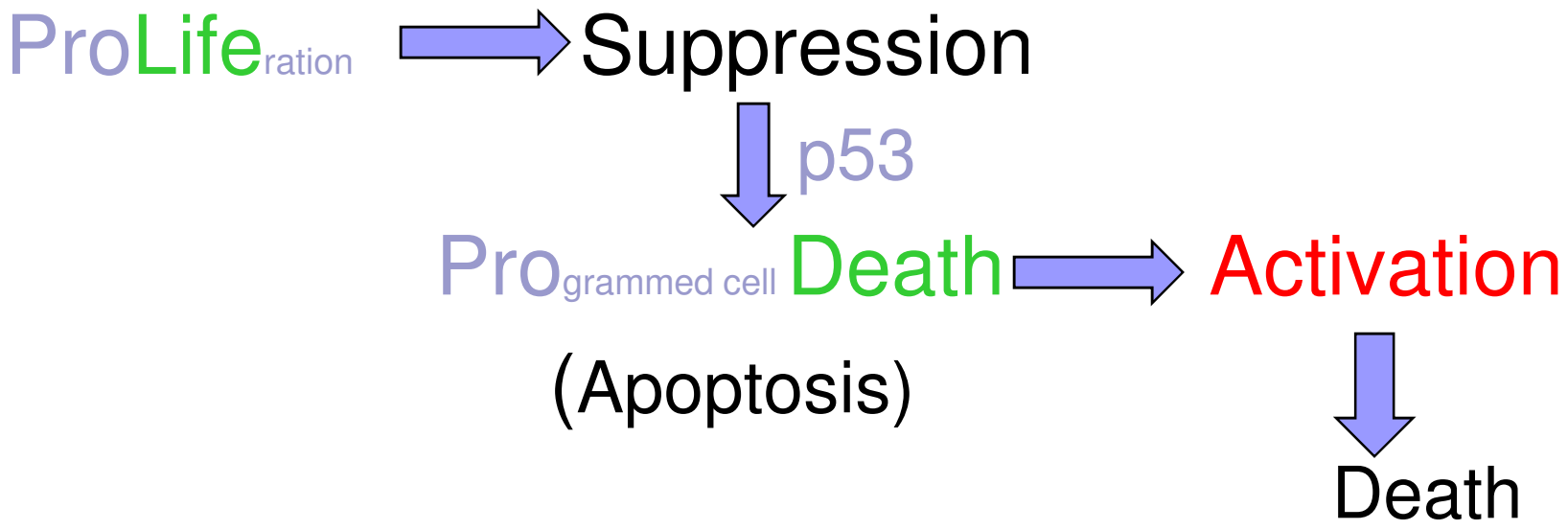
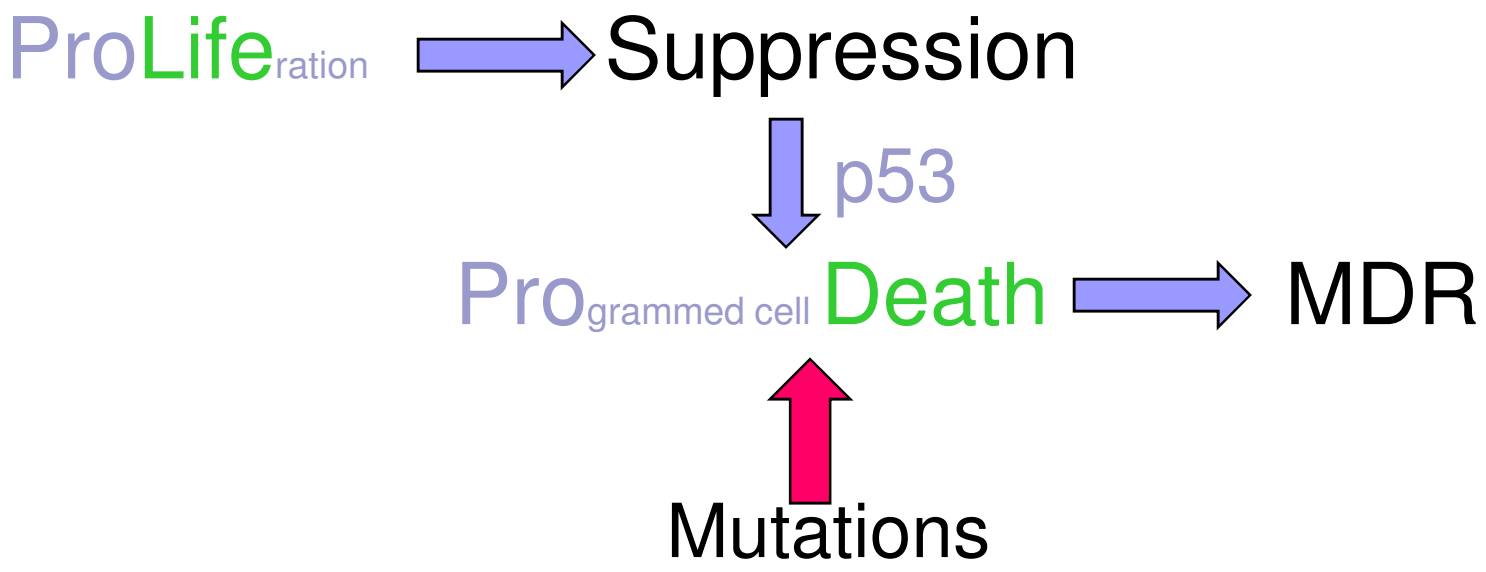
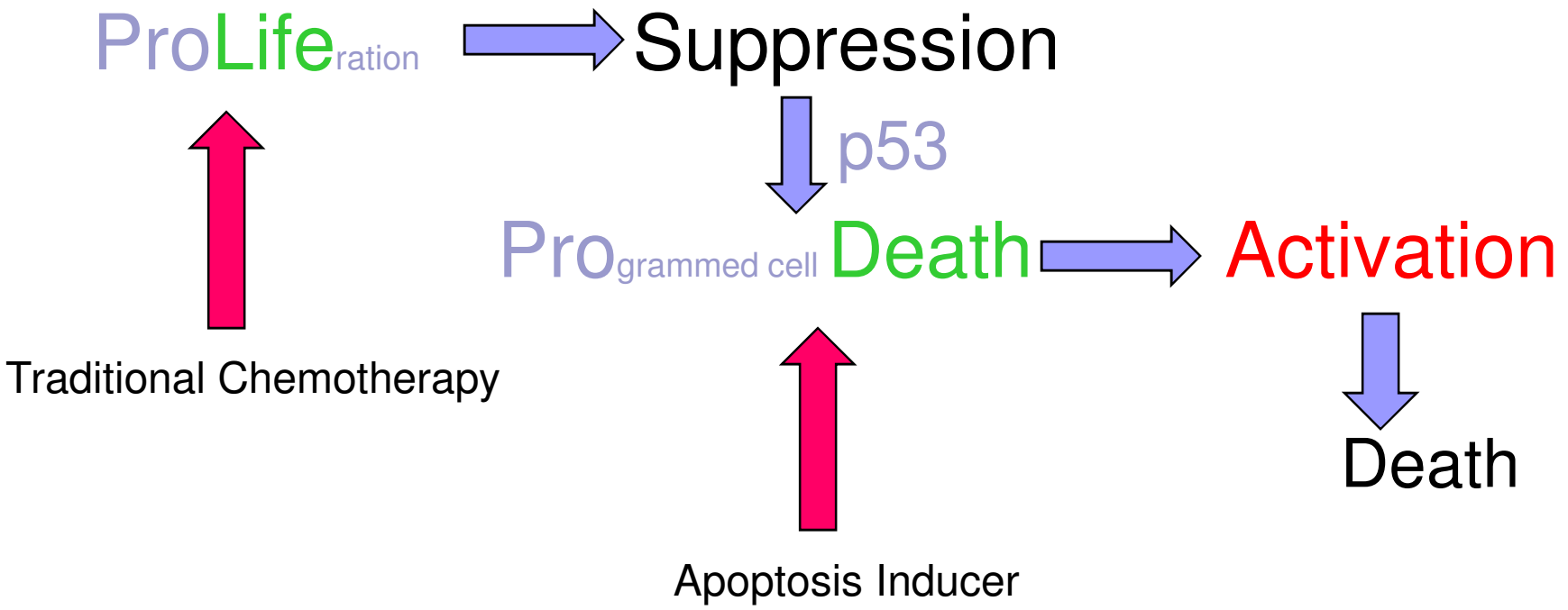
Cell Wrong Proliferation Control



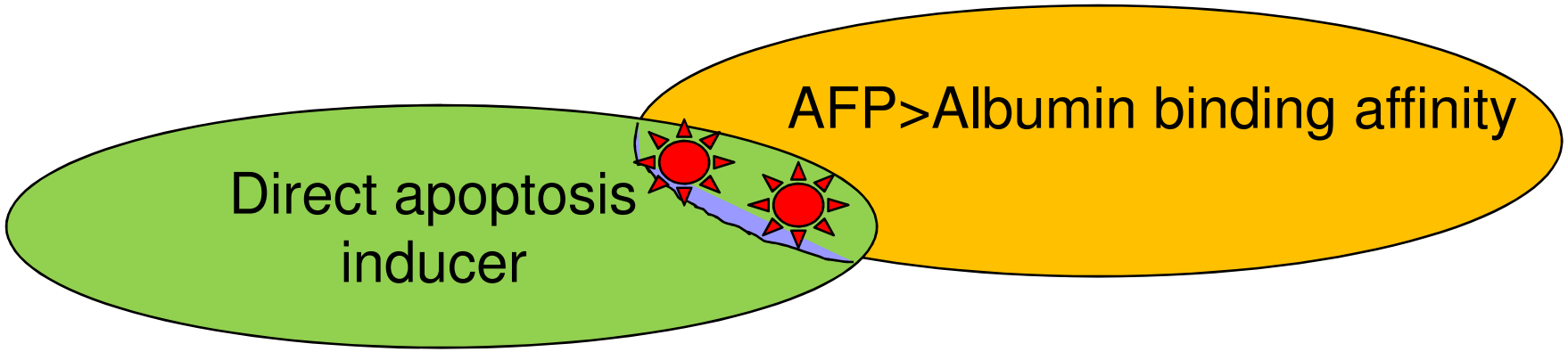
Multiple Drug Resistance



Apoptosis Inducer vs Traditional Chemotherapy



Drug Selection Criteria



Registered drug, or NCI 60 cancer lines panel tested

Low effective dose

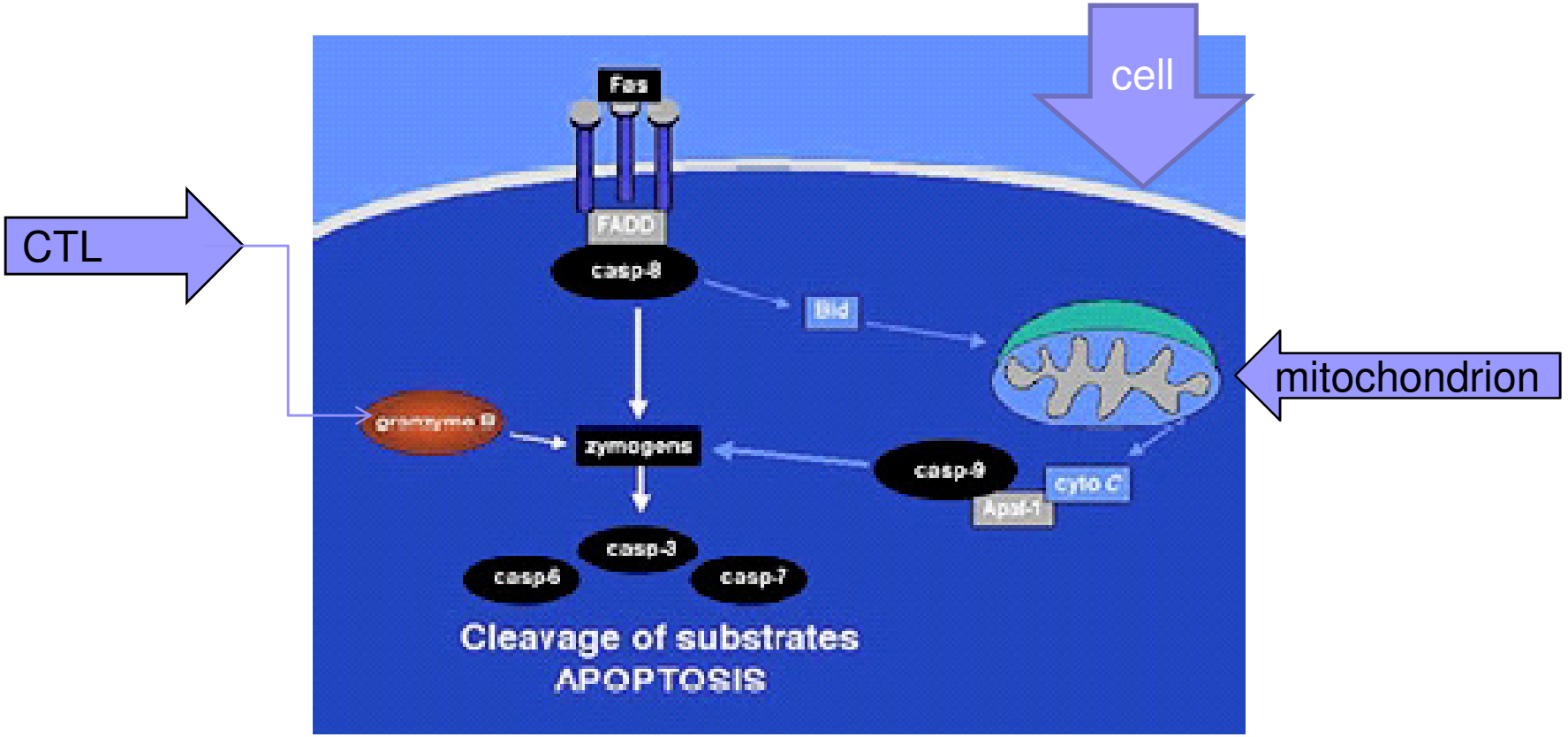
No mutagen/carcinogen effect

Analytical assay developed

Chemical stability

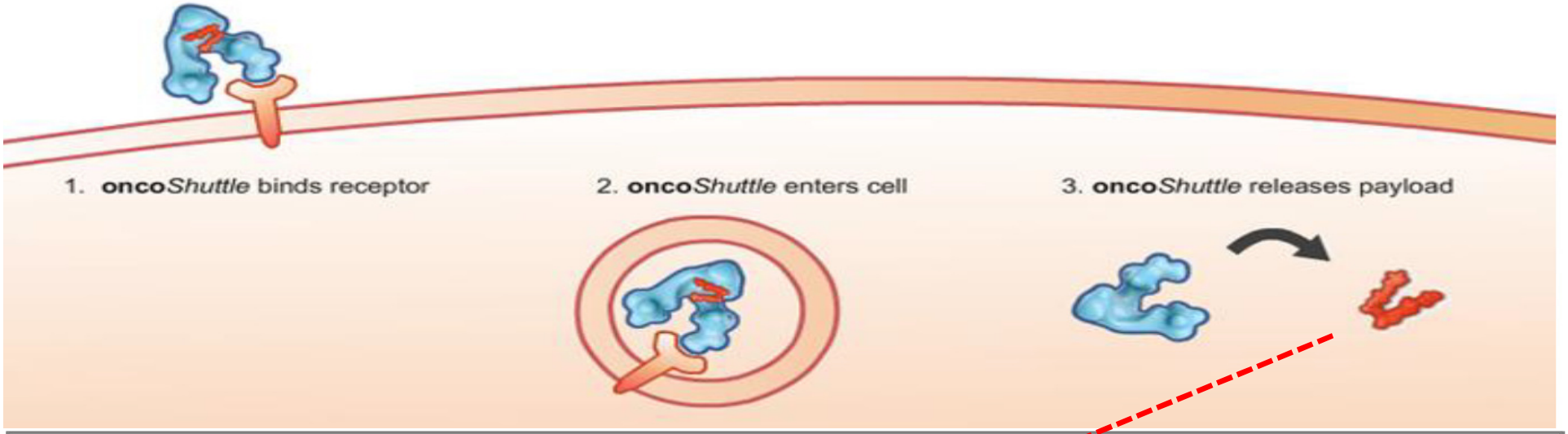
Low price

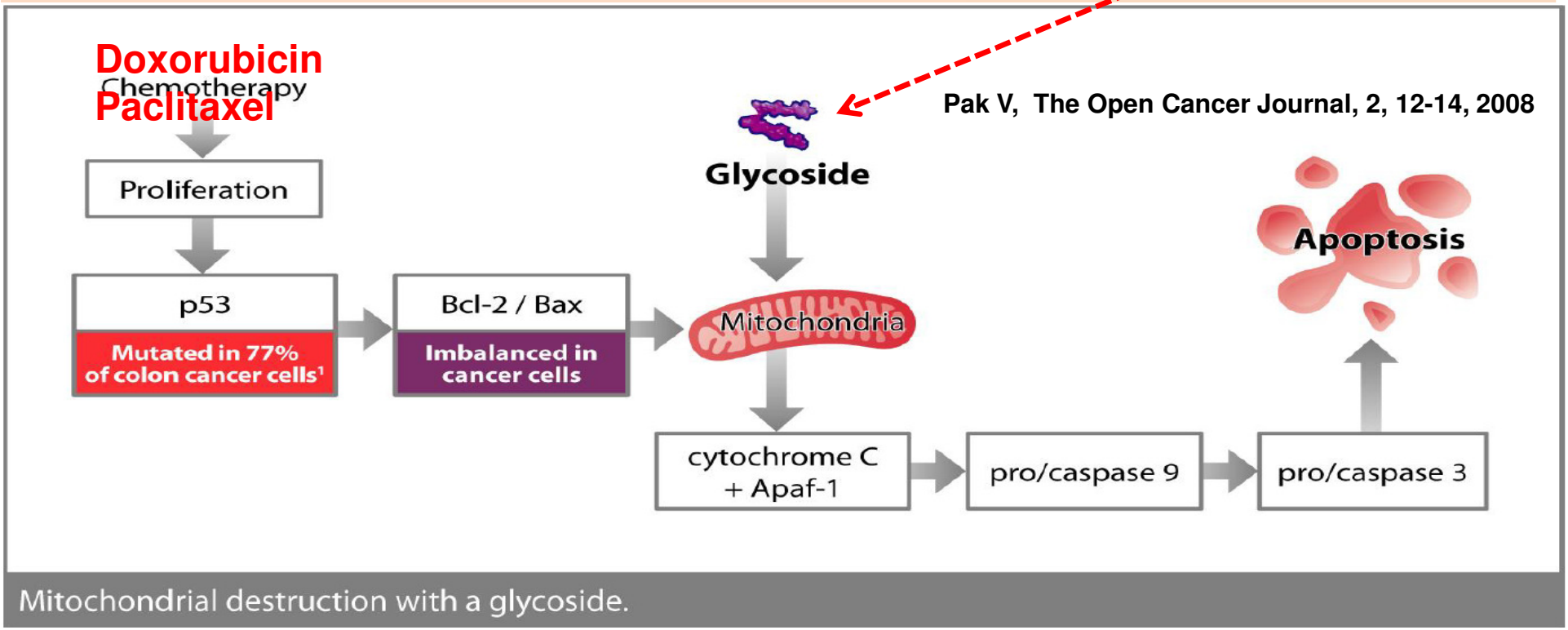
Double Targeting as an Effective Anti-Cancer Strategy



CTL – cytotoxic lymphocyte

Double Targeting + Toxin Efficacy





Mitochondrial destruction with a glycoside.

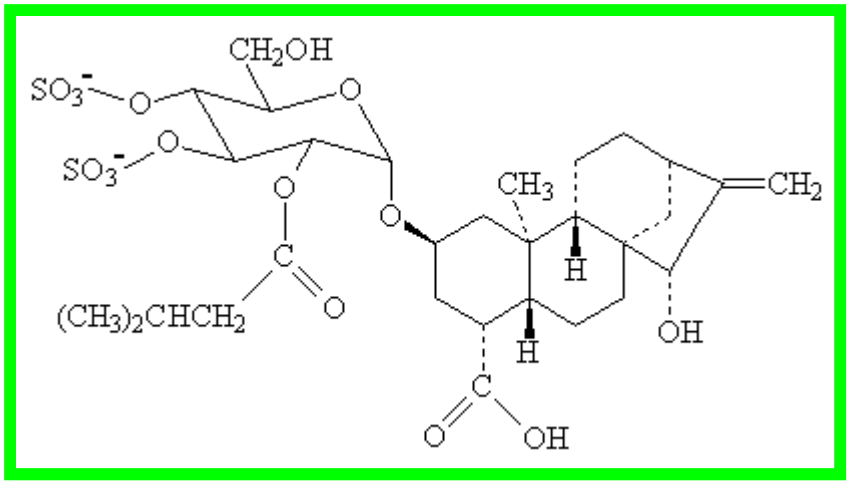
Direct Apoptosis Inducer Atractyloside

- Binds to the inner mitochondrion membrane
  Vignais PV, et al, FEBS Lett., 8(6):328-332, 1970.

- Induces Apoptosis via a p53-independent pathway (no MDR)
  Stewart MJ, et al, Hum. Exper. Toxicol. 21:643-647, 2002.

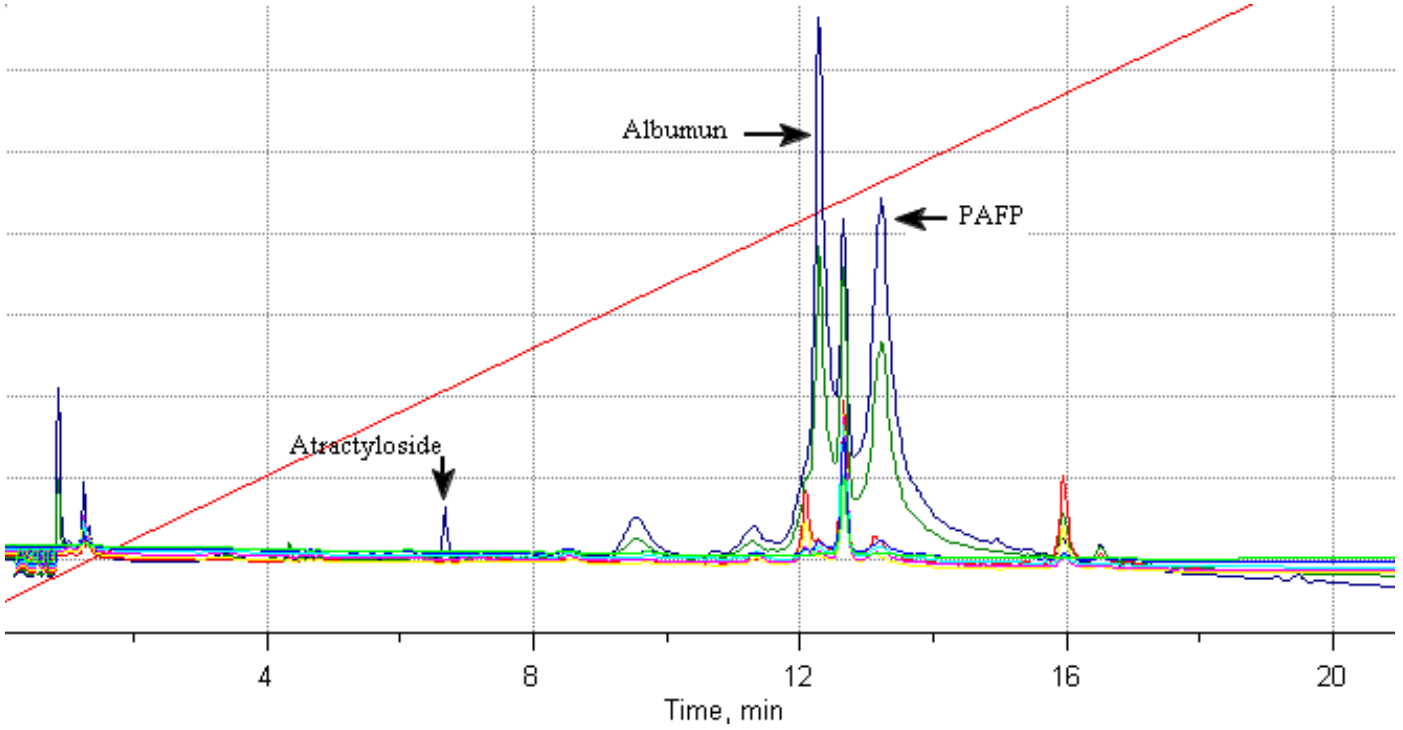
- Like Paclitaxel inhibits tubulin assembly in addition to its effects on mitochondria

  Stewart MJ, Steenkamp V., Ther. Drug Monitoring 22(6):641-649, 2000.



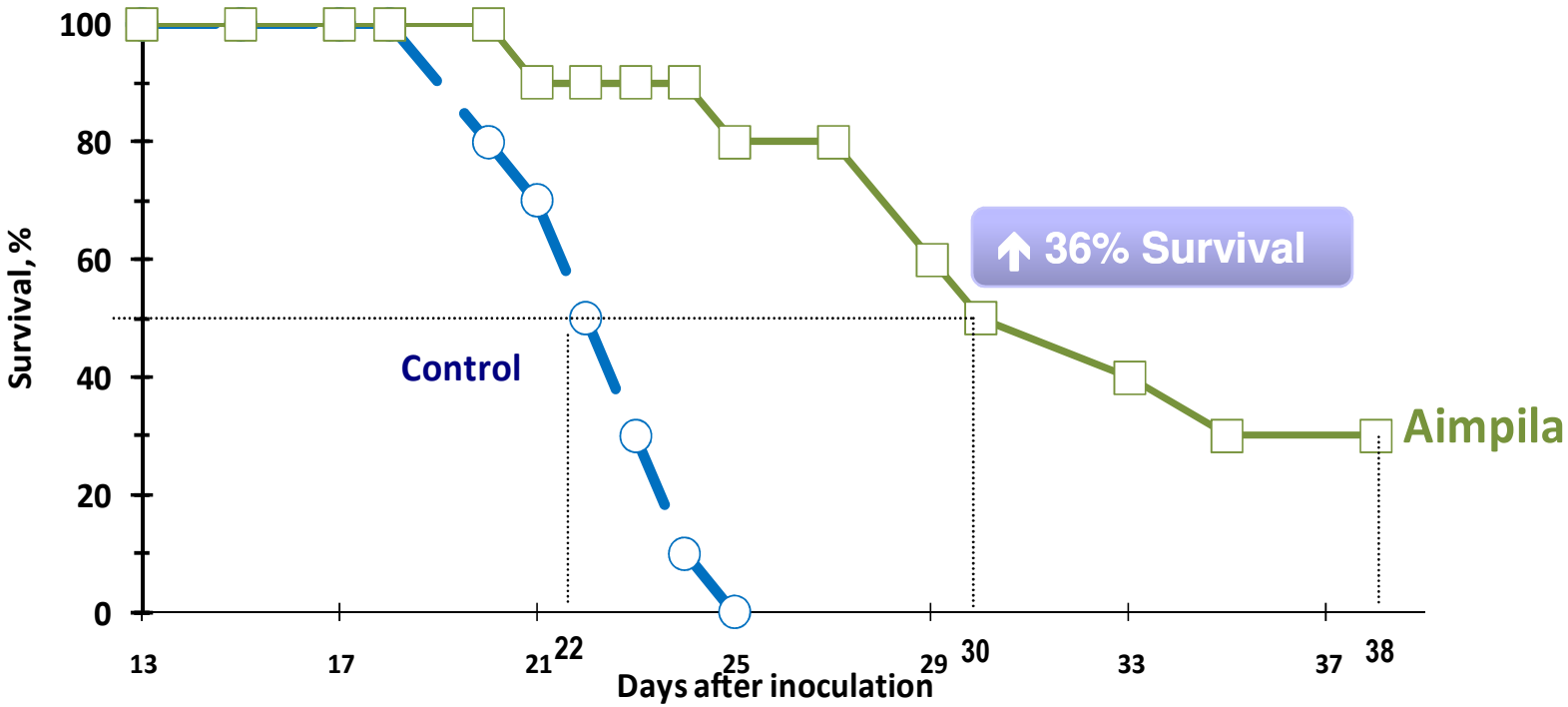
Glycoside Atractyloside, MW=803

HPLC of Aimpila (AFP+Atractyloside)



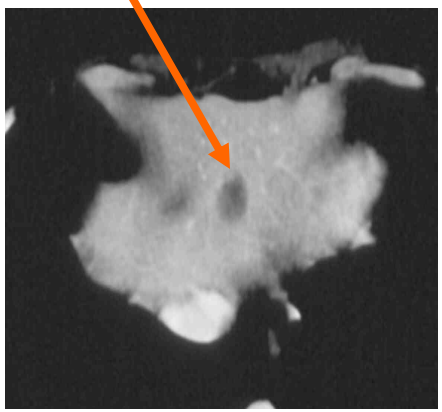
Improved Survival

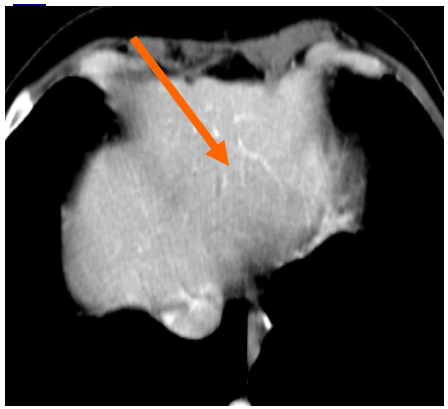
- 10 DBA_2 mice in each group
- Inoculation of 20,000 P-388 cells
- Fed with 0.012mg/kg Atractyloside (Aimpila) starting day 1 after inoculation



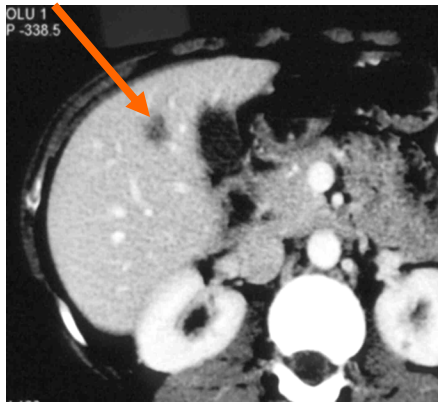
Liver Mts elimination after 8 weeks with Aimpila

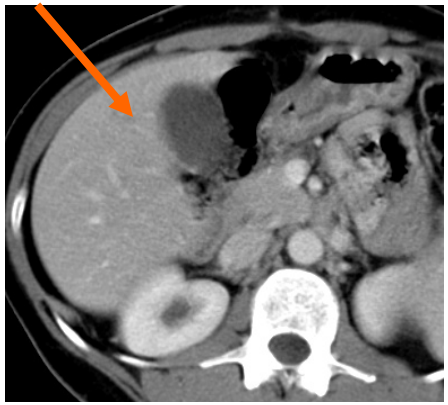
Before





After





Compositions of AFP and inducers of apoptosis for the treatment of cancer
US Patent # 8,071,547 Dec. 6, 2011

Summary

- High cancer cells versus normal cells specificity
- Personalized medicine (>90% are AFP receptor -positive)
- Cancer cell targeted delivery and toxin internalization
- Apoptosis inducer as an effective cytotoxin
- Overcomes MDR
- Quick metastasis reduction
- Improves survival and quality of life