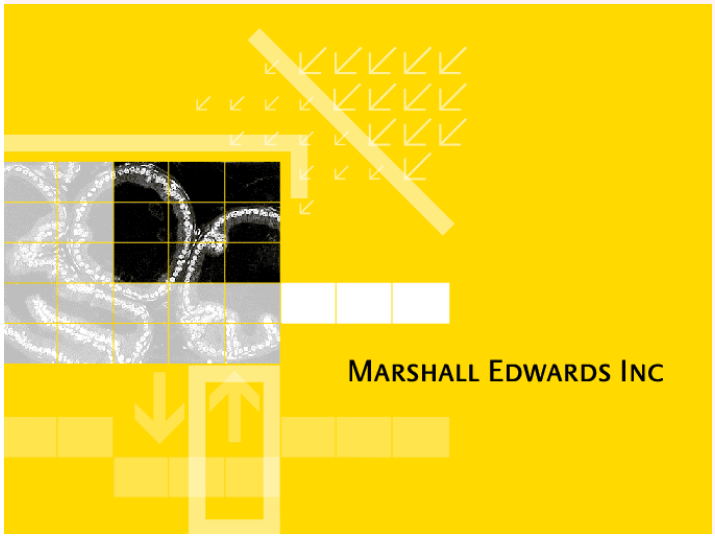
Marshall Edwards Inc (MEI)

- Phenoxodiol technology licensed to Marshall Edwards Inc
  - Safety & Low toxicity demonstrated
  - IND approved by US FDA Jan 2001
  - FDA approval to expedite trial progress granted
  - Now in Phase II human clinical trials in the US and Australia
  - Oral and IV dose forms

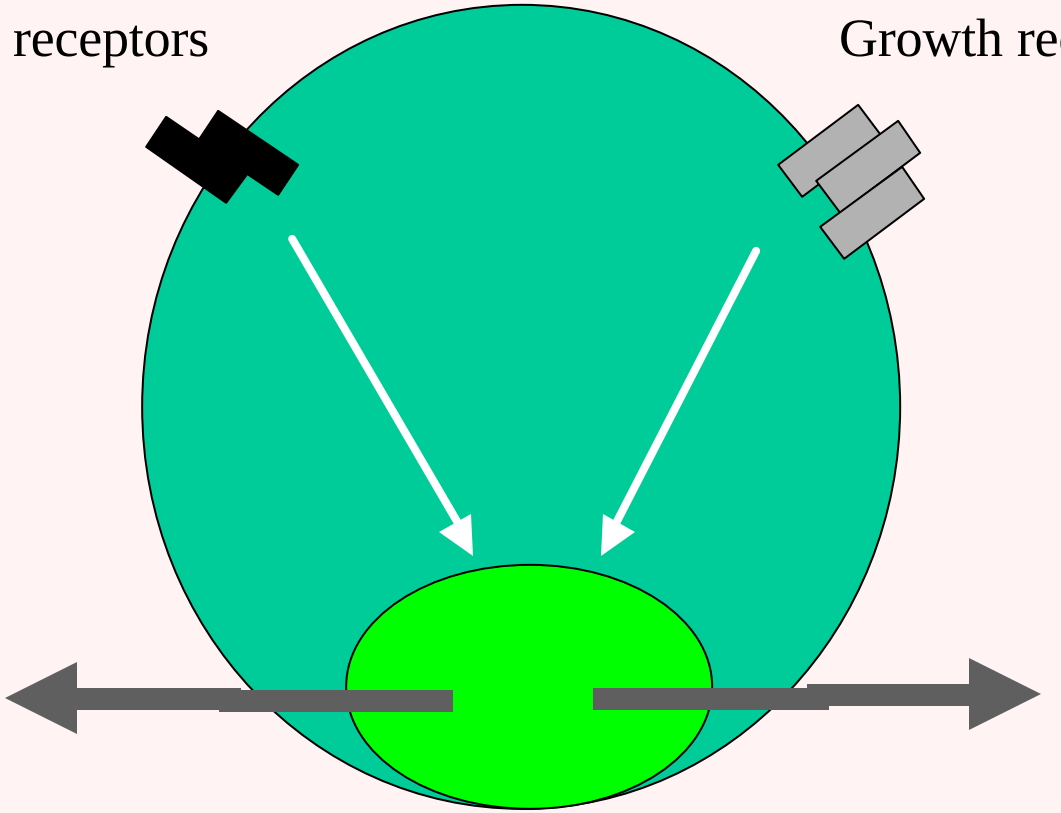


Phenoxodiol: Mechanisms of action

- A multiple signal transduction regulator
- Molecular targets…
  - Fas-ligand/FLIP (Death Receptor)
  - Sphingosine kinase (Growth Receptor)
  - t-NOX (Upstream Regulator)
  - Topoisomerase (DNA Replication)

Normal cell

Balance of growth and death receptors determines life span of cell

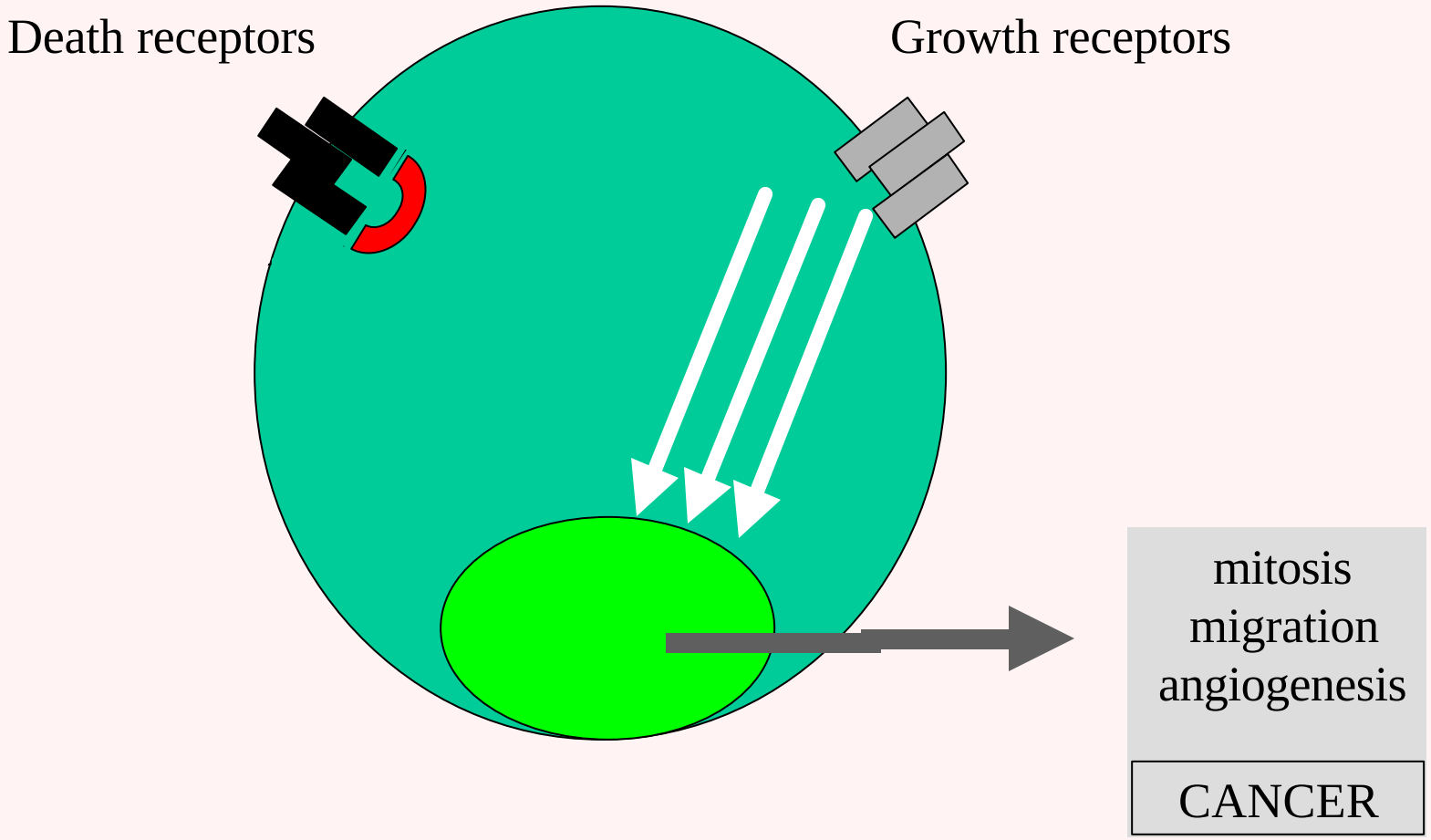


DEATH
- apoptosis

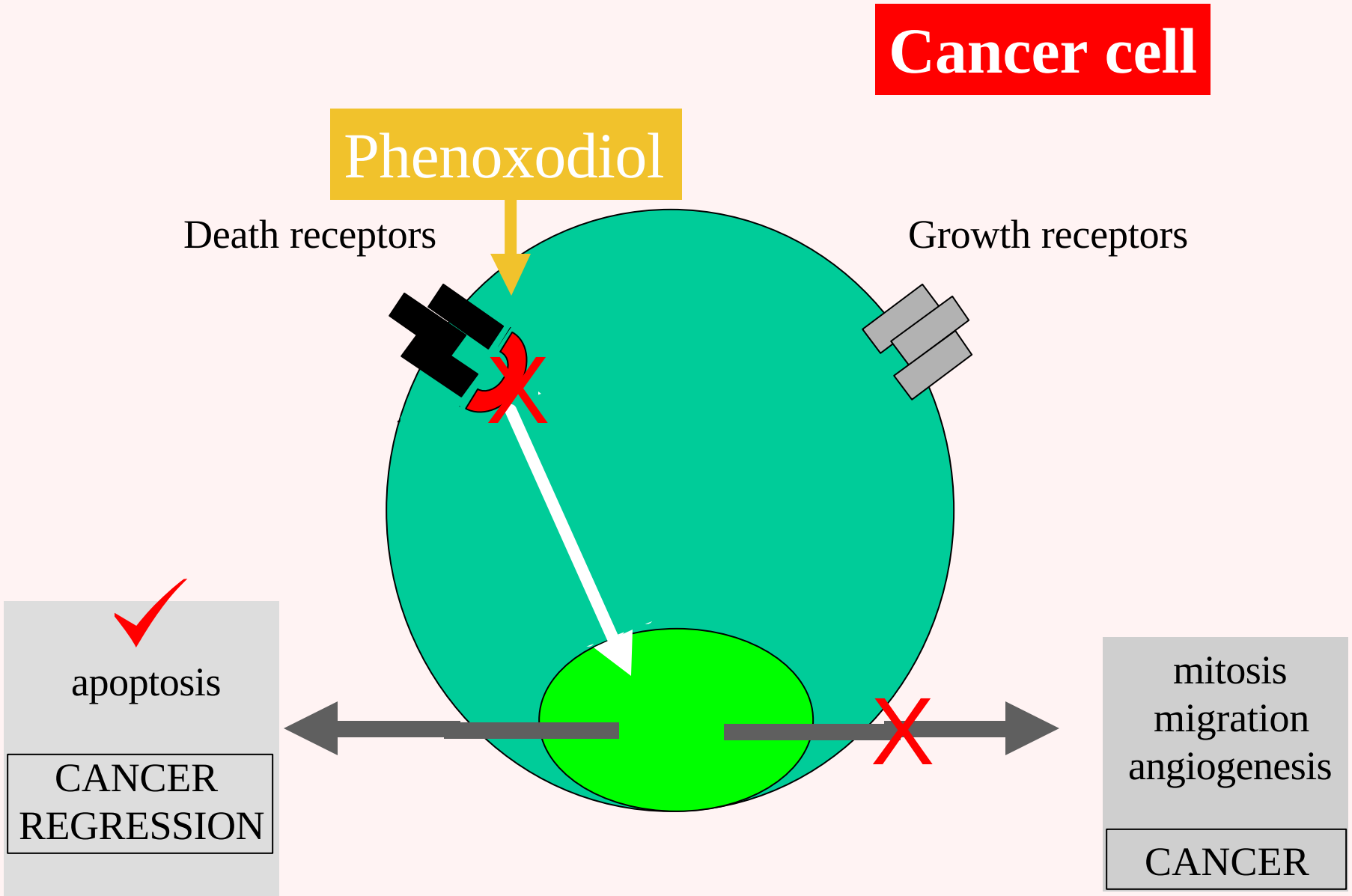
GROWTH
- mitosis
- migration
- angiogenesis

Cancer cell

Upregulated growth and death receptor blockade via FLIP

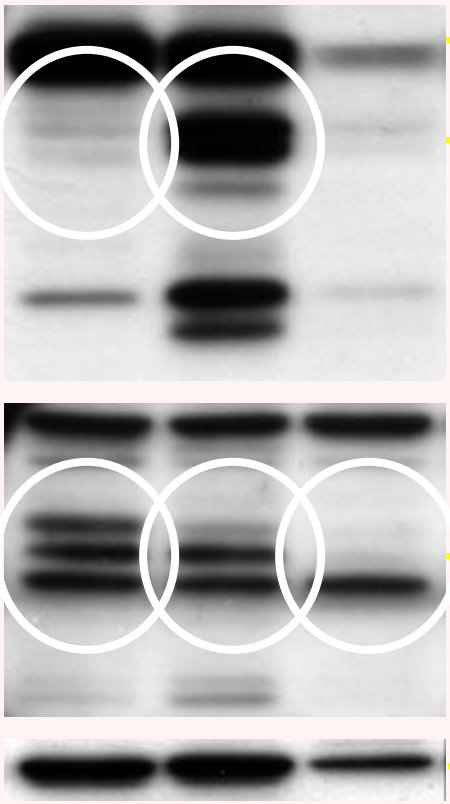


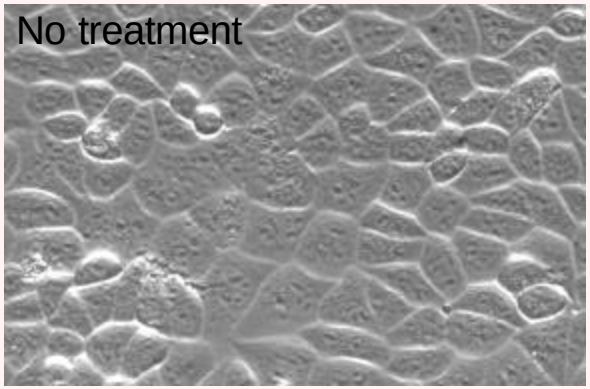
Phenoxodiol inhibits FLIP

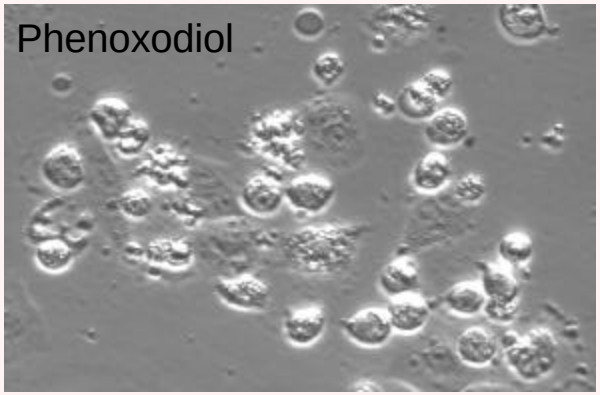


Phenoxodiol inhibits FLIP

CP70 cisplatin resistant human ovarian cancer cells







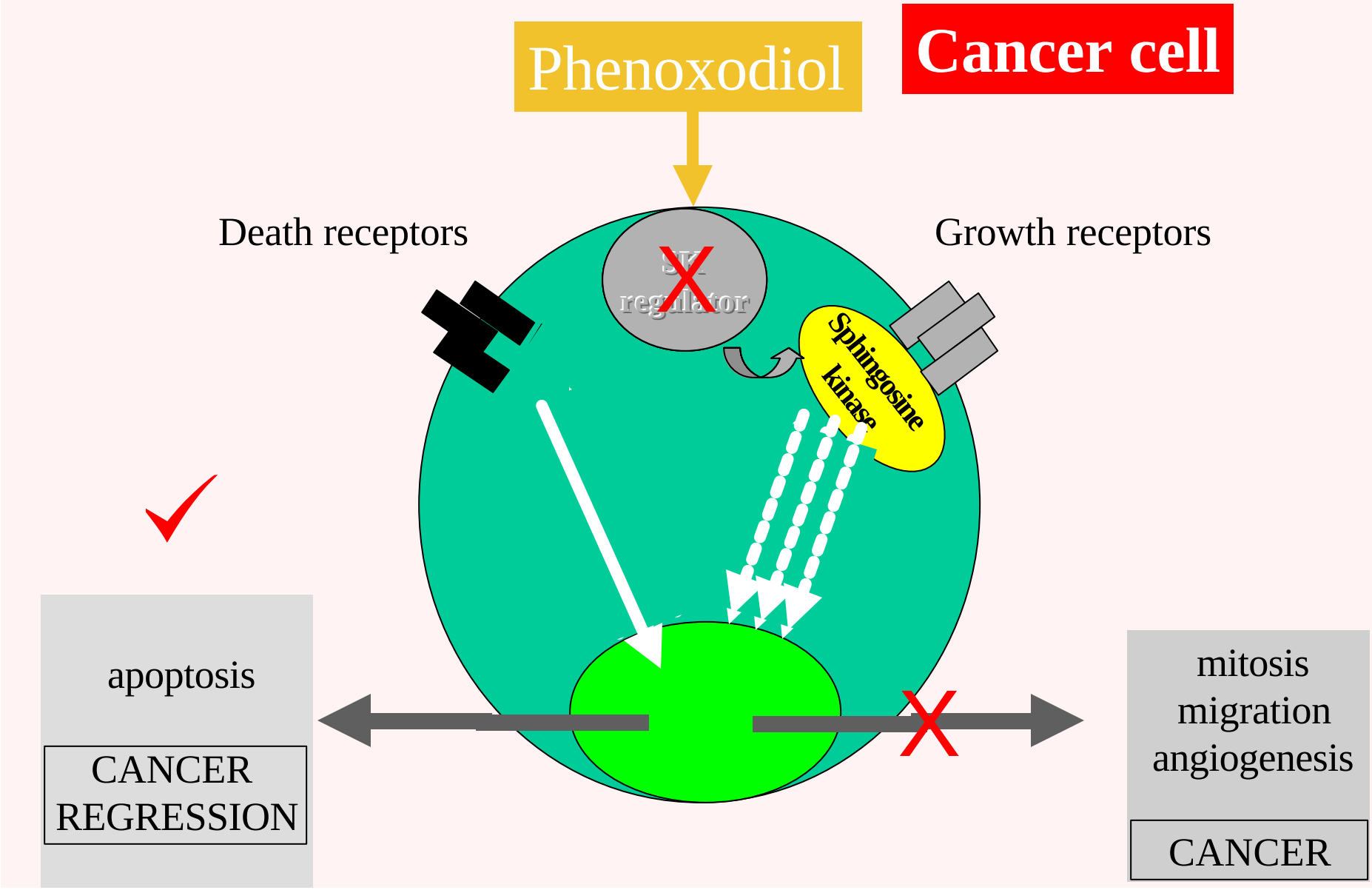
Phenoxodiol - an isoflavone analogue – induces apoptosis in chemo-resistant ovarian cancer cells. Marijke Kamsteeg, Thomas Rutherford, Eva Sapi, Bozena Hanczaruk, Shoreh Shahabi,Maryann Flick, David Brown* and Gil Mor. *Oncogene* (2003) **22**, 2611–2620

Phenoxodiol inhibits SK

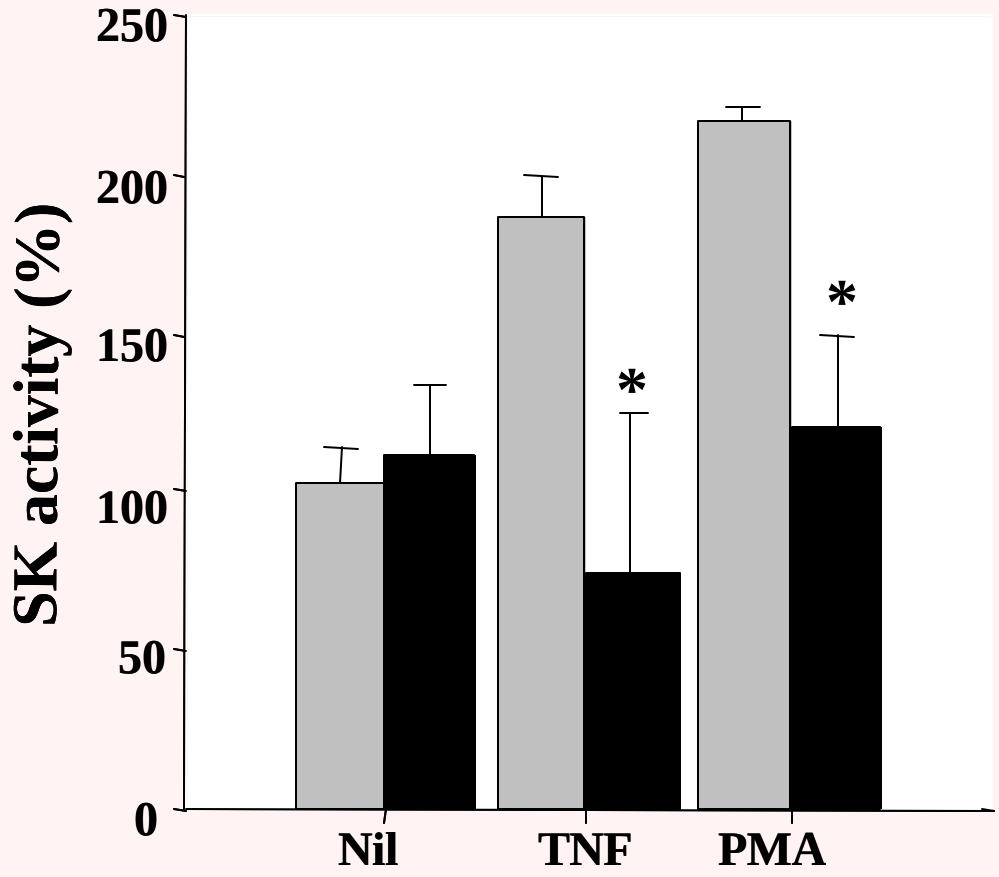
- Sphingosine kinase (SK) is a highly conserved lipid kinase, which phosphorylates sphingosine to form sphingosine-1-phosphate.
- SK is known to regulate cellular functions such as cell growth , proliferation and survival , endothelial cell activation and angiogenesis
- SK has been shown to have oncogenic activity*

* P. Xia, J. R. Gamble, L. Wang, S. M. Pitson, P. A. Moretti, B. W. Wattenberg, R. J. D'Andrea, and M. A. Vadas. An oncogenic role of sphingosine kinase. *Curr.Biol.* **10** (23):1527-1530, 2000.

Phenoxodiol inhibits SK

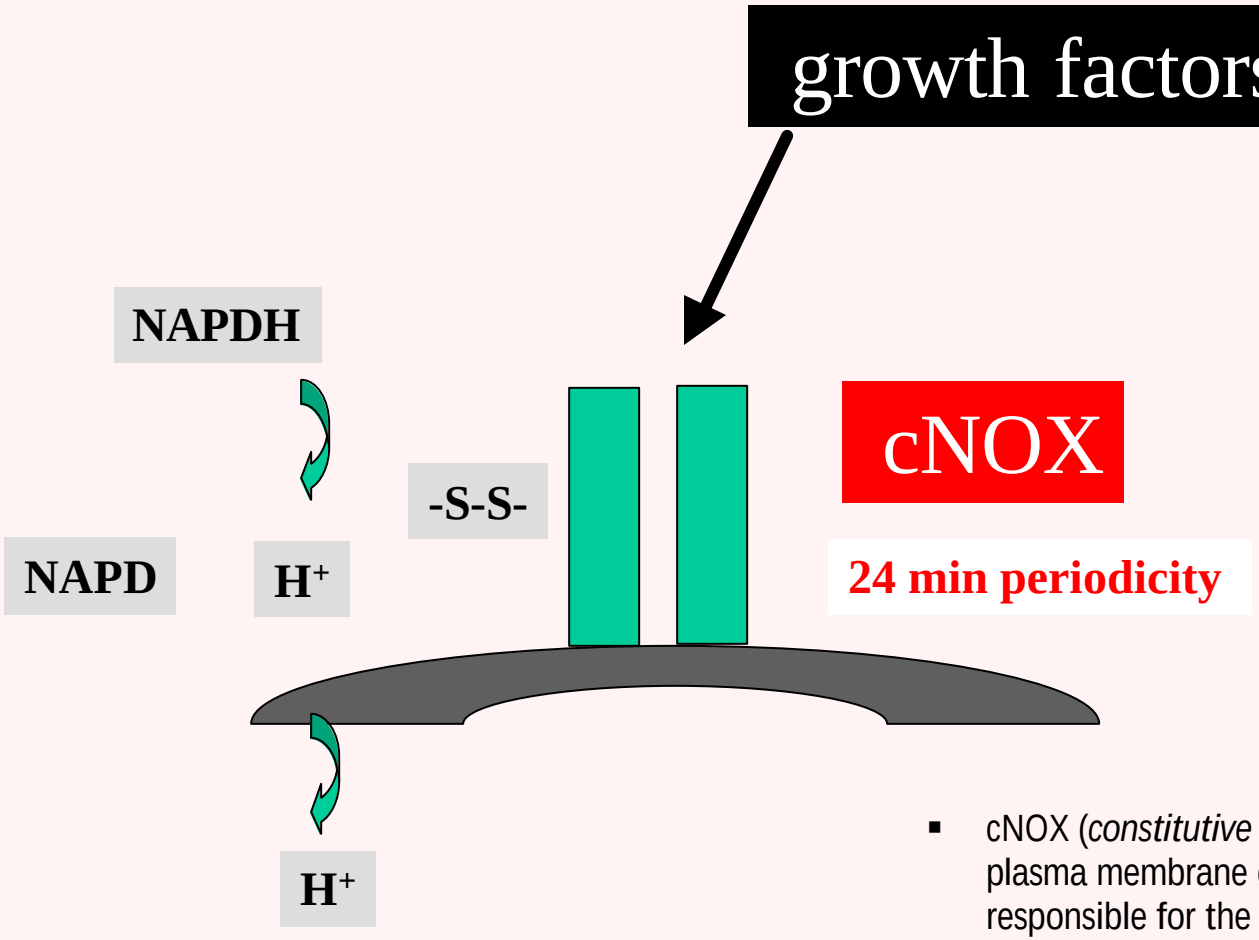


Phenoxodiol inhibits SK



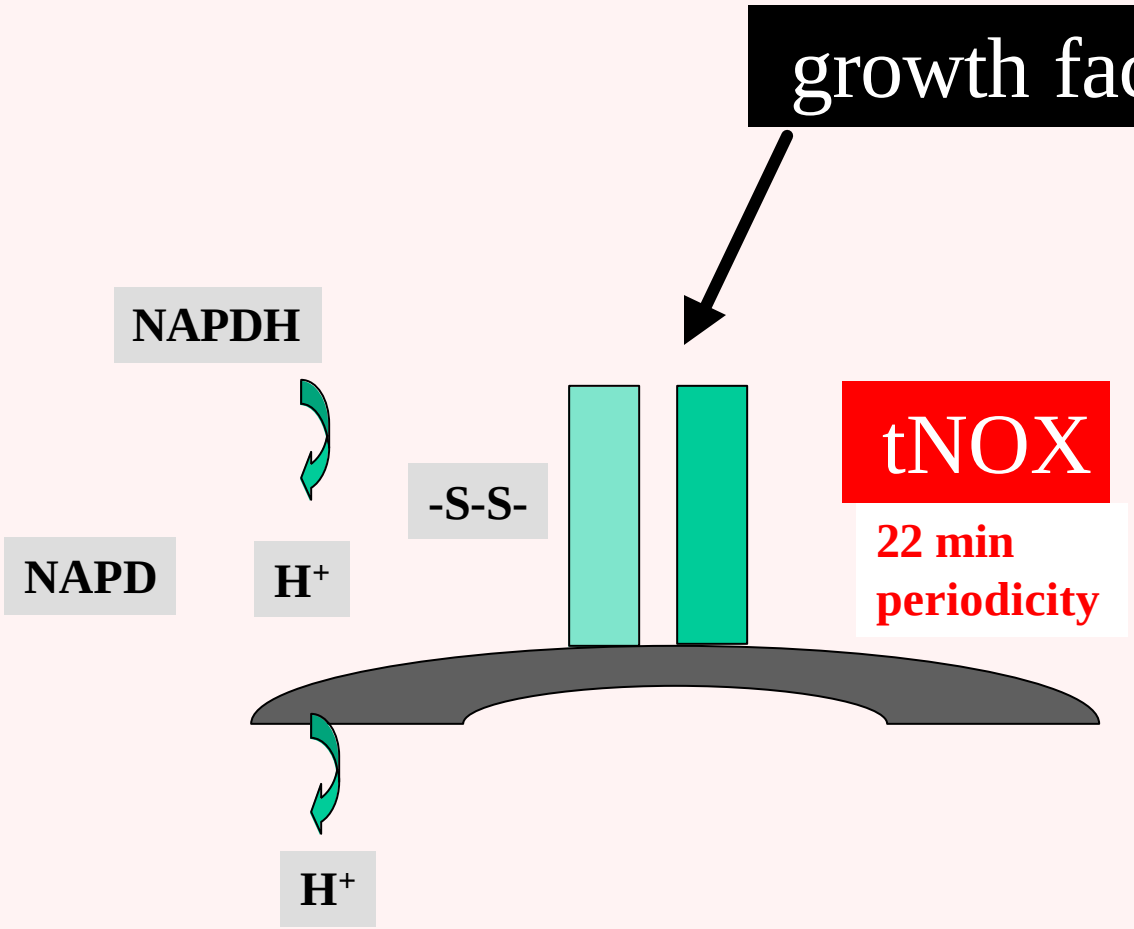
Phenoxodiol, a derivative of plant flavonoids, shows potent anti-tumour and anti-angiogenic properties. J.R. Gamble, P. Xia, C.N. Hahn, S.M. Pitson, J.J. Drew, C.J. Drogemuller, C.J. Carter, D. Brown, C.Walker and M.A. Vadas. *Cancer Research* (Submitted)

A novel signal transduction target



- cNOX (*constitutive* NAPH oxidase) is present on the plasma membrane of all plant and animal cells and is responsible for the trans-membrane H+ pump. It also has a second, putative function – control of ATP-ase activity.
- It comprises two proteins, each of which delivers H+ by a separate chemical process, one of which is a di-thiol process. These two proteins alternate their activity, but are interdependent.

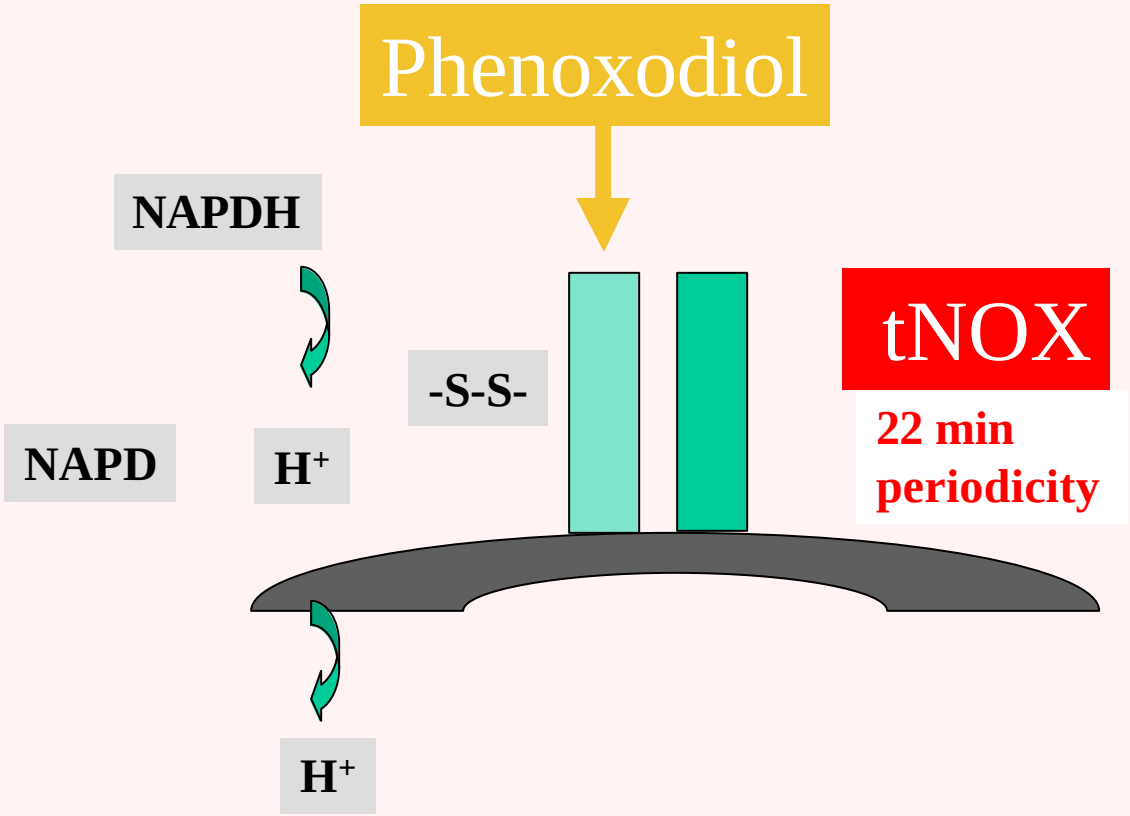
A novel signal transduction target



- tNOX (tumour NAPH oxidase) is a variant of cNOX that differs in the 30 terminal amino acids on the paired protein that operates the di-thiol H+ transfer mechanism.
- tNOX is expressed by human cancer cells in all forms of human cancer tested to date. cNOX also is expressed by cancer cells, but is subordinate to tNOX (that is, cNOX activity is suppressed by tNOX even after tNOX inhibited by drugs).
- tNOX has a periodicity of 22 minutes, producing an abnormal intra-cellular biorhythm. This is hypothesised to be a fundamental error that is a prerequisite of the carcinogenic process, by inhibiting triggering of the apoptotic mechanism.

N. Cho, P. J. Chueh, C. Kim, S. Caldwell, D. M. Morre, and J. Morre. Monoclonal antibody to a cancer-specific and drug-responsive hydroquinone (NADH)\ oxidase from the sera of cancer patients. *Cancer Immunol.Immunother*. **51** (3):121-129, 2002.

A novel signal transduction target

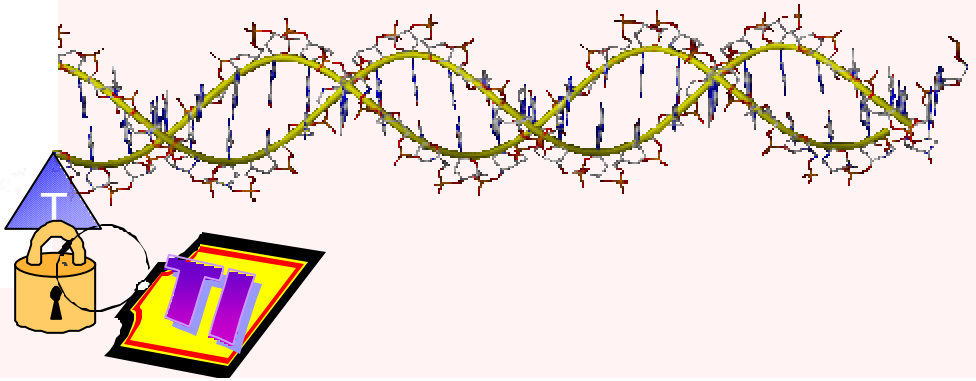


- Phenoxodiol specifically inhibits the paired protein in tNOX that operates the dithiol H+ pump mechanism.
- Phenoxodiol has no effect on cNOX.

Phenoxodiol inhibits topoisomerase

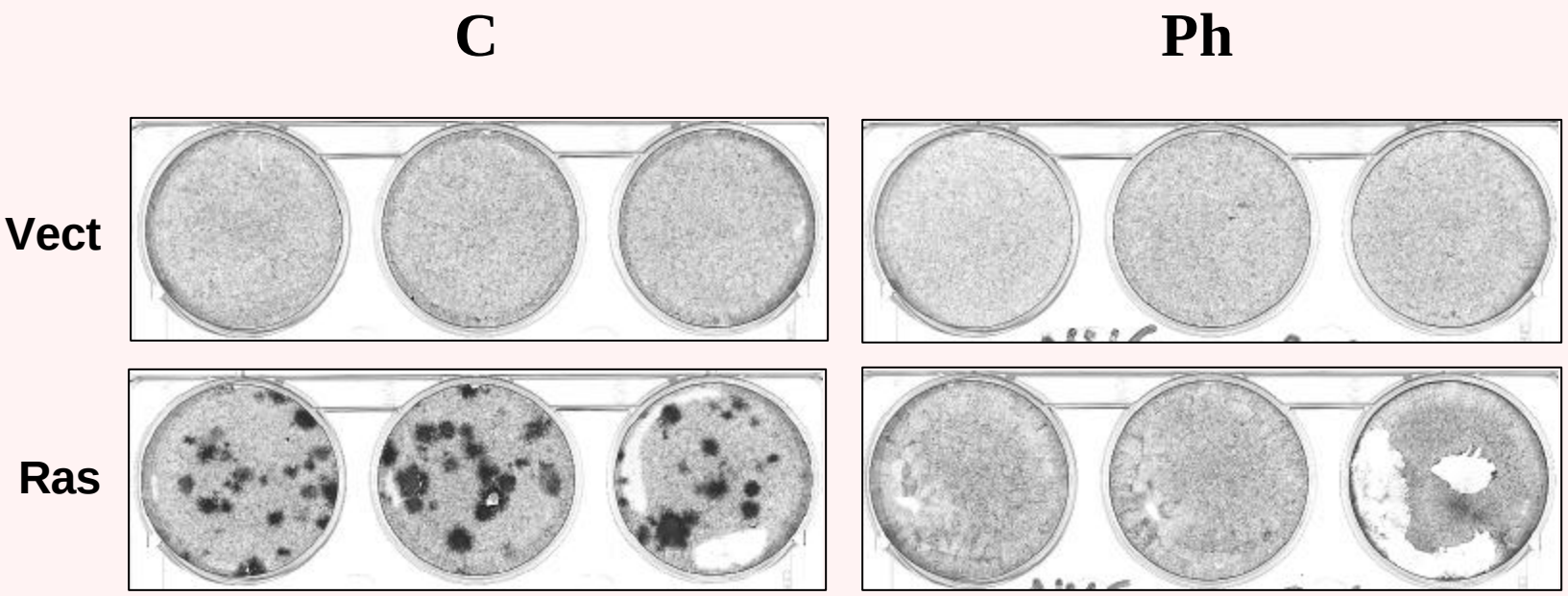
- Phenoxodiol is a topo poison: stabilizes the normally transient reaction between topoisomerase and DNA preventing re-joining of the cut ends to repair the break

Phenoxodiol (2H-1-Benzopyran-7-0, 1,3-(4-hydroxyphenyl), a novel isoflavone derivative, inhibits DNA topoisomerase II by stabilizing the cleavable complex. Andreas Constantinou and Alan Husband *Anticancer Res*. **22,** 2581-2586, 2002.



Phenoxodiol: Pre-clinical Data

- Phenoxodiol has no effect on NIH 3T3 cells (Normal cells)

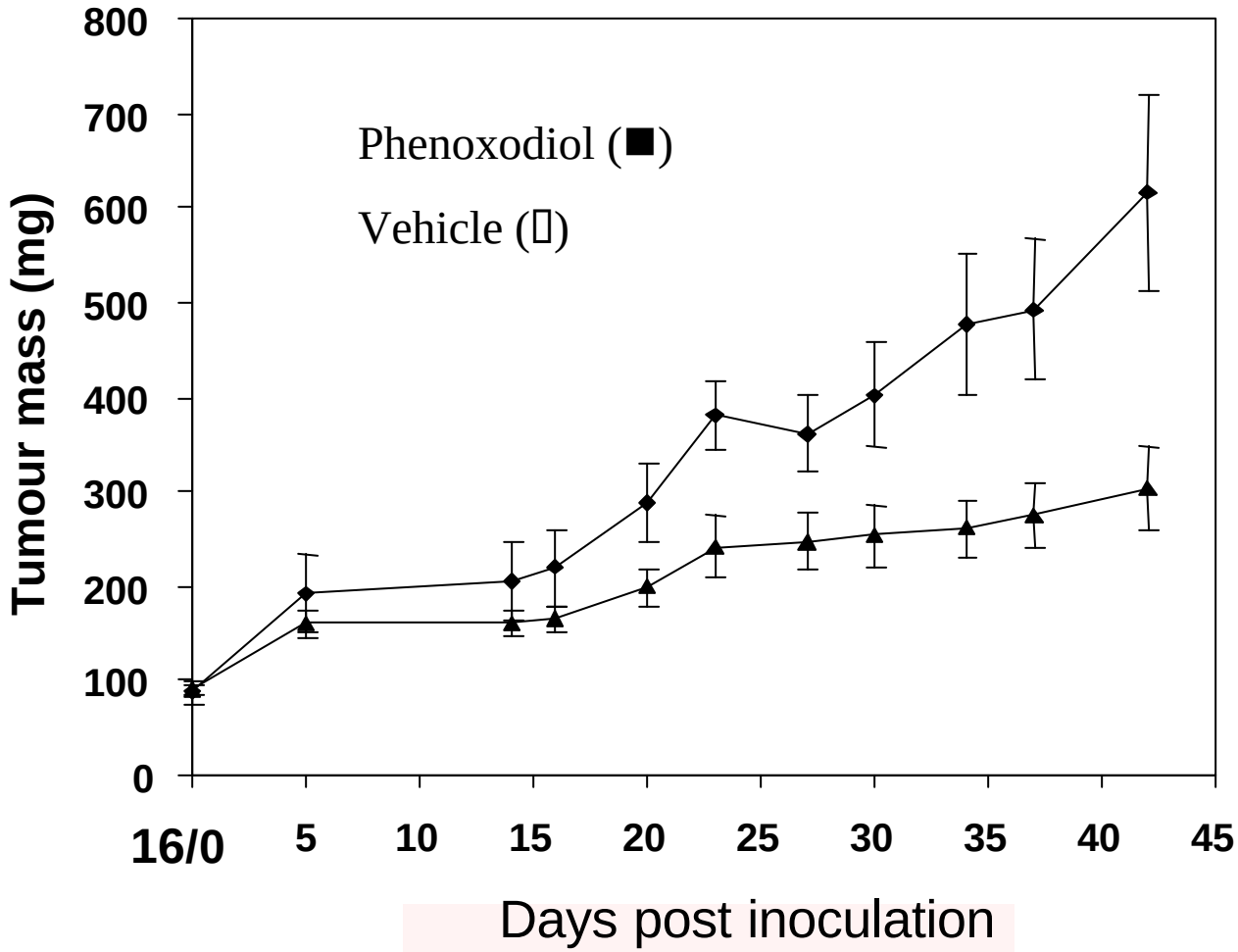


- Phenoxodiol inhibits growth of Ras-transformed NIH 3T3 cells (Cancer cells)

Gamble, et al. 2003

Phenoxodiol: In vivo animal data

- Phenoxodiol inhibits growth of human xenografts in mice

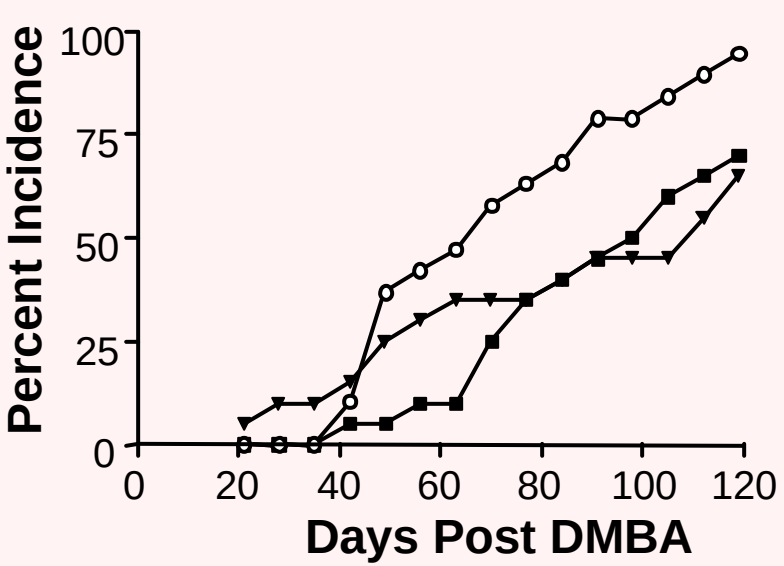


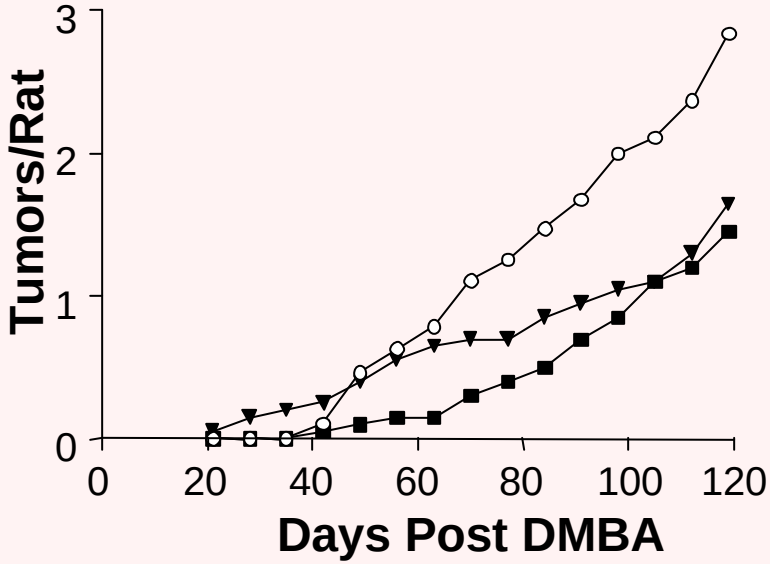
Phenoxodiol or vehicle orally administered for 5 days per week from the time of inoculation of LNCaP cells. Tumour mass assessed over 58 days.

Gamble, et al. 2003

Phenoxodiol: In vivo animal data

- Phenoxodiol inhibits DMBA-induced mammary tumour multiplicity and incidence





Rats (n = 20) were started on basal diet (o) or diets containing 50 mg/kg phenoxodiol (▼), or 75 mg/kg phenoxodiol (■) one week prior to administration of DMBA

Phenoxodiol, a novel isoflavone derivative, inhibits DMBA-induced mammary carcinogenesis in female Sprague-Dawley rats. Constantinou, A.I., Mehta, R. and Husband, A.J. (2003) *Europ. J. Cancer.*(In Press).

Phenoxodiol clinical program

Phase Ia Studies Complete

– #NV06-0012: single intravenous injection at a dosage of 5 mg/kg to 6 patients

- ·a mean 90.1% (range 85.9 - 94.7%) of plasma phenoxodiol was conjugated as glucuronide or sulphate forms
- · the mean half-life of the unconjugated phenoxodiol was 40.1 minutes
- · the AUC was 3.21 µg.hr/mL.

– #NV06-0013: continuous intravenous infusion at a dosage of 1 mg/kg/hour to 5 subjects

- steady state levels of free (unconjugated) phenoxodiol were achieved in the range 0.6 – 1.1 µg/ML for the duration of infusion
- · free plasma phenoxodiol levels reached a steady state of 0.78 ug/mL after 90 minutes (16.2 µ g/mL for conjugated phenoxodiol)
- · free phenoxodiol was cleared at a rate of 22 mL/min/kg.

Phenoxodiol clinical program

Phase Ib Studies Complete

– #NV06-0022 (St. George): dose-escalating (1, 2, 5, 10, 15, 20, 25 and 30 mg/kg) treatment regime of single bolus injections once per week for 12 consecutive weeks in patients with late-stage solid tumors that were refractory to standard therapies

- 5/21 patients had Grade 1 nausea that was managed with metoclopramide;
- 2/21 patients had hypersensitivity reactions (flushing, back pain). One of these patients also had transient thrombocytopenia and was withdrawn from the study. The other patient was adequately managed with steroid and anti-histamine pre-medication;
- no other toxicities were encountered and the MTD was not reached;
- 14/21 patients experienced disease progression and were withdrawn from the study prior to completion; 7/21 patients were able to complete 12 weeks of treatment without disease progression, and 2 of these (renal carcinoma, pancreatic carcinoma) completed 18 weeks of treatment without disease progression.

Phase I and pharmacokinetic study of weekly phenoxodiol, a novel isoflav-3-ene, in patients with advanced, solid cancer. Liauw W, Links M, Pirabhahar S, Husband A, Kelly G, and de Souza P. ASCO Miami, October 2001

Phenoxodiol clinical program

Phase Ib Studies Complete

- #NV06-0023 (RPAH): dose-escalating (1.3, 5, 10, 16, 25, 33 mg/kg) Continuous intravenous infusion (CIV) treatment regime for 7 days, then 7 days no treatment for min 3 cycles (6 weeks total) in patients with late-stage solid tumors that were refractory to standard therapies
- 24 patients target
  - 6/21 patients were able to complete 6 weeks of treatment without disease progression

Phenoxodiol clinical program

Phase Ib Studies Complete

– #NV06-0024 (Cleveland USA): dose-escalating (0.65, 1.3, 2.2, 3.3, 4.6, 20, 27 mg/kg) treatment regime of CIV treatment regime for 7 days, then 7 days no treatment for min 3 cycles (6 weeks total) in patients with late-stage solid tumors that were refractory to standard therapies, total 21 patients

- 12/21 patients were able to complete 6 weeks of treatment without disease progression
- 4/21 remained stable after 12 weeks

Phase I study of phenoxodiol given by constant intravenous infusion in patients with solid neoplasms refactory to standard therapy. Hutson T.E., Mekhail, T., Weiss, P., Roman, S., Dreicer, R., Peereboom, D., Olencki, T., Kelly, G., Ganapathi, R., Bukowski, R. AACR, San Francisco, April, 2002

Phenoxodiol clinical program

Phase 2 – Prostate Cancer Oral Therapy

- #NV06-0025 (Monash Medical Centre, Sir Charles Gairdner Hospital): oral dose regime: Multi-centre Phase 2 study in patients with stage 4 hormone-refractory prostatic adenocarcinoma
- 3 capsules per day (each treatment cycle lasts 4 weeks, 3 weeks daily treatment, 1 week no treatment)
- Min 3 cycles per patient (continued beyond 3 if no toxicity or progression)
- Each cohort will commence on progressively higher dose strata (24 patients total)
- 12 patients recruited

Phenoxodiol clinical program

Phase 2 - Leukaemia

- #NV-0028; extension of NV-0027 (Royal North Shore Hospital, Sydney) IV dose regime
- minimum 1 cycles (each cycle 6 weeks, bolus IV on 3 consecutive days per week)
- Leukaemias (refractory to standard therapy)
- Each cohort will remain on a particular dose stratum for repeat cycles unless toxicity or disease progression occurs (30 patients total)
- 0.2, 1.0, 5.0 mg/kg/injection

Phenoxodiol clinical program

Phase 2 – Ovarian cancer IV Therapy

– #NV-0028 (Yale University, New Haven, CT; RPAH Sydney) IV dose regime: Multi-centre study in patients with recurrent ovarian cancer that is resistant to standard chemotherapy

–IV escalating dose (1, 3, 10, 20 mg/kg) dose on 2 consecutive days each week (each treatment cycle lasts 12 weeks)

–Each cohort commences on progressively higher dose strata (40 patients total)

–Response will be measured by:

- tumour mass (assessed by imaging, where applicable);
- tumour marker (CA125) (where applicable);

–Progress:

- 28 patients to date

"In some of these women, disease regression or stabilization has been realized,"

Professor Thomas Rutherford M.D., Department of Obstetrics and Gynecology, Yale University School of Medicine at Society of Gynecologic Oncologists Annual meeting January 31 - February 4, 2003, New Orleans, LA

Phenoxodiol clinical program

FDA Approvals increasingly in Phase II for small clinical effects

- Sept, 1998: Herceptin (Mab to human epidermal growth factor receptor 2 (HER2)): Breast cancer: 469 patients, remission extended by 7.3 months (std chemotherapy extends for 4.5 months) only suitable for 25% of breast cancer patients (tumors with excess HER2)
- May 5, 2003: Iressa (EGFR specific TK inhibitor): NSCLC: 216 patients, 10% responded
- May 13, 2003: Velcade (proteasome inhibitor): Multiple myeloma: 188 patients 28% responded, FDA approval in 4 months.
- FDA Commissioner Mark B. McClellan, "The approval of Velcade illustrates FDA's commitment to providing patients with access to safe and effective drugs as quickly as possible."

Phenoxodiol clinical program

- Phase 2 – SCC/BCC Oral Therapy
  - Oral dose regime: Low Dose Oral Phenoxodiol in Patients with Cutaneous Squamous Cell Carcinoma and/or Basal Cell Carcinoma (Royal North Shore Hospital, Sydney)
  - 50 mg t.i.d. for a treatment cycle comprising 12 weeks. If at 12 weeks the lesions have clinically responded (ie >50% reduction in tumour size) but have not disappeared completely, then therapy may be continued for up to 8 further weeks before the area is excised.
  - Total of 30 patients. The 1st 15 patients will have the tumour (or tumour site) excised at the conclusion of 12-20 weeks of treatment for assessment of histological tumour response.
  - If histological analysis confirms complete histological disappearance of lesions in the 1st 15 patients then a second group of 15 patients will be enrolled.
  - At the completion of 12-20 weeks of therapy the lesions have disappeared clinically then therapy will be ceased and they will be observed at 1-2 monthly intervals for clinical evidence of recurrence. If at the end of up to 20 weeks therapy there is clinical evidence of tumour the area will be treated surgically.

Phenoxodiol clinical program

- Phase 2 – Cervical SCC (Yale University Hospital)
  - IND under submission for oral dose form
  - Squamous cell reproductive cancers

Phenoxodiol clinical program

Timelines

2002	2003

Phase Ib/IIa trials

Phase II trials

Phase I (phenoxodiol analogues)