



**Anticancer Potential of a Research
Product (CSE17)**
Azadirachta indica in treatment of
Aerodigestive Cancers

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And

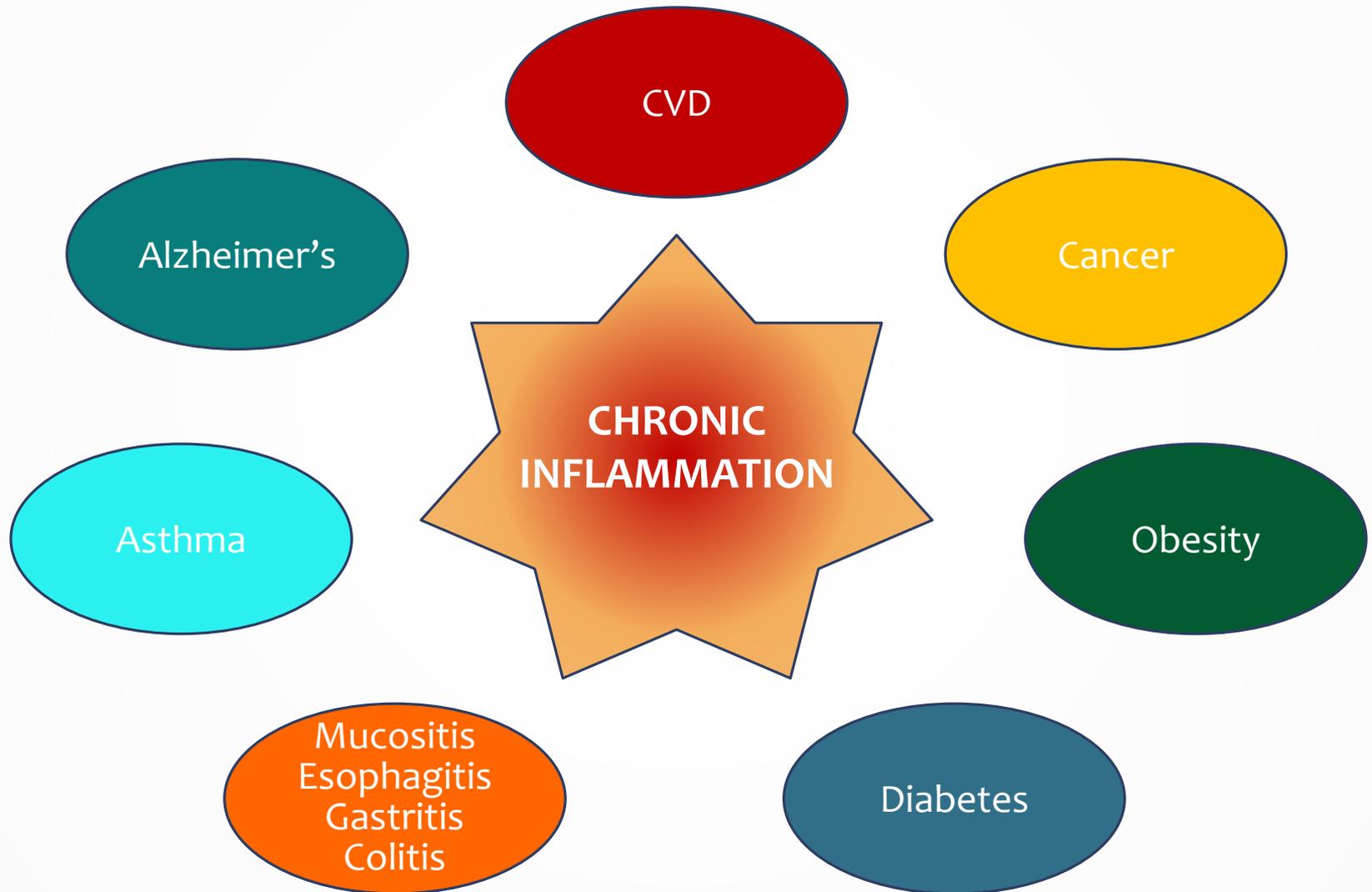
Girish Soman RD MTech
NuAxon Research Inc

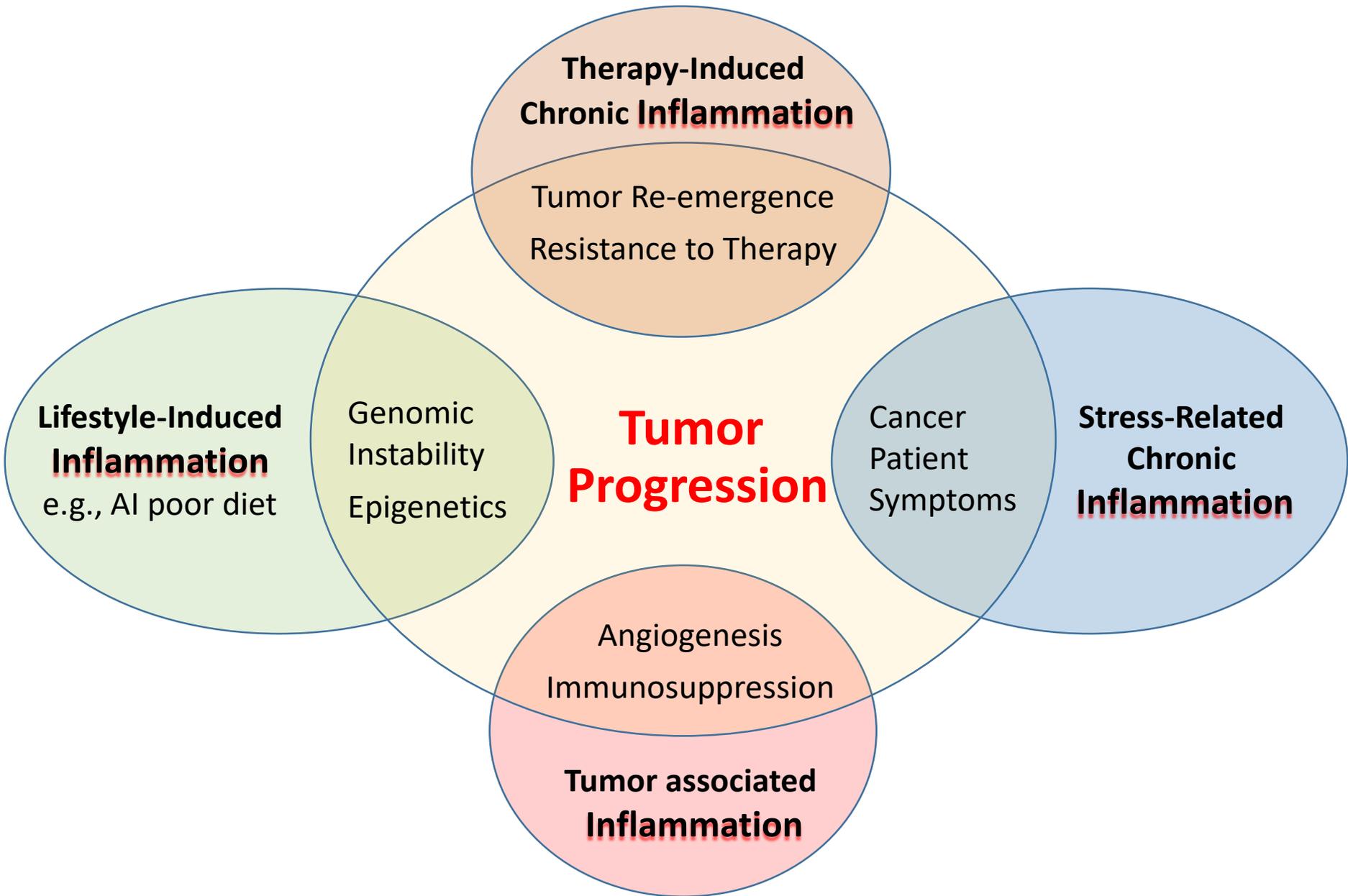
Inflammation and Cancer



- Chronic, clinically invisible, unresolved **inflammation** may create a very high risk for common cancers.
- Tumors corrupt the inflammatory pathway to survive.

A Common Nexus





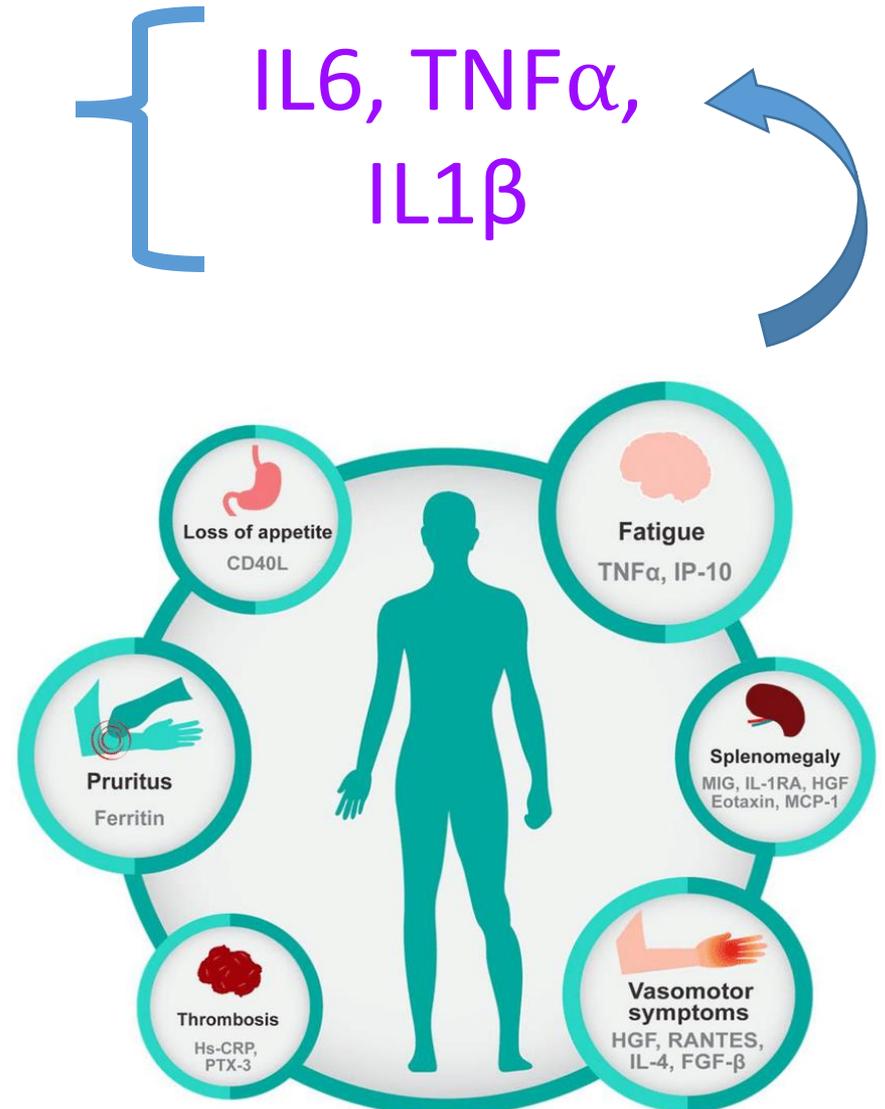
Manifestations of Stress In the Cancer Patient

- Symptom clusters (PTSD-like):

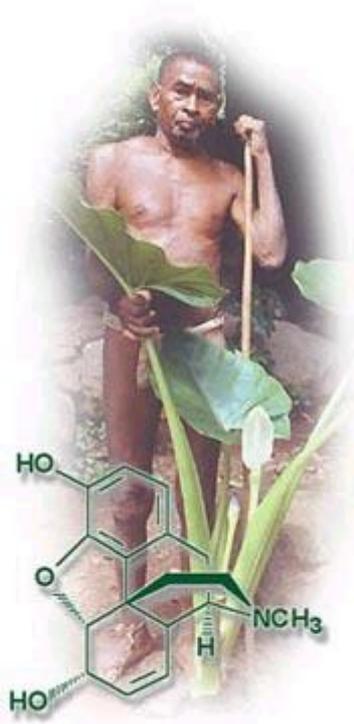
- Anxiety
- Sleep disruption
- Depression
- Fatigue
- CICD

- Physiological

- Lymphedema
- Esophagitis
- Mucositis
- Inflamed Membranes



Ethnopharmacology: Learning from Other Cultures

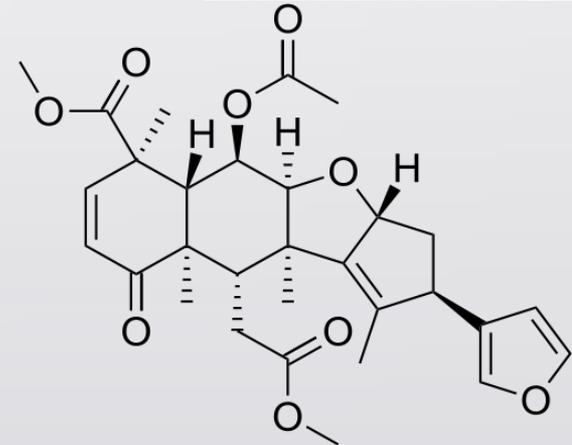


- ▶ A discipline of research which investigates the role of natural products in healthcare. It places a special emphasis on **maintaining linkage** to lifestyle practices in other cultures.
- ▶ A major concern is that with acculturation in the developing world we will soon lose this knowledge.



Azadirachta indica (Neem)

- Used in Ayurvedic medicine (India)
- Indicated to maintain oral health
- “Poor man’s toothbrush”
 - Bioactives are **liminoids**
 - Bitter tasting and highly anti-inflammatory

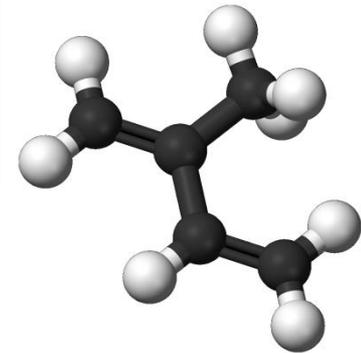
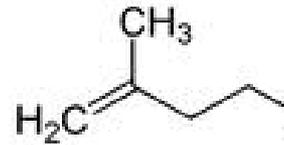


Neem: A pluripotent botanical!

135 compounds

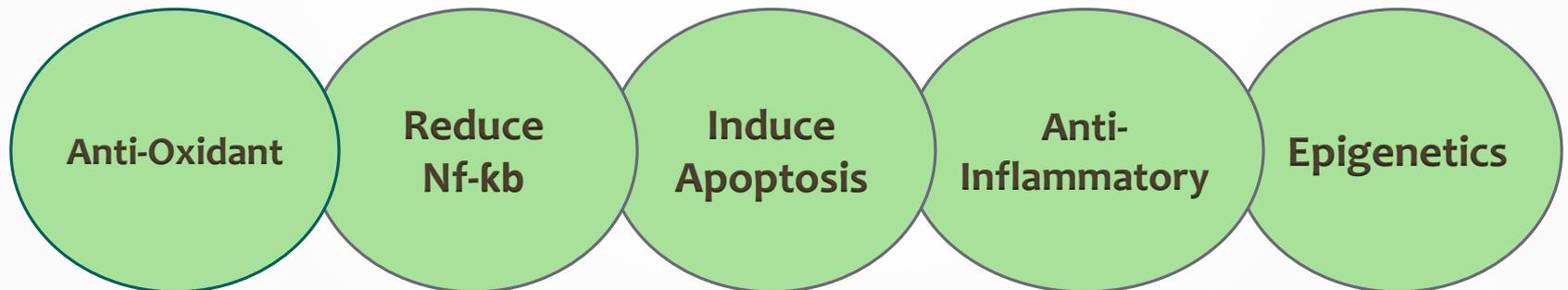
- isoprenoids
- non-isoprenoids

Isoprene



Nimbin, Nimbidin, Ninbidol, Gedunin, Quercetin, Salannin, Azadirachtin A and B, and

Nimbolide



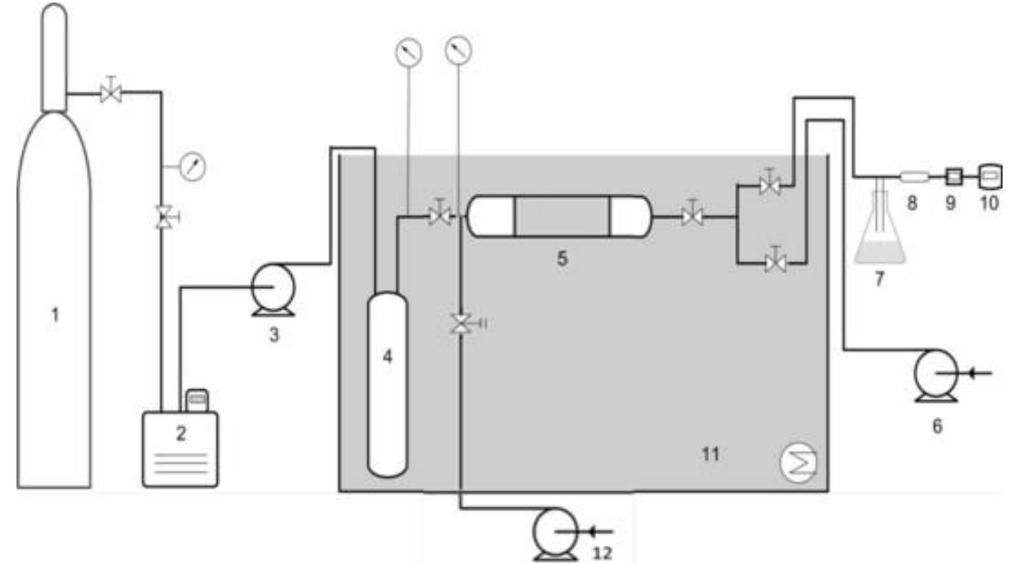
Use of Neem in Human Research Published Reports

Health Topic (N)	Plant Part	Publication Year(s)	Study Design
Insect Repellence (16)	seed	1993 - 2010	field trials (controlled)
Vaginal health (10)	leaf, seed	1995 - 2011	clinical trials (various designs)
Oral health (7)	leaf	1998 - 2010	clinical trials (mostly RCTs)
Contraception (5)	leaf, seed	1984 - 2006	clinical trials (various designs)
Congestive heart failure (2)	seed	1958	case series
Dermal infestation (2)	leaf, seed	1992, 2007	clinical trials (non-controlled)
Diabetes (2)	leaf, seed	1973, 2006	case series; clinical trial (controlled)
Gastrointestinal ulcer (2)	bark, seek	1985	clinical trials (non-controlled)
HIV / AIDS (2)	leaf	2004, 2007	clinical trials (non-controlled)
Wound healing (2)	seed	1925, 2012	case series
Malaria (1)	not specified	2001	clinical trial (controlled)
Topical Allergenicity (3)	seed, pollen, ns	2000 - 2008	case reports, clinical tests
Poisoning: Children (8)	seed	1981 - 2009	case reports, case series
Poisoning: Adults (4)	leaf, bark, seed	1984 - 2011	case reports

(66 English language publications as of 2014)

ns = not specified; RCT = randomized clinical trial

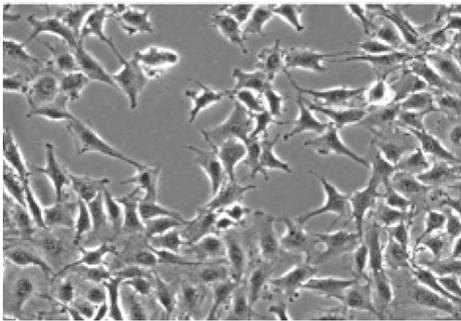
Supercritical CO₂ Extraction



- (1) CO₂ cylinder
- (2) Refrigerated bath
- (3) High-pressure pump for CO₂
- (4) Supply tank
- (5) Extractor
- (6) Peristaltic pump
- (7) Collection flask
- (8) Porapak trap Q
- (9) Gas flow meter
- (10) Volume totalizer
- (11) Heater
- (12) High-pressure pump for ethanol/water

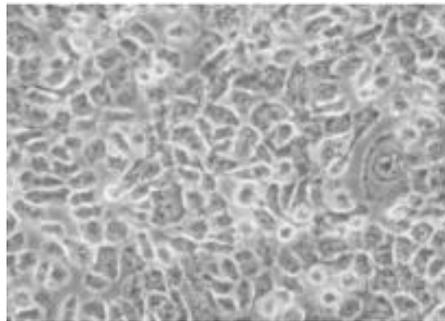
Can SCNE/Nimbolide inhibit OSCC
through disruption of inflammatory
markers?

Human OSCC cell line characteristics



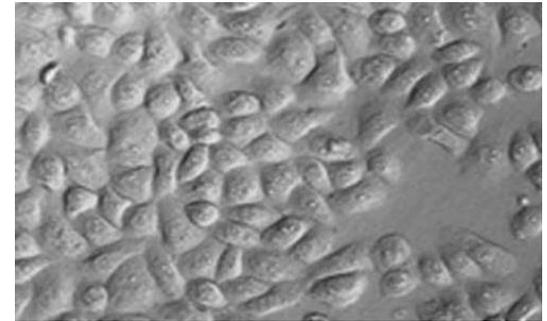
SCC-4

Slow growing
Adherent, non-mobile



Cal27

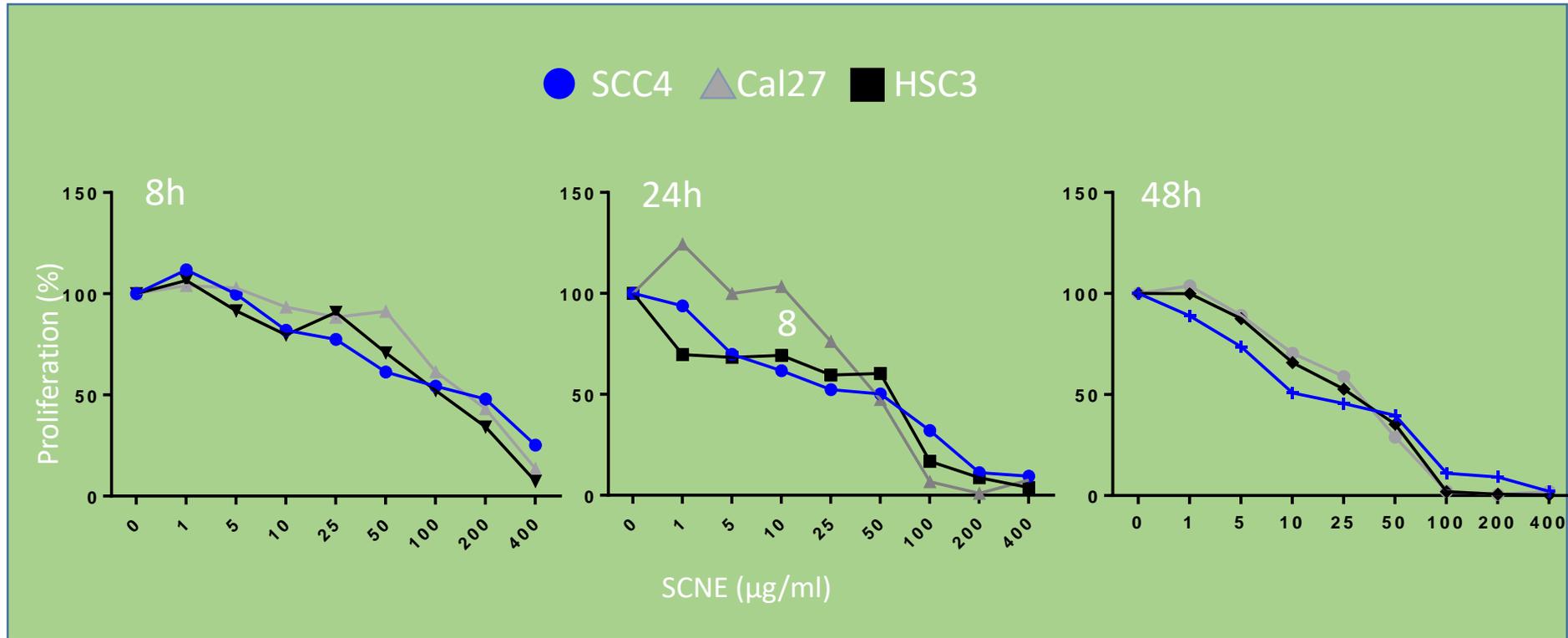
Fast growing
Slowly invasive



HSC-3

Fast growing
Highly invasive

(CSE17) inhibits OSCC cell growth



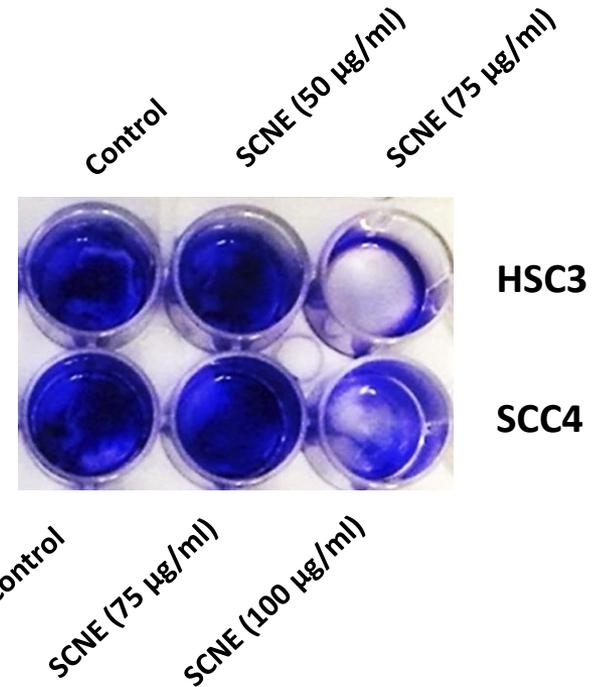
(CSE17) promotes G2 stall & decreases colony formation

(CSE17)

Tables 1 and 2. Cell Cycle Analysis of OSCC Cell Lines.

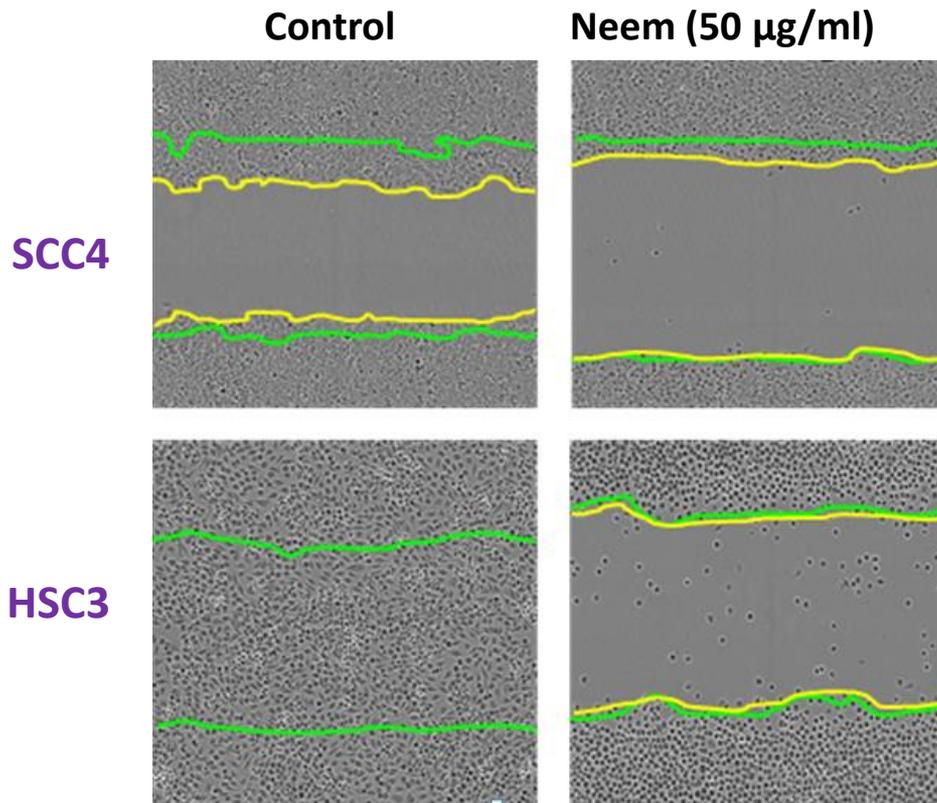
Table 1.	Treatment	%G1	%S	%G2	%SubG1
HSC-3	Control	56.34	15.49	16.66	-0.2
	Neem	65.91	8.67	21.36	4.74

Table 2.	Treatment	%G1	%S	%G2	%SubG1
SCC-4	Control	68.12	15.49	16.66	-1.07
	Neem	48.71	14.29	36.7	0.26



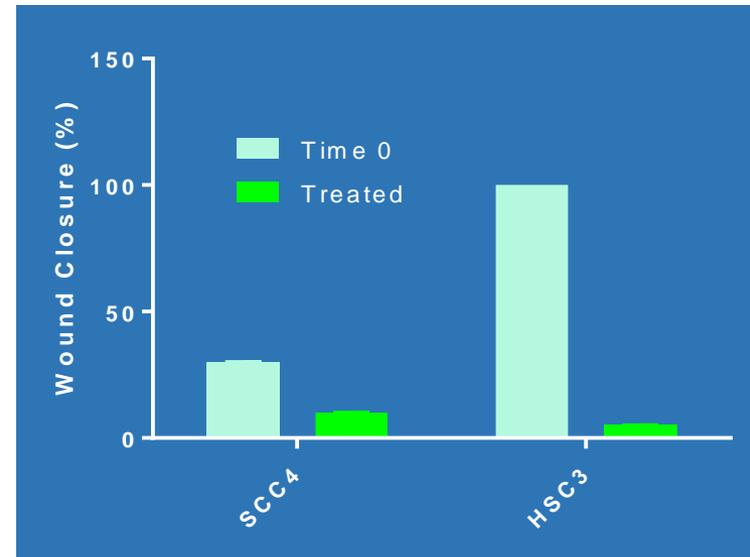
G2 checkpoint arrest creates radiosensitive cells:
fluorouracil, cisplatin, taxanes

(CSE17) inhibits migration of OSCC cell lines in Scratch Wound Healing Assay



24 h

4 h



Animal Models for Oral Cancer

Xenograft mice using OSCC cell lines

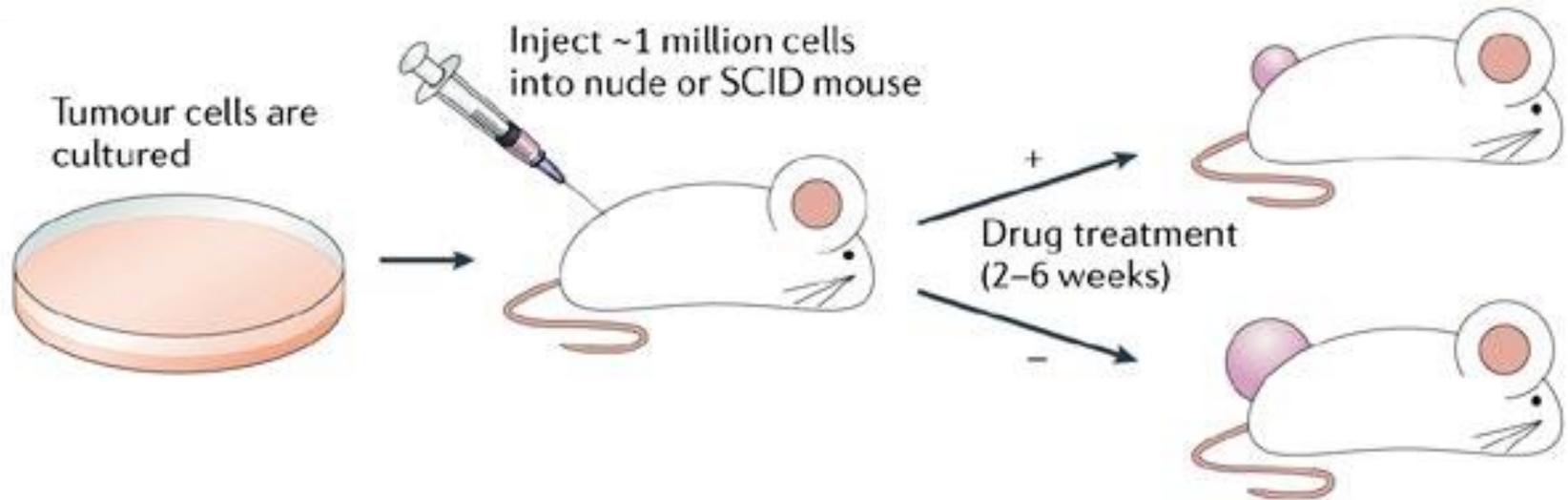
DMBA Hamster Cheek Pouch Model

- Historical
- Carcinogen painting; no mechanistic reagents; behavior

4NQO Models in Mouse/Rat

- Mouse model: carcinogen painting and drinking water methods
- Good reagents
- Rat gives larger tissue acquisition and ideal for dietary studies
- But mouse allows for genetic approaches

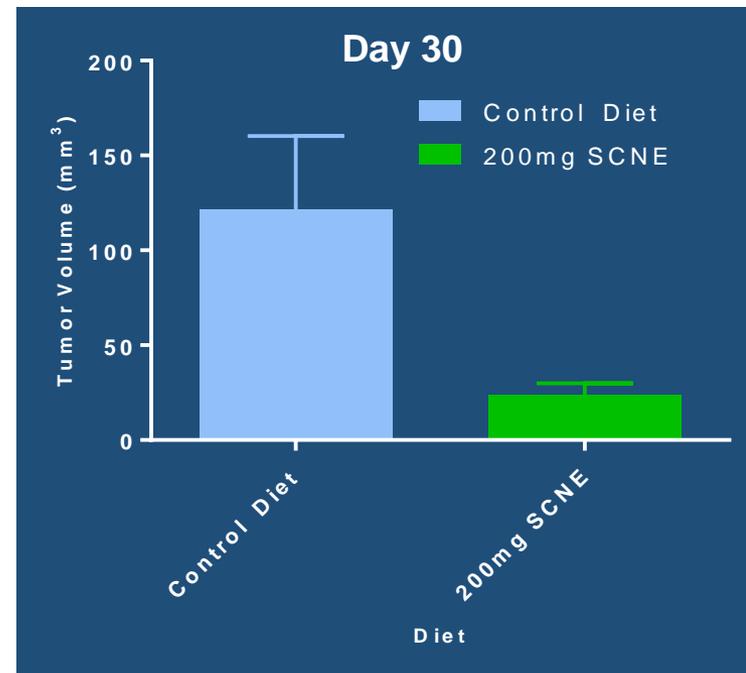
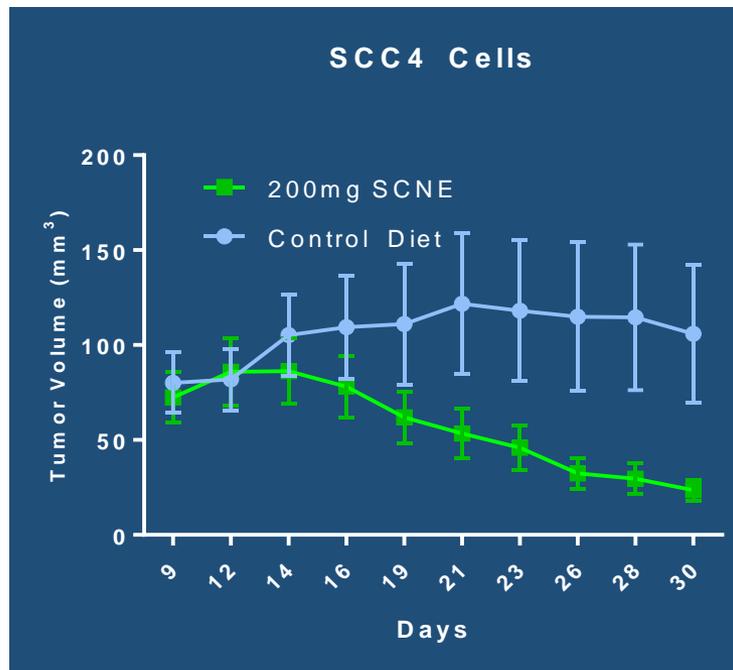
Is Neem effective in *in vivo* models?



Control arm: standard chow

Treatment arm: 200 mg/kg neem chow

SCNE inhibits SCC-4 tumor growth in xenograft mice

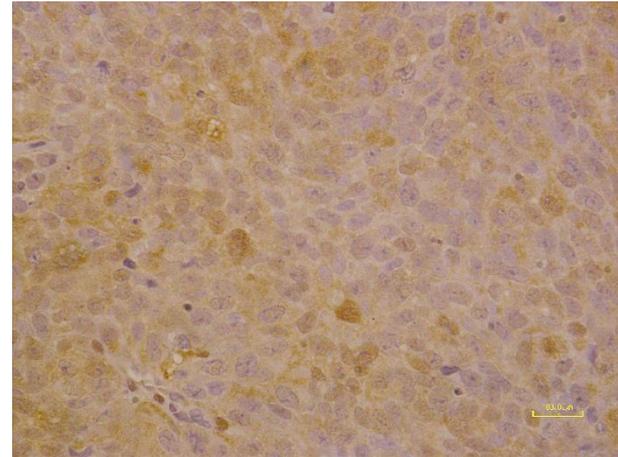
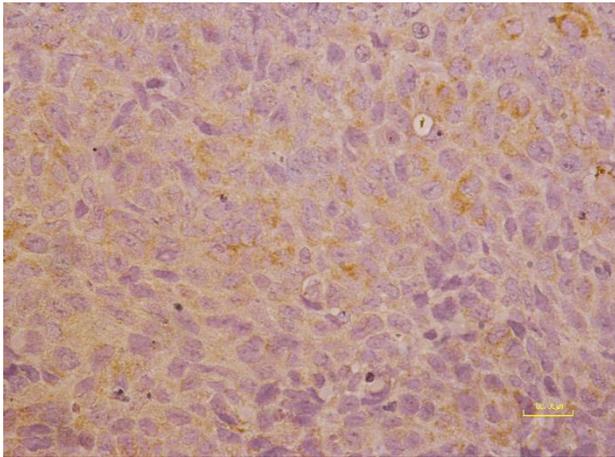


(CSE17) inhibits markers of proliferation in SSC4 Xenograft mice

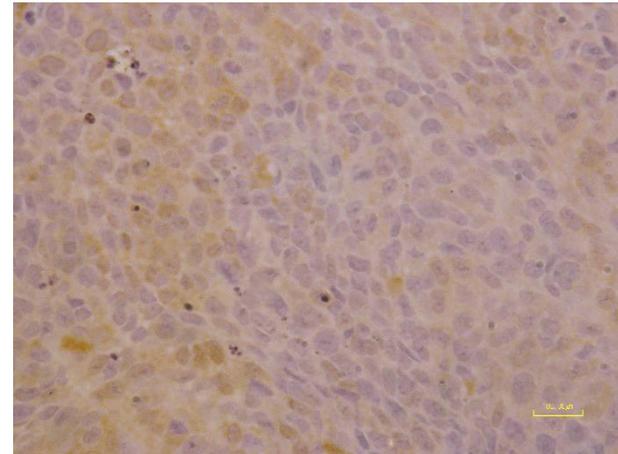
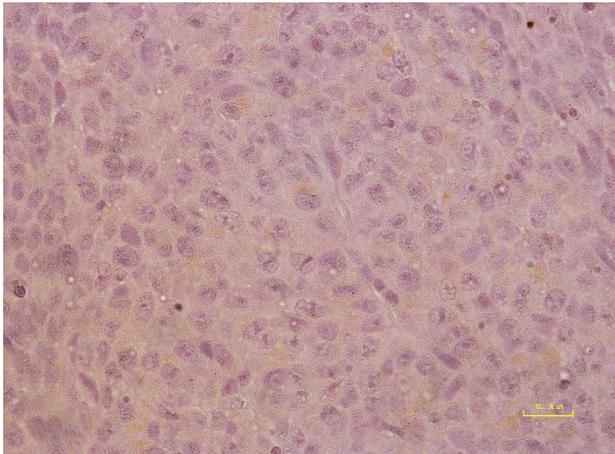
EGFR

STAT3

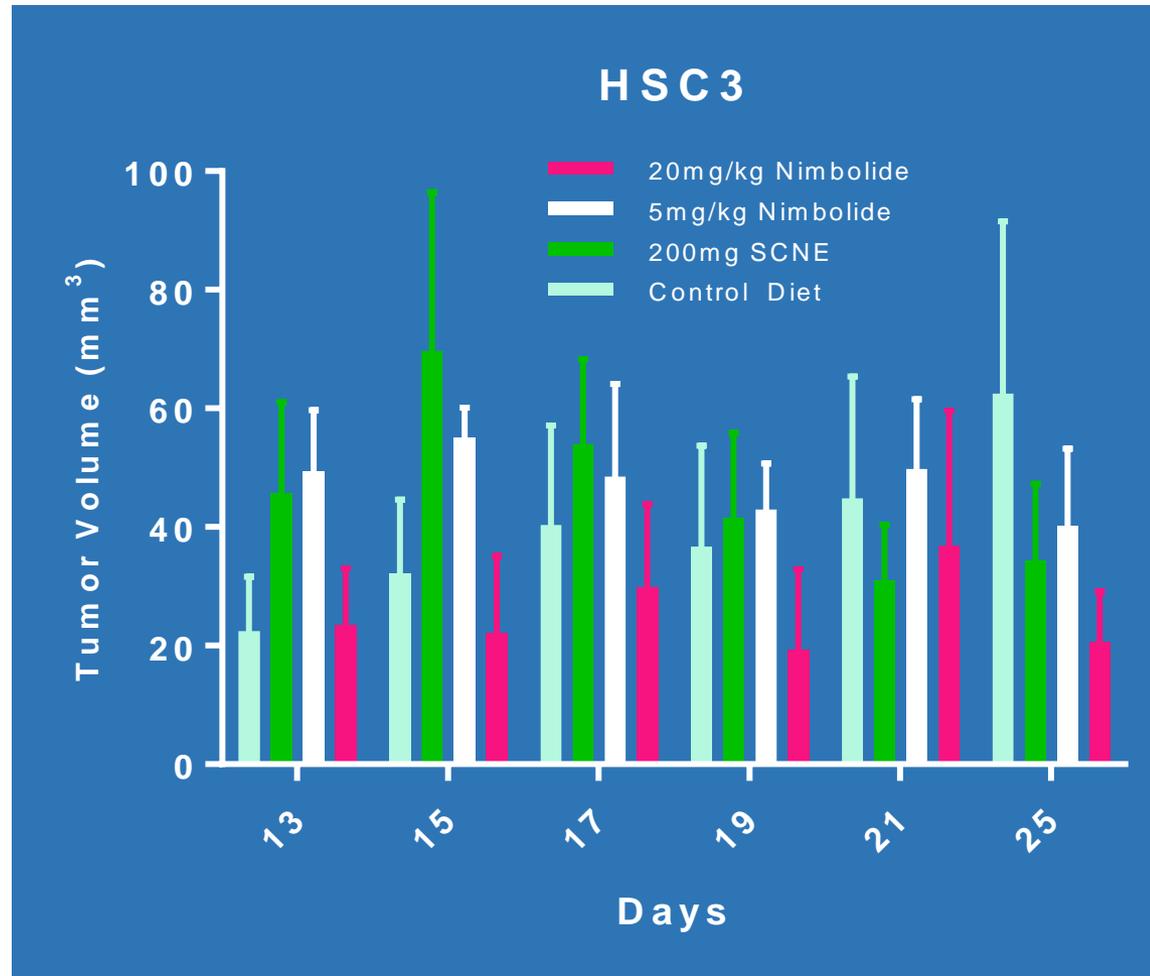
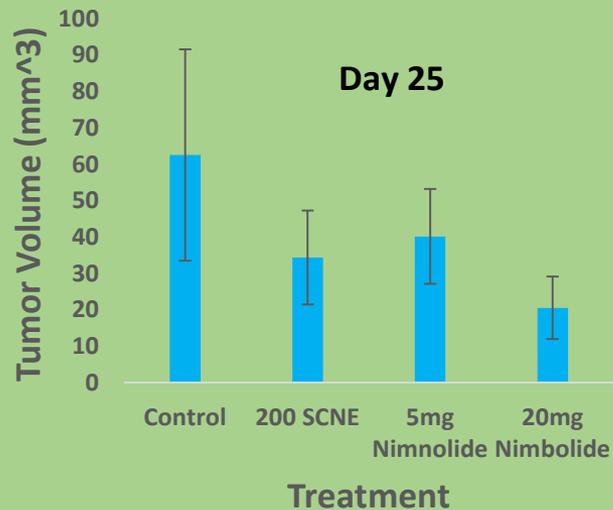
Control



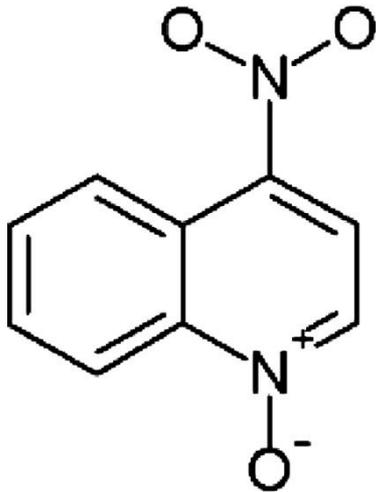
SCNE



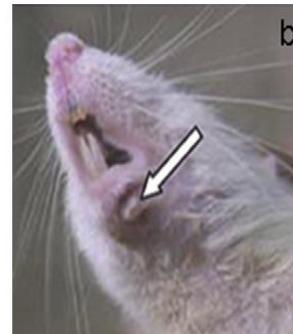
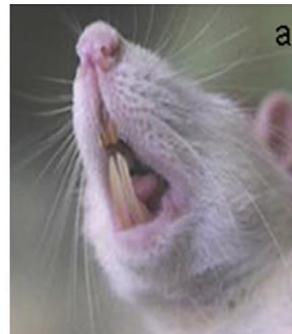
(CSE17) inhibits HSC-3 tumor growth in xenograft mice



4-nitro-quinoline-1 oxide (4NQO) tongue cancer model



Thought to elicit its carcinogenicity by producing DNA adducts after being metabolized to 4-hydroxyaminoquinoline-1 oxide, which forms 8-hydroxydeoxyguanosine (8OHdG), and causes oxidative damage.

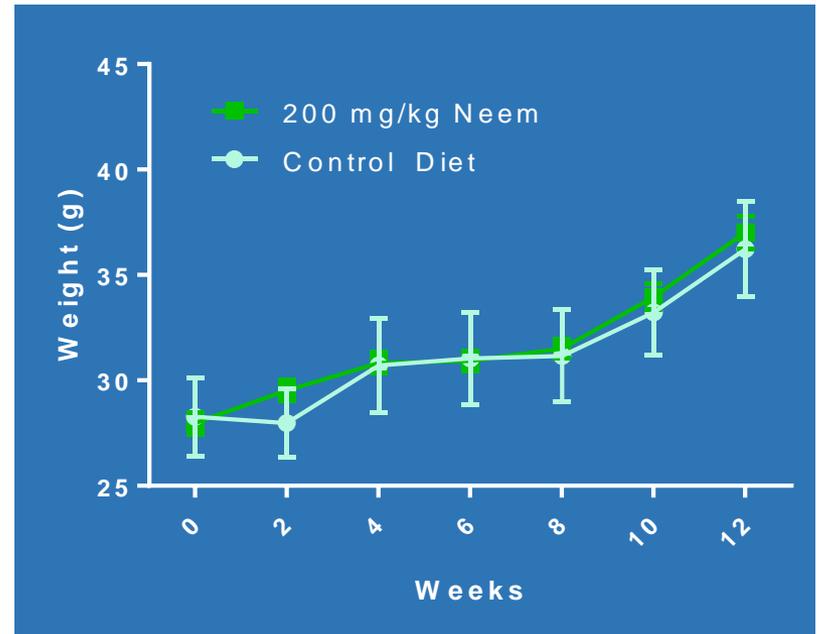
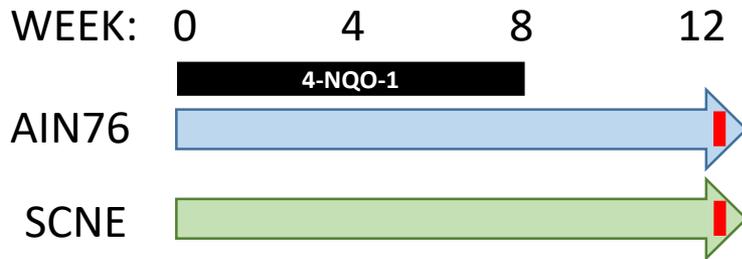




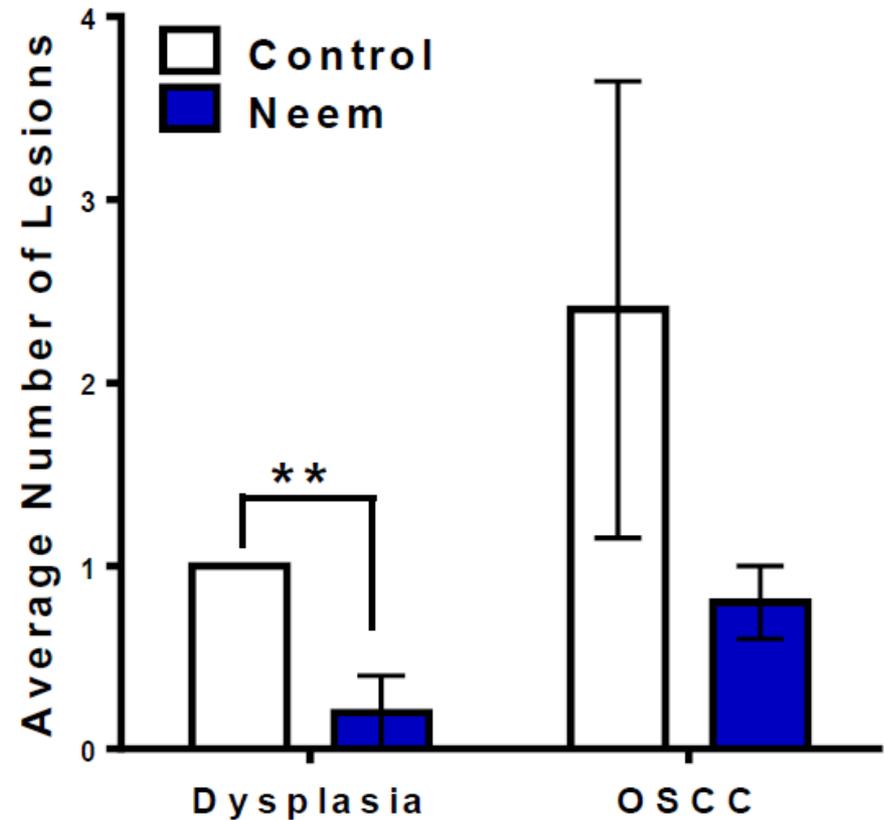
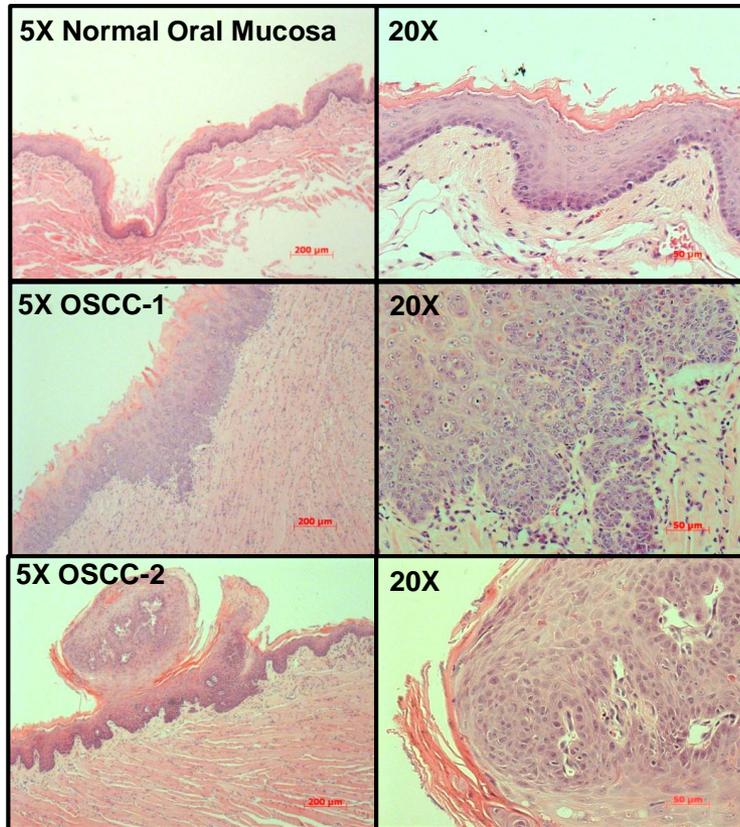
CBA Strain:

Bagg Albino ♀ x DBA ♂

Greater sensitivity to carcinogens



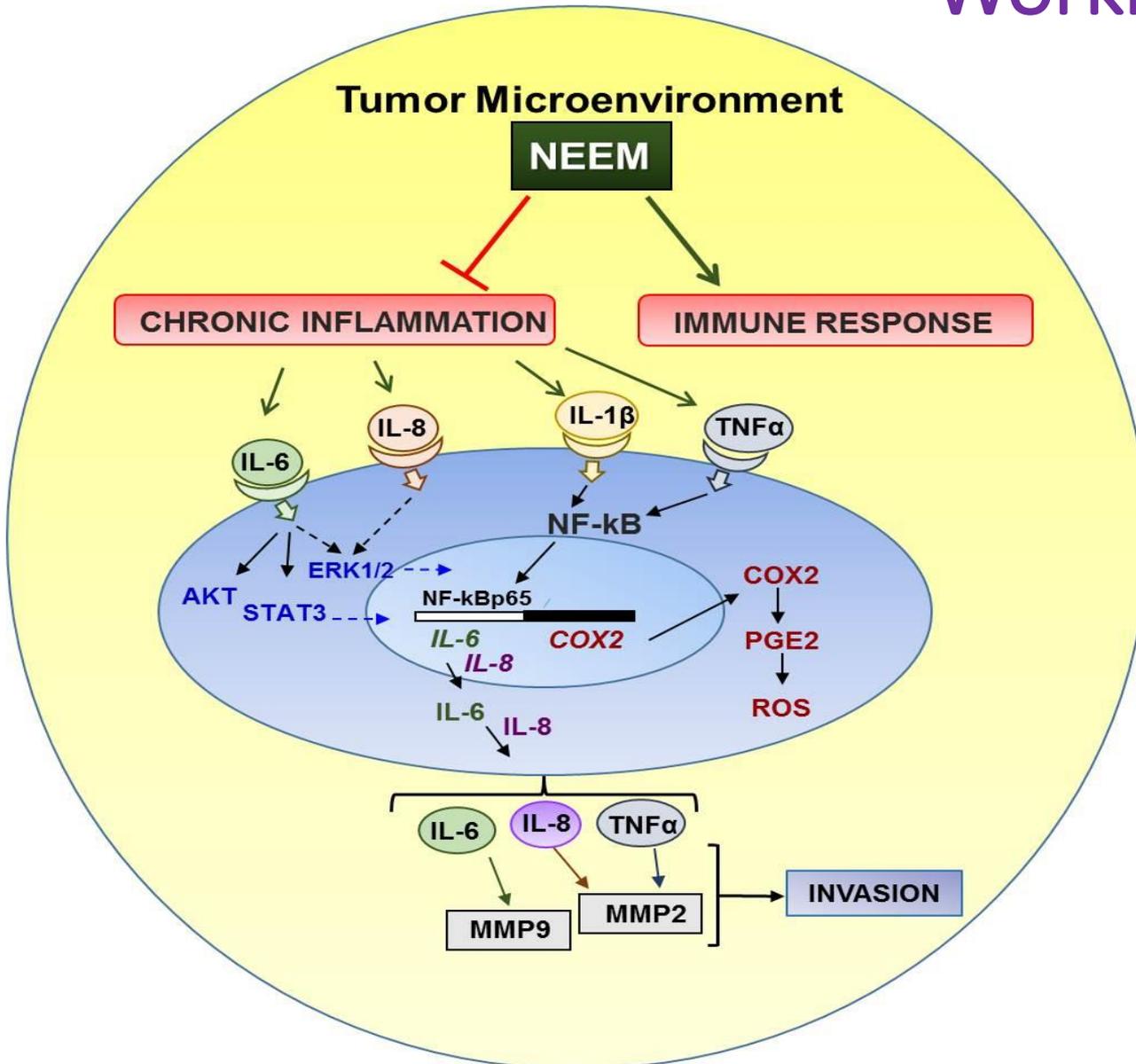
(CSE17) inhibits dysplasia & OSCC in CBA mice



Advantages of Supercritical CO₂ Extraction

- Replaces **conventional organic solvents** with SCFs in extraction procedures
- Allows for waste separation and minimization, as well as solvent recycling.
- High efficiency, high extraction rates and more selectivity.

Working Model



(CSE17) - Conclusions

1. (CSE17) on its own has significant anticancer activity
2. CSE 17 inhibits OSCC cell growth and causes G2 stall.
3. CSE 17 reduces COX2 levels and is ANTI-INFLAMMATORY
4. In human xenograft mouse model and tongue cancer mouse model si CSE17 has anticancer activity
5. CSE17 reduced proliferation markers and circulating inflammatory cytokine levels.

TAKE HOME FOR THE PRACTICING ONCOLOGIST

- Cancer patient symptomology may be driven by **CHRONIC INFLAMMATION**
- As such it is a clinical target
- **NSAIDS** are unsafe for the long-term
- Naturally occurring supplements with **AI** activity could be beneficial in relieving:
 - Anxiety
 - Depression
 - Sleep disruption
 - Chemotherapy induced cognitive decline
 - Pain
- **Neem-based (CSE17)** is the first wave of such efforts to help the cancer patients and survivors to improve their quality of life.

THANK YOU