American Institute of Integrative Oncology
RESEARCH & EDUCATION

Foundations of Integrative Oncology
COURSE

GRAND ROUNDS CALL
With Dr. Nalini Chilkov
November 13th, 2019
Second Wednesday of Every Month
5:30 PM Pacific / 6:30 PM Mountain / 7:30 PM Central / 8:30 PM Eastern

Agenda

- **Clinical Pearl**
  - CRP Serum Levels and Cancer: A Diagnostic and Prognostic Biomarker
- **Case Study**
  - 59yo F Basal cell carcinoma - 13 Moh surgeries
- **Clinical Question:**
  - How do we approach multidrug resistance MDR?
  - Is there a different approach to treat non-solid cancer than solid cancer?
  - Are there special tests or biomarkers for non-solid cancer?
  - Do they have different traits or characteristics? Are there special cautions we should be aware of?
  - Regarding Osteosarcoma
- **Research Highlights:**
  - Pretreatment Vitamin D Deficiency Is Associated With Impaired Progression-Free and Overall Survival in Hodgkin Lymphoma

Clinical Pearl: CRP Serum Levels and Cancer: A Diagnostic and Prognostic Biomarker
LINK to SLIDES: https://aiiore-members-only.s3-us-west-1.amazonaws.com/Grand+Rounds/2019+11+13+Clinical+Pearl+Slides+-+CRP+Serum+Levels+and+Cancer+-+A+Diagnostic+and+Prognostic+Biomarker+.pdf

Case Study: 59yo F Basal cell carcinoma - 13 Moh surgeries
Submitted by: Judy Pruzinsky L.Ac
Overview: 59-year old woman, who has numerous basal cell carcinomas. **She has not been able to bring herself to a dermatologist for 2.5 years, after having her last 4 Mohs**

surgeries (and 9 surgeries prior to that). She now feels she probably has several new sites on her face (always on her face) and is wondering if there is any new wisdom in the world of integrative oncology for skin cancers (non-melanoma).

Judy Pruzinsky, L.Ac.: 2017 Case Notes

What is most important in managing the terrain of basal cell carcinomas? Although one of the most benign forms of cancer, after 13 Mohs surgeries, the patient doesn't feel that way.

Most basal cell carcinomas: DNA damage occurred 10-20+ years prior

- Assess Tumor Microenvironment: ALWAYS assess factors identified

- Consider new immune therapies-
  - Topical - such as ZYCLARA Imiquimod which can also reveal subclinical lesions (approved for Actinic Keratoses and HPV Skin lesions (warts but also many studies on BCC) increases Interferon-*a,* CD3, CD4, CD8, CD11c, and CD68 T cells
  - Oral-Systemic Therapy Odomzo® (sonidegib) Tyrosine Kinase inhibitor: inhibits Hedgehog signaling pathway involved in BCC (many adverse SE) ***Resveratrol and Curcumin and Oridonin from Rabdosia rubescens also inhibit Hedgehog Signaling Pathway***

- Nutraceutical-Botanical Systemic Therapy must include inflammation control. Decrease NFkB (curcumin, Boswellia, Scutellaria baicalensis, O3FA), inhibit Hedgehog signalling pathway (Yu Jin Curcuma longa>curumin, resveratrol, Rabdosia rubescens), immune modulation (Ganoderma, astragalus, Coriolus, cordyceps), support for epithelial repair (Vit A), and specific targeted botanicals and phytochemicals (Parthenolide -Tanacetum parthenium -Feverfew), EGCG from Green Tea (Camellia sinensis, Tanshinones and Salvionolic acid: Salvia Milthiorhizza-Dan Shen), Scutellaria baicalensis-Huang Qin

- Acupuncture LI 4, LI 11, Sp10, St 36, GB 41, SJ5 (Lu 1, Lu 10)

- Example of Custom Compounded Formula from CHILKOV CLINIC

240ml	480ml	
20	40	**Salvia Red Sage** (Dan Shen)
40	80	**Tumeric Yu Jin** Curcuma longa
40	80	**Scutellaria baicalensis** Huang Qin
10	20	Oldenlandia(aka Heydotis) Bai Hua She She Cao
30	60	Gotu Kola Centella Asiatica
10	20	**Green Tea** Camellia Sinensis Cha Ye
10	20	**Tangerine peel** Citrus Reticulata Chen Pi
20	40	**Rabdosia rubescens Dong Ling Cao (oridonin)**
20	40	Cordyceps fungus Dong Chong Xia Cao
20	40	Ganoderma lucidum fungus Ling Zhi Reishi
10	20	Astragalus membranaceus Huang Qi

Recommendations:
See PDF Case Study Notes & Articles

Questions & Answers

Ana Komazec: **MULTIDRUG RESISTANCE**

- **How do we approach multidrug resistance MDR?**
- **I assume we are using botanicals that inhibit**
  - **PI3K/Akt signaling (Honokiol)**
  - **LDHA inhibitors (of which I don't have a wide grasp of what is available to us except EGCG, Scutellaria baicalensis),**
  - **Inhibitors of glycolytic enzymes and so forth?**
- **How do you communicate available options with the patients who reach the MDR stage?**
  - ***It is rare to REVERSE MDR. Our botanical, phytochemical and nutriceutical, as well as dietary interventions, serve to reduce MDR***

Dr. Chilkov:

See webinar slides from Dr. Daniel Weber on Multidrug Resistance and Chinese Herbal Medicine in Resource Library for a detailed explanation of the physiologic processes that lead to multidrug resistance and multiple phytochemical interventions that prevent MDR while some phytochemicals also are synergistic and enhance drug therapies.

Examples of phytochemicals discussed: Curcuma yu jin, Salvia milthiorrhiza, dan shen, Lisgusticum chuan xiong, Glycyrrhiza Gan Cao, Chrysanthemum Huang Ju, Schizandra wu wei zi, Silybum silymarin, Resveratrol, Soy Isoflavones,-Genistein-Daidzein, Citrus Bioflavinoids: Naringin, Hesperitin, Tangeritin.

Questions & Answers

Man-Ling Lai: **Is there a different approach to treat non-solid cancer than solid cancer? Are there special tests or biomarkers for non-solid cancer? Do they have different traits or characteristics? Are there special cautions we should be aware of?**

Dr. Chilkov:

- ***Solid tumor*** *An abnormal mass of tissue that usually does not contain cysts or liquid areas. Solid tumors may be benign, or malignant. Different types of solid tumors are named for the type of cells that form them. Examples of solid tumors are sarcomas, carcinomas, and lymphomas.*

- ***THE BONE MARROW*** *is the primary microenvironment for Hematologic (Myeloid) Malignancies (Lymphomas, Myelomas, Leukemias) which involve functional cells of bone marrow and immune system More inflammatory, more hypercoagulation and fibrosis*

Blood. 2017 Feb 16; 129(7): 811–822.

Myeloid malignancies and the microenvironment

https://www.ncbi.nlm.nih.gov/pubmed/28064238

Claudia Korn and Simón Méndez-Ferrer

- *In order to individualize care each unique cancer, cell line and its characteristics, as well as stage and grade, must be understood along with the tumor microenvironment*

Man-Ling Lai: **What do you think about taking a high dosage of green tea/EGCG without food could raise liver enzymes?**

Dr. Chilkov:

There is a subset of patients with COMT (catechol - o - methyl - transferase) polymorphisms that will experience elevated LFTs with EGCG...it is not related to taking with or without food…..

Questions & Answers

Victoria Woods: **Do you have experience with osteosarcoma? Have a teenager who had radiotherapy when he was 2 for rhabdomyosarcoma, now has metastatic osteosarcoma. Prognosis poor, not responding to treatment. I sent them to Nagourney for sensitivity resistance testing.**

Dr. Chilkov:

Sarcomas in general and osteosarcomas, in particular, are very very treatment-resistant. Sample tx plan for one of my current liposarcoma-osteosarcoma patients.

*****Restrict/Limit Carbohydrates, Starches, Sweets, Grains, Fruit to lower HgbA1c*****
this lowers insulin and blood sugar to reduce growth signaling

TM (Tetrathiomolybdate) 20 mg with each meal, 60 mg on empty stomach in the evening

Supplements	***one month supply***
DFH Twice Daily Multi (iron free copper free)	***1/2x/day (1 bottle 120 caps)***
DFH Omegavail TG 1000	***2/2x/day replaces Artic Cod Liver (2 bottles 120 caps)***
DFH Curcumevail	***2/2x/day (2 bottles 120 caps)***
DFH EGCG	***2/2x/day (2 bottles 120 caps)***
DFH Annatto Tocotrienols+Nigella Seed Oil	***2/2x/day (2 bottles 120 caps)***
DFH Osteoben	***2/2x/day (1 bottle 120 caps) replaces Osteomatrix***
PUR Boswellia AKBA	***2/2x/day (1 bottle 120 caps)***
DFH Probiophage	***1/2/xday probiotic (120 caps =2months)***
DFH Vitamin D Supreme 5000 iu	***1/2x/day (180 caps=3 months) or 10 drops=10,000 iu********
DFH Zinc Supreme	***1/2x/day (60 caps = 1 month)***
CS PURE HONOKIOL	***2/2x/day—- PLUS 2 caps PURE HONOKIOL at bedtime***

CS Modified Citrus Pectin -Professional Dissolve in very hot water 1 level teaspoon twice daily at least 30 MIN AWAY from meals, supplements, herbs

****RED SAGE extract 2 teaspoons daily (reduce D Dimer and microscopic activity)****

DFH-Designs for Health PUR -Pure Encapsulations

Daily Therapeutic Shake 2 scoops=40 grams protein (total daily protein goal 60-80 grams protein)

TAKE WITH PROTEIN DIGESTIVE ENZYMES
DFH 2 CAPS HYDROLYZYME
(1 CAP PER 10G PROTEIN 1 CAP PER SCOOP OF POWDER)

DFH Pure Paleo Protein (collagen) or Pure Pea Protein (vegan) 2 scoops Vanilla or Plain
choose your protein powder and your flavor OR your protein powder
DFH Paleo Fiber 1 heaping teaspoon
DFH Paleo Reds 1 heaping teaspoon
DFH Carnitine Tartrate 1/2 tsp(preserve muscle and mitochondrial function)
MRL Coriolous (active against sarcoma) 2 scoops
mix with unsweetened coconut milk, almond milk or coconut water
(So Delicious and Pacific brand are free of carrageenan)

Optional: organic fresh or frozen berries, mango, spinach, avocado, mint, parsley, cinnamon, vanilla, cardamom, ginger, yogurt or kefir, chia seeds, almond butter, 1/2 organic lemon with peel and seeds

SELENIUM: Eat 2 organic Brazil Nuts Daily

DFH Magnesium Citrate Powder 1 - 1 1/2 t 1-2x/day with 8 oz water to soften stool and prevent constipation as needed

Custom Herbal Formula (Fight Tumor, reduce inflammation and fibrosis)
2 teaspoons daily
shake well Dilute in Ginger Tea or water
take with food in the stomach

8oz	16oz	
250 ml	500ml	
20	40	Astragalus and Ganoderma Extract
30	60	Pinellia and Magnolia Formula
25	50	Scutellaria Baicalensis extract
20	40	Oldenlandia extract
15	30	Andrographis
12.5	25	Taxus brev
12.5	25	Catharanthus
10	20	Camptotheca
10	20	Green Tea
10	20	Feverfew
20	40	Polygonatum Yu Zhu
20	40	Red Ginseng Extract* (increased dose)
10	20	Milk Thistle Extract
10	20	Ginger root extract
10	20	Tangerine extract Chen Pi
15	30	Pau D Arco extract

Kidney Support Formula. UPDATED 08-02-19
1 teaspoon daily

(you can mix with your other tonic and take at the same time)

480 ml 240ml
200 100 Nettle Seed Extract
80 40 Polygonum multiflorum extract
80 40 Poria Fu Ling Pi Extract
80 40 Oat Seed Extract
40 20. Cooked Rehmannia
40 20. Fr Cornii

Naturopathic Oncology Considerations

oral Low Dose Naltrexone 4.5mg at bedtime

IV Therapies that are toxic to tumor cells
IV VITAMIN C
IV Artesunate
IV Curcumin

IV or Subcutaneous (inject under the skin..you can do this yourself at home)
Mistletoe an immunotherapy

geneticgenie.org Methylation and Detoxification reports

Research:

Pretreatment Vitamin D Deficiency Is Associated With Impaired Progression-Free and Overall Survival in Hodgkin Lymphoma

ASCO ORIGINAL REPORTS Hematologic Malignancy DOI: 10.1200/JCO.19.00985 Journal of Clinical Oncology - published online before print October 17, 2019, PMID: 31622132

Sven Borchmann, MD1,2,3; **Melita Cirillo**, MD1,4; **Helen Goergen**, Dipl. Math1; **Lydia Meder**, PhD1,2; **Stephanie Sasse**, MD1; **Stefanie Kreissl**, MD1;

https://doi.org/10.1200/JCO.19.00985

PURPOSE

Vitamin D deficiency is described as a modifiable risk factor for the incidence of and mortality in many common cancers; however, data in Hodgkin lymphoma (HL) are lacking.

PATIENTS AND METHODS

We thus performed a study measuring pretreatment vitamin D levels in prospectively treated patients with

HL and correlated this with clinical outcomes. A total of 351 patients from the German Hodgkin Study Group clinical trials (HD7, HD8, and HD9) were included.

RESULTS

Fifty percent of patients were vitamin D deficient (< 30 nmol/L) before planned chemotherapy. Pretreatment vitamin D deficiency was more common in relapsed/refractory patients than matched relapse-free controls (median baseline vitamin D, 21.4 nmol/L *v* 35.5 nmol/L; proportion with vitamin D deficiency, 68% *v* 41%; *P* < .001). **Vitamin D–deficient patients had impaired progression-free survival (10-year difference, 17.6%; 95% CI, 6.9% to 28.4%; hazard ratio, 2.13; 95% CI, 1.84 to 2.48; *P* < .001) and overall survival (10-year difference, 11.1%; 95% CI, 2.1% to 20.2%; hazard ratio, 1.82; 95% CI, 1.53 to 2.15; *P* < .001), consistent across trials and treatment groups**. **We demonstrated that vitamin D status is an independent predictor of outcome and hypothesized that vitamin D status might be important for the chemosensitivity of HL. We subsequently performed experiments supplementing physiologic doses of vitamin D (calcitriol) to cultured HL cell lines and demonstrated increased antiproliferative effects in combination with chemotherapy.** In an HL-xenograft animal model, we showed that **supplemental vitamin D (dietary supplement, cholecalciferol) improves the chemosensitivity of tumors by reducing the rate of tumor growth compared with vitamin D or chemotherapy alone.**

CONCLUSION

On the basis of our clinical and preclinical findings, **we encourage that vitamin D screening and replacement be incorporated into future randomized clinical trials to properly clarify the role of vitamin D replacement therapy in HL.**

Research:

A Phase 3 Randomized Trial of Nicotinamide for Skin-Cancer Chemoprevention
Andrew C. Chen, et al n engl j med 373;17 nejm.org October 22, 2015

1000 mg/day
Nonmelanoma skin cancers, such as basal cell carcinoma and squamous-cell carcinoma, are common cancers that are caused principally by ultraviolet (UV) radiation.

Nicotinamide (vitamin B3) has been shown to have protective effects against dam-
age caused by UV radiation and to reduce the rate of new premalignant actinic
keratoses.

In conclusion, among high-risk patients, nicotinamide was associated with a lower rate of new nonmelanoma skin cancers than was placebo and had an acceptable safety profile. Nicotinamide is widely accessible as an inexpensive over-the-counter vitamin supplement and presents a new opportunity for the chemoprevention of nonmelanoma skin cancers that is readily translatable into clinical practice.

References:

1. Allin, K. H., Nordestgaard, B. G., Flyger, H., & Bojesen, S. E. (2011). **Elevated pre-treatment levels of plasma C-reactive protein are associated with poor prognosis after breast cancer: a cohort study.** ***Breast Cancer Research***, *13*(3). doi: 10.1186/bcr2891

2. Borchmann, S., Cirillo, M., Goergen, H., Meder, L., Sasse, S., Kreissl, S., … Engert, A. (2019). **Pretreatment Vitamin D Deficiency Is Associated With Impaired Progression-Free and Overall Survival in Hodgkin Lymphoma.** *Journal of Clinical Oncology*. doi: 10.1200/jco.19.00985

3. Chen, A. C., Martin, A. J., Choy, B., Fernández-Peñas, P., Dalziell, R. A., Mckenzie, C. A., … Damian, D. L. (2015). **A Phase 3 Randomized Trial of Nicotinamide for Skin-Cancer Chemoprevention.** *New England Journal of Medicine*, *373*(17), 1618–1626. doi: 10.1056/nejmoa1506197

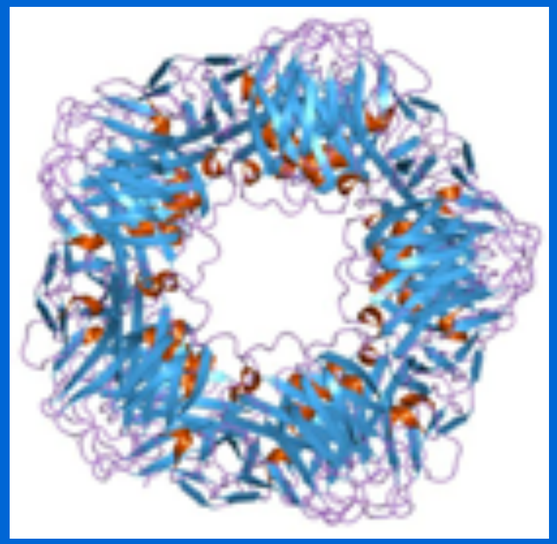
4. Grivennikov, S. I., & Karin, M. (2010). **Inflammation and oncogenesis: a vicious connection.** ***Current Opinion in Genetics & Development*****,** *20*(1), 65–71. doi: 10.1016/j.gde.2009.11.004

5. Korn, C., & Méndez-Ferrer, S. (2017). **Myeloid malignancies and the microenvironment.** ***Blood*****,** *129*(7), 811–822. doi: 10.1182/blood-2016-09-670224

6. Shrotriya, S., Walsh, D., Bennani-Baiti, N., Thomas, S., & Lorton, C. (2015). **C-Reactive Protein Is an Important Biomarker for Prognosis Tumor Recurrence and Treatment Response in Adult Solid Tumors: A Systematic Review**. *Plos One*, *10*(12). doi: 10.1371/journal.pone.0143080

7. Zhang, J., Zhang, C., Li, Q., Zhang, J., Gu, X., Zhao, W., … Cheng, W. (2019). C-Reactive Protein/Albumin Ratio Is an Independent Prognostic Predictor of Survival in Advanced Cancer Patients Receiving Palliative Care. *Journal of Palliative Medicine*. doi: 10.1089/jpm.2019.0102

CRP Serum Levels and Cancer: A Diagnostic and Prognostic Biomarker



Dr. Nalini Chilkov, L.Ac., O.M.D., Founder

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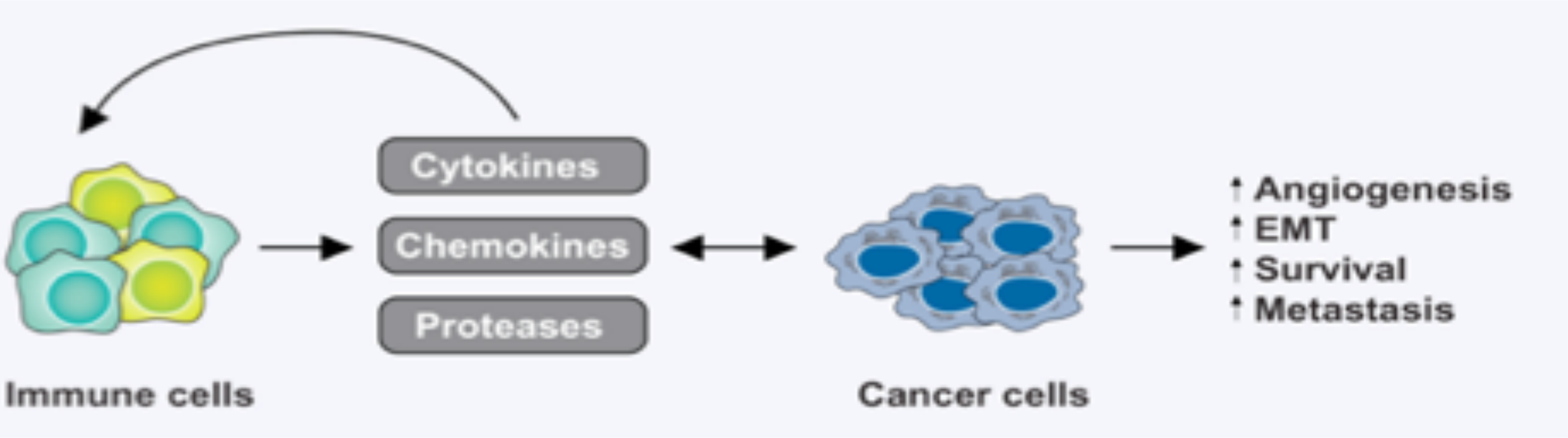
ONCO INFLAMMATION
Inflammation in the Tumor Microenvironment



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CANCER RELATED INFLAMMATION PROMOTES

- Tumor Growth
- Proliferation
- Angiogenesis
- Metastasis
- Immune Suppression
- Cancer Related Fatigue
- Depression
- Pain

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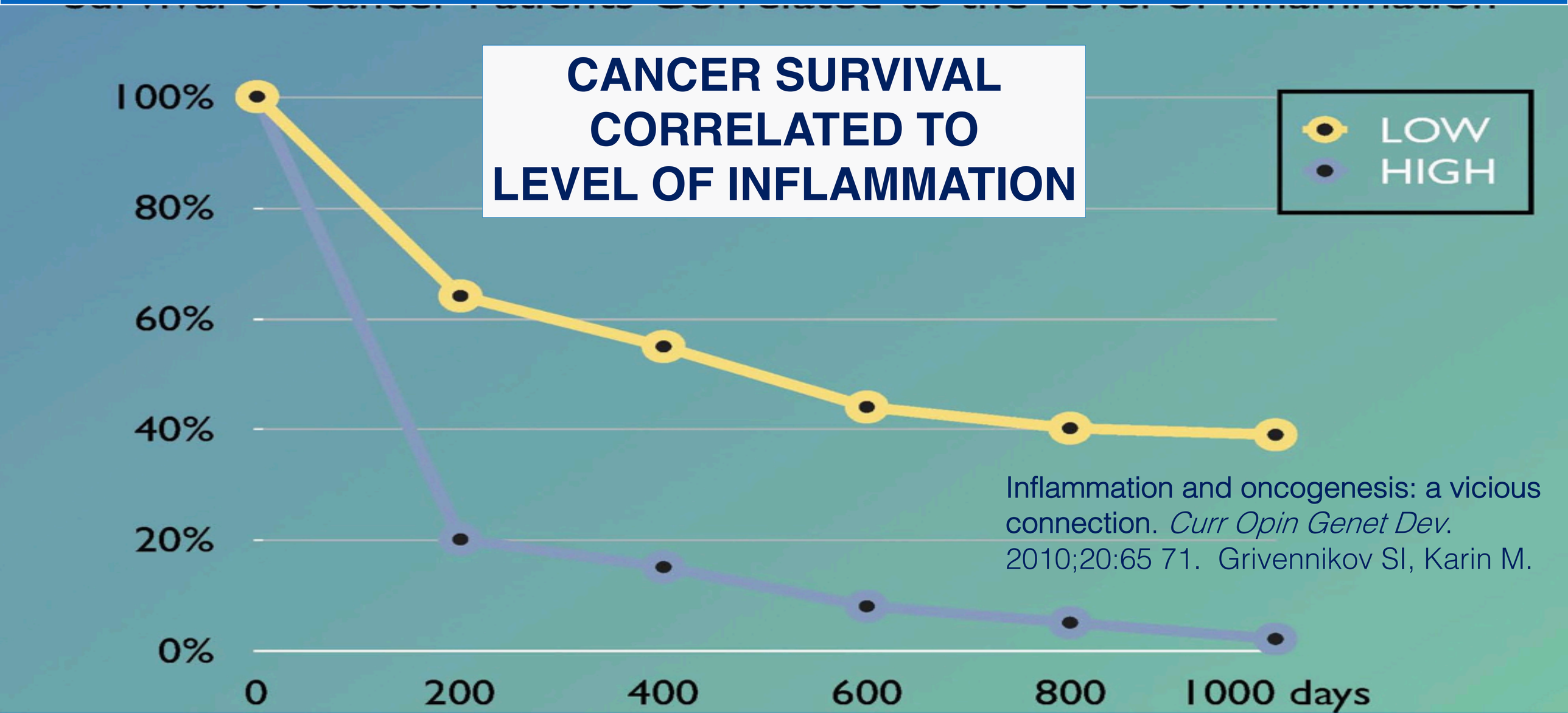
CHRONIC INFLAMMATION
TUMOR ASSOCIATED INFLAMMATION
THERAPY INDUCED INFLAMMATION



Inflammation and oncogenesis: a vicious connection. *Curr Opin Genet Dev*. 2010;20:65 71. Grivennikov SI, Karin M.

INFLAMMATION
C-REACTIVE PROTEIN

TARGET 1.0-3.0 mg/L

- Prognostic Marker in A Wide Variety of Cancers
- Increased Risk of Carcinogenesis, Angiogenesis,
- Metastasis, Advanced Stage, Mortality
- Poor Prognosis

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CRP: Prognosis, Treatment Response and Survival

C-Reactive Protein (CRP) is a well-established inflammatory marker.

It is also a biomarker of **cancer survival**.

CRP is elevated in patients with solid tumors, and high levels predict **poor prognosis**, **blunted treatment response,** as well as **tumor recurrence.**

X

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C-Reactive Protein, Cardiac Results confirmed on dilution.	63.24	High	mg/L	0.00 - 3.00	03

Relative Risk for Future Cardiovascular Event

Low	<1.00
Average	1.00 - 3.00
High	>3.00

GGT	76	High	IU/L	0 - 65	03
Sedimentation Rate-Westergren	55	High	mm/hr	0 - 15	03
Vitamin B12	>2000	High	pg/mL	232 - 1245	03
Ceruloplasmin	43.6	High	mg/dL	16.0 - 31.0	03
Copper, Serum [A]	189	High	ug/dL	72 - 166	03
D-Dimer	1.19	High	mg/L FEU	0.00 - 0.49	03
Fibrinogen Activity	561	High	mg/dL	180 - 383	
Zinc, Plasma or Serum [A]	122		ug/dL Detection Limit = 5	56 - 134	
Ferritin, Serum	632	High	ng/mL	16 - 124	

16 yo male
Aggressive
Treatment
Resistant
Osteo-
Sarcoma

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C-Reactive Protein Is an Important Biomarker for Prognosis Tumor Recurrence and Treatment Response in Adult Solid Tumors: A Systematic Review

As part of the systemic inflammatory response to a tumor, the body releases pro-inflammatory cytokines and growth factors.

Interleukin-6, produced by the tumor or surrounding cells, stimulates liver production of acute-phase reaction proteins that increase C-reactive protein (CRP) and fibrinogen.

Elevated CRP correlates with disease stage and increased cancer mortality

(Shrotriya S, et al. *PloS One*. 2015: 10(12), e0143080).

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Crit Rev Clin Lab Sci. 2011 Jul-Aug;48(4):155-70

Elevated C-reactive protein in the diagnosis, prognosis, and cause of cancer.

Allin KH et al.

Among individuals diagnosed with cancer during the study period, individuals with a high baseline **CRP (>3mg/L) had an 80% greater risk of early death** compared with those with low CRP levels (<1 mg/L).

Patients with invasive breast cancer and
CRP levels**>3mg/L at diagnosis had a 1.7-fold increased risk of death** from breast cancer compared to patients with CRP levels<1mg/L at diagnosis.

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Elevated pre-treatment levels of plasma C-reactive protein are associated with poor prognosis after breast cancer: a cohort study.

Individuals with a high baseline **CRP (>3 mg/L)** have an **80% greater risk of early death** compared with those with low CRP levels (<1 mg/L).

Patients with invasive breast cancer and **CRP levels>3 mg/L at diagnosis have a 1.7 fold increased risk of death** compared to those with CRP levels<1 mg/L at diagnosis

(Allin KH, et al. *Breast Cancer Res.* 2011: 13(3), R55).

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Curr Opin Pharmacol. 2009 August ; 9(4): 351 369.

Inflammation and Cancer: How Friendly Is the Relationship For Cancer Patients? B.B. Aggarwal

CRP is emerging as an important prognostic marker in a wide variety of cancers.

C-reactive protein (CRP), an NF-κB-regulated gene product has been **linked with prognosis of cancers of the breast, colon, kidney , ovary, lung and stomach, and multiple myeloma, melanoma, and non-Hodgkin's lymphoma.**

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CRP : ALBUMIN RATIO

CRP/Alb >0.095 POOR PROGNOSIS
Higher levels are associated with
- Larger tumor size
- Poorer differentiation
- Deeper tumor invasion
- More lymph node metastasis
- More distant metastasis
- More advanced TNM stage
- Early identification of cachexia

J Palliat Med. 2019 Jun 12. doi: 10.1089/jpm.2019.0102. C-Reactive Protein/Albumin Ratio Is an Independent Prognostic Predictor of Survival in Advanced Cancer Patients Receiving Palliative Care. Zhang J

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2-4 g/d

ONCO INFLAMMATION- CRP

- Curcuma Longa
- Scutellaria baicalensis
- Salvia milthiorrhiza
- Boswellia serrata
- Andrographis paniculate
- Tanacetum parthenium
- Resveratrol
- Berberine
- Omega 3 Fatty Acids

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CASE STUDY SUBMISSION

Important: In observance of HIPAA and the sacred trust between care giver and patient, absolutely no patient names or identifying information is to be disclosed. Patient privacy is to be preserved. If you attach any medical records, pathology, surgical or laboratory reports, all names are to be removed.

Date	5/9/17 RESUBMITTED ON 10/14/19 ****
Clinician Name & Credentials	Judy Pruzinsky, L.Ac.
Email	judy@judypruzinsky.com

Describe Your Patient (Please SUMMARIZE and use economy of words. You will have 15 minutes to present)

Age, Gender & Ethnicity	59 yr old woman of Scottish and Slovakian descent
Body Type	5'7" medium build
Values *What is most important to this patient? (Quality of Life, Decision Making, Side Effects?)*	Feeling good - clear, healthy. Understanding when something is not "right" in body, mind
Stress Resilience	She can take on a lot, and let go of a lot when stress builds up too much
Other	
Primary Diagnosis & Date *(ex. Breast Cancer L, T3 N1 M0, BRCA1 positive, grade 3, Ki67 > 45%)*	4 Mohs surgeries, March 2017, after almost two years of not going to Dermatologist. Two yrs ago patient hit a wall, after hx of 9 Mohs and didn't feel surgeries were sustainable health care, but couldn't deny suspected growths. SHE HAS NOT BEEN TO THE DERMATOLIGIST FOR 2.5 YEARS AND NOW FEELS SHE PROBABLY HAS SEVERAL NEW SITES ON HER FACE. ****
Secondary Diagnosis *(ex. Diabetes Type 2, Obesity)*	Background hx: -1999 to present 13 Mohs surgeries for basal and one squamous cell carcinoma, all on face, the majority were within 2011 and 2017, usually having at least one every six months around 1993, first basal cell - treated with alternative therapy (Compound X - a formula of Native American botanicals) biopsy negative twice then positive one year later - 1st Mohs surgery

Patient Status

☐ New Diagnosis ☐ Recurrence ☐ In Treatment ☐ In Recovery ☐ In Remission ☐ At Risk

Concomitant and/or Complicating Factors *(ex: poorly controlled diabetes, insomnia, poor support system)*	Even maintaining good diet, struggles with gas and bloating and cravings. Recently did Organix test, shows high bacterial growth, energy markers (citrate, succinate, hydroxymethyglutarate), ox. stress (8-Hydroxy-2-deoxyguanosine) and NT (5-hydroxyindoleacetate) Will be starting on gut cleanse soon. Hx of osteoporosis, maybe first dx 10 yrs ago. Has tried TRF, now on Osteoben
Adverse Effects of Cancer or Cancer Treatments *(ex. anxiety-depression, diarrhea, peripheral neuropathy)*	With dx of last 4 basal cells patient went into a fair amount of fear and resistance. First time I saw this to such an extent
Relevant Laboratory, Pathology & Medical Reports *(attach a PDF with patient identifying information removed or summarize)*	Standard blood test- low Vit D, even taking 5K IU daily, low Total Protein and Free T3 (first time)

Brief Summary of Recent History

Mostly paleo since 2011
(Mostly) gave up sugar, deeply into good nutrition mid '90s
Vegetarian (some of the years as vegan) 18 years, completing in mid '90s

Brief Summary of Additional Relevant Health, Medical, Psycho-Social and/or Family History

Lots of sunbathing as pre-teen into teens, whole body tanned easily except face (and to a lesser extent chest)
lots of sugar, and some fried foods as a child even through early 20s teen year snacks: french fries and milkshake
smoked cigarettes from age 12-22 (one to two packs per day)

Other Relevant Information

Such as Chinese or Ayurvedic diagnosis, Naturopathic/Homeopathic Information, etc. *(ex. Liver Qi Stagnation, Dysbiosis)*

Earth- Sp/St imbalance-dampness leading to yin deficient, some Liver-Gallbladder involvement, more in past presenting as stagnation (don't know if earlier in life this might have presented as Liver fire rising - easy to anger, rebellious)

Brief Summary of Relevant Past Oncology or Medical Treatments

(ex. surgery, radiotherapy, chemotherapy, immunotherapy, hormone therapy, drug therapy)

Summary of Recent and Current Treatments

Medical Oncology Care *(surgery, radiotherapy, chemotherapy, immunotherapy, hormone therapy, drug therapy)*

Integrative Oncology Care *(nutraceutical, botanical, phytochemical, acupuncture, energy medicine, other)*

Metabolic Synergy 4-6, Osteoben 2, B Supreme 1, Vit D Synergy 2, NeuroMag 2, Iodine Synergy 1, Brain Vitale 2, Digestyzme and HCl 1 each w meal, Omega Synergy 2 At Night: melatonin 2, magnesium malate 2 Recently: Niacinamide 500 mg TID
Rotates in / out: Immunitone, Broccoprotect, Zinc, PMeal or Cleanse and Greens Recently Acnutrol - thinks caused on-going diarrhea

Your 2 Core Questions (stated clearly and succinctly)

1. IS WONDERING IF THERE IS ANY NEW WISDOM IN THE WORLD OF INTEGRATIVE ONCOLOGY FOR SKIN CANCERS THAT ARE NOT MELANOMAS. ***
2. I SEE THE IMIQUIMOD CREAM TX NOW HAS FIVE YEAR FOLLOW-UP STUDIES SHOWING LOW RECURRENCE. IS THER MUCH NEGATIVE SIDE EFFECTS FROM THIS TREATMENT AND IF SO, WHICH BOTANICALS / NUTRACEUTICALS WOULD BE BEST TO MINIMIZE SUCH? *** I ALSO FOUND CLINICAL STUDIES CURRENTLY GOING ON AT SLOAN KETTERING. DO YOU

Attached Medical Records for Reference (with patient identifying information removed)

Case study submitted by Judy Pruzinsky L.Ac

Overview:

59-year old woman, who has numerous basal cell carcinomas.
She has not been able to bring herself to a dermatologist for 2.5 years, after having her last 4 Mohs surgeries (and 9 surgeries prior to that). She now feels she probably has several new sites on her face (always on her face) and is wondering if there is any new wisdom in the world of integrative oncology for skin cancers (non-melanoma).

Core Question:

1. Is wondering if there is any new wisdom in the world of integrative oncology for skin cancers that are not melanomas
2. I see the imiquimod cream tx now has five year follow up studies showing low recurrence. Are there much negative side effects from this treatment and if so which botanicals/nutraceuticals would be best to minimize such?
3. How would you view the benefits and disadvantages to certain forms of radiotherapy currently being piloted - Electronic Skin Surface Brachytherapy for Treating Basal Cell and Squamous Cell Skin Cancers and Photo Beam Radiotherapy?
4. **Lastly have you ever heard about Novadermy?** I am trying to get the exact steps to the treatment, but there are proprietary aspects that are not released. It is a process starting with a deep peel, followed by stem cells as part of the rejuvenating process. Unlike most deeper peels that I have heard of taking weeks to heal, complete with scabs, this process is a 10 day intensive and leave one only with some redness and sometimes swelling.

Judy Pruzinsky, L.Ac.: 2017 Case Notes

What is of most important in managing the terrain of basal cell carcinomas? Although one of the most benign forms of cancer, after 13 Mohs surgeries, the patient doesn't feel that way.

Most basal cell carcinomas: DNA damage occurred 10-20+ years prior

- Assess Tumor Microenvironment: ALWAYS assess factors identified

- Consider new immune therapies-
  - Topical - such as ZYCLARA Imiquimod which can also reveal subclinical lesions (approved for Actinic Keratoses and HPV Skin lesions (warts but also many studies on BCC) increases Interferon-*a,* CD3, CD4, CD8, CD11c, and CD68 T cells
  - Oral-Systemic Therapy Odomzo® (sonidegib) Tyrosine Kinase inhibitor: inhibits Hedgehog signaling pathway involved in BCC (many adverse SE) ***Resveratrol and Curcumin and Oridonin from Rabdosia rubescens also inhibit Hedgehog Signaling Pathway***

- Nutraceutical-Botanical Systemic Therapy must include inflammation control. Decrease NFkB (curcumin, Boswellia, Scutellaria baicalensis, O3FA), inhibit Hedgehog signalling pathway (Yu Jin Curcuma longa>curumin, resveratrol,

- Rabdosia rubescens), immune modulation (Ganoderma, astragalus, Coriolus, cordyceps), support for epithelial repair (Vit A), and specific targeted botanicals and phytochemicals (Parthenolide -Tanacetum parthenium -Feverfew), EGCG from Green Tea (Camellia sinensis, Tanshinones and Salvionolic acid: Salvia Milthiorhizza-Dan Shen), Scutellaria baicalensis-Huang Qin

- Acupuncture LI 4, LI 11, Sp10, St 36, GB 41, SJ5 (Lu 1, Lu 10)

- Example of Custom Compounded Formula from CHILKOV CLINIC

240ml	480ml	
20	40	**Salvia Red Sage** (Dan Shen)
40	80	**Tumeric Yu Jin** Curcuma longa
40	80	**Scutellaria baicalensis** Huang Qin
10	20	Oldenlandia(aka Heydotis) Bai Hua She She Cao
30	60	Gotu Kola Centella Asiatica
10	20	**Green Tea** Camellia Sinensis Cha Ye
10	20	**Tangerine peel** Citrus Reticulata Chen Pi
20	40	**Rabdosia rubescens Dong Ling Cao (oridonin)**
20	40	Cordyceps fungus Dong Chong Xia Cao
20	40	Ganoderma lucidum fungus Ling Zhi Reishi
10	20	Astragalus membranaceus Huang Qi

https://www.aad.org/media/stats-skin-cancer

Incidence rates

- Skin cancer is the most common cancer in the United States.[1-2]
- Current estimates are that one in five Americans will develop skin cancer in their lifetime[3-4]
- It is estimated that approximately 9,500 people in the U.S. are diagnosed with skin cancer every day.[5-7]
- Research estimates that nonmelanoma skin cancer, including basal cell carcinoma and squamous cell carcinoma, affects more than 3 million Americans a year.[5, 8]
- Research indicates that the overall incidence of BCC increased by 145 percent between 1976-1984 and 2000-2010, and the overall incidence of SCC increased 263 percent over that same period.[9]
    - Women had the greatest increase in incidence rates for both types of NMSC.[9]
    - NMSC incidence rates are increasing in people younger than 40.[9]
- More than 1 million Americans are living with melanoma.[10]
- It is estimated that 192,310 new cases of melanoma, 95,830 noninvasive (in situ) and 96,480 invasive, will be diagnosed in the U.S. in 2019.[6-7]
    - Invasive melanoma is projected to be the fifth most common cancer for both men (57,220 cases) and women (39,260 cases) in 2019.[6-7]
- Melanoma rates in the United States doubled from 1982 to 2011 and have continued to increase.[1, 7]
- Caucasians and men older than 50 have a higher risk of developing melanoma than the general population.[6-7, 11]
    - The incidence in men ages 80 and older is three times higher than women of the same age.[6]
    - The annual incidence rate of melanoma in non-Hispanic Caucasians is 26 per 100,000, compared to 4 per 100,000 in Hispanics and 1 per 100,000 in African Americans.[6]
- Skin cancer can affect anyone, regardless of skin color.
    - Skin cancer in patients with skin of color is often diagnosed in its later stages, when it's more difficult to treat.[12]
        - Research has shown that patients with skin of color are less likely than Caucasian patients to survive melanoma.[13]
        - Twenty-four percent of melanoma cases in African American patients are diagnosed at the regional stage, while 16 percent are diagnosed at the distant stage.[7]
    - People with skin of color are prone to skin cancer in areas that aren't commonly exposed to the sun, like the palms of the hands, the soles of the feet, the groin and the inside of the mouth. They also may develop melanoma under their nails.[12]
- Before age 50, melanoma incidence rates are higher in women than in men; however, rates in men are twice as high by age 65 and three times as high by age 80.[6]
    - It is estimated that melanoma will affect 1 in 27 men and 1 in 40 women in their lifetime.[7]
- Melanoma is the second most common form of cancer in females age 15-29.[14]
    - Melanoma incidence is increasing faster in females age 15-29 than in males of the same age group.[15]
- Research indicates that the incidence of melanoma in women 18-39 increased 800 percent from 1970 to 2009.[16]

    - Melanoma in Caucasian women younger than 44 has increased 6.1 percent annually, which may reflect recent trends in indoor tanning.[11]

Survival rates

- Basal cell and squamous cell carcinomas, the two most common forms of skin cancer, are highly curable if detected early and treated properly.[6, 17]
- The five-year survival rate for people whose melanoma is detected and treated before it spreads to the lymph nodes is 99 percent.[6-7]
- Five-year survival rates for regional and distant stage melanomas are 64 percent and 23 percent, respectively.[6-7, 15]

Mortality rates

- The vast majority of skin cancer deaths are from melanoma.[6]
- Nearly 20 Americans die from melanoma every day. In 2019, it is estimated that 7,230 deaths will be attributed to melanoma — 4,740 men and 2,490 women.[6-7]
    - Research indicates that men diagnosed with melanoma between the ages of 15 and 39 were 55 percent more likely to die from melanoma than females diagnosed with melanoma in the same age group.[18]
- People with SCC have a higher risk of death from any cause than the general population.[19]
- An estimated 4,420 deaths from skin cancers other than melanoma and NMSC are expected to occur in the United States in 2019.[6-7]

Risk factors

- Exposure to natural and artificial ultraviolet light is a risk factor for all types of skin cancer.[6]
- The majority of melanoma cases are attributable to UV exposure.[20-22]
- Increasing intermittent sun exposure in childhood and during one's lifetime is associated with an increased risk of squamous cell carcinoma, basal cell carcinoma and melanoma.[23]
    - Research suggests that regular sunscreen use reduces melanoma risk.[24-25]
    - Higher melanoma rates among men may be due in part to lower rates of sun protection.[1, 26]
- Even one blistering sunburn during childhood or adolescence can nearly double a person's chance of developing melanoma.[27]
    - Experiencing five or more blistering sunburns between ages 15 and 20 increases one's melanoma risk by 80 percent and nonmelanoma skin cancer risk by 68 percent.[27]
- Exposure to tanning beds increases the risk of melanoma, especially in women 45 and younger.[29-30]
    - Researchers estimate that indoor tanning may cause upwards of 400,000 cases of skin cancer in the U.S. each year.[31-32]
- Risk factors for all types of skin cancer include skin that burns easily; blond or red hair; a history of excessive sun exposure, including sunburns; tanning bed use; a weakened immune system; and a history of skin cancer.[6]
- People with more than 50 moles, atypical moles or large moles are at an increased risk of developing melanoma, as are sun-sensitive individuals (e.g., those who

sunburn easily, or have natural blond or red hair) and those with a personal or family history of melanoma.[6, 33]
- Melanoma survivors have an approximately nine-fold increased risk of developing another melanoma compared to the general population.[34]
- Men and women with a history of nonmelanoma skin cancer are at a higher risk of developing melanoma than people without a nonmelanoma skin cancer history.[35]
    - Women with a history of nonmelanoma skin cancer are at a higher risk of developing leukemia, breast, kidney and lung cancers, and men with a history of nonmelanoma skin cancer are at a higher risk of developing prostate cancer.[35]
- Caucasian individuals who have had more than one melanoma have an increased risk of developing both subsequent melanomas and other cancers, including those of the breast, prostate and thyroid.[36]

Prevention and detection

- Because exposure to UV light is the most preventable risk factor for all skin cancers, the American Academy of Dermatology encourages everyone to stay out of indoor tanning beds and protect their skin from the sun's harmful UV rays by seeking shade, wearing protective clothing and using a broad-spectrum, water-resistant sunscreen with an SPF of 30 or higher.
    - Because severe sunburns during childhood may increase one's risk of melanoma, children should be especially protected from the sun.[6]
- Skin cancer warning signs include changes in size, shape or color of a mole or other skin lesion, the appearance of a new growth on the skin, or a sore that doesn't heal. If you notice any spots on your skin that are different from the others, or anything changing, itching or bleeding, the American Academy of Dermatology recommends that you make an appointment with a board-certified dermatologist.
- The American Academy of Dermatology encourages everyone to perform regular skin self-exams to check for signs of skin cancer.
    - About half of melanomas are self-detected.[37-41]
- A dermatologist can make individual recommendations as to how often a person needs a skin exam from a doctor based on individual risk factors, including skin type, history of sun exposure and family history.
- Individuals with a history of melanoma should have a full-body exam by a board-certified dermatologist at least annually and perform regular self-exams to check for new and changing moles.[42]

Cost

- About 4.9 million U.S. adults were treated for skin cancer each year from 2007 to 2011, for an average annual treatment cost of $8.1 billion.[2]
    - This represents an increase over the period from 2002 to 2006, when about 3.4 million adults were treated for skin cancer each year, for an annual average treatment cost of $3.6 billion.[2]
- The annual cost of treating nonmelanoma skin cancer in the U.S. is estimated at $4.8 billion, while the average annual cost of treating melanoma is estimated at $3.3 billion.[2]
- Researchers estimate that there were nearly 34,000 U.S. emergency department visits related to sunburn in 2013, for an estimated total cost of $11.2 million.[43]

Learn more about skin cancer:

Basal cell carcinoma Melanoma Squamous cell carcinoma Skin Cancer Resource Center

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Multi-Drug Resistance

USING CHINESE BOTANICALS AND BOTANICAL ISOLATES IN CONJUNCTION WITH CHEMOTHERAPY TO OVERCOME MDR IN CANCER TREATMENT

APP.BOTANICALONCOLOGY.COM

Introduction

WHAT IS MDR?

MDR

- MDR is defined as insensitivity of cancer cells to cytotoxic and cytostatic actions of a number of structurally and functionally unrelated drugs. Cancer cells are intrinsically resistant to anti-cancer agents because of genetic and epigenetic heterogeneity.
- Also, there are some host factors which include poor absorption, rapid metabolism and excretion that can result in low serum drug levels. The mechanisms include alteration in the expression of proteins involved in the apoptotic signalling such as p53, Bcl2 family of proteins.
- Drug efflux proteins (P-gp, MRPs) and metabolising enzyme (CYP450) are major factors in drug interactions. Overlapping substrate specificities of these proteins result in complex and sometimes perplexing pharmacokinetic profiles of multidrug regimens.(Pal & Mitra, 2006).

MDR

- Multidrug-resistance (MDR) is the chief limitation to the success of chemotherapy. According to the National Cancer Institute, multidrug-resistance is a phenomenon where cancer cells adopt to anti-tumour drugs in such a way that makes the drugs less effective. Studies have shown that 40% of all human cancers develop MDR.
- Deaths due to cancer occur in most of the cases when the tumour metastasises. Chemotherapy is the only choice of treatment in metastatic cancer, and MDR limits that option.
- Cancer defends itself against chemotherapeutic regimes by several mechanisms including MDR. Therefore, a detailed understanding of ABC-(ATP-binding cassette) transporters mediated drug resistance would help to formulate strategies to overcome this problem.

MDR

- Chemotherapy kills drug-sensitive cells, but leaves behind a higher proportion of drug-resistant cells. As the tumour begins to grow again, chemotherapy may fail because the remaining tumour cells are now resistant.
- That cells have mechanisms to transport a variety of molecules out of the cytoplasm has been known for decades. For example, organic cation transporters were some of the earliest such mechanisms identified, and the kidney's secretory capability in this regard was first demonstrated in 1947.
- The presence of ATP-binding cassette, ABC-transport proteins in tumour cells circumvents an intracellular accumulation of chemotherapeutic drugs.

MDR prevention

- Recent investigations have shown that preventing the emergence of MDR at the onset of chemotherapy treatment, rather than reversing MDR once it has developed, may assist in overcoming drug resistance.
- Recent studies have demonstrated that several small-molecule inhibitors, including Pgp inhibitors, are capable at preventing the development of MDR when co-treated with cytotoxic drugs in different *in vitro* and *in vivo* model systems. Preventing or delaying the emergence of drug resistance is likely to enhance the effectiveness of chemotherapy and improve clinic outcomes for patients with cancer.

- A prevention strategy for circumventing drug resistance in cancer chemotherapy. Curr Pharm Des. 2001 Nov; 7(16):1595-614.
- Novel strategies to prevent the development of multidrug resistance (MDR) in cancer. Oncotarget. 2017 Oct 13; 8(48): 84559–84571. doi: 10.18632/oncotarget.19187

Gut Microbiome & Chemotherapy Metabolism

- The significance of the gut microbiota as a determinant of drug pharmacokinetics and accordingly therapeutic response is of increasing importance with the advent of modern medicines characterised by low solubility and/or permeability, or modified-release.
- These physicochemical properties and release kinetics prolong drug residence times within the gastrointestinal tract, wherein biotr
- Modulating the microbiome, either through exogenous replacement (probiotics) or curtailing interventions, such as antibiotics or specific inhibitors, affords exciting opportunities to improve healthcare outcomes and advance personalised medicine. Transformation by commensal microbes can occur.
- The Impact of the Gut Microbiota on Drug Metabolism and Clinical. Yale J Biol Med. 2016 Sep; 89(3): 375–382.

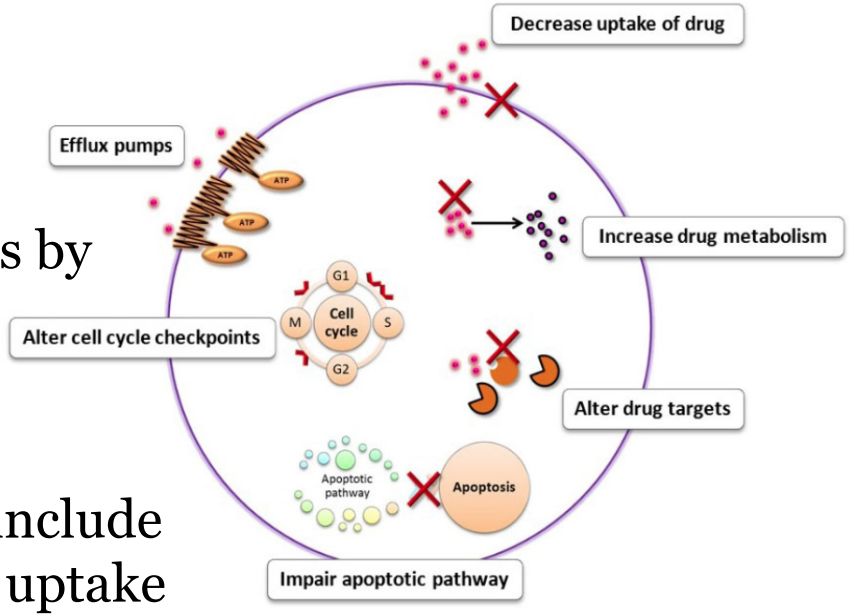
Chemotherapy-driven Dysbiosis

- Montassier et al., (2015) found that faecal samples collected after chemotherapy exhibited significant decreases in abundances of Firmicutes and Actinobacteria and significant increases in abundances of Proteobacteria compared to samples collected before chemotherapy.
- Following chemotherapy, patients had reduced capacity for nucleotide metabolism, energy metabolism, metabolism of co-factors and vitamins, and increased capacity for glycan metabolism, signal transduction and xenobiotics biodegradation.
- Chemotherapy-driven dysbiosis in the intestinal microbiome. AP&T. Volume42, Issue5.September 2015 Pages 515-528 https://doi.org/10.1111/apt.13302

Mechanisms of MDR towards cancer chemo-therapeutic drugs

The MDR cancer cells may subsequently develop cross-resistance to several unexposed and structurally unrelated chemotherapeutic agents

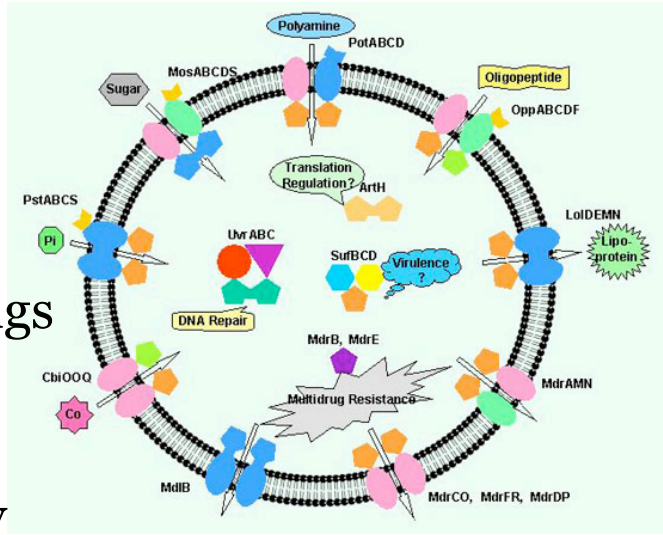
- Cancer cells can develop resistance to multiple drugs by various mechanisms as depicted.



- Mechanisms include (a) decreased uptake of drug, (b) reduced intracellular drug concentration by efflux pumps, (c) altered cell cycle checkpoints, (d) altered drug targets, (e) increased metabolism of drug and (f) induced emergency response genes to impair apoptotic pathway.

ATP-Binding Cassette (ABC)

ABC transporters are transmembrane proteins that utilise the energy of adenosine triphosphate (ATP) hydrolysis to carry out certain biological processes including translocation of various substrates across membranes and non-transport-related processes such as translation of RNA and DNA repair.



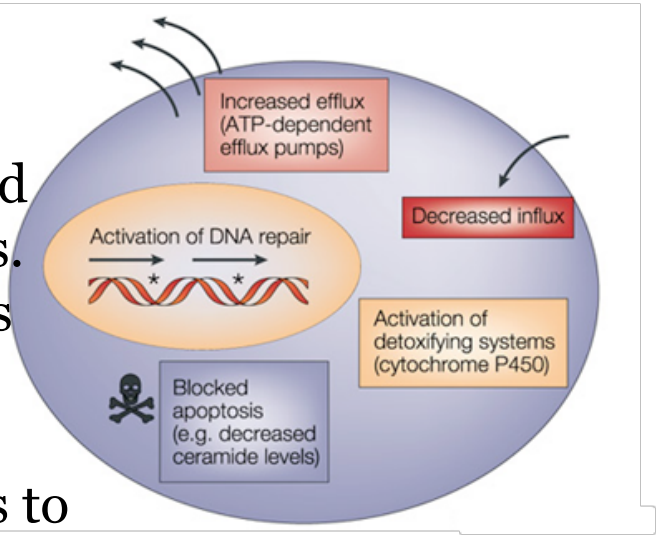
- When the ABC transporter proteins are overexpressed in cancer cells they can export anticancer drugs and render tumours resistant
- Nine ABCC subfamily members, the so-called Multidrug Resistance Proteins (MRPs) 1-9, have been implicated in mediating multidrug resistance in tumour cells.

MDR

Multidrug-resistance (MDR) is the chief limitation to the success of chemotherapy. According to the National Cancer Institute, multidrug-resistance is a phenomenon where cancer cells adopt measures to anti-tumour drugs in such a way that makes the drugs less effective.

Studies have shown that 40% of all human cancers develop MDR.

- Tumour cells adopt several mechanisms to evade death induced by anti-tumour agents. These include changes in apoptotic pathways and activation of cell-cycle check points to increase DNA repair.



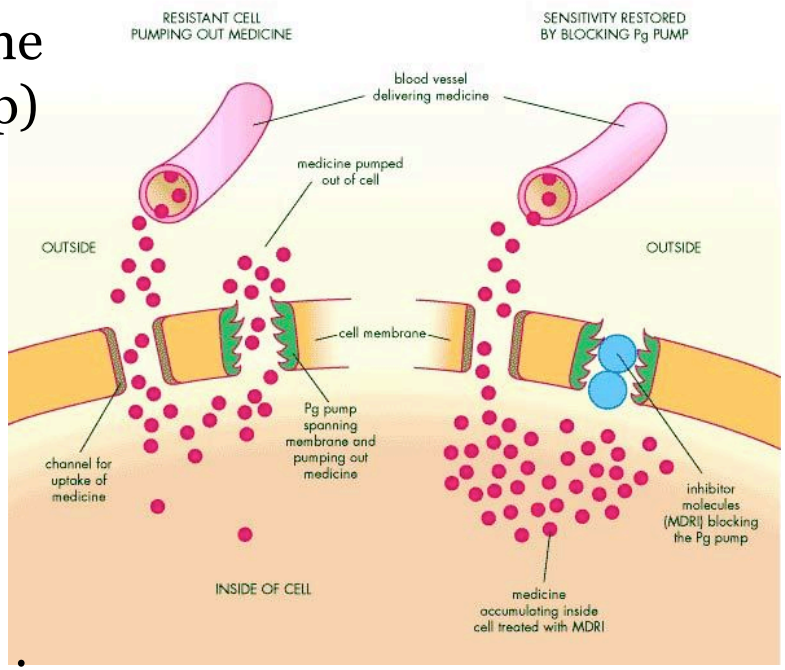
- Cancer cells develop resistance by increased expression of multidrug-resistant proteins and altered anti-tumour drug transport mechanisms.

MDR1
(Pgp)

P-gp plays an important role in altering the pharmacokinetics of a wide variety of drugs.

Tumours with detectable levels of P-gp are 3-4 fold more susceptible to chemotherapeutic failure than P-gp negative tumours. Therefore the role of P-gp in the development of MDR is very significant.

- Plasma membrane glycoprotein (Pgp) was the first ABC-transporter detected in various cancers exerting resistance to a variety of chemically unrelated cytotoxic agents including anti-tumour drugs such as doxorubicin, vinblastine, ritonavir, indinavir and paclitaxel. It works as an energy-dependent efflux pump and can recognise a wide range of substrates.

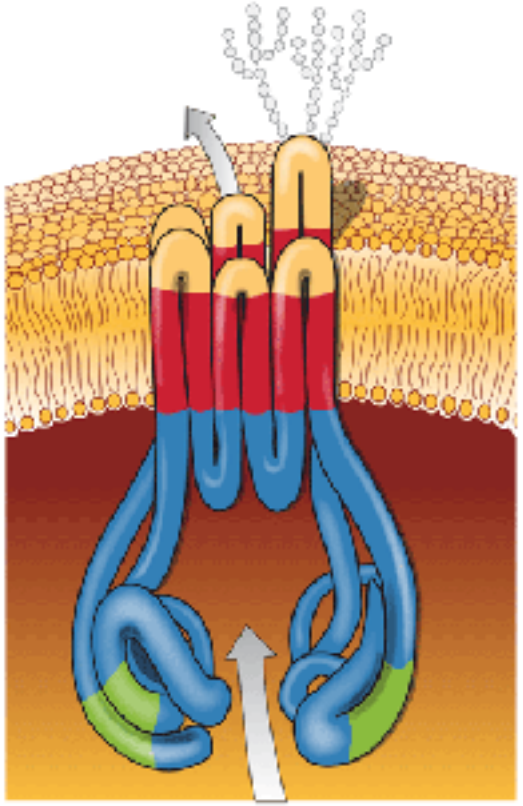


Pgp Efflux

The Pgp is a 170 kDa intrinsic membrane protein that effluxes a wide range of drugs from the cell.

The membrane lipid bilayer plays and important role in Pgp function and may regulate both binding and transport of drugs.

- Pgp expression has been linked to the efflux of chemotherapeutic drugs in human cancers leading to multidrug resistance.
- Pgp activity can also result in low oral absorption and poor brain penetration.
- Interaction of drugs with the Pgp may also cause an increase in toxicity of co-administered compounds.



Multidrug Resistance Protein 1 (MRP1)

Like Pgp, MRP1 is also overexpressed in tumour cells and represents a major obstacle to drug delivery.

- High levels of expression of MRP1-7 proteins were observed in non-small-cell lung cancer. In breast cancer, there is also a significant expression of this protein which may increase the chance of treatment failure.
- Studies have also shown that over expression of MRP causes resistance to methotrexate (MTX), also known as BCRP, ABCP and ABCG2 and anti-foliates such as ZD1694 in colorectal cancer.

Breast Cancer Resistance Protein (BCRP)

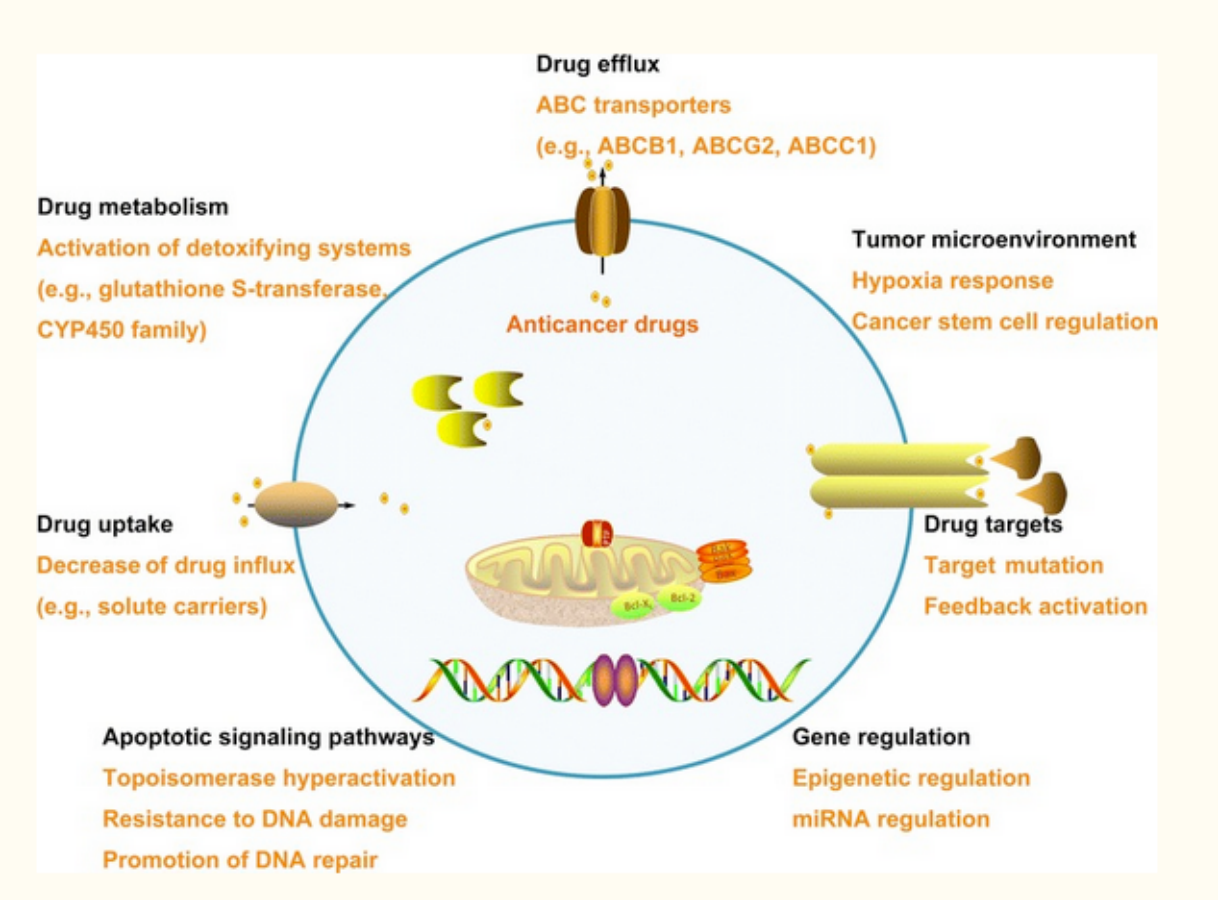
Overexpression of BCRP was reported in the plasma membrane of drug-resistant ovarian, breast, colon, gastric cancer, and fibrosarcoma cell lines.

- Since BCRP is expressed in the gastrointestinal tract, it is thought that this protein may affect the bioavailability of the drugs. Its overexpression in several types of cancer makes it a relevant target of strategies aimed at defeating multidrug-resistance.
- BCRP belongs to a novel branch of the ABC-transporter family. The members of this subfamily are about half the size of the full-length ABC transporters, thus known as half-transporters.

The main mechanism of MDR is overexpressing ATP-binding cassette (ABC) transporters to increase drug efflux, resulting in a decrease in intracellular drug concentration.

Other mechanisms of MDR are reducing drug uptake by influx transporters, boosting drug metabolism, blocking apoptotic signalling pathways, elevating adaptability by epigenetic regulation and microRNA regulation, mutation in drug targets or feedback activation of other targets and signalling pathways, and change of tumour microenvironment.

ABCB1, ATP-binding cassette subfamily B member 1; ABCG2, ATP-binding cassette subfamily G member 2; ABCC1, ATP-binding cassette subfamily C member 1; CYP450, cytochrome P450



Li et al., 2017

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Botanicals & MDR

HERBS AND ISOLATES IN MDR

Chinese Botanicals and Isolates

- Chinese botanicals and isolates (CBIs) consist of a large number of components and have been safely used in humans for thousands of years, they could serve as a natural chemical pool for screening of MDR modulators. A considerable portion of anti-cancer agents currently used in the clinic were isolated from medicinal plants.
- Certain CBIs not only have anticancer properties, but may also alter the expression or function of drug transporters. The combined use of CBIs with conventional chemotherapeutic drugs may increase the efficacy and reduce the side effects (Wang Zj et al, 2010).

Chinese Botanicals and Isolates

- P-gp is vulnerable to inhibition, activation or induction by many compounds as well as CBIs (Breier et al, 2005).
- Modulation of P-gp activity with selective inhibitors could also be a useful strategy to increase the oral bioavailability of P-gp substrate drugs, in particular, to develop oral formulations of anticancer drugs transported by P-gp .

Multi-Drug Resistance (MDR)

- Ginsenosides
  - Rg3 reversed multi-drug resistance and restored the sensitivity of resistant KBV20 cell line to various anticancer drugs. Increased animal life span in an in vivo MDR model in a dose-independent manner (Kim SH et al, 2003; Yue et al, 2006).
  - Combination of purified saponins containing Rb1, Rb2, Rc, Rd, Re and Rg1 reversed MDR (Liu et al, 2008; Si & Tien, 2005)
- Quercetin/Kaempferol
  - Quercetin is less potent than kaempferol but more effective than genistein and daidzein in reversing MDR (Kioka et al, 1992).
  - Quercetin reverses MDR through action on mitochondrial membrane potential and the induction of apoptosis (Kothan et al, 2004)
- Puerarin
  - Down-regulates MDR1 expression via nuclear factor κ-B and CRE (cAMP response element) transcriptional activity (Hien et al, 2010)

Plasma Membrane Glycoprotein (Pgp)

- Curcumin
  - Enhances sensitivity to vincristine by the inhibition of P-gp in SGC7901/VCR cell line (Chang et al, 2006).
  - Curcumin derivatives reversed MDR by inhibiting P-gp efflux (Tang et al, 2005)
  - Bisdemethoxycurcumin modified from curcumin resulted in greater inhibition of P-gp expression (Um et al, 2008).
  - Curcumin derivatives reversed MDR by inhibiting P-gp efflux (Limtrakul et al, 2004).
- Honokiol
  - Down-regulates expression of P-glycoprotein at mRNA and protein levels in MCF-7/ADR, a human breast MDR cancer cell line (Xu et al, 2006)
- Quercetin
  - Inhibits overexpression of P-gp mediated by arsenite (Kioka et al, 1992).

P-glycoprotein

- Pheophorbide a (Pa) from Scutellaria barbata could significantly inhibit the growth of R-HepG2 cells with an IC50 value at 25.0 microM after 48 hours treatment (Tang et al, 2007).
- Scutellaria barbata and low dose 5-FU can significantly inhibit the tumour growth both in vitro and in vivo (Xu et al, 2013).
- Scutellaria barbata (SB) elevated expression of Bax, p53, Akt, and JNK by up-regulating the apoptotic pathway and down-regulating the survival pathway in prostate cancer cells (Wong et al, 2009)

P-glycoprotein

- Steroidal saponin from *Trillium tschonoskii* (TTS) could reverse the MDR in HCC cells and significantly enhance chemosensitisation. TTS inhibited HepG2 and R-HepG2 cells survival in a dose-dependent manner by 75% and 76%, respectively ($p<0.01$), as well as colony formation 77% and 81% ($p<0.01$).
- TTS also repressed expression of many other MDR genes, such as MRP1, MRP2, MRP3, MRP5, MVP and GST-π. In vivo, TTS dose-dependently reduced R-HepG2 cells xenografts tumour formation by inhibiting tumour cells proliferation (Wang et al, 2013)

P-glycoprotein

- Kampo extract medicines, Senkyu-cha-cho-san (SCCS) and Sokei-kakketsu-to (SKKT), effected intestinal absorption of P-glycoprotein (P-gp) in vivo.
- Concomitant administration of each Kampo extract medicine unexpectedly showed the tendency to decrease Cmax and AUC of talinolol. Decreased intestinal absorption of talinolol might be caused, not by the inhibition of P-gp, but by the inhibition of organic anion transporting peptides by both Kampo extract medicines (Iwanaga et al, 2012).

Formulas

- SCCS: Chuan Xiong Cha Tiao San
  - Menthae Haplocalycis (bo he)
  - Ligusticum chuanxiong (chuan xiong)
  - Angelicae Dahuricae (bai zhi)
  - Notopterygium incisum (qiang huo)
  - Asarum heterothropoides (xi xin)
  - Cyperus Rotundi carbonized (chao xiang fu)
  - Schizonepeta tenuifolia (jing jie)
  - Ledebouriellae Divaricatae (fang feng)
  - Glycyrrhizae Uralensis Preparata (zhi gan cao)
  - Cameliae (lu cha)
- SKKT: Shu Jing Huo Xue Tang
  - Paeoniae Alba (bai shao)
  - Angelicae Sinensis (dang gui)
  - Ligusticum chuanxiong (chuan xiong)
  - Rehmanniae Glutinosae (sheng di)
  - Pruni Persicae (tao ren)
  - Atractylodes lancea (cang zhu)
  - Poriae Cocos (fu ling)
  - Achyranthis Bidentatae (niu xi)
  - Clematis chinensis (wei ling xian)
  - Stephaniae Tetrandrae (han fang ji)
  - Notopterygium incisum (qiang huo)
  - Ledebouriellae Divaricatae (fang feng)
  - Gentianae Longdancao (long dan cao)
  - Angelicae Dahuricae (bai zhi)
  - Citri Reticulatae (chen pi)
  - Glycyrrhizae Uralensis (gan cao)
  - Zingiberis Recens (sheng jiang)

Diosgenin

- Diosgenin is a naturally occurring steroidal sapogenin and is one of the major bioactive compounds found in Trigonella foenum-graecum seeds. In addition to being a lactation aid, diosgenin has been shown to be hypocholesterolaemic, gastro-and hepato-protective, anti-oxidant, anti-inflammatory, anti-diabetic, and anti-cancer. Diosgenin has a unique structural similarity to oestrogen.
- Diosgenin has also been reported to reverse multi-drug resistance in cancer cells and sensitise cancer cells to standard chemotherapy. (Sethi, Shanmugam, Warrier et al., 2018

Diosgenin

- The anticancer mode of action of diosgenin has been demonstrated via modulation of multiple cell signalling events involving critical molecular candidates associated with growth, differentiation, apoptosis, and oncogenesis.
- Altogether, these preclinical and mechanistic findings strongly implicate the use of diosgenin as a novel, multitarget-based chemo-preventive or therapeutic agent against several cancer types. (Raju & Mehta 2009)

Diosgenin

- Akt signalling has gained recognition for its functional role in more aggressive, therapy-resistant malignancies and is frequently constitutively active in cancer cells. Diosgenin inhibits pAkt expression another functional downstream target of Akt, was inhibited by Diosgenin in ER(+) but not in ER(-) BCa cells.
- Additionally, we found that diosgenin caused G1 cell cycle arrest by downregulating cyclin D1, cdk-2 and cdk-4 expression in both ER(+) and ER(-) BCa cells resulting in the inhibition of cell proliferation and induction of apoptosis. and XIAP. The Raf/MEK/ERK pathway.
- Studies indicate diosgenin significantly inhibits tumour growth in both MCF-7 and MDA-231 xenografts in nude mice. (Srinivasan, Koduru, Kumar et al., 2009)

Breast Cancer Resistance Protein (BCRP)

- Flavonoids, a major class of natural compounds widely present in foods and herbal products, have been shown to be human BCRP inhibitors (Zhang S et al, 2005).
- The flavones Retusin (Origanum vulgare) and Ayanin (Croton schiedeanus) were found to be highly potent inhibitors of BCRP (Pick et al, 2011)
- Chrysin (Passiflora caerulea) and biochanin A (Trifolium pratense) were the most potent BCRP inhibitors, producing significant increases in mitoxantrone accumulation at concentrations of 0.5 or 1.0μM and in mitoxantrone cytotoxicity at a concentration of 2.5μM (Zhang S et al, 2004; Wang & Morris, 2007).

Apoptosis pathways in cancer

All: Cancer Letters. Volume 332, Issue 2 May 2013

- Emodin and Rhein from *Rheum* (Dahuang) and *Polygonum cuspidatum* (Huzhang) produce ROS and ROS-induced apoptosis.
- Artemisinin from *Artemisia annua* (Qinghao) been shown to kill tumour cells through a ROS-dependent mechanism.
- Berberine from *Coptis chinensis* (Huanglian) induces ROS-mediated apoptosis.
- The flavones Chrysin and Apigenin from *Scutellaria Baicalensis* (Huangqin) potentiate the cytotoxicity of anti-cancer drugs by depleting cellular GSH.
- Wogonin from *Scutellaria Baicalensis* (Huangqin) can sensitise TNFα-resistant tumour cells to undergo TNFα-induced apoptosis.

Apoptosis pathways in cancer

- Evodiamine, a constituent from Chinese herb Evodiae fructus (Wuzhuyu) promoted the phosphorylations of Raf-1 kinase and Bcl-2 and was found to be superior to that of paclitaxel (Carcinogenesis 2005; 26:968-75).
- Kanglaite (KLT) injection is an anti-tumour new drug which extracts from Chinese medicine-*coix seed* and could inhibit some anti-apoptotic gene and activate some pro-apoptotic genes. KLT may induce the apoptosis of tumour cell by way of up-regulate the expression of p53 genes (Lu et al, 2008).
- Several studies show that phytochemicals, such as curcumin, EGCG, genistein, quercetin, and reseveratrol, can reverse chemo-resistance to cisplatin and/or paclitaxel by modulating NF-κB or/and MDR-transporter proteins both in vitro and in vivo (Surh, 2003; Aggarwal et al, 2004; Weir et al, 2007).

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Chinese Botanical Studies

HERBS, ISOLATES AND COMPOUNDS

Chinese Medicinals and MDR

- Efferth et al. (2002) investigated the activity of 22 drugs from traditional Chinese medicine toward sensitive and MDR1- or MRP1-overexpressing multidrug-resistant human CCRF-CEM leukaemia cells.
- These herbal drugs included artesunate, artemisinin, baicalein, baicalin, berberine, bufalin, cantharidin, cephalotaxine, curcumin, daidzein, daidzin, diallyl disulfide, ginsenoside Rh2, glycyrrhizic acid, isonardosinon, homoharringtonine, nardosinon, nardofuran, puerarin, quercetin, tannic acid, and tetrahydronardosinon.
- TCM-derived compounds can modulate multidrug resistance. Artesunate and bufalin revealed both high anti-leukaemic activity if applied alone as well as modulation effects in combination with daunorubicin. Homoharringtonine, artesunate, and bufalin have potent anticancer activity. The latter two also increased the accumulation of daunorubicin in the multidrug resistant cells.

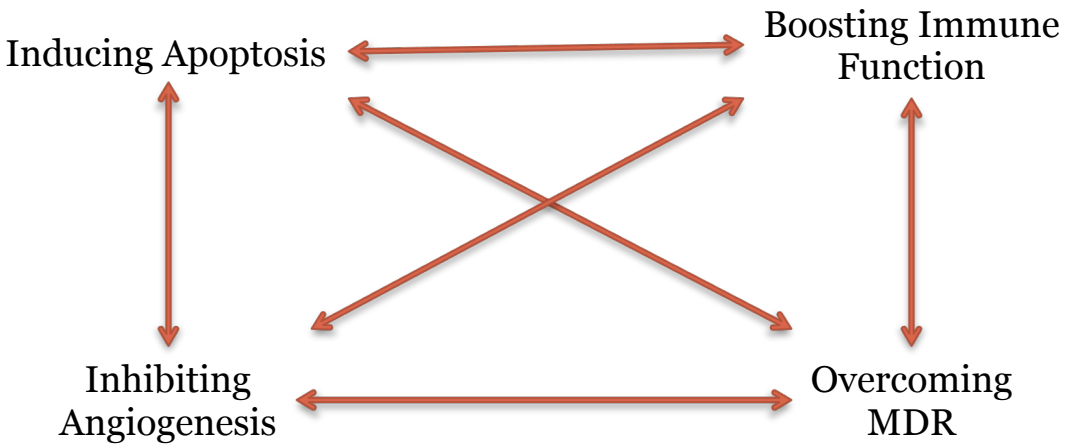
Chinese Medicinals and MDR

- Fractions from the PB group (herbs with the ability to promote blood circulation and remove blood stasis) showed more significant effects than fractions from the CH group (herbs with the ability to clear away heat and toxic materials). Fractions from dichloromethane (CH_2Cl_2) extracts were more effective than fractions from ethyl acetate (EtOAc) extracts (Yang L et al, 2011).
- Aqueous extracts of 12 Chinese medicinal herbs, Anemarrhena asphodeloides, Artemisia argyi, Commiphora myrrha, Duchesnea indica, Gleditsia sinensis, Ligustrum lucidum, Rheum palmatum, Rubia cordifolia, Salvia chinensis, Scutellaria barbata, Uncaria rhychophylla and Vaccaria segetalis demonstrated growth inhibitory activity on some or all of the cancer cell lines, but only two showed activity against the normal mammary epithelial cells (Shoemaker et al, 2005).

Traditional Chinese Medicine in Cancer Therapy

There is a molecular basis for the reported synergies of TCM herbs when administered alongside so-called 'conventional therapies' in tumour cell regulation, which help in bringing about homeostasis (Parekh et al, 2009).

- By identifying potent bio-actives derived from TCM, and tailoring formulations that encapsulate/ incorporate them into cutting-edge drug delivery systems for parenteral administration, one can envision overcoming the shortfalls that have prevented TCM being accepted by the West as a real adjunct/alternative to conventional cancer therapies.
- With a library of over 250,000 individual therapeutic compounds at our disposal, many of which have yet to be successfully isolated and tested for both safety and efficacy, there are certainly challenges that lay ahead.



Sheng Mai Injection

- 'Shengmai Injection' (SMI), consisting of *Panax ginseng* (renshen) and *Ophiopogon japonicus* (maidong), down-regulated P-gp expression in peripheral blood lymphocyte membrane. When used together with oxaliplatin, 5-fluorouracil or folinic acid, the injection prolonged the survival rate of colon cancer patients (Kaye, 1999).
- The injection also enhanced the efficacy of tamoxifen and nifedipine in combination therapy (Wang & Yang, 2001).
- SMI might improve human immune function to facilitate the chemotherapy of patients with stomach cancer (Lin et al, 1995).
- SMI injection would not influence the efficacy of chemotherapy on advanced NSCLC patients, while it could improve the quality of life, increase the body weight of patients, alleviate adverse reactions of chemotherapy as myelosuppression so as to improve the tolerance of organism to chemotherapy (Cao et al, 2008).

Kanglaite Injection

- Kanglaite (KLT) was effective in reversing MDR of cells and increasing the sensitivity of cancer cells to chemotherapeutic agents by a quite small effective dosage (2-8μl/ml) which was far below its IC50 for K562/vcr cells (Yang H et al, n.d.).
- KLT has been proven to play its anticancer role through inhibition of the mitosis of tumour cells during G2/M phases, induction of apoptosis and inhibition of the formation of newly generated blood vessels (Li, 2001).
- Standard treatment course for KLT is 200 ml (2 bottles) per day via intravenous drip x 42 days (84 bottles). Clinical experiences in China and Russia suggest 2 treatment courses (Ruan et al, 2006).

Artemisinin, Artesunate and Di-hydroartemisinin

- A, A & D-h increased cytotoxicity of pirarubicin and doxorubicin in P-glycoprotein-overexpressing K562/adr cells, and in MRP1-overexpressing GLC4/adr cells (Reungpatthanaphong & Mankhetkorn , 2002).
- Artesunate all showed blockade of rhodamine (Rho)-123 transport and decreased basal-to-apical (B-A) P-gp-mediated DIG transport at concentrations of 100 μM and 1 mM (Oga et al, 2012).
- Artesunate induced apoptosis and reactive oxygen species in neuroblastoma cells that over-express P-glycoprotein (Michaelis et al, 2009). Downregulates expression of Survivin mRNA (Wang, 2010).
- More than 70 cell lines from different tumour types have been reported to be inhibited by artesunate and its related compound artemisinin (Efferth et al, 2001; Efferth et al, 2003).

Schisandra lignans extract (SLE)

- 10μM verapamil (a known P-gp inhibitor) and SLE (0.5, 2.0, and 10.0μg/ml) were observed to significantly enhance the uptake and inhibit the efflux ratio of P-gp substrates in Caco-2 and L-MDR1 cells.
- A single-dose SLE at 500mg/kg could increase the area under the plasma concentration time curve of digoxin and vincrisine significantly without affecting terminal elimination half-time.
- Long-term treatment with SLE for continuous 10 days could also increase the absorption of P-gp substrates with greatly down regulation of P-gp expression in rat intestinal and brain tissues (Liang et al, 2013).

Species (pin yin name)	Family	Active Components	In Vitro and In Vivo Activity	Refs
Fructus Schizandrae (wu wei zi)	Magnoliaceae	schizandrin A schizandrin B schizandrin C schizandrol A schizandrol B	25 μg/ml (KBv200, MCF-7/Dox, vincristine)	Huang M et al, 2008
Silybum marianum	Asteraceae	silymarin	Inhibited OATP1B1- and BCRP-mediated rosuvastatin (BCRP) transport	Deng et al, 2008
Isolate & Source	Grapevines, pine, soy and legumes	Resveratrol	30 μM (MCF7, mitoxantrone, bodipy-FL-prazosin)	Cooray et al, 2004
	Soy	Daidzein (8-hydroxydaidzein)	Decreased mRNA expression levels of MDR1 and MRP 1	Lo, 2012
	Citrus fruits	Hesperetin & Naringin	Significant inhibition of OATP2B1	Shirasaka et al, 2013
	Soy	Genistein	3 μM-10 μM (K562/BCRP, SN-38 and mitoxantrone)	Imai et al, 2004

Species (pin yin name)	Family	Active Components	In Vitro and In Vivo Activity	Refs
Curcuma wenyujin (yu jin)	Zingiberaceae	Sesquiterpenes Diterpenoids	Markedly increased doxorubicin accumulation in MCF-7/ADR cells.	Yang L et al (1), 2011
Chrysanthemum indicum (ye huang ju)	Asteraceae	Pyrethrins	Sensitised resistant cancer cells at a non-toxic concentration (10 μg mL^{-1})	
Salvia chinensis (dan shen)	Lamiaceae	Sesquiterpenes	Enhanced apoptosis induced by doxorubicin in MCF-7/ADR cells, and restore the effect of docetaxel on the induction of G2/M arrest in A549/Taxol cells	Yang L et al (2), 2011
Ligusticum chuanxiong (chuan xiong)	Apiaceae	Ligustrazine Tetramethylpyrazine		
Cassia tora (jue ming zi)	Fabaceae	Cinnamaldehyde	Salvia reduced microvessel density (MVD)	Liu F et al, 2012
Glycyrrhiza glabra (gan cao)	Leguminosae	Glycyrrhetinic acid	100 μM(KB/MRP, doxorubicin) Targets both the proteasome and peroxisome proliferator-activated receptors (PPARs)	Nabekura et al, 2008; Lallemand et al, 2011

Source	Isolate	In Vitro and In Vivo Activity	Refs
Citrus , tomato	Naringenin	3 μM-10 μM (K562/BCRP, SN-38 and mitoxantrone)	Imai et al, 2004
Citrus	Tangeretin	Inhibited P-glycoprotein and increased doxorubicin accumulation	Wesołowska et al, 2012
Scutellaria polyodon, Cirsium rhinoceros	Acacetin	1 -3 μM (K562/BCRP, SN-38 and mitoxantrone)	Imai et al, 2004; Shim et al, 2007
Ginger	Kaempferol (Zingiber zerumbet)	2 -3 μM (K562/BCRP, SN-38 and mitoxantrone).	Imai et al, 2004
Ginger	Kaempferol (Zingiber zerumbet)	Showed a potent P-gp inhibitory effect	Chung et al, 2007
Grapes, onions, tea	Myricetin	25 μM (MDCKII-MRP1, vincristine) Inhibited MRP1 by perturbation of the lipid phase of membranes.	van Zanden et al, 2005; Zhang Sz et al, 2004; Wesołowska et al, 2009

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Botanicals and Chemotherapy

ADVANTAGES, LIMITS AND CONCERNS

Complementary Oncology

- Scientifically-based therapies of complementary medicine cannot replace the well studied conventional cancer-destructive therapies such as surgery, chemo-, radio- or hormone therapy. Accordingly, they are by no means "alternative therapies".
- Complementary approaches in oncology that are recommended as additional to standard cancer destructive therapies claim to optimise this therapy. A great body of data emerging from scientifically sound clinical trials prove that defined complementary procedures are beneficial for the patients (Beuth & Schierholz, 2007).

Drug/Herb Interaction

- P-glycoprotein (P-gp) and cytochrome P450 3A4 (CYP3A4) together constitute a highly efficient barrier for many orally absorbed drugs. Available literature and clinical reports indicate that many drugs and herbal active constituents are substrates for both P-gp and CYP3A4.
- Exposure with pure herbal agents such as hypericin, kaempferol and quercetin or extract of SJW resulted in higher uptake or influx of ritonavir and erythromycin. Hypericin, kaempferol and quercetin also caused a remarkable inhibition of cortisol metabolism. (Pal & Mitra, 2006).
- Multiple doses of baicalin decreased the expression of hepatic CYP3A2 by approximately 58% (Tian et al, 2013)

Drug/Herb Interactions

- Inhibition or induction of P-gp by co-administered drugs or food as well as herbal constituents may result in pharmacokinetic interactions leading to unexpected toxicities or under-treatment. On the other hand, modulation of P-gp expression and/or activity may be a useful strategy to improve the pharmacological profile of anticancer P-gp substrate drugs.
- Several variants in the ABCB1 coding regions result in amino acid change and potentially affect P-gp expression and activity. Hoffmeyer et al (2000) reported an association among a SNP in exon 26 (C3435T) of ABCB1, reduction in duodenal P-gp levels, and higher peak plasma concentrations of the P-gp substrate digoxin in healthy volunteers.
- Also, genetic variability in the MDR1 gene affects absorption and tissue distribution of P-gp substrate drugs (Marchetti et al, 2007).

Drug/Herb Interactions

- The prevalence of CAM use among cancer patients receiving conventional therapy is 54%– 77%, and that about 72% of patients do not inform their treating physician. CAM use significantly increases the risk for interactions with anticancer drugs, especially because of the small therapeutic range and steep dose–toxicity curve of these drugs (Lee et al, 2012).
- CAM use substantially increases the risk for interactions with anticancer drugs, especially because of the narrow therapeutic window of these compounds. However, for most CAMs, it is unknown whether they affect metabolising enzymes and/or drug transporter activity (Marchetti et al, 2007).
- It is important to note that various methods have been used to conduct in vivo and in vitro assessments of herb-drug interactions (Zhou et al, 2003). Yet, many of these studies were conducted in animals but not in humans (Lee et al, 2012).

CYP 3A4

- P-glycoprotein (P-gp), multiple drug resistance associated proteins (MRPs), and cytochrome P450 3A4 together constitute a highly efficient barrier for many orally absorbed drugs.
- Drug efflux proteins (P-gp, MRPs) and metabolising enzyme (CYP450) are major factors in drug interactions. Overlapping substrate specificities of these proteins result in complex and sometimes perplexing pharmacokinetic profiles of multidrug regimens (Pal & Mitra, 2006).

COP & BER

- Coptis extract (COP) and its major constituent, Berberine (BER) increase ROS production, reduce MDR, and enhance the inhibitory effects of chemotherapeutic agents on A549 cell growth.
- Combinations of COP or BER with chemotherapeutic agents (5-FU, CPT, and TAX) exhibited a stronger inhibitory effect on A549 cell growth (He et al, 2012).
- Repeated administration of Berberine (300 mg, t.i.d., p.o.) decreased CYP2D6, 2C9, and CYP3A4 activities (Guo et al, 2011). CYP2D6 was inhibited by tetrahydropalmatine (THP) from Corydalis genus or Stephania rotunda and BER (Zhao Y et al, 2012).

COP & BER

- BER caused CYP3A4 and P-glycoprotein inhibition in the liver and gut wall, respectively, and because of an increase in gastric-emptying time causing increased cyclosporine A bioavailability and reduced metabolism (Cicero & Ertek, 2009).
- Cyclosporin A, a circumventor of MDR, markedly decreases glucosylceramide levels (Lavie et al, 1997). Glucosylceramide has clinical significance for the early identification of drug-resistant tumours (Lucci et al, 1998).
- Levels of glucosylceramide synthase mRNA, glucosylceramide synthase protein, and P-glycoprotein (P-gp) increased in parallel (Gouazé et al, 2004).

Tetrandrine

- Tetrandrine (TET) reversed MDR in vitro and modulated Pgp-mediated drug efflux (Tian & Pan, 1997; Choi et al, 1998). A combination of tetrandrine with doxorubicin or vincristine in vitro demonstrated synergistic anticancer effects (Sun et al, 1999). Tetrandrine reduced P-gp expression (Li et al, 2006).
- TET at the tested dose of combination treatment could achieve plasma concentrations that reversed MDR in experimental models and it had no apparent effect on doxorubicin pharmacokinetics in mice and CYP 3A4 activity in human liver microsomes (Dai et al, 2007).

Tetrandrine

- Co-administration of TET restores the sensitivity of MDR cancer cells to doxorubicin, paclitaxel, docetaxel, and vincristine (Fu LW et al, 2002; Zhu X et al, 2005) through the inhibition of P-gp.
- In mice with MDR MCF-7/adr or KBv200 cell xenografts, co-administration of TET increases the anticancer activity of doxorubicin and vincristine without a significant increase in toxicity (Fu L et al, 2004).
- **Cytotoxicity:** Dogs administered tetrandrine at low dose (3 mg/kg) and medium dose (10 mg/kg) levels showed no toxic changes. Administration of the drug at a high dose level (40 mg/kg) for 2 months may induce focal necrosis of liver cells, abnormal liver function, and acceleration of erythrocyte sedimentation rate. After the dogs had received tetrandrine for 6 months continuously, necrosis of liver tissue appeared (Tainlin et al, 1982).

Herb	Anti-Cancer Effect	Reference	CYP 450 Enzyme	Reference
Salvia miltiorrhiza (dan shen)	Diminution of cell proliferation. Neo-tanshinlactone is 10-fold more potent than tamoxifen citrate	Lee CY et al, 2008 Wang et al, 2004.	Inhibits CYP 1A2, 2C9, 2D6 and 3A4.	Chan, 2001; Qui et al, 2008
Panax ginseng (ren shen)	Ginsenoside Rp1 inhibits insulin-like growth factor 1 receptor (IGF-1R)/Akt pathway and cancer cell proliferation.	Kang et al, 2011	Inhibits CYP3A4, 2C9, 2C19, 2D6, 2E1	Henderson et al, 1999; Foster et al, 2002
Angelica sinensis (dang gui)	Acetone extract could induce G1/S arrest and activate the mechanism of apoptosis in human cancer cells	Cheng YL et al, 2004	Inhibits CYP 1A2, 2C9, 2D6, 2E1 and 3A	Lo et al , 1995; Sevior et al, 2010
Rheum officinale (da huang)	Emodin reverses multi-drug resistance in MCF-7/Adr cells and down-regulates ERCC1 protein expression. Suppressed the tumour growth derived from Side population (SP) cells through down-regulating ABCG2 expression. Down-regulates MRP1	Fu JM et al, 2012; Li XX et al, 2013	Inhibits CYP1A1, CYP1B1, CYP2E1	Sun et al, 2000; Shimpo et al, 2003; Mahadevan et al, 2007

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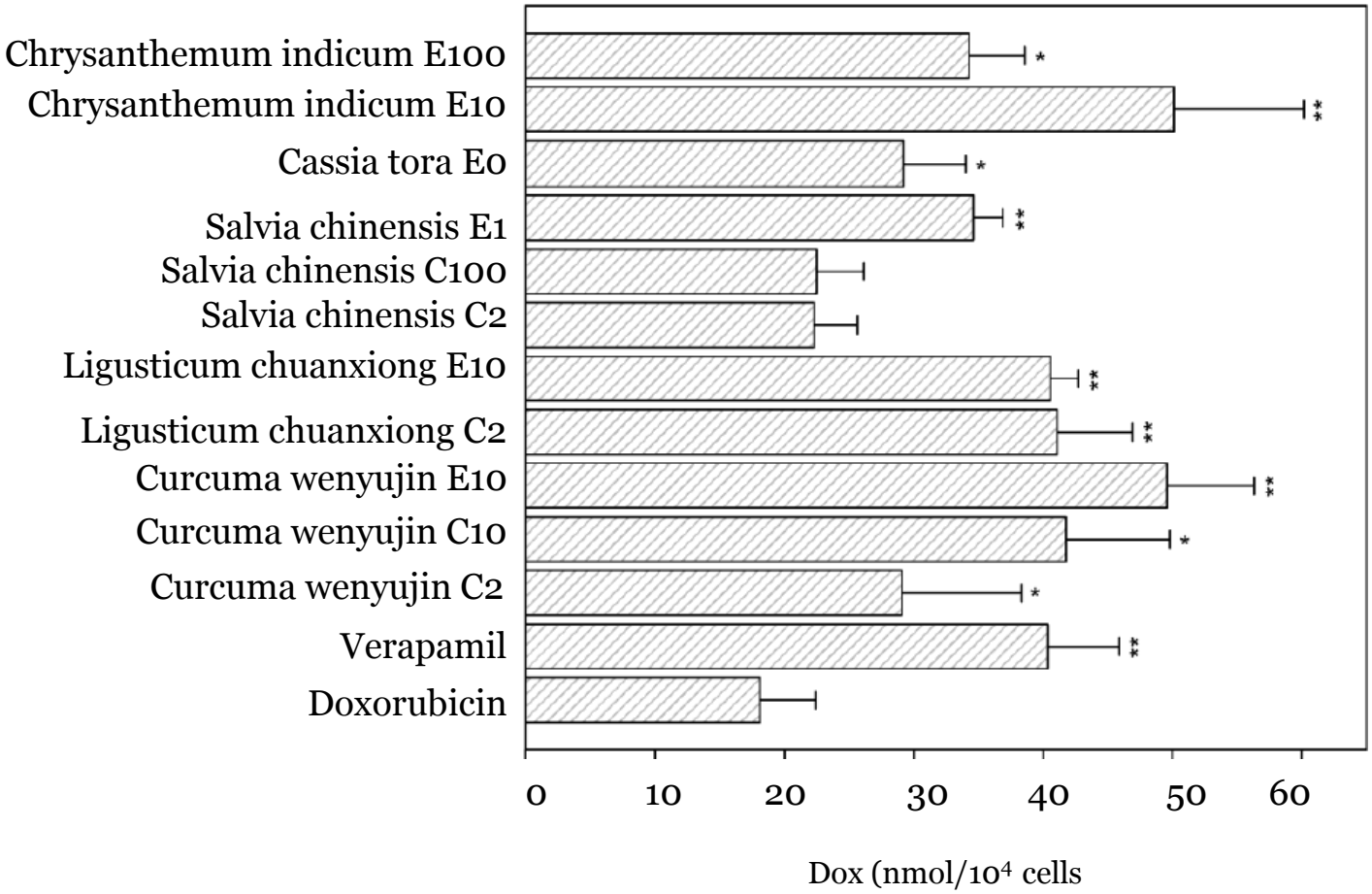
Additional Studies

PROTOCOLS

Effects of fractions on doxorubicin (Dox) accumulation in MCF-7/ADR cells.



Notes: Bars represent means ± standard deviations (SD) of triplicate determinations; ** and * represent $p < 0.01$ and 0.05, respectively, compared with the control group (doxorubicin) in the MCF-7/ADR cells.

Notes

- The dried and powdered herbal materials (100 g) were extracted with 95% EtOH (500 mL) for 3 h thrice at 80°C. The EtOH extracts were concentrated under reduced pressure, suspended in 200 mL water, and then partitioned exhaustively with equal volume of CH2Cl2 and EtOAc successively.
- The CH2Cl2 extracts were chromatographed respectively over a silica gel column with gradient elution by MeOH/CH2Cl2 (0:100, 1:99, 2:98, 5:95, 10:90, 20:80, 100:0, each four column volume) to yield 7 fractions (C0, C1, C2, C5, C10, C20, C100); the EtOAc extracts were followed in the same treatment to obtain another 7 fractions (E0, E1, E2, E5, E10, E20, E100). Each herb yielded 14 fractions, denoted by species name combined with fraction number.
- All fractions were dried and dissolved in DMSO to a final concentration of 40 mg mL–1.

Notes

- Cells were incubated with fractions for 48 h. IC50 and IC10 values (μg mL–1) in MTT test experiment were expressed as means ± standard deviations of three experiments.
- Reversing fold in MDR reversal activity experiment is the IC50 ratio of doxorubicin alone to doxorubicin with sample in MCF-7/ADR cells. (The IC50 of doxorubicin alone for MCF-7/ADR cells and MCF-7 cells used here were 1.14 and 0.13 μg mL–1, respectively.
- The IC50 of doxorubicin in the presence of 5 μg mL-1 verapamil for the MCF-7/ADR cells and the MCF-7 cells were 0.14 and 0.18 μg mL–1, respectively.)

Source: Department of Natural Medicinal Chemistry, China Pharmaceutical University, Nanjing, People's Republic of China; School of Traditional Chinese Pharmacy, China Pharmaceutical University, Nanjing, People's Republic of China (2010)

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Protocols

- Fu LW, et al (2001) MTT cell proliferation assay in combination with other in vitro drug evaluation assays to screen potential MDR modifiers from a series of naturally occurring bis-benzylisoquinoline alkaloids (BBIs) that were isolated from natural plants. These include berbamine, oxyacanthine, tetrandrine, guattegaumerine and constitute a series of almost 400 phenylalanine-derived metabolites with a rich and varied chemistry and pharmacology.
- In Vitro screening showed potent activities to restore sensitivity of resistant tumour cells, such as MCF-7/adr and KBv200 cells, to many antitumour drugs including doxorubicin and vincristine.
- Measurement of radioactive [3H]-Vincristine indicated that these BBIs increased intracellular drug accumulation in MDR cells, but had little effect on drug-sensitive cells.

Protocols

- The mechanism of these compounds to reverse MDR was associated with the increase in the intracellular drug accumulation through inhibiting the activity of P-gp. Another important feature is that the in vitro cytotoxic effect of these naturally occurring BBIs themselves on tumour cells was very low.
- Tetrandrine potentiated the cytotoxicity of Dox; a 20.4-fold reversal of resistance was achieved in the presence of 2.5 μmol/l of TET. Accumulation and efflux studies with the P-gp substrates, Dox and Fura-2, demonstrated that TET inhibited the P-gp-mediated drug efflux. In addition, TET lowered cell membrane fluidity in a concentration-dependent manner (Fu LW et al, 2002).
- In vitro studies showed that co-administration of TET at 2.5 μM, which has little cytotoxicity alone, reversed the sensitivity of KBv200 cells to paclitaxel and docetaxel around 10-fold (Zhu XM et al, 2005).

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- Zuo Jin Wan (ZJW), comprised of Rhizoma Coptidis and Fructus Evodiae in the ratio of 6 : 1, has been identified to have anticancer activity. ZJW could increase the concentration of chemotherapeutic drugs in HCT116/L-OHP cells in a dose-dependent manner. ZJW could also reverse drug resistance of colorectal cancer cells by decreasing P-gp level in vitro and in vivo.
- ZJW reversed MDR via increasing the sensitivity of MDR cells to chemotherapeutic agents. Second, ZJW reversed MDR through down-regulation of P-gp in vitro and in vivo. And third, combination of chemotherapy with ZJW prolonged the overall survival time of xenograft model and reduced the tumour volume (Sui et al, 2013).
- Recent findings have found that berberine and coptisine, which are the major active constituents of Coptis, were found to reverse ABCB1-mediated MDR in human MDR cancer cells (Min et al, 2006; He et al, 2012).

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Myeloid malignancies and the microenvironment

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Abstract

Research in the last few years has revealed a sophisticated interaction network between multiple bone marrow cells that regulate different hematopoietic stem cell (HSC) properties such as proliferation, differentiation, localization, and self-renewal during homeostasis. These mechanisms are essential to keep the physiological HSC numbers in check and interfere with malignant progression. In addition to the identification of multiple mutations and chromosomal aberrations driving the progression of myeloid malignancies, alterations in the niche compartment recently gained attention for contributing to disease progression. Leukemic cells can remodel the niche into a permissive environment favoring leukemic stem cell expansion over normal HSC maintenance, and evidence is accumulating that certain niche alterations can even induce leukemic transformation. Relapse after chemotherapy is still a major challenge during treatment of myeloid malignancies, and cure is only rarely achieved. Recent progress in understanding the niche-imposed chemoresistance mechanisms will likely contribute to the improvement of current therapeutic strategies. This article discusses the role of different niche cells and their stage- and disease-specific roles during progression of myeloid malignancies and in response to chemotherapy.

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Disclosures

CME questions author Laurie Barclay, freelance writer and reviewer, Medscape, LLC, owns stock, stock options, or bonds from Pfizer. Associate Editor David M. Bodine and the authors declare no competing financial interests.

Learning objectives

Upon completion of this activity, participants will be able to:

1. Identify main niche alterations in acute myeloid leukemia and Philadelphia chromosome–negative myeloproliferative neoplasms, based on a review.
2. Identify main niche alterations in chronic myelogenous leukemia.
3. Identify main niche alterations in myelodysplastic syndrome.

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Introduction

Myeloid malignancies are clonal hematopoietic disorders characterized by excessive proliferation, abnormal self-renewal, and/or differentiation defects of hematopoietic stem cells (HSCs) and myeloid progenitor cells. They mainly consist of myeloproliferative neoplasms (MPNs), myelodysplastic syndrome (MDS), and acute myeloid leukemia (AML), caused by different genetic and epigenetic changes in HSCs and functional changes in bone marrow (BM) niche cells. Genetic and epigenetic modifications have also been noted in BM mesenchymal stromal cells (BMSCs) in MDS and AML. The end results of these alterations are phenotypically distinct diseases that likely require the design of specific treatments. However, the use of selective inhibitors is challenging, because they sometimes also affect the normal hematopoietic counterparts, and clones carrying other mutations often cause relapse after chemotherapy. In this case, overcoming common mechanisms of resistance might be more likely to succeed therapeutically.

The World Health Organization subdivided MPNs into four distinct diseases: chronic myelogenous leukemia (CML), characterized by the BCR-ABL oncogene fusion (Philadelphia chromosome [Ph^+]) protein and the three Ph^- disorders named polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF).[1] Whereas PV is primarily associated with high erythrocyte counts, patients with ET have high platelet counts, and PMF is mainly related to BM failure as a result of fibrotic BM degeneration. These different clinical symptoms suggest that each MPN subtype is an independent disease, but transitions among them are observed in some patients.

Alterations of BM niches contribute to the progression of myeloid malignancies

Streaming from the discovery of driver mutations, myeloid malignancies were initially regarded as primarily driven by leukemic cell–autonomous mechanisms. However, cumulative evidence indicates that leukemic cells can exploit physiological niche signals and can overcome control by the normal microenvironment and/or remodel BM niches into permissive/self-reinforcing environments that support disease progression at the expense of normal hematopoiesis.

During leukemogenesis, malignant clones become progressively independent of niche-imposed physiological control mechanisms. In early leukemogenesis, BM homing and spatial localization of early leukemic stem cells (LSCs), which are also called pre-LSCs, are similar to that of normal HSCs. However, at later stages, LSCs home similarly to committed progenitors and become progressively independent of microenvironmental WNT signals.[2] Some LSC alterations can simultaneously stimulate proliferation and myeloid skewing without affecting self-renewal. For instance, reduced JunB expression observed in many myeloid malignancies diminishes the responsiveness of LSCs to Notch and transforming growth factor β (TGF-β) niche signals.[3] LSCs can become progressively insensitive to TGF-β during disease evolution from chronic to acute leukemia.[4]

The first indications of microenvironmental contribution to myeloid malignancies derive from reciprocal BM transplantation experiments showing that myeloid malignancies can arise from originally nonmutated hematopoietic cells in an altered microenvironment. For instance, MPN-like disease is observed in mice carrying a constitutive nonhematopoietic retinoic acid receptor γ deletion.[5] MPN-like disease also develops after combined retinoblastoma protein deletion in nonhematopoietic and myeloid cells.[6,7] Similarly, Notch pathway inhibition by the deletion of ubiquitin E3 ligase Mind bomb 1 (*Mib1*) in nonhematopoietic cells causes nontransplantable MPN-like disease, which can be reverted by microenvironmental Notch activation.[8] Altogether, these pioneering studies represent strong evidence that the microenvironment exerts more than a mere bystander effect in myeloid malignancies.

Role of the BM vasculature

Several myeloid malignancies, including AML, MPN, and MDS, have been correlated with increased BM angiogenesis.[9-13] BM vascularization in MPN patients correlates with janus kinase (JAK2) allele burden and stage, being highest in PMF, followed by CML, PV, and ET patients.[11,12,14] MDS is characterized by lower vascularization, but blood vessel density similarly increases with disease aggressiveness and specifically correlates with progression to fibrosis.[13,15] In addition to the increased vascular density, BM vessel morphology is disorganized and irregular in MPN and AML.[11,16] Hence, increased and disorganized BM vascularization is a common niche alteration of myeloid malignancies.

Progression of myeloid malignancies is supported by synergistic crosstalk between malignant blasts and endothelial cells (ECs). Enhanced BM vascularization correlates with upregulation of angiogenic factors, including vascular endothelial growth factor (VEGF)–A and interleukins (ILs).[10,12,17-20] Whereas blasts secrete proangiogenic molecules, ECs release angiocrine factors that promote blast survival and proliferation.[9,17] Blast-derived angiogenic factors act in a paracrine manner and also stimulate leukemic cell survival and proliferation via autocrine pathways.[19,21,22] Similar to solid tumors, leukemic cells produce the key proangiogenic factor VEGF-A, which stimulates angiogenesis by paracrine mechanisms and increases blast survival and proliferation via autocrine VEGFR2-dependent pathways.[17,19,21,23] Endothelial VEGF signaling stimulates blood vessel formation and also induces angiocrine factor (such as granulocyte-macrophage colony-stimulating factor [GM-CSF], macrophage CSF [M-CSF], granulocyte CSF [G-CSF], IL-6, and stem cell factor) production in ECs, which promotes proliferation of malignant cells (Figure 1).[24-26] VEGF-dependent EC activation similarly increases EC-AML cell adhesion and AML aggressiveness.[26]

Leukemic cell–derived proangiogenic and proinflammatory factors such as IL-1β and basic fibroblast growth factor (bFGF) can stimulate ECs to release VEGF-C, thereby supporting blast survival and proliferation.[22] Megakaryocytes represent an alternative VEGF source in MDS and might also stimulate angiogenesis in ET and PMF.[13] High VEGF-A and VEGF-C plasma concentrations are associated with adverse prognosis in AML and CML, and VEGF-A levels correlate with MPN disease stage (PMF>PV>ET).[10-12,18,27] Targeting VEGF signaling with bevacizumab has not been successful so far, but

adjuvant treatment with different tyrosine kinase inhibitors (TKIs) in AML and MPN might normalize the microenvironment and eradicate malignant cells. However, patient response to therapy is heterogeneous and identifying susceptible subgroups is crucial.[18,28-30]

The angiopoietin 1 (ANG)/TIE signaling system—a master regulator of solid tumor angiogenesis—is also gaining recognition in myeloid malignancies. High *ANG2* levels in AML patients do not correlate with changes in vessel density but may be of prognostic value because they correlate with improved survival when VEGF expression is low.[31-33] Abnormal ANG/TIE signaling has been detected in ECs and also in leukemic cells.[34,35] Autocrine ANG1/TIE2 signaling in blasts induces signal transducer and activator of transcription 1 (STAT1)/3/5/6 and ERK pathways, which support leukemic cell proliferation,[34,36] and TIE2/IP-3 kinase signaling increases AML cell survival.[35]

Other proangiogenic factors such as bFGF and HGF are also upregulated in AML, CML, and MDS.[10] Likewise, proinflammatory cytokines, including tumor necrosis factor α (TNF-α), IL-6, and IL-1β, are increased when AML blasts are cocultured with ECs. These cytokines stimulate EC proliferation and G-CSF and GM-CSF production, thereby promoting leukemic cell expansion.[25,37] Secretion of TNF-α and IL-1β by AML blasts upregulates endothelial adhesion receptors such as selectins VCAM-1 and ICAM-1 to support vascular adhesion and proliferation.[38] EC activation by inflammatory cytokines might compromise vascular integrity and favor thrombosis, further aggravating the proinflammatory environment.

Alterations in ECs might be a predisposing factor for the development of myeloid malignancies. MPN-like disease has also been observed in response to deletion of endothelial-specific *Rbpj*.[39] Loss of endothelial Notch signaling upregulates microRNA 155 (miR-155), which de-represses nuclear factor κB (NF-κB) leading to G-CSF and TNF-α overexpression and myeloid expansion. The potential relevance of this pathway is emphasized by an increased miR-155 level in human PMF BM.[39] CML cells and ECs also communicate via exosomes by shuttling miR-126 to downregulate VCAM-1 and CXCL12 in ECs and decrease CML adhesion and migration.[40]

Despite the release of multiple proangiogenic factors in the tumor microenvironment, hypoxia represents a common feature of myeloid malignancies and can influence LSC cycling, quiescence, differentiation, metabolism, and chemotherapy resistance. However, the role of hypoxia and downstream hypoxia-inducible factor 1α (HIF-1α) signaling in leukemia remains controversial, with published evidence for both supporting and inhibitory roles. In some studies, hematopoietic HIF-1α deletion promotes AML and MPN progression in mice.[41,42] Similarly, combined deletion of HIF-1α and HIF-2α can accelerate AML initiation, but it is dispensable for disease maintenance.[43] In contrast, other studies indicate that HIF-1α and HIF-2α support LSC survival by inducing p16 and p19 signaling and reducing reactive oxygen species (ROS) levels and endoplasmic reticulum stress, respectively.[44,45] Hypoxia-induced VEGF production in a mouse model of CML correlates with increased clonogenicity, maintenance, repopulation capacity, and TKI resistance of BCR-ABL$^+$ cells.[46] Cytarabine and doxorubicin resistance is partly conferred by HIF-1α signaling, which induces quiescence in AML subclones by interfering with apoptosis and supporting survival signaling.[47,48] Hypoxia might also favor leukemogenic niche metabolism and cytokine secretion. In addition to hypoxia, cytokines, interferon alfa, hormones, and genetic modifications can stimulate HIF-1α signaling, and their deregulation in a leukemic niche might similarly control this pathway in a hypoxia-independent manner.[49] HIF-1α might be a prognostic marker for high-risk AML and CML patients and a valuable therapeutic target. These divergent results on the role of hypoxia in the LSC niche call for further studies on this particular aspect of environmental control.

Intense morphologic and functional remodeling of BM vessels has been observed in myeloid malignancies and generally result in increased but dysfunctional vasculature. A synergistic crosstalk is established between ECs and leukemic cells, which stimulates the growth of both. Increased permeability of an

activated endothelium might also favor adhesion and mobilization of both inflammatory and leukemic cells, further aggravating inflammation, invasion of peripheral organs, and resistance (discussed below).

Role of BMSCs

Although initial in vitro studies did not observe major alterations in BMSCs, recent in vivo characterization has identified BMSCs as essential components of the HSC niche that are deregulated in a disease-specific manner in myeloid malignancies. The niche might have disparate roles in various myeloid malignancies, and these roles might also change during disease evolution (Figure 2).

The first evidence of a possible role for BMSCs in myeloid malignancies arose from studies that identified chromosomal abnormalities such as hypodiploidy and chromosomal translocations, duplications, and deletions in hematopoietic cells of patients with myeloid malignancies and also in BMSCs.[50-56] These cytogenetic abnormalities have been observed in 30% to 70% of the BMSCs from patients with MDS or AML and are different from hematopoietic mutations in the same individuals.[50,52] Yet stromal genomic alterations are associated with unfavorable prognostic chromosomal abnormalities in hematopoietic cells.[51,52] Likewise, 55% of the BMSCs from patients with MDS showed abnormal karyotypes, and 57% of the BMSCs from patients with AML who had trisomy 8 and monosomy 7 in hematopoietic cells showed cytogenetic aberrations.[54] Similarly, trisomy 8 mosaicism is associated with increased incidence of myeloid leukemia and MDS, and stromal cells in these patients favor leukemic cell proliferation.[57] Chromosomal and epigenetic abnormalities in BMSCs from patients with MDS and AML have been linked to certain disease subtypes and distinctive gene-expression programs.[53,56,58] These genetic abnormalities in BMSCs suggest enhanced genetic susceptibility and an active role of BMSCs in the progression of MDS or AML.

The functional consequences of these BMSC alterations are still debated. Several studies have noted normal differentiation, adhesion, expression and survival and the ability to support hematopoiesis ex vivo in BMSCs from patients with MDS, CML, or AML.[50-52,54,59-62] In contrast, other studies showed abnormal differentiation, defective hematopoietic supportive capacity, reduced expression of adhesion molecules, increased apoptosis, and increased production of IL-1β and stem cell factor in BMSCs of patients with myeloid malignancies.[55,56,63-75]

These in vitro studies proposed some divergent views on BMSC alterations and contributions to disease, but more recent in vivo studies on several myeloid malignancies have reported altered BMSC growth and differentiation and the production of cytokine and HSC retention factor. Whether these BMSC alterations were predisposing or initiating factors for disease has remained elusive so far. The first evidence that alterations of BMSCs can drive myeloid malignancies arose from the deletion of the RNA processing enzyme Dicer1 in osteoprogenitor cells, which caused MDS-like disease with sporadic transformation to AML. The disease could be reverted by transplanting leukemic cells from mice with *Dicer1*-deleted osteoprogenitors into wild-type mice. Loss of DICER1 in osteoprogenitor cells (but not in mature osteoblasts) resulted in downregulation of the ribosome maturation protein SBDS, which is mutated in human Shwachman-Bodian-Diamond syndrome and is associated with congenital BM failure and leukemic predisposition.[76] Reduced expression of DICER, DROSHA, and SBDS has been noted in BMSCs from patients with MDS,[77] emphasizing the potential clinical relevance of these findings. A recent study has provided mechanistic insight on genotoxic stress caused by mutations in BMSCs, which can impair normal hematopoiesis and favor leukemogenesis. Loss of *Sbds* in BMSCs in mice recapitulates the characteristic osteoporosis found in human Shwachman-Bodian-Diamond syndrome. It also stimulates BMSC p53 signaling and secretion of the inflammatory molecules S100A8 and S100A9. S100A8/A9 activates toll-like receptor 4 on normal hematopoietic stem and progenitor cells (HSPCs), which leads to inflammatory damage, including hyperpolarized mitochondria, which triggers increased ROS production

and DNA double-strand breaks. The potential relevance of niche S100A8/A9 expression in human leukemogenesis is emphasized by the correlation of S100A8/A9 expression in BMSCs and bone lining cells and the leukemic evolution of patients with MDS.[78]

Patients with Noonan syndrome often carry a mutation in the RAS signaling mediator *PTPN11* and are at increased risk for developing childhood MPNs. A recent study has now shown that leukemogenic effects of activating *PTPN11* mutations are not solely hematopoietic cell autonomous, but that *PTPN11* mutations in BMSCs and osteoprogenitor cells can similarly drive MPN progression. Excessive CCL3 production by PTPN11-activated BMSCs results in the recruitment of monocytes to BMSCs, which hyperactivate HSCs by secreting inflammatory cytokines, including IL-1β, thereby exacerbating disease progression.[79]

Recent studies using mouse models of CML, MPNs, and AML demonstrate that specific BMSC-leukemic cell interactions are important for leukemogenesis (Table 1).[16,80,81] In an inducible BCR-ABL mouse model, CML cells support BMSC proliferation and abnormal differentiation, which generate functionally altered and inflammatory osteoblasts. BMSCs in CML failed to maintain normal HSCs because of reduced *Cxcl12* expression, favoring the expansion of less niche-dependent LSCs. Osteoblastic cells in CML secrete proinflammatory cytokines (IL-1β and TNF-α) that amplify disease progression by triggering myeloid cell proliferation and creating a self-reinforcing niche.[81] CML cells also instruct BMSCs to secrete PIGF, which stimulates angiogenesis and promotes CML proliferation and metabolism, in part independently of BCR-ABL1 signaling.[82]

Progression of Ph^- MPN also disrupts normal BMSC function, thus promoting disease progression. Proinflammatory cytokines produced by $JAK2^{V617F}$ hematopoietic cells, such as IL-1β, can cause local neuropathy and microenvironmental damage that leads to disease manifestation. In this inflammatory environment, MPN-associated neuropathy sensitizes $nestin^+$ BMSCs to undergo apoptosis and reduces their HSC niche properties (including *Cxcl12* expression). Genetic depletion of $nestin^+$ cells can worsen myelofibrosis and thrombocytosis, which is also observed in mice lacking the β_3-adrenergic receptor. In contrast, neural protection by neurotrophic factors or neural stimulation of the microenvironment by chronic treatment with β_3-adrenergic agonists rescues $nestin^+$ cells and improves Cxcl12 and IL-1β expression, neutrophilia, thrombocytosis, and myelofibrosis.[80]

Similar neuropathy-driven microenvironmental deregulation has been reported in AML.[16] Yet the neuropathy might be comparatively less relevant in AML because neural interventions did not significantly affect leukemogenesis in that study. Whether neuropathy-driven microenvironmental changes are more broadly relevant in other hematologic malignancies remains to be investigated.

Reciprocal leukemic-niche interactions have also been highlighted in MDS. On one hand, patient-derived MDS cell engraftment is dependent on niche factors, such as LIF, VEGF, IGF-BP2, and N-cadherin. On the other hand, exposure of normal BMSCs to MDS renders them malignant-like, which highlights MDS-induced BMSC reprogramming.[83] Alterations of WNT signaling in BMSCs have been associated with defective BMSC proliferation in MDS,[84,85] partially because of the induction of senescence.[86]

Communication between BMSCs and MDS cells is partly mediated by extracellular vesicles.[87] Exosome-mediated crosstalk between CML cells and human BMSCs triggers IL-8–dependent survival.[88,89] Similarly, primary leukemic cells and cell lines release microvesicles containing RNAs that alter the secretion of niche-reprograming factors.[90]

Together, these studies highlight the role of BMSCs as key elements of predisposition, manifestation, and evolution of myeloid malignancies. Whereas functionally or genetically altered BMSCs increase inflammation and genotoxic stress, mutated hematopoietic cells critically compromise the normal function

of BMSCs in the HSC niche, hampering normal hematopoiesis and favoring leukemogenesis. Whether these changes in BMSCs initiate disease in humans and/or select for particular mutated clones is a subject of intense research.

Anchoring of myeloid leukemic cells to their niches

Adhesion molecules are important for LSC engraftment and interaction with the niche. CD44 is a glycoprotein receptor for hyaluronan, selectins, and osteopontin. A specialized glycoform of CD44 named HCELL is a BM homing receptor.[91] CD44 is overexpressed in CML,[92] and homing and engraftment of CML and AML LSCs to their BM niches is much more dependent on CD44 compared with normal HSCs or B-cell acute lymphoblastic leukemia cells. Anti-CD44 treatment reduces CML incidence and AML burden in xenografts.[93,94] In addition to directing LSC homing, CD44 also maintains LSCs in a primitive state,[94,95] and high CD44 levels correlate with AML induction and relapse in AML mouse models.[96,97]

CD44 is a potent E-selectin receptor in CML, and E-selectin blockade can also reduce LSC numbers in CML.[98] E-selectin is overexpressed on BM endothelium in AML, and antagonizing E-selectin can sensitize AML cells to chemotherapy.[99] CD44 cooperates with other adhesion molecules, such as CD49d[100] and integrin β1, which inhibit CML proliferation.[101] Treatment with INF-α can restore impaired integrin β1–mediated adhesion of CML cells and inhibit their proliferation.[102,103] This interaction might be relevant in other malignancies because integrin β1–mediated adhesion influences chemotherapy sensitivity in AML and increased fibronectin secretion in early fibrotic stages of MPN.[104,105]

Downregulation of BM *Cxcl12* helps different myeloid malignancies[16,80,106] and correlates with HSPC mobilization and extramedullary hematopoiesis. GCSF produced by CML cells decreases *Cxcl12* expression by BMSCs and directly impairs normal hematopoiesis in an inducible BCR-ABL transgenic model. Long-term LSCs show reduced homing and retention in the BM because of increased G-CSF and decreased CXCL12 levels.[106]

Leukemic cells can further highjack normal BM vascular niches dependent on CXCL12 and E-selectin.[107] Activation of CXCL12 receptor CXCR4 in leukemic cells is important for AML cell survival and BM retention. Neutralizing CXCR4 antibodies decreases human AML cell numbers in xenografts.[108]

Role of osteoblasts

Reduced trabecular bone mass in retinoic acid receptor γ– or retinoblastoma protein–deficient mice correlates with aggravated MPN, suggesting that endosteal niche alterations can promote MPN progression.[5,6] The role of osteoblasts in leukemia progression seems to be disease specific, because constitutive parathyroid hormone receptor activation in osteoblasts increases bone remodeling and attenuates CML progression but stimulates MLL-AF9 AML progression. Increased bone remodeling in mice with constitutively active parathyroid hormone signaling causes TGF-β release from bone, reducing LSC proliferation and maintenance in CML but not in AML, probably because of reduced TGFBR1 expression or higher constitutive pSMAD2/3 signaling in AML.[4] These differences suggest that the niche or niches might play different roles at various stages of leukemogenesis and/or in a disease-specific manner (Figure 2).

The increased osteogenic potential of BMSCs can contribute to PMF.[109] Similarly, increased osteoblastic priming has been observed in BMSCs from childhood MDS.[110] Osteoblasts also expand during the chronic phase of CML,[81] when they negatively regulate normal and malignant HSC proliferation.[111] This is reminiscent of the role of nestin$^+$ BMSCs in Ph$^-$ MPN, in which depletion of nestin$^+$ cells or their Cxcl12 production can accelerate MPN progression.[80] Therefore, MPN preleukemic cells seem to retain

sensitivity to normal cues from the microenvironment, and protecting the niche might be beneficial at this stage. In contrast, during the blast crisis of this disease, which resembles acute leukemia, osteoblasts are markedly reduced,[112] suggesting that osteoblasts are differentially affected in AML and CML. AML has been associated with increased bone remodeling and accumulation of osteoblast-primed BMSCs, which do not seem to be able to mature into osteoblasts, correlating with decreased mineralized bone.[16]

Strikingly, expression of a constitutively activated form of β-catenin in osteoblasts might be sufficient for driving AML-like disease. Activated β-catenin signaling increases osteoblastic *Jagged* expression, leading to aberrant Notch signaling in HSCs. Inhibition of osteoblastic Notch signaling by Jagged deletion or pharmacologic treatment with γ-secretase inhibitors prevents AML development in mice.[113] The same group has shown that 38% of patients with MDS, AML, or MDS with leukemic transformation have increased nuclear β-catenin in *Runx2*-expressing osteoblastic cells associated with increased Notch activity in $CD34^+$ HSPCs.[114] Osteoblasts are decreased in patients with MDS or AML, and osteoblast recovery correlates with better prognosis.[115] Overall, this represents another example of potential mechanisms of niche-driven oncogenesis in myeloid malignancies.

The niche in response to chemotherapy

High-dose chemotherapy (HDC) is used to eradicate leukemic cells in AML and advanced MDS. Cytotoxic agents can damage the BM microenvironment and compromise niche function, regeneration, and maintenance of normal hematopoiesis (Figure 3). Chronic stromal damage by HDC is manifested by reduced BMSCs and CD44 expression in allogenic BM transplantation recipients, which is associated with slower hematopoietic recovery.[116,117] Myelosuppression can cause endothelial regression that leads to a discontinuous, hemorrhagic endothelium accompanied by endothelial denudation. Sinusoidal vessels are particularly sensitive to irradiation, and subsequent EC regeneration via VEGFR2 signaling is critical for hematopoietic reconstitution.[118] Chemotherapy-triggered BM sympathetic neuropathy can lead to loss of $nestin^+$ cells and ECs, which interferes with hematopoietic regeneration. Neuroprotective agents have been reported to protect nerves from chemotherapy-induced injury and to support the survival of blood vessels and associated $nestin^+$ cells, which leads to accelerated hematopoietic recovery.[119] Osteoblasts are reduced after multiple rounds of chemotherapy, and osteoprogenitor numbers are decreased in response to HDC, eventually causing osteopenia.[120,121] Adipocyte accumulation in aplastic BM might compromise niche function by negatively influencing hematopoietic recovery after myeloablation.[122] Therefore, the damage inflicted by chemotherapy in the BM microenvironment can interfere with normal hematopoiesis and eventually result in BM failure.

HSC transplantation used in relapsed or high-risk AML can rapidly induce neoplasia from malignant or premalignant donor HSCs.[123] A dysfunctional host microenvironment resulting from mutations or HDC might also promote transformation of donor-derived HSCs into malignant cells. Likewise, growth of LSCs can alter the microenvironment and compromise normal HSC growth after allogenic HSC transplantation.

Although multiple LSC-intrinsic mechanisms of chemoresistance have been described, the microenvironment has recently attracted attention in protecting LSCs from chemotherapy (Figure 3). Early coculture studies showed that cytarabine treatment of BMSCs interferes with apoptosis and enhances survival of AML cells.[124,125] BMSC-derived TGF-β1 is a mediator of resistance during cytarabine treatment of AML.[126] Another key chemotherapy resistance–conferring pathway is CXCL12/CXCR4. Chemotherapy upregulates CXCR4 in AML cells, and imatinib enhances CXCR4 expression in $BCR\text{-}ABL^+$ cells, which results in increased CXCL12/CXCR4 survival signaling and lodgment into protective niches.[127-130] Adjuvant treatment of AML and CML with CXCR4 inhibitors decreases BMSC-induced survival pathways and sensitizes AML and CML cells to chemotherapy and imatinib treatment,

respectively.[127-130] Co-recruitment of CXCR4 and its downstream mediator Lyn into lipid rafts is another imatinib-induced chemotherapy resistance mechanism in CML.[131] In fact, pharmacologic targeting of lipid rafts in combination with CXCR4/TGF-β1 can further sensitize CML cells to therapy.[126,131]

BMSC-induced CML chemotherapy resistance also occurs via upregulation of galectin-3, which stimulates leukemic cell proliferation, protection from apoptosis, and BM lodgment.[132] Likewise, N-cadherin–dependent interaction of stromal and CML cells has been proposed to activate β-catenin signaling in CML cells, thereby shielding leukemic cells from TKI treatment.[133] Reciprocal activation of NF-κB signaling via VCAM-1/very late antigen 4 (VLA-4) interaction occurs in BMSCs and AML cells, and blockade of stromal NF-κB signaling can sensitize AML cells to chemotherapy.[134] Likewise, leukemic and stromal cell interaction via VLA-4 and fibronectin interferes with drug-induced apoptosis. Combined treatment of cocultures with VLA-4–specific antibodies and cytarabine improves survival, and patients with VLA-4–negative AML have a favorable prognosis.[135] Human AML cells preferentially home and engraft in the endosteal BM of immunodeficient mice where they remain more quiescent and protected from chemotherapy.[136]

ECs can also confer chemotherapy resistance to AML cells, and blocking VEGFR2 signaling can increase the susceptibility of leukemic cells to chemotherapy.[26,137] Leukemic cell adhesion to the vasculature has been proposed to induce quiescence, resistance to chemotherapy, and relapse.[138] One study observed that AML cells acquired EC-like features and integrated into the blood vessel where they can become quiescent and evade chemotherapeutic treatment.[139] Alternatively, ECs can protect AML cells from chemotherapy by producing high levels of VEGF and platelet-derived growth factor (PDGF) in response to cytarabine.[140]

Emerging evidence indicates that BMSCs shield LSCs from therapy by affecting their energy metabolism. Coculture of AML cells and BMSCs upregulates the mitochondrial proteins BCL2 and UCP2, which modify cellular energy metabolism by uncoupling leukemic mitochondria, suppressing ROS level, increasing the apoptotic threshold, and supporting aerobic glycolysis (Warburg effect). The increased apoptotic threshold resulting from decreased mitochondrial membrane potential and reduced ROS level can also protect LSCs from chemotherapy.[141,142] A recent study has shown that BMSCs can modify LSC metabolism by directly transferring mitochondria to AML blasts in a cell-cell contact- and endocytosis-dependent manner. Mitochondrial uptake by the leukemic blasts increases their adenosine triphosphate production and protects them from mitochondrial depolarization after chemotherapy, thereby providing a survival advantage.[143]

BMSCs cocultured with AML cells promote chemotherapy resistance by increasing c-myc levels in AML cells, and c-myc inhibition can rescue AML cells from microenvironment-mediated drug resistance.[144] Conditioned medium from BMSCs has been shown to support Stat3 survival signaling in CML cells in response to imatinib.[145] Combining CML targeting by TKIs with the JAK2 inhibitor ruxolitinib can overcome resistance by blocking JAK/STAT signaling activated by BMSC-derived cytokines.[146] Evidence for BMSC-induced chemotherapy resistance has been obtained mainly from studies on AML and CML, but other cytokines produced by BMSCs (including IL-6, FGF, and CXCL10) can also promote $JAK2^{V617F+}$ cell resistance to atiprimod. Cytokine neutralizing antibodies may effectively restore apoptosis in Ph^{-} MPN cells.[147]

Treating the AML subtype acute promyelocytic leukemia (APL) with all-*trans*-retinoic acid induces LSC differentiation and improves patient survival. Non-APL AML is unresponsive to differentiation therapy, and APL patients eventually relapse.[148] Several leukemic cell–intrinsic resistance mechanisms have been

identified, but upregulation of the all-*trans*-retinoic acid–metabolizing enzyme cytochrome P450 gene CYP26 by stromal cells contributes to development of minimal residual disease.[149] Likewise, CYP3A4 upregulation in stromal cells confers resistance during etoposide treatment of AML.[150]

The understanding of niche-controlled resistance is still in its infancy, and despite the continuous development of novel treatment options, evading resistance mechanisms might arise. An important additional challenge is to avoid the elimination of normal HSCs. Therefore, identifying factors released by tumor cells that trigger resistance mechanisms conferred by niche cells is of importance to eventually interfere with disease relapse (Figure 3). Development of more selective drugs that act only on the mutated hematopoietic cells and/or combined treatment targeting not only the mutated cells but also the microenvironment might improve the outcome. However, the therapeutic strategies targeting the microenvironment should discriminate phases of normal HSPC niche damage vs advanced niche transformation. At early-stage disease and/or to diminish the damage caused by chemotherapy on normal niches, preventive strategies aiming at protecting the normal microenvironment might boost normal hematopoiesis and help to control preleukemic cells. However, at advanced disease stages, when the microenvironment has been profoundly transformed to support leukemogenesis, different niche-targeting strategies will be needed.

Future directions

Significant progress over the past few years has revealed important roles of the BM microenvironment in the pathogenesis of myeloid malignancies. However, the underlying mechanisms are only beginning to be elucidated, and an increasing complexity is becoming apparent, with different roles in distinct diseases and disease stages. Therefore, caution should be taken when extrapolating conclusions. Future focus on the complex interaction of neoplastic and microenvironmental cells will improve the development of niche-targeting strategies. Important questions remain for the future. For instance, do LSCs reside within specific niches? Do they interact with specific cell types? And are these cell types different from those interacting with normal HSCs? Do other cells regulating normal HSCs also play a role in leukemogenesis? Immune cells such as macrophages play key roles in the microenvironment of solid tumors and lymphoid malignancies, but their contribution to the myeloid malignancies remains much less explored. Do distinct niches for progenitor cells contribute to specific subtypes of malignancies? Are there key niche alterations during leukemogenesis leading to leukemic transformation (from preleukemia to leukemia)? Do somatic mutations in nonhematopoietic cells contribute to hematologic malignancies and how? Adjuvant therapies targeting the contribution of the microenvironment to leukemogenesis and resistance will likely be needed to fully eradicate LSCs. However, rational design of novel treatment strategies first requires proper understanding of the normal niches and their alterations in myeloid malignancies. Because the incidence of these diseases increases with age, parallel study of the aging process is of major importance. The reduced tolerance to chemotherapy, which contributes to the increased lethality of myeloid malignancies in the elderly, might be partially the result of impaired niche recovery during aging.

The current state-of-the art literature highlights the importance of the BM niche in contributing to myeloid malignancy progression by inducing or facilitating disease development, as well as conferring resistance to chemotherapy. The emerging recognition of the environment as a crucial player in multiple steps of the leukemic cascade lays the foundation for tackling leukemia from a different angle to improve current treatments.

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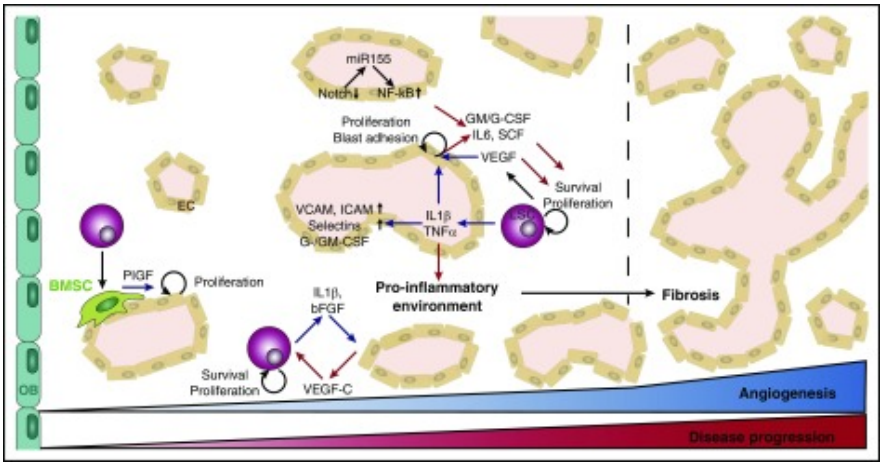
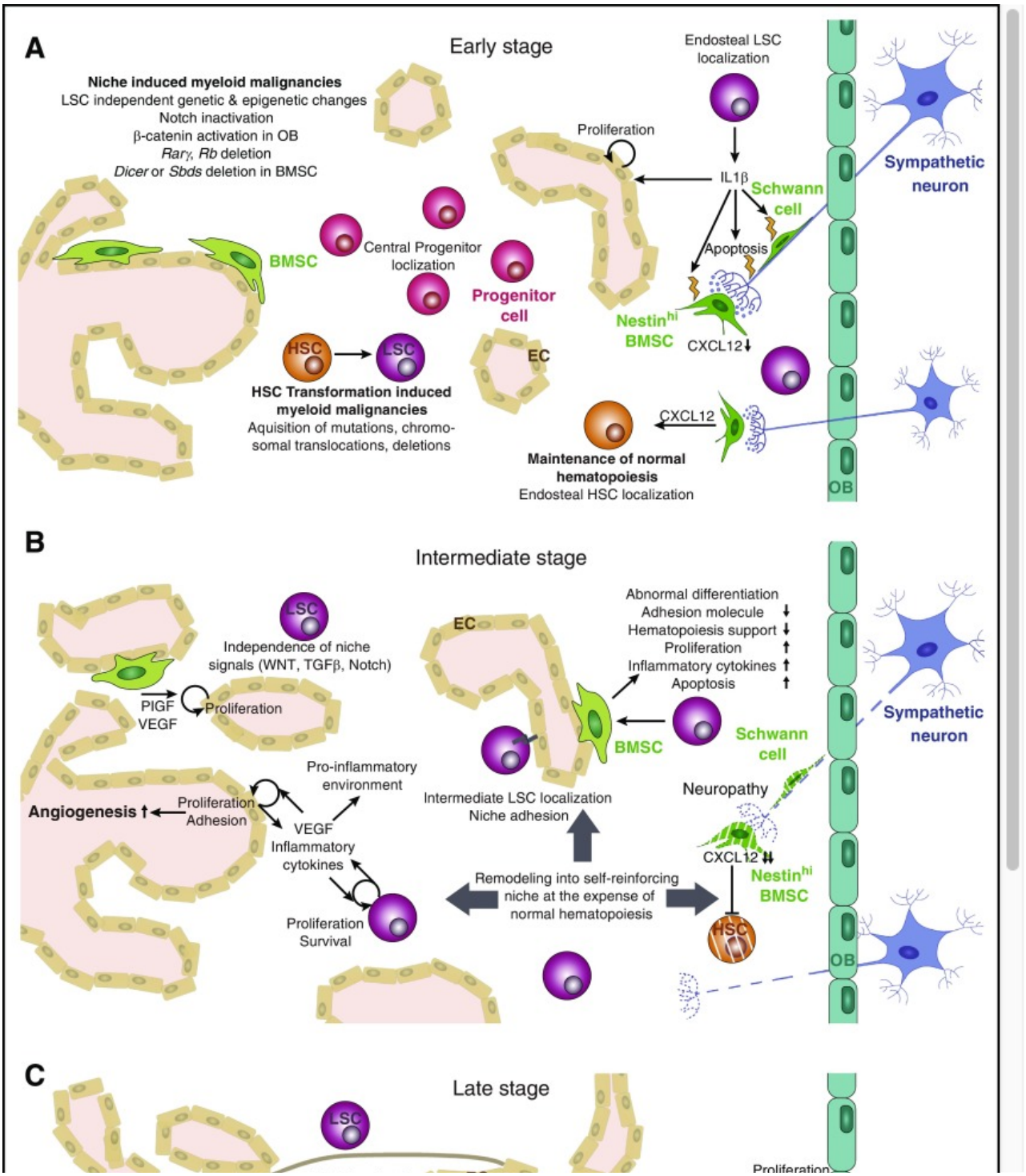
Figures and Tables

Figure 1.



Role of BM blood vessels in myeloid malignancies. Angiogenesis increases during progression of myeloid malignancies and is particularly associated with fibrotic stages of the disease. Leukemic cells produce angiogenic factors such as VEGF and inflammatory cytokines (blue arrows) to stimulate proliferation of ECs, expression of adhesion molecules, and secretion of angiocrine factors. EC-derived angiocrine factors (red arrows) stimulate leukemic cell proliferation and survival, triggering a vicious cycle to remodel the BM into a self-reinforcing niche. OB, osteoblast.

Figure 2.



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Microenvironmental changes during leukemogenesis. In the BM niche, HSC function is tightly controlled by a specialized microenvironment comprising sympathetic neurons, BMSCs, OBs, and ECs. (A) During early stages of myeloid malignancies, HSPCs acquire genetic alterations that transform them into LSCs. These mutations also create a proinflammatory environment that damages sensitive elements of the microenvironment, such as Schwann cells and their associated nerve terminals. (B) During intermediate stages of the disease, the environment remodels into a self-reinforcing niche that interferes with normal hematopoiesis. LSCs become independent of niche signals and localize more centrally in the BM. MSCs acquire an abnormal phenotype, and angiogenesis increases as a result of high VEGF and cytokine levels. (C) Late stages of the disease are characterized by a proinflammatory environment and myelofibrosis, high blood vessel density, and central LSC localization. Rarγ, retinoic acid receptor γ; Rb, retinoblastoma protein; TPO, thrombopoeitin.

Table 1.

Main niche alterations in different myeloid malignancies

Disease name		Alteration
AML	Disease phenotype	Hyperproliferation and impaired differentiation of HSC and myeloid progenitors
		Acute rapidly progressing disease
	Genetic LSC alterations	Chromosomal translocations, inversions, mutations in *NPM1*, *CEBPA*, *KIT*, *RUNX1*, *FLT-IDT*, or epigenetic factors
	Niche alterations	Neuropathy correlating with altered microenvironment[16]
		PTH activation in osteoblasts accelerates AML.[4]
		CD44 and E-selectin are important for LSC niche adhesion and maintaining LSC primitive state.[94,95,99]
		β-catenin activation in osteoblasts stimulates *Jag1* expression which activates Notch signaling in HSCs to induce AML.[113,114]
Ph–MPN	Disease phenotype	Clonal HSC disorder with hyperproliferation and expansion of myeloid cells
		Erythrocythemia (PV), thrombocythemia (ET), BM fibrosis (PMF)
		Slow progression, chronic disease stage, possible transformation to AML
	Genetic LSC alterations	Mutations in *JAK2* (PV, ET, PMF), *MPL* (ET, PMF), *CALR* (ET, PMF)
	Niche alterations	LSCs secrete IL-1β, which damages Schwann cells and sympathetic nerve terminals causing early neuropathy. This results in apoptosis of nestin^{+} BMSCs, reduces Cxcl12 production, and results in thrombocytosis and fibrosis.[80]
		Reduced osteoblast numbers at late stage of the disease.[112]
		Inactivation of Notch or deletion of retinoic acid receptor γ or retinoblastoma protein can cause niche-induced MPN.[5,6,8]
		PTPN11 activation in BMSCs induces CCL3-mediated monocyte recruitment and subsequent IL-1β–dependent HSC hyperactivation driving MPN progression.[79]
CML	Disease phenotype	Clonal HSC disorder with hyperproliferation and expansion of myeloid cells
		Slow progression, chronic disease stage, possible transformation to AML
	Genetic LSC alterations	Chromosomal translocation resulting in BCR-ABL gene fusion

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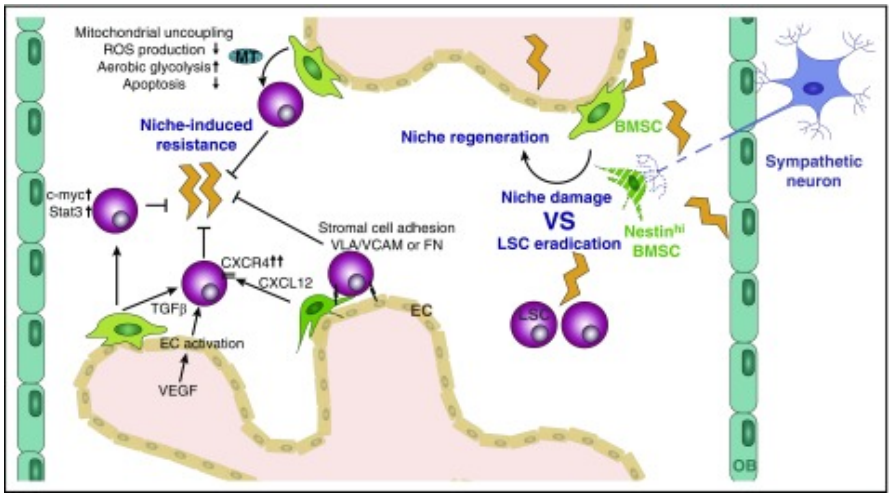
PTH, parathyroid hormone; TLR, toll-like receptor.

Figure 3.



Protection of LSCs from chemotherapy by the microenvironment. Chemotherapy eradicates LSCs but at the same time damages multiple cell types of the niche and triggers subsequent niche regeneration. Prolonged treatment induces the development of resistance mechanisms, some of which are mediated by niche cells, including BMSCs and ECs. MT, mitochondria; FN, fibronectin.

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ORIGINAL ARTICLE

A Phase 3 Randomized Trial of Nicotinamide for Skin-Cancer Chemoprevention

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ABSTRACT

BACKGROUND

Nonmelanoma skin cancers, such as basal-cell carcinoma and squamous-cell carcinoma, are common cancers that are caused principally by ultraviolet (UV) radiation. Nicotinamide (vitamin B_3) has been shown to have protective effects against damage caused by UV radiation and to reduce the rate of new premalignant actinic keratoses.

METHODS

In this phase 3, double-blind, randomized, controlled trial, we randomly assigned, in a 1:1 ratio, 386 participants who had had at least two nonmelanoma skin cancers in the previous 5 years to receive 500 mg of nicotinamide twice daily or placebo for 12 months. Participants were evaluated by dermatologists at 3-month intervals for 18 months. The primary end point was the number of new nonmelanoma skin cancers (i.e., basal-cell carcinomas plus squamous-cell carcinomas) during the 12-month intervention period. Secondary end points included the number of new squamous-cell carcinomas and basal-cell carcinomas and the number of actinic keratoses during the 12-month intervention period, the number of nonmelanoma skin cancers in the 6-month postintervention period, and the safety of nicotinamide.

RESULTS

At 12 months, the rate of new nonmelanoma skin cancers was lower by 23% (95% confidence interval [CI], 4 to 38) in the nicotinamide group than in the placebo group (P=0.02). Similar differences were found between the nicotinamide group and the placebo group with respect to new basal-cell carcinomas (20% [95% CI, −6 to 39] lower rate with nicotinamide, P=0.12) and new squamous-cell carcinomas (30% [95% CI, 0 to 51] lower rate, P=0.05). The number of actinic keratoses was 11% lower in the nicotinamide group than in the placebo group at 3 months (P=0.01), 14% lower at 6 months (P<0.001), 20% lower at 9 months (P<0.001), and 13% lower at 12 months (P=0.001). No noteworthy between-group differences were found with respect to the number or types of adverse events during the 12-month intervention period, and there was no evidence of benefit after nicotinamide was discontinued.

CONCLUSIONS

Oral nicotinamide was safe and effective in reducing the rates of new nonmelanoma skin cancers and actinic keratoses in high-risk patients. (Funded by the National Health and Medical Research Council; ONTRAC Australian New Zealand Clinical Trials Registry number, ACTRN12612000625875.)

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Nonmelanoma skin cancers, mainly basal-cell carcinomas and squamous-cell carcinomas, are the most common cancers in white populations.[1] In Australia, nonmelanoma skin cancers are four times as common as all other cancers combined,[2,3] and in the United States, the annual total cost of treating nonmelanoma skin cancers is estimated to be $4.8 billion.[4] Basal-cell carcinomas rarely metastasize but are locally invasive and can be disfiguring.[5] Squamous-cell carcinomas, especially less well-differentiated tumors on the head and neck, have metastatic potential and may originate from premalignant actinic keratoses.[6]

Nonmelanoma skin cancers and actinic keratoses are caused primarily by exposure to ultraviolet (UV) radiation.[7] The use of sunscreens can reduce the incidence of squamous-cell carcinoma[8] and actinic keratosis[9] and may also reduce the incidence of basal-cell carcinoma and melanoma after prolonged use.[10] However, adherence to the application of sunscreens is often suboptimal, even among high-risk persons.[11] The increasing incidence of nonmelanoma skin cancer worldwide[14] highlights the need for additional preventive measures.

UV radiation increases the risk of skin cancer by damaging DNA, suppressing cutaneous antitumor immunity,[12] and inhibiting DNA repair by depleting cellular ATP.[13] Nicotinamide is an amide form of vitamin B_3 and the precursor of nicotinamide adenine dinucleotide (NAD^+), an essential cofactor for ATP production. Nicotinamide prevents ATP depletion and glycolytic blockade induced by UV radiation,[13] thereby boosting cellular energy and enhancing DNA repair.[14,15] Nicotinamide also reduces the level of immunosuppression induced by UV radiation, which is triggered by DNA damage,[16] without altering baseline immunity.[17,18] Therapy with nicotinamide, administered orally in healthy volunteers at daily doses of 500 mg or 1500 mg, resulted in similar levels of protection against immunosuppression induced by UV radiation.[18] Two phase 2, double-blind, randomized, placebo-controlled trials showed that among Australians with sun-damaged skin, the number of actinic keratoses at 4 months was 29% lower among those who received 500 mg of nicotinamide administered orally once daily and 35% lower among those who received 500 mg of nicotinamide twice daily than among those who received placebo.[19] Given the activity of nicotinamide in these preclinical and early clinical studies, we conducted a multicenter, phase 3, double-blind, randomized, placebo-controlled trial (Oral Nicotinamide to Reduce Actinic Cancer [ONTRAC]) to assess the efficacy of oral nicotinamide for the chemoprevention of nonmelanoma skin cancer in a high-risk population.

METHODS

STUDY DESIGN AND OVERSIGHT

The ONTRAC study was conducted at the Royal Prince Alfred and Westmead Hospitals in Sydney. The protocol of the study was approved by the human ethics committees of the University of Sydney and of each participating center, and all the study participants provided written informed consent. All the authors participated in the design of the study, collected the data, and contributed to the analysis or interpretation of the data (or both). All the authors vouch for the completeness and accuracy of the data and analyses and for the fidelity of the study to the protocol, which is available with the full text of this article at NEJM.org. The first author wrote the first draft of the manuscript; no one who was not an author contributed to the writing of the manuscript. The decision to submit the manuscript for publication was made by all the authors. There were no agreements regarding data confidentiality between the sponsor (University of Sydney) and the authors. The nicotinamide and placebo tablets used in the study were donated by the manufacturer (Blackmores), which had no role in the design of the study, in the accrual or analysis of data, in reviewing the manuscript, or in the decision to submit the manuscript for publication.

STUDY PARTICIPANTS

Eligible participants were 18 years of age or older and had had at least two histologically confirmed nonmelanoma skin cancers in the previous 5 years. Participants were ineligible if they were immunosuppressed; were pregnant or breastfeeding; had notably impaired liver or kidney function; had active peptic ulcer disease, a recent myocardial infarction, hypotension, a genetic skin-cancer syndrome, or large areas of confluent skin cancer (i.e., individual lesions that could not be counted); or had used nicotinamide supplements, oral retinoids, or field treatments for actinic keratosis, such as topical fluorouracil, in

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the previous 4 weeks. Participants were also excluded if they had had metastatic cancer, invasive melanoma, or an internal malignant condition in the previous 5 years.

STUDY PROCEDURES

We randomly assigned participants in a 1:1 ratio to receive either 500 mg of nicotinamide (Insolar, Blackmores) twice daily or matched placebo. Randomization was performed centrally with stratification according to 5-year history of nonmelanoma skin cancer (<6 vs ≥6 nonmelanoma skin cancers), sex, and study site. Nicotinamide and placebo were administered in identical coated tablets. Participants received either nicotinamide or placebo for 12 months, and adherence was monitored by two of the authors who counted the remaining tablets at each visit through 12 months. Skin-cancer checks were performed by dermatologists, who were unaware of the study-group assignments, at baseline and at visits at 3-month intervals (hereafter referred to as 3-month visits) for 18 months. Detected lesions that did not immediately warrant biopsy were monitored at subsequent visits, and if they were later found to be malignant on biopsy, the date of their initial detection was assigned as the date of detection for analyses. Actinic keratoses on the face, scalp, forearms, and hands were counted by means of palpation and observation at baseline and at the 3-month visits through 12 months by a single author at each site, who was unaware of the study-group assignments.

The histologic diagnosis of skin cancer was made by histopathologists at each site according to routine clinical practice. All new squamous lesions, including invasive squamous-cell carcinoma, keratoacanthomas, Bowen's disease (squamous-cell carcinoma in situ [full-thickness epidermal dysplasia]), and actinic keratoses (partial-thickness epidermal dysplasia),[20] and new high-risk subtypes of basal-cell carcinoma (morpheic, infiltrating, and micronodular)[20] were additionally reviewed by a single histopathologist, who was unaware of the study-group assignments, to ensure consistent classification of the types of squamous-cell carcinoma and subtypes of basal-cell carcinoma. New melanomas and severely dysplastic nevi were reviewed by a single histopathologist with subspecialty expertise in melanocytic neoplasms, who was unaware of the study-group assignments. Assessments for adverse events were performed over the course of the entire 12-month intervention period and for 30 days thereafter. Blood samples were obtained at baseline and at 12 months for full blood counts and for assessment of electrolyte levels and renal and liver function.

STUDY END POINTS

The primary end point was the number of new, histologically confirmed nonmelanoma skin cancers (i.e., basal-cell carcinomas plus squamous-cell carcinomas, including invasive and in situ squamous-cell carcinoma) through the end of the 12-month intervention period. Secondary end points included the number of new basal-cell carcinomas, new squamous-cell carcinomas, and actinic keratoses during the 12-month intervention period, the number of new nonmelanoma skin cancers in the 6-month postintervention period, and the safety of nicotinamide as assessed by the numbers and types of adverse events. Because previous studies have suggested a benefit from nicotinamide[21,22] with respect to cognitive function and transepidermal water loss, these variables were also prespecified as secondary end points, but the results are not presented here.

STATISTICAL ANALYSIS

We estimated that with a sample size of 386, the study would have 90% power to detect a 33% lower rate of new nonmelanoma skin cancers with nicotinamide than with placebo at 12 months at a 5% level of significance, assuming that nonmelanoma skin cancer counts would follow a Poisson distribution and that a mean of 1.0 new nonmelanoma skin cancers per person would be detected in the placebo group, and allowing for an average rate of nonadherence of up to 10%. Analyses were prespecified in a statistical analysis plan (see the protocol) and were performed according to the intention-to-treat principle. In accordance with the provision specified in the statistical analysis plan, a negative binomial model was used for the analysis of data on nonmelanoma skin cancer because of overdispersion that rendered the Poisson model inappropriate. Models included an offset term to account for variation in the duration of follow-up. The primary analysis of nonmelanoma skin cancers included center and 5-year nonmelanoma skin cancer history as covariates; these covariates were omitted in a secondary sensitivity analysis. The same approach was used for the analysis of

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basal-cell carcinomas and squamous-cell carcinomas.

The consistency of the treatment effect with respect to the primary end point was investigated by means of a series of prespecified subgroup analyses that tested for an interaction between study-group assignment and age, sex, 5-year nonmelanoma skin cancer history, actinic keratosis count at baseline, smoking, nonsteroidal antiinflammatory drug use at baseline,[23] and statin use at baseline.[24] With an assumption of independence among the seven tests of interaction, there was a 30% probability that at least one P value would be less than 0.05 by chance alone. We explored the consistency of the treatment effect on the rate of nonmelanoma skin cancer over time by using generalized estimating equations to fit a negative binomial model for repeated measures to the data on nonmelanoma skin cancer collected at the 3-month visits. The data on actinic keratosis counts collected at the 3-month visits were analyzed with the use of a mixed-effects model for repeated measures. Models for repeated measures included study group, center, baseline value, time point, and the interaction between time and study group as covariates. No formal adjustment for multiple comparisons was made to P values from secondary analyses.

RESULTS

PATIENT CHARACTERISTICS

During the period from July 2, 2012, to June 14, 2014, we evaluated 432 patients (321 at the Royal Prince Alfred Hospital and 111 at the Westmead Hospital in Sydney) to determine eligibility for enrollment. We randomly assigned, in a 1:1 ratio, 386 of these patients (292 at the Royal Prince Alfred Hospital and 94 at the Westmead Hospital) to receive either nicotinamide or placebo (193 patients in each study group) (Fig. 1). The baseline characteristics were similar in the two groups (Table 1). The median rate of adherence to the nicotinamide or placebo regimen over the 12-month intervention period was 96% (mean, 88%) in the placebo group and 94% (mean, 89%) in the nicotinamide group, with no between-group differences in adherence at any of the 3-month visits (Table S1 in the Supplementary Appendix, available at NEJM.org). Follow-up rates were high, and skin assessments after baseline were available for all but 3 participants (Fig. 1).

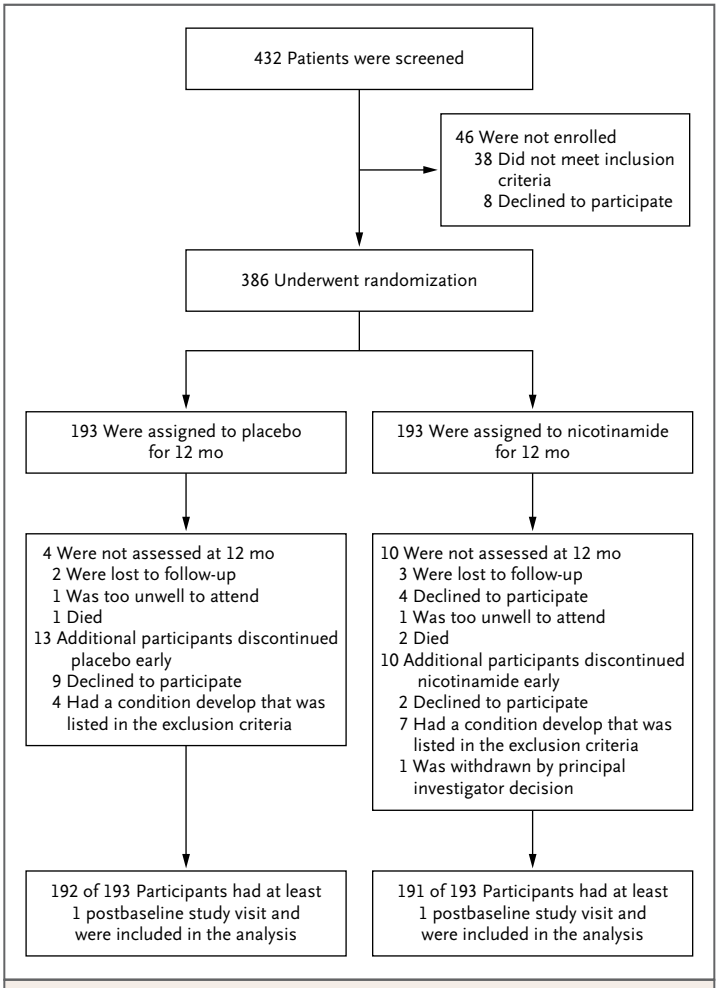

Figure 1. Screening, Randomization, and Follow-up.

OUTCOMES OF NONMELANOMA SKIN CANCERS AND ACTINIC KERATOSES

Figure 2 shows the results for the development of new nonmelanoma skin cancers, basal-cell carcinomas, and squamous-cell carcinomas. The mean number of new nonmelanoma skin cancers per person through the 12-month intervention period was significantly lower in the nicotinamide group than in the placebo group (1.8 [total of 336 cancers] vs. 2.4 [total of 463 cancers]), representing a rate that was lower by an estimated 23% (95% confidence interval [CI], 4 to 38) with nicotinamide after adjustment for center and 5-year nonmelanoma skin-cancer history (P=0.02) and by an estimated 27% (95% CI, 5 to 44) with no adjustment (P=0.02). At each 3-month visit during the 12-month intervention period, the estimated rate of new nonmelanoma skin cancers was lower in the nicotinamide group than in the

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Table 1. Baseline Characteristics.*

Characteristic	Placebo (N=193)	Nicotinamide (N=193)
Age — yr		
Mean	66.4±11.8	66.4±11.8
Range	30–91	30–89
Sex — no. (%)		
Male	121 (63)	122 (63)
Female	72 (37)	71 (37)
Never smoked — no. (%)	88 (46)	92 (48)
Skin cancers in previous 5 years — no.		
Nonmelanoma skin cancers		
Mean	8.2±7.4	7.9±8.0
Range	2–52	2–61
Basal-cell carcinomas		
Mean	6.1±7.0	5.7±6.9
Range	0–49	0–59
Squamous-cell carcinomas		
Mean	2.1±3.2	2.1±3.5
Range	0–23	0–31
Actinic keratoses at baseline		
Mean	46.2±42.9	47.7±43.2
Range	0–214	0–205
Medical history — no. (%)		
Hypertension	84 (44)	86 (45)
Hypercholesterolemia	82 (42)	79 (41)
Asthma	21 (11)	37 (19)
Ischemic heart disease	23 (12)	32 (17)
Osteoporosis	20 (10)	19 (10)
Diabetes	15 (8)	16 (8)
Cancer other than skin	10 (5)	14 (7)
Stroke or transient ischemic attack	13 (7)	5 (3)
Sunscreen use in the past week — no. (%)	98 (51)	90 (47)
Statin use — no. (%)	71 (37)	75 (39)
Nonsteroidal antiinflammatory drug use — no. (%)	48 (25)	53 (27)

* Plus–minus values are means ±SD. There were no significant differences between the groups at baseline except for a more frequent history of asthma (P=0.03) in the nicotinamide group.

placebo group: relative difference, 25% (95% CI, −7 to 48) at 3 months (P=0.11), 27% (95% CI, −5 to 50) at 6 months (P=0.09), 18% (95% CI, −18 to 43) at 9 months (P=0.29), and 29% (95% CI, −6 to 52) at 12 months (P=0.09).

The effect of nicotinamide on nonmelanoma skin cancers was not maintained into the 6-month follow-up period after the drug was discontinued (relative difference, nicotinamide vs. placebo, −17%; 95% CI, −59 to 14; P=0.33) or modified by age, baseline actinic keratosis count, sex, smoking status, nonsteroidal antiinflammatory drug use, or statin use (Fig. S1 in the Supplementary Appendix). There was a trend toward greater effectiveness of nicotinamide among patients who had had a higher number of nonmelanoma skin cancers in the 5 years before baseline. The interaction term was significant (P=0.02) when the nonmelanoma skin cancer count in the previous 5 years was treated as a continuous covariate, but was not significant (P=0.18) when 5-year history of nonmelanoma skin cancer was treated as a categorical covariate (i.e., <6 vs. ≥6 nonmelanoma skin cancers). There was no significant difference between the groups in the number of recurrent nonmelanoma skin cancers (13 in the placebo group and 17 in the nicotinamide group).

The mean number of basal-cell carcinomas per person through the 12-month intervention period was 1.3 in the nicotinamide group (total of 239 cancers) and 1.7 in the placebo group (total of 327 cancers), representing a rate that was lower by an estimated 20% (95% CI, −6 to 39) with nicotinamide after adjustment for center and 5-year basal-cell carcinoma history (P=0.12). This relative difference with respect to basal-cell carcinomas appeared to be associated primarily with a lower number of superficial basal-cell carcinomas in the nicotinamide group than in the placebo group (Table 2). The number of basal-cell carcinomas was similar in the two groups in the 6-month postintervention period (relative difference, nicotinamide vs. placebo, −6%; 95% CI, −53 to 26; P=0.73).

The mean number of squamous-cell carcinomas per person through the 12-month intervention period was 0.5 in the nicotinamide group (total of 97 cancers) and 0.7 in the placebo group (total of 136 cancers), representing a rate that was lower by an estimated 30% (95% CI, 0 to 51) with nicotinamide after adjustment for center and 5-year squamous-cell carcinoma history (P=0.05). The effect of nicotinamide appeared to be independent of the differentiation of squamous-cell carcinoma (well differentiated, moderately differentiated, or poorly differentiated) (Table 2). There was a nonsignificant trend toward the development of more squamous-cell carcinomas in the nicotinamide group than in

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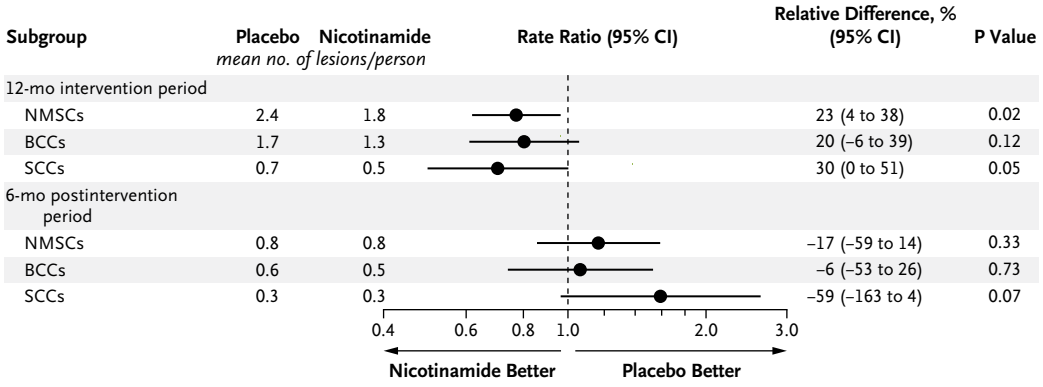

Figure 2. Incidence of New Nonmelanoma Skin Cancers, Basal-Cell Carcinomas, and Squamous-Cell Carcinomas.

The mean numbers of lesions per participant reflect simple averages, whereas the rate ratios (the ratio of the estimated rates in the two study groups), relative differences (1 minus the rate ratio, multiplied by 100), and P values are estimated from a model that includes center and skin cancer count in the previous 5 years as covariates. When the covariates of center and 5-year nonmelanoma skin cancer history were omitted from the models in secondary analyses, the conclusions remained unchanged and the estimates corresponded directly to the ratio of mean numbers per participant in the two groups. The rate of new nonmelanoma skin cancers (NMSCs) during the 12-month intervention period was significantly lower with nicotinamide than with placebo (relative difference, 23%; P=0.02), with similar relative differences observed for both basal-cell carcinomas (BCCs) and squamous-cell carcinomas (SCCs). This benefit with nicotinamide was not observed during the 6-month postintervention period. Because of rounding, the mean number of SCCs per patient appears identical in the two study groups; the actual values are 0.250 for the placebo group and 0.325 for the nicotinamide group. Although the between-group comparison for the postintervention period is unbiased, the annualized rates for this period cannot be compared directly with those from the 12-month intervention period because of variance in the duration of surveillance of detected lesions that did not immediately warrant biopsy (e.g., a lesion identified at month 3 could be monitored for many months before being confirmed as a nonmelanoma skin cancer on biopsy, whereas a lesion identified at month 18 could not be monitored for many months).

the placebo group in the 6-month postintervention period (relative difference, nicotinamide vs. placebo, –59%; 95% CI, –163 to 4; P=0.07).

The number of actinic keratoses was 11% lower in the nicotinamide group than in the placebo group at 3 months (P=0.01), 14% lower at 6 months (P<0.001), 20% lower at 9 months (P<0.001), and 13% lower at 12 months (P=0.001). This equated to 3 to 5 fewer actinic keratoses, on average, from the baseline count in the nicotinamide group than in the placebo group (Fig. 3). The rate of sunscreen use in the week before baseline and at the 3-month visits through 12 months was lower in the nicotinamide group than in the placebo group (Table S2 in the Supplementary Appendix).

SAFETY

No clinically significant between-group differences were found with respect to the number or types of adverse events that occurred in the study groups (Table S3 in the Supplementary Appendix). The terms for the most common serious adverse events, reported according to the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.03, that occurred in the two groups combined included neoplasm (12 patients), cardiac chest pain (9 patients), fall (7 patients), lung infection (6 patients), atrial fibrillation (6 patients), injury (6 patients), heart failure (5 patients), and hematoma (5 patients). Two internal cancers were diagnosed in the placebo group (duodenal carcinoma diagnosed at month 1 of the study and lung cancer at month 3) and five internal cancers were diagnosed in the nicotinamide group (non-Hodgkin's lymphoma diagnosed at month 1 of the study, colorectal cancer at month 2, lung cancer at month 2, prostate cancer at month 7, and bladder cancer at month 9). Four new invasive melanomas and six new melanomas in situ were diagnosed during the 12-month intervention period and were evenly distributed between the two groups. A microcystic adnexal carcinoma developed as a collision tumor (two originally separate tumors that have developed in close

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Table 2. Subtypes of Basal-Cell Carcinoma and Differentiation of Squamous-Cell Carcinoma at 12 Months.

Variable	Placebo (N=193)	Nicotinamide (N=193)
	number	
Basal-cell carcinoma		
Total	327	239
Subtype		
Superficial	181	98
Nodular	128	115
Micronodular	6	6
Infiltrating	12	18
Morpheic	0	2
Squamous-cell carcinoma		
Total	136	97
Differentiation		
Bowen's disease (squamous-cell carcinoma in situ)	69	47
Well differentiated	43	31
Moderately differentiated	24	16
Poorly differentiated	0	3

proximity) with a squamous-cell carcinoma in a patient receiving nicotinamide. There were no clinically or statistically significant differences between the study groups with respect to changes in weight, blood pressure, hemoglobin, white-cell count, platelet count, or levels of creatinine, alkaline phosphatase, γ-glutamyl transferase, alanine aminotransferase, or aspartate aminotransferase.

DISCUSSION

The rate of new nonmelanoma skin cancers was lower in the nicotinamide group than in the placebo group (relative difference, 23%; P=0.02), with similar differences in the rates of new basal-cell carcinomas and new squamous-cell carcinomas. There was a trend toward increasing effectiveness of nicotinamide among patients who had had higher numbers of nonmelanoma skin cancers in the preceding 5 years; however, the statistical evidence was limited, given the multiple tests performed, and is insufficient to warrant restricting treatment to a particular subgroup of high-risk patients, particularly in light of the favorable safety profile and low cost of nicotinamide. The possible increased efficacy among participants with higher numbers of nonmelanoma skin cancers may reflect the immunoprotective effects of nicotinamide.[18] Patients with previous skin cancers have a greater susceptibility to the immunosuppressive effects of sunlight,[18] and it may be that this susceptibility is more pronounced in patients with higher numbers of nonmelanoma skin cancers, who thus may have a greater potential to benefit from nicotinamide.

Sunscreen is effective in reducing the number of actinic keratoses and the incidence of squamous-cell carcinoma,[8] but even in our high-risk study population, only half the patients had used sunscreen in the week before baseline. Hence, potential exists for oral chemopreventive agents to become an effective component in the prevention of skin cancers. By chance, there was a lower rate of sunscreen use from baseline to 12 months in the nicotinamide group than in the placebo group. The lower number of new nonmelanoma skin cancers with nicotinamide treatment than with placebo observed in our study is therefore not attributable to chance differences in sunscreen use. Other agents with evidence of clinical efficacy for the prevention of nonmelanoma skin cancer include oral retinoids, topical DNA repair enzymes, and oral nonsteroidal antiinflammatory drugs. In a randomized, controlled trial involving 2297 participants, the risk of new squamous-cell carcinomas was lower among those who received oral administration of 25,000 IU of retinol daily than among those who received placebo (hazard ratio, 0.74; P=0.04), but the risk of basal-cell carcinomas was not lower with retinol (hazard ratio, 1.06; P=0.36).[25] A number of smaller studies showed that oral retinoids such as acitretin and isotretinoin significantly reduced the risk of new nonmelanoma skin cancers,[26-29] although these agents are associated with substantial adverse effects including dry skin, increased lipid levels, hepatotoxic effects, and teratogenicity.[30] Treatment with topical DNA repair enzymes was associated with a lower rate of new actinic keratoses and basal-cell carcinomas than was placebo in patients with xeroderma pigmentosum,[31] but the usefulness of these DNA repair enzymes in the broader population is limited by cost and availability. A randomized, controlled trial involving 240 patients showed that treatment with

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the nonsteroidal antiinflammatory drug celecoxib was associated with a significantly lower rate of new nonmelanoma skin cancers than was placebo, but this was not a primary or secondary end point of the trial.[23]

Nicotinamide was significantly associated with lower actinic keratosis counts than those with placebo as early as the first 3-month visit and at each subsequent 3-month visit up to 12 months, a finding that is consistent with the results from our previous phase 2 studies.[19] Similarly, there was a relatively constant, although nonsignificant, trend toward lower rates of new nonmelanoma skin cancers in the nicotinamide group than in the placebo group at each 3-month visit during the 12-month intervention period. This benefit was not maintained in the postintervention period. The trend toward lower rates of new nonmelanoma skin cancers with nicotinamide than with placebo starting from 3 months after the start of intervention suggests that nicotinamide suppresses the progression of nascent, preexisting cancers. The chemopreventive effect of nicotinamide was lost shortly after discontinuation, a finding that was also seen with respect to oral retinoids in other studies.[26-28,32]

Nicotinamide appeared to reduce the incidence of new superficial basal-cell carcinomas more than that of other subtypes, whereas it had a relatively constant effect across strata of differentiation of squamous-cell carcinoma. However, interpretation was limited by small numbers of high-risk subtypes of basal-cell carcinoma (micronodular, infiltrating, and morpheic) and of poorly differentiated squamous-cell carcinomas. The characteristics of superficial basal-cell carcinomas differ from those of nodular basal-cell carcinomas at the molecular level,[33] and superficial basal-cell carcinomas are proportionally more common in immunosuppressed transplant recipients,[34] which suggests that different biologic pathways underlie the pathogenesis of superficial basal-cell carcinomas and may enable them to be more readily prevented by nicotinamide.

We found that nicotinamide had a good safety profile. Nicotinamide has been used at pharmacologic doses (up to 3 g daily) over many years with minimal side effects[35] and is used clinically to treat autoimmune blistering disorders such as bullous pemphigoid, usually at doses of 1.5 g daily.[36] Unlike nicotinic acid (niacin), nicotinamide does not cause vasodilatory side effects such as flushing, itching, hypotension, and headaches.[37] Our decision to use a dose of 1000 mg daily was based on the results of our phase 2 studies that showed a reduction in actinic keratosis counts at this dose.[19] Previous studies on immunosuppression induced by UV radiation suggested that there is no greater efficacy with 1500 mg than with 500 mg daily,[18] but the minimum and maximum effective chemopreventive doses are as yet unknown.

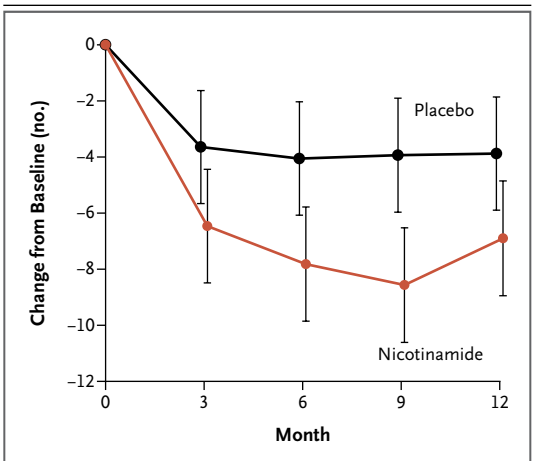

Figure 3. Change from Baseline to Month 12 in Number of Actinic Keratoses.

The change from baseline in the number of actinic keratoses was adjusted for center and number of actinic keratoses at baseline. The number of actinic keratoses was 11% lower in the nicotinamide group than in the placebo group at 3 months (P=0.01), 14% lower at 6 months (P<0.001), 20% lower at 9 months (P<0.001), and 13% lower at 12 months (P=0.001).

In conclusion, among high-risk patients, nicotinamide was associated with a lower rate of new nonmelanoma skin cancers than was placebo and had an acceptable safety profile. Nicotinamide is widely accessible as an inexpensive over-the-counter vitamin supplement and presents a new opportunity for the chemoprevention of nonmelanoma skin cancers that is readily translatable into clinical practice.

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