Small-Molecule Enhancers of the Antileukemic Activity of Vitamin D Derivatives (VDDs) in AML Models

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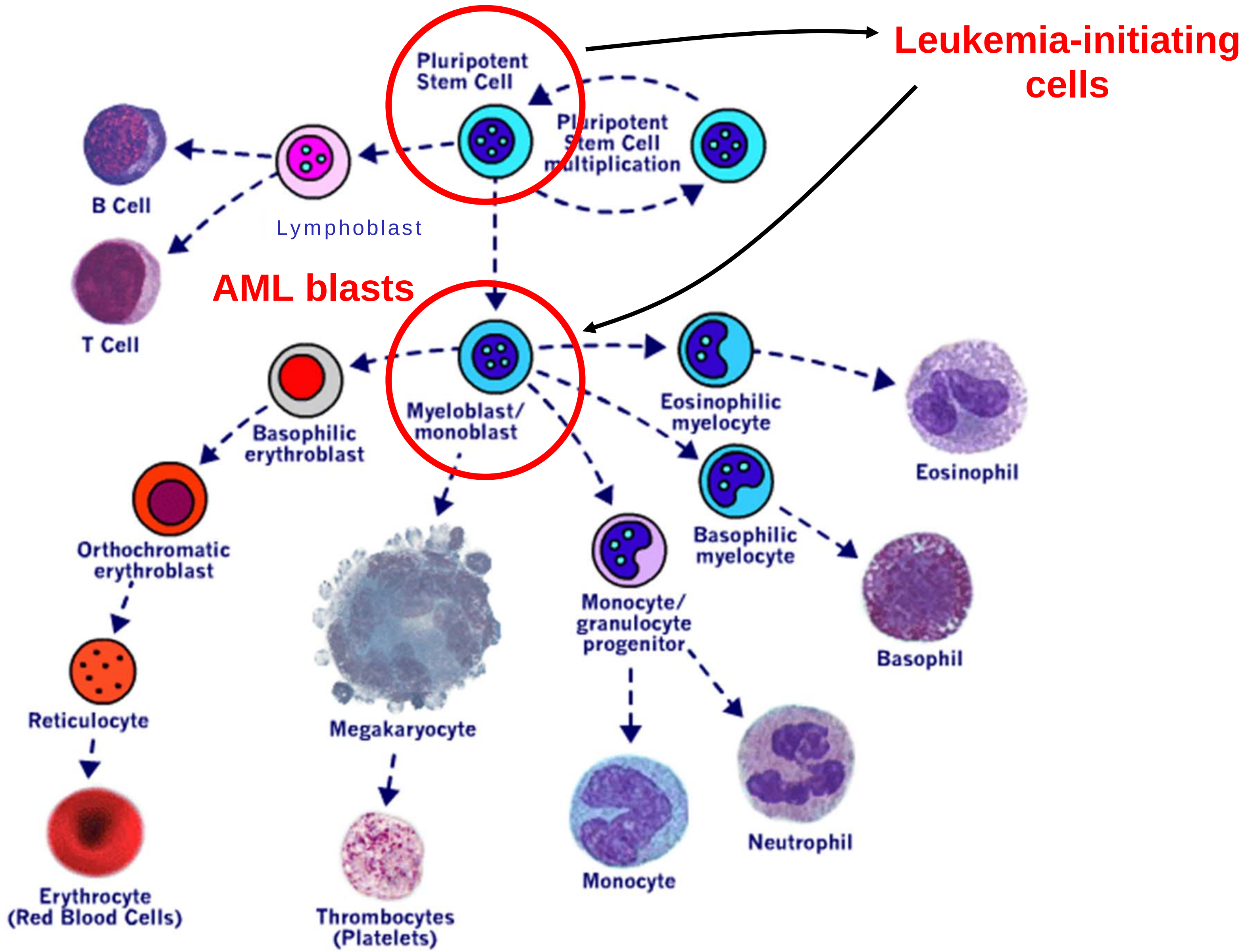
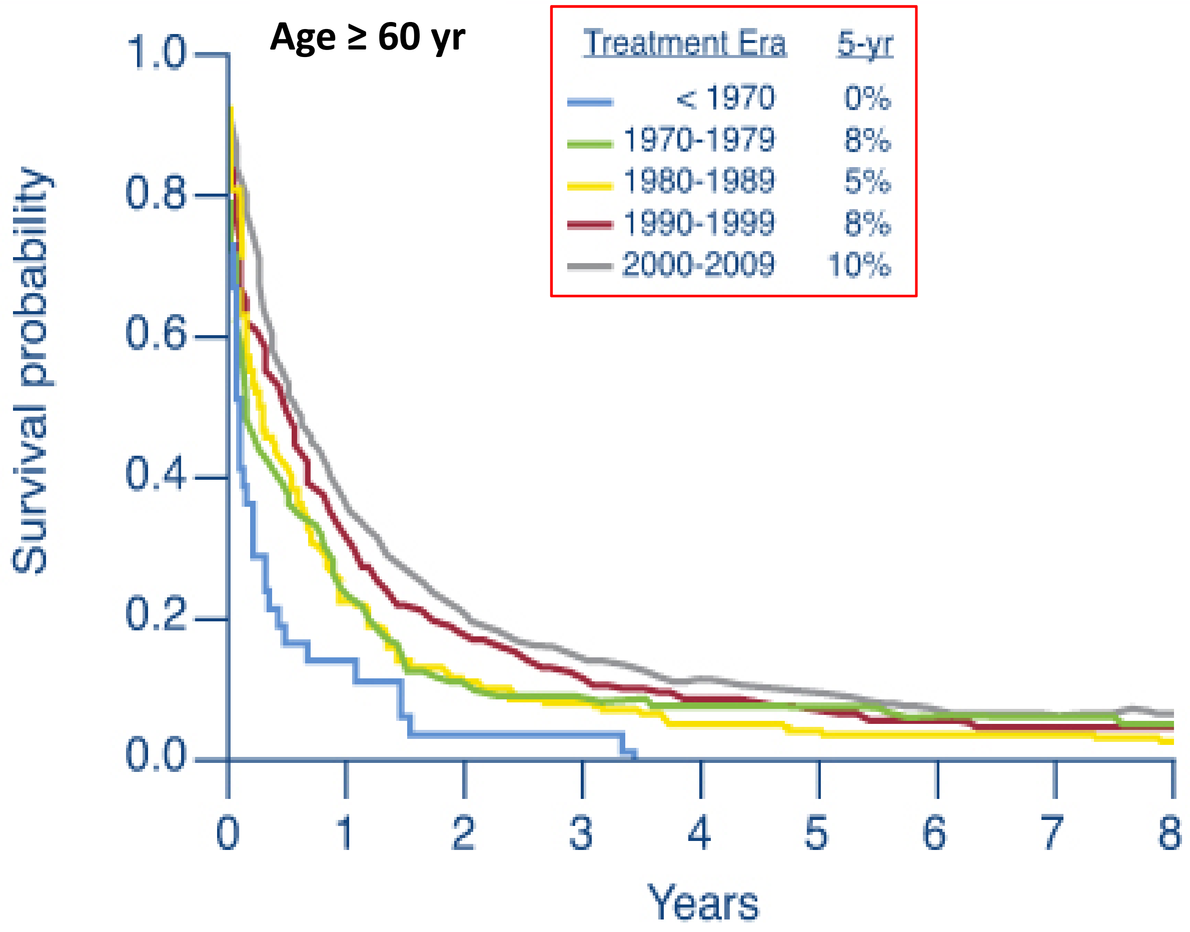
BGU

Acute Myeloid Leukemia (AML)



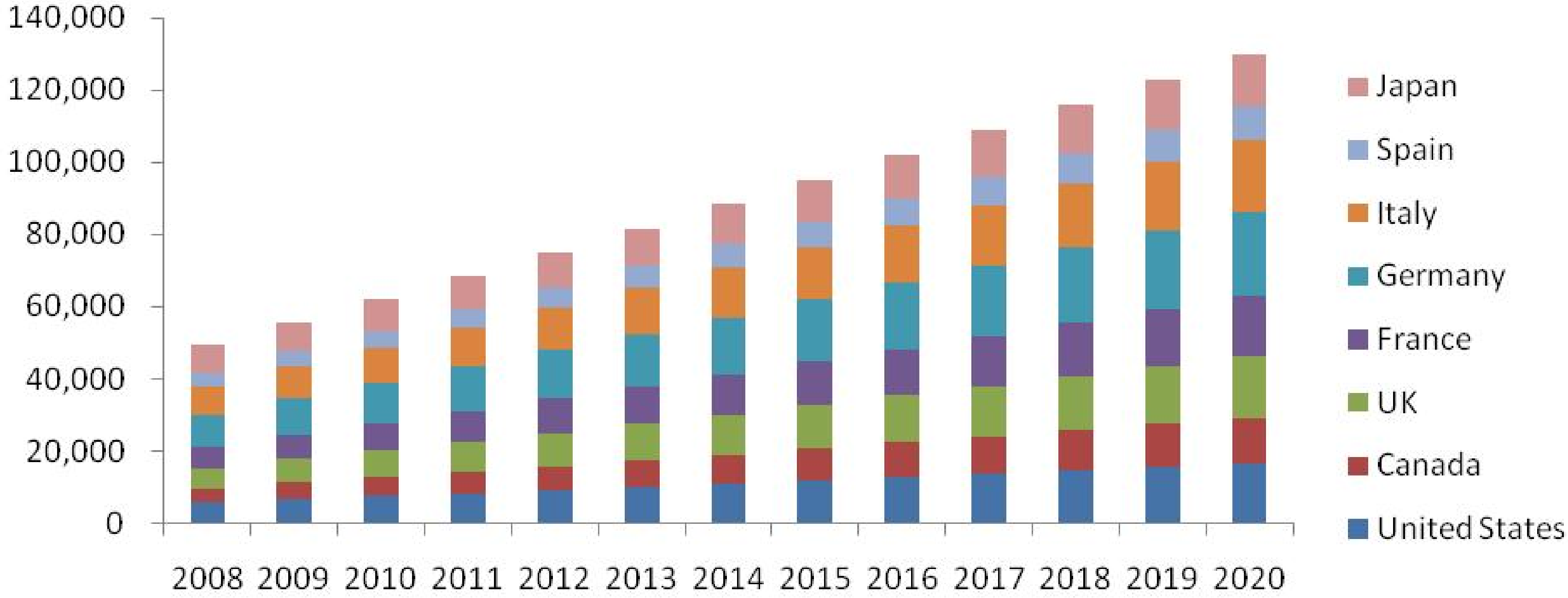
Acute Myeloid Leukemia: Incidence & Survival



Limited progress in treatment of older patients with AML (MD Anderson Cancer Center Database)

Novel therapeutic strategies are needed

Acute Myeloid Leukemia diagnosed population in developed countries (2008 - 2020)



Source: *MarketsAndMarkets* analysis

AML prevention becomes an issue

THERAPY of AML

Chemotherapy

↓

Cell death (apoptosis)

Cytarabine + Anthracycline "7+3"

- 50-70% CR → inevitable recurrence
- Toxicity, esp. in older patients

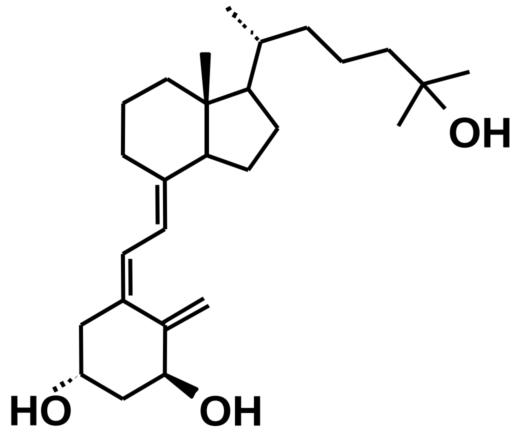
Differentiation therapy

↓

Cell maturation

AML-M3 (APL):
All *trans*-retinoic acid (ATRA)
+ Chemo or As_2O_3
~95% CR – 80% DFS

1α,25-dihydroxyvitamin D_3 (1,25D) as an anticancer agent



- **Powerful inducer of differentiation, growth arrest and/or apoptosis in cancer models in vitro & in vivo**
- **Causes severe *hypercalcemia* at pharmacologically effective doses *in vivo***
- **Synthetic low-calcemic analogs – limited progress in cancer clinical trials**

Combination approach:

Low (tolerated) dose of 1,25D or analog
+ Enhancer / Sensitizer

Initial findings showing cooperation of 1,25D and other compounds:

1. Miyaura C, Abe E, Honma Y, Hozumi M, Nishii Y, Suda T. **Cooperative effect of 1α,25-dihydroxyvitamin D_3 and dexamethasone in inducing differentiation of mouse myeloid leukemia cells**. *Arch Biochem Biophys*, **1983,** 227: 379-385.

2. Olsson I, Gullberg U, Ivhed I, Nilsson K. **Induction of differentiation of the human histiocytic lymphoma cell line U-937 by 1α,25-dihydroxycholecalciferol**. *Cancer Res*, **1983**, 43: 5862-5867.

Differentiation-inducing agents cooperating with VDDs

Compound	Cell type	Comments
Retinoids: ATRA 9-cis-RA Synthetic retinoids	Hematopoietic, Prostate, Breast, Pancreas, Ovary, Neuroblastoma, Lung, Colon, Melanoma	Synergistic, additive or antagonistic effects depending on cell type. Role of androgen receptor is suggested.
PKC activators: TPA Bryostatin	Hematopoietic Breast	Involvement of NFκB nuclear targets and enhanced VDR expression is suggested.
HDAC inhibitors: Sodium butyrate Trichostatin A	Hematopoietic, Colon, Prostate Breast, Prostate	Cooperation is associated with upregulation of VDR.
TGF-β	Hematopoietic, Breast, Colon Bone, Multiple myeloma	Cooperation involves upregulation of TGF-β receptors and VDR.
GM-CSF	Hematopoietic	Synergistic differentiating effect is associated with induction of c-fos and downregulation of c-myc.
Dimethyl sulfoxide	Hematopoietic	DMSO-induced G1 arrest is required for synergy

Danilenko & Studzinski (2004) *Exp Cell Res*

Various drugs and other agents cooperating with VDDs

Compound	Cell type	Comments
Dexamethasone	Hematopoietic, Myeloma, Breast, Ovary, Squamous cell carcinoma	Dexamethasone reduces hypercalcemia induced by 1,25D_3. The enhancing effect is attributed to VDR upregulation and reduction in ERK and Akt activities.
<u>Cytochrome P450 inhibitors:</u> Ketoconazole Liarozole VID400	Hematopoietic, Prostate, Breast Ovary	Enhancement is cell type-dependent. The mechanism of potentiation appears to be due to inhibition of 24-hydroxylase activity, which results in the reduced vitamin D_3 metabolism.
NSAIDs	Hematopoietic	The potentiating effect is mediated by inhibition of aldoketoreductase (AKR1C3).
<u>Cytokines</u>: TNFα IL-1β, IL-4, IL-6 Interferon	Hematopoietic, Breast, Kidney	Involvement of ROS is suggested. Enhance ICAM-1-dependent adhesion. Confer monocytic phenotype.
<u>Chemotherapeutic agents</u>: ***Anti-microtubule drugs*** Docetaxel (Taxotere) Paclitaxel (Taxol) ***Topoisomerase inhibitors*** Camptothecin Doxorubicin Etoposide ***Platinum drugs*** Cisplatin Carboplatin ***Nucleoside analogs*** 1-β-D-arabinofuranosylcytosine 5-aza-2'-deoxycytidine	Prostate cancer, Breast, Bone, Prostate, Squamous cell carcinoma	Pretreatment with deltanoids lowers the threshold for chemotherapy agents. Enhanced growth arrest and CD14 expression. p53 and ROS are involved in cooperation. Sequence of treatments is critical to the effect. Restore 1,25D_3 effect by DNA demethylation. Myeloid and monocytoid cells have different sensitivities to pyrimidine nucleoside analogs. Combinations enhance VDR/RXR binding to VDRE.

Danilenko & Studzinski (2004) *Exp Cell Res*

Phytochemicals and protein kinase inhibitors as functional enhancers of VDDs' effects

- **Plant polyphenols**:

  Carnosic acid, Curcumin, Silibinin

- **Kinase inhibitors**:

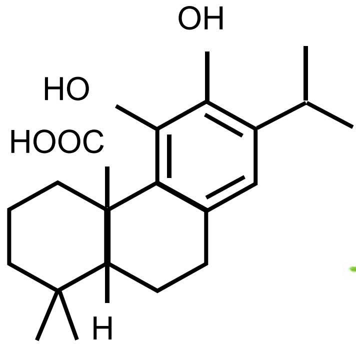
  p38, Cot1 and ERK5 kinases

Danilenko et al. (2001) *JNCI*
Danilenko et al (2003) *Cancer Res*
Wang et al. (2005) *J Cell Physiol*
Shabtay et al (2008) *Oncology*
Bobilev et al. (2011) *Cancer Biol Ther*
Wassermann et al. (2012) *Leuk Res Treatment*

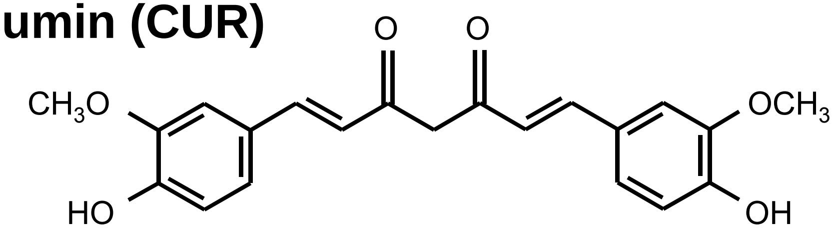
Zhang et al. (2007) *J Steroid Biochem Mol Biol*
Wang et al. (2010) *Cell Cycle*
Wang et al. (2014) *J Steroid Biochem Mol Biol*
Wang et al. (2014) *J Cell Physiol*
Wang et al. *Exp Cell Res (in revision)*

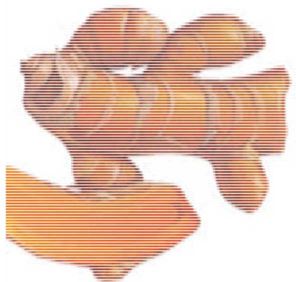
Plant polyphenols – potential anticancer agents

Carnosic acid (CA)

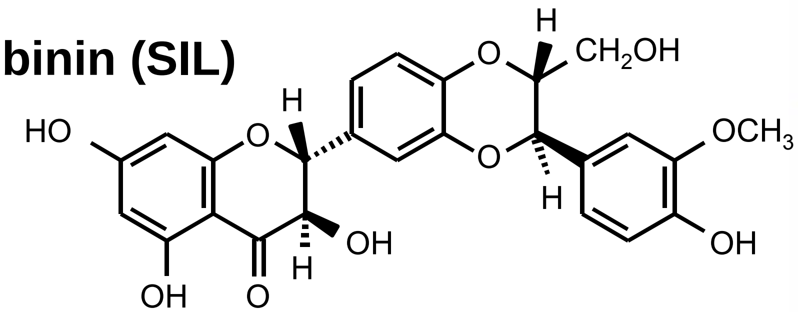


Curcumin (CUR)

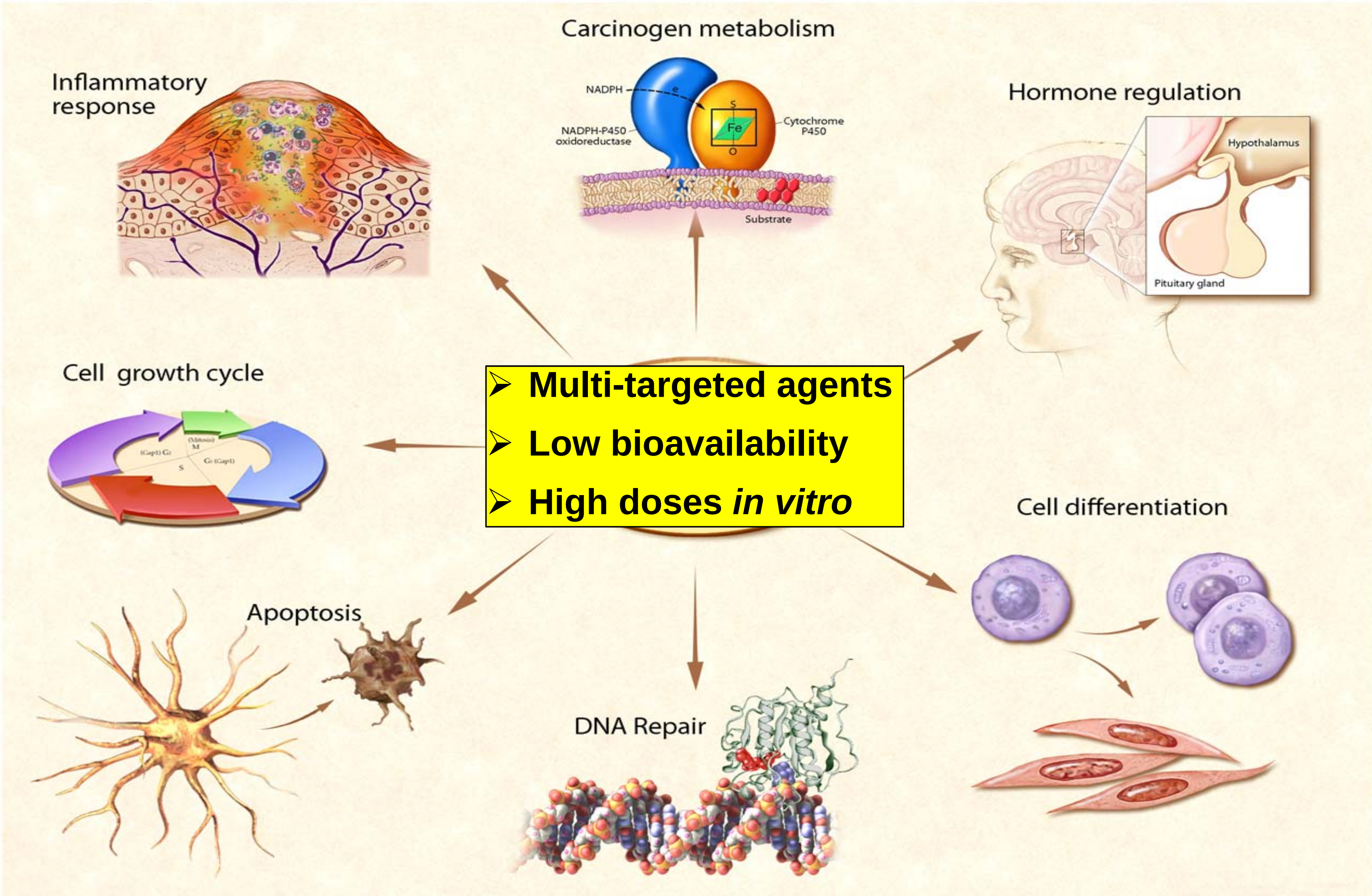




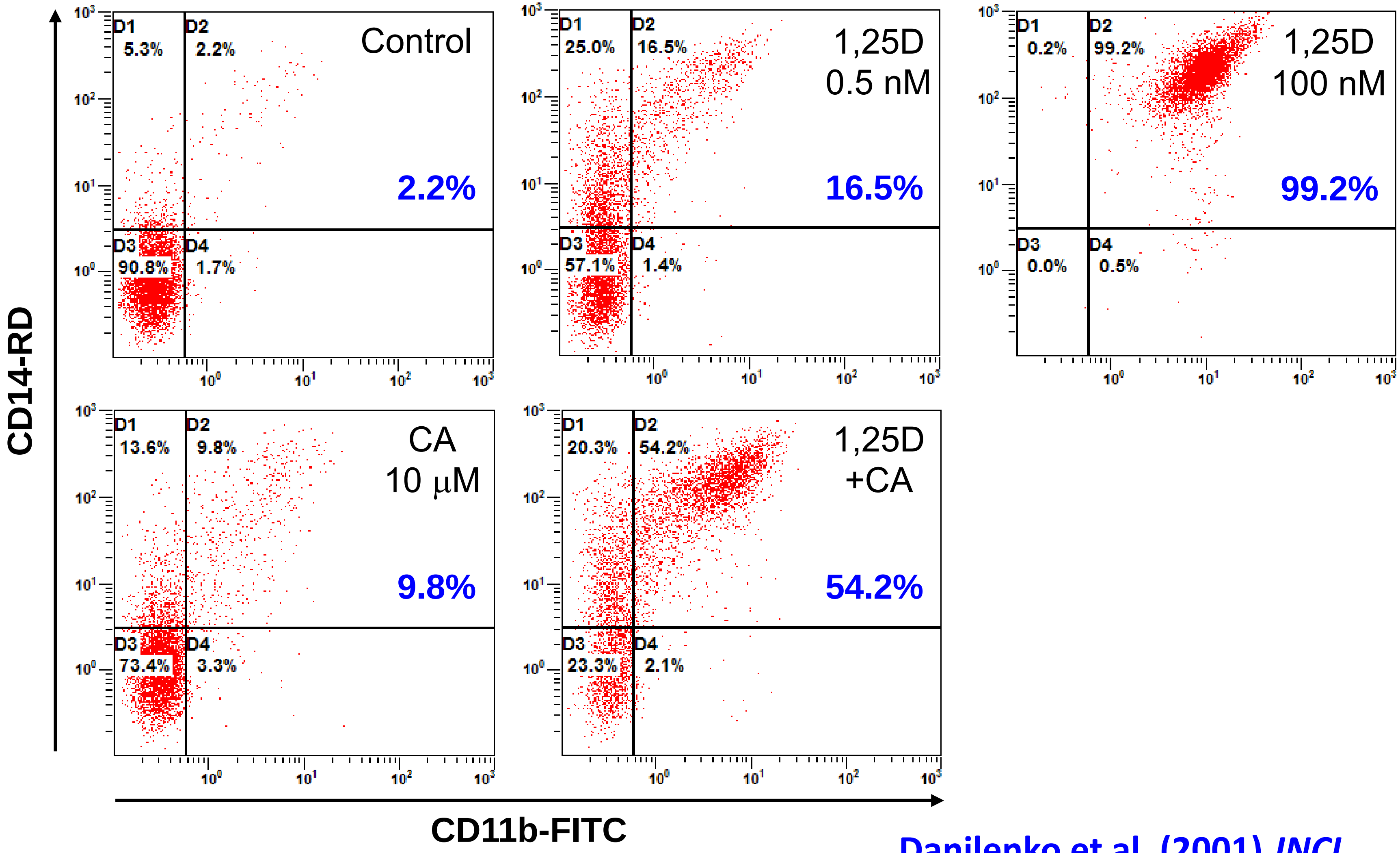
Silibinin (SIL)



Mechanisms Involved in the multi-targeted anticancer effects of phytochemicals



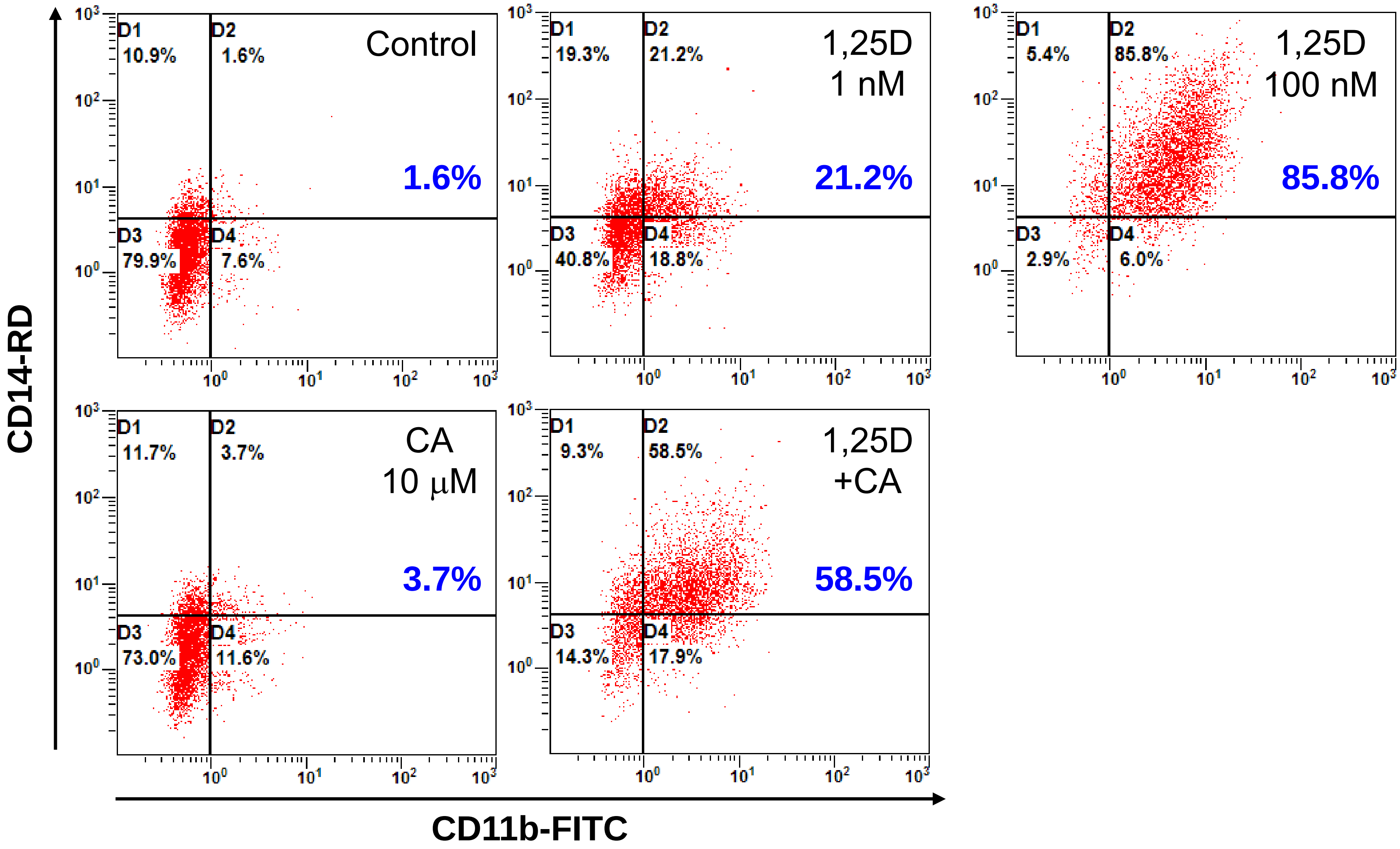
Synergistic induction of differentiation by 1,25D and carnosic acid in HL60-G cells (96 h)



Danilenko et al. (2001) *JNCI*

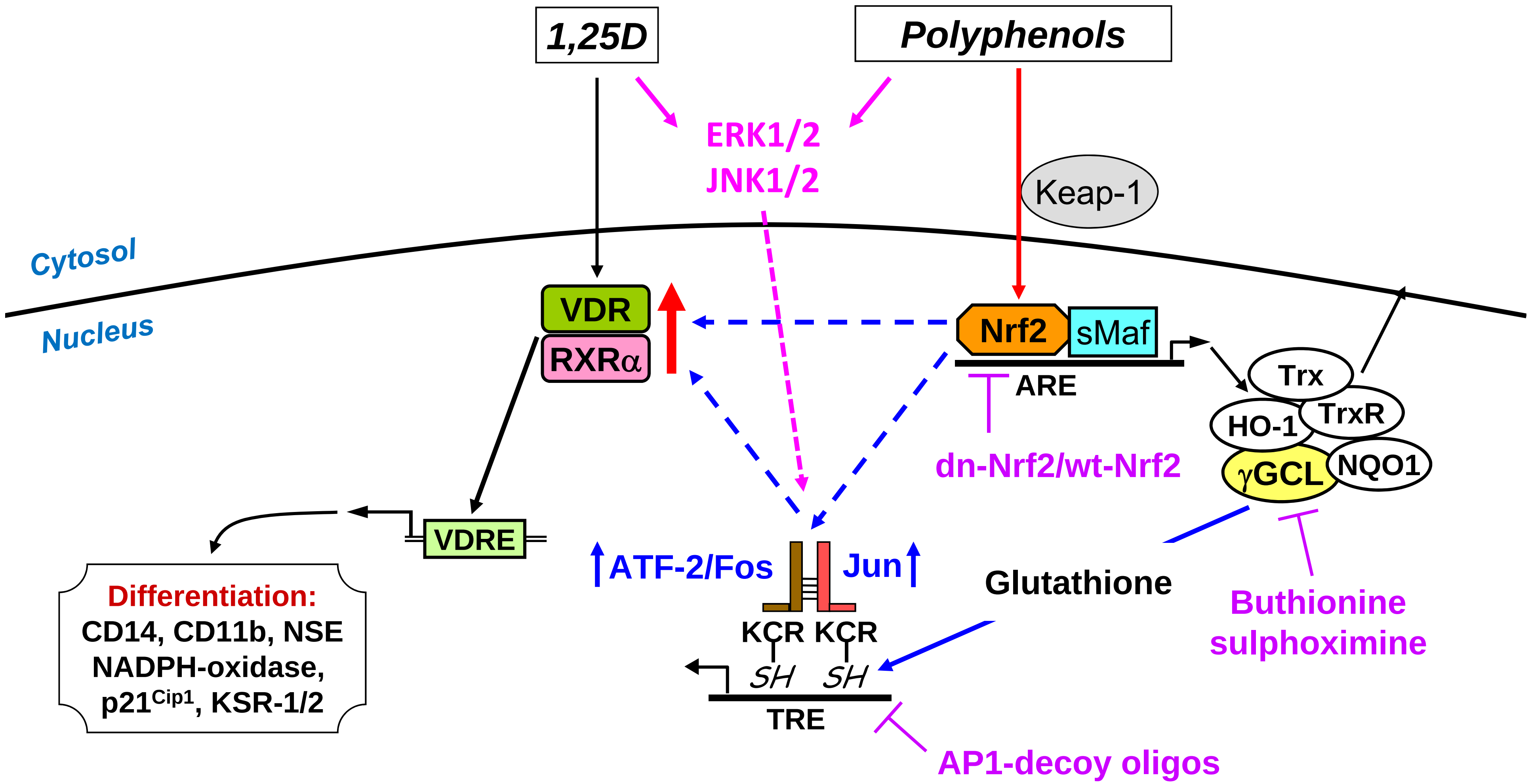
Danilenko et al (2003) *Cancer Res*

Synergistic induction of differentiation by 1,25D and carnosic acid in U937 cells (96 h)



Bobilev et al. (2011) *Cancer Biol Ther*

Putative mode of synergy between 1,25D and polyphenols



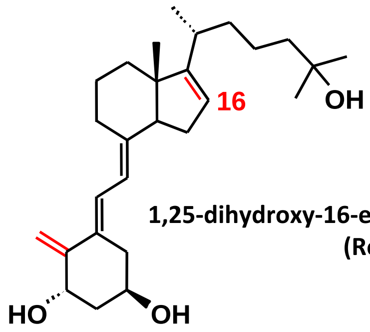
Bobilev et al. (2011) *Cancer Biol Ther*

Wassermann et al. (2012) *Leuk Res Treatment*

Danilenko et al. (2003) *Cancer Res*

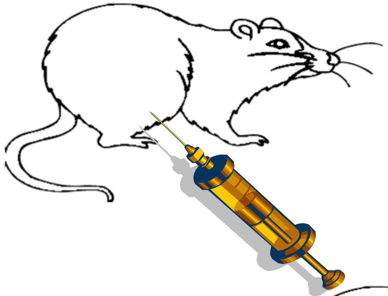
Wang et al. (2005) *J Cell Physiol*

Syngeneic Tumor Model of AML

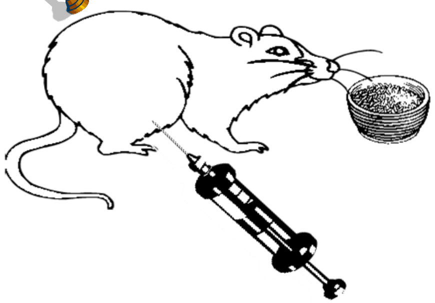


1,25-dihydroxy-16-ene-5,6-trans-cholecalciferol
(Ro25-4020)

Healthy Balb/c mice



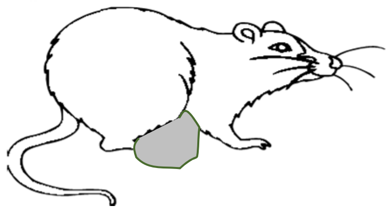
Day 0: Inoculation i.p. with 1.0 × 10^5 WEHI-3B D^- cells



Day 1: Injections of 1,25D_3 analogs i.p. every 3 days.

Oral treatment with a dried ethanolic rosemary extract (~35% CA) mixed with a powdered food

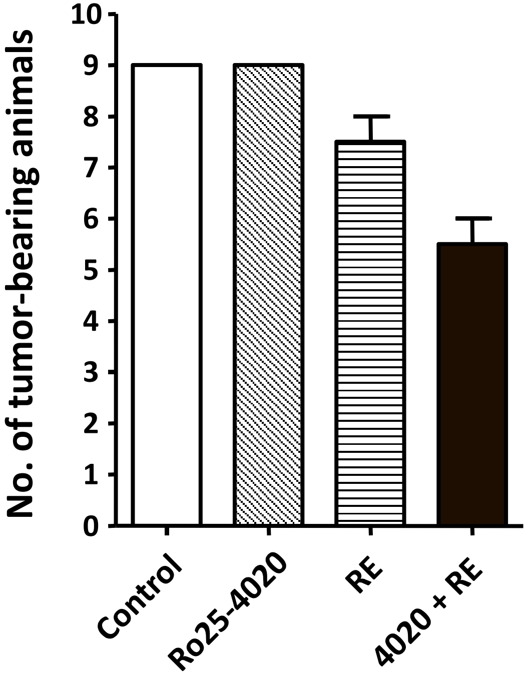
Tumor

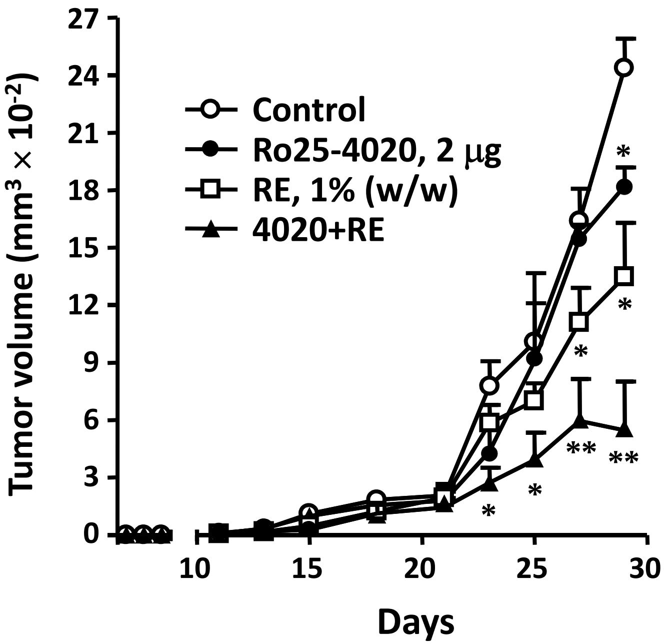


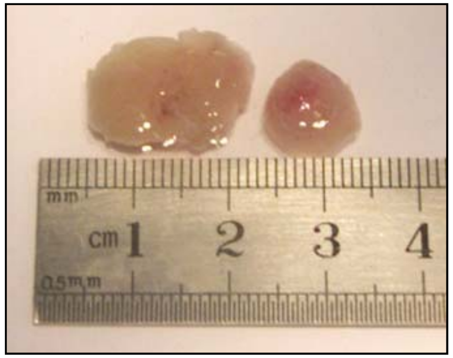
Inoculation of WEHI 3B D^- cells resulted in the formation of solid tumors on the anterior abdominal wall

Sharabani et al. (2006) *Int J Cancer*

Cooperative Antileukemic Effects of Ro25-4020 and Rosemary Extract in the AML Tumor Model

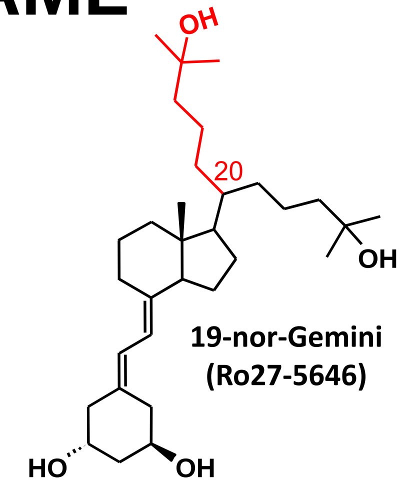






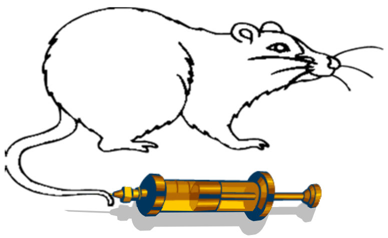
Sharabani et al. (2006) *Int J Cancer*

Syngeneic Model of Systemic AML

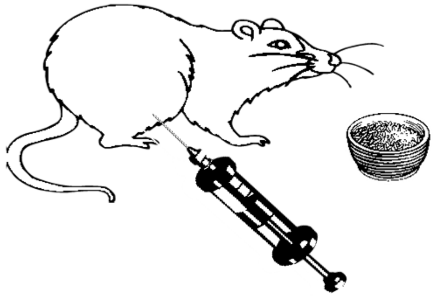


19-nor-Gemini
(Ro27-5646)

Healthy Balb/c mice



Day 0: Inoculation i.v. with 2.0 × 10^{6} WEHI 3B D^{-} cells

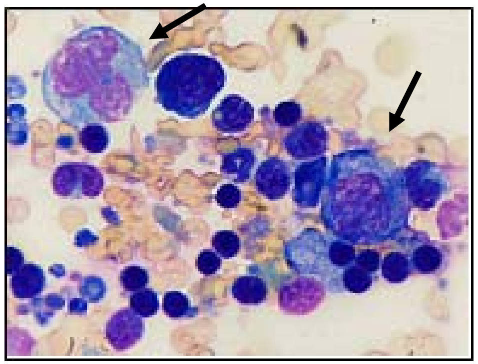


Day 7: Injections of Ro27-5646 i.p. every 3 days.

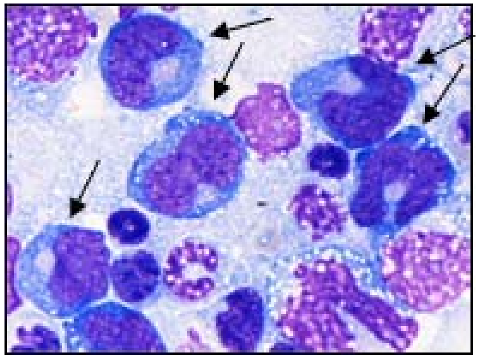
Oral treatment with a dried ethanolic rosemary extract mixed with a powdered food

Day 21: Leukemia development

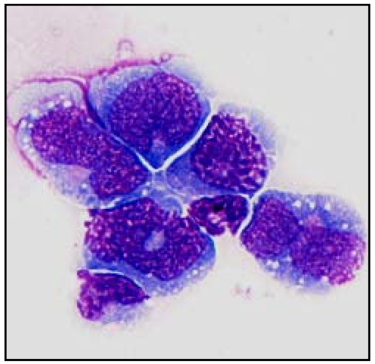
A. Peripheral blood



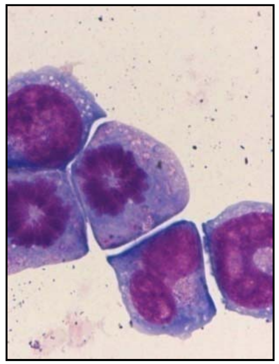
B. Bone marrow



C. Blast cluster in BM

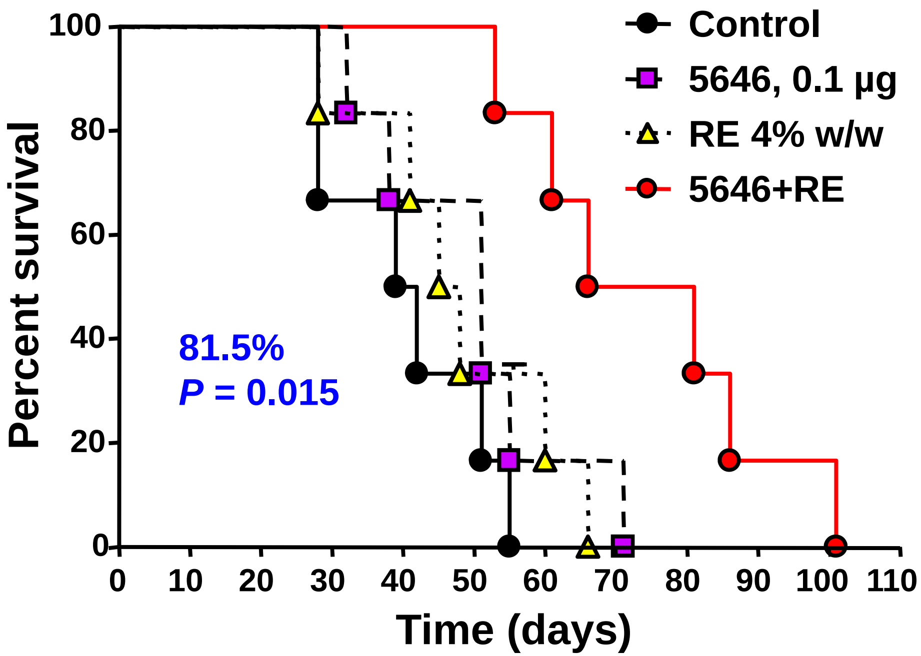


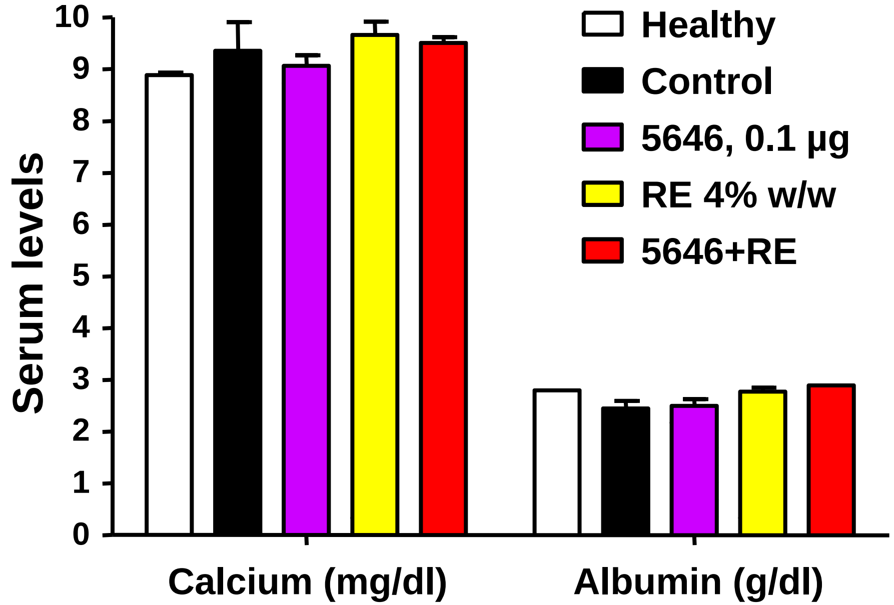
D. WEHI-3B D^{-} cells



Shabtay et al. (2008) *Oncology*

Synergistic antileukemic effects of rosemary extract (RE) and Ro27-5646 in a syngeneic mouse model of AML

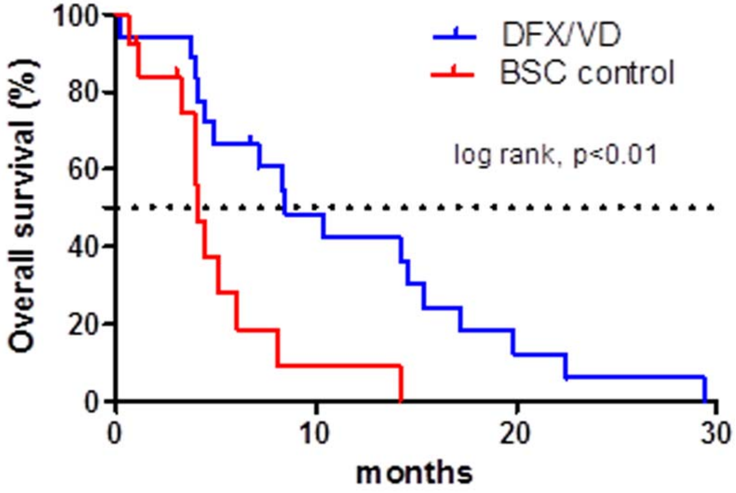




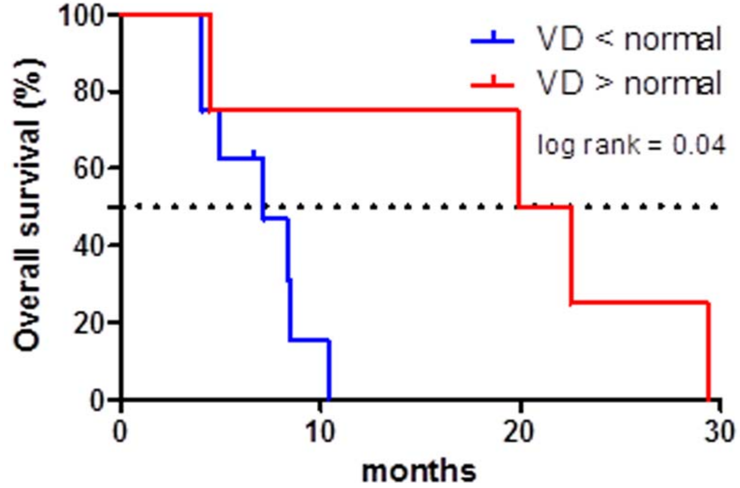
Shabtay et al. (2008) *Oncology*

$25(OH)D_3$ – Deferasirox combination increases overall survival in elderly AML patients

BSC, best supportive care - 13 patients (~ 76 yo)
DFX, deferasirox (1-2 g/d) + **VD**, $25(OH)D_3$ (100,000 IU/week) – 17 patients (~ 71 yo)



Median survival:
10.4 months vs. 4 months



Only serum levels of $25(OH)D_3$ prior to treatment was able to predict patients' outcome:
21.2 vs. 7.1 months

Paubelle E et al. (2013) PLOS One 8: e65998

Cancer 2014;120:521-9.

Original Article

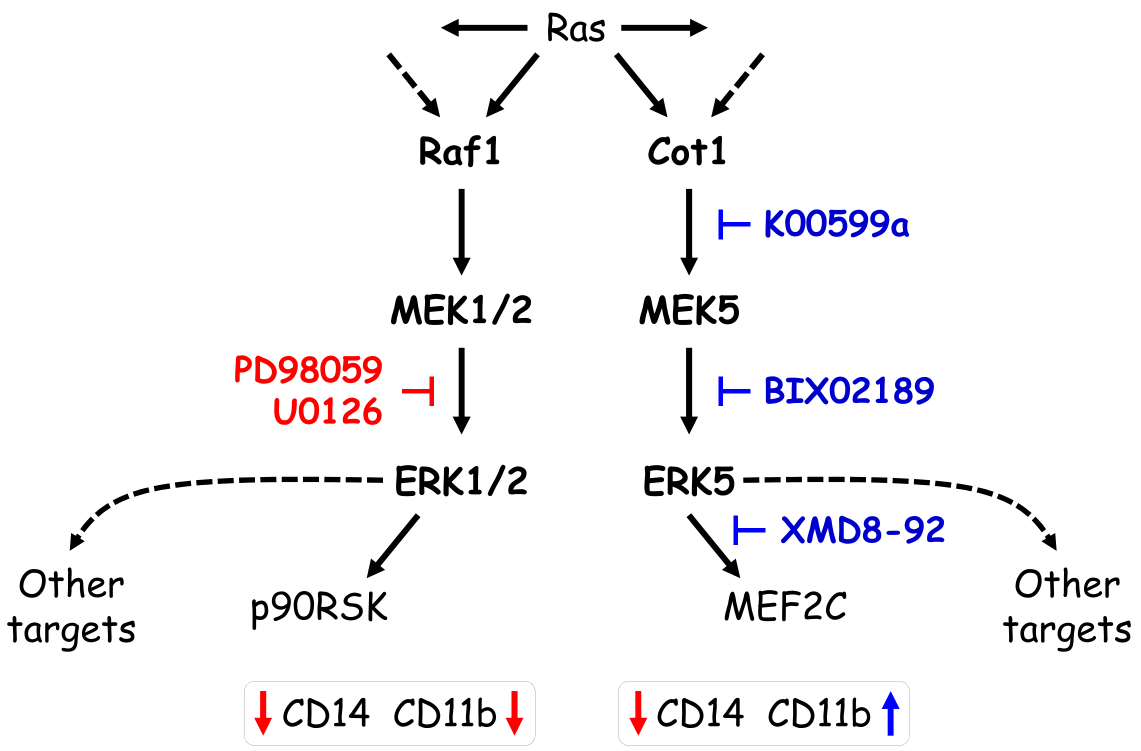
Low 25(OH) Vitamin D_3 Levels Are Associated With Adverse Outcome in Newly Diagnosed, Intensively Treated Adult Acute Myeloid Leukemia

Hun Ju Lee, MD[1]; Josephia R. Muindi, MD, PhD[2]; Wei Tan, MA[3]; Qiang Hu, PhD[3]; Dan Wang, MA[3]; Song Liu, PhD[3]; Gregory E. Wilding, PhD[3]; Laurie A. Ford, BS[1]; Sheila N. J. Sait, PhD[4]; Annemarie W. Block, PhD[4]; Araba A. Adjei, PhD[2]; Maurice Barcos, MD, PhD[5]; Elizabeth A. Griffiths, MD[1]; James E Thompson, MD[1]; Eunice S. Wang, MD[1]; Candace S. Johnson, PhD[2]; Donald L. Trump, MD[6]; and Meir Wetzler, MD[1]

BACKGROUND: Several studies have suggested that low 25(OH) vitamin D_3 levels may be prognostic in some malignancies, but no studies have evaluated their impact on treatment outcome in patients with acute myeloid leukemia (AML). **METHODS:** Vitamin D levels were evaluated in 97 consecutive, newly diagnosed, intensively treated patients with AML. MicroRNA expression profiles and single nucleotide polymorphisms (SNPs) in the 25(OH) vitamin D_3 pathway genes were evaluated and correlated with 25(OH) vitamin D_3 levels and treatment outcome. **RESULTS:** Thirty-four patients (35%) had normal 25(OH) vitamin D_3 levels (32-100 ng/mL), 34 patients (35%) had insufficient levels (20-31.9 ng/mL), and 29 patients (30%) had deficient levels (<20 ng/mL). Insufficient/deficient 25(OH) vitamin D_3 levels were associated with worse relapse-free survival (RFS) compared with normal vitamin D_3 levels. In multivariate analyses, deficient 25(OH) vitamin D_3, smoking, European Leukemia Network genetic group, and white blood cell count retained their statistical significance for RFS. Several microRNAs and SNPs were associated with 25(OH) vitamin D_3 levels, although none remained significant after multiple test corrections; one 25(OH) vitamin D_3 receptor SNP, rs10783219, was associated with a lower complete remission rate ($P = .0442$) and with shorter RFS ($P = .0058$) and overall survival ($P = .0011$). **CONCLUSIONS:** It remains to be determined what role microRNA and SNP profiles play in contributing to low 25(OH) vitamin D_3 level and/or outcome and whether supplementation will improve outcomes for patients with AML. *Cancer* 2014;120:521-9. © *2013 American Cancer Society.*

Roswell Park Cancer Institute, Elm and Carlton Streets, Buffalo, NY, 14263;

Roles of ERK1/2 and ERK5 signaling in $1,25D_3$-induced differentiation of AML cells



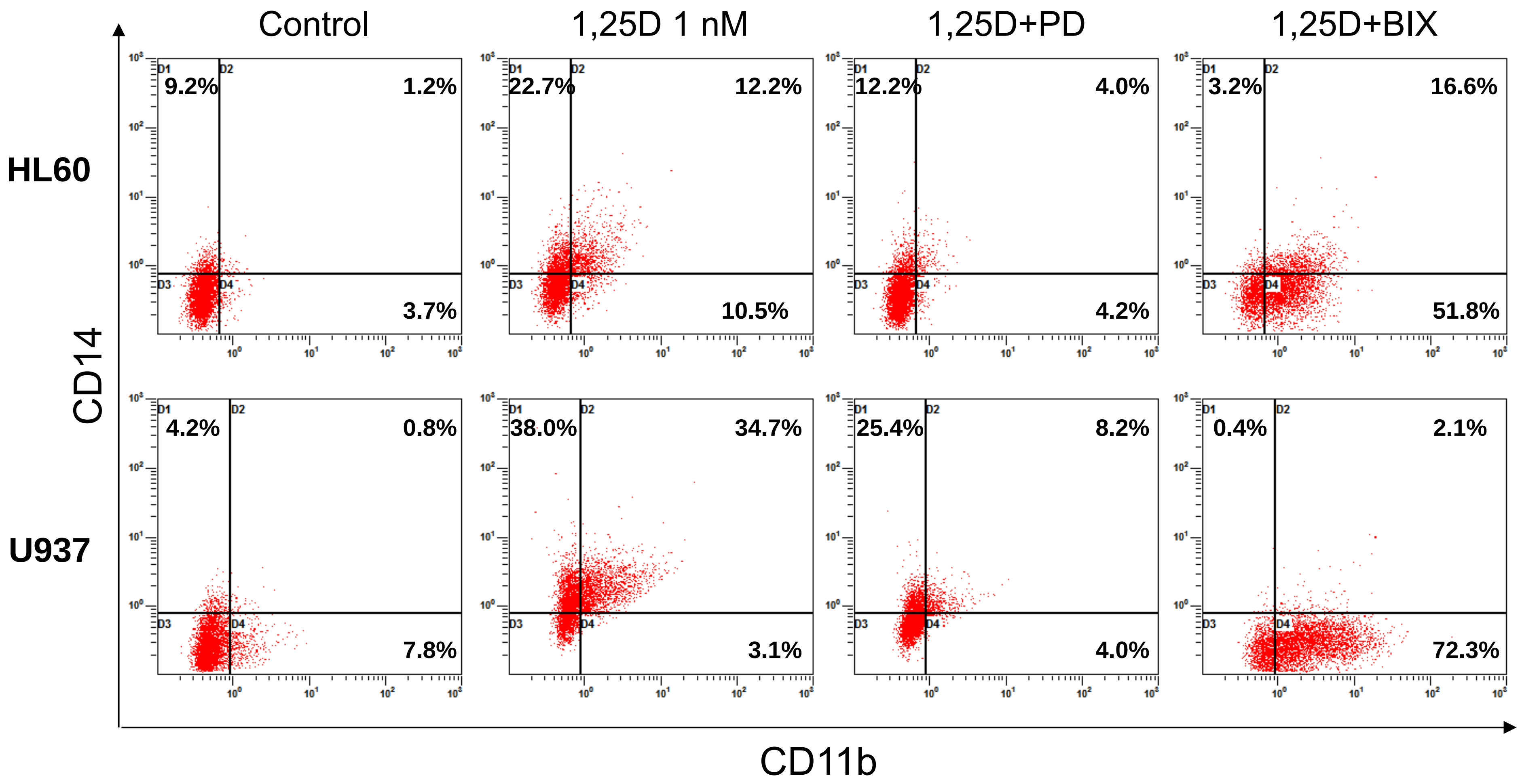
Wang et al. (2010) *Cell Cycle*

Wang et al. (2014) *J Steroid Biochem Mol Biol*

Wang et al. (2014) *J Cell Physiol*

Wang et al. (2014) *Exp Cell Res (accepted)*

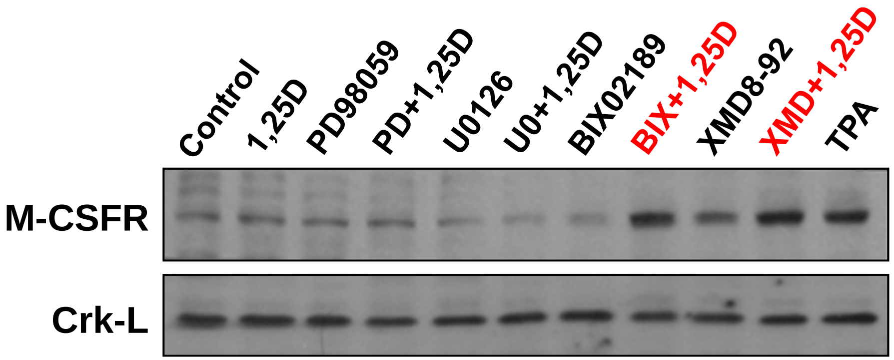
MEK5/ERK5 inhibition modulates CD14 and CD11b expression

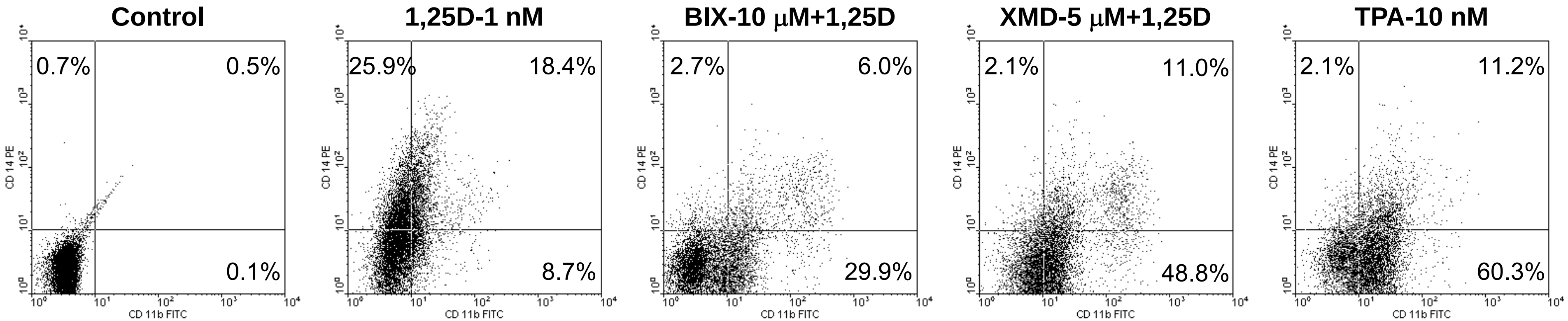


Wang et al. (2014) *J Steroid Biochem Mol Biol*

Wang et al. (2014) *J Cell Physiol*

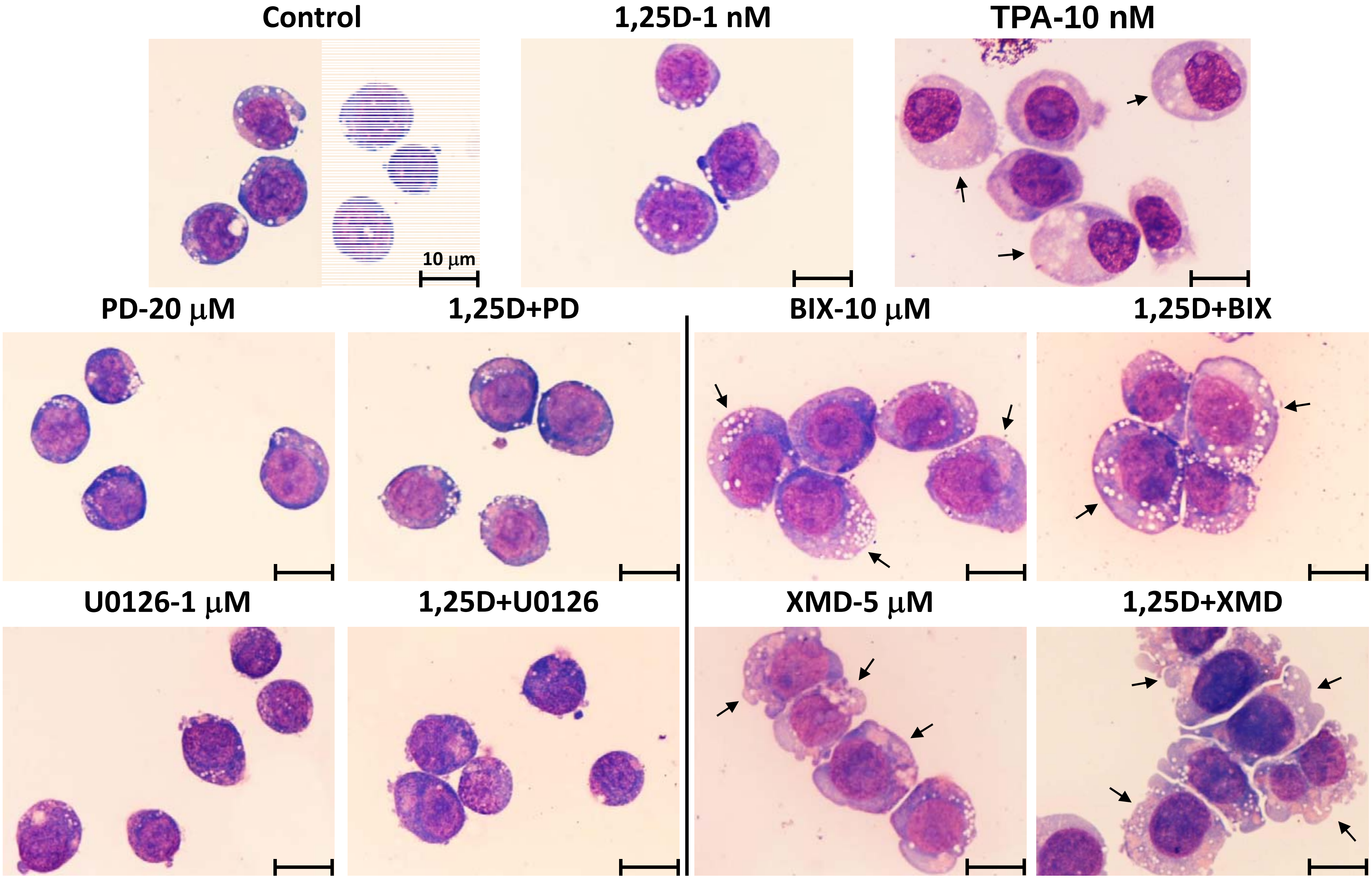
MEK5/ERK5 inhibition potentiates M-CSF receptor expression





Wang et al. (2014) *Exp Cell Res (accepted)*

HL60 cells treated with ERK5 inhibitors acquire macrophage-like morphology



Wang et al. (2014) *Exp Cell Res (accepted)*

Conclusion:

- **Synergistically acting combinations of VDDs and small-molecule sensitizing agents demonstrate promising antileukemic activity, which is mediated by distinct molecular mechanisms.**

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Xuening Wang

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