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

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Acute Myeloid Leukemia (AML)

Leukemia-initiating cells

AML blasts

Pluripotent Stem Cell

Lymphoblast

B Cell

T Cell

Myeloblast/monoblast

Orthochromat

ic erythroblast

Reticulocytes

Erythrocyte (Red Blood Cells)

Megakaryocyte

Thrombocytes (Platelets)

Monocyte/mononuclear progenitor

Monocyte

Neutrophil

Eosinophilic myelocyte

Basophilic myelocyte

Basophil
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

- Cytarabine + Anthracycline “7+3”
  - 50-70% CR → inevitable recurrence
  - Toxicity, esp. in older patients

Differentiation therapy

- AML-M3 (APL):
  - All trans-retinoic acid (ATRA)
  - + Chemo or As$_2$O$_3$
  - ~95% CR – 80% DFS
$1\alpha,25$-dihydroxyvitamin $D_3$ (1,25D) as an anticancer agent

- Powerful inducer of differentiation, growth arrest and/or apoptosis in cancer models \textit{in vitro} & \textit{in vivo}
- Causes severe \textit{hypercalcemia} at pharmacologically effective doses \textit{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:**


Differentiation-inducing agents cooperating with VDDs

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

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<tr>
<th>Compound</th>
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<tbody>
<tr>
<td>Dexamethasone</td>
<td>Hematopoietic, Myeloma, Breast, Ovary, Squamous cell carcinoma</td>
<td>Dexamethasone reduces hypercalcemia induced by 1,25D₃. The enhancing effect is attributed to VDR upregulation and reduction in ERK and Akt activities.</td>
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<td>Cytochrome P450 inhibitors:</td>
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<td>Ketoconazole</td>
<td>Hematopoietic, Prostate, Breast, Ovary</td>
<td>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 D₃ metabolism.</td>
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<td>Liarozole</td>
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<td>VID400</td>
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<tr>
<td>NSAIDs</td>
<td>Hematopoietic</td>
<td>The potentiating effect is mediated by inhibition of aldoketoreductase (AKR1C3).</td>
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<td>Cytokines:</td>
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<tr>
<td>TNFα</td>
<td>Hematopoietic, Breast, Kidney</td>
<td>Involvement of ROS is suggested. Enhance ICAM-1-dependent adhesion. Confer monocytic phenotype.</td>
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<tr>
<td>IL-1β, IL-4, IL-6 Interferon</td>
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<td>Chemotherapeutic agents:</td>
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<td>Anti-microtubule drugs</td>
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<tr>
<td>Docetaxel (Taxotere)</td>
<td>Prostate cancer, Breast, Bone, Prostate, Squamous cell carcinoma</td>
<td>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 1,25D₃ effect by DNA demethylation. Myeloid and monocytoid cells have different sensitivities to pyrimidine nucleoside analogs. Combinations enhance VDR/RXR binding to VDRE.</td>
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<td>Paclitaxel (Taxol)</td>
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<td>Topoisomerase inhibitors</td>
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<td>Camptothecin</td>
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<td>Doxorubicin</td>
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<td>Etoposide</td>
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<td>Platinum drugs</td>
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<td>Cisplatin</td>
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<td>Carboplatin</td>
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<td>Nucleoside analogs</td>
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<tr>
<td>1-β-D-arabinofuranosylcytosine</td>
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<td>5-aza-2'-deoxycytidine</td>
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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

- Multi-targeted agents
- Low bioavailability
- High doses *in vitro*
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

**1,25D**
- ERK1/2
- JNK1/2

**Polyphenols**
- Keap-1
- Keap-1

**Differentiation:**
- CD14, CD11b, NSE
- NADPH-oxidase, p21Cip1, KSR-1/2

**Glutathione**
- Buthionine sulfoximine
- AP1-decoy oligos

**References:**
- 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

Healthy Balb/c mice

Day 0: Inoculation i.p. with $1.0 \times 10^5$ WEHI-3B D⁻ cells

Day 1: Injections of 1,25D₃ analogs i.p. every 3 days.
Oral treatment with a dried ethanolic rosemary extract (~35% CA) mixed with a powdered food

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

Healthy Balb/c mice

**Day 0:** Inoculation i.v. with $2.0 \times 10^6$ 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)D₃ – 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)D₃ (100,000 IU/week) – 17 patients (~ 71 yo)

Only serum levels of 25(OH)D₃ prior to treatment was able to predict patients’ outcome:
- 21.2 vs. 7.1 months

Median survival:
- 10.4 months vs. 4 months

Paubelle E et al. (2013) PLOS One 8: e65998
Low 25(OH) Vitamin D₃ Levels Are Associated With Adverse Outcome in Newly Diagnosed, Intensively Treated Adult Acute Myeloid Leukemia

Hun Ju Lee, MD¹; Josephia R. Muindi, MD, PhD²; Wei Tan, MA³; Qiang Hu, PhD³; Dan Wang, MA³; Song Liu, PhD³; Gregory E. Wilding, PhD³; Laurie A. Ford, BS¹; Sheila N. J. Salt, PhD⁴; Annemarie W. Block, PhD⁴; Araba A. Adjei, PhD²; Maurice Barcos, MD, PhD⁵; Elizabeth A. Griffiths, MD¹; James E Thompson, MD¹; Eunice S. Wang, MD¹; Candace S. Johnson, PhD²; Donald L. Trump, MD⁶; and Meir Wetzler, MD¹

BACKGROUND: Several studies have suggested that low 25(OH) vitamin D₃ 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 D₃ pathway genes were evaluated and correlated with 25(OH) vitamin D₃ levels and treatment outcome. RESULTS: Thirty-four patients (35%) had normal 25(OH) vitamin D₃ 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 D₃ levels were associated with worse relapse-free survival (RFS) compared with normal vitamin D₃ levels. In multivariate analyses, deficient 25(OH) vitamin D₃, 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 D₃ levels, although none remained significant after multiple test corrections; one 25(OH) vitamin D₃ receptor SNP, rs10783219, was associated with a lower complete remission rate (P = 0.0442) and with shorter RFS (P = 0.0058) and overall survival (P = 0.0011). CONCLUSIONS: It remains to be determined what role microRNA and SNP profiles play in contributing to low 25(OH) vitamin D₃ level and/or outcome and whether supplementation will improve outcomes for patients with AML. Cancer 2014;120:521-9. © 2013 American Cancer Society.
Roles of ERK1/2 and ERK5 signaling in 1,25D₃-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)
Synergistically acting combinations of VDDs and small-molecule sensitizing agents demonstrate promising antileukemic activity, which is mediated by distinct molecular mechanisms.

Conclusion:
Acknowledgements

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Joseph Levy

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Xuening Wang

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