New Treatment for Cancer and Metabolic Diseases: Vitamin D Receptor Coregulator Inhibitors

(OTT ID 1250)

Inventor: Leggy A. Arnold, Department of Chemistry and Biochemistry and Milwaukee Institute UW-Milwaukee

For further information please contact:

Jessica Silvaggi
Licensing Manager
1440 East North Ave.
Milwaukee, WI 53202
Tel: 414-906-4654
jessica@uwmrf.org

Shortfalls of current therapies for Cancer and Metabolic Disease

Problems/Unmet Needs:

- Leukemia (57.1%) estimated deaths 24,090
- Ovarian cancer (44.6%) estimated deaths 14,270
- Surgery not possible/difficult; radiation not applicable
- Current chemotherapy drugs are non-specific/toxic
- Poor quality of life outcomes/survival

Technological Solution:

- The inventor has discovered novel small molecules (VDRCI) that can disrupt the interaction between the vitamin D receptor (VDR) and coregulator proteins that modulate VDR-mediated gene transcription
- Focus on a new tissue-selective cancer treatment
- Reprogramming the cancer cells to stop growing
- VDRCI are expected to be more effective and safer than current treatments with less side effects
- VDRCI are expected to be more metabolically stable than current secosteroid-based ligands enabling systemic treatments
- The inhibitors can be easily generated in a one-step synthesis

Road to Commercialization: Market, Intellectual Property, and Partnering

Market

- Ave Ca drug price - >$10,000/mo; $70,000-115,000 annually
- Avail mkt (ovarian Ca) – 30,000 patients x $100,000/an = $300MM
- Ovarian cancer diagnostics and treatment market is forecast to reach $35 billion in 2018 (BCC Research)
- The current market for nuclear receptor targeted drugs is estimated to be 10%-15% of the global pharmaceutical market of US $400 billion

Intellectual Property

- **US Utility Patent application filed February 2014:**
- Continue to aggressively pursue US protection

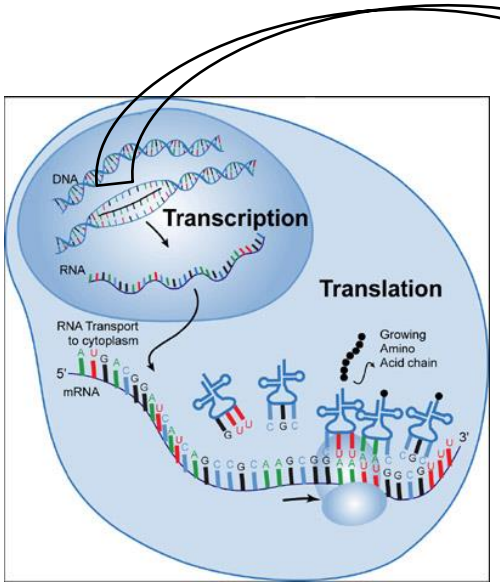
Partnering

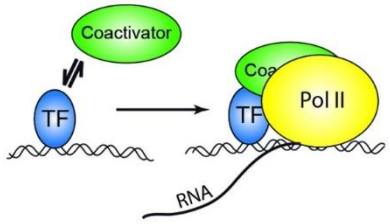
- Looking for a development partner to:
  - Support pharmacokinetic and pharmacodynamic analysis for IND filing
  - License novel compounds
  - Conduct clinical trials for effective lead compounds

Vitamin D Receptor –Gene regulation

VDR Function

- VDR is a transcription factor in the nuclear receptor family
- VDR forms a heterodimer with the retinoid-X receptor and is is activated by vitamin D analogs
- Coregulators can bind to the VDR heterodimer regulated by the vitamin D ligand to either activate or repress transcription of target genes
- **VDR-coregulator inhibitors** have the potential to cell-specifically inhibit the activation or repression of genes



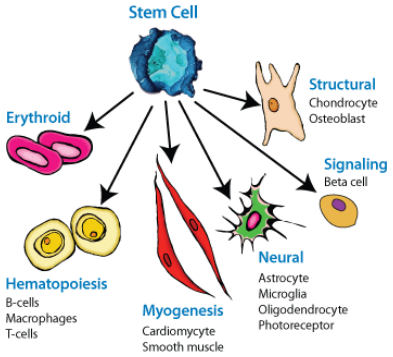


TF = vitamin D receptor (VDR)

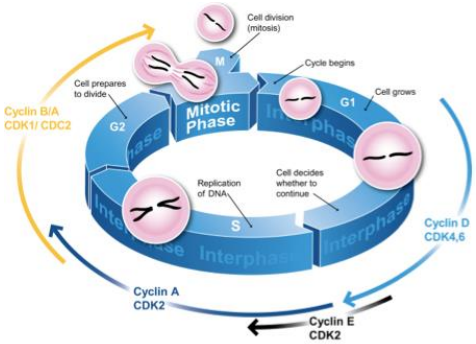
Compounds that inhibit the interaction between VDR and coactivators

Vitamin D Receptor Function

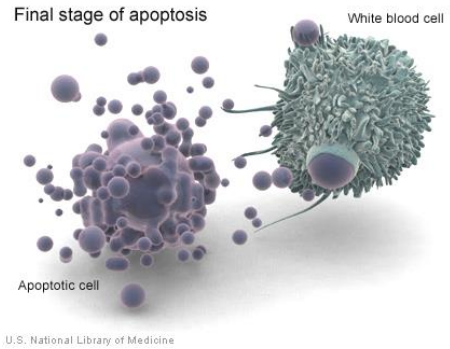
Three main areas to fight cancer



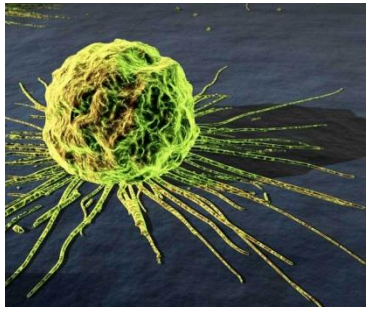
Differentiation



Proliferation



Apoptosis



Cancer

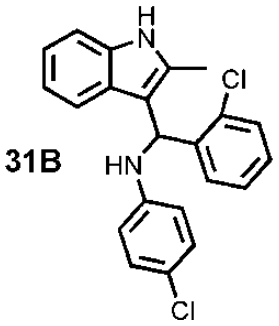
Disease Targets

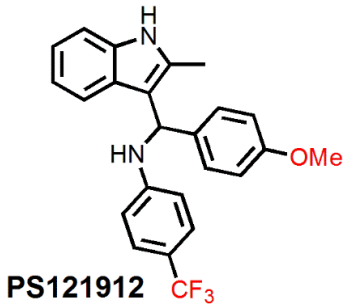
New Compounds Developed

- Developed a compound 31B against ovarian cancer
- Developed a compound PS121912 against leukemia
- In vivo activity was demonstrated

Vitamin D-based treatments

- Limited systemic application because of high risk to cause hypercalcemia and hypercalciurea
- Limited tissue selectivity because of a general expression profile of VDR
- The majority of these drugs are used topically





31B to Treat Ovarian Cancer

31B is inducing apoptosis in three different ovarian cancer cells lines

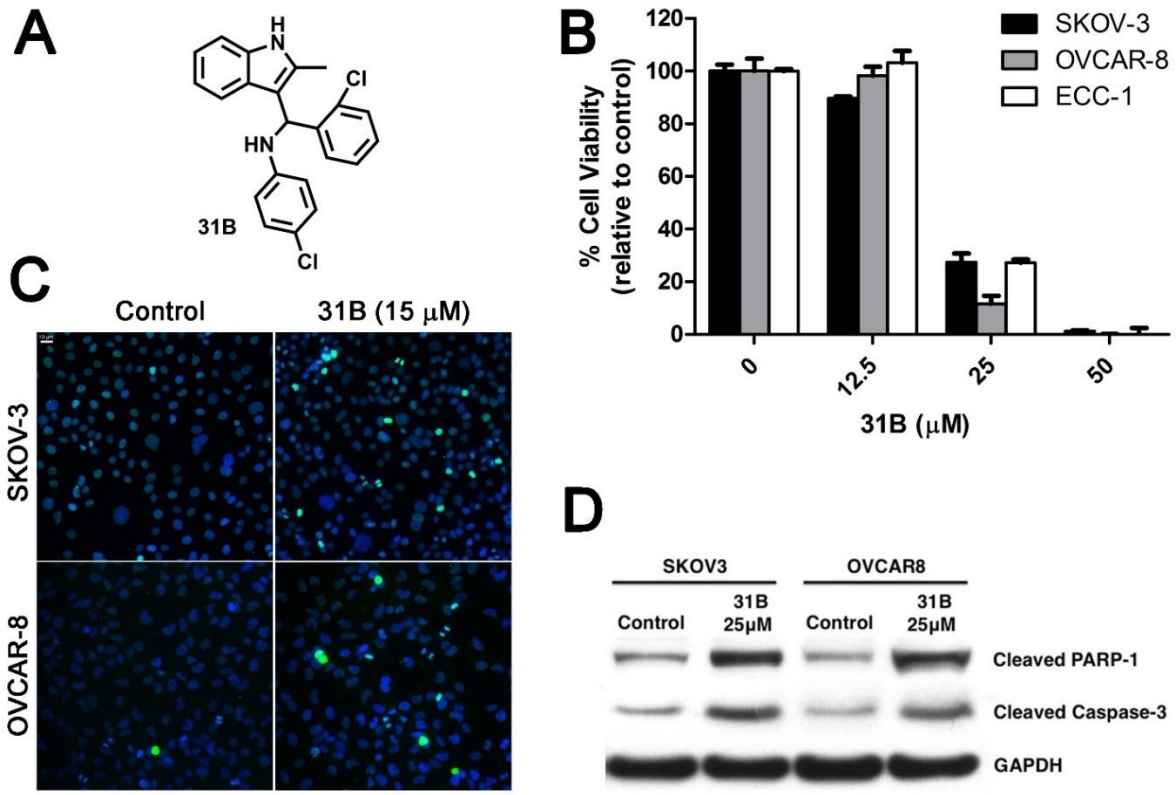


Fig-1: Anti-proliferative effects of 31B in a panel of gynecologic cancer cells. (A) Chemical structure of 31B. **(B)** SKOV3, OVCAR8, and ECC-1 were treated for 18 hours with different concentrations of 31B. Cell viability was determined by MTS assay. **(C)** SKOV3 and OVCAR8 cells were treated with 15 µM 31B or DMSO vehicle control for 18 hours. TUNEL assay was carried out by co-staining with fluorescein-12-dUTP (labeling of DNA nicks in apoptotic cells) and of chromatin with DAPI. Representative fluorescent images were taken, and apoptotic stain (green) and nuclear stain (blue) overlaid. TUNEL positive nuclei due to DNA fragmentation appear as green areas **(D)** SKOV3 and OVCAR8 cells were treated with 25 µM 31B and analyzed by immunoblotting using primary antibodies against cleaved PARP-1, cleaved caspase-3, and GAPDH.

31B to Treat Ovarian Cancer

31B anti-proliferation is VDR dependent

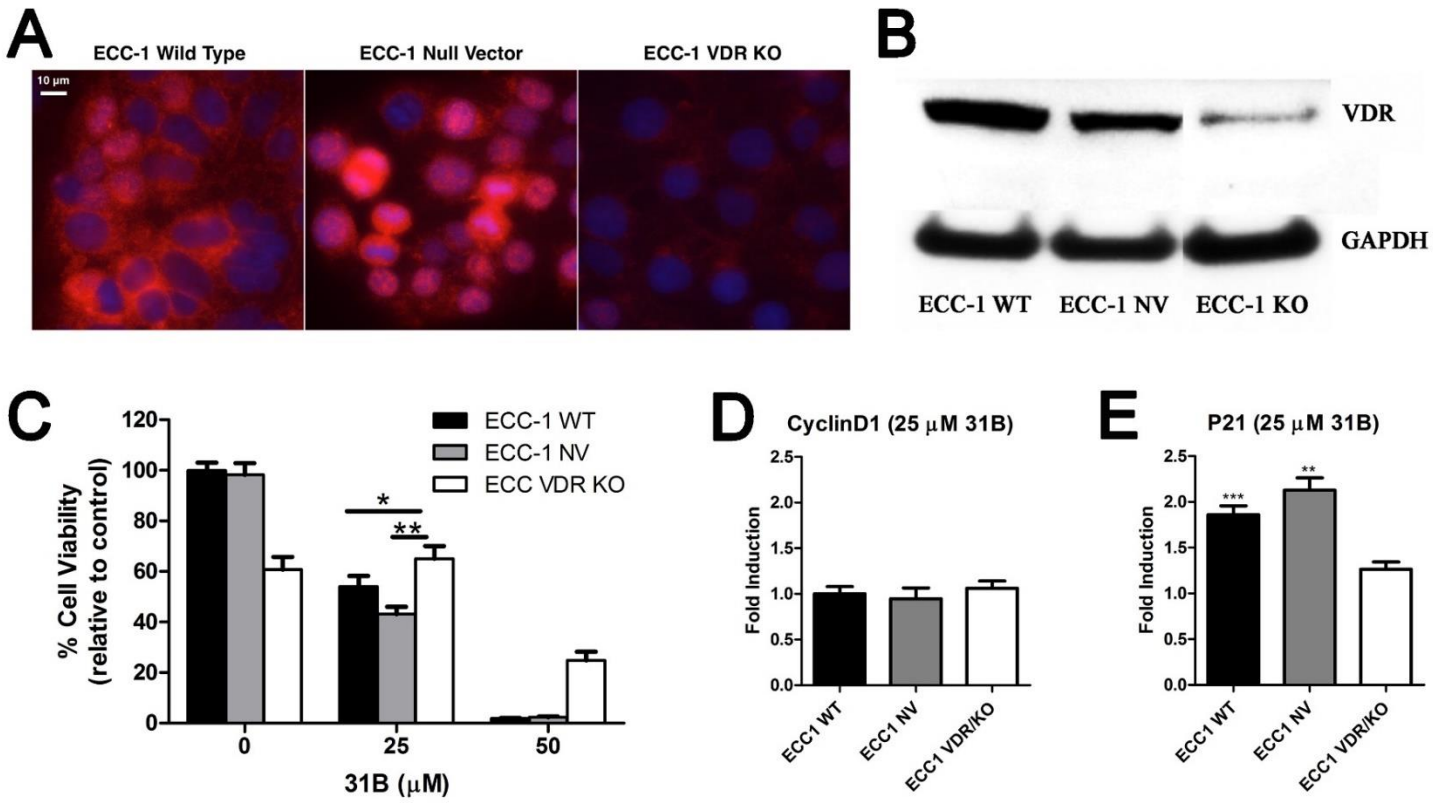


Fig-2: VDR-dependent physiological effects of 31B. (A) ECC-1 wild type (WT), null vector (NV), and VDR/KO cells were fixed, and VDR expression was visualized by fluorescent IHC. **(B)** Cell lysates of ECC-1 WT, ECC-1 NV, and ECC-1 VDR/KO were separated by SDS page and analyzed by Western blot for expression of VDR and GAPDH as a control. **(C)** BrdU incorporation assay was used to determine the anti-proliferative effect of 31B in ECC-1 WT, ECC-1 NV and ECC-1 VDR/KO cells after 18h. (**D and E**) mRNA levels of cyclin D1 and p21 from vehicle and 31B-treated ECC-1 WT, ECC-1 NV, and ECC-1 VDR/KO cells were quantified after 18h by qRT-PCR. GAPDH was used as housekeeping gene. The ΔΔCt method was used to measure the fold change in gene expression of cyclin D1 and p21.

31B Treats Ovarian Cancer in Mice

31B as anti-tumor activity, is non-toxic and non-calcemic

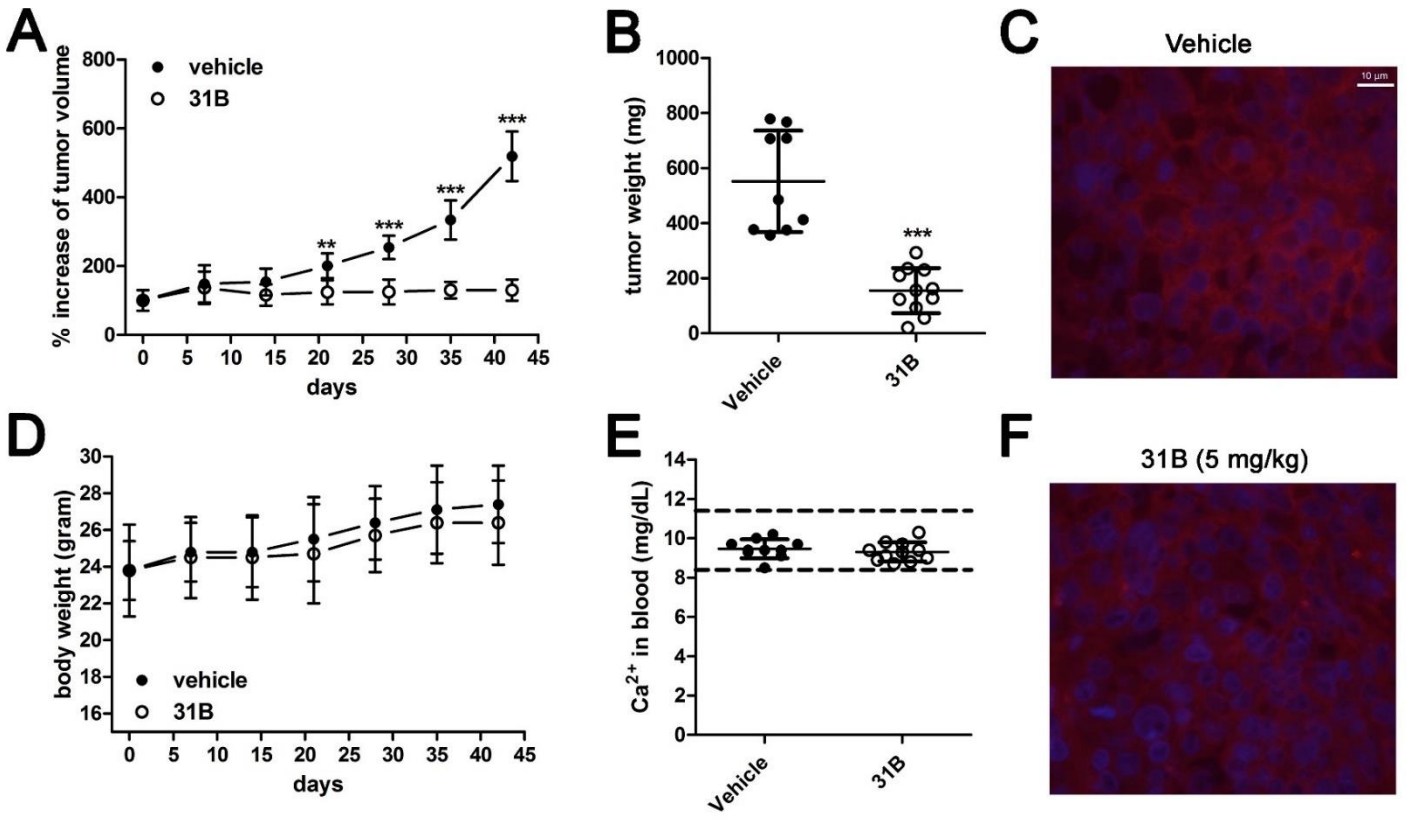


Fig-4: Anti-cancer activity of 31B in a cisplatin-resistant ovarian cancer mouse xenograft model. Nude mice bearing SKOV3 derived tumor xenografts were dosed (IP) with either vehicle control (n = 10) or 31B (n = 11) (5 mg/kg) 5 times a week. Tumor volume **(A)** and animal weight **(D)** and was determined each week. The tumor weight **(B)** and calcium concentration **(E)** in the blood was determined at the 42nd day of the study. Paraffin-embedded SKOV3 xenograft tumor tissue was stained for DAPI and expression of FASN (red fluorescence) for vehicle **(C)** and 31B-treated animals **(F)**.

31B to Treat Ovarian Cancer

31B reduced the metabolism of ovarian cancer cells

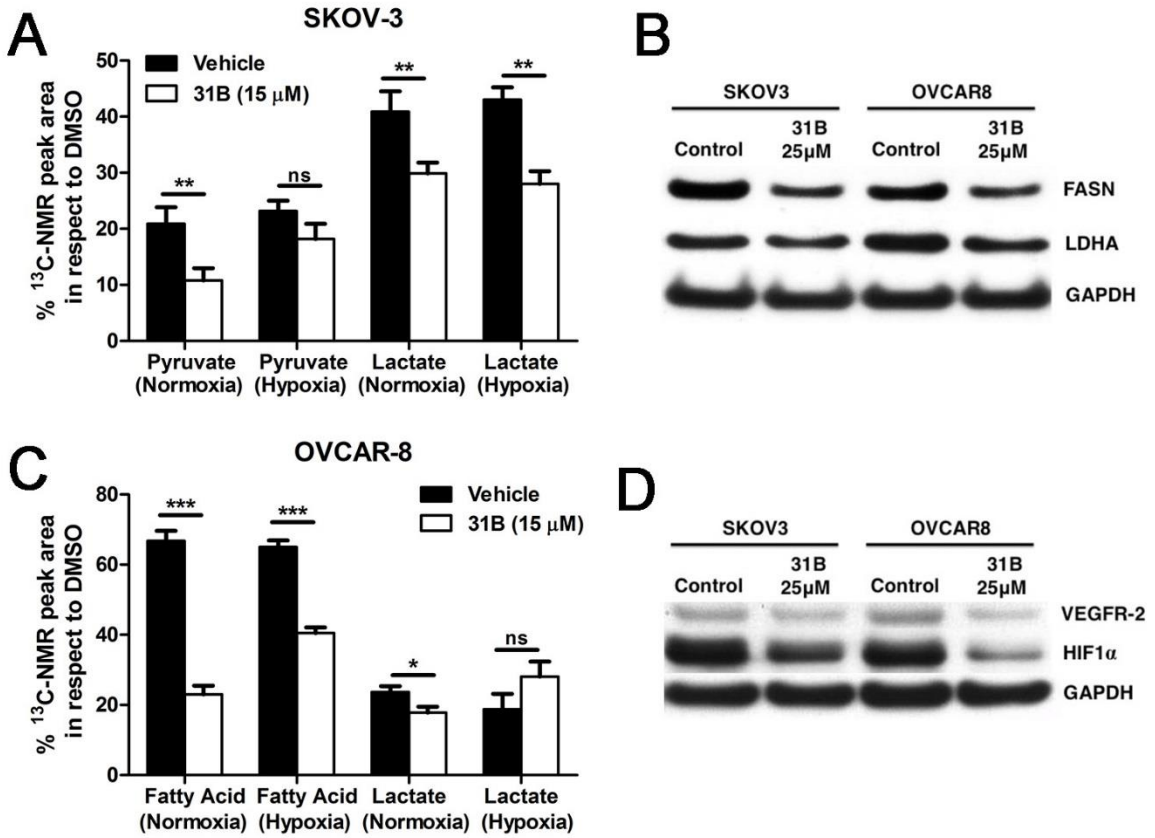
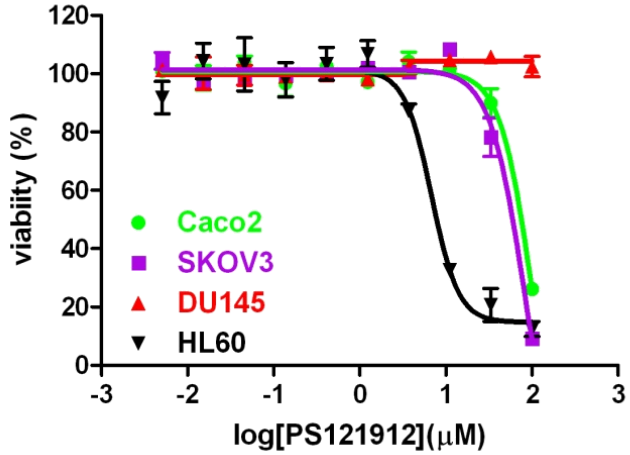
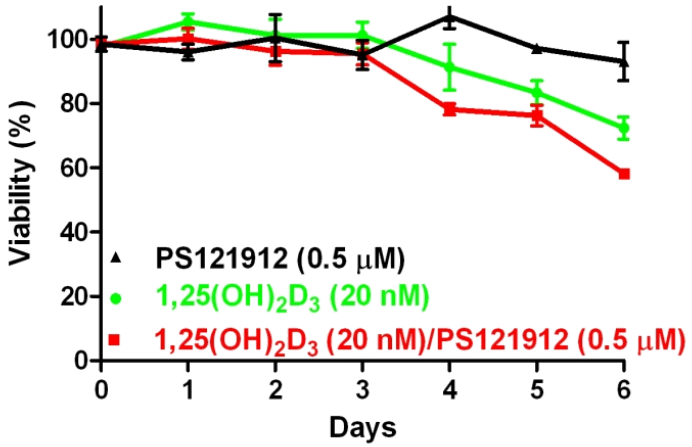


Fig-5: Modulation of ovarian cancer cell glycolysis and lipogenesis in the presence of 31B. SKOV3 and OVCAR8 cells were grown in glucose-deprived media containing [1,6]^{13}C-glucose. 31B or vehicle was added, and plates were incubated either under normoxia or hypoxia for 6 hrs. The media was collected, and D_2O and DMSO reference were added for analysis by ^{13}C-NMR. The area under peaks for key metabolites pyruvate, lactate, and fatty acid were obtained in triplicate and calculated as a proportion of the DMSO control peak area, and plotted in graphs **(A and C)**. SKOV3 and OVCAR8 cells were treated with 25 μM 31B or vehicle, and lysates were probed via Western blot with primary antibodies against FASN and LDHA **(B)** and VEGFR-2 and HIF1α **(D)**.

PS121912 Shows Anti-Proliferative Effects

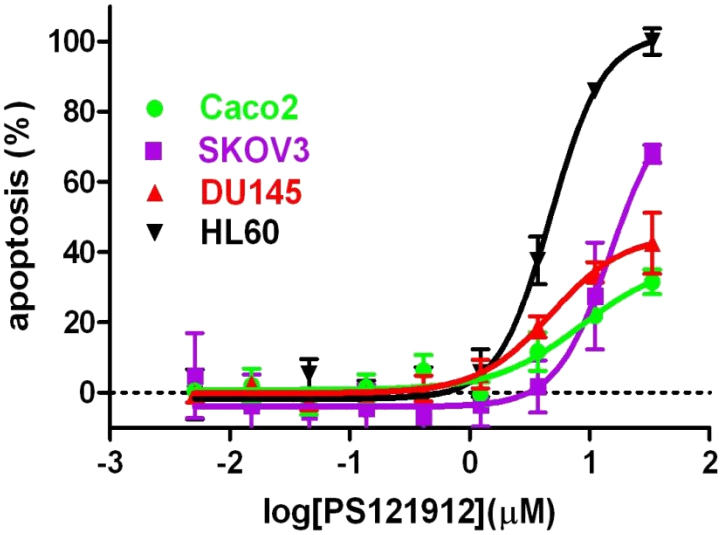




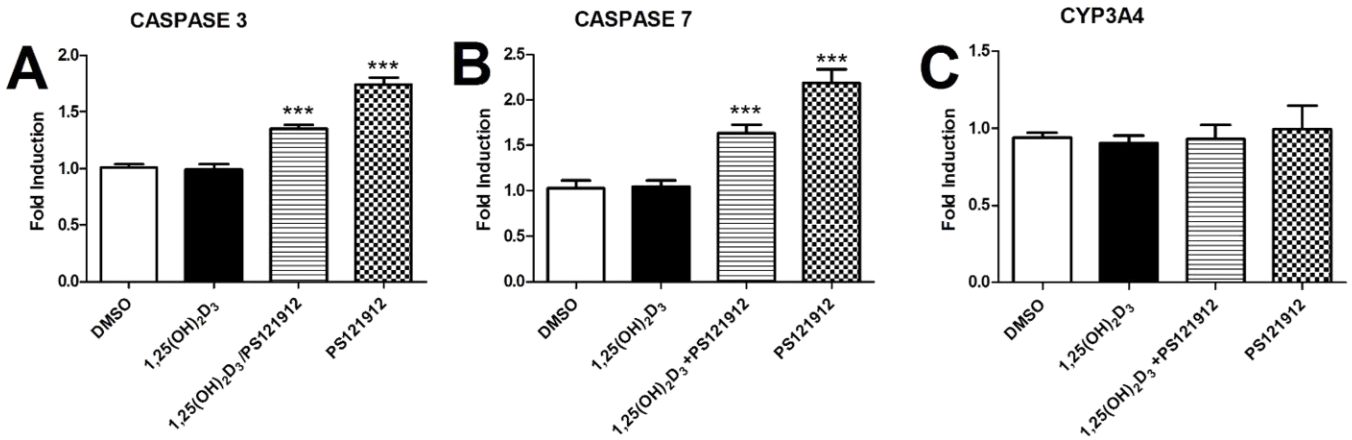
Proliferation studies with PS121912 and 1,25(OH)$_2$D$_3$. Anti-proliferation induced by PS191219 after 18 hours with different cancer cells; **B/C**) Long term anti-proliferation study in DU145 and HL60. Cell viability was determined using Cell Titer-Glo (Promega).

- The anti-proliferation effect of 1,25-(OH)$_2$D$_3$ was significantly amplified for all cancer cells in the presence of 0.5 μM PS121912 for HL60 and 2 μM for all other cell lines.
- The crucial influence of 1,25-(OH)$_2$D$_3$ in respect to cell viability is strong evidence that VDR is mediating the anti-proliferation effects of PS121912.

PS121912 Induces Apoptosis



- Cancer cell lines were treated with PS121912 in a dose-dependent manner and caspase 3/7 activity was quantified using Apo-Glo after 18 hrs (left)
- PS121912 increase the mRNA levels of caspase 3 and caspase 7 after 18h in HL60.



PS121912 Regulates Coregulator Recruitment

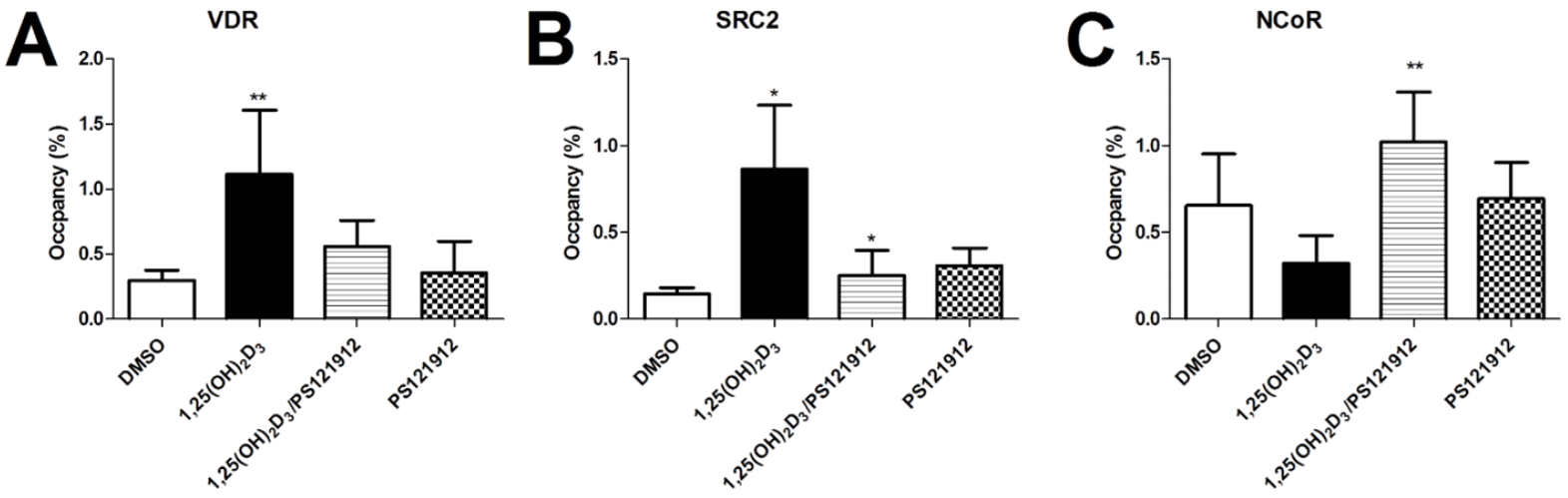


Fig.3 Chromatin immunoprecipitation assay (ChIP) in HL-60 cells at the CYP24A1 promoter. Cells were incubated with 20 nM $1,25(OH)_2D_3$, 0.5 µM PS121912, or the combination thereof. A) IP using VDR antibody; B) IP using SRC2 antibody, and C) IP using NCoR antibody.

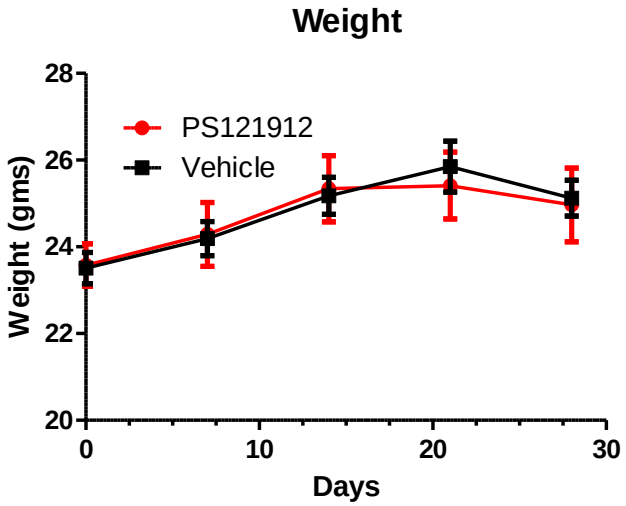
$1,25(OH)_2D_3$ induced the DNA occupancy of VDR at the CYP24A1 promoter site in addition to the recruitment of coactivator SRC2. In respect to corepressor recruitment, $1,25(OH)_2D_3$ reduced the interaction between VDR and NCoR. Importantly, the interaction between DNA-bound VDR and SRC2 was reduced in the presence of PS121912, whereas PS121912 promoted the interaction between VDR and NCoR. Finally, low concentrations of PS121912 by itself had no significant effect of the VDR-coregulator recruitment.

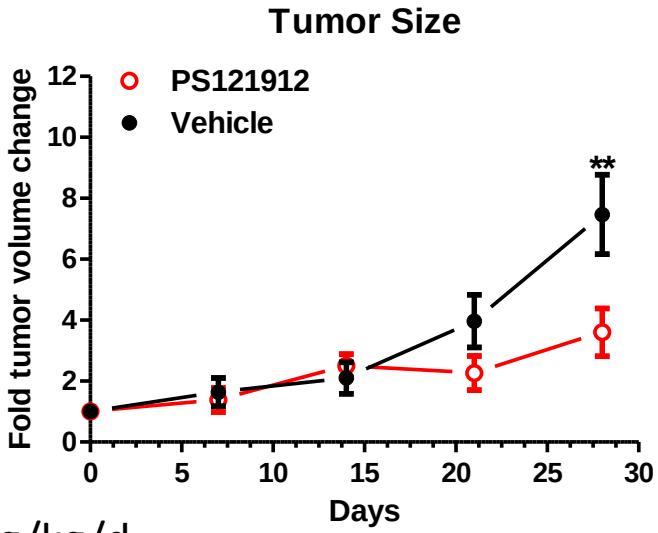
PS121912 Regulates Cell Cycle Proteins

Gene	Description	1,25D3[a]	1,25D3[b] PS121912	Gene	Description	1,25D3[a]	1,25D3[b] PS121912
GUSB	glucuronidase, beta	0.99±0.08	0.92±0.1	CDKN2C	cyclin-dependent kinase inhibitor 2C	1.16±0.33	0.86±0.19
ATM	ataxia telangiectasia mut.	1.23±0.11	0.87±0.30	CDKN2D	cyclin-dependent kinase inhibitor 2D	1.17±0.44	0.81±0.19
ATR	ataxia telangiectasia	0.90±0.15	0.63±0.15	E2F1	E2F transcription factor 1	0.61±0.06	0.67±0.19
CCNA1	cyclin A1	0.37±0.01	0.47±0.24	E2F2	E2F transcription factor 2	1.41±0.35	1.13±0.29
CCNA2	cyclin A2	1.05±0.01	0.78±0.07	E2F3	E2F transcription factor 3	0.94±0.25	0.70±0.22
CCNB1	cyclin B1	1.07±0.08	0.73±0.11	E2F4	E2F transcription factor 4,	0.69±0.13	0.52±0.17
CCNB2	cyclin B2	1.01±0.23	0.73±0.06	GSK3B	glycogen synthase kinase 3 beta	0.90±0.42	0.57±0.12
CCND1	cyclin D1	0.20±0.01	0.18±0.08	HDAC1	histone deacetylase 1	0.80±0.14	0.73±0.07
CCND2	cyclin D2	0.44±0.03	0.41±0.22	HDAC2	histone deacetylase 2	0.79±0.03	0.74±0.09
CCND3	cyclin D3	1.13±0.04	0.97±0.18	HDAC3	histone deacetylase 3	0.76±0.13	0.73±0.23
CCNE1	cyclin E1	0.74±0.02	0.78±0.08	HDAC4	histone deacetylase 4	0.82±0.12	0.73±0.07
CCNE2	cyclin E2	0.84±0.05	0.70±0.07	HDAC5	histone deacetylase 5	0.90±0.08	0.77±0.12
CCNH	cyclin H	0.91±0.04	0.82±0.15	HDAC6	histone deacetylase 6	0.72±0.17	0.55±0.05
CDC2	cell division cycle 2,	0.81±0.05	0.62±0.07	HDAC7	histone deacetylase 7	0.85±0.32	0.64±0.14
CDC25A	cell division cycle 25A	1.28±0.14	1.67±0.37	HDAC9	histone deacetylase 9	0.53±0.02	0.41±0.16
CDK2	cyclin-dependent kinase 2	0.81±0.11	0.61±0.03	PPP2CA	protein phosphatase 2	0.86±0.01	0.80±0.15
CDK4	cyclin-dependent kinase 4	0.93±0.21	0.70±0.05	RAF1	v-raf-1 leukemia viral gene	0.86±0.04	0.74±0.09
CDK6	cyclin-dependent kinase 6	0.61±0.12	0.39±0.09	RB1	retinoblastoma 1	0.97±0.03	0.77±0.15
CDK7	cyclin-dependent kinase 7	1.11±0.04	0.62±0.07	TGFB1	transforming growth factor, beta 1	0.91±0.01	0.74±0.12
CDKN1A	cyclin-dependent kinase inhibitor 1A	1.59±0.08	2.15±0.44	TGFB2	transforming growth factor, beta 2	0.29±0.08	0.36±0.11
CDKN1B	cyclin-dependent kinase inhibitor 1B	0.84±0.01	0.69±0.21	TGFB3	transforming growth factor, beta 3	0.65±0.25	0.48±0.12
CDKN2A	cyclin-dependent kinase inhibitor 2A	0.66±0.09	0.57±0.10				±

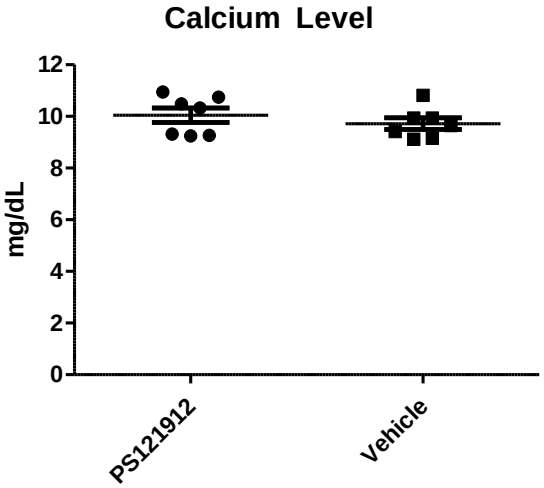
HL-60 were treated with compounds for 18 hours followed by mRNA extraction, RT qPCR. The fold induction was calculated in respect to vehicle using the ΔΔCt method. [a]20 nM $1,25(OH)_2D_3$, [b]20 nM $1,25(OH)_2D_3$/ 0.5 μM PS121912. ■ > 1.25, ■ < 0.6.

PS121912 Treats Leukemia in Mice

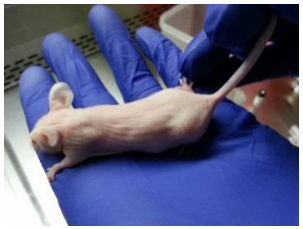




PS121912 1 mg/kg/d



Untreated (10)



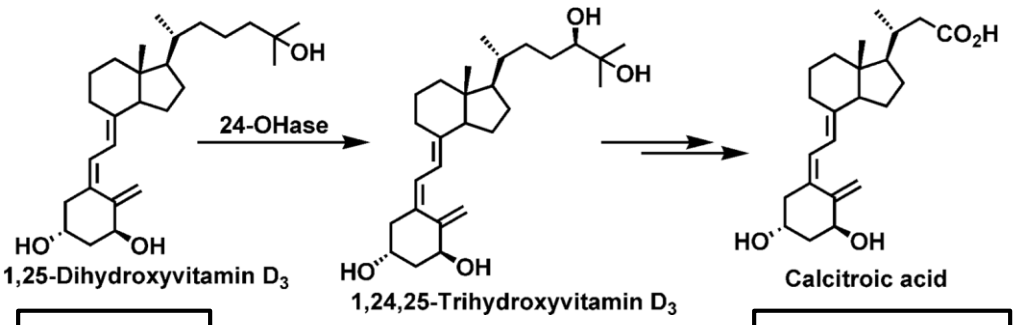
Treated (10)

VDR-mediated Gene Regulation

Regulation of cytochrome P_{450} enzyme 24-hydroxylase

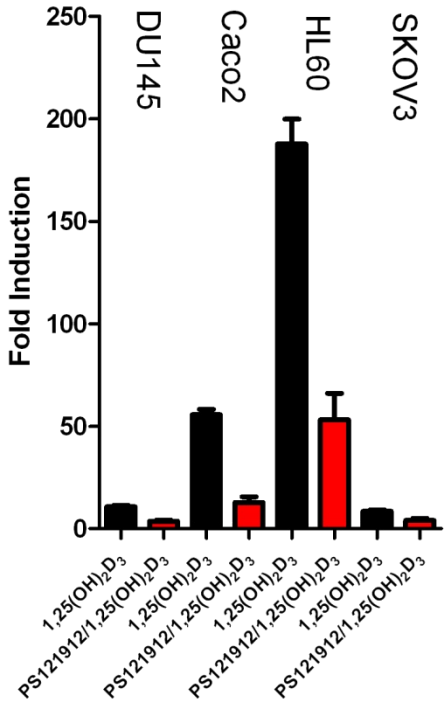
- CYP24A1 (24-Hydroxylase gene) is regulated by VDR
- 24-Hydroxylase regulates the function VDR through metabolic degradation of vitamin D ligands
- CYP24A1 is over-expressed in many cancers
- Cancer patients with elevated CYP24A1 levels have poor prognoses

➢ Application of VDR-coregulator inhibitors as transcriptional regulators of CYP24A1 represents a novel strategy to fight cancer proliferation and differentiation



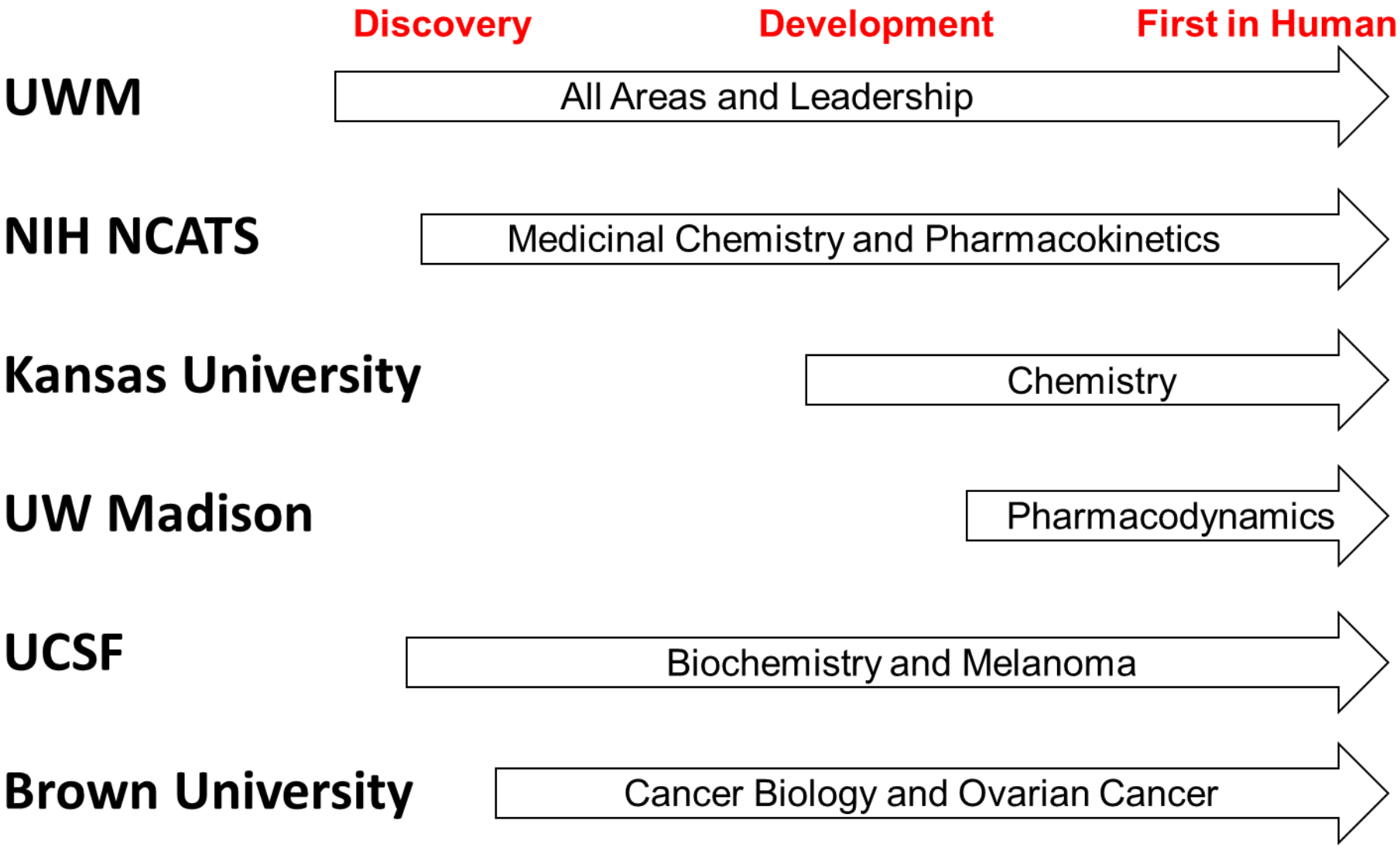
active

Not active



Methods: rt-PCR in HL60 cell after 18h treatments (20/100 nM $1,25(OH)_2D_3$ and 0.5/2 uM PS121912)

Collaborative Projects



Next Steps

Path A

1. Intensify the search for pharmaceutical partners **to license** IP
2. **Pharmacokinetic** analysis (how is the drug candidate distributed *in vivo*)
3. **Pharmacodynamics** analysis (discreet effects *in vivo*)
4. **IND filing** with commercial partner

Path B

1. **Grant** application (current pending R01)
2. **In house pharmacokinetics/pharmacodynamics**
3. Apply for **SBIR** funding and **venture capital**
4. **IND filing** at UWM

New Treatment for Cancer and Metabolic Diseases: Vitamin D Receptor Coregulator Inhibitors

(OTT ID 1250)

Inventor: Leggy A. Arnold, Department of Chemistry and Biochemistry
UW-Milwaukee

For further information please contact:

Jessica Silvaggi
Licensing Manager
1440 East North Ave.
Milwaukee, WI 53202
Tel: 414-906-4654
jessica@uwmrf.org