A Novel Fluorobenzyl Polyethylene Glycol Conjugated Tetraiodothyroacetic Acid (fb-PMT), Targeting Thyrointegrin $\alpha\text{v}\beta 3$ in Treatment Acute Myeloid Leukemia

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Research

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Abstract

Background: Acute myeloid leukemia (AML) is associated with poor long-term survival, even with newer therapeutic agents. Here we show the results of our pre-clinical study, in which we evaluate the efficacy of a new thyrointegrin $\alpha\text{v}\beta 3$ antagonist, named **fluorobenzy**l Polyethylene glycol conjugated tetraiodothyroacetic acid (fb-PMT).

Methods and Results: fb-PMT effectively suppresses the malignant growth of human acute myeloid leukemia (AML) after successful engraftment in transgenic NSG-S xenograft mouse models of either established human AML cell line or primary AML cells. Daily treatment with fb-PMT (1-10 mg/kg body weight) subcutaneously (s.c.) for 3-4 weeks was associated with marked regression of leukemogenesis and extended survival in both models. The efficiency of the fb-PMT therapy was verified using IVIS imaging, flowcytometry and histopathological examination to monitor the engraftment of leukemic cells in the bone marrow and other organs. fb-PMT therapy for 3-4 weeks at 3 and 10 mg/kg daily doses exhibited significant reduction ($P < 0.0001$) of leukemic cell burden of 74% and >95%, respectively. All fb-PMT-treated mice in the 10 mg/kg treatment arm successfully maintained remission after discontinuing the daily treatment. Comprehensive fb-PMT safety assessments demonstrated excellent safety and tolerability at multiple folds above the anticipated human therapeutic doses. Lastly, our genome-wide microarray screens demonstrated that fb-PMT works through the molecular interference mechanism with multiple signaling pathways contributing to growth and survival of leukemic cells.

Conclusion: our preclinical findings of the potent anticancer activities of fb-PMT and its favorable safety profiles warrant its clinical investigation for the effective and safe management of AML.

Background

Acute myeloid leukemia (AML) is one of the most aggressive malignant hematological disorders. More than 19,000 new cases were estimated to occur in the United States in 2020 (1.1% of all new cancer cases) [1]. In 2020, the estimated number of deaths from AML is 11,180, which represent 56% of new cases (1.8% of all cancer deaths), with an overall 5-year survival rate of 27.4% [1]. Current treatment regimens for AML include traditional chemotherapy, allogeneic hematopoietic cell transplantation, and targeted therapies for specific mutations in limited numbers of AML patients, all of whom still suffer from adverse effects and relapse. New broad spectrum effective and safe treatment options are urgently needed for the different types of AML.

AML is now curable in up to 40% of patients under the age of 60 years and in up to 15% of patients over the age of 60. In elderly patients who are unable to tolerate the available chemotherapy, mean survival is 5-to-10 months [2]. Important advances in defining the 'genomic landscape' of AML have enabled the prognostic classification of the disease and, to some extent, have facilitated the choice of chemotherapeutic agents. Among the mutations of particular interest and importance in AML are those of the *FLT3*, *NPM*, *CEBPA*, *KIT*, *NRAS*, *IDH1/2*, and *TP53* genes [2]. Targeted therapies for specific mutations in treating AML were recently approved by the FDA based on the genetic basis of the disease in different subsets of AML patients. However, those targeted therapies treat only a small percentage of the AML population and patients suffer from relapse upon discontinuation of treatment[3].

On the other hand, crosstalk between leukemic cells and the bone marrow microenvironment is recognized as one of important factors that keeps leukemic cells in quiescent state and helps them to escape the different therapies. One of the functionally important entities in the bone marrow microenvironment is represented by the integrins. Integrins are a family of heterodimeric structural proteins of the plasma membrane that are important to cell-cell interactions and cell motility [4, 5]. They are critical to leukocytes' function and to the angiogenesis process, particularly local release of growth factors that are generously overexpressed and produced by cancer cells and by dividing endothelial cells of tumor-relevant blood vessels. There are different members of Integrin family including $\alpha\text{v}\beta 3$, $\alpha\text{v}\beta 5$, and $\alpha 5\beta 1$ which involved in angiogenesis and have high affinity to arginine-glycine-aspartate (RGD) extracellular matrix (ECM)- containing angiogenesis modulators such as fibronectin, fibrinogen and osteopontin [6–8]. $\alpha\text{v}\beta 3$ (thyrointegrin) is generously expressed by cancer cells and by dividing endothelial cells. Mechanistically, thyrointegrin $\alpha\text{v}\beta 3$ enhances β -catenin signaling and the tyrosine kinase activities in AML [9, 10]. Integrin $\alpha\text{v}\beta 3$ was reported to be highly expressed in AML and associated with poor outcomes [11, 12].

This study focused on comprehensive preclinical evaluations of anticancer activities of a novel thyrointegrin $\alpha\text{v}\beta 3$ antagonist, fb-PMT in models of AML. Mechanistically, fb-PMT and similar molecules bind to a specific site on thyrointegrin $\alpha\text{v}\beta 3$ receptors that can be blocked with high affinity by various triazole tetrac derivatives [13–16]. Our genome-wide microarray screens demonstrated that fb-PMT appears to exert its potent anticancer actions on human AML through a molecular interference mechanism with multiple signaling pathways supporting growth and survival of leukemic cells.

Materials And Methods

Tumor cells and test compound

AML cell lines (K562-Luc; human erythroleukemia cells and KG1a; human myelocytic leukemia cells) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). These cell lines are commonly used in research studies as experimental AML models. Primary human AML cells (De novo AML 6373, harboring FLT3-ITD mutation) were collected by leukapheresis from AML patients at the University Hospital, University of Pennsylvania, with informed consents (IRB protocol 703185).

The K562 cells were established from a chronic myeloid leukemia female patient in blastic crisis and expressing P210 bcr-abl mutation [17, 18]. Morphologically, K562 cells represent undifferentiated, nongranulated blast cells with a basophilic cytoplasm with two or more prominent nucleoli. The K562 cells are strongly reactive for acid phosphatase while negative for other cytochemical markers. K562-Luc cells were maintained in RPMI 1640 (Roswell Park Memorial Institute) media supplemented with 10% fetal bovine serum (FBS). To maintain luciferase labeled cells, blasticidin antibiotic was added at a concentration of 8 µg/mL.

KG1a cells were first isolated from a male patient with AML in 1980 [19]. KG1a cells are unable to differentiate into functionally mature cells and remain in an early or primitive state of differentiation. Cytogenetically, KG1a cells harbor the chromosome 7 deletion. In our experiments, KG1a cells were cultured in RPMI 1640 media supplemented with 10% fetal bovine serum. Both K562-Luc and KG1a cells were grown in a humidified chamber with 5% CO₂ and 95% humidity at 37°C.

fb-PMT, (fluorobenzyl Polyethylene glycol Mono-Triazole tetraiodothyroacetic acid), was synthesized at our facility (Pharmaceutical Research Institute, Rensselaer, NY, USA) and GMP scaleup was done at Dalton Pharmaceuticals (Toronto, Canada) [7, 16]. fb-PMT (molecular weight 2478.9 daltons) is a white powder that is soluble in Tris buffer at pH 8.0, ~ 100 mg/mL, with a final pH of 7.4.

Competitive Binding of fb-PMT to Purified αvβ3

The binding affinity of fb-PMT to purified αvβ3 was assessed using a previously described methods [7, 20]. Purified αvβ3 (1 µg/mL) was coated to polystyrene microtiter plate wells at 4°C overnight, and then the wells were blocked with 3% bovine serum albumin (BSA) for 2 h at room temperature. The wells were washed with buffer A (50 mM Tris/HCl, 100 mM NaCl, 1 mM CaCl₂, 1 mM MgCl₂, 1% BSA), and anti-αvβ3 conjugated with biotin (1:1000 in buffer A) was added and incubated for 1 h at room temperature. Increasing concentrations of compounds were added in the presence or absence of fibrinogen and incubated for 2 h at room temperature, and then wells were washed three times with buffer A and incubated with a streptavidin – Horseradish peroxidase (HRP) conjugate (1:1000 in buffer A) for 1 h at room temperature. Finally, wells were washed three times with buffer A, and 100 µL peroxidase substrate 3,3',5,5'-tetramethylbenzidine (TMB) was added, and the reaction was terminated after 30 min with 50 µL of 450 nm stop solution for TMB. Absorbance was determined at 450 nm with a plate reader.

Animals and treatment protocols

Mice were used in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals and approved by the Albany VA Medical Center (Albany, NY, USA) institutional animal care and use committee (IACUC) (protocol number 545017). Eighty male NSG-S mice (6–8 weeks of age) were purchased from Jackson Laboratories (Bar Harbor, ME). Preconditioning was done by intraperitoneal injection of busulfan (30 mg/kg, Otsuka America Pharmaceutical Inc., Hayward, CA, USA) 24 hours prior to cell injections. K562-Luc cells and primary AML cells (6373) (5 to 10 × 10⁶ per mouse) were transplanted via tail vein injection into mice.

For the K562-Luc animal model (40 mice), in vivo imaging system (IVIS) (PerkinElmer, Waltham, MA, USA) scans and the peripheral blood smears examination were performed on animals once a week. The fb-PMT treatment schedule was initiated on day 10 post-implantation when increased counts of blast cells became evident in peripheral blood smears and confirmed by IVIS signals. fb-PMT was administered subcutaneously (s.c.) daily at 3 different doses (1, 3, and 10 mg/kg body weight) or vehicle, PBS (control) for 21 days for both the ON arm (21 days treatment and then 20 mice have been sacrificed) and the ON + OFF arm (21 days treatment followed by 14 days treatment discontinuation and then the remaining 20 mice have been sacrificed). Control animals were administered with vehicle (phosphate buffered saline (PBS), pH 7.4) daily.

In experiments using the primary AML cell animal model (40 mice), the treatment protocol was initiated on animals after confirmation of successful engraftment. Treatment was initiated on day 40 post-implantation with fb-PMT (1, 3, and 10 mg/kg) or control (vehicle, PBS) daily s.c. for 28 days. Twenty animals (ON arm) were humanely sacrificed after 28 days of treatment, and peripheral blood smears

and bone marrow aspirates were examined histologically at the end of the 28 days of treatment. To evaluate the relapse after treatment, the remaining 20 mice (ON + OFF arm) were maintained without treatment for an additional 14 days, then they were humanely sacrificed and processed to obtain samples of peripheral blood smears, bone marrow aspirates and organs for histological examination. The maintaining for 14 days (ON + OFF) was established based on the animals' condition in the control groups.

Assessment of Leukemic cells engraftment by flow cytometry and immunohistostaining

Human AML engraftment was assessed by flowcytometry and defined as the percentage of human CD45+/CD33 + cells in total live mononuclear cells. Fresh bone marrow cells from NSG-S mice engrafted with K562-Luc and primary AML cells (6373) were collected at day 10 and 40 post-engraftment, respectively, once the blast cells detected in peripheral blood smear. samples were stained with antibodies for cell surface markers: anti-human CD45-PE, anti-human CD33-FITC (BD Biosciences, San Jose, CA, USA). Cells were incubated with monoclonal antibodies for 15 min at room temperature, washed once in PBS containing 0.1% human serum albumin (HSA), and analyzed by flow cytometry. Data acquisition was performed using a FACS Aria III (BD Biosciences) equipped with an argon and red diode laser, and analysis was performed using Cell Quest software (BD Biosciences).

As the K562-Luc cells express dim CD34, immunohistochemistry was performed for Formalin-fixed decalcified femurs from primary AML cells (6373) transplanted mice were paraffin-embedded and sectioned at 5 µm sections. Slides were stained using human anti-CD34 primary antibody (R&D system, Minneapolis, MN USA) then with HRP conjugated secondary antibody (Cell Signaling Technology Inc. Danvers, MA, USA). HRP activity was detected by diaminobenzidine tetrahydrochloride (DAB) and the slide were counterstain by Methyl green.

Safety study in animal models

The objective of this study was to evaluate the safety of fb-PMT after a period of daily s.c injections for 28 days in multiple species. Thirty-two cynomolgus monkeys (CM) were administered fb-PMT (2.5, 7.5 and 15 mg/kg) or vehicle daily s.c for 28 days (8 CM/ group). Clinical chemistry, biomarkers and histopathological examination were carried out (**data not shown**).

RNA isolation from AML cells and microarrays

K562-Luc and KG1a cells were cultured in 50 cm² cell culture flasks with 10 mL phenol red free RPMI media containing 10% FCS to 75% confluence. K562-Luc and KG1a cells were treated (at 50% confluence) with 30 µM fb-PMT for 48 hours. Total RNA was immediately isolated from harvested cells using Trizol and checked for quality using an Agilent Bioanalyzer (Agilent Technologies, Santa Clara, CA) before being used for microarray analysis. The quality and the concentrations of the extracted RNA were analyzed using a NanoDrop (Thermo Fisher Scientific, Waltham, MA) and the Agilent Bioanalyzer. RNA samples (100 ng) deemed to be of sufficient quality (RIN greater than 8) were processed according to the standard Affymetrix RNA labeling protocol. At least two independent biological replicates of control and treated samples were concurrently interrogated in microarray analyses. In preliminary experiments, the treatment dose and duration were carefully selected so as to not significantly affect growth and survival of target cells for the duration of experiments.

Microarray Analysis. Labeled RNA samples were processed for hybridization employing the Clariom™ S human array platform (Affymetrix, Santa Clara, CA) at the Center for Functional Genomics, University at Albany, Rensselaer, NY. Briefly, 100 ng of total RNA was processed using the WT Plus Reagent kit (Affymetrix). Sense target Complementary DNA (cDNAs) were generated using the standard Affymetrix WT protocol and hybridized to Affymetrix Human Clariom S arrays. Arrays were washed, stained, and scanned on a GeneChip 3000 7G scanner using Affymetrix GeneChip Command Console Software (AGCC). Transcriptome Analysis Console Software (TAC v3.0.1.5) was used to identify differentially expressed genes (DEGs). Briefly, the CEL files were summarized using the SST-RMA algorithm in TAC and the normalized data were subjected to one-way ANOVA with a Benjamin Hochberg False Discovery Rate correction included ($P < 0.05$). A 1.5-fold expression change cut-off was used to select entities that were statistically differentially expressed between the conditions being compared (treated and untreated groups). In the standard workflow protocol, the fragmented biotin-labeled cDNAs were hybridized for 16 h to Affymetrix Arrays, scanned on an Affymetrix Scanner 3000 7G using AGCC software, and processed as described above. Alternatively, CEL files after QC screening using Affymetrix Expression Console software were imported into GeneSpring GX11.5 (Agilent Technologies). The data was then quantile normalized using PLIER and baseline transformed to the median of the control samples. The probe sets were further filtered to exclude the bottom 20th percentile across all samples. The resulting entity lists were subjected to an unpaired T-test with the Benjamini-Hochberg False Discovery rate correction and a 1.5-fold

expression changes filter to identify differentially expressed transcripts between the control and test conditions at a p-value < 0.05. All analyzed and reported data are MIAME compliant and the raw data have been deposited in Gene Expression Omnibus (GEO; GSE95790) as detailed on the Microarray Gene Expression Data Society (MGED) website (<http://www.mged.org/Workgroups/MIAME/miame.html>). Overall, the workflow of the microarray analyses was modeled based on previously published contributions [21].

Gene set enrichment analyses of DEGs were done using the Enrichr bioinformatics platform, which enables the interrogation of nearly 200,000 gene sets from more than 100 gene set libraries. The Enrichr API (January 2018 through October 2020 releases) [22, 23] was used to test genes of interest for significant enrichment in numerous functional categories. When technically and analytically feasible, different sets of DEGs defined at multiple significance levels of statistical metrics and comprising from dozens to several thousand individual genetic loci were analyzed using differential Gene set enrichment analysis (GSEA) to gain insights into biological effects of DEGs and infer potential mechanisms of anticancer activities. This approach was successfully implemented for identification and characterization of human-specific regulatory networks governed by human-specific transcription factor-binding sites [24] and functional enhancer element [25], 13,824 genes associated with 59,732 human-specific regulatory sequences [26], 8,405 genes associated with 35,074 human-specific neuroregulatory single-nucleotide changes [27]. Initial GSEA entail interrogations of each specific set of DEGs using 29 distinct genomic databases, including comprehensive pathway enrichment Gene Ontology (GO) analyses followed by in-depth analyses of the selected genomic databases deemed most statistically informative. In all tables and plots (unless stated otherwise), in addition to the nominal p values and adjusted p values, the “combined score” calculated by Enrichr software is reported, which is a product of the significance estimate and the magnitude of enrichment (combined score $c = \log(p) * z$, where p is the Fisher's exact test p-value and z is the z-score deviation from the expected rank).

Statistical analysis

An overall comparison of the means for all groups was carried out using a one-way ANOVA. Tukey confidence intervals were used to test for differences in means for each experimental group versus the control group. Results are presented as means $\pm$ S.D. A value of $P < 0.05$ indicated a statistically significant difference.

Results

In Vitro Binding Affinity of fb-PMT with the thyrointegrin $\alpha v \beta 3$.

In binding affinity experiments of fb-PMT, we confirmed that fb-PMT has a high affinity for the thyrointegrin $\alpha v \beta 3$ receptors with a lower IC_{50} (50% inhibitory concentration) of 0.23 nM (Fig. 1).

Preclinical in vivo therapy experiments revealed potent fb-PMT anticancer activities.

Effects of fb-PMT therapy on K562-Luc human leukemic cell line FLT3-ITD primary human AML cells engrafted in transgenic mice.

In the K562-Luc engrafted in transgenic mice, blast cells appeared in the blood smears of NSG-S mice 10 days after engraftment, with an average value of 40%. After 21 days of fb-PMT daily s.c. injection, blast cell counts continually and consistently decreased in a dose-dependent manner in treated versus control groups, while animals in the control group showed increased blast cells in the peripheral blood. No blast cells could be detected in fb-PMT-treated animals (10 mg/kg) at the end of the treatment. Furthermore, there was no rebound increase in peripheral blast cells at 1 and 3 mg/kg with full sustained remission at fb-PMT dose of 10 mg/kg at 1–2 weeks post-discontinuation of treatment (Fig. 2, Supplementary Figure S1A).

On the other hand, primary AML cells (6373-FLT3-ITD) cells appeared in the blood smears of NSG-S mice 40 days after engraftment, with an average value of 26%. After 28 days of daily s.c. treatment, peripheral smears of treated animals were entirely normal at fb-PMT dose of 10 mg/kg. Daily s.c. injections of fb-PMT at 1, 3, and 10 mg/kg doses prevented blast cell expression/reproduction compared to controls by 54%, 75%, and 90.5%, respectively (Fig. 2 and Supplemental Figure S1B).

After termination, the bone marrow K562-Luc engrafted mice with a daily treatment of fb-PMT at 3 mg/kg manifested 30–40% infiltration with blast cells, while 70% maturation could be detected. fb-PMT-treated animals at 10 mg/kg dose presented bone marrows with blast cell counts < 5% and > 95%, normal maturation have been documented (segmented neutrophils). The remission was maintained in all treated mice at least 2 weeks after fb-PMT therapy discontinuation (Fig. 3A, 3B and Supplemental Figure S2A).

IVIS scans and histopathological results at sacrifice showed a dose-dependent decrease of brain, lung, liver, and spleen infiltration with the leukemic cells in the group of fb-PMT-treated mice (10 mg/kg) in comparison to control group Supplemental Figure S3. The fb-PMT therapy at 10 mg/kg dose in the ON + OFF treatment group resulted in successfully maintained remission in all animals 2 weeks after withdrawal of the daily treatment. The sustained remission was confirmed using blood smear analyses, IVIS scans, flowcytometry and histopathological examinations.

Regarding the bone marrow samples from mice engrafted with primary AML cells (FLT3-ITD), the fb-PMT-treated group (10 mg/kg) restored the normal bone marrow maturation with abundant megakaryocytes in comparison to control animals (Fig. 3C and 3D). The results were confirmed with flowcytometry and immunohistochemistry analysis (Supplemental Figure S2)

Furthermore, we evaluated the splenic infiltration in our animal models. Histopathological results showed a marked decrease of splenic metastases of the leukemic cells in the group treated with fb-PMT (10 mg/kg) compared to control group (Fig. 4). Similarly, to the K562-Luc AML experiments, the primary AML model (ON + OFF) group (10 mg/kg) manifested the successful maintenance of remission 2 weeks after withdrawal of daily therapy. The splenic weight showed marked decrease (80%) even with the low dose (1 mg/kg). The ON + OFF groups maintained normal splenic weight in comparison to control, which may reflect successful prevention of engraftment (Fig. 4A). Treatment with fb-PMT daily for 28 days (ON treatment) or 28 days ON and 14 days off treatment (ON + OFF treatment) resulted in maximal suppression of primary AML infiltration into the spleen of AML engrafted transgenic mice (Fig. 4B).

Microarray analyses of fb-PMT effects on gene expression in human AML cells

Previous contributions have established that one of the principal molecular mechanisms of anticancer activities of thyrointegrin $\alpha\beta 3$ antagonists is exemplified by significant changes in expression of genes essential for growth and survival of malignant cells [28–32]. Therefore, to gain insights into mechanisms of the fb-PMT activities against cancer cells, it was of interest to do genome-wide gene expression profiling experiments. Following 2 days of a single-dose treatment with 30 μM of fb-PMT, RNA samples were extracted from biological replicates of control and treated human AML cells and subjected to microarray analyses (Methods). Results of these analyses are reported in Figs. 5–7 and Tables 1–3.

Table 1

Examples of mimicry of pathway activation and pathway interference gene expression signatures (GES) identified in fb-PMT-treated K562 cells.

fb-PMT pathway activation GES	Genes	P-value*	Odds Ratio*	Combined Score*
RB1 pathway signature (n = 10)	STARD4;TMEM2;SESN3;SPIN4;SLX4IP;CD109;CENPE;CEP135;TCP11L2;PLD1	3.70E-04	6.266319	49.51741
IRF9 pathway signature (n = 6)	CENPE;EID3;SESN3;CD109;ASB7;TCP11L2	5.12E-04	7.54717	57.18613
MAML1 pathway signature (n = 6)	CENPE;PTAR1;STARD4;TMEM30A;CD109;TCP11L2	0.001655	4.696673	30.07697
RAP1A pathway signature (n = 5)	CENPE;SPIN4;ACSL1;ELOVL7;IL18R1	0.001067	6.410256	43.86473
GATA4 pathway signature (n = 5)	ERRFI1;ACSL1;LRRC28;CD9;ELOVL7	4.15E-04	7.905138	61.56706
fb-PMT pathway interference GES	Genes	P-value*	Odds Ratio*	Combined Score*
MYC pathway signature (n = 8)	ERRFI1;CENPE;BLOC1S6;TMEM30A;CD109;SESN3;ZNF17;KDSR	2.04E-04	9.21659	78.29477
HIF1A pathway signature (n = 6)	ERRFI1;ERV3-1;TCP11L2;SH3BGRL2;ELOVL7;IL18R1	1.48E-04	7.430341	65.52441
TWIST1 pathway signature (n-5)	EID3;SESN3;CD109;TCP11L2;ASB7	0.00152	5.91716	38.39501
TFAP2C pathway signature (n = 5)	CENPE;SPIN4;ACSL1;CD109;ELOVL7	5.21E-04	7.518797	56.84253
Legend: GES were identified based on the analyses of top 50 down-regulated genes in fb-PMT-treated K562 cells; *, Statistical metrics were defined by the Enrichr bioinformatics platform (Methods).				

Table 2

Mimicry of pathway activation and pathway interference gene expression signatures (GES) identified in fb-PMT-treated K562 cells.

Enriched Terms	P-value*	Adjusted P-value*	Odds Ratio*	Combined Score*	Genes
IRF9 OE HUMAN GSE50002 CREEDSID GENE 1659 DOWN	2.89E-07	5.65E-04	31.44654	473.5	<i>CENPE;EID3;SESN3;CD109;ASB7</i>
TFAP2C SIRNA HUMAN GSE15481 CREEDSID GENE 2895 DOWN	2.94E-07	2.88E-04	31.32832	471.2	<i>CENPE;ACSL1;SPIN4;CD109;ELOVL7</i>
TFAP2C KD HUMAN GSE15481 CREEDSID GENE 2970 DOWN	3.60E-07	2.35E-04	30.08424	446.4	<i>CENPE;ACSL1;SPIN4;CD109;ELOVL7</i>
TWIST1 OE MOUSE GSE50002 CREEDSID GENE 1075 UP	9.62E-07	4.71E-04	24.65483	341.6	<i>EID3;SESN3;CD109;ASB7;TCP11L2</i>
IRF9 OE HUMAN GSE50002 CREEDSID GENE 1663 DOWN	1.16E-06	4.54E-04	23.74169	324.5	<i>EID3;SESN3;CD109;ASB7;TCP11L2</i>
MYC OE U2OS HUMAN GSE59819 RNASEQ UP	6.23E-06	0.002034	30.72197	368.2	<i>CENPE;ERRFI1;TMEM30A;CD109</i>
MYC OE U2OS HUMAN GSE66789 RNASEQ UP	6.23E-06	0.001743	30.72197	368.2	<i>CENPE;ERRFI1;TMEM30A;CD109</i>
IRF9 OE HUMAN GSE50002 CREEDSID GENE 1656 DOWN	8.26E-06	0.002021	28.6123	334.9	<i>SESN3;CD109;ASB7;TCP11L2</i>
IRF9 OE HUMAN GSE50002 CREEDSID GENE 1653 DOWN	1.43E-05	0.00312	24.87562	277.4	<i>SESN3;CD109;ASB7;TCP11L2</i>
IRF9 OE HUMAN GSE50002 CREEDSID GENE 1657 DOWN	2.20E-05	0.004317	22.29654	239.1	<i>SESN3;CD109;ASB7;TCP11L2</i>
IRF9 OE HUMAN GSE50002 CREEDSID GENE 1654 DOWN	2.45E-05	0.004353	21.71553	230.6	<i>SESN3;CD109;ASB7;TCP11L2</i>
HIF1A KO MOUSE GSE35111 CREEDSID GENE 1406 DOWN	2.98E-05	0.004868	20.63983	215.1	<i>ERRFI1;TCP11L2;ELOVL7;IL18R1</i>
Legend: GES were identified based on the analyses of 12 down-regulated genes in fb-PMT-treated K562 cells; *, Statistical metrics were defined by the Enrichr bioinformatics platform (Methods).					

Table 3
Examples of functionally-significant genes down-regulated by the fb-PMT treatment in KG1A human AML cells.

Description	Gene Symbol	Fold Change	P-value
X-linked inhibitor of apoptosis, E3 ubiquitin protein ligase	<i>XIAP</i>	-2.9	0.0185
osteoclast stimulating factor 1	<i>OSTF1</i>	-2.86	0.0331
signal transducer and activator of transcription 2	<i>STAT2</i>	-2.81	0.0202
thymopoietin	<i>TMPO</i>	-2.59	0.0069
signal transducer and activator of transcription 4	<i>STAT4</i>	-2.54	0.0451
YES proto-oncogene 1, Src family tyrosine kinase	<i>YES1</i>	-2.49	0.0315
polymerase (RNA) II (DNA directed) polypeptide B, 140kDa	<i>POLR2B</i>	-2.44	0.0354
cyclin-dependent kinase 14	<i>CDK14</i>	-2.21	0.0402
TTK protein kinase	<i>TTK</i>	-2.2	0.046
topoisomerase (DNA) III alpha	<i>TOP3A</i>	-2.12	0.0116
prohibitin 2; small Cajal body-specific RNA 12	<i>PHB2</i>	-2.03	0.042
cyclin-dependent kinase 17	<i>CDK17</i>	-1.97	0.0303
phosphoglycerate kinase 2	<i>PGK2</i>	-1.85	0.0471
polymerase (DNA directed), epsilon 2, accessory subunit	<i>POLE2</i>	-1.83	0.0441
B-Raf proto-oncogene, serine/threonine kinase	<i>BRAF</i>	-1.81	0.0315
growth factor receptor bound protein 2	<i>GRB2</i>	-1.81	0.0301
Pim-1 proto-oncogene, serine/threonine kinase	<i>PIM1</i>	-1.71	0.038
serine/threonine kinase 4	<i>STK4</i>	-1.69	0.0135
prostaglandin E synthase 3 (cytosolic)	<i>PTGES3</i>	-1.56	0.0429
B-cell CLL/lymphoma 9	<i>BCL9</i>	-1.52	0.0334

Overall, there were 518 significantly down-regulated gene expression records and 283 significantly up-regulated gene expression records, expression of which were changed at least 1.5-fold in fb-PMT-treated K562 cells. In fb-PMT-treated KG1a cells, 223 significantly down-regulated gene expression records and 191 significantly up-regulated gene expression records were identified, expression of which were changed at least 1.5-fold in fb-PMT-treated cells. All DEG identified by the Affymetrix Expression Console software were subjected to GSEA employing a panel of 29 genomic databases (Methods). Analyses of fb-PMT treatment-induced DEGs in both K562 and KG1a cells identified sets of down-regulated genes and a genomic database of Transcription Factor (TF) Perturbations Followed by Expression as the most informative setting among comparison records. GSEA of down-regulated DEGs using the TF Perturbations Followed by Expression database identified 60 and 84 significantly enriched records (adjusted p value < 0.05) for fb-PMT-treated K562 and KG1a cells, respectively.

GSEA of fb-PMT effects on gene expression revealed signatures of the molecular mimicry of both activation of and interference with multiple transcriptional pathways:

Follow-up analyses of down-regulated DEGs using the TF Perturbations Followed by Expression database identified multiple examples of the molecular mimicry of pathway activation and pathway interference gene expression signatures (GES) identified in fb-PMT-treated human AML cells (Table 1; Fig. 5). Notable examples of the fb-PMT-induced GES of transcriptional pathway's activation include *RB1*, *IRF9*, *MAML1*, *RAP1A*, and *GATA4* pathways, and examples of the fb-PMT-induced GES of pathway's interference include *MYC*, *HIF1A*, *TWIST1*, and *TFAP2C* pathways. Integrations of DEGs comprising fb-PMT-induced GES listed in Table 1 identified a total of 25 genes, differential expression of which appears to define molecular signals of either activation of or interference with transcriptional pathways

in fb-PMT-treated human AML cells (Fig. 5). GSEA of genes comprising the 25-gene and 12-gene expression signatures validated their significance in defining observations of the molecular mimicry of transcriptional pathways' activation and interference induced by fb-PMT treatment in human AML cells (Figs. 5–7, Table 2). GSEA of all significant DEGs confirmed and extended these findings.

Interestingly, GSEA identified the *SNAI*/transcriptional pathway as the most significantly enriched pathway of the molecular interference observed in K562 cells treated with fb-PMT among either down-regulated or up-regulated DEGs (Supplemental Fig. 4). Additional examples of the specific genes and pathways of potential functional significance revealed by the GSEA of 233 genes down-regulated in KG1a cells after fb-PMT treatment are shown in Table 3. Of note, GSEA of the LINCS L1000 Ligand Perturbations database of up-regulated genes revealed evidence of molecular interference with functions of multiple growth factors in human cancer cell lines (Fig. 7).

GSEA of fb-PMT effects on gene expression revealed signatures of the molecular interference with a regulatory crosstalk of estrogen pathway and multi-kinase transcriptional matrix of cell cycle progression.

GSEA of databases of Ligand perturbations from GEO focused on up-regulated genes and Ligand perturbations from GEO focused on down-regulated genes revealed multiple examples of molecular interference with transcriptional signaling induced by many endogenous ligands, among which the enrichment of genes implicated in estrogen signaling was particularly apparent (Fig. 6 and Supplemental Fig. 4). Similarly, GSEA of the ARCHS4 Kinases Co-expression database revealed evidence of the molecular interference with functions of multiple kinases. GSEA of the integrated 69-gene signature of the fb-PMT interference with estrogen signaling in human AML cells identified 16 genes that appear implicated in transcriptional regulatory crosstalk of estrogens with multiple kinases in human tissues (Supplemental Fig. 5). Integration of 16 estrogen-regulated genes with 35 genes encoding kinases engaged in regulatory crosstalk in human tissues generated the 50-gene signature of estrogen pathway/multi-kinase gene expression regulatory matrix, which appears engaged in the cell cycle progression pathway (Supplemental Fig. 5). Intriguingly, GSEA of the 50-gene interference signature with estrogen-kinase regulatory matrix using the DisGeNET database of human disorders revealed that these genes were implicated in a remarkably broad spectrum of human malignancies (Supplemental Fig. 5),

Discussion

The present study assessed the efficacy of a novel thyrointegrin $\alpha\beta3$ antagonist (fb-PMT) against human AML cells. fb-PMT proved to be a highly effective anticancer agent in vivo. Experiments using different human AML models in mice documented eradication of the leukemic cells' engraftment after 3–4 weeks of continuous treatment with fb-PMT. Experimental therapy ON + OFF studies showed the significant efficacy of fb-PMT in preventing relapse, thus confirming the curative effects in vivo of fb-PMT treatment in clinically relevant animal models of human AML. The role of integrin in leukemogenesis was indicating by Yi et al. who reported that binding of leukemia cells to the bone marrow extracellular matrix (ECM) through integrins might influence drug response and the survival of leukemic cells [9]. Integrin $\alpha\beta3$ has been reported to be more expressed in AML cells especially CD34-positive cells, monocytic leukemias, patient with NPM, FLT3-ITD [12].

Consistent with the previous reports on mechanisms of anticancer actions of thyrointegrin $\alpha\beta3$ antagonists [29, 33–37], genome-wide microarray screens reported here demonstrated that fb-PMT appears to exert its potent anticancer actions on human AML cells through the molecular interference mechanism with multiple signaling pathways supporting growth and survival of leukemic cells. We detected significant molecular signals of transcriptional interference with gene expression induced in human cancer cells in response to multiple growth factors such as EGF, IGF-1, TGFA, and many others.

Other significant examples of the fb-PMT-induced GES of pathway's interference include *SNAI*, *MYC*, *HIF1A*, *TWIST1*, and *TFAP2C*. Notably, inference of potential contribution to the fb-PMT anticancer activity of the interference with these pathways seems highly congruent with their known biological functions such as cell cycle control (*MYC*), survival and maintenance of stem cells (*HIF1A*, *TFAP2C*), and essential features of the malignant phenotype (*TWIST1*, *SNAI*) [38–52].

Consistently, examples of the fb-PMT-induced GES of transcriptional pathway's activation include *RB1*, *IRF9*, *MAML1*, *RAP1A*, and *GATA4* pathways, known biological functions of which appear highly consistent with the hypothesis that activation of these pathways may contribute to fb-PMT anticancer activity [53–56].

Finally, consistent with our previous reports on the crosstalk between integrin $\alpha\beta3$ and estrogen receptor α (ER α), which contributes to the induced proliferation of cancer cells [57–60], we found that fb-PMT interfered with estrogen signaling in human AML cells. The $\alpha\beta3$

agonist (thyroid hormone) was associated with increased phosphorylation and nuclear enrichment of ER α . Confocal microscopy indicated that both T4 and estradiol (E2) caused nuclear translocation of integrin α v and phosphorylation of ER α . The specific ER α antagonist (ICI 182,780; fulvestrant) blocked T4-induced ERK1/2 activation, ER α phosphorylation, PCNA expression, and proliferation [57].

Furthermore, GLP toxicity study showed that fb-PMT is safe and well tolerated at > 60 fold higher than the effective anticancer doses after daily s.c injection for 4 weeks in rat and monkey models (data not shown).

fb-PMT is an effective anticancer agent against solid tumors and hematological malignancies, with broad spectrum, potent anti-angiogenic activity against all known growth factors and other pro-angiogenesis stimuli [28, 61, 62]. Collectively, preclinical findings of fb-PMT warrant its clinical investigation for the effective and safe management of AML.

Conclusion

Our novel thyrointegrin α v β 3 antagonist, fb-PMT, is preferable for potential clinical use because of its efficacy against human xenograft models of AML as well as its safety, even at high doses. Our genomic data demonstrated the potent anticancer actions on human AML through the molecular interference mechanism with multiple signaling pathways supporting growth and survival of leukemic cells. fb-PMT could have a broader application because it could be utilized, either alone or in combination with chemotherapeutic agents, to treat AML cancer or other cancers.

Abbreviations

AGCC: Affymetrix GeneChip Command Console Software

AML: Acute myeloid leukemia

Akt: Protein kinase B

BCR-ABL: Breakpoint cluster region gene/ Abelson proto-oncogene

BSA: Bovine serum Albumin

CEBPA: CAAT enhancer-binding protein alpha.

DEGs: Differentially expressed genes

ECM: Extracellular matrix

Er α : Estrogen receptor α

fb-PMT: fluorobenzyl Polyethylene glycol conjugated tetraiodothyroacetic acid

FBS: Fetal bovine serum

RPMI: Roswell Park Memorial Institute Medium

FLT3-ITD: FMS-like tyrosine kinase-3-internal tandem duplication

GEO: Gene Expression Omnibus

cDNA: Complementary DNA

PBS: Phosphate-buffered saline

HRP: Horseradish peroxidase

GES: Gene expression signatures

GSEA: Gene set enrichment analysis

HIF1A: Hypoxia Inducible Factor 1 Subunit Alpha

HSA: Human serum albumin

IACUC: Institutional animal care and use committee

IDH1/2: Isocitrate dehydrogenases 1 and 2

IRF9: Interferon Regulatory Factor 9

IVIS: In Vivo Imaging Systems

MAML1: Mastermind Like Transcriptional Coactivator 1

MGED: Microarray Gene Expression Data

NPM: Nucleophosmin

RAP1A: Ras-related protein Rap-1A

RB1: Retinoblastoma protein-1

RGD: Arginine-glycine-aspartate

S.C.: Subcutaneously

SNAIL: Snail Family Transcriptional Repressor 1

TF: Transcription Factor

TFAP2C: Transcription Factor AP-2 Gamma

TMB: Tetramethylbenzidine

TP53: Tumor protein p53

TWIST1: Twist Family BHLH Transcription Factor 1

Declarations

Ethics approval and consent to participate

Primary human AML cells (De novo AML 6373, harboring FLT3-ITD mutation) were collected by leukapheresis from AML patients at the University Hospital, University of Pennsylvania, with informed consents (IRB protocol 703185).

Mice were used in accordance with Public Health Service Policy on Humane Care and Use of Laboratory Animals and approved by the Albany VA Medical Center (Albany, NY, USA) IACUC (protocol number 545017).

Consent for publication

“Not applicable”

Availability of data and materials

All data generated or analysed during this study are included in this published article and its supplementary information files. For further inquiries regarding the current study data, the corresponding author can provide any required explanations on reasonable request.

Competing interests

S.A. Mousa holds several patents on anticancer compounds assigned to NanoPharmaceuticals LLC and founder of the company. G.V. Glinsky is a paid consultant to NanoPharmaceuticals LLC. The remaining authors have no conflicts to declare.

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Author Contributions

S.A Mousa designed the experiment and is the Principal Investigator. Noureldien Darwish and Sudha Thangirala conducted the experiment. Noureldien Darwish did the data analysis. G.V. Glinsky contributed to the genetic analysis. Noureldien Darwish contributed to the manuscript write up and data interpretation. All authors have approved the final version of the manuscript.

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References

1. **Key Statistics for Acute Myeloid Leukemia (AML)** [<https://www.cancer.org/cancer/acute-myeloid-leukemia/about/key-statistics.html>]
2. Döhner H, Weisdorf DJ, Bloomfield CD: **Acute Myeloid Leukemia**. *New England Journal of Medicine* 2015, **373**(12):1136-1152.
3. Yu J, Jiang PYZ, Sun H, Zhang X, Jiang Z, Li Y, Song Y: **Advances in targeted therapy for acute myeloid leukemia**. *Biomarker Research* 2020, **8**(1):17.
4. Ciciarello M, Corradi G, Loscocco F, Visani G, Monaco F, Cavo M, Curti A, Isidori A: **The Yin and Yang of the Bone Marrow Microenvironment: Pros and Cons of Mesenchymal Stromal Cells in Acute Myeloid Leukemia**. *Frontiers in Oncology* 2019, **9**(1135).
5. Forte D, Krause DS, Andreeff M, Bonnet D, Mendez-Ferrer S: **Updates on the hematologic tumor microenvironment and its therapeutic targeting**. *Haematologica* 2019, **104**(10):1928-1934.
6. Akwii RG, Sajib MS, Zahra FT, Mikelis CM: **Role of Angiopoietin-2 in Vascular Physiology and Pathophysiology**. *Cells* 2019, **8**(5).
7. Rajabi M, Godugu K, Sudha T, Bharali DJ, Mousa SA: **Triazole Modified Tetraiodothyroacetic Acid Conjugated to Polyethylene Glycol: High Affinity Thyrointegrin $\alpha\beta 3$ Antagonist with Potent Anticancer Activities in Glioblastoma Multiforme**. *Bioconjugate Chemistry* 2019, **30**(12):3087-3097.
8. Bridoux A, Khan RA, Chen C, Chevé G, Cui H, Dyskin E, Yasri A, Mousa SA: **Design, synthesis, and biological evaluation of bifunctional thyrointegrin inhibitors: new anti-angiogenesis analogs**. *Journal of enzyme inhibition and medicinal chemistry* 2011, **26**(6):871-882.
9. Yi H, Zeng D, Shen Z, Liao J, Wang X, Liu Y, Zhang X, Kong P: **Integrin $\alpha\text{v}\beta 3$ enhances β -catenin signaling in acute myeloid leukemia harboring Fms-like tyrosine kinase-3 internal tandem duplication mutations: implications for microenvironment influence on sorafenib sensitivity**. *Oncotarget* 2016, **7**(26):40387-40397.
10. Jiang X, Mak PY, Mu H, Tao W, Mak DH, Kornblau S, Zhang Q, Ruvoilo P, Burks JK, Zhang W *et al*: **Disruption of Wnt/ β -Catenin Exerts Antileukemia Activity and Synergizes with FLT3 Inhibition in FLT3-Mutant Acute Myeloid Leukemia**. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2018, **24**(10):2417-2429.
11. Hosseini A, Ghorbanmehr N, Valojerdi MR, Bakhtiyari M, Movaghgar B: **Leukaemia inhibitory factor increases $\alpha\text{v}\beta 3$ integrin expression in cultured mouse blastocysts**. *Reproduction, Fertility and Development* 2020, **32**(13):1116-1124.
12. Kuželová K, Obr A, Marková J, Gašová Z: **Integrin expression and adhesivity to fibronectin in primary acute myeloid leukemia cells: Impact of NPM1 and FLT3 mutations**. *European Journal of Haematology* 2020, **105**(5):578-587.
13. Cheng SY, Leonard JL, Davis PJ: **Molecular aspects of thyroid hormone actions**. *Endocr Rev* 2010, **31**(2):139-170.
14. Davis PJ, Davis FB, Mousa SA, Luidens MK, Lin HY: **Membrane receptor for thyroid hormone: physiologic and pharmacologic implications**. *Annual review of pharmacology and toxicology* 2011, **51**:99-115.
15. Davis PJ, Goglia F, Leonard JL: **Nongenomic actions of thyroid hormone**. *Nature reviews Endocrinology* 2016, **12**(2):111-121.
16. Hay BA, Godugu K, Darwish NHE, Fujioka K, Sudha T, Karakus OO, Mousa SA: **New Thyrointegrin $\alpha\text{v}\beta 3$ Antagonist with a Scalable Synthesis, Brain Penetration, and Potent Activity against Glioblastoma Multiforme**. *Journal of Medicinal Chemistry* 2021.
17. Wu SQ, Voelkerding KV, Sabatini L, Chen XR, Huang J, Meisner LF: **Extensive amplification of bcr/abl fusion genes clustered on three marker chromosomes in human leukemic cell line K-562**. *Leukemia* 1995, **9**(5):858-862.

18. Tang C, Schafraneck L, Watkins DB, Parker WT, Moore S, Prime JA, White DL, Hughes TP: **Tyrosine kinase inhibitor resistance in chronic myeloid leukemia cell lines: investigating resistance pathways.** *Leuk Lymphoma* 2011, **52**(11):2139-2147.
19. Koefler HP, Billing R, Lusic AJ, Sparkes R, Golde DW: **An undifferentiated variant derived from the human acute myelogenous leukemia cell line (KG-1).** *Blood* 1980, **56**(2):265-273.
20. Kouns WC, Hadvary P, Haering P, Steiner B: **Conformational modulation of purified glycoprotein (GP) IIb-IIIa allows proteolytic generation of active fragments from either active or inactive GPIIb-IIIa.** *J Biol Chem* 1992, **267**(26):18844-18851.
21. Glinsky GV, Berezovska O, Glinskii AB: **Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer.** *J Clin Invest* 2005, **115**(6):1503-1521.
22. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, Clark NR, Ma'ayan A: **Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool.** *BMC bioinformatics* 2013, **14**:128.
23. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, Koplev S, Jenkins SL, Jagodnik KM, Lachmann A *et al*: **Enrichr: a comprehensive gene set enrichment analysis web server 2016 update.** *Nucleic acids research* 2016, **44**(W1):W90-97.
24. Glinsky GV: **Human-specific genomic features of pluripotency regulatory networks link NANOG with fetal and adult brain development.** *bioRxiv* 2017:022913.
25. Glinsky G, Barakat TS: **The evolution of Great Apes has shaped the functional enhancers' landscape in human embryonic stem cells.** *Stem Cell Research* 2019, **37**:101456.
26. Glinsky GV: **A Catalogue of 59,732 Human-Specific Regulatory Sequences Reveals Unique-to-Human Regulatory Patterns Associated with Virus-Interacting Proteins, Pluripotency, and Brain Development.** *DNA Cell Biol* 2020, **39**(1):126-143.
27. Glinsky GV: **Impacts of genomic networks governed by human-specific regulatory sequences and genetic loci harboring fixed human-specific neuro-regulatory single nucleotide mutations on phenotypic traits of modern humans.** *Chromosome research : an international journal on the molecular, supramolecular and evolutionary aspects of chromosome biology* 2020:1-24.
28. Rajabi M, Godugu K, Sudha T, Bharali DJ, Mousa SA: **Triazole Modified Tetraiodothyroacetic Acid Conjugated to Polyethylene Glycol: High Affinity Thyrointegrin $\alpha(v)\beta(3)$ Antagonist with Potent Anticancer Activities in Glioblastoma Multiforme.** *Bioconjug Chem* 2019, **30**(12):3087-3097.
29. Mousa SA, Glinsky GV, Lin H-Y, Ashur-Fabian O, Hercbergs A, Keating KA, Davis PJ: **Contributions of Thyroid Hormone to Cancer Metastasis.** *Biomedicines* 2018, **6**(3):89.
30. Mousa S, Sudha T, Godugu K, Rajabi M, Sell S, Davis P: **Abstract 1300: Novel thyrointegrin $\alpha v \beta 3$ antagonist in the treatment of glioblastoma multiforme.** *Cancer Research* 2019, **79**:1300-1300.
31. Chin Y-T, He Z-R, Chen C-L, Chu H-C, Ho Y, Su P-Y, Yang Y-CSH, Wang K, Shih Y-J, Chen Y-R *et al*: **Tetrac and NDAT Induce Anti-proliferation via Integrin $\alpha v \beta 3$ in Colorectal Cancers With Different K-RAS Status.** *Front Endocrinol (Lausanne)* 2019, **10**:130-130.
32. Sudha T, Bharali DJ, Sell S, Darwish NHE, Davis PJ, Mousa SA: **Nanoparticulate Tetrac Inhibits Growth and Vascularity of Glioblastoma Xenografts.** *Horm Cancer* 2017, **8**(3):157-165.
33. Davis PJ, Tang H-Y, Hercbergs A, Lin H-Y, Keating KA, Mousa SA: **Bioactivity of Thyroid Hormone Analogs at Cancer Cells.** *Front Endocrinol (Lausanne)* 2018, **9**:739-739.
34. Lin H-Y, Glinsky GV, Mousa SA, Davis PJ: **Thyroid hormone and anti-apoptosis in tumor cells.** *Oncotarget* 2015, **6**(17):14735-14743.
35. Yalcin M, Bharali DJ, Lansing L, Dyskin E, Mousa SS, Hercbergs A, Davis FB, Davis PJ, Mousa SA: **Tetraiodothyroacetic acid (tetrac) and tetrac nanoparticles inhibit growth of human renal cell carcinoma xenografts.** *Anticancer research* 2009, **29**(10):3825-3831.
36. Yalcin M, Lin HY, Sudha T, Bharali DJ, Meng R, Tang HY, Davis FB, Stain SC, Davis PJ, Mousa SA: **Response of human pancreatic cancer cell xenografts to tetraiodothyroacetic acid nanoparticles.** *Horm Cancer* 2013, **4**(3):176-185.
37. Bharali DJ, Yalcin M, Davis PJ, Mousa SA: **Tetraiodothyroacetic acid-conjugated PLGA nanoparticles: a nanomedicine approach to treat drug-resistant breast cancer.** *Nanomedicine (Lond)* 2013, **8**(12):1943-1954.
38. Ma P, Xing M, Han L, Gan S, Ma J, Wu F, Huang Y, Chen Y, Tian W, An C *et al*: **High PD-L1 expression drives glycolysis via an Akt/mTOR/HIF-1 α axis in acute myeloid leukemia.** *Oncol Rep* 2020, **43**(3):999-1009.
39. Jabari M, Allahbakhshian Farsani M, Salari S, Hamidpour M, Amiri V, Mohammadi MH: **Hypoxia-Inducible Factor1-A (HIF1 α) and Vascular Endothelial Growth Factor-A (VEGF-A) Expression in De Novo AML Patients.** *Asian Pac J Cancer Prev* 2019, **20**(3):705-710.
40. Abdul-Aziz AM, Shafat MS, Sun Y, Marlein CR, Piddock RE, Robinson SD, Edwards DR, Zhou Z, Collins A, Bowles KM *et al*: **HIF1 α drives chemokine factor pro-tumoral signaling pathways in acute myeloid leukemia.** *Oncogene* 2018, **37**:2676-2686.

41. Raffel S, Falcone M, Kneisel N, Hansson J, Wang W, Lutz C, Bullinger L, Poschet G, Nonnenmacher Y, Barnert A *et al*: **BCAT1 restricts α KG levels in AML stem cells leading to IDHmut-like DNA hypermethylation.** *Nature* 2017, **551**(7680):384-388.
42. Migliavacca J, Percio S, Valsecchi R, Ferrero E, Spinelli A, Ponzoni M, Tresoldi C, Pattini L, Bernardi R, Coltella N: **Hypoxia inducible factor-1 α regulates a pro-invasive phenotype in acute monocytic leukemia.** *Oncotarget* 2016, **7**(33):53540-53557.
43. Gao XN, Yan F, Lin J, Gao L, Lu XL, Wei SC, Shen N, Pang JX, Ning QY, Komeno Y *et al*: **AML1/ETO cooperates with HIF1 α to promote leukemogenesis through DNMT3a transactivation.** *Leukemia* 2015, **29**(8):1730-1740.
44. Wang X, Sun D, Tai J, Chen S, Yu M, Ren D, Wang L: **TFAP2C promotes stemness and chemotherapeutic resistance in colorectal cancer via inactivating hippo signaling pathway.** *Journal of Experimental & Clinical Cancer Research* 2018, **37**(1):27.
45. Chen D, Liu W, Zimmerman J, Pastor WA, Kim R, Hosohama L, Ho J, Aslanyan M, Gell JJ, Jacobsen SE *et al*: **The TFAP2C-Regulated OCT4 Naive Enhancer Is Involved in Human Germline Formation.** *Cell Rep* 2018, **25**(13):3591-3602.e3595.
46. Kang J, Kim W, Lee S, Kwon D, Chun J, Son B, Kim E, Lee JM, Youn H, Youn B: **TFAP2C promotes lung tumorigenesis and aggressiveness through miR-183- and miR-33a-mediated cell cycle regulation.** *Oncogene* 2017, **36**(11):1585-1596.
47. Do H, Kim D, Kang J, Son B, Seo D, Youn H, Youn B, Kim W: **TFAP2C increases cell proliferation by downregulating GADD45B and PMAIP1 in non-small cell lung cancer cells.** *Biol Res* 2019, **52**(1):35-35.
48. Pastor WA, Liu W, Chen D, Ho J, Kim R, Hunt TJ, Lukianchikov A, Liu X, Polo JM, Jacobsen SE *et al*: **TFAP2C regulates transcription in human naive pluripotency by opening enhancers.** *Nat Cell Biol* 2018, **20**(5):553-564.
49. Kim W, Kim E, Lee S, Kim D, Chun J, Park KH, Youn H, Youn B: **TFAP2C-mediated upregulation of TGFBR1 promotes lung tumorigenesis and epithelial-mesenchymal transition.** In: *Exp Mol Med*. vol. 48; 2016: e273.
50. Shousha WG, Ramadan SS, El-Saiid AS, Abdelmoneim AE, Abbas MA: **Expression and clinical significance of SNAI1 and ZEB1 genes in acute myeloid leukemia patients.** *Molecular biology reports* 2019, **46**(4):4625-4630.
51. Carmichael CL, Wang J, Nguyen T, Kolawole O, Benyoucef A, De Maziere C, Milne AR, Samuel S, Gillinder K, Hediye-Zadeh S *et al*: **The EMT modulator SNAI1 contributes to AML pathogenesis via its interaction with LSD1.** *Blood* 2020, **136**(8):957-973.
52. Carmichael CL, Wang J, Nguyen T, Kolawole O, Benyoucef A, De Mazière C, Milne AR, Samuel S, Gillinder K, Hediye-Zadeh S *et al*: **The EMT modulator SNAI1 contributes to AML pathogenesis via its interaction with LSD1.** *Blood* 2020, **136**(8):957-973.
53. Tian WL, Guo R, Wang F, Jiang ZX, Tang P, Huang YM, Sun L: **The IRF9-SIRT1-P53 axis is involved in the growth of human acute myeloid leukemia.** *Experimental cell research* 2018, **365**(2):185-193.
54. Patnaik MM, Lasho T, Howard M, Finke C, Ketterling RL, Al-Kali A, Pardananani A, Droin N, Gangat N, Tefferi A *et al*: **Biallelic inactivation of the retinoblastoma gene results in transformation of chronic myelomonocytic leukemia to a blastic plasmacytoid dendritic cell neoplasm: shared clonal origins of two aggressive neoplasms.** *Blood Cancer Journal* 2018, **8**(9):82.
55. Su L, Tan Y, Lin H, Liu X, Yu L, Yang Y, Liu S, Bai O, Yang Y, Jin F *et al*: **Mutational spectrum of acute myeloid leukemia patients with double CEBPA mutations based on next-generation sequencing and its prognostic significance.** *Oncotarget* 2018, **9**(38):24970-24979.
56. Watanabe T, Oyama T, Asada M, Harada D, Ito Y, Inagawa M, Suzuki Y, Sugano S, Katsube K-i, Karsenty G *et al*: **MAML1 enhances the transcriptional activity of Runx2 and plays a role in bone development.** *PLoS Genet* 2013, **9**(1):e1003132-e1003132.
57. Hsieh MT, Wang LM, Changou CA, Chin YT, Yang YSH, Lai HY, Lee SY, Yang YN, Whang-Peng J, Liu LF *et al*: **Crosstalk between integrin α v β 3 and ER α contributes to thyroid hormone-induced proliferation of ovarian cancer cells.** *Oncotarget* 2017, **8**(15):24237-24249.
58. Davis PJ, Mousa SA, Cody V, Tang HY, Lin HY: **Small molecule hormone or hormone-like ligands of integrin α v β 3: implications for cancer cell behavior.** *Horm Cancer* 2013, **4**(6):335-342.
59. Lin HY, Su YF, Hsieh MT, Lin S, Meng R, London D, Lin C, Tang HY, Hwang J, Davis FB *et al*: **Nuclear monomeric integrin α v in cancer cells is a coactivator regulated by thyroid hormone.** *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* 2013, **27**(8):3209-3216.
60. Meng R, Tang HY, Westfall J, London D, Cao JH, Mousa SA, Luidens M, Herbergs A, Davis FB, Davis PJ *et al*: **Crosstalk between integrin α v β 3 and estrogen receptor- α is involved in thyroid hormone-induced proliferation in human lung carcinoma cells.** *PloS one* 2011, **6**(11):e27547.
61. M MSaR: **Non-cleavable polymer conjugated with alpha V beta 3 integrin thyroid antagonists.** . In: *USA*. Edited by Patent U, vol. 10,695,436; 2020.
62. M MSaR: **Non-cleavable polymer conjugated with .alpha.V.beta.3 integrin thyroid antagonists.** In. Edited by Patent U. USA; 2019.

Figures

Figure 1

Figure 1

Binding Affinity of fb-PMT to human Integrin $\alpha v \beta 3$ Receptors. fb-PMT high affinity for the thyrointegrin $\alpha v \beta 3$ receptors (K_i 0.23 nM)

Figure 2

Figure 2

Blood smear from transgenic mice engrafted with leukemic cells A) K562 cells in peripheral blood (PB) cells, control blood smears versus fb-PMT treated mice at different doses. Blast cells appeared in the blood smears of NSG-S mice after 10 days. After 21 days of fb-PMT daily subcutaneous injection, the control group showed many blast cells in the peripheral blood, while fb-PMT (1, 3, and 10 mg/kg) treated mice showed a significant decrease in the blast cells by 35%, 65%, and >95%, respectively. The decrease of blast cell percentage in the peripheral blood was dose-dependent. Left side shows blast cells in peripheral blood in fb-PMT treated animals at different doses ON 21 Days and ON+OFF treatment for 14 days and right side shows representative K562-Luc AML control blood smears versus fb-PMT (10 mg/kg) treated animal cells. Control group showed immature cells with prominent nucleoli (blast cells) in the peripheral blood. fb-PMT (10 mg/kg) treated animal showed segmented neutrophils with absent blast cells, after 21 days of daily subcutaneous injection. fb-PMT (10 mg/kg) maintained remission after 2 weeks discontinuation. B) Primary AML cells (6373-FIT3-ITD), fb-PMT treated at 1, 3, and 10 mg/kg subcutaneous daily for 28 days and 14 days OFF treatment prevented blast cell expression/reproduction by 54%, 75%, and 90.5%, respectively, compared to controls. Right side shows representative image from control group with immature cells and prominent nucleoli (blast cells) in the peripheral blood where fb-PMT (10 mg/kg) treated animal shows segmented neutrophils with absent blast cells. OFF study, 14 days post-treatment, fb-PMT (10 mg/kg) showed successful maintained remission.

Figure 3

Figure 3

Blast cells in bone marrow. A) Myeloblast in bone marrow of transgenic mice engrafted with K562-Luc cells and after 21 days of treatment (ON treatment) and 14 days post-treatment (ON + OFF treatment). Bone marrow of fb-PMT (3 mg/kg) treated group was associated with 30-40% infiltration with blast cells, and 70% maturation could be detected. fb-PMT (10 mg/kg) treated group presented with blast cells <5% and 90-95% normal maturation (segmented neutrophils). ON+OFF treatment, fb-PMT (10 mg/kg) showed maintained remission. B) Histopathological evaluation of bone marrow of transgenic mice engrafted with K562-Luc cells (ON+OFF treatment). Myeloblast in bone marrow of transgenic mice after 21 days of treatment. Bone marrow of fb-PMT (3 mg/kg) treated group was associated with 30-40% infiltration with blast cells, and 70% maturation could be detected. fb-PMT (10 mg/kg) treated group presented with blast cells <5% and 90-95% normal maturation (segmented neutrophils). C) Myeloblast in bone marrow from transgenic mice engrafted with primary AML cells (6373-FIT3-ITD) control versus fb-PMT treated animal through 28 days and 14 days post-treatment. More than 20% immature blast cells in marrow smears is characteristic of AML. Treated group (10 mg/kg) was associated with >90% decrease in leukemic cells burden. The ON+OFF group showed sustained remission with fb-PMT (10 mg/kg) after 14 days. D) Histopathological evaluation of bone marrow in transgenic mice engrafted with primary AML cells (6373-FIT3-ITD) control versus fb-PMT treated animal through 28 days and 14 days post-treatment. Bone marrow of fb-PMT (3 mg/kg) treated group was associated with 35% infiltration with blast cells, and 60-70% maturation could be detected. The fb-PMT (10 mg/kg) treated group presented with blast cells <5% and >95% normal maturation (segmented neutrophils), (**P<0.0001, **P<0.001, *P<0.01).

Figure 4

Figure 4

Evaluation of leukemic cells infiltration of spleen. A) Evaluation of splenic infiltration of leukemic cells in transgenic mice engrafted with primary AML cells (6373-FIT3-ITD) control versus fb-PMT treated animal through 28 days and 14 days post-treatment. The splenic weight shows marked decrease in weight (80%) even with the low dose fb-PMT (1 mg/kg). ON+OFF groups showed maintained normal splenic weight in comparison to control, which may reflect successful prevention of engraftment. B) Histopathological evaluation of splenic infiltration with primary AML cells in fb-PMT treated (10 mg/kg) versus control group. Upper row (control group) shows 90-95 % Infiltration with leukemic cells. Lower row (fb-PMT, 10 mg/kg) shows multiple normal lymphoid follicles with leukemic cells infiltration 5-10%.

Figure 5

Figure 5

Identification and characterization of the 25-gene fb-PMT-induced expression signature in K562 cells. A) Integration into different functional categories of fb-PMT treatment-induced gene expression signatures (GES) as reported in Table 1. B) GSEA of 25-gene GES using the TF perturbations followed by expression database. C) GSEA of 25-gene GES using the Gene perturbations from gene expression omnibus (GEO) database focused on up-regulated genes. D) GSEA of 25-gene GES using the GEO database focused on down-regulated genes. Numbers shown next to the bar graphs report the values of Cumulative scores (Methods).

Figure 6

Figure 6

Networks and Ligands associated with the 25-gene fb-PMT-induced expression signature in K562 cells. A) Affected genomic regulatory networks revealed by the GSEA of the Gene perturbations from gene expression omnibus (GEO) database focused on up-regulated (left) and down-regulated (right) genes. B) GSEA of the Ligand perturbations from GEO database focused on up-regulated genes. C) GSEA of the Ligand perturbations from GEO database focused on down-regulated genes. Numbers shown next to the bar graphs report the values of Cumulative scores (Methods). D) fb-PMT gene expression signature in K562 cells

Figure 7

Figure 7

Examples of the specific genes and pathways of potential functional significance revealed by the GSEA of 233 genes down-regulated in KG1a cells after fb-PMT treatment. A) Functionally significant genes down-regulated by fb-PMT treatment in KG1a human AML cells. B) GSEA of the TF Perturbations Followed by Expression database (top 30 of 84 significant records). C) GSEA of the LINCS L1000 Ligand Perturbations database of up-regulated genes revealed evidence of molecular interference with functions of multiple growth factors in human cancer cell lines. D) GSEA of the KEGG 2019 Human database revealed evidence of targeting multiple cancer pathways (including AML).

Supplementary Files

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