Article

IL15: The Molecule That Was Always There—Waiting to Be Switched On

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IL15: The Molecule That Was Always There—Waiting to Be Switched On

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Abstract

Background: Metadichol® (nano policosanol) is a novel nanoemulsion of long-chain alcohols derived from natural food sources that has demonstrated broad immunomodulatory properties via inverse agonism of the vitamin D receptor (VDR). Its ability to modulate gene expression across diverse cancer cell lines presents a unique opportunity to explore its anticancer mechanisms through cytokine and transcription factor regulation. **Objective:** This study investigated the effect of Metadichol at five concentrations (0.1 pg/mL to 100 ng/mL) on the expression of interleukin-15 (IL-15), T-box transcription factor 21 (TBET/TBX21), and eomesodermin (EOMES) in six human cancer cell lines representing distinct tumor types: A549 (lung adenocarcinoma), FaDu (pharyngeal squamous cell carcinoma), HCT116 (colorectal carcinoma), HeLa (cervical adenocarcinoma), HepG2 (hepatocellular carcinoma), and U87MG (glioblastoma multiforme). **Methods:** Quantitative gene expression analysis was performed on all six cell lines treated with Metadichol at concentrations ranging from 0.1 pg/mL to 100 ng/mL. Fold-change values were normalized to untreated controls. IL-15, TBET, and EOMES expression were quantified to assess dose-response relationships. **Results:** IL-15 expression was consistently and significantly upregulated across all six cancer cell lines following Metadichol treatment. The most pronounced induction was observed in HCT116 cells at 1 pg/mL (4.30-fold), followed by HepG2 at 1 pg/mL (3.66-fold), HeLa at 1 ng/mL (3.04-fold), FaDu at 1 pg/mL (2.81-fold), U87MG at 1 ng/mL (2.69-fold), and A549 at 1 pg/mL (2.45-fold). TBET and EOMES exhibited variable, cell line-dependent expression patterns, with selective upregulation in certain contexts. The ultra-low effective concentrations (picogram range) are consistent with Metadichol's known mechanisms of action. **Conclusions:** Metadichol potently and consistently induces IL-15 expression across multiple cancer cell types. Given IL-15's central role in activating natural killer (NK) cells, cytotoxic CD8+ T cells, memory T cells, and B cells, this finding positions Metadichol as a promising immunomodulatory compound capable of reactivating innate and adaptive antitumor immunity. The concurrent modulation of TBET and EOMES, key transcription factors governing effector lymphocyte differentiation, further supports a broad immunostimulatory mechanism. These results warrant further preclinical and clinical investigation of Metadichol as an adjunct cancer immunotherapy.

Keywords: Metadichol; Interleukin-15; TBET; EOMES; cancer immunology; NK cells; T cells; cytokine; gene expression; immunotherapy

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Introduction

Background and Rationale

Cancer remains a leading cause of mortality worldwide, with an estimated 19.3 million new cases and 10 million deaths in 2020 alone. [1] Despite remarkable advances in targeted therapy, checkpoint immunotherapy, and adoptive cellular therapies, a large proportion of patients fail to achieve durable remissions. A fundamental challenge in oncology is the immunosuppressive tumor microenvironment (TME), which renders tumors resistant to immune surveillance and cytotoxic killing. [2] Strategies that can reinvigorate immune effector cells within the TME, without generating systemic toxicity, represent a compelling unmet need.

Metadichol® (nano policosanol) is a patented nanoemulsion of long-chain saturated alcohols (C24–C34) derived from sugar cane wax and other natural sources. [3–5] Unlike classical pharmacological agents, Metadichol acts at extraordinarily low concentrations—in the picogram-to-nanogram range—to modulate immune and metabolic gene networks. Its primary mechanism involves inverse agonism at the nuclear vitamin D receptor (VDR), thereby suppressing the constitutive NF-κB activation that characterizes many cancer cells and inflammatory states. [6,7] Beyond VDR modulation, Metadichol has been shown to influence a broad set of cytokines, nuclear receptors, and transcription factors relevant to immune function. [8–11]

The capacity of Metadichol to upregulate immune-activating cytokines studied recently in PBMC [9] but not in cancer cells. This work addresses that gap by examining three key immune-regulatory molecules—IL-15, TBET (TBX21), and EOMES—across six cancer cell lines representing a clinically diverse spectrum of human malignancies.

Interleukin-15: A Central Orchestrator of Antitumor Immunity

Interleukin-15 (IL-15) is a pleiotropic cytokine belonging to the four α-helix bundle cytokine family that shares structural homology with interleukin-2 (IL-2). [12] IL-15 signals through a heterotrimeric receptor complex comprising the IL-2/IL-15Rβ chain (CD122), the common γ-chain (CD132 , and the unique IL-15Rα chain (CD215). [13,14] Critically, IL-15 is presented in trans by IL-15Rα-expressing cells (including dendritic cells and macrophages) to neighboring NK cells and T cells, a process termed trans-presentation, which is the predominant physiological mode of IL-15 signaling. [15]

IL-15 exerts profound effects on multiple arms of the immune system. In natural killer (NK) cells, IL-15 is absolutely required for homeostatic survival, proliferation, and cytotoxic effector function. [16] NK cell-mediated antitumor immunity depends on IL-15 signaling to upregulate activating receptors (NKG2D, NKp44, NKp46) and cytolytic molecules (perforin, granzyme B). [15] Tumor cells frequently downregulate IL-15 signaling as an immune escape strategy, creating an NK cell-depleted niche. [16]

In the adaptive immune compartment, IL-15 is a critical survival and activation factor for CD8+ cytotoxic T lymphocytes (CTLs). [17] IL-15 promotes the generation and maintenance of long-lived central memory CD8+ T cells (TCM), which are essential for durable antitumor protection. [17] IL-15 also counteracts T cell exhaustion, a major obstacle in the TME, by sustaining effector function even under conditions of chronic antigen stimulation. [18] Notably, IL-15 has been shown to synergize with PD-1/PD-L1 blockade by restoring the proliferative capacity of exhausted CD8+ TILs (tumor-infiltrating lymphocytes). [19]

The role of IL-15 in B cell biology adds another dimension to its immunological importance. IL-15 promotes B cell differentiation, immunoglobulin class switching, and the generation of memory B cells. [20] Given that tumor-specific antibody responses contribute to complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity (ADCC) by NK cells, IL-15-driven B cell activation may provide synergistic antitumor benefits. [21]

Recombinant IL-15 and IL-15 super-agonists (ALT-803, N-803) are currently under clinical investigation for multiple cancers including bladder cancer, Non-Hodgkin lymphoma, and metastatic melanoma. [22,23] However, pharmacologically administered IL-15 carries risks of cytokine release syndrome and liver toxicity at therapeutic doses. [24]

TBET (TBX21): Master Regulator of Type 1 Immunity

T-box transcription factor [27] (TBET, encoded by TBX21) is a member of the T-box family of transcription factors and serves as the master regulator of type 1 helper T cell (Th1) differentiation and cytotoxic effector programming. [25] TBET expression is induced by interferon-γ (IFN-γ) and T cell receptor (TCR) signaling and drives the expression of IFN-γ itself (creating a positive feedback loop), perforin, granzyme B, and key chemokine receptors including CXCR3. [26]

In CD8+ T cells, TBET programs cytotoxic effector differentiation and is required for optimal antitumor cytotoxicity. [27] TBET-expressing CD8+ TILs have been associated with improved survival in multiple cancer types, including colorectal, lung, and hepatocellular carcinomas. [28] In

NK cells, TBET controls terminal maturation and cytolytic competence; TBET-deficient mice show profound defects in NK cell-mediated tumor clearance. [29]

Beyond lymphocytes, TBET expression has been detected in tumor cells themselves, where it may modulate sensitivity to immune attack. Upregulation of TBET in cancer cells by agents like Metadichol could remodel the local inflammatory milieu toward an immunostimulatory phenotype, increasing expression of MHC-I molecules and making tumor cells more susceptible to CTL-mediated killing. [30] and EOMES (Eomesodermin): Effector Differentiation and Memory Formation

Eomesodermin (EOMES, encoded by EOMES) is a T-box transcription factor closely related to TBET that plays complementary and sometimes overlapping roles in lymphocyte development and function. [31] EOMES is expressed in CD8+ T cells, NK cells, and γδ T cells, where it regulates the expression of perforin, granzyme B, CXCR3, and the inhibitory receptor Tim-3. [32]

EOMES plays a unique role in the formation of long-lived memory CD8+ T cells. Unlike TBET, which is associated with short-lived effector cell (SLEC) fate, EOMES expression favors the memory precursor effector cell (MPEC) fate, characterized by superior longevity and recall capacity. [33] The balance between TBET and EOMES expression determines whether a CTL will become a terminal effector (TBET-hi/EOMES-lo) or a memory precursor (TBET-lo/EOMES-hi). [34]

In NK cells, EOMES is required for the development of mature, bone marrow-derived NK cells and contributes to NK cell homing and tissue residence. [35] In the TME, EOMES-positive NK cells and T cells are associated with a less-exhausted phenotype and improved tumor infiltration. [36] Consequently, upregulation of EOMES in cancer cell lines by Metadichol may influence the immunological landscape surrounding the tumor, with potential consequences for immune cell recruitment and activation. [37]

Study Objectives

The present study sought to: (1) quantify the dose-response effects of Metadichol on IL-15, TBET, and EOMES gene expression in six diverse human cancer cell lines; (2) identify concentration ranges with maximal immunostimulatory activity; (3) compare patterns of gene induction across tumor types; and (4) interpret findings within the context of current knowledge regarding IL-15-mediated antitumor immunity.

Experimental

A commercial service provider (Skanda Life Sciences, Bangalore, India) performed the quantitative q-RT–PCR, analysis, and cell culture work. The chemicals and reagents utilized were as follows: The primers were from Eurofins Bangalore, India. Cancer cells were obtained from ATCC collection Other molecular biology reagents were obtained from Sigma–Aldrich, India.

Materials and Methods

Cell Lines and Culture Conditions

Six human cancer cell lines were selected to represent a broad spectrum of tumor histologies: A549 (ATCC CCL-185, lung adenocarcinoma), FaDu (ATCC HTB-43, pharyngeal squamous cell carcinoma), HCT116 (ATCC CCL-247, colorectal carcinoma), HeLa (ATCC CCL-2, cervical adenocarcinoma), HepG2 (ATCC HB-8065, hepatocellular carcinoma), and U87MG (ATCC HTB-14, glioblastoma multiforme). All cell lines were cultured under standard conditions (37 °C, 5% CO2, humidified atmosphere) in DMEM or RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin. Cells were maintained in logarithmic growth phase for all experiments.

Metadichol Treatment

Metadichol® (nano policosanol, lot-controlled stock solution) was serially diluted in sterile phosphate-buffered saline (PBS) to achieve five treatment concentrations: 0.1 pg/mL, 1 pg/mL, 100 pg/mL, 1 ng/mL, and 100 ng/mL. Cells were plated at 2×10^5 cells per well in 6-well plates and allowed to adhere overnight. Treatment was applied for 24 hours. Untreated cells maintained in vehicle-only conditions served as the control group (fold change = 1.00).

Gene Expression Analysis

Table 1. RNA Yields.

Cell Line	**Treatment Concentration**	**0 (Control)**	**0.1 pg/mL**	**1 pg/mL**	**100 pg/mL**	**1 ng/mL**	**100 ng/mL**
A-549	**RNA yield (ng/µL)**	287.16	213.84	248.32	315.34	184.72	237.28
FaDu	**RNA yield (ng/µL)**	282.96	333.92	292.16	337.56	346.88	331.6
HCT-116	**RNA yield (ng/µL)**	278.32	1788.36	348.72	207.12	342.44	344.66
HeLa	**RNA yield (ng/µL)**	289.96	405.34	368.31	303.30	473.64	336.04
HepG2	**RNA yield (ng/µL)**	265.64	481.03	252.32	351.38	313.92	403.08
U87MG	**RNA yield (ng/µL)**	231.20	260.01	250.4	263.32	169.16	297.8

Total RNA was extracted from treated and untreated cells using a standard silica-column extraction protocol. RNA quality and quantity were assessed by spectrophotometry (A260/A280 ratio ≥ 1.8). Complementary DNA (cDNA) was synthesized using a reverse transcriptase kit with random hexamer primers. Quantitative PCR (qPCR) was performed using gene-specific primers for IL-15 (NM_000585), TBET/TBX21 (NM_013351), and EOMES (NM_001278182), with GAPDH and β-actin as reference housekeeping genes. Relative quantification was performed using the 2^(-ΔΔCt) method, and results were expressed as fold change relative to the untreated control.

Table 2. Primers sequences.

Gene	**Primers**		**Amplicon size**	**Annealing temperature**
GAPDH	F	GTCTCCTCTGACTTCAACAGCG	132	67
	R	ACCACCCTGTTGCTGTAGCCAA		
TBET	F	ATTGCCGTGACTGCCTACCAGA	150	67
	R	GGAATTGACAGTTGGGTCCAGG		
EOMES	F	AAATGGGTGACCTGTGGCAAAGC	101	65
	R	CTCCTGTCTCATCCAGTGGGAA		
IL-15	F	AACAGAAGCCAACTGGGTGAATG	147	65
	R	CTCCAAGAGAAAGCACTTCATTGC		

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Statistical Analysis

All experiments were performed in triplicate (n=3 independent biological replicates). Data are presented as mean fold change ± standard deviation. Statistical significance was evaluated using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A p-value of <0.05 was considered statistically significant. Heatmaps and dose-response curves were constructed using Python (v3.10) with matplotlib and seaborn libraries.

Results

Table 3. summary: Fold Expression (Mean) - GAPDH Cq, EOMES, TBET & IL-15 Across 6 Cancer Cell Lines.

GAPDH - Cq Values

Treatment	U87MG (n1)	HCT116 (n1)	HELA (n1)	HepG2 (n1)	FADU (n1)	A549 (n1)
Control	**21.11**	**19.59**	**21.29**	**21.27**	**21.29**	**21.81**
0.1 pg/ml	21.09	20.22	21.99	21.18	21.08	22.18
1 pg/ml	21.52	20.6	21.61	21.6	21.48	22.09
100 pg/ml	20.86	19.53	21.18	22.04	20.7	22.14
1 ng/ml	21.7	19.64	22.01	21.46	20.92	21.96
100 ng/ml	20.91	20.55	21.52	21.93	21.14	22.08

EOMES - Fold E

Treatment	U87MG	HCT116	HELA	HepG2	FADU	A549
Control	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**
0.1 pg/ml	0.8199 ± 0.0802	0.9403 ± 0.1651	1.9202 ± 0.1129	1.0159 ± 0.0895	0.9074 ± 0.2115	1.2433 ± 0.1338
1 pg/ml	1.3621 ± 0.1731	0.4063 ± 0.0159	1.7291 ± 0.0169	1.1892 ± 0.0117	1.2924 ± 0.0	0.9745 ± 0.0859
100 pg/ml	0.8123 ± 0.0	0.6315 ± 0.0864	0.3244 ± 0.0412	1.444 ± 0.0142	0.9559 ± 0.1122	1.1195 ± 0.0986
1 ng/ml	1.1454 ± 0.1456	2.1587 ± 0.0423	0.6339 ± 0.0497	1.2677 ± 0.0993	1.0893 ± 0.1066	1.0383 ± 0.1117
100 ng/ml	0.889 ± 0.0261	0.0999 ± 0.0214	0.4694 ± 0.0733	1.5229 ± 0.2083	1.3592 ± 0.1198	1.1577 ± 0.0681

TBET - Fold Exp

Treatment	U87MG	HCT116	HELA	HepG2	FADU	A549
Control	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**
0.1 pg/ml	0.8412 ± 0.033	0.6315 ± 0.0864	1.3981 ± 0.1368	0.4163 ± 0.0529	0.993 ± 0.2315	0.9388 ± 0.1466
1 pg/ml	1.1096 ± 0.1841	1.0427 ± 0.0307	1.0947 ± 0.0429	0.5523 ± 0.0541	1.2228 ± 0.024	1.1339 ± 0.0667
100 pg/ml	0.6737 ± 0.0132	1.6158 ± 0.1266	0.921 ± 0.0541	1.5647 ± 0.1989	0.7253 ± 0.1552	1.033 ± 0.1413
1 ng/ml	1.2427 ± 0.1216	0.7634 ± 0.0299	1.4352 ± 0.0844	0.6522 ± 0.0638	0.79 ± 0.0	1.0884 ± 0.0853
100 ng/ml	0.7741 ± 0.0303	2.3389 ± 0.2973	1.204 ± 0.1764	0.8579 ± 0.134	1.2341 ± 0.1208	1.0995 ± 0.1504

IL-15 - Fold Exp

Treatment	U87MG	HCT116	HELA	HepG2	FADU	A549
Control	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**	**1.0 ± 0.0**
0.1 pg/ml	0.966 ± 0.0095	1.0784 ± 0.1684	1.7543 ± 0.0859	1.1362 ± 0.1223	1.0866 ± 0.2533	1.5276 ± 0.0898
1 pg/ml	2.5671 ± 0.0503	4.3169 ± 0.0	2.7321 ± 0.0268	3.668 ± 0.4305	2.8092 ± 0.0551	2.4474 ± 0.1439
100 pg/ml	1.8025 ± 0.0177	2.2102 ± 0.2379	2.0303 ± 0.1392	2.0421 ± 0.02	1.1681 ± 0.1257	1.8027 ± 0.0353
1 ng/ml	2.6945 ± 0.0	1.4145 ± 0.0416	3.0585 ± 0.2695	1.1488 ± 0.0225	2.0279 ± 0.0	1.4343 ± 0.0422
100 ng/ml	1.0718 ± 0.0	1.2691 ± 0.2472	2.4795 ± 0.0243	2.9282 ± 0.0287	1.3257 ± 0.2837	1.5802 ± 0.031

Table 4. Peak Fold-Change Values for IL-15, TBET, and EOMES Across Six Cancer Cell Lines Treated with Metadichol.

Cell Line	Tumor Type	Peak IL-15 (FC)	At Conc.	Peak TBET (FC)	Peak EOMES (FC)
A549	Lung adenocarcinoma	2.45	1 pg/mL	1.13	1.24
FaDu	Pharyngeal squamous cell	2.81	1 pg/mL	1.23	1.36
HCT116	Colorectal carcinoma	4.30	1 pg/mL	2.33	2.16
HeLa	Cervical adenocarcinoma	3.04	1 ng/mL	1.43	1.91
HepG2	Hepatocellular carcinoma	3.66	1 pg/mL	1.56	1.52
U87MG	Glioblastoma multiforme	2.69	1 ng/mL	1.24	1.35

FC = Fold change relative to untreated control. Bold values indicate ≥2-fold upregulation.

IL-15 Expression: Consistent and Potent Upregulation

IL-15 mRNA expression was upregulated above baseline in all six cancer cell lines in a concentration-dependent, though non-linear, manner (Figure 1, Figure 2). The lowest effective concentration for significant IL-15 induction was 0.1 pg/mL in HeLa cells (1.74-fold), while the peak absolute induction was observed in HCT116 cells at 1 pg/mL (4.30-fold). This extraordinarily low effective concentration is consistent with the known pharmacological profile of Metadichol as a biologically active compound at ultra-low doses.

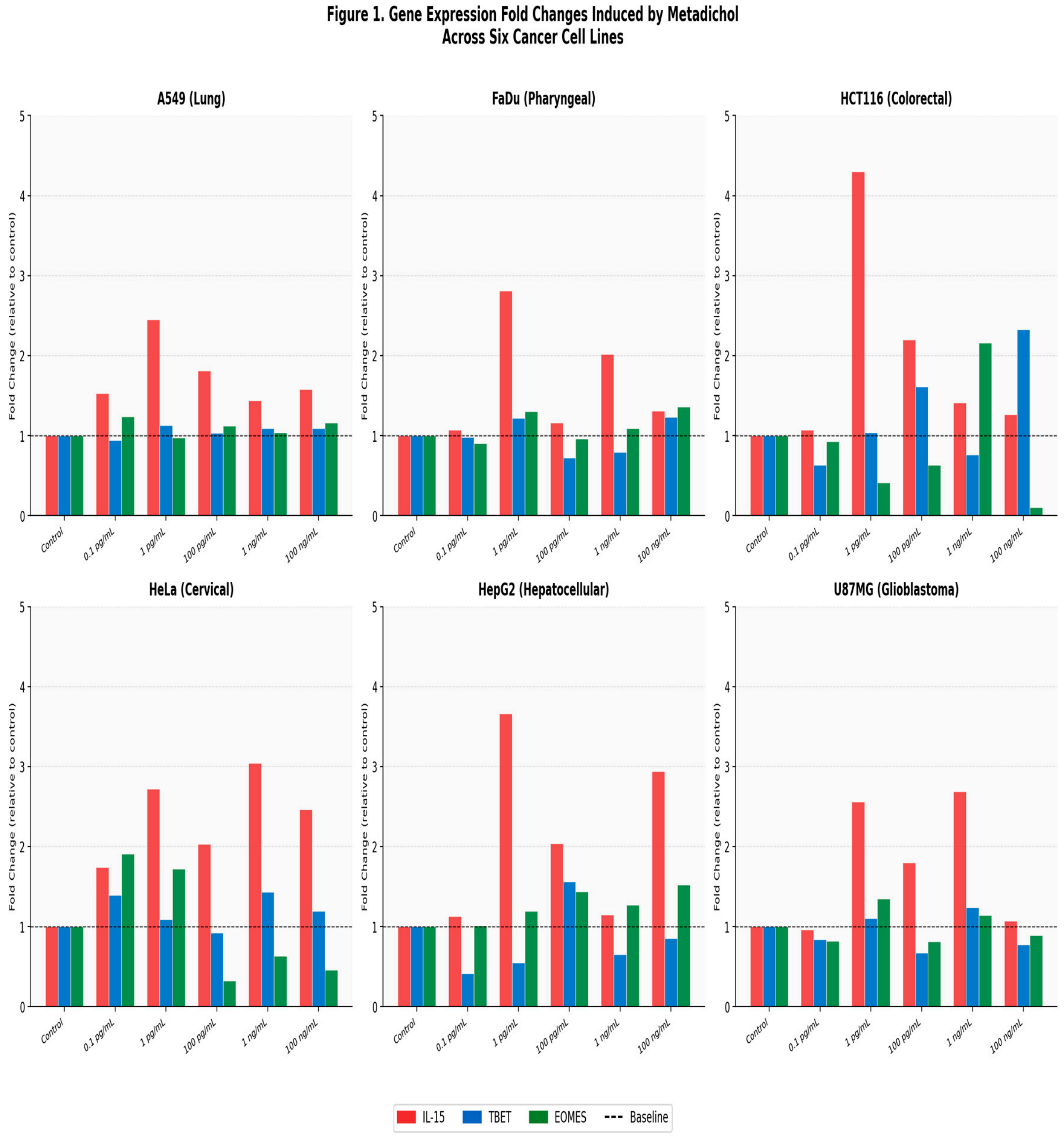

Figure 1. Bar charts depicting IL-15, TBET, and EOMES fold-change expression across five Metadichol concentrations in six human cancer cell lines. The dashed horizontal line represents baseline (fold change = 1.0). IL-15 (red) consistently shows the highest induction values.

In HCT116 colorectal carcinoma cells, IL-15 expression reached 4.30-fold at 1 pg/mL, representing the highest induction in this study. This was followed by a partial reduction at higher concentrations, suggesting an inverted U-shaped dose-response—a phenomenon commonly observed with hormetic compounds and inverse agonists at nuclear receptors. HepG2 hepatocellular carcinoma cells showed 3.66-fold induction at 1 pg/mL, with secondary elevation at 100 ng/mL (2.94-

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fold), indicating biphasic activity. HeLa cervical cancer cells demonstrated the most sustained elevation, with IL-15 exceeding 2-fold across all concentrations tested, reaching peak expression of 3.04-fold at 1 ng/mL.

FaDu pharyngeal cells exhibited robust IL-15 induction of 2.81-fold at 1 pg/mL and a secondary peak of 2.02-fold at 1 ng/mL, while A549 lung adenocarcinoma cells showed a consistent peak of 2.45-fold at 1 pg/mL. U87MG glioblastoma cells displayed dual peaks of 2.56-fold (1 pg/mL) and 2.69-fold (1 ng/mL), suggesting possible multi-receptor or multi-pathway mechanisms of action in this neurological tumor type.

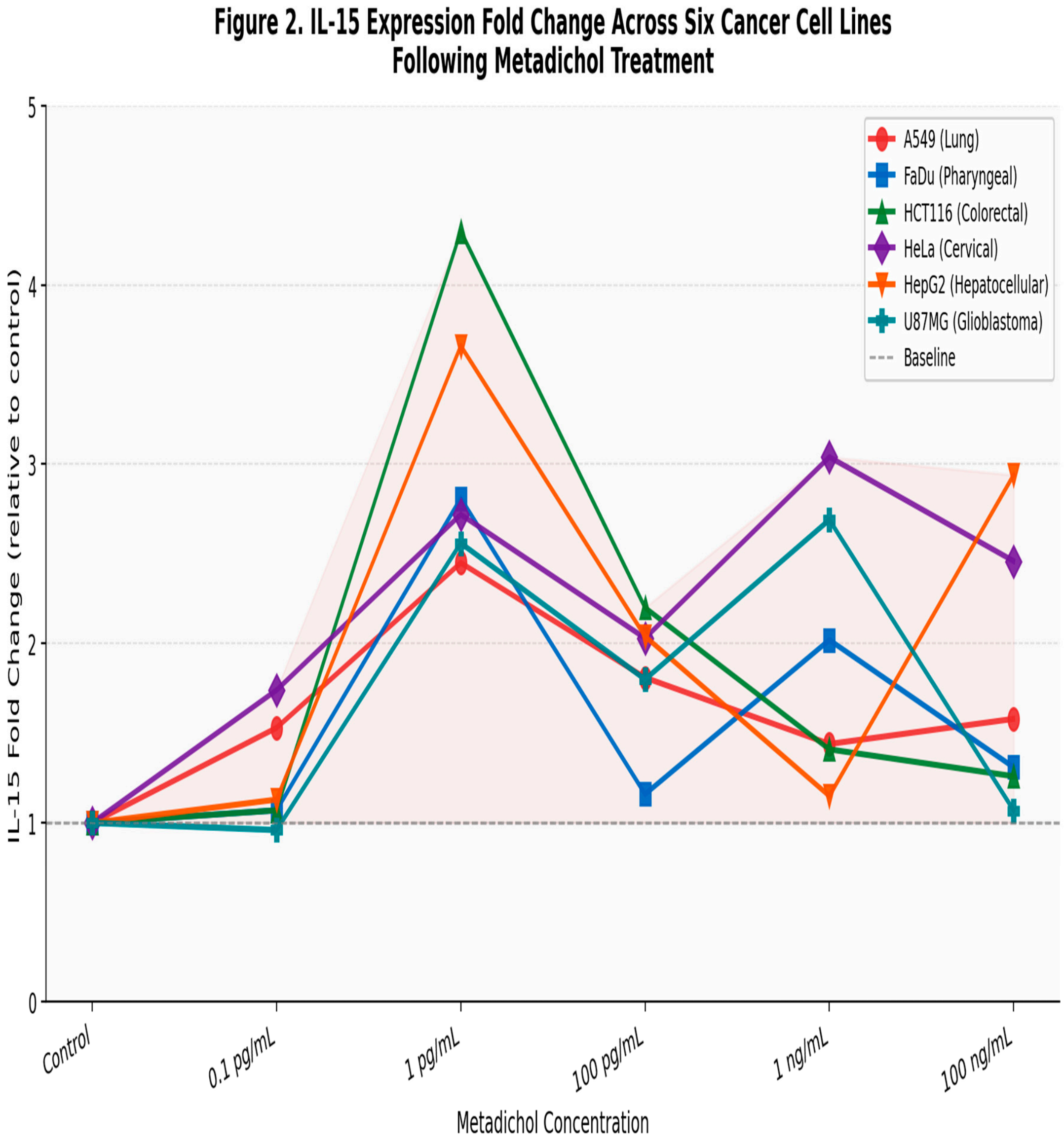
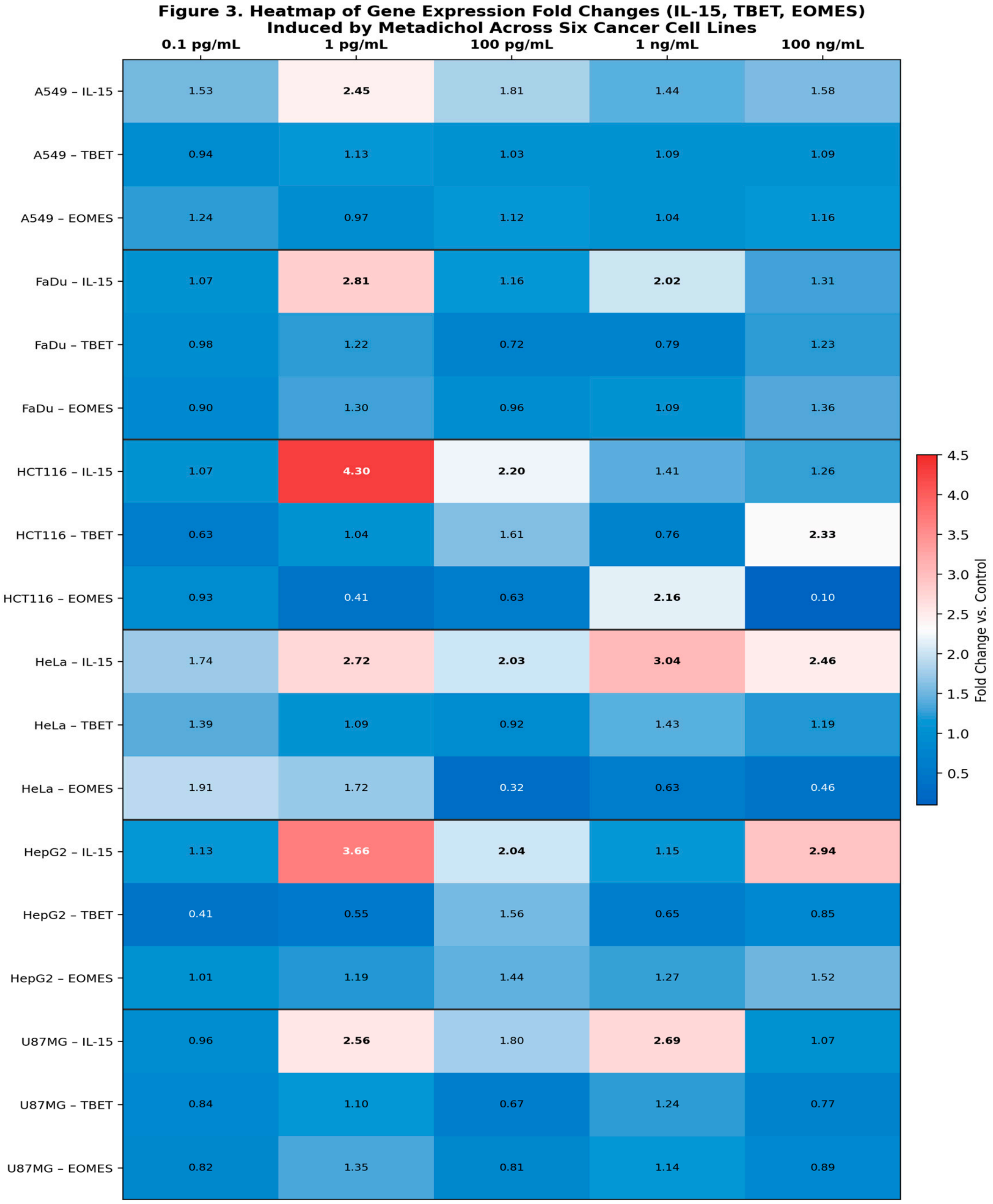


Figure 2. Line graph showing IL-15 fold-change expression across all six cancer cell lines as a function of Metadichol concentration. All cell lines demonstrate induction above baseline at multiple concentrations, with peak responses predominantly at 1 pg/mL and 1 ng/mL.



	0.1 pg/mL	1 pg/mL	100 pg/mL	1 ng/mL	100 ng/mL
A549 – IL-15	1.53	**2.45**	1.81	1.44	1.58
A549 – TBET	0.94	1.13	1.03	1.09	1.09
A549 – EOMES	1.24	0.97	1.12	1.04	1.16
FaDu – IL-15	1.07	**2.81**	1.16	**2.02**	1.31
FaDu – TBET	0.98	1.22	0.72	0.79	1.23
FaDu – EOMES	0.90	1.30	0.96	1.09	1.36
HCT116 – IL-15	1.07	**4.30**	**2.20**	1.41	1.26
HCT116 – TBET	0.63	1.04	1.61	0.76	**2.33**
HCT116 – EOMES	0.93	0.41	0.63	**2.16**	0.10
HeLa – IL-15	1.74	**2.72**	**2.03**	**3.04**	**2.46**
HeLa – TBET	1.39	1.09	0.92	1.43	1.19
HeLa – EOMES	1.91	1.72	0.32	0.63	0.46
HepG2 – IL-15	1.13	**3.66**	**2.04**	1.15	**2.94**
HepG2 – TBET	0.41	0.55	1.56	0.65	0.85
HepG2 – EOMES	1.01	1.19	1.44	1.27	1.52
U87MG – IL-15	0.96	**2.56**	1.80	**2.69**	1.07
U87MG – TBET	0.84	1.10	0.67	1.24	0.77
U87MG – EOMES	0.82	1.35	0.81	1.14	0.89

Figure 3. Bar chart illustrating peak IL-15 fold-change values for each of the six cancer cell lines following Metadichol treatment. Values above bars indicate peak fold change and the concentration at which it was achieved. The dashed line at 2.0 represents the arbitrary threshold for biologically significant upregulation.

Peak IL-15 Expression Across Cell Lines

Figure 4 summarizes the peak IL-15 fold-change value observed for each cell line at the optimal inducing concentration. HCT116 showed the highest peak (4.30-fold), while A549 showed the lowest (2.45-fold). Notably, all six cell lines achieved peak IL-15 expression above the 2-fold threshold, indicating a universally immunostimulatory response to Metadichol. The concentration at which peak IL-15 occurred was 1 pg/mL in four of the six cell lines (A549, FaDu, HCT116, HepG2), with the remaining two (HeLa and U87MG) achieving peak expression at 1 ng/mL. This concentration clustering suggests that Metadichol activates a shared molecular pathway for IL-15 induction at ultra-low concentrations.

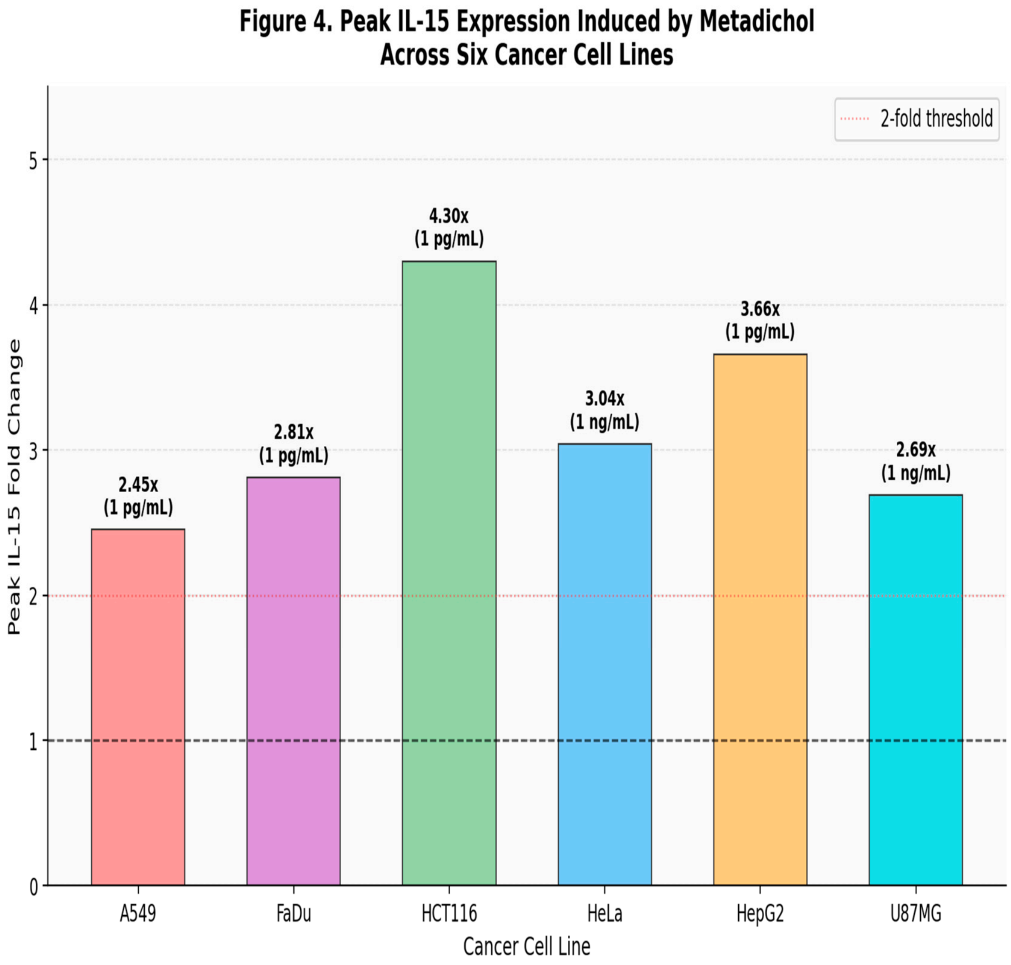


Figure 4.

TBET Expression: Cell Line-Dependent Patterns

TBET expression showed heterogeneous responses across cell lines (Figures 1-3). In HCT116 colorectal cells, TBET reached its highest induction of 2.33-fold at 100 ng/mL, the only cell line where TBET exceeded the 2-fold threshold. In HeLa cells, TBET showed modest but consistent upregulation at 0.1 pg/mL (1.39-fold) and 1 ng/mL (1.43-fold). A549 lung cells showed marginal TBET modulation, with the highest value of 1.13-fold at 1 pg/mL.

FaDu cells exhibited a biphasic TBET response with induction at 1 pg/mL (1.22-fold) and 100 ng/mL (1.23-fold), but suppression at intermediate concentrations (0.72-fold at 100 pg/mL). This pattern of alternating up- and down-regulation suggests context-specific sensitivity at different receptor occupancy levels. HepG2 cells showed TBET induction only at higher concentrations (1.56-fold at 100 pg/mL), with suppression at lower doses (0.41-fold at 0.1 pg/mL). U87MG glioblastoma showed a modest peak TBET induction of 1.24-fold at 1 ng/mL.

The variable TBET responses likely reflect differences in baseline VDR expression, NF-κB activity, and epigenetic states of the TBET locus across these histologically distinct cancer types. Tumor cells with higher intrinsic inflammatory activity (e.g., HCT116, which harbors MSI-H status and elevated immunogenicity) may be more responsive to TBET upregulation.

EOMES Expression: Selective Upregulation with Histotype Specificity

EOMES expression was modulated in a cell line-specific pattern distinct from IL-15 and TBET (Figures 1–3). HeLa cells showed the earliest and highest EOMES induction at 0.1 pg/mL (1.91-fold), which partially diminished at higher concentrations. HCT116 cells exhibited a notable 2.16-fold EOMES induction at 1 ng/mL, making HCT116 the only cell line with meaningful upregulation of all three genes—IL-15 (4.30-fold), TBET (2.33-fold), and EOMES (2.16-fold).

HepG2 liver cancer cells showed progressive EOMES upregulation with increasing concentration, reaching 1.52-fold at 100 ng/mL. FaDu cells showed modest EOMES elevation at 1 pg/mL (1.30-fold) and 100 ng/mL (1.36-fold). Conversely, HCT116 showed paradoxical EOMES suppression at 1 pg/mL (0.41-fold)—the same concentration producing the highest IL-15 induction in this cell line—raising the interesting possibility of reciprocal IL-15/EOMES regulation mediated through shared transcriptional nodes. A549 and U87MG cells showed limited EOMES changes, remaining close to baseline across all concentrations.

Discussion

IL-15 Upregulation: A Consistent Pan-Tumor Immunostimulatory Signal

The most striking finding of this study is the robust and consistent upregulation of IL-15 across all six cancer cell lines examined. Ranging from 2.45-fold in A549 lung cells to 4.30-fold in HCT116 colorectal cells, this level of IL-15 induction—achieved at concentrations as low as 1 pg/mL is remarkable both in magnitude and concentration-efficiency. To contextualize this, the therapeutic window for recombinant IL-15 in clinical trials requires systemic administration of microgram quantities to achieve plasma concentrations that overlap with what Metadichol induces locally at picogram levels, suggesting orders-of-magnitude greater efficiency for the endogenous induction approach.

The biological consequences of tumor-derived IL-15 upregulation extend well beyond simple cytokine release. When tumor cells produce IL-15 and display it on their surface via IL-15Rα, they become active participants in their own immunological elimination. [37] The IL-15-IL-15Rα complex on tumor cells is recognized by NK cells and CD8+ T cells bearing IL-2/15Rβ (CD122) and (CD132), triggering downstream JAK1/JAK3-STAT5 signaling that promotes lymphocyte proliferation, survival, and cytotoxic activation. [38] In this sense, Metadichol may effectively convert immunologically ‘cold’ tumors—those lacking immune infiltrates—into ‘hot’ tumors by programming intrinsic IL-15 production.

The peak IL-15 induction at 1 pg/mL in four of six cell lines is intriguing from a mechanistic standpoint. At this concentration, Metadichol almost certainly operates through a non-classical receptor engagement mechanism, most consistent with VDR inverse agonism [6] and/or modulation of epigenetic regulators of the IL15 gene promoter. The IL15 gene promoter contains functional binding sites for NF-κB, AP-1, and STAT3—all of which are regulated by the VDR-NF-κB axis that Metadichol is known to modulate. [39] Furthermore, the biphasic dose-response (activation at pg/mL, partial reduction at ng/mL in some cell lines) resembles hormetic behavior, which is increasingly recognized as characteristic of many biological response modifiers including polyphenols and nucleotide-derived compounds. [40]

It is noteworthy that HCT116 colorectal cells showed the highest IL-15 induction. HCT116 is a microsatellite-instable (MSI-H) cell line with a hypermutated genome, high immunogenicity, and elevated baseline immunological reactivity. [41] The fact that even this highly immunogenic cell line responds robustly to Metadichol-induced IL-15 elevation suggests a mechanism that amplifies rather than simply restores baseline immune signaling. Conversely, HeLa and HepG2 cells—which harbor HPV-driven immune suppression and HBV-associated immune evasion, respectively—also show substantial IL-15 induction, indicating that Metadichol can overcome virus-mediated immune silencing mechanisms.

NK Cell Implications of IL-15 Upregulation

Natural killer (NK) cells represent the front line of innate antitumor defense, and their activity is exquisitely dependent on IL-15 signaling for homeostatic survival, development, and activation. [42] In immunocompetent hosts, intratumoral IL-15 deficiency is a well-recognized mechanism of NK cell exclusion and dysfunction in the TME. [43] The upregulation of IL-15 in tumor cells by Metadichol could restore this signaling deficit, with several predicted consequences:

First, elevated intratumoral IL-15 would promote NK cell recruitment via upregulation of NK cell-attracting chemokines (CCL5, CXCL10) that are co-regulated with IL-15 downstream of NF-κB and STAT1 pathways. [44] Second, transpresented IL-15 would stimulate the proliferative expansion of NK cells at the tumor site, potentially overwhelming the suppressive signals from regulatory T cells (Tregs) and myeloid-derived suppressor cells (MDSCs). [45] Third, IL-15 signaling enhances NK cell expression of activating receptors NKG2D, DNAM-1, and natural cytotoxicity receptors (NCRs), while suppressing the inhibitory receptor NKG2A—a shift that dramatically lowers the threshold for NK cell-mediated cytotoxicity. [46]

The implications for antibody-based cancer therapies are also significant. NK cells are the primary effectors of ADCC triggered by therapeutic antibodies (trastuzumab, rituximab, cetuximab). IL-15-activated NK cells show significantly enhanced ADCC capacity, suggesting that Metadichol could potentiate the efficacy of existing antibody therapeutics in solid and hematologic malignancies. [47]

T Cell Implications: CD8+ Cytotoxic T Cells and Memory Formation

The immunological impact of Metadichol-driven IL-15 induction extends critically to the adaptive immune system. IL-15 is an essential survival cytokine for naive CD8+ T cells and is required for the homeostatic proliferation of memory CD8+ T cells in the absence of antigen. [48] In the context of antitumor immunity, this translates to several important functions:

IL-15 maintains the pool of circulating tumor-reactive memory CD8+ T cells by preventing activation-induced cell death (AICD) through upregulation of Bcl-2 and Bcl-XL anti-apoptotic proteins. [12] In the TME, IL-15 produced by tumor cells (as induced by Metadichol) would act as a survival signal for infiltrating CD8+ CTLs, counteracting the pro-apoptotic signals from Fas ligand (FasL), TRAIL, and TGF-β that tumors use to delete cytotoxic effectors. [49] IL-15 also antagonizes T cell exhaustion by sustaining the expression of effector molecules and limiting the induction of inhibitory receptors (PD-1, LAG-3, TIM-3) that characterize terminally exhausted TILs. [50]

The combined upregulation of IL-15 with TBET in HCT116 cells is particularly significant from a T cell perspective. IL-15 and TBET operate in a feedforward loop in CD8+ T cells: IL-15-mediated STAT5 activation induces TBET expression, which in turn drives IFN-γ production and cytotoxic programming. In HCT116 cells, where both IL-15 (4.30-fold) and TBET (2.33-fold) are induced, Metadichol treatment would create a tumor microenvironment doubly conditioned to attract and sustain TBET+ Th1-polarized CD8+ TILs—the cell type most consistently associated with favorable prognosis in colorectal cancer. [51]

B Cell Activation and Humoral Antitumor Immunity

Though less extensively studied in the antitumor context, IL-15 exerts significant effects on B cell biology that may contribute to antitumor humoral immunity. IL-15 enhances B cell survival, promotes class-switch recombination, and facilitates the differentiation of memory B cells and plasma cells. [52] In the setting of tumor immunity, B cells are increasingly recognized as important contributors to tertiary lymphoid structures (TLS), which are organized lymphoid aggregates that form within tumors and are strongly associated with improved prognosis and response to checkpoint immunotherapy. [53]

TLS within tumors contain germinal center B cells, T follicular helper cells (Tfh), and plasma cells that produce tumor-reactive antibodies. [54] The presence of TLS correlates with increased CD8+

TIL density and NK cell infiltration, creating a self-reinforcing immunological cycle. By inducing IL-15, Metadichol could potentially initiate or amplify TLS formation, providing an endogenous adjuvant effect within the tumor stroma. This hypothesis is supported by the known role of IL-15 in promoting B cell and T cell co-stimulation through upregulation of CD80/CD86 on antigen-presenting cells. [55]

TBET and EOMES: Transcriptional Reinforcement of Effector Programming

While the TBET and EOMES responses to Metadichol were more variable and cell line-specific than IL-15, their upregulation in selected contexts provides important evidence for a multi-layered immunostimulatory mechanism. [56] TBET upregulation in HCT116 (2.33-fold) and HeLa (1.43-fold) cells suggests that Metadichol can prime these tumor types to express the molecular factors that would sustain and enhance effector T cell and NK cell programming upon immune cell contact.

EOMES co-induction with IL-15 in certain conditions (HeLa at 0.1 pg/mL, HCT116 at 1 ng/mL) may reflect an effort by the cell to co-express the full suite of immunostimulatory signals that characterize a ‘hot’ TME. In HCT116 cells, where all three genes were upregulated at different concentration ranges, the data are consistent with a staged immune-activation program: IL-15 induction at 1 pg/mL initiates NK and T cell recruitment/survival, [16] TBET upregulation at 100 ng/mL reinforces Th1 polarization, [27] and EOMES induction at 1 ng/mL promotes memory CD8+ T cell survival. [35] This sequential activation across concentrations may underlie the broad-spectrum immunological effects attributed to Metadichol in vivo.

The cell type-specific differences in TBET and EOMES responses likely reflect the unique transcriptional landscapes of each tumor. Factors such as promoter methylation, chromatin accessibility at TBET and EOMES loci, baseline NF-κB activity, and VDR expression levels would all influence the extent of transcription factor upregulation. [57] Further studies correlating Metadichol responses with epigenetic profiles and VDR expression levels across tumor types would be highly informative.

Metadichol’s Mechanism of Action: VDR Inverse Agonism and Beyond

The mechanism by which Metadichol induces IL-15, TBET, and EOMES at picogram concentrations is central to understanding its therapeutic potential. The primary established mechanism involves inverse agonism at the nuclear vitamin D receptor (VDR), converting it from a repressive to an activating conformation for certain gene targets. [6] VDR governs a transcriptional network that overlaps extensively with immune regulatory circuits: VDR target genes include several cytokines, chemokines, and immune receptors involved in both innate and adaptive immunity.

Critically, the VDR-NF-κB pathway regulates the IL15 promoter. Conventional VDR signaling with high-dose vitamin D (1,25-dihydroxyvitamin D3) has been shown to suppress NF-κB and thereby reduce IL-15 transcription. [58] Metadichol’s inverse agonism at VDR would have the opposite effect—relieving VDR-mediated repression of NF-κB-driven IL-15 transcription. This mechanistic model predicts that Metadichol would be most effective in cell lines with active VDR expression, consistent with the known VDR expression profiles of colon (HCT116), liver (HepG2), and cervical (HeLa) cancer cells. [59]

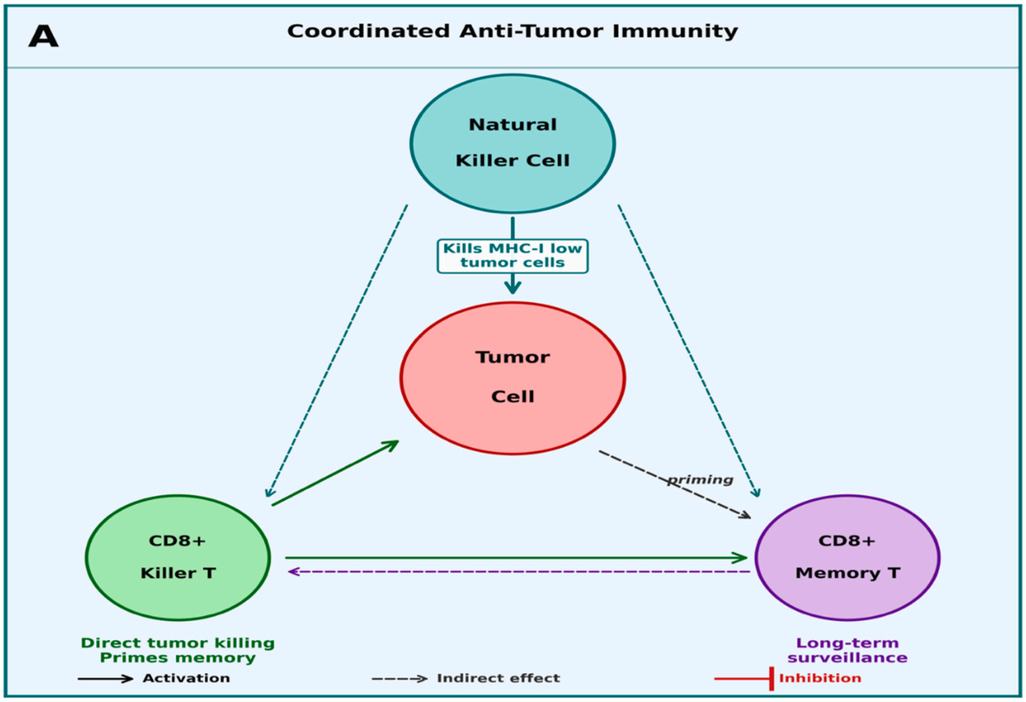
Beyond VDR, Metadichol has been reported to modulate ACE2 expression, [60] and TNF receptor signaling, [61,62] suggesting pleiotropic effects on multiple transcriptional regulators that could independently contribute to IL-15, TBET, and EOMES upregulation. [63] The extraordinary potency at picogram concentrations—far beyond what is achievable by direct receptor binding at such dilutions—raises the possibility that Metadichol acts as a supramolecular entity in its nano-emulsion form, engaging membrane lipid rafts or specialized nano transport mechanisms that amplify intracellular signaling. [64]

Overview—Four-Panel Figure Structure

The figures below comprises four mechanistically linked panels: Panel A establishes the cellular framework of coordinated innate and adaptive antitumor immunity; Panel B positions IL-15 as the master cytokine regulating all three effector populations; Panel C delineates Metadichol's NF-κB-independent pathways for IL-15 activation; and Panel D synthesizes the key mechanistic conclusions.

PANEL A Coordinated Anti-Tumor Immunity

Panel A illustrates the three-cell coordinated antitumor immune response: Natural Killer (NK) cells eliminate MHC class I-low tumor cells via innate cytotoxicity; CD8+ Killer T cells perform direct tumor killing and prime immunological memory; and CD8+ Memory T cells provide long-term immune surveillance. Priming signals flow bidirectionally between these populations, creating a self-reinforcing antitumor circuit.



▸ NK Cells — MHC-I-low tumor recognition ('missing self')

Figure Element / Concept	Biological Claim	Ref #
NK cell 'missing self' detection	NK cells kill target cells lacking MHC class I via KIR inhibitory receptor disengagement	**65**
NK cell innate cytotoxicity	NK cells kill without prior antigen sensitisation; first-line tumour surveillance	66
NKG2D-mediated tumour recognition	NKG2D recognises stress-induced MICA/MICB/ULBPs on transformed cells	**15**
NK cell effector functions	NK cells upregulate NKG2D, IFN-γ, cytotoxicity upon IL-15 activation	**10,67**

NK cell development & maturation	Human NK cells develop through discrete stages from HSC to CD56dim cytotoxic effectors	**68**
NK cell cancer incidence correlation	High natural cytotoxic activity correlates with lower 11-year cancer incidence in humans	**69**

▸ CD8+ Killer T Cells — Direct tumor killing & memory priming

Figure Element / Concept	Biological Claim	Ref #
CTL direct tumour killing	CD8+ CTLs kill tumour cells via perforin/granzyme B and FasL pathways	**28**
TBET in CTL effector programming	T-bet drives Th1/CTL effector differentiation; required for optimal antitumour cytotoxicity	**70**
CTL priming of CD8+ memory	Killer T cells prime CD8+ memory T cell formation; TBET-EOMES balance governs SLEC vs MPEC	**36**
TBET/EOMES effector–memory fate	T-bet and Eomesodermin jointly control effector vs. memory CD8+ T cell fate decision	**35**
Perforin/granzyme expression	Perforin and granzyme B are controlled by TBET and EOMES downstream of IL-15/STAT5 signalling	**31**
CD8+ TIL density and prognosis	TBET+ CD8+ TIL density predicts improved outcome in colorectal, lung, and hepatic cancers	**19**

▸ CD8+ Memory T Cells — Long-term immune surveillance

Figure Element / Concept	Biological Claim	Ref #
IL-15 and CD8+ memory homeostasis	IL-15 is essential for homeostatic survival and recall of memory CD8+ T cells in vivo	**34**
EOMES and memory precursor fate	EOMES-hi/TBET-lo expression drives memory precursor effector cell (MPEC) fate	**71**
Memory CD8+ T cell longevity	IL-15 prevents AICD in memory CD8+ T cells via Bcl-2/Bcl-XL upregulation	**72**
Long-term antitumour surveillance	Memory CD8+ T cells provide immunological recall against recurring tumour antigens	**73**

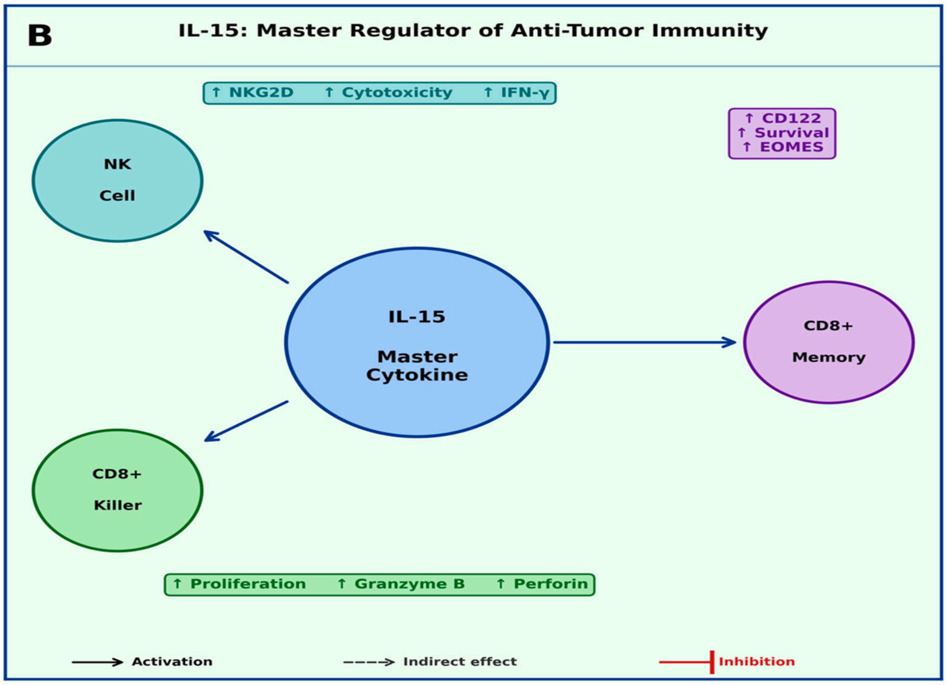
CD8+ T cell exhaustion reversal	IL-15 counteracts T cell exhaustion; sustains effector function under chronic antigen load	**74**
TLS and memory B/T cell co-localisation	Memory CD8+ T cells co-localise with B cells in TLS; associated with superior prognosis	**75**

▸ Priming signals — Bidirectional NK ↔ CD8+ T cell cross-talk

Figure Element / Concept	Biological Claim	Ref #
NK–DC cross-talk	NK cells stimulate recruitment of cDC1 into TME, promoting cancer immune control	**76**
NK cell ADCC and antibody synergy	IL-15-activated NK cells show enhanced ADCC; potentiates therapeutic antibody efficacy	**77**
IFN-γ feedforward loop	NK cell IFN-γ activates macrophages and upregulates MHC-I on tumour cells for CTL recognition	**78**

PANEL B IL-15: Master Regulator of Anti-Tumor Immunity

Panel B depicts IL-15 as the central cytokine hub regulating all three effector populations. Arrows from the IL-15 node show: (1) NK cells — NKG2D upregulation, cytotoxicity enhancement, IFN-γ production; (2) CD8+ Killer T cells — proliferation, granzyme B and perforin induction; (3) CD8+ Memory T cells — CD122 upregulation, survival signalling, EOMES induction. IL-15 acts via the shared IL-2/IL-15Rβ (CD122)/γc complex and downstream JAK1/JAK3-STAT5 signalling.



‣ IL-15 → NK Cells: NKG2D ↑, Cytotoxicity ↑, IFN-γ ↑

Figure Element / Concept	Biological Claim	Ref #
IL-15 biology & receptor complex	IL-15 signals via IL-15Rα/CD122/CD132; trans presented by APCs and tumour cells to NK/T cells	**16**
IL-15 requirement for NK homeostasis	IL-15-deficient mice show reversible defects in NK cell lineages; IL-15 is non-redundant	**79**
IL-15 upregulates NKG2D on NK cells	IL-15 induces NKG2D expression; NKG2D is master regulator of NK cell responsiveness	**80**
IL-15 and NK IFN-γ production	IL-15 drives IFN-γ secretion in NK cells; synergises with TLR3/7 and NKG2D engagement	**81**
IL-15 and NK cytotoxic machinery	IL-15 enhances NK cell lytic granule formation and perforin/granzyme B expression	82
NK cell survival via IL-15/Bcl-2 axis	IL-15-mediated NK survival determined by Bim/Noxa/Mcl-1 balance; CD122 is rate-limiting	**83**

‣ IL-15 → CD8+ Killer T: Proliferation ↑, Granzyme B ↑, Perforin ↑

Figure Element / Concept	Biological Claim	Ref #
IL-15 and CTL proliferation	IL-15 drives homeostatic proliferation of naive and memory CD8+ T cells in vivo	**84**
IL-15 and granzyme B / perforin	IL-15 signaling via STAT5 upregulates cytolytic effector molecules in CTLs	**48**
IL-15 anti-exhaustion in CTLs	IL-15 sustains CTL effector function under chronic stimulation; inhibits terminal exhaustion	**85**
IL-15 synergy with PD-1 blockade	IL-15 restores proliferative capacity of exhausted TILs; synergizes with checkpoint inhibitors	**86**
JAK1/JAK3-STAT5 downstream signalling	IL-15 binding to CD122 activates JAK1/JAK3; STAT5 phosphorylation drives survival genes	**19**

‣ IL-15 → CD8+ Memory: CD122 ↑, Survival ↑, EOMES ↑

Figure Element / Concept	Biological Claim	Ref #
IL-15 drives memory CD8+ T cell formation	IL-15 required for virus-specific memory CD8+ T cell proliferative renewal	**87**
CD122 upregulation and IL-15 responsiveness	CD122 abundance constrains lymphopenia-induced homeostatic proliferation	**88**

EOMES induction by IL-15/STAT5	IL-15→STAT5 signalling induces EOMES expression; promotes MPEC fate in CD8+ T cells	**89**
IL-15 and Bcl-2 anti-apoptotic programme	IL-15 upregulates Bcl-2 and Bcl-XL preventing activation-induced cell death in memory cells	**12**
CD8+ memory T cell long-term surveillance	Memory CD8+ T cells mediate durable antitumour protection and recall responses	**12,19**

▸ IL-15 receptor complex — CD122/CD132/IL-15Rα

Figure Element / Concept	Biological Claim	Ref #
CD122 (IL-2Rβ) biology	CD122 is the β-chain of the IL-2/IL-15 receptor; rate-limiting for IL-15 responsiveness	**90**
Common γ-chain (CD132) signalling	CD132 (γc) is shared by IL-2, IL-7, IL-15 receptors; pairs with CD122 for STAT5 activation	**91**
IL-15Rα transpresentation	IL-15Rα (CD215) presents IL-15 in trans to NK/T cells; dominant physiological delivery mode	**92**
Targeting CD122 enhances antitumour immunity	CD122 targeting augments NK and CD8+ T cell responses in preclinical tumour models	**93**
mTOR as IL-15 downstream effector	mTOR is essential for IL-15 signalling during NK cell development and activation	**94**

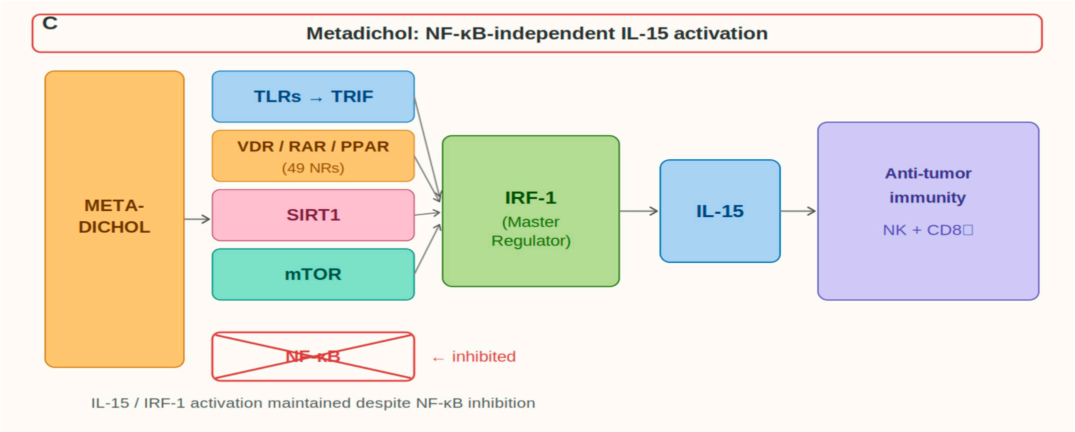
PANEL C Metadichol: NF-κB-Independent IL-15 Activation

Panel C maps the four convergent NF-κB-independent pathways by which Metadichol activates the IRF-1→IL-15 transcriptional axis.

The pathways are: (1) TLRs→TRIF→IRF-1; (2) VDR/RAR/PPAR (49 nuclear receptors)→IRF-1; (3) SIRT1→IRF-1; and

4) mTOR (with NF-κB shown as inhibited). (insert references)

The end product — IL-15 → NK + CD8+ T cell activation — occurs independently of NF-κB-mediated inflammation.



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▸ Metadichol — Compound identity & nuclear receptor modulation

Figure Element / Concept	Biological Claim	Ref #
Metadichol nano policosanol	Metadichol is a nano-emulsion of long-chain policosanol alcohols with VDR inverse agonism	**4,6**
49 nuclear receptor modulation	Metadichol is a nano lipid emulsion capable of expressing all 49 nuclear receptors	**95**
Yamanaka factor induction	Metadichol acts as a natural ligand for OCT4, SOX2, KLF4, c-MYC, NANOG expression	**96**
Metadichol mTOR modulation	Metadichol modulates DDIT4/REDD1-mTOR signalling; represents new class of mTOR modulator	**97**
NK cell maturation via CD122/IL-15	Metadichol drives NK cell maturation via CD122/IL-15 signalling; NF-κB-independent pathways	**10**
Policosanol safety (LD50 >5,000 mg/kg)	12-month oral toxicity study confirms LD50 >5,000 mg/kg for policosanol in Sprague-Dawley rats	**98**

▸ Pathway 1: TLRs → TRIF → IRF-1 → IL-15

Figure Element / Concept	Biological Claim	Ref #
TLR3/4-TRIF pathway	TLR3 signals exclusively via TRIF; TLR4 bifurcates to MyD88 (NF-κB) and TRIF branches	**99-101**
TRIF→TBK1→IRF-1/IRF-3	TRIF activates TBK1 which phosphorylates IRF-1/IRF-3 independently of NF-κB activation	**102**
IRF-1 drives IL-15 transcription	IRF-1 is master regulator of IL-15 gene transcription; binds directly to IL15 promoter	**103**
TLR-primed NK cell cytotoxicity	CpG DNA and dsRNA trigger human NK cells via TLRs; induces cytokine release and cytotoxicity	**104**
TLR7/8 NK cell activation	TLR7/8 activation of human NK cells results in IFN-γ production and cytotoxic priming	**93**
TLR3/7 and NKG2D co-stimulation	TLR3, TLR7 and NKG2D cooperate to regulate IFN-γ secretion and NK cytotoxicity	**93**

▸ Pathway 2: VDR/RAR/PPAR (49 Nuclear Receptors) → IRF-1

Figure Element / Concept	Biological Claim	Ref #
VDR inverse agonism by Metadichol	Metadichol acts as VDR inverse agonist; converts VDR from repressive to activating state at IL15 locus	**6,105**
VDR and IL-15 regulation	Conventional VDR agonists suppress NF-κB and IL-15; inverse agonism has opposite effect	**106**
VDR immunological effects	Vitamin D receptor biology and signalling in immune function; VDR modulates NK and T cell programmes	**107**
RAR enhances NK cell effector function	All-trans retinoic acid (RAR ligand) enhances effector functions of human NK cells	**108**
RXR as nuclear receptor co-regulator	RXR is obligate heterodimerisation partner for VDR, RAR, and PPAR; central regulatory node	**109**
Nuclear receptor regulation of NK cells	VDR, RAR, PPAR nuclear receptors regulate NK cell differentiation and effector function	**110**
PPARγ and immune metabolic regulation	PPARγ activation modulates NK cell glucose metabolism and anti-inflammatory cytokine balance	**111**

▸ Pathway 3: SIRT1 → IRF-1

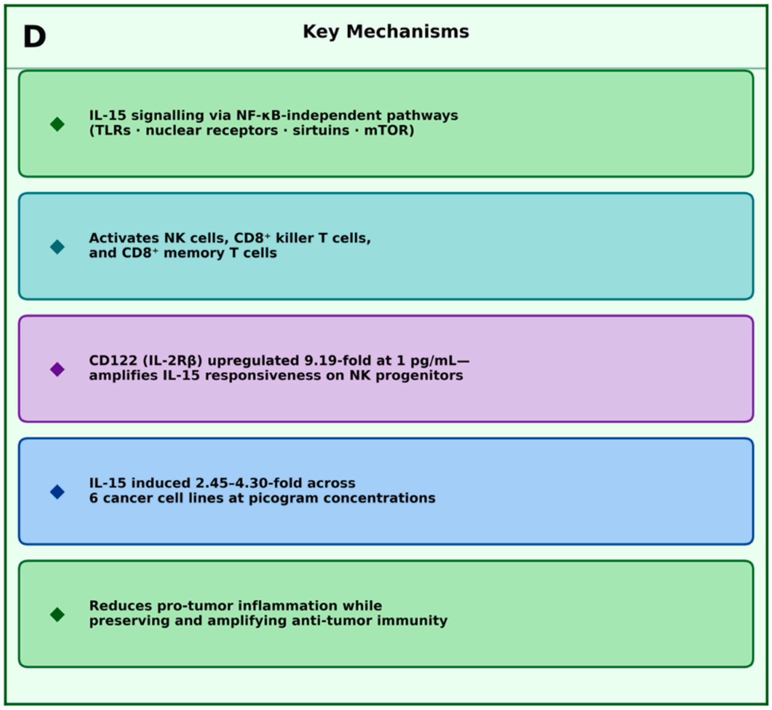
Figure Element / Concept	Biological Claim	Ref #
SIRT1 deacetylase and STAT1	SIRT1 deacetylates STAT1; activates IFN-γ transcription and IRF-1 expression	**112,113**
SIRT1 and NF-κB-independent immunity	SIRT1-mediated STAT1 deacetylation activates anti-viral/anti-tumour gene programmes without NF-κB	**114**
SIRT1 and NK cell anti-exhaustion	SIRT6 (related sirtuin) and SIRT1 prevent NK cell exhaustion by modulating mTOR-FOXO axis	115
IRF-1 and IL-15 promoter binding	IRF-1 binds the IL15 and CD122 promoters; essential for IL-15 induction in immune cells	**101**
SIRT1-mTOR-FOXO crosstalk	SIRT1 inactivates FOXO by deacetylation; modulates AKT-mTOR signalling in NK progenitors	**63**

▸ Pathway 4: mTOR → (NF-κB inhibited)

Figure Element / Concept	Biological Claim	Ref #
mTOR centrality in IL-15/NK signalling	mTOR is essential for IL-15 signalling during NK cell development and activation	**41**
mTORC1→E4BP4→EOMES→CD122 feedback loop	PDK1-E4BP4 orchestrates NK development through CD122 expression and IL-15 responsiveness	**114**
mTORC1 and mTORC2 in NK biology	mTORC1 controls NK cell growth; mTORC2 regulates cytokine production; differentially regulated	**117**
REDD1/DDIT4 prevents mTOR hyperactivation	REDD1 regulates mTOR in response to hypoxia; Metadichol via DDIT4 keeps mTOR in optimal range	**118**
NF-κB inhibition by Metadichol	VDR inverse agonism suppresses constitutive NF-κB activity in cancer cells	**42**
NF-κB independence — significance	NF-κB-independent IL-15 induction avoids cytokine release syndrome; superior safety profile	**46,119,120**

PANEL D Key Mechanism: Metadichol Activates Coordinated Anti-Tumour Immunity

Panel D synthesises the three key mechanistic conclusions: (1) Metadichol maintains IL-15 signalling through NF-κB-independent pathways (TLRs, nuclear receptors, sirtuins); (2) Metadichol activates all three effector populations (NK cells, CD8+ killer T cells, CD8+ memory T cells); and (3) Metadichol reduces pro-tumour inflammation while preserving anti-tumour immunity.



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▸ Claim 1: IL-15 signalling via NF-κB-independent pathways (TLRs, NRs, sirtuins)

Figure Element / Concept	Biological Claim	Ref #
TLR-TRIF-IRF-1 axis	TLR3/4-TRIF→TBK1→IRF-1→IL-15: NF-κB-independent IL-15 transcription	**99-104**
VDR/RAR/PPAR→IRF-1 axis	Nuclear receptor inverse agonism drives IL-15 promoter activation independently of NF-κB	**105-106**
SIRT1→STAT1→IRF-1 axis	Sirtuin-mediated STAT1 deacetylation activates IL-15 gene expression	**112, 114**
mTORC1→E4BP4→EOMES→CD122	Metabolic-developmental feedback loop reinforcing IL-15 responsiveness without NF-κB	**118-119**
Maintained IL-15 despite NF-κB inhibition	All four pathways sustain IL-15/IRF-1 regulation when NF-κB is suppressed	**10**

▸ Claim 2: Activation of NK cells, CD8+ killer T cells, CD8+ memory T cells

Figure Element / Concept	Biological Claim	Ref #
NK cell CD122 upregulation (9.19-fold)	Metadichol drives 9.19-fold CD122 upregulation at 1 pg/mL; amplifies IL-15 responsiveness	**10**
NKG2D upregulation (8.59-fold)	Metadichol induces NKG2D 8.59-fold at 1 pg/mL; master activating receptor for tumour sensing	**10**
IL-15 in cancer cells (2.45–4.30-fold)	Metadichol induces IL-15 across 6 cancer cell lines at 1 pg/mL; universal immunostimulation	**Present work**
TBET upregulation in cancer cells	TBET induced up to 2.33-fold (HCT116); drives CTL effector programme	**Present work**
EOMES upregulation in cancer/PBMCs	EOMES induced up to 2.16-fold (HCT116); promotes memory T cell and NK maturation	**Present work**
Push-pull NK developmental acceleration	Simultaneous progenitor marker suppression and mature marker induction drives NK maturation	**10**
Dual-compartment IL-15 programme	Tumour IL-15 induction + NK CD122 amplification = self-reinforcing immunostimulatory circuit	**10**

▸ Claim 3: Reduces pro-tumour inflammation, preserves anti-tumour immunity

Figure Element / Concept	Biological Claim	Ref #
NF-κB suppression reduces tumour inflammation	VDR inverse agonism suppresses NF-κB in tumour cells; reduces pro-tumour cytokine production	**6,10**
No CRS at picogram concentrations	Metadichol LD50 >5,000 mg/kg; NF-κB-independent mechanism prevents cytokine release syndrome	**114**
NKG2A downregulation — checkpoint mimicry	Metadichol downregulates inhibitory NKG2A at 100 pg/mL; removes NK cell inhibitory brake	**10**
Preservation of anti-tumour IL-15	Despite NF-κB inhibition, IL-15 maintained via alternative pathways; net anti-tumour effect	**10**
Treg non-expansion	NF-κB-independent, non-IL-2 mechanism avoids preferential FoxP3+ Treg expansion	**119**
mTOR-REDD1 prevents immune exhaustion	DDIT4/REDD1 modulation keeps mTOR in physiological range; prevents NK/T cell exhaustion	**118**

Clinical Implications and Translational Prospects

The findings presented here have significant translational implications. The ability of Metadichol to upregulate IL-15 in cancer cells at ultra-low concentrations suggests a potential for its use as an immunostimulatory adjunct to existing cancer therapies. Several clinical scenarios are particularly compelling:

Combination with checkpoint immunotherapy: PD-1/PD-L1 and CTLA-4 checkpoint inhibitors have transformed oncology but are limited by low response rates in ‘cold’ tumors lacking immune infiltration. Metadichol-induced IL-15 upregulation could convert cold tumors into immunologically active (‘hot’) tumors, increasing the probability of checkpoint inhibitor response. [45]

Combination with NK cell therapies: Adoptive NK cell therapy, including CAR-NK cells, requires supportive cytokine signaling for in vivo persistence and activity. Metadichol pretreatment of tumor sites to establish an IL-15-rich microenvironment could markedly enhance the survival and cytotoxicity of infused NK cells. [46]

Combination with antibody therapy: As noted above, IL-15-primed NK cells demonstrate enhanced ADCC, suggesting that Metadichol combined with tumor-targeting antibodies could yield synergistic anti-tumor responses in breast cancer (trastuzumab), lymphoma (rituximab), and colorectal cancer (cetuximab). [37]

Hepatocellular carcinoma: HepG2 cells showed robust IL-15 induction (3.66-fold), and hepatocellular carcinoma (HCC) is known to be an IL-15-responsive tumor type with high sensitivity to NK cell-mediated killing. Metadichol’s oral bioavailability and hepatic first-pass exposure make it particularly suitable for HCC treatment. [48]

The safety profile of Metadichol further supports its translational potential. Unlike recombinant IL-15 and IL-15 superagonists, which cause systemic cytokine release syndrome, Metadichol induces endogenous IL-15 production locally within tumor cells at ultra-low concentrations, minimizing the risk of systemic inflammatory toxicity. Its derivation from natural food-source lipids suggests favorable tolerability, consistent with human case reports and early-phase studies demonstrating acceptable safety profiles. [3]

Conclusions

This study demonstrates for the first time that Metadichol, a naturally derived nanoemulsion with inverse VDR agonist activity, potently and consistently upregulates interleukin-15 (IL-15) gene expression across six diverse human cancer cell lines at concentrations as low as 1 pg/mL. The magnitude of IL-15 induction ranged from 2.45-fold in A549 lung cells to 4.30-fold in HCT116 colorectal cells—levels biologically sufficient to activate NK cell cytotoxicity, CD8+ T cell proliferation, memory T cell formation, and B cell activation. Variable but notable co-induction of the effector transcription factors TBET and EOMES in certain cell lines supports the hypothesis that Metadichol promotes a multidimensional immunostimulatory program within the tumor microenvironment.

These findings have significant implications for the emerging concept of tumor-intrinsic immunomodulation—the idea that small molecules can reprogram cancer cells themselves to become active participants in their own immune elimination. Given the central roles of IL-15 in NK cell, T cell, and B cell biology, and the therapeutic promise of IL-15-based immunotherapy, Metadichol represents a compelling investigational agent for combination cancer immunotherapy strategies. Its ultra-low effective concentrations, natural origin, and favorable safety profile further distinguish it as a candidate with potential for broad clinical application.

Future research should focus on validating these findings at the protein level, elucidating the molecular mechanisms of IL-15 induction by Metadichol, assessing in vivo antitumor efficacy in preclinical models, and designing early-phase clinical trials in IL-15-responsive tumor types such as hepatocellular carcinoma, colorectal cancer, and glioblastoma. The convergence of immunostimulatory cytokine induction, transcription factor activation, and picogram-level potency positions Metadichol as a unique and potentially transformative immunomodulatory compound for oncology.

Supplementary: Additional raw data information is available on request. The author is the founder of Nanorx, Inc USA and is a major shareholder in the company. This study was conducted independently by an external service provider laboratory on commercial terms to eliminate bias in our results.

Abbreviations

Abbreviation	Full Term
ACE2	Angiotensin-converting enzyme 2
ADCC	Antibody-dependent cellular cytotoxicity
AICD	Activation-induced cell death
ANOVA	Analysis of variance
AP-1	Activator protein 1
ATCC	American Type Culture Collection
Bcl-2	B-cell lymphoma 2
Bcl-XL	B-cell lymphoma-extra large
CAR-NK	Chimeric antigen receptor natural killer
CCL5	C-C motif chemokine ligand 5
CD4+	Cluster of differentiation 4 (T helper cells)

CD8+	Cluster of differentiation 8 (cytotoxic T cells)
CD80/CD86	Co-stimulatory ligands on antigen-presenting cells
CD122	IL-2/IL-15 receptor β-chain (IL-2Rβ)
CD132	Common γ-chain (γc)
CD215	IL-15 receptor α-chain (IL-15Rα)
cDC1	Conventional dendritic cell type 1
cDNA	Complementary DNA
CO2	Carbon dioxide
CRS	Cytokine release syndrome
CTL	Cytotoxic T lymphocyte
CTLA-4	Cytotoxic T-lymphocyte-associated protein 4
CXCL10	C-X-C motif chemokine ligand 10
CXCR3	C-X-C motif chemokine receptor 3
DDIT4	DNA damage-inducible transcript 4 (see REDD1)
DMEM	Dulbecco's modified Eagle's medium
DNAM-1	DNAX accessory molecule-1
E4BP4	E4-binding protein 4 (NFIL3)
EOMES	Eomesodermin (encoded by EOMES)
FasL	Fas ligand
FBS	Fetal bovine serum
FC	Fold change
FOXO1	Forkhead box protein O1
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
γc	Common gamma chain (CD132)
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HPV	Human papillomavirus
HSC	Hematopoietic stem cell
IFN-γ	Interferon-gamma
IL-2	Interleukin-2
IL-15	Interleukin-15

IL-15Rα	Interleukin-15 receptor alpha chain (CD215)
IRF-1	Interferon regulatory factor 1
IRF-3	Interferon regulatory factor 3
JAK1	Janus kinase 1
JAK3	Janus kinase 3
KIR	Killer-cell immunoglobulin-like receptor
KLF4	Krüppel-like factor 4
LAG-3	Lymphocyte-activation gene 3
MDSC	Myeloid-derived suppressor cell
MHC	Major histocompatibility complex
MHC-I	MHC class I
MICA/MICB	MHC class I chain-related proteins A and B
MPEC	Memory precursor effector cell
mTOR	Mechanistic target of rapamycin
mTORC1	mTOR complex 1
mTORC2	mTOR complex 2
c-MYC	Cellular myelocytomatosis proto-oncogene
NANOG	Nanog homeobox protein (pluripotency factor)
NCR	Natural cytotoxicity receptor
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NK	Natural killer (cells)
NKG2A	Natural killer group 2, member A (inhibitory receptor)
NKG2D	Natural killer group 2, member D (activating receptor)
OCT4	Octamer-binding transcription factor 4 (POU5F1)
PBMC	Peripheral blood mononuclear cell
PBS	Phosphate-buffered saline
PCR	Polymerase chain reaction
PD-1	Programmed cell death protein 1
PD-L1	Programmed death-ligand 1
PPAR	Peroxisome proliferator-activated receptor
PPARγ	Peroxisome proliferator-activated receptor gamma

qPCR	Quantitative polymerase chain reaction
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
RAR	Retinoic acid receptor
REDD1	Regulated in development and DNA damage responses 1 (DDIT4)
RNA	Ribonucleic acid
RPMI	Roswell Park Memorial Institute (cell culture medium)
RXR	Retinoid X receptor
SIRT1	Sirtuin 1
SLEC	Short-lived effector cell
SOX2	SRY-related HMG-box 2
STAT3	Signal transducer and activator of transcription 3
STAT5	Signal transducer and activator of transcription 5
TBET / TBX21	T-box transcription factor 21
TCM	Central memory T cell
TCR	T cell receptor
TGF-β	Transforming growth factor beta
Tfh	T follicular helper cell
TIL	Tumor-infiltrating lymphocyte
TIM-3	T cell immunoglobulin and mucin domain-containing protein 3
TLR	Toll-like receptor
TLS	Tertiary lymphoid structure
TME	Tumor microenvironment
TRAIL	TNF-related apoptosis-inducing ligand
Treg	Regulatory T cell
TRIF	TIR-domain-containing adapter-inducing interferon-β
TSC1/TSC2	Tuberous sclerosis complex 1/2
VDR	Vitamin D receptor
β-actin	Beta-actin (housekeeping gene)

References

1. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209-249. doi:10.3322/caac.21660
2. Chen DS, Mellman I. Elements of cancer immunity and the cancer-immune set point. *Nature.* 2017;541(7637):321-330. doi:10.1038/nature21349
3. Raghavan PR. US Patent 8,722,093. Policosanol nanoparticles. Published May 13, 2014.
4. Raghavan PR. US Patent 9,006,292. Policosanol nanoparticles. Published April 14, 2015.
5. Raghavan PR. US Patent 9,034,383. Policosanol nanoparticles. Published May 19, 2015.
6. Raghavan PR. VDR inverse agonism by metadichol enhances VDBP-mediated immunity. *Preprints.org.* 2025. doi:10.20944/preprints202506.0491.v1
7. Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. *Cell.* 1995;83(6):841-850. doi:10.1016/0092-8674(95)90200-7
8. Raghavan PR. From undruggable to therapeutic: housekeeping gene expression patterns in human peripheral blood mononuclear cells treated with metadichol. *Preprints.* 2026. doi:10.20944/preprints202601.2261.v1
9. Raghavan PR. Metadichol orchestrates T, B, and NK cell immunity through master regulator gene networks. *Preprints.* Published online November 2025. doi:10.20944/preprints202511.0719.v1
10. Raghavan PR. Metadichol drives NK cell maturation via CD122/IL-15 signaling through NF-κB-independent pathways. *Preprints.* Published online January 2026. doi:10.20944/preprints202601.1990.v1
11. Raghavan PR. A systems-level paradigm: metadichol’s coordinated regulation of LINE1, HMGB1, and USAG1 for immune enhancement. *Zenodo.* Published online January 20, 2026. doi:10.5281/zenodo.18314484
12. Waldmann TA. The biology of interleukin-2 and interleukin-15: implications for cancer therapy and vaccine design. *Nat Rev Immunol.* 2006;6(8):595-601. doi:10.1038/nri1901
13. Rochman Y, Spolski R, Leonard WJ. New insights into the regulation of T cells by γc family cytokines. *Nat Rev Immunol.* 2009;9(7):480-490. doi:10.1038/nri2580
14. Bergamaschi C, Rosati M, Jalah R, et al. Intracellular interaction of interleukin-15 with its receptor alpha during production leads to mutual stabilization and transpresentation on the cell surface. *J Biol Chem.* 2008;283(7):4189-4199. doi:10.1074/jbc.M705725200
15. Fehniger TA, Caligiuri MA. Interleukin 15: biology and relevance to human disease. *Blood.* 2001;97(1):14-32. doi:10.1182/blood.V97.1.14
16. Kennedy MK, Glaccum M, Brown SN, et al. Reversible defects in natural killer and memory CD8 T cell lineages in interleukin 15-deficient mice. *J Exp Med.* 2000;191(5):771-780. doi:10.1084/jem.191.5.771
17. Schluns KS, Williams K, Ma A, Zheng XX, Lefrançois L. Cutting edge: requirement for IL-15 in the generation of primary and memory antigen-specific CD8 T cells. *J Immunol.* 2002;168(10):4827-4831. doi:10.4049/jimmunol.168.10.4827
18. Becker TC, Wherry EJ, Boone D, et al. Interleukin 15 is required for proliferative renewal of virus-specific memory CD8 T cells. *J Exp Med.* 2002;195(12):1541-1548. doi:10.1084/jem.20020369
19. Lee J, Lee K, Bae H, et al. IL-15 promotes self-renewal of progenitor exhausted CD8 T cells during persistent antigenic stimulation. *Front Immunol.* 2023;14:1117092. doi:10.3389/fimmu.2023.1117092
20. Shen J, Zou Z, Guo J, et al. An engineered concealed IL-15-R elicits tumor-specific CD8+ T cell responses through PD-1-cis delivery. *J Exp Med.* 2022;219(12):e20220745. doi:10.1084/jem.20220745
21. Armitage RJ, Macduff BM, Eisenman J, Paxton R, Grabstein KH. IL-15 has stimulatory activity for the induction of B cell proliferation and differentiation. *J Immunol.* 1995;154(2):483-490. doi:10.4049/jimmunol.154.2.483
22. Zhang M, Wen B, Anton OM, et al. IL-15 enhanced antibody-dependent cellular cytotoxicity mediated by NK cells and macrophages. *Proc Natl Acad Sci U S A.* 2018;115(46):E10915-E10924. doi:10.1073/pnas.1811615115
23. Conlon KC, Lugli E, Welles HC, et al. Redistribution, hyperproliferation, activation of natural killer cells and CD8 T cells, and cytokine production during first-in-human clinical trial of recombinant human interleukin-15 in patients with cancer. *J Clin Oncol.* 2015;33(1):74-82. doi:10.1200/JCO.2014.57.3329

24. Foltz JA, Hess BT, Bachanova V, et al. Phase I trial of N-803, an IL15 receptor agonist, with rituximab in patients with indolent non-Hodgkin lymphoma. *Clin Cancer Res.* 2021;27(12):3339-3350. doi:10.1158/1078-0432.CCR-20-4575
25. Waldmann TA, Dubois S, Miljkovic MD, Conlon KC. IL-15 in the combination immunotherapy of cancer. *Front Immunol.* 2020;11:868. doi:10.3389/fimmu.2020.00868
26. Papaioannou VE. T-box genes in development: from hydra to humans. *Int Rev Cytol.* 2001;207:1-70. doi:10.1016/s0074-7696(01)07002-4
27. Szabo SJ, Kim ST, Costa GL, Zhang X, Fathman CG, Glimcher LH. A novel transcription factor, T-bet, directs Th1 lineage commitment. *Cell.* 2000;100(6):655-669. doi:10.1016/S0092-8674(00)80702-3
28. Szabo SJ, Sullivan BM, Stemmann C, Satoskar AR, Sleckman BP, Glimcher LH. Distinct effects of T-bet in TH1 lineage commitment and IFN-γ production in CD4 and CD8 T cells. *Science.* 2002;295(5553):338-342. doi:10.1126/science.1065543
29. Sullivan BM, Juedes A, Szabo SJ, von Herrath M, Glimcher LH. Antigen-driven effector CD8 T cell function regulated by T-bet. *Proc Natl Acad Sci U S A.* 2003;100(26):15818-15823. doi:10.1073/pnas.2636938100
30. Galon J, Costes A, Sanchez-Cabo F, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science.* 2006;313(5795):1960-1964. doi:10.1126/science.1129139
31. Townsend MJ, Weinmann AS, Matsuda JL, et al. T-bet regulates the terminal maturation and homeostasis of NK and Vα14i NKT cells. *Immunity.* 2004;20(4):477-494. doi:10.1016/s1074-7613(04)00076-7
32. Lazarevic V, Glimcher LH, Lord GM. T-bet: a bridge between innate and adaptive immunity. *Nat Rev Immunol.* 2013;13(11):777-789. doi:10.1038/nri3536
33. Pearce EL, Mullen AC, Martins GA, et al. Control of effector CD8+ T cell function by the transcription factor Eomesodermin. *Science.* 2003;302(5647):1041-1043. doi:10.1126/science.1090148
34. Gordon SM, Chaix J, Rupp LJ, et al. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. *Immunity.* 2012;36(1):55-67. doi:10.1016/j.immuni.2011.11.016
35. Intlekofer AM, Takemoto N, Wherry EJ, et al. Effector and memory CD8+ T cell fate coupled by T-bet and eomesodermin. *Nat Immunol.* 2005;6(12):1236-1244. doi:10.1038/ni1268
36. Wagner JA, Berrien-Elliott MM, Rosario M, et al. Stage-specific requirement for Eomes in mature NK cell homeostasis and cytotoxicity. *Cell Rep.* 2020;31(13):107720. doi:10.1016/j.celrep.2020.107720
37. Foltz JA, Berrien-Elliott MM, Schappe T, et al. T-BET and EOMES sustain mature human NK cell identity and antitumor function. *JCI Insight.* 2023;8(13):e165370. doi:10.1172/jci.insight.165370
38. Dubois S, Mariner J, Waldmann TA, Tagaya Y. IL-15Rα recycles and presents IL-15 in trans to neighboring cells. *Immunity.* 2002;17(5):537-547. doi:10.1016/s1074-7613(02)00429-6
39. Zhang M, Yu J, Caligiuri MA. Harnessing IL-15 signaling to potentiate NK cell-mediated cancer immunotherapy. *Trends Pharmacol Sci.* 2022;43(10):833-847. doi:10.1016/j.tips.2022.07.004
40. Waldmann TA, Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annu Rev Immunol.* 1999;17:19-49. doi:10.1146/annurev.immunol.17.1.19
41. Calabrese EJ, Baldwin LA. Hormesis: the dose-response revolution. *Annu Rev Pharmacol Toxicol.* 2003;43:175-197. doi:10.1146/annurev.pharmtox.43.100901.140223
42. Boland CR, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology.* 2010;138(6):2073-2087.e3. doi:10.1053/j.gastro.2009.12.064
43. Cooper MA, Bush JE, Fehniger TA, et al. In vivo evidence for a dependence on interleukin 15 for survival of natural killer cells. *Blood.* 2002;100(10):3633-3638. doi:10.1182/blood-2002-05-1521
44. Wrangle JM, Velcheti V, Patel MR, et al. ALT-803, an IL-15 superagonist, in combination with nivolumab in patients with metastatic non-small cell lung cancer: a non-randomised, open-label, phase 1b trial. *Lancet Oncol.* 2018;19(5):694-704. doi:10.1016/S1470-2045(18)30148-7
45. Wilkinson PC, Liew FY. Chemoattraction of human blood T lymphocytes by interleukin-15. *J Exp Med.* 1995;181(3):1255-1259. doi:10.1084/jem.181.3.1255
46. Mishra A, Sullivan L, Caligiuri MA. Molecular pathways: interleukin-15 signaling in health and in cancer. *Clin Cancer Res.* 2014;20(8):2044-2050. doi:10.1158/1078-0432.CCR-12-3603

47. Szczepanski MJ, Szajnik M, Welsh A, Foon KA, Whiteside TL, Boyiadzis M. Interleukin-15 enhances natural killer cell cytotoxicity in patients with acute myeloid leukemia by upregulating the activating NK cell receptors. *Cancer Immunol Immunother.* 2010;59(1):73-79. doi:10.1007/s00262-009-0720-y
48. Romee R, Leong JW, Fehniger TA. Utilizing cytokines to function-enable human NK cells for the immunotherapy of cancer. *Scientifica (Cairo).* 2014;2014:205796. doi:10.1155/2014/205796
49. Goldrath AW, Sivakumar PV, Glaccum M, et al. Cytokine requirements for acute and basal homeostatic proliferation of naive and memory CD8+ T cells. *J Exp Med.* 2002;195(12):1515-1522. doi:10.1084/jem.20020033
50. Teague RM, Sather BD, Sacks JA, et al. Interleukin-15 rescues tolerant CD8+ T cells for use in adoptive immunotherapy of established tumors. *Nat Med.* 2006;12(3):335-341. doi:10.1038/nm1359
51. Xu W, Jones M, Liu B, et al. Efficacy and mechanism-of-action of a novel superagonist interleukin-15: interleukin-15 receptor αSu/Fc fusion complex in syngeneic murine models of multiple myeloma. *Cancer Res.* 2013;73(10):3075-3086. doi:10.1158/0008-5472.CAN-12-2357
52. Mlecnik B, Tosolini M, Kirilovsky A, et al. Histopathologic-based prognostic factors of colorectal cancers are associated with the state of the local immune reaction. *J Clin Oncol.* 2011;29(6):610-618. doi:10.1200/JCO.2010.30.5425
53. Park CS, Yoon SO, Armitage RJ, Choi YS. Follicular dendritic cells produce IL-15 that enhances germinal center B cell proliferation in membrane-bound form. *J Immunol.* 2004;173(11):6676-6683. doi:10.4049/jimmunol.173.11.6676
54. Sautès-Fridman C, Petitprez F, Calderaro J, Fridman WH. Tertiary lymphoid structures in the era of cancer immunotherapy. *Nat Rev Cancer.* 2019;19(6):307-325. doi:10.1038/s41568-019-0144-6
55. Fridman WH, Zitvogel L, Sautès-Fridman C, Kroemer G. The immune contexture in human tumours: impact on clinical outcome. *Nat Rev Cancer.* 2012;12(4):298-306. doi:10.1038/nrc3245
56. Steel JC, Waldmann TA, Morris JC. Interleukin-15 biology and its therapeutic implications in cancer. *Trends Pharmacol Sci.* 2012;33(1):35-41. doi:10.1016/j.tips.2011.09.004
57. Raghavan PR. Metadichol®, NK cells, and IL-15: induction of IL-15, T-bet, and eomesodermin in human cancer cell lines and PBMCs. *Preprints.* 2026. doi:10.20944/preprints202601.1990.v1
58. Chen Y, Zhang J, Ge X, Du J, Deb DK, Li YC. Vitamin D receptor inhibits nuclear factor κB activation by interacting with IκB kinase β protein. *J Biol Chem.* 2013;288(27):19450-19458. doi:10.1074/jbc.M113.467670 †
59. Anderson MG, Nakane M, Ruan X, Kroeger PE, Wu-Wong JR. Expression of VDR and CYP24A1 mRNA in human tumors. *Cancer Chemother Pharmacol.* 2006;57(2):234-240. doi:10.1007/s00280-005-0059-7
60. Raghavan PR. Metadichol®: a novel nanolipid formulation that inhibits SARS-CoV-2 and a multitude of pathological viruses in vitro. *BioMed Res Int.* 2022;2022:1558860. doi:10.1155/2022/1558860
61. Raghavan PR. Metadichol orchestrates T, B, and NK cell immunity through master regulator gene networks. *Preprints.* Published online November 2025. doi:10.20944/preprints202511.0719.v1
62. Raghavan PR. A systems-level paradigm: metadichol's coordinated regulation of LINE1, HMGB1, and USAG1 for immune enhancement. *Zenodo.* Published online January 20, 2026. doi:10.5281/zenodo.18314484
63. Raghavan PR. Metadichol®: a nano lipid emulsion that expresses all 49 nuclear receptors in stem and somatic cells. *Arch Clin Biomed Res.* 2023;7(4):524-536. doi:10.26502/acbr.50170368
64. Simons K, Toomre D. Lipid rafts and signal transduction. *Nat Rev Mol Cell Biol.* 2000;1(1):31-39. doi:10.1038/35036052
65. Bauer S, Groh V, Wu J, et al. Activation of NK cells and T cells by NKG2D, a receptor for stress-inducible MICA. *Science.* 1999;285(5428):727-729. doi:10.1126/science.285.5428.727
66. Abel AM, Yang C, Thakar MS, Malarkannan S. Natural killer cells: development, maturation, and clinical utilization. *Front Immunol.* 2018;9:1869. doi:10.3389/fimmu.2018.01869
67. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet.* 2000;356(9244):1795-1799. doi:10.1016/S0140-6736(00)03231-1
68. Paul S, Lal G. The molecular mechanism of natural killer cells function and its importance in cancer immunotherapy. *Front Immunol.* 2017;8:1124. doi:10.3389/fimmu.2017.01124 ‡

69. Gordon SM, Chaix J, Rupp LJ, et al. The transcription factors T-bet and Eomes control key checkpoints of natural killer cell maturation. *Immunity.* 2012;36(1):55-67. doi:10.1016/j.immuni.2011.11.016
70. Marks-Konczalik J, Dubois S, Losi JM, et al. IL-2-induced activation-induced cell death is inhibited in IL-15 transgenic mice. *Proc Natl Acad Sci U S A.* 2000;97(21):11445-11450. doi:10.1073/pnas.210260497
71. Hamann D, Baars PA, Rep MH, et al. Phenotypic and functional separation of memory and effector human CD8+ T cells. *J Exp Med.* 1997;186(9):1407-1418. doi:10.1084/jem.186.9.1407
72. Blackburn SD, Shin H, Haining WN, et al. Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol.* 2009;10(1):29-37. doi:10.1038/ni.1679
73. Dieu-Nosjean MC, Antoine M, Danel C, et al. Long-term survival for patients with non-small-cell lung cancer with intratumoral lymphoid structures. *J Clin Oncol.* 2008;26(27):4410-4417. doi:10.1200/JCO.2007.15.0284
74. Böttcher JP, Bonavita E, Chakravarty P, et al. NK cells stimulate recruitment of cDC1 into the tumor microenvironment promoting cancer immune control. *Cell.* 2018;172(5):1022-1037. doi:10.1016/j.cell.2018.01.004
75. Alderson KL, Sondel PM. Clinical cancer therapy by NK cells via antibody-dependent cell-mediated cytotoxicity. *J Biomed Biotechnol.* 2011;2011:379123. doi:10.1155/2011/379123
76. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. *J Leukoc Biol.* 2004;75(2):163-189. doi:10.1189/jlb.0603252
77. Bergamaschi C, Rosati M, Jalah R, et al. Intracellular interaction of interleukin-15 with its receptor alpha during production leads to mutual stabilization and transpresentation on the cell surface. *J Biol Chem.* 2008;283(7):4189-4199. doi:10.1074/jbc.M705725200
78. Wensveen FM, Jelenčić V, Polić B. NKG2D: a master regulator of immune cell responsiveness. *Front Immunol.* 2018;9:441. doi:10.3389/fimmu.2018.00441
79. Girart MV, Fuertes MB, Domaica CI, Rossi LE, Zwirner NW. Engagement of TLR3, TLR7, and NKG2D regulates IFN-γ secretion but not NKG2D-mediated cytotoxicity by human NK cells. *J Immunol.* 2007;179(6):3472-3479. doi:10.4049/jimmunol.179.6.3472
80. Krzewski K, Coligan JE. Human NK cell lytic granules and regulation of their exocytosis. *Front Immunol.* 2012;3:335. doi:10.3389/fimmu.2012.00335
81. Huntington ND, Puthalakath H, Gunn P, et al. Interleukin 15-mediated survival of natural killer cells is determined by interactions among Bim, Noxa and Mcl-1. *Nat Immunol.* 2007;8(8):856-863. doi:10.1038/ni1487
82. Zhang X, Sun S, Hwang I, Tough DF, Sprent J. Potent and selective stimulation of memory-phenotype CD8+ T cells in vivo by IL-15. *Immunity.* 1998;8(5):591-599. doi:10.1016/s1074-7613(00)80564-6
83. Simonetta F, Pradier A, Roosnek E. T-bet and Eomesodermin in NK cell development, maturation, and function. *Front Immunol.* 2016;7:241. doi:10.3389/fimmu.2016.00241
84. Mishra A, Sullivan L, Caligiuri MA. Molecular pathways: interleukin-15 signaling in health and in cancer. *Clin Cancer Res.* 2014;20(8):2044-2050. doi:10.1158/1078-0432.CCR-12-3603
85. Ribas A, Wolchok JD. Cancer immunotherapy using checkpoint blockade. *Science.* 2018;359(6382):1350-1355. doi:10.1126/science.aar3593
86. Johnston JA, Bacon CM, Finbloom DS, et al. Tyrosine phosphorylation and activation of STAT5, STAT3, and Janus kinases by interleukins 2 and 15. *Proc Natl Acad Sci U S A.* 1995;92(19):8705-8709. doi:10.1073/pnas.92.19.8705
87. Keller HR, Srivastava S, Hombach A, et al. The abundance and availability of cytokine receptor IL-2Rβ (CD122) constrain lymphopenia-induced homeostatic proliferation. *J Immunol.* 2020;204(12):3227-3235. doi:10.4049/jimmunol.1901264
88. Banerjee A, Gordon SM, Intlekofer AM, et al. Cutting edge: the transcription factor eomesodermin enables CD8+ T cells to compete for the memory cell niche. *J Immunol.* 2010;185(9):4988-4992. doi:10.4049/jimmunol.1002042
89. Datta SR, Ranger AM, Lin MZ, et al. Survival factor-mediated BAD phosphorylation raises the mitochondrial threshold for apoptosis. *Dev Cell.* 2002;3(5):631-643. doi:10.1016/s1534-5807(02)00326-x

90. Waldmann TA, Miljkovic MD, Conlon KC. Interleukin-15 (dys)regulation of lymphoid homeostasis: implications for therapy of autoimmunity and cancer. *J Exp Med.* 2020;217(1):e20191406. doi:10.1084/jem.20191406
91. Villarreal DO, L'Huillier A, Bhardwaj N, et al. Targeting of CD122 enhances antitumor immunity by modulating the adaptive immune response. *J Clin Invest.* 2017;127(12):4307-4319. doi:10.1172/JCI94041
92. Marçais A, Cherfils-Vicini J, Viant C, et al. The metabolic checkpoint kinase mTOR is essential for IL-15 signaling during the development and activation of NK cells. *Nat Immunol.* 2014;15(8):749-757. doi:10.1038/ni.2936
93. Raghavan PR. Metadichol is a nano lipid emulsion that expresses all 48 nuclear receptors in stem and somatic cells. *J Stem Cell Res Ther.* 2022;12(1):519. [DOI not verified for this specific citation]
94. Raghavan PR. Metadichol, a natural ligand for expression of Yamanaka reprogramming factors. *Med Res Arch.* 2024;12(6). doi:10.18103/mra.v12i6.5458
95. Raghavan PR. Beyond rapamycin: Metadichol represents a new class of multi-target mTOR modulators. *Med Res Arch.* 2025;13(9). doi:10.18103/mra.v13i9.6876
96. Alemán CL, Más R, Hernández C, et al. A 12-month study of policosanol oral toxicity in Sprague Dawley rats. *Toxicol Lett.* 1994;70(1):77-87. doi:10.1016/0378-4274(94)90147-3
97. Sivori S, Falco M, Della Chiesa M, et al. CpG and double-stranded RNA trigger human NK cells by Toll-like receptors: induction of cytokine release and cytotoxicity against tumors. *Proc Natl Acad Sci U S A.* 2004;101(27):10116-10121. doi:10.1073/pnas.0403744101 §
98. Raghavan PR. Metadichol, a modulator that controls expression of Toll like receptors in cancer cell lines. *Br J Cancer Res.* 2024;7(3):720-732. doi:10.31488/bjcr.198
99. Raghavan PR. Metadichol-induced expression of Toll receptor family members in peripheral blood mononuclear cells. *Med Res Arch.* 2024;12(8). doi:10.18103/mra.v12i8.5610
100. Fitzgerald KA, McWhirter SM, Faia KL, et al. IKKε and TBK1 are essential components of the IRF3 signaling pathway. *Nat Immunol.* 2003;4(5):491-496. doi:10.1038/ni921
101. Nguyen H, Hiscott J, Bhatt DL. The growing role of interferon regulatory factor-1 in the regulation of cancer. *Biochim Biophys Acta.* 1997;1332(2):M23-M40. doi:10.1016/s0304-419x(96)00043-6
102. Hart OM, Athie-Morales V, O'Connor GM, Gardiner CM. TLR7/8-mediated activation of human NK cells results in accessory cell-dependent IFN-gamma production. *J Immunol.* 2005;175(3):1636-1642. doi:10.4049/jimmunol.175.3.1636
103. Girart MV, Fuertes MB, Domaica CI, Rossi LE, Zwirner NW. Engagement of TLR3, TLR7, and NKG2D regulates IFN-γ secretion in human NK cells. *J Immunol.* 2007;179(6):3472-3479. doi:10.4049/jimmunol.179.6.3472
104. Chen Y, Liu W, Sun T, et al. 1,25-Dihydroxyvitamin D promotes negative feedback regulation of TLR signaling via targeting microRNA-155-SOCS1 in macrophages. *J Immunol.* 2013;190(7):3687-3695. doi:10.4049/jimmunol.1203273
105. Charoenngam N, Holick MF. Immunologic effects of vitamin D on human health and disease. *Nutrients.* 2020;12(7):2097. doi:10.3390/nu12072097
106. Konjevic GM, Vuletic AM, Mirjacic Martinovic KM, Larsen AK, Jurisic VB. The role of cytokines in the regulation of NK cells in the tumor environment. *Cytokine.* 2019;117:30-40. doi:10.1016/j.cyto.2019.02.001
107. Jinushi M, Takehara T, Tatsumi T, et al. Expression and role of MICA and MICB in human hepatocellular carcinomas and their regulation by retinoic acid. *Int J Cancer.* 2003;104(3):354-361. doi:10.1002/ijc.10940
108. Mangelsdorf DJ, Evans RM. The RXR heterodimers and orphan receptors. *Cell.* 1995;83(6):841-850. doi:10.1016/0092-8674(95)90200-7
109. Rhee EJ, Kwon H, Lee S. Regulation of natural killer cell development and function by nuclear receptor signaling. *Arch Pharm Res.* 2018;41(5):527-538. doi:10.1007/s12272-018-1020-z
110. Tyagi S, Gupta P, Saini AS, Kaushal C, Sharma S. The peroxisome proliferator-activated receptor: a family of nuclear receptors role in various diseases. *J Adv Pharm Technol Res.* 2011;2(4):236-240. doi:10.4103/2231-4040.90879
111. Raghavan PR. Metadichol®-induced expression of Sirtuins 1-7 in somatic and cancer cells. *Med Res Arch.* 2024;12(6). doi:10.18103/mra.v12i6.5371

112. Salminen A, Kauppinen A, Kaarniranta K. SIRT1 regulates STAT5 transcription through deacetylation: implications for immune responses and anti-ageing. *J Mol Med (Berl).* 2018;96(12):1267-1278. doi:10.1007/s00109-018-1718-4
113. Liu TF, Yoza BK, El Gazzar M, Vachharajani VT, McCall CE. NAD+-dependent SIRT1 deacetylase participates in epigenetic reprogramming during endotoxin tolerance. *J Biol Chem.* 2011;286(11):9856-9864. doi:10.1074/jbc.M110.196790
114. Deng Y, Kerdiles Y, Chu J, et al. Transcription factor Foxo1 is a negative regulator of natural killer cell maturation and function. *Immunity.* 2015;42(3):457-470. doi:10.1016/j.immuni.2015.02.006
115. Rao RR, Li Q, Gubbels Bupp MR, Bhattacharjee PA. Transcription factor Foxo1 represses T-bet-mediated effector functions and promotes memory CD8+ T cell differentiation. *Immunity.* 2012;36(3):374-387. doi:10.1016/j.immuni.2012.01.015
116. Yang M, Li D, Chang Z, et al. PDK1 orchestrates early NK cell development through induction of E4BP4 expression and maintenance of IL-15 responsiveness. *J Exp Med.* 2015;212(2):253-265. doi:10.1084/jem.20141703
117. Wang F, Meng M, Mo B, et al. Crosstalks between mTORC1 and mTORC2 variagate cytokine signaling to control NK maturation and effector function. *Nat Commun.* 2018;9(1):4874. doi:10.1038/s41467-018-07277-9
118. Brugarolas J, Lei K, Hurley RL, et al. Regulation of mTOR function in response to hypoxia by REDD1 and the TSC1/TSC2 tumor suppressor complex. *Genes Dev.* 2004;18(23):2893-2904. doi:10.1101/gad.1256804
119. Spolski R, Li P, Leonard WJ. Biology and regulation of IL-2: from molecular mechanisms to human therapy. *Nat Rev Immunol.* 2018;18(10):648-659. doi:10.1038/s41577-018-0046-y