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REVIEW

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GSK-J4: An H3K27 histone demethylase inhibitor, as a potential anti-cancer agent

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Abstract

Aberrant epigenetic modifications are emerging as potent drivers of tumor initiation and progression. The deregulation of H3K27me3 marks has shown to play an important role in cancer progression in several cancers. The H3K27me3 mark is associated with gene silencing. The reversible nature of these epigenetic aberrations makes them an important target for treating cancer. GSK-J4 is a histone demethylase inhibitor that inhibits the JMJD3/UTX enzyme, which results in the upregulation of H3K27me3 levels. In this review, the anti-cancer properties of GSK-J4 have been summarized, the various molecular pathways targeted, in-vivo studies, and drug combination studies in different cancer models. GSK-J4 targeted pathways like apoptosis, cell cycle, invasion, migration, DNA damage repair, metabolism, oxidative stress, stemness, etc. GSK-J4 is a promising candidate alone and in combination with other conventional anti-cancer drugs against different cancer types.

KEYWORDS
cancer, combinatorial studies, GSK-J4, histone modifications, In-vivo studies

Abbreviations: ADP, adenosine di-phosphate; ALDH+, aldehyde dehydrogenase positive; AML, acute myeloid leukemia; AR+, androgen receptor positive; ATF4, activating transcription factor 4; Bcl2, B-cell lymphoma 2; CCND-1, Cyclin D1; CCNU, 1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (ie, lomustine); CD24+, cluster of differentiation 24; CEMIP, cell migration inducing hyaluronidase; CRC, colorectal cancer; CRPC, castration-resistant prostate cancer; CTBP1, C-terminal binding protein 1; CXCL9, CXC motif chemokine ligand 9; DIPG, diffuse intrinsic pontine glioma; DNA, deoxyribonucleic acid; EMT, epithelial to mesenchymal transition; EPHB2, Ephrin type-B receptor 2; ER, endoplasmic reticulum; EZH2, enhancer of zeste homolog 2; FDA, food and drug administration; FEN1, flap structure-specific endonuclease 1; GRP78, glucose-regulated protein 78; GSC, glioma stem cells; H2-D1, histocompatibility 2, D region locus 1; HOX, homeobox genes; HSP90AA1, heat shock protein 90 alpha family class A member 1; JIB04, Jumonji histone demethylase inihibitor; JMJD3, Jumonji domain-containing protein-3; KDM, lysine demethylase; KDM4C, lysine demethylase 4C; KDM5B/5C, lysine demethylase5B/5C; KDM6A/6B, lysine demethylase 6A/6B; KRAS, kirsten rat sarcoma virus; LNCaP, lymph node metastasis of a Caucasian patient; LuAC, lung adenocarcinoma; MCM3, minichromosome maintenance complex component 3; MCM4, minichromosome maintenance complex component 4; MDA-MB-231, M.D. Anderson-Metastatic Breast 231; MHC, major histocompatibility complex; NF2, neurofibromatosis type 2; NOTCH, neurogenic locus notch homolog protein; p21CIP1, p21 cdk-interacting protein; PARP, Poly (ADP-ribose) polymerase; PCNA, Proliferating cell nuclear antigen; PKC-a, protein kinase C alpha; PLAUR, plasminogen activator, urokinase receptor; POLD1, DNA polymerase delta; PUMA, p53 upregulated modulator of apoptosis; RA, retinoic acid; RNA, ribonucleic Acid; SOX2, sex-determining region Y(SRY)-box transcription factor 2; SUMO, small ubiquitin-like modifier; TGFβ, transforming growth factor beta; TIC, tumor-initiating cells; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labelling; UTX, ubiquitously transcribed tetratricopeptide repeat, X chromosome; VP-16, Vepesid 16; XRCC1, X-ray repair cross-complementing protein 1.

1 | INTRODUCTION

When cells accumulate mutations that affect their ability to regulate the cell cycle and start dividing uncontrollably, forming tumors, that is when cancer develops. The disease is so dynamic that it has intrigued scientists worldwide to find a cure to eradicate it without affecting normal cell functions. The existing treatment strategies are either associated with several side effects or are ineffective once cancer has metastasized. In a report by GLOBOCAN, there were 19.3 million new cancer cases and 10 million deaths in 2020.[1] The cases are only going to increase year after year owing to the increase in globalization-associated risk factors. Moreover, metastasis or spread of cancer from the primary site to secondary tumor formation in distant body parts is responsible for 90% of cancer-associated deaths.[2] It is, therefore, essential to come up with treatment strategies that can target cancer cells specifically and, more importantly, prevent the process of cancer metastasis.

Epigenetic changes are being extensively studied as they have been established as a cancer hallmark. These changes include histone modifications, DNA methylation and non-coding RNAs. Their reversible nature makes them an ideal treatment option.[3] Histone

modifications such as acetylation, methylation, phosphorylation, ubiquitination and SUMOylation (Small ubiquitin-like modifier) occur at the N-terminal tail of histones.[4] Some other modifications include citrullination, ADP-ribosylation, deamination, formylation, O-GlcNAcylation, propionylation, butyrylation, crotonylation and proline isomerization at over 60 amino acid residues.[5] These modifications can regulate gene expression by altering the microstructure of DNA or proteins associated with chromatin structure.[6]

Histone methylations are responsible for several gene-activating or gene-repressing events by regulating the accessibility of transcription factors to target genes. A group of enzymes known as histone methyltransferases add methyl groups to the histone tails, whereas histone demethylases remove those methyl marks. Lysines and arginines are the most commonly methylated amino acids on the histone tails,[7] and methylations on histone H3 and H4 N-terminal tails are the most commonly studied.[8] Histone methylations on H3K9, H3K27 and H4K20 are associated with heterochromatin formation.[9] H3K4me3, H3K36me3, H3K79me3 and H3K4me1 are associated with gene expression.[10]

H3K27 methylation associated with heterochromatin formation is catalyzed by the enzyme EZH2 (Enhancer of zeste homolog 2). EZH2 is involved in stem cell proliferation and differentiation in adipocytes, keratinocytes and neurons.[11] The enzymes JMJD3 (Jumonji domain-containing protein-3) and UTX (Ubiquitously transcribed tetratricopeptide repeat, X chromosome) remove the di- and tri-methyl marks on H3K27. Aberrant H3K27 methylation is associated with cancer progression.[12] The expression of JMJD3 has been found to be upregulated in prostate cancer, and the expression is even higher in metastatic prostate cancer.[13] JMJD3 has also been found to be upregulated in cancers like glioblastomas, breast carcinoma, melanoma, renal cell carcinoma and Hodgkin's lymphoma.[14-17] The inhibition of JMJD3, either by knocking it down or using specific small molecule inhibitors, can be a promising cancer therapy approach.

GSK-J4 is a small molecule inhibitor of JMJD3/UTX. It has been effectively used as an anti-cancer agent against several types of cancers, including acute myeloid leukemia,[18] glioma,[19] breast cancer,[20] thyroid cancer,[21] lung cancer,[22] colorectal cancer,[23] prostate cancer,[24] neuroblastoma,[25] hemangiosarcoma[26] and ovarian cancer.[27] It has been shown to target different signaling molecules via inhibiting JMJD3, which gives GSK-J4 its anti-cancer properties. The in-vivo and in-vitro studies from pre-clinical data show that GSK-J4 has therapeutic potential in diseases ranging from cancer, inflammation, autoimmune diseases and infectious diseases.[28] In this review, the aim is to summarize the existing literature on the effect of GSK-J4, focusing on different cancer types, the in-vivo studies, and studies involving the combination of GSK-J4 with a conventional anti-cancer drug.

2 | MODE OF ACTION OF GSK-J4

GSK-J4 is an ethyl ester derivative of GSK-J1. It has higher cell permeability than GSK-J1 and is a pro-drug of GSK-J1. It is a dual inhibitor of JMJD3/KDM6B (Lysine demethylase 6B) and UTX/KDM6A (Lysine demethylase 6A).[29] Both of these enzymes catalyze the demethylation of H3K27me3. The co-crystal structure of GSK-J1 bound to the JMJD3 enzyme revealed interactions of GSK-J1 within the active site of the enzyme.[29] These methylases are dependent on Fe2+ and α-ketoglutarate as cofactors, and GSK-J1 is a competitive inhibitor of the two cofactors.[30] As a result, GSK-J4 is not a specific inhibitor of KDM6A/6B; it also inhibits other demethylases like KDM5B/5C and KDM4C.[30] Some studies show that GSK-J4 treatment increases the trimethylation of H3K27, while others reported no change in H3K27me3 levels after GSK-J4 treatment. GSK-J4 increased H3K27me3 levels in embryoid bodies,[31] ovarian cancer stem cells,[32] glioma cell lines,[19] primary acute myeloid leukemia (AML) cells,[18] and dendritic cells.[33] Only a minor accumulation of H3K27me3 was observed in the prostate cancer cell line R1-D567, and no accumulation was observed in R1-AD1 or CWR22Rv-1 prostate cancer cells[34] upon GSK-J4 treatment.

3 | GSK-J4 AS AN ANTI-CANCER DRUG

Several studies, both in-vitro and in-vivo, have shown GSK-J4 as an anti-cancer agent. GSK-J4 treatment results in an accumulation of H3K27me3 marks which in turn leads to tumor suppression (Figure 1). In this review, the concentration, molecular targets, and functional outcomes of GSK-J4 treatment in different cancer types have been summarized (Table 1). Studies that have conducted combinatorial studies in which GSK-J4 has been used to sensitize cancer cells either to radiation or to FDA-approved anti-cancer drugs has also been briefly summarized.

3.1 | In-vitro studies

3.1.1 | Blood cancer

Leukemia, lymphoma and myeloma are the three main types of blood cancers. 544 352 new cases of Non-Hodgkin Lymphoma, 474 519 new cases of leukemia, 176 404 new cases of multiple myeloma, and 83 087 new cases of Hodgkin lymphoma have been reported in 2020.[1] 711 840 deaths due to these cancers were reported in 2020.[1] GSK-J4 inhibits cell proliferation, induces apoptosis, inhibits colony formation and arrests the cell cycle (at G0/G1 phase) of the acute myeloid leukemia (AML) cell line, Kasumi-1, at a concentration range of 1.25-10 μM.[18] GSK-J4 downregulated the expression of *FEN1*, *HSP90AA1*, *MCM3*, *MCM4* and *PCNA*, which are known to regulate DNA replication and cell cycle.[18] It also abrogates the expression of several *HOX* genes like *HOXA5*, *HOXA7*, *HOXA9* and *HOXA11*, all of which are involved in cancer progression.[18] In the AML KG-1a cells, it reduces cell viability and induces cell cycle arrest by upregulating p21 and downregulating CyclinD1 and CyclinA2 at a dose range of 2-10 μM.[35] It also induced apoptosis by inducing cleaved caspase 9 and Bax and inhibiting the PKC-a/p-Bcl2 pathway.[35] Moreover, the ER-stress-related proteins, caspase-12, GRP78 (Glucose-regulated protein 78) and ATF4 (Activating transcription factor 4) were also

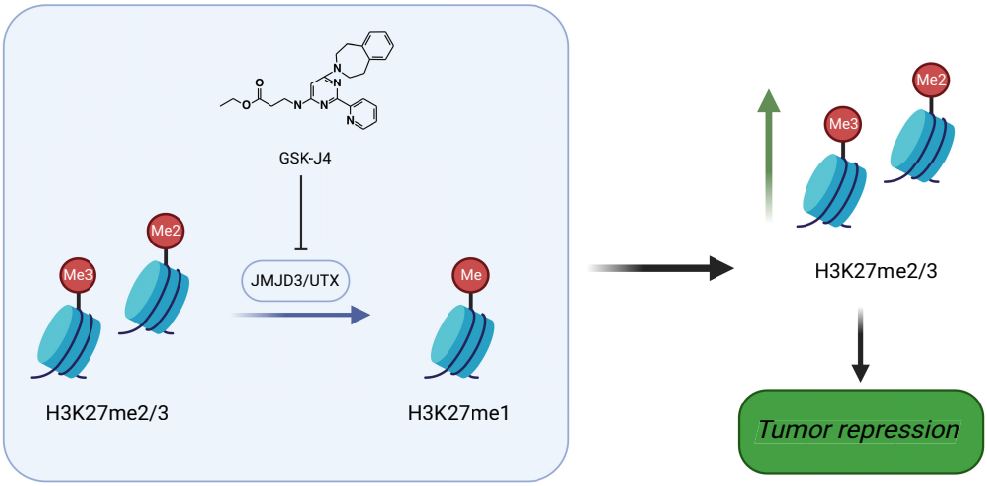

FIGURE 1 Schematic of the mode of action of GSK-J4 as an anti-cancer drug. Created with BioRender.com [Color figure can be viewed at wileyonlinelibrary.com]

upregulated after GSK-J4 treatment.[35] Another study showed that GSK-J4 abolishes genes involved in homologous recombination and non-homologous end-joining pathways.[36]

Overall, GSK-J4 mainly targets cell cycle regulators, well-conserved Homeobox genes, apoptosis markers, and DNA repair pathways to inhibit uncontrolled blood cancer cell proliferation.

3.1.2 | Brain cancer

Cancers of the brain include meningioma, astrocytoma, glioblastoma, medulloblastoma, pituitary adenoma, schwannoma, craniopharyngioma and many others. They are generally incurable and difficult to target because of the presence of the blood-brain barrier. Almost 308 102 new cases and 251 329 deaths due to cancers of the brain and central nervous system have been reported in 2020.[1] The glioma cell lines U87 and U251, when treated with GSK-J4 in the dose range of 4-8 μM, showed inhibition of cell proliferation and migration and induction of apoptosis. In contrast, there was no effect on normal human brain microvascular endothelial cells.[19] There was a clear increase in the H3K27me3 levels and an inhibition of JMJD3 expression after treatment with GSK-J4 in the U87 and U251 cell lines.[19] Another study on diffuse intrinsic pontine glioma (DIPG) cells showed that GSK-J4 treatment indirectly modifies the structure of the DNA such that it becomes inaccessible to DNA repair enzymes.[37] It also sustains *γH2AX* and *53BP1* expression at high levels in irradiated DIPG cells, thus inhibiting DNA repair via the homologous recombination pathway.[37] It also reduced the clonogenic survival of the glioma cells at a concentration of 6 μmol/L.[37] When GSK-J4 treatment was given to temozolomide-resistant U251, GBM3 and DBTRG glioblastoma cell lines, it decreased cell proliferation, blocked cell cycle progression, decreased clonogenicity and induced apoptosis in these cell lines.[38] In a study where GSK-J4 was used as a KDM2B inhibitor against glioma stem cells (GSC), GSK-J4 reduced cell viability and induced double-strand breaks in a dose-dependent manner.[39] There was a corresponding increase in H3K36me2, and as a result, the p21CIP1/WAF1 expression increased along with the induction of caspase 3 and cleavage of PARP (Poly (ADP-ribose) polymerase). It also reduced the self-renewal and survival capacity of glioma stem cells. At concentrations of 2.5 and 5 μM, it reduced the levels of EZH2 and SOX2 (Sex-determining region Y(SRY)-box transcription factor 2), both of which are glioma stem cells (GSC) regulators.[39]

Since GSK-J4 targets glioma cancer cells, cancer stem cells and drug-resistant cancer cells, it is a promising approach to treating brain cancer, the deadliest of all cancers.

3.1.3 | Lung cancer

The second most commonly diagnosed cancer after breast cancer and the leading cause of death in men worldwide is lung cancer.[1] Deregulated epigenetic modifications have been shown to be involved in lung cancer.[40] A study on the effect of the treatment of GSK-J4 on several lung adenocarcinoma cell lines showed that some lung adenocarcinoma (LuAC) cell lines were sensitive while others were resistant to GSK-J4 treatment. GSK-J4 also reduced the colony formation ability and tumor growth in-vivo of the GSK-J4 sensitive cell lines. At a concentration of 10 μmol/L, GSK-J4 induced cell cycle arrest and apoptosis in the sensitive cell lines. Moreover, there was an induction of oxidative stress due to *ATF4* induction and metabolic stress due to increased glutamine/glutamate transport and metabolism in the GSK-J4 sensitive cells, which led to decreased cell viability. They also found that *KRAS*-mutant cell lines are not sensitive to GSK-J4, and the overexpression of *KRAS* sensitizes the cells to GSK-J4.[22] GSK-J4 also inhibited TGFβ induced migration and EMT in BZR, A549 and H1299 lung cancer cells by downregulating the expression of vimentin and slug. GSK-J4 also significantly reduced syntenin transcript and protein expression in the lung cancer cell lines.[41]

3.1.4 | Breast cancer

Breast cancer has the highest incidence rate, with almost 2 261 419 cases diagnosed in 2020.[1] The overexpression of cell migration

TABLE 1 Summary of GSK-J4 dose used, pathway targeted and molecules targeted in studies involving different types of cancer.

SR no.	Type of cancer	Dose	Pathway	Molecular target	References
1.	Glioma	4-8 μM	↓Cell proliferation ↑Apoptosis ↓Migration	↓JMJD3	19
		6 μmol/L	↓DNA damage repair ↓Clonogenic survival	↑Levels of gH2AX and 53BP1	37
2.	Thyroid cancer	1.502 mM	↓Cell proliferation (cells blocked in G_2M-S stage) ↓Migration and invasion ↓Clonogenic survival	↓JMJD3	21
3.	Breast cancer	0-10 μM	↓Cell proliferation ↓Migration and invasion	↓CEMIP	20
	Breast cancer stem cells	6-20 μM	↓Cell proliferation ↓Self-renewal ↓Tumorigenicity (In-vivo)	↓JMJD3 ↓UTX	42
4.	Colorectal cancer	3-5 μM	↓Cell proliferation	↓JMJD3 ↑H2-D1 ↑CXCL9 ↑Psmb8	23
		7.5-15 μM	↓Cell viability ↓Migration ↓Colony formation ↓Subcutaneous CRC tumor growth in vivo ↓TIC properties ↓Stemness associated gene signatures	↓KDM6	46
5.	Prostate cancer	4-30 μM	↓Cell proliferation	↓c-MYC ↓NF2 ↓CTBP1 ↓EPHB2 ↓PLAUR	24
		50 mg/kg	↓Tumor growth (In-vivo)	↓JMJD3	51
		4-20 μM	↓Cell proliferation ↓Colony formation	↓JMJD3/UTX	34
		1 μM	↓Cell viability ↓Tumor growth velocity and volume ↑Apoptosis	↓KDM6B	48
6.	Acute myeloid leukemia	1.25-10 μM	↓Cell proliferation ↑Apoptosis Cell cycle arrest ↓Colony formation ↓Tumorigenicity (In-vivo)	↓FEN1, ↓HSP90AA1, ↓MCM3, ↓MCM4 and ↓PCNA, ↓HOXA5, ↓HOXA7, ↓HOXA9, ↓HOXA11	18
		2-10 μM (cell cycle arrest)	Cell cycle arrest, ↓Cell proliferation ↑Apoptosis Induced ER stress	↑P21 ↓CyclinD1/A2 ↑Bax, cle-caspase9	35
7.	Neuroblastoma	1 μM	↓Cell viability ↓Tumorigenicity (In-vivo)	↑PUMA ↑IRE1α ↑ATF4	25
8.	Lung cancer	10 μmol/L	↓Cell viability ↓Tumorigenicity (In-vivo) ↓Colony formation ↑Oxidative stress ↑Metabolic stress ↑Apoptosis Cell cycle arrest	↑ATF4	22
		10-30 μM	↓Migration	↓Syntenin	41

(Continues)

TABLE 1 (Continued)

SR no.	Type of cancer	Dose	Pathway	Molecular target	References
9.	Myeloma	0.5-12 μM	↓Cell viability ↑Apoptosis	↓KDM5B ↓KDM6A ↓KDM6B	52
10.	Hemangiosarcoma	22.3-136 nM	↓Cell viability ↑Apoptosis ↓Tumor size (in vivo)	↓KDM2B	26
11.	Ovarian cancer	4 μM	↓TGF-β induced EMT	↓JMJD3/UTX	27

inducing hyaluronidase (CEMIP) protein is associated with a poor prognosis of breast cancer.[20] GSK-J4 treatment in MDA-MB-231 breast cancer cell lines suppressed *CEMIP* expression by enhancing H3K27me3 on the promoter of the *CEMIP* gene.[20] GSK-J4 also reduced the cell viability and inhibited the invasive and migratory capabilities of the breast cancer cells at concentrations ranging from 2.5 to 10 μM.[20] A study on breast cancer stem cells reported that inhibition of JMJD3 and the corresponding increase in H3K27me3 due to GSK-J4 treatment not only reduced cancer cell proliferation but also suppressed the breast cancer cell population by inhibiting their expansion and self-renewal ability as well as reduced the expression of stem-cell markers.[42] GSK-J4, at concentrations of 1 and 3 μM, also prevented tolerance to chemotherapy and colony formation ability of triple-negative breast cancer cells.[43] Therefore, GSK-J4 is a reliable molecule for breast cancer therapy.

3.1.5 | Colorectal cancer

Colon and rectal cancer occur at the lower end of the digestive tract and comprise 10% of newly diagnosed cancer cases, and 9.4% of deaths have occurred in the year 2020 due to colorectal cancer (CRC).[1] Cancer recurrence and development of resistance to therapy mainly occur because of tumor-initiating cells.[44] Moreover, stem cells are mainly responsible for the progression and metastasis of CRC.[45] GSK-J4 effectively inhibited the proliferation of CRC of different origins at IC50 concentrations ranging from about 0.75 to 21.41 μM. GSK-J4 also inhibited colony formation and migratory potential of different CRC cell lines.[46] In addition, GSK-J4 reduced the population of ALDH+ and CD24 + CD44+ cells, which represent tumor initiating cells (TICs) and stem-like cells, respectively. It also decreased total β-catenin and MYC protein levels, along with the downregulation of signature genes of TICs.[46] GSK-J4 also inhibited the proliferation of the MC38 colon cancer cell line at concentrations of 3 μM, 4 to 5 μM in a time-dependent manner. GSK-J4, at a concentration of 5 μM, increased the transcript levels of MHC-class I component H2-D1, chemokine CXCL9 (CXC motif chemokine ligand 9) and immunoproteasome Psmb8 in this cell line.[23] GSK-J4 effectively targets the tumor-initiating populations in colorectal cancer, which would prevent its recurrence and improve treatment outcomes.

3.1.6 | Prostate cancer

Prostate cancer is the fourth most commonly diagnosed cancer and the second leading cause of death in men worldwide.[1] Reduced global H3K27me3 levels are correlated with prostate cancer aggressiveness.[47] A study on the effect of GSK-J4 on castration-resistant prostate cancer cells showed that GSK-J4 effectively reduced the proliferation and colony formation ability of the cancer cells at concentrations ranging from 4 to 20 μM, as compared with normal cells.[34] GSK-J4 was also found to downregulate metastasis-associated genes like *c-MYC*, *NF2*, *CTBP1*, *EPHB2* and *PLAUR*, along with inhibiting cell proliferation and viability of the LNCaP cell line at 30 μM concentration.[24] Another study showed that GSK-J4 inhibits cell proliferation, arrests cells at the sub-G0-G1 phase, and induces apoptosis in PC-3 and C42B cell lines. This is achieved by downregulating the expression of *CCND-1* by preventing the binding of KDM6B and smad2/3 to the cyclin D1 promoter.[48] A recent study on GSK-J4 (20 μM) showed that it could inhibit (transforming growth factor beta) TGFβ induced epithelial to mesenchymal transition (EMT), migration and invasion in prostate cancer cells by inhibiting the phosphorylation of smad3 in the LNCaP cells and that of c-jun in the PC3 cells.[49] GSK-J4 can thus be considered a promising therapeutic option for prostate cancer.

3.1.7 | Other types of cancers

GSK-J4 also inhibited the proliferation of Kirsten rat sarcoma virus (KRAS)-mutant thyroid cancer cells and arrested the cells at the G2M and S phase.[21] GSK-J4 inhibited TGFβ induced EMT in ovarian cancer cells by inhibiting the expression of mesenchymal markers like fibronectin and vimentin. It, however, did not alter the expression of the epithelial marker E-cadherin with or without TGFβ treatment.[27] There was also inhibition of cell proliferation and induction of apoptosis at 22.3-136 nM of GSK-J4 in canine hemangiosarcoma along with a decrease in tumor volume.[26] GSK-J4 also induced differentiation of neuroblastoma cells and endoplasmic reticulum stress along with upregulation of *PUMA* and cell death induction. High-risk neuroblastoma models that were chemorefractory and produced from patient xenografts could be effectively stopped from growing in vivo by GSK-J4. The growth of neuroblastomas resistant to either treatment alone was also constrained by the combination of GSK-J4 and retinoic

acid (RA), which enhanced differentiation and ER stress over GSK-J4 effects. This high-risk subtype of neuroblastoma can be effectively treated with a combination of epigenetic-targeted treatments and B cell lymphoma 2 (BCL-2) homology domain three mimetics by inducing *PUMA*, which increases tumor sensitivity to the BCL-2 inhibitor venetoclax, in *MYCN*-amplified neuroblastoma.[25]

3.2 | In-vivo studies

GSK-J4 significantly reduced hCD45+ mCD45− human leukemic cells engrafted in mice. The treatment did not induce toxicity to the livers and kidneys of these mice.[18] The in vivo study suggests that GSK-J4 reduces the leukemia burden in a Kasumi-1 xenograft mouse model, providing evidence for the potential application of GSK-J4 in leukemia treatment clinically.[18] A study on mice implanted with MDA-MB-231 breast cancer cells showed that GSK-J4 increased the H3K27me3 levels in the xenografts, with a corresponding decrease in tumor volume and inhibition of metastasis of cancer cells. Moreover, they did not observe an overall decrease in body weight of the tumor-implanted, GSK-J4 treated mice which indicates that GSK-J4 is well tolerated.[20] A study involving organoid culture from the mouse intestine showed that GSK-J4 significantly inhibited the ability of intestinal organoid formation. The study also showed that GSK-J4 could inhibit subcutaneous colorectal cancer (CRC) tumor growth in vivo.[46] GSK-J4 also increased the sensitivity of chemoresistant CRC cells to oxaliplatin by reducing the tumor growth rate.[50] A study involving androgen-positive (AR+) prostate xenografts in mice showed that GSK-J4 reduced tumor growth in AR+ xenografts, whereas there was no significant effect on AR- xenografts.[51]

3.3 | GSK-J4 synergism with other anti-cancer agents

A list of studies also showed that GSK-J4 acts in synergism with anti-cancer drugs (Table 2). AML cells co-treated with GSK-J4 were significantly more sensitive to olaparib, as indicated by dose-response analysis.[36] GSK-J4 also showed synergism with cytosine arabinoside (Ara-C), one of the most commonly used anti-leukemic agents, in

TABLE 2 List of anti-cancer drugs showing synergism with GSK-J4.

GSK-J4 in combination with	Cancer type	Pathway/process targeted	References
Cytosine arabinoside	AML	↓Cell proliferation ↓Colony formation	18
Decitabine	AML	↑Apoptosis ↑Bax and cleaved caspase 9	35
Olaparib	AML	↓Cell viability	52
Venetoclax	AML	↑Bcl2 induction by GSK-J4 sensitizes cells to Venetoclax	52
Cabazitaxel	CRPC	↓Cell viability	34
MDV3100	CRPC	↓Cell proliferation	48
Radiation	Glioma	↓DNA repair by downregulating PCNA, XRCC1, POLD1 ↑Apoptosis ↓Cell proliferation	37
CCNU	Glioma stem cells	↓Cell viability ↓Self-renewal capacity of Glioma stem-cells	39
VP-16	Glioma stem cells	↓Cell viability ↓Self-renewal capacity of Glioma stem-cells	39
JIB 04	Glioblastoma	↓Cell proliferation ↓Clonogenic activity	38
Capecitabine	Breast cancer	↓Persister cell proliferation	43
Hesperetin	Prostate cancer	↓Cell proliferation ↓TGF-β induced EMT, migration and invasion ↓Phosphorylation of smad3 and c-jun Alters H3K27me3, H3K9me3, H3K4me3 marks	49
Doxorubicin	Thyroid cancer	↓Cell proliferation ↓Invasion and migration ↑Apoptosis by increasing caspase3 levels	21
Oxaliplatin	Colorectal cancer	↓Colony formation ↑Apoptosis ↑NOTCH2 expression induced by Oxaliplatin is suppressed by GSK-J4 treatment	50

inhibiting cell proliferation and reducing the number of colony-forming units.[18] A combination of decitabine and GSK-J4 significantly reduced cell proliferation and induced apoptosis by significantly upregulating the expression of Bax and cleaved caspase-9 in KG-1a leukemic cells.[35] AML cell lines also showed increased sensitivity to venetoclax treatment when given in combination with GSK-J4, as GSK-J4 induced Bcl2 expression in certain AML subtypes.[36] GSK-J4, in combination with radiotherapy, increased the survival of mice with orthotopic human DIPG xenografts.[37] The irradiated SF8628 K27M DIPG cells had sustained increased γH2AX and 53BP1 expression after treatment with GSK-J4 thereby inhibiting DNA repair.[37] GSK-J4 downregulated the expression of DNA repair proteins like *PCNA*, *XRCC1* and *POLD1*, of the irradiated cells.[37] The expression of Ki-67, a cell proliferation marker, went down and the number of apoptotic cells (TUNEL+ [Terminal deoxynucleotidyl transferase dUTP nick end labeling]) increased considerably after the combination of GSK-J4 with radiation thereby inhibiting cell proliferation and inducing cell death.[37] Clonogenic assay showed that 72 h of GSK-J4 treatment also greatly enhanced the radiation effect in the K27M DIPG cells by reducing clonal growth of these cells as comapred to irradiated cells alone.[37] Jumonji histone demethylase inihibitor (JIB 04), a multi-KDM inhibitor in combination with GSK-J4, showed synergism against temozolomide-resistant glioblastoma cell lines by inhibiting the clonogenic activity of the cells.[38] In glioma stem cell lines 4121 and 1587, a high-dose combination of GSK-J4 with chemotherapeutic drugs like CCNU (1-(2-chloroethyl)-3-cyclohexyl-1-nitrosourea (ie, lomustine)) and VP-16 (Vepesid 16) showed synergistic inhibition of cell viability and decreasing the self-renewal capacity of glioma stem cells.[39] GSK-J4, in combination with capecitabine, decreases the persister cell population and thereby delays recurrence when the combination treatment is given at the onset of chemotherapy exposure in mice with xenografts of triple-negative breast cancer cells.[43] Oxaliplatin and GSK-J4 treatment in colorectal cancer cells drastically reduced colony formation, and GSK-J4 enhanced oxaliplatin-induced apoptosis in these cells by upregulating H3K27me3 levels and regulating the *NOTCH* signaling pathway.[50] GSK-J4 and Cabazitaxel treatment showed synergism at low doses against castration-resistant prostate cancer cell lines (CRPC).[34] KDM6B has been shown to be involved in

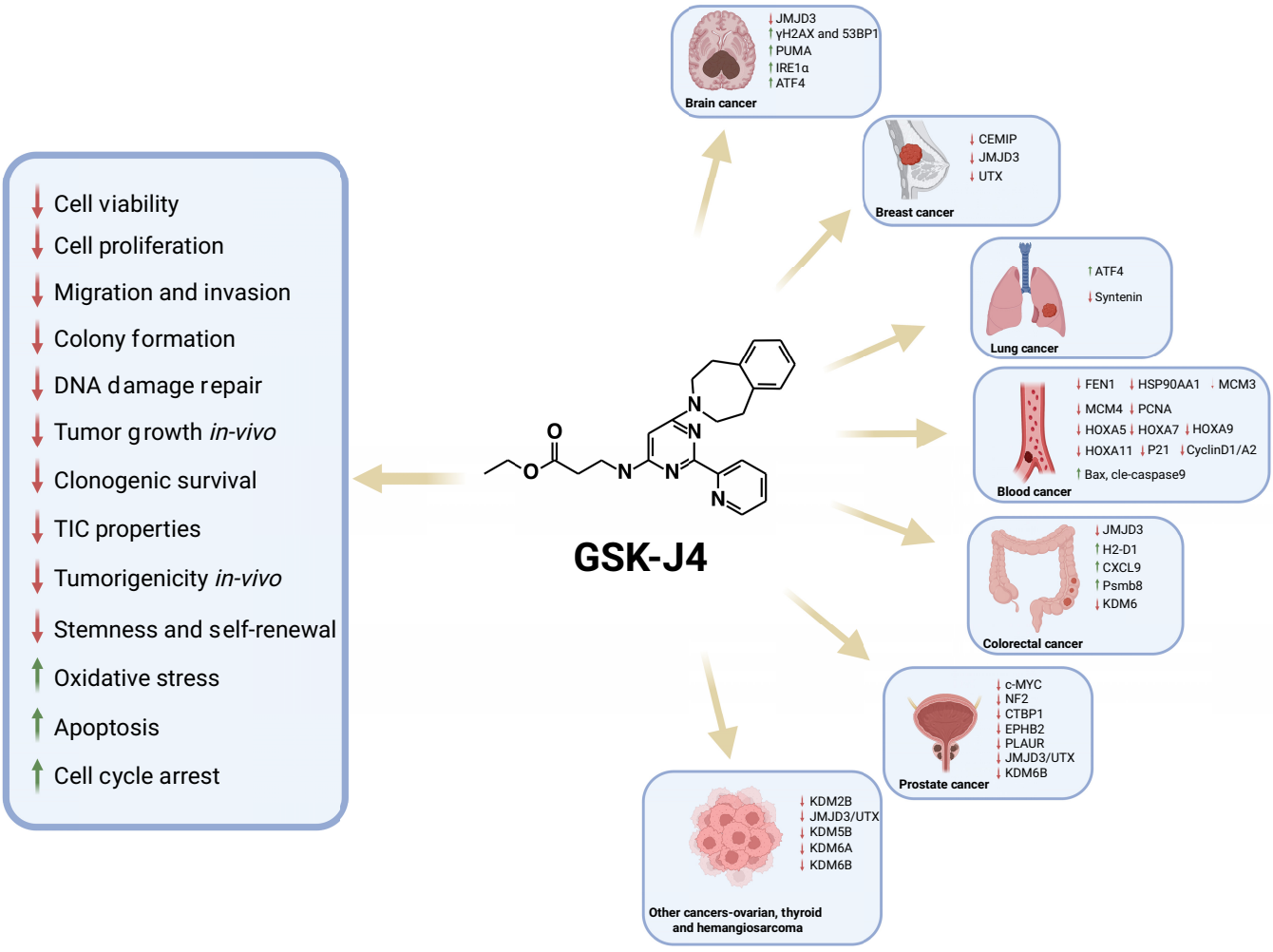

FIGURE 2 Schematic summarizing the molecular factors targeted by GSK-J4 resulting in anti-cancer activities of GSK-J4 against numerous cancers. Created with BioRender.com [Color figure can be viewed at wileyonlinelibrary.com]

the development of CRPC, and the combination of MDV3100 and GSK-J4 is effective against MDV3100-resistant CRPC as the combination treatment had a higher proliferation inhibition efficiency.[48] Another recently published study showed that a combination of GSK-J4 with hesperetin inhibited TGFβ induced EMT, migration and invasion in prostate cancer cells by inhibiting the phosphorylation of both smad3 and c-jun as well as inducing changes in the methylation of H3K4, H3K9 and H3K27.[49] A study on thyroid KRAS-mutant anaplastic thyroid cancer showed that doxorubicin and GSK-J4 treatment could significantly increase the inhibition of the proliferation of thyroid cancer cells along with the inhibition of invasion and migration of these cells. It induced apoptosis in the cells by increasing the caspase 3 levels thereby inhibiting sphere formation. The combination also inhibited the growth of Cal-62 thyroid cancer cell xenografts in nude mice.[21]

4 | CONCLUSION

GSK-J4, being an H2K37 demethylase inhibitor, changes the epigenetic landscape in cells and consequently targets different cellular functions ranging from cell proliferation, apoptosis, cell cycle, invasion, migration, stem-cell properties, differentiation, DNA repair, colony formation and organoid formation in different cancers (Figure 2). Its effectiveness has been proven against cancer growth and progression in vitro and in vivo. Moreover, in-vivo studies show that GSK-J4 is tolerated well by mice. GSK-J4 also enhances the effect of known anti-cancer drugs when given in combination. Studies involving overcoming immune evasion, and the role of the tumor microenvironment during GSK-J4 treatment can be the next step in this direction. In-vivo studies involving the treatment of late-stage cancers or cancers that have metastasized, with GSK-J4, need to be carried out to see if changing the epigenetic landscape can be the solution towards treating advanced-stage cancers. Along with effective treatment, it is important that the side effects be minimized to improve the quality of life of the patient. Extensive animal studies and clinical trials need to be carried out to pursue GSK-J4 in combination with other drugs to minimize toxicity and maximize the efficacy of treatment.

AUTHOR CONTRIBUTIONS

Nidhi Dalpatraj: Conceptualization and writing the original draft. Ankit Naik: Figures. Noopur Thakur: Review and Editing. The work reported in the paper has been performed by the authors, unless clearly specified in the text.

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CONFLICT OF INTEREST

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