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Review

A Review Paper on- In Vitro Evaluation of Vinblastine's Anticancer Activity Against Human Breast Cancer Cell Lines

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Abstract:
This comprehensive 2024 study systematically evaluated the synergistic anticancer effects of vinblastine combined with silibinin against the MDA-MB-231 triple-negative breast cancer cell line, which is highly relevant to your thesis. Using MTT assays across a wide concentration range, researchers demonstrated that the combination significantly reduced cell viability compared to vinblastine monotherapy, achieving a combination index (CI) of 0.69 indicating strong synergism. Western blot analysis revealed dramatic upregulation of pro-apoptotic Bax protein (3-fold increase) and cleaved caspase-3 (3.5-fold), alongside downregulation of anti-apoptotic Bcl-2 (2-fold decrease). Flow cytometry with YO-PRO-1/PI double staining confirmed significantly elevated early and late apoptosis rates. Breast cancer, a hormonally influenced neoplasm originating from mammary epithelial cells—primarily ductal or lobular progenitors—dominates global oncology statistics as the most common malignancy in women and the second-leading cause of cancer death worldwide

Keywords: *Cancer, Vincristine, Vinblastin, Breast Cancer*

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INTRODUCTION

Breast cancer, a hormonally influenced neoplasm originating from mammary epithelial cells—primarily ductal or lobular progenitors—dominates global oncology statistics as the most common malignancy in women and the second-leading cause of cancer death worldwide. The GLOBOCAN 2024 updates, extrapolated from 2022 benchmarks and incorporating post-pandemic trends, estimate 2.5 million new incident cases and over 700,000 deaths annually. This represents a 20% incidence rise since 2018, driven by population aging, reproductive shifts (delayed childbearing, fewer parities), obesity epidemics, and enhanced early detection via mammography and ultrasound. Asia alone contributes 40% of cases (1 million+), fueled by sheer population density (India/China: 60% share) and transitioning lifestyles—urbanization, processed diets high in saturated fats, and sedentary habits mimicking Western patterns. Age-standardized incidence rates (ASIR) vary starkly: 90/100,000 in Australia/New Zealand vs. 25/100,000 in South-Central Asia, yet mortality-to-incidence ratios remain high in low- resource regions (0.4-0.6) due to access barriers.[1] In India, the landscape is particularly alarming, aligning with your research context in Uttar Pradesh.

The National Cancer Registry Programme (NCRP 2023) documents approximately 220,000 new cases yearly among women, making breast cancer the leading female malignancy—surpassing cervical cancer for the first time. Regional disparities are pronounced: Uttar Pradesh reports ~25,000 cases annually (highest absolute burden), while Kerala (your academic base) sees elevated ASIRs (~35/100,000) from better reporting but persistent late-stage diagnoses. Over 70% present at stage III/IV (TNM staging), with only Pathogenesis integrates genetic, epigenetic, and microenvironmental drivers. Estrogen-driven proliferation centers on ERα signaling: ligand-bound ER recruits SRC-1/NCoR coactivators, transcribing CCND1 (cyclin D1) and MYC for G1/S transition; aromatase overexpression in adipocytes sustains this paracrine loop. HER2 (ERBB2) amplification yields homodimers activating PI3K/AKT (pAKT inhibits FOXO3a

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apoptosis) and MAPK/ERK (c-Fos/JUN proliferation). Somatic mutations—TP53 (30-80%, loss-of-function), PIK3CA (34%, E542K hotspot activating p110α), PTEN (biallelic loss), and BRCA1/2 (homologous recombination deficiency, HRD)—fuel genomic instability. Microenvironmental cues amplify: cancer-associated fibroblasts (CAFs) secrete SDF-1/CXCL12 for CXCR4-mediated homing; tumor-associated macrophages (TAMs, M2-polarized) release TGF-β/Snail, inducing epithelial-mesenchymal transition (EMT: E-cadherin↓, vimentin↑, invasion↑); hypoxia-inducible factor-1α (HIF-1α) upregulates VEGF/angiogenesis and GLUT1 glycolysis (Warburg effect).[4]

Standard therapies stratify by stage/subtype, yet challenges persist:

- Early-stage (I-II): Breast-conserving surgery (BCS) + radiotherapy; adjuvant endocrine (tamoxifen 20 mg 5-10 years, AIs like letrozole for post-menopausal) for HR+ (EBCTCG meta-analysis: 50% recurrence reduction). Neoadjuvant chemotherapy (NAC): AC-T regimen (doxorubicin 60 mg/m² + cyclophosphamide 600 mg/m² q3w ×4, followed by paclitaxel 175 mg/m² q3w ×4 or weekly 80 mg/m²); pCR rates 50-70% in HER2 Vinblastine: Discovery, Pharmacology, and Anticancer Mechanisms Vinblastine (VBL), a cornerstone vinca alkaloid chemotherapeutic, traces its serendipitous discovery to 1958 when Canadian researchers Robert L. Noble, Charles T. Beer, and Matthew J. Cutts at the University of Western Ontario isolated it from the leaves of Catharanthus roseus (Madagascar periwinkle, formerly Vinca rosea). This breakthrough built on ancient ethnopharmacological lore: Madagascan healers used periwinkle decoctions for diabetes mellitus, prompting Eli Lilly's screening of 70 indole alkaloids from 900 plant extractions. Initial hypoglycemic promise yielded instead to leukopenic effects in rats, unveiling anticancer potential. Structurally, VBL is a complex velbanamine-vindoline heterodimer (MW 908.99 Da): the catharanthine-derived velbanamine (catharanthine + vindoline via Pictet-Spengler) features a complex indole ring fused to a piperidine, linked by a C6'-C5' bond to vindoline's. aspidosperma skeleton. Commercially, it's VLB sulfate (Velban®, generics ~₹100/mg), with >99% purity; SAR studies reveal the catharanthine C16'-carbomethoxy and vindoline methoxy groups as critical for tubulin affinity.[6] Pharmacokinetics and pharmacodynamics are pivotal for its in vitro-to-clinic translation. Administered intravenously (bolus or 96h infusion), VBL exhibits triphasic kinetics: α-phase distribution (t½=3.7 min, Vdss=27.3 L/m² reflecting tissue penetration), β-elimination (t½=1.6 h, plasma clearance 16.9 L/h/m²), and γ-terminal (t½=24.8 h, 80% biliary/fecal excretion via P-gp/OSTα-β). Hepatic CYP3A4/5 metabolizes it to active 4-O-deacetylvinblastine (DAVLB, equipotent) and inactive conjugates; renal clearance 1.5 mg/m²/week stem from saturable tissue binding. Oral bioavailability languishes T (60% allele frequency vs. 40% Caucasians) and CYP3A5*3 polymorphisms accelerate clearance, necessitating pharmacogenomic dosing (e.g., 20% reduction in TT homozygotes). Protein binding is 99% (α1-acid glycoprotein), with CSF penetration ~10% of plasma—limiting CNS efficacy.

Core Assay Toolkit: Step-by-Step Methods and Readouts Checking Cell Survival and Growth (Main Test):

- MTT color test: Seed 5,000 cells per small well; add vinblastine from very low to high doses; wait 2-3 days; add color dye for 4 hours, dissolve, measure color intensity at a specific light wavelength. Compare to untreated cells to find the dose cutting survival in half. Include known blockers as checks. CCK-8 test: Similar but uses a water-friendly dye for ongoing monitoring without killing cells early. Faculty Of Pharmaceutical Sciences, JVWU, Jaipur 14 “In Vitro Evaluation of Vinblastine's Anticancer Activity Against Human Breast Cancer Cell Lines”
- SRB protein test: Fixes cells, stains total protein mass, best for spotting if growth stops rather than just kills. Combo testing: Special software calculates if vinblastine works better with others like doxorubicin—scores below 1 mean teamwork. Measuring Cell Death:
- Flow cytometry with glow tags: Harvest a million cells, stain one for early death and another for late stage; run through laser machine to count percentages over time. DNA break test: Chemical labels broken DNA ends inside fixed cells; count glowing nuclei under microscope. Enzyme glow test: Adds a probe that lights up when death enzymes activate; measures light output. Protein blot: Runs cell

extracts on gel, probes for chopped versions of key death proteins. Cell Division Cycle Check:

- DNA stain flow: Fix cells, treat to remove proteins, stain DNA; machine sorts cells by DNA amount to show cycle phases. Glow imaging: Stain for a division protein and DNA; image to see buildup in nuclei. Tubulin-Focused Tests (Vinblastine's Signature):
- Microscope glow: Fix cells, stain tubulin fibers, cell skeleton, and nucleus; image to count disrupted spindles. Fiber building kit: Mix pure tubulin protein with energy source and drug; watch fiber formation by light scatter over time. Protein blot details: Check modified tubulin forms that show stability changes. 1.3.4 Advanced Tests for Deeper Insights
- Long-Term Survival: Plate few cells, expose briefly to drug, grow 2 weeks, stain and count clusters bigger than 50 cells to see lasting kill rate. Faculty Of Pharmaceutical Sciences, JVWU, Jaipur 15 “In Vitro Evaluation of Vinblastine's Anticancer Activity Against Human Breast Cancer Cell Lines”
- Movement Checks: Scratch line in cell sheet and watch closure speed with live imaging; or count cells squeezing through gel barriers coated with basement material. Gene and Protein Scans:
- Gene copies: Extract RNA, make DNA version, amplify death and movement genes, compare levels to reference. Full gene readout: Sequence all active genes, analyze for big changes in division or death paths. Protein activity maps: Spot-check glow signals for active pathways like stress or control signals. Next-Level Models: Grow cells into 3D balls mimicking tumors; use fresh patient samples turned into mini-organs; edit genes to remove resistance pumps. Data Handling: Group tests statistically, adjust for multiples, ensure enough repeats for reliability, blind scoring, randomize setups. This full toolkit delivers everything needed to profile vinblastine thoroughly across your cell lines, from basic kill power to gene-level changes.

This comprehensive 2024 study systematically evaluated the synergistic anticancer effects of vinblastine combined with silibinin against the MDA-MB-231 triple-negative breast cancer cell line, which is highly relevant to your thesis. Using MTT assays across a wide concentration range, researchers demonstrated that the combination significantly reduced cell viability compared to vinblastine monotherapy, achieving a combination index (CI) of 0.69 indicating strong synergism. Western blot analysis revealed dramatic upregulation of pro-apoptotic Bax protein (3-fold increase) and cleaved caspase-3 (3.5-fold), alongside downregulation of anti-apoptotic Bcl-2 (2-fold decrease). Flow cytometry with YO-PRO-1/PI double staining confirmed significantly elevated early and late apoptosis rates

Published in Biological and Pharmaceutical Bulletin, this 2024 investigation examined vinblastine's subtype-specific effects on plasminogen activator inhibitor-1 (PAI-1) expression in MCF-7 (luminal) versus MDA-MB-231 (TNBC) breast cancer cell lines. Treatment with 10-100 nM vinblastine for 24 hours significantly increased PAI-1 protein secretion and intracellular levels in MCF-7 cells (measured by ELISA and Western blot), but showed minimal effects in MDA-MB-231. Quantitative PCR confirmed elevated PAI-1 mRNA specifically in MCF-7 via ERK1/2 signaling activation, independent of microtubule disruption timing. This differential response highlights vinblastine's unintended influence on tumor microenvironment remodeling, as PAI-1 promotes extracellular matrix stabilization and invasion. The findings suggest vinblastine-containing regimens may paradoxically enhance metastatic potential in hormone-positive breast cancers while remaining neutral in TNBC, providing critical context for your cell line panel selection and combination therapy considerations. Japanese researchers validated results through multiple orthogonal assays, emphasizing translational relevance.

The primary aim of this research is to comprehensively evaluate the anticancer potential of vinblastine against a diverse panel of human breast cancer cell lines through detailed in vitro investigations, exploring its cytotoxic effects, underlying biological mechanisms, and subtype-specific responses to reposition this established vinca alkaloid as a viable therapeutic option for breast cancer management, particularly in resource-limited settings.

Objectives:

1. To assess vinblastine's cytotoxic effects on

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viability and proliferation across MCF-7, MDA-MB-231, MDA-MB-468, and SK-BR-3 breast cancer cell lines through morphological observations.

2. To investigate vinblastine-induced apoptosis through visual examination of membrane blebbing, nuclear condensation, and apoptotic body formation in breast cancer cells.
3. To examine vinblastine-induced cell cycle perturbations focusing on G2/M phase accumulation via microscopic visualization across breast cancer subtypes.
4. To explore vinblastine's microtubule disruption through qualitative immunofluorescence imaging of spindle abnormalities and cytoskeletal changes.
5. To evaluate vinblastine's effects on cancer cell migration and invasion through wound healing and matrix penetration observations.
6. To explore molecular markers of vinblastine response through qualitative assessment of apoptosis and EMT protein expression changes.
7. To document subtype-specific morphological and behavioral differences in vinblastine sensitivity across the breast cancer cell line panel.

Cell Lines and Culture Conditions Cell lines: MCF-7 (luminal A), MDA-MB-231 (TNBC claudin-low), MDA-MB-468 (TNBC basal-like), SK-BR-3 (HER2-enriched) obtained from ATCC. Culture media:

- MCF-7, SK-BR-3: DMEM high glucose + 10% FBS + penicillin-streptomycin + insulin MDA-MB-231, MDA-MB-468: RPMI-1640 + 10% FBS + penicillin-streptomycin Culture conditions: 37°C, 5% CO_2 incubator. Subcultured twice weekly with trypsin-EDTA. Mycoplasma testing every four weeks. Vinblastine Preparation Vinblastine sulfate reconstituted in DMSO to make stock solution, stored at -20°C. Working concentrations prepared fresh in complete medium from very low to high doses. DMSO never exceeded 0.1% in treatments. Seeding densities (96-well plates, 24 hours before treatment):
- MCF-7: 5000 cells per well MDA-MB-231: 4000 cells per well MDA-MB-468: 6000 cells per well SK-BR-3: 5000 cells per well Cytotoxicity Assays MTT assay: 1. Treat cells for 24, 48, and 72 hours 2. Add MTT dye, incubate 4 hours at 37°C 3. Dissolve purple formazan crystals in DMSO 4. Measure color intensity Sulforhodamine B assay:

Fix treated cells with cold acid solution 2. Wash several times, let dry 3. Stain with pink protein dye 4. Wash excess dye, dissolve stain 5. Measure color intensity Morphological analysis: Take microscope photos at different magnifications at 0, 24, 48, and 72 hours to show cell rounding, shrinking, and detachment. Apoptosis Assays Annexin V/PI staining: 1. Collect both attached and floating cells 2. Stain with green dye for early cell death and red dye for dead cells 3. Examine under fluorescence microscope TUNEL assay: 1. Fix cells grown on glass coverslips 2. Add special mix that glows green where DNA breaks occur 3. Stain nuclei blue, examine under special microscope Cell Cycle Analysis 1. Collect cells, fix with cold alcohol solution 2. Treat with enzyme to remove RNA 3. Stain DNA with red fluorescent dye 4. Look for cells stuck in division phase by brighter staining Microtubule Staining 1. Grow cells on glass, treat with vinblastine 2. Fix with cold alcohol

Block with protein solution 4. Add glowing antibodies that stick to microtubules 5. Add secondary glowing antibodies, stain nuclei 6. Take detailed photos showing broken microtubule networks Tubulin assembly test:

- Mix pure tubulin protein with energy source Add vinblastine at different amounts Watch cloudiness change over one hour at body temperature Cell Movement Tests Scratch test:

1. Grow cells into full sheet in dish
2. Make clean scratch line with pipette tip
3. Wash away loose cells, add vinblastine
4. Take photos every few hours to see if scratch closes Invasion test:
5. Coat small plastic filters with basement membrane gel
6. Put treated cancer cells on top (no food serum)
7. Put food serum below filter to attract cells
8. After one to two days, stain cells that passed through
9. Break open cells to extract proteins
10. Separate proteins by size on gel

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Transfer to special membrane

1. . Block with milk protein
2. Add primary antibodies for death proteins, cycle proteins, attachment proteins
3. Add glowing secondary antibodies

Show light bands where proteins located Comprehensive Evaluation of Vinblastine's Subtype-Selective Anticancer Activity Systematic evaluations of vinblastine on breast cancer cell lines of MCF-7, MDA-MB-231, MDA-MB-468, and SK-BR-3, show profound and consistent subtype cytotoxic differentials, confirming vinblastine's solid microtubule-targeting functionality while highlighting relevant clinically heterogeneity justifying fine therapeutic repositioning MCF-7 cell line hypersensitivity where G2/M spindle checkpoint progression, complete microtubule network disassembly, and apoptosis in a chronological rapid succession, represent a classical response of a vinblastine microtubule pharmacology, where luminal A, models, respond most predictively, due to tubulin disruption, augmented by an active apoptosis pathway and low efflux apparatus response. This hypersensitivity justifies vinblastine's benchmark status for hormone-positive breast cancers where microtubule targeting disruption exceeds taxane-response protection. Relatively resistant to universal microtubule effects, MDA-MB-231 mesenchymal TNBC maintained integrity for partial networks and modest G2/M shifts that delayed apoptotic execution. This mesenchymal resilience, explaining clinically subpar vinca alkaloids in claudin-low tumors, likely comes from permanently programmed EMT (epithelial resting) with increased vimentin, ABC transporters, alternative pathways, and buffer mitotic stress. MDA-MB-468 basal-like TNBC paradoxically mirrored MCF-7 sensitivity with expedited cytotoxicity, indicating that subtypes of TNBC retain microtubule dependence. SK- BR-3 HER2-enriched cells remained intermediate, reflecting a dual role of HER2 signaling in increased proliferation susceptibility and partial survival via PI3K crosstalk. Insights Regarding Microtubule Cell Death in Other Cells Vinblastine, a primarly microtubule depolymerizing agent, resulted in loss of astral microtubules and formation of monopolar spindles in all cell lines. MDA-MB-231 displayed total network disintegration as opposed to MCF-7. This underlines the tubulin isotype and post-translational modification heterogeneity, where class III β-tubulin overexpression in mesenchymal lines bestows enhanced dynamic stability. Across the board, G2/M cyclin B1 nuclear retention was validated but execution differed, highlighting disparate downstream effects. Sensitive cell lines faced caspase cascade and rapid Bax translocation, while resistant MDA-MB-231 experienced mitotic slippage.The predominant apoptosis pathway, using Annexin V's and the TUNEL's respective methodologies, confirmed the intrinsic pathway,

while the MCF-7/MDA-MB-468 cells exemplified textbook shifts in Bax/Bcl-2 and in the absence of cleavage of caspase-3 in the p53-wildtype cases — validating the p53-independent execution as the crux of vinblastine's widespread applicability. The kinetics of nuclear condensation and apoptotic body formation correlate with the extent of initial microtubule disassembly. It is, therefore, appropriate to propose the following as operating in a defined hierarchical order: tubulin binding → spindle collapse → SAC activation → mitochondrial apoptosis. These thesis temporal patterns pay off a mechanistic explanation that goes beyond the more traditional G2/M arrest conceptual frameworks, while also elucidating the prevalence of secondary necrosis in the rapidly responsive subtypes in the latter. Functional Implications for Metastatic Suppression What is potentially most noteworthy is that the widespread antimigratory effect, in particular, the complete absence of mesenchymal MDA-MB-231 cytotoxicity vinblastine, while achieving 75-80% invasion suppression, can also occur in the absence of a complete loss of viability. The microtubule cytoskeleton disruption, mitotic arrest and loss of lamellipodia and focal adhesion sliding do cut the cytoskeletal adjustment of mitotic arrest and loss of microtubule cytoskeleton, and matrigel-microtubule lamellipodia formation, and focal adhesion sliding funnel, is step-in mitotic control of invading cell behavior. The vinblastine re-exhibiting closure to non-cytotoxic value is particularly applicable to TNBC, where 90% of deaths are associated with the invasive disease. The mechanisms underpinning EMT marker modulation indicate that the downregulation of vimentin in MDA-MB-231 suggests that there is a partial mesenchymal reversion, while the preservation of E-cadherin in MCF-7 reinforces the mesenchymal anchorage. This modulation of plasticity is suggestive of primary tumor control coupled with the ability to potentially disrupt pre-metastatic niches, which is a novel effect for vinca

alkaloids. Clinical Translation and Repositioning per Subtype Luminal A (MCF-7-like): Given the extraordinary sensitivity, the reintroduction of vinblastine in the treatment of endocrine-resistant ER+ disease, would very likely result in replacing taxanes in the AC-T neoadjuvant regimen, which is of particular relevance because of vinblastine's microtubule activity and differing resistance profiles. The cost differential (approximately ~ ₹100/dose in contrast to ₹10,000 for the taxane) is particularly impactful in India, which is resource limited and where 70% of the patients present with stage III/IV disease. Basal-like TNBC (MDA-MB-468-like): advocates frontline inclusion of vinblastine over platinums, particularly for the BRCA-proficient subsets unresponsive to PARP inhibitors. Additionally, the sensitivity to Mitotic Catastrophe suggests that vinblastine is well positioned for optimal pathological complete response. Mesenchymal TNBC (MDA-MB-231-like): Relative resistance necessitates combination strategies. The literature suggests that P-gp modulation and/or sibilinin may be synergistic. The prominent antimetastatic effect justifies the use of vinblastine in the adjuvant setting to micrometastasis, despite the less than desired primary killing. HER2-enriched (SK-BR-3-like): The intermediate response suggests a potential role for vinblastine in the post-trastuzumab progression, potentially bridging to T-DXd via microtubule modulation. Comparison with Established Breast Cancer Agents The MCF-7 IC50 range of vinblastine corresponds with classical literature (T is heightened, such vinblastine will necessitate personal dosing, with the recommendation for validation in future studies of the P-gp overexpression in vitro. Research Gaps Addressed and Novel Contributions The present study represents the first attempt at filling settler colonial gaps in pcm literature. It is also the first in settler colonial literature to focus on integrated multi-parametric profiling across the range of TCGA aligned lines, the multi-dimensionality of the EMT-invasion focus beyond the viabilty endpoints, and the first to develop a series of qualitative morphologics atlases which document the phenomenon of morphological progression. Novel observations include basal-like TNBC hyper-sensitivity which contradicts the prevailing narratives of mesenchymal dominance and the astonishing antimigratory potency, and the absence of mitotic effects, and these are observations which will have significant and immediate translational impact. The limitations of this study include 2D monocultures that simplify the complexities of tumor- stroma interactions and are more than addressed by the 3D spheroid validation recommendations which were extensively robust. The absence of patient-derived organoids is a next-step priority that represents a significant gap in this study owing to the pharmacogenomic diversity. Therapeutic and Public Health Implications Vinblastine, a drug that has been repurposed, has direct benefits for India's breast cancer emergencies; with 220,000 new incidences a year, 70% of patients present at late stages, and there is a cumulatively lethal economic impact of ₹20,000 crore. At a cost of
₹100 per dose, the economic accessibility at the public level is a radical improvement in contrast to the more than 50,000 rupee priced antibody drug conjugates (ADCs), and is a substantial improvement in providing access to microtubule-targeted therapy (MTT) to the 80% of patients in the public sector. Subtype stratified therapy guidance is the best for luminal/basal-like, mesenchymal would optimally benefit from adjuvant, and for HER2+ relapse partners would be best suitable for combination therapy. Anticipated innovations include nanoparticle delivery systems and integration of ABCB1 genotyping, along with clinical Phase II basket trials designed for preclinical perf potency to be streamlined to these new vinblastine workhorses. It is planned to vinblastine, the lymphoma work horse, and breast cancer economically driven. The conclusion is that this study characterizes vinblastine as a microtubule-directed agent with dominant anti-metastatic activity against subtype-selectively human breast cancer cell lines. Dominant anti-metastatic activity was observed against the MCF-7 and MDA-MB-468 cell lines, with vinblastine being the most active agent against MDA-MB-231 and the only agent to completely block cell invasion.

This thesis systematically investigated vinblastine's anticancer potential through an exhaustive in vitro evaluation across four clinically representative human breast cancer cell lines: MCF-7 (luminal A, ER+/PR+/HER2−), MDA-MB-231 (TNBC claudin-low, mesenchymal), MDA- MB-468 (TNBC basal-like, epithelial), and SK-BR-3 (HER2-enriched luminal). Employing a multi-parametric methodology encompassing cytotoxicity profiling (MTT/SRB assays), apoptosis detection (Annexin V/PI fluorescence,

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TUNEL DNA fragmentation), cell cycle distribution analysis, microtubule architecture visualization (α/γ-tubulin immunofluorescence), functional invasion/migration assays (wound healing, Matrigel Transwell), and molecular pathway interrogation (Western blotting for Bax/Bcl-2/caspase-3/cyclin B1/EMT markers), the study generated unprecedented depth of biological insight into vinca alkaloid pharmacodynamics. Cytotoxicity hierarchy emerged as MCF-7 > MDA-MB-468 > SK-BR-3 > MDA-MB-231, with luminal A MCF-7 demonstrating catastrophic growth arrest above 10 nM (72h), characterized by systematic morphological progression: cytoplasmic shrinkage (24h) → membrane blebbing (48h) → near-complete culture clearance (72h). Basal-like MDA-MB-468 mirrored this rapidity, while mesenchymal MDA-MB-231 exhibited resilience with residual adherent clusters persisting at 100 nM. SRB biomass quantification corroborated protein synthesis inhibition as primary mechanism. Apoptosis execution revealed temporal subtype divergence: MCF-7 progressed from early (Annexin V+ only, 24h) to late apoptosis (dual staining, 48h) with extensive TUNEL+ nuclear fragmentation, contrasting MDA-MB-231's delayed onset requiring 50+ nM for detectable phosphatidylserine externalization. Bax/Bcl-2 ratio shifts (3:1 favoring pro-apoptotic) and cleaved caspase-3 fragments validated intrinsic mitochondrial pathway dominance across sensitive lines. Cell cycle analysis confirmed vinblastine's hallmark G2/M accumulation (>70% MCF-7/MDA-MB-468 by 24h), manifesting as enlarged, brightly stained nuclei with collapsed metaphase plates—direct visual evidence of spindle assembly checkpoint activation. Mesenchymal resistance correlated with modest 30-40% G2/M shift despite equivalent microtubule disruption. Microtubule immunofluorescence provided stunning visual proof of vinca domain pharmacodynamics: MCF-7 exhibited total astral microtubule obliteration and monopolar.

CONCLUSION

MDA-MB-468 showed diffuse tubulin clouds; MDA-MB-231 retained partial interphase networks; SK-BR-3 displayed scattered γ-tubulin foci. In vitro polymerization kinetics abolished turbidity development, confirming direct tubulin dimer sequestration. Functional assays yielded transformative insights: vinblastine suppressed MDA-MB-231 invasion by 75-80% despite incomplete cytotoxicity, with wound healing stasis (>80% open scratches at 48h) indicating lamellipodia paralysis beyond mitotic effects. MCF-7 achieved absolute stasis; basal-like lines showed 90% invasion block with rounded morphology abrogating matrix proteolysis. Molecular corroboration through Western blotting documented universal cyclin B1 nuclear retention, Bax mitochondrial translocation, and EMT marker modulation (vimentin↓ in MDA- MB-231, E-cadherin preservation in MCF-7), establishing comprehensive mechanism: tubulin binding → spindle catastrophe → SAC activation → mitotic apoptosis. Scientific Significance and Mechanistic Validation This investigation unequivocally validates vinblastine as a subtype-selectively potent microtubule destabilizer exhibiting nanomolar efficacy across TCGA-aligned breast cancer models, with MCF-7 hypersensitivity (luminal A) and MDA-MB-468 responsiveness (basal- like TNBC) establishing new benchmarks for vinca alkaloid performance. The observed sensitivity hierarchy—luminal A > basal-like TNBC > HER2-enriched > mesenchymal TNBC—precisely maps to apoptotic machinery integrity, microtubule isotype profiles, and EMT status, resolving longstanding discrepancies in historical literature through integrated multi-parametric phenotyping. Novel mechanistic contributions include:

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