CONCOMITANT DELIVERY OF BCL-2 PROTEIN FAMILY INHIBITORS AND CHEMOTHERAPEUTIC DRUGS FOR CANCER THERAPY

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Concomitant Delivery of BCL-2 Protein Family Inhibitors and Chemotherapeutic Drugs for Cancer Therapy

by

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Dedicated to the pursuit of knowledge…

CERTIFICATE

This is to certify that the thesis entitled **"Concomitant Delivery of BCL-2 Protein Family Inhibitors and Chemotherapeutic Drugs for Cancer Therapy"** submitted by **Ms. Neha Mehrotra** to **Indian Institute of Technology Delhi** for the award of degree of Doctor of Philosophy in Biomedical Engineering is a record of bona fide research work carried out by her. Ms. Mehrotra has worked under our supervision and has fulfilled the requirements for submission of this thesis. The results contained in this thesis are original and have not been submitted in any form to any other university or institute for the award of a degree or diploma.

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Abstract

Cancer is a major global health issue with approximately 19.3 million cases diagnosed and 10 million deaths in the year 2020. Current standards of cancer treatment – surgery, chemotherapy, radiation therapy, hormone therapy and immunotherapy have increased overall survival rates in most cancer types. However, their response has not been optimal as cancer therapy is an intricate process which needs to cover all facets of tumor growth. Newer approaches that combine two therapeutic modalities such as chemotherapy with radiation, targeted therapy, hormone or immune therapy have emerged to overcome the limitations of the current clinical practices. Combination therapy approaches, especially those based on two or more chemo drugs or chemotherapeutics with biological molecules have shown remarkable potential by being able to target multiple pathways simultaneously and therefore reducing the chances of resistance and relapse. However, these approaches are based on systemic delivery of free drugs which still suffers from issues such as low aqueous solubility, poor stability and bioavailability in systemic circulation, complicated dosage regimes and sub-optimal biodistribution profile. In recent years, nanoparticle-based drug delivery systems for combination therapies have gained a lot of momentum. Nanodelivery vehicles can overcome poor solubility, bioavailability and stability issues which would in turn improve biodistribution and tumor accumulation of the combination drugs, making nanomaterial-based delivery systems promising candidates for improved combination cancer therapy.

The present research work is focused on the development of polylactic acid-based block copolymeric nanoparticles for concomitant delivery of BCL-2 family inhibitors with chemotherapeutic drugs. PLA based block copolymers with different molecular weights and hydrophobic/hydrophilic block ratios were synthesized and evaluated for preparation of anticancer agent loaded nanoparticles. A hybrid block copolymer, synthesized via ring opening polymerization using L-Lactide, with methoxy polyethylene glycol 5000 and Pluronic L-61 as

macroinitiators was chosen as the final polymer. The synthesized block copolymer was characterized using GPC, proton NMR and FTIR spectroscopy. Block copolymer was found to have an average molecular weight of ~25kDa. Proton NMR spectrum showed characteristics peaks of PLA at 5.2 and 1.6 ppm while peaks at 3.6 ppm and 1.2 ppm confirmed the incorporation of PEG and PPG blocks into the hybrid block copolymers. The synthesized copolymer was used to prepare MS1 peptide and Navitoclax loaded single and dual nanoparticles with a high encapsulation efficiency, spherical morphology, and uniform dispersion. The use of PLA based copolymer for concomitant delivery of MS1/Paclitaxel and Navitoclax/Decitabine resulted in sustained release with rapid cellular internalization and localization at target intracellular site. Biological activity of MS1 NPs was evaluated in five different breast cancer cell lines and a moderate inhibition of cellular proliferation was observed with IC_{50} ranging from 142–612nM for different cell lines (MCF-7, MDA-MB-468, SUM-149, ZR-75-1, SK-BR-3). The synergistic potential of MS1 with Paclitaxel, a widely used drug for breast cancer treatment, was evaluated for breast cancer therapy. However, out of the five breast cancer cell lines chosen for the study, synergistic effect of MS1/PTX dual NPs was observed only in MCF-7 cell line, which is a hormone-dependent breast cancer. No significant improvement in cytotoxicity was observed in the other cell lines.

Navitoclax/Decitabine dual NPs with a NAV/DCB molar ratio of 1:1 exhibited significant improvement in IC_{50} over single NPs in breast cancer and AML cell lines. Treatment with single drug loaded NPs led to IC_{50} values in the range of 0.055-7μM for NAV NPs and 0.092-5.5μM for DCB NPs in breast cancer and AML cell lines. Exposure to NAV/DCB dual NPs led to synergistic cytotoxicity and caused a reduction of 4 to 13-fold in IC_{50} values depending on the cell line. *In vivo* biological activity of NAV/DCB NPs evaluated in AML xenograft model in NOD-SCID mice demonstrated 43.6% reduction in tumor on treatment with dual NPs as compared to control group. Pharmacokinetics and biodistribution studies of NAV/DCB NPs

conducted in EAC tumor bearing BALB/c mice confirmed prolonged blood circulation and significant accumulation in tumor tissue. The dual NPs showed good cytocompatibility and hemocompatibility with reduced *in vivo* platelet toxicity. Tumor inhibition studies in murine syngeneic breast cancer model with dual NPs resulted in 94% reduction in tumor size as compared to control while treatment with single NAV NPs and DCB NPs resulted in 45% and 13.5% reduction respectively. Concomitant delivery of Navitoclax and Decitabine using PLA based hybrid block copolymeric nanoparticles presents a novel, safe and effective nanoformulation for combination cancer therapy especially for breast cancer.

सार

कैंसर एक प्रमुख वैश्विक स्वास्थ्य समस्या है। वर्ष 2020 में लगभग 19.3 मिलियन मामलों का निदान किया गया और कैंसर के कारण 10 मिलियन मौतें हुईं। कैंसर के उपचार के वर्तमान मानकों - सर्जरी, कीमोथेरेपी, विकिरण चिकित्सा, हार्मोन थेरेपी और इम्यूनोथेरेपी ने अधिकांश कैंसर प्रकारों में समग्र जीवित रहने की दर में वृद्धि की है। हालांकि, उनकी प्रतिक्रिया इष्टतम नहीं रही है क्योंकि कैंसर चिकित्सा एक जटिल प्रक्रिया है जिसमें ट्यूमर के विकास के सभी पहलुओं को शामिल करने की आवश्यकता होती है। नए दृष्टिकोण जो दो चिकित्सीय तौर-तरीकों जैसे कि कीमोथेरेपी को विकिरण, लक्षित चिकित्सा, हार्मोन या प्रतिरक्षा चिकित्सा के साथ जोड़ते हैं, वर्तमान नैदानिक प्रथाओं की सीमाओं को दूर करने के लिए उभरे हैं। संयोजन चिकित्सा दृष्टिकोण ने, विशेष रूप से दो या दो से अधिक कीमोथेरेपी दवाओं या जैविक अणुओं के साथ कीमोथेराप्यूटिक्स पर आधारित, एक साथ कई मार्गों को लक्षित करने में सक्षम होने और इसलिए प्रतिरोध और रिलेप्स की संभावना को कम करने में उल्लेखनीय क्षमता दिखाई है। हालांकि, ये दृष्टिकोण वाहक मुक्त दवाओं के प्रणालीगत वितरण पर आधारित हैं जो अभी भी कम जलीय घुलनशीलता, खराब स्थिरता और प्रणालीगत परिसंचरण में जैव उपलब्धता, जटिल खुराक व्यवस्था और उप-इष्टतम जैव वितरण प्रोफ़ाइल जैसे मुद्दों से ग्रस्त हैं। हाल के वर्षों में, संयोजन चिकित्सा के लिए नैनोपार्टिकल-आधारित दवा वितरण प्रणाली ने बहुत गति प्राप्त की है। नैनोडिलीवरी वाहन खराब घुलनशीलता, जैवउपलब्धता और स्थिरता के मुद्दों को दूर कर सकते हैं जो बदले में संयोजन दवाओं के जैव वितरण और ट्यूमर संचय में सुधार करेंगे, जिससे नैनोमटेरियल-आधारित डिलीवरी सिस्टम बेहतर संयोजन कैंसर चिकित्सा के लिए आशाजनक उम्मीदवार बनेंगे। वर्तमान शोध

कार्य कीमोथेराप्यूटिक दवाओं के साथ बीसीएल-2 परिवार अवरोधकों के सहवर्ती वितरण के लिए पॉलीलैक्टिक एसिड-आधारित ब्लॉक कोपोलिमेरिक नैनोकणों के विकास पर केंद्रित है। विभिन्न आणविक भार और हाइड्रोफोबिक/हाइड्रोफिलिक ब्लॉक अनुपात वाले पीएलए आधारित ब्लॉक कॉपोलिमर को एंटीकैंसर एजेंट लोडेड नैनोकणों की तैयारी के लिए संश्लेषित और मूल्यांकन किया गया था। एल-लैक्टाइड का उपयोग करके रिंग ओपनिंग पोलीमराइजेशन के माध्यम से संश्लेषित एक हाइब्रिड ब्लॉक कॉपोलीमर, मेथॉक्सी पॉलीइथाइलीन ग्लाइकॉल 5000 और प्लुरोनिक एल -61 के साथ मैक्रोइनिटिएटर के रूप में अंतिम बहुलक के रूप में चुना गया था। संश्लेषित ब्लॉक कॉपोलीमर को जीपीसी, प्रोटॉन एनएमआर और एफटीआईआर स्पेक्ट्रोस्कोपी का उपयोग करने की विशेषता थी। ब्लॉक कॉपोलीमर का औसत आणविक भार ~ 25kDa पाया गया। प्रोटॉन एनएमआर स्पेक्ट्रम ने पीएलए की विशेषताओं को 5.2 और 1.6 पीपीएम पर दिखाया, जबकि 3.6 पीपीएम और 1.2 पीपीएम पर चोटी ने हाइब्रिड ब्लॉक कॉपोलिमर में पीईजी और पीपीजी ब्लॉकों को शामिल करने की पुष्टि की। संश्लेषित कॉपोलीमर का उपयोग MS1 पेप्टाइड और Navitoclax लोडेड सिंगल और डुअल नैनोपार्टिकल्स को उच्च एनकैप्सुलेशन दक्षता, गोलाकार आकारिकी और समान फैलाव के साथ तैयार करने के लिए किया गया था। MS1/Paclitaxel और Navitoclax/Decitabine के सहवर्ती वितरण के लिए PLA आधारित कॉपोलीमर के उपयोग के परिणामस्वरूप लक्ष्य इंट्रासेल्युलर साइट पर तेजी से सेलुलर आंतरिककरण और स्थानीयकरण के साथ निरंतर रिलीज हुई। MS1 NPs की जैविक गतिविधि का मूल्यांकन पांच अलग-अलग स्तन कैंसर सेल लाइनों में किया गया था और विभिन्न सेल लाइनों (MCF-7, MDA-MB-468, SUM-149, ZR) के लिए 142-612nM से लेकर IC50 के साथ सेलुलर प्रसार का एक मध्यम निषेध देखा गया था। -75-1, एसके-बीआर-3)। स्तन कैंसर के इलाज के लिए व्यापक रूप से इस्तेमाल की

जाने वाली दवा पैक्लिटैक्सेल के साथ MS1 की सहक्रियात्मक क्षमता का मूल्यांकन स्तन कैंसर चिकित्सा के लिए किया गया था। हालांकि, अध्ययन के लिए चुनी गई पांच स्तन कैंसर सेल लाइनों में से, MS1 / PTX दोहरे NPs का सहक्रियात्मक प्रभाव केवल MCF-7 सेल लाइन में देखा गया, जो एक हार्मोन-निर्भर स्तन कैंसर है। अन्य सेल लाइनों में साइटोटोक्सिसिटी में कोई महत्वपूर्ण सुधार नहीं देखा गया। 1:1 के NAV/DCB मोलर अनुपात के साथ Navitoclax/Decitabine दोहरे NPs ने स्तन कैंसर और AML सेल लाइनों में एकल NPs पर IC50 में महत्वपूर्ण सुधार प्रदर्शित किया। सिंगल ड्रग लोडेड एनपी के साथ उपचार ने एनएवी एनपी और 0.092- के लिए 0.055-7μM की सीमा में IC50 मान का नेतृत्व किया। स्तन कैंसर और एएमएल सेल लाइनों में डीसीबी एनपी के लिए 5.5μM। NAV/DCB दोहरे NPs के संपर्क में आने से सहक्रियात्मक साइटोटोक्सिसिटी हुई और सेल लाइन के आधार पर IC50 मानों में 4 से 13 गुना की कमी आई। एनओडी-एससीआईडी चूहों में एएमएल ज़ेनोग्राफ़्ट मॉडल में मूल्यांकन किए गए एनएवी / डीसीबी एनपी की विवो जैविक गतिविधि में नियंत्रण समूह की तुलना में दोहरे एनपी के साथ उपचार पर ट्यूमर में 43.6% की कमी का प्रदर्शन किया। ईएसी ट्यूमर असर बीएएलबी/सी चूहों में किए गए एनएवी/डीसीबी एनपी के फार्माकोकाइनेटिक्स और बायोडिस्ट्रीब्यूशन अध्ययनों ने लंबे समय तक रक्त परिसंचरण और ट्यूमर के ऊतकों में महत्वपूर्ण संचय की पुष्टि की। दोहरी एनपी ने विवो प्लेटलेट विषाक्तता में कमी के साथ अच्छी साइटोकम्पैटिबिलिटी और हेमोकंपैटिबिलिटी दिखाई। दोहरी एनपी के साथ म्यूरिन सिनजेनिक स्तन कैंसर मॉडल में ट्यूमर निषेध अध्ययन के परिणामस्वरूप ट्यूमर के आकार में नियंत्रण की तुलना में 94% की कमी आई, जबकि एकल एनएवी एनपी और डीसीबी एनपी के साथ उपचार के परिणामस्वरूप क्रमशः 45% और 13.5% की कमी आई। पीएलए आधारित हाइब्रिड ब्लॉक कोपोलिमेरिक नैनोपार्टिकल्स का उपयोग करते हुए

नेवीटोक्लैक्स और डेसिटाबाइन का सहवर्ती वितरण विशेष रूप से स्तन कैंसर के लिए संयोजन कैंसर चिकित्सा के लिए एक उपन्यास, सुरक्षित और प्रभावी नैनोफॉर्म्यूलेशन प्रस्तुत करता है।

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ABBREVIATIONS

%	Percentage
µg	Microgram
µl	Microliter
5-FU	5-Flourouracil
Abx	Abraxane
ACN	Acetonitrile
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AML	Acute Myeloid Leukemia
ASO	Anti-sense Oligonucleotide
AST	Aspartate Amino Transferase
ATP	Adenosine Triphosphate
ATR-FTIR	Attenuated Total Reflection-Fourier transform Infrared Spectroscopy
BAD,	BCL-2 Associated Death Promoter
BAK	BCL-2 Homologous Antagonist Killer
BAX	BCL-2 Associated X Protein
BCA	Bicinchonic Assay
BCL-2	B-cell Lymphoma 2
BFL-1/BCL2A1	BCL-2 Related Protein A1
BH3	BCL-2 Homology 3
BH4	BCL-2 Homology 4
BID	BH-3 Interacting Domain Death Agonist

BIK	BCL-2 Interacting Killer
BIM	BCL-2 like Protein 11
BMF	BCL-2 Modifying Factor
BOK	BCL-2 Related Ovarian Killer
BRAF	V-RAF Murine Sarcoma Viral Oncogene Homolog B1
BUN	Blood Urea Nitrogen
°C	Degree Centigrade
CAR	Chimeric Antigen Receptor
CBC	Complete Blood Count
CEBPA	CCAAT Enhancer Binding Protein Alpha
CI	Combination Index
CLL	Chronic Lymphocytic Leukemia
CLSM	Confocal Laser Scanning microscope
CTLA-4	Cytotoxic T-Lymphocyte–Associated Antigen 4
DAPI	4′,6-diamidino-2-phenylindole
DCB	Decitabine
DCB NPs	Decitabine loaded copolymeric nanoparticles
DCC	N, N-dicyclohexuylcarbodimide
DMAP	4-dimethylaminopyridine
DLS	Dynamic Light Scattering
DMEM	Dulbecco’s modified eagle’s media
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic Acid

EAC	Ehrlich Ascites Carcinoma
EDTA	Ethylene-diamine-tetra acetic acid
EE%	Encapsulation Efficiency %
EGFR	Epidermal Growth Factor Receptor
FACS	Fluorescence-Activated Cell Sorting
FBS	Fetal Bovine Serum
FDA	Food and Drug Administration
FE-SEM	Field emission – scanning electron microscopy
FITC	Fluorescein isothiocyanate
FLT3	FMS-Like Tyrosine Kinase 3
GM-CSF	Granulocyte-Macrophage Colony Stimulating Factor
gm	Gram
GPC	Gel Permeation Chromatography
HEK 293	Human Embryonic Kidney 293
HER2	Human Epidermal Growth Factor Receptor 2
HGF	Hepatocyte Growth Factor
HIF 1	Hypoxia Inducible Factor 1
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
HRK	Harakiri, BCL-2 Interacting Protein
HR-TEM	High Resolution- Transmission Electron Microscopy
hrs	Hour
H & E	Hematoxylin and Eosin stain
IAEC	Institutional Animal Ethics Committee

IC_{50}	50% Inhibitory Concentration
INF	Interferon
IP	Intraperitoneal
ITD	Internal Tandem Duplication
IV	Intravenous
IU	International Unit
K	Kelvin
kDa	Kilo Dalton
Kg	Kilogram
KRAS	Kirsten Rat Sarcoma Viral Oncogene Homolog
Kv	Kilo Volt
MCL-1	Myeloid Cell Leukemia-1
MCF-7	Michigan Cancer Foundation-7
MDA-MB-468	M.D. Anderson Metastatic Breast Cancer 468
MDR	Multi drug resistance
mg	Milligram
mm	Millimeter
mM	Millimole
MM	Multiple Myeloma
Mn	Number Average Molecular Weight
MOMP	Mitochondrial Outer Membrane Potential
MS1 NPs	MS1 Peptide Loaded Copolymeric Nanoparticles
Mw	Weight Average Molecular Weight
MWCO	Molecular Weight Cut-Off

MTT	3-(4,5-dimethylthiazol-2-yl)-2,5diphenyltetrazolium bromide
MUC-1	Mucin-1
MYC	Human Homolog of Avian Viral Myelocytomatosis Gene
NAV	Navitoclax
NAV NPs	Navitoclax loaded copolymeric nanoparticles
NC	Negative Control
NCT	National Clinical Trial
nm	Nanometer
nM	Nano mole
NMR	Nuclear Magnetic Resonance
NOD	Non-Obese Diabetic
NPs	Nanoparticles
NSCLC	Non-Small Cell Lung Cancer
NuBCP-9	Nur 77 Derived BCL-2 Converting Peptide-9
OD	Optical Density
PARP	Poly Adenosine Diphosphate-Ribose Polymerase
PBS	Phosphate Buffer Saline
PC	Positive Control
PCL	Polycaprolactone
PD	Pharmacodynamics
PD-L1	Programmed Death -Ligand 1
PDI	Polydispersity Index
PEG	Polyethylene glycol
PGA	Polyglycolic Acid

P-gp 1	Poly-glycoprotein 1
pH	Logarithmic value of hydrogen
PI	Propidium Iodide
PI3K	Phosphoinositide 3-kinase
PK	Pharmacokinetics
PLA	Polylactic acid
PLGA	Poly (lactic-co-glycolic acid)
PPG	Polypropylene glycol
ppm	Parts per Million
PROTAC	Proteolysis Targeting Chimera
PS	Phosphatidylserine
p-TSA	p-Toluenesulfonic acid
PTX	Paclitaxel
PTX NPs	Paclitaxel loaded copolymeric nanoparticles
PUMA	p53 Upregulated Modulator of Apoptosis
RBC	Red Blood Cells
RhoB	Rhodamine B
RNA	Ribodeoxynucleic Acid
ROP	Ring Opening Polymerization
RPM	Rotation per minute
RT	Room Temperature
SAHB	Stabilized α-Helices of BCL-2 Domains
SC	Subcutaneous
SCID	Severe Combined Immune Deficient

SCLC	Small Cell Lung Cancer
SD	Standard Deviation
SGOT,	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
TFA	Trifluoroacetic Acid
TKI	Tyrosine Kinase Inhibitor
TME	Tumor Microenvironment
TNBC	Triple Negative Breast Cancer
TOR	Target of Rapamycin
TUSC2	Tumor Suppressor Candidate 2
UV	Ultraviolet
VEGF	Vascular Endothelial Growth Factor
WBC	White Blood Cells
WHO	World Health Organization