

**DEVELOPMENT OF POLYLACTIC ACID BASED BIODEGRADABLE  
NANOPARTICLES FOR CONCOMITANT DELIVERY OF CANCER  
STEM CELL INHIBITOR AND PRIMARY CHEMOTHERAPEUTIC  
DRUGS FOR CANCER THERAPY**

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INDIAN INSTITUTE OF TECHNOLOGY DELHI**

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by

**MOHD ANEES**

**Centre for Biomedical Engineering**

Submitted

in fulfillment of the requirements of the degree of Doctor of Philosophy

to the



**INDIAN INSTITUTE OF TECHNOLOGY DELHI**

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*Dedicated to My  
Family....*



# **CERTIFICATE**

This is to certify that the thesis entitled “**DEVELOPMENT OF POLYLACTIC ACID BASED BIODEGRADABLE NANOPARTICLES FOR CONCOMITANT DELIVERY OF CANCER STEM CELL INHIBITOR AND PRIMARY CHEMOTHERAPEUTIC DRUGS FOR CANCER THERAPY**” submitted by **Mr. Mohd Anees** 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 him. **Mr. Mohd Anees** has worked under my supervision and has fulfilled the requirements for the submission of this thesis.

The results contained in this thesis are original and have not been submitted in partial or full, to any other university or institute for the award of any degree or diploma.

**Prof. Harpal Singh**

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## ABSTRACT

Cancer is a major global health issue with approximately 20 million diagnosed cases in year 2020. As per WHO reports, cancer caused approximately 10 million deaths this year which is continuously rising with an estimation to exceed around 12.4 million cancer death per year by the end of 2030. Chemotherapy is one of the widely used approach for cancer treatment and provided to the patient either alone or in combination with other therapeutic strategies in adjuvant or neoadjuvant setups. However, development of drug resistance, metastasis and cancer relapse are some of the serious limitations associated with chemotherapy in almost all cancer types. Clinical advancement has revealed that the existence of cancer stem cells (CSCs) within the tumor is the major reason for these constrains and cause treatment failures in the cancer patients. Therefore, eradication of CSCs along with the whole bulk tumor is a compulsive task to achieve highest therapeutic efficacy of the treatment. Salinomycin (SAL), a small molecule based ionophore antibiotic, has potential to specifically target and kill CSCs of various cancer types through blocking their multiple pathways simultaneously. However, direct administration of SAL in the body is not approved for the human uses due to their high off targeted toxicity & poor pharmaceutical properties. Therefore, the present work is mainly focused on the development of a novel biodegradable polymeric nanoparticles (NPs) as an efficient drug delivery vehicle for systemic delivery of SAL with an aim to overcome all limitations associated with the conventional approaches of chemotherapy and improve therapeutic efficacy of the cancer treatment.

Herein, we have developed a biodegradable polylactic acid (PLA) based block copolymer consisting of an appropriate mixture of mPEG-PLA & PLA-Pluronic L61-PLA and optimize it as a drug delivery nano-platform for the cancer therapy. PLA based block copolymeric platform was synthesized by ring opening polymerization of L-lactide using mPEG & Pluronic

L-61 as the macroinitiators for the reaction. Synthesized block copolymer was then characterised for their physiochemical properties through GPC, NMR, ATR-FTIR, DSC & XRD and used to prepare SAL encapsulated PLA based block copolymeric NPs employing emulsion solvent evaporation technique. Prepared SAL loaded NPs showed hydrodynamic size of around 92 nm with a narrow polydispersity index. Also, developed PLA based nanopatform showed high encapsulation efficiency (>70 %) and sustained release profile (~ 35 % in 15 days in PBS, pH 7.4) for SAL. Furthermore, *in-vitro* cytotoxicity evaluation of SAL NPs was carried out against various cancer types and result of the analysis suggested that SAL NPs exhibits its cytotoxicity in a dose dependent manner against all treated cancer cell lines. IC<sub>50</sub> values of SAL NPs were found to be same as free SAL which confirmed that encapsulation of SAL in PLA based block copolymeric NPs have no negative effect on its anti-cancer activity and therapeutic efficacy. Additionally, prepared SAL loaded NPs were also found to be highly effective against chemo-resistant cancer cells as well as CSCs derived from the cancer patient. Most importantly, the cytotoxic effect of SAL NPs against chemo-resistant cancer cells and CSCs was significantly higher as compared to the free SAL indicating the chemo-sensitising ability of the Pluronic L-61 present in the developed block copolymeric platform. Encapsulation of SAL in PLA based block copolymeric NPs also improved its *in-vivo* pharmacokinetics & biodistribution profile and SAL NPs showed prolonged circulatory shelf-life, reduced non-specific biodistribution and higher tumor accumulation as compared to free SAL. Consequently, undesired toxicity of SAL was significantly reduced after its encapsulation in the NPs, which in-turn increased the dose tolerability in healthy BALB/c mice as compared to free SAL. However, treatment of EAC tumor bearing BALB/c mice with single SAL loaded NPs resulted in moderate tumor regression and provided no survival benefits to the treated mice. The most possible reason for this result may be that SAL is a specific inhibitor of CSCs and have moderate cytotoxicity against non-CSCs of the tumor. CSCs represents very

small fraction and usually resides inside the core of whole bulk tumor surrounded by heterogenous daughter cells. Therefore, debulking the tumor mass is a necessity to expose the core of the tumor to access & kill the CSCs. Since SAL was found to be non-efficient to debulk the tumor, a primary chemotherapeutic drug having potent anti-tumor efficacy was also analysed in a combination therapy with SAL for the cancer treatment. This thesis explores various Taxane (Paclitaxel) and Anthracycline (Pirarubicin & Doxorubicin) based small molecules as primary chemotherapeutic drugs and evaluated them for their tumor debulking potential for the cancer therapy. Since direct administration of all these chemo drugs in the body have been reported to cause significant off targeted toxicity, primary chemo drugs were also encapsulated in prepared PLA based block copolymeric NPs with an aim to reduce their undesired toxic effects and simultaneously improve their therapeutic efficacy for the treatment. Among all, Pirarubicin (PIRA) was found to be the most promising candidate with superior anti-cancer potential and hence selected as the best primary chemo drug for further studies. Pirarubicin (PIRA) is a semi-synthetic derivative of anthracycline that is reported to have lesser toxicity and better clinical outcomes as compared to its parental form doxorubicin (DOX). Prepared PIRA NPs showed hydrodynamic size of ~130 nm with uniform particle size distribution, high encapsulation efficiency (~ 95 %) and sustained drug release profile (~ 20 % in 15 days) at physiological conditions. *In-vitro* cytotoxicity evaluation against triple negative breast cancer (TNBC) cell lines and three dimensionally grown SUM-149 mammospheres showed that PIRA NPs exhibits a superior anti-cancer activity over DOX NPs. Additionally, primary chemo drugs loaded NPs showed several folds higher dose tolerability in BALB/c mice which confirmed that, off targeted toxicity of free chemo drugs was significantly reduced after their encapsulation in prepared PLA based block copolymeric NPs. Moreover, treatment of EAC tumor harbouring mice with PIRA NPs resulted in highest tumor regression as compared to the groups treated with free PIRA, free DOX or DOX NPs. Altogether, the results

conclude that prepared PIRA NPs exhibits an excellent anti-cancer efficacy and has a strong potential for the cancer therapy. However, group treated with free as well as NPs loaded primary chemo drugs including PIRA NPs showed significant tumor regrowth after a temporary pause in all the treated mice. These results conclude that treatment of EAC tumor with primary chemo drug alone is not sufficient to prevent cancer relapse may be due to the escape of CSCs from the treatment and hence primary chemo drug loaded NPs were required to be combined with CSCs inhibitor to achieve highest therapeutic efficacy of the treatment. Considering high potential for tumor debulking, both primary chemo drugs PIRA & DOX were selected for combination therapy and their different weight ratios were co-encapsulated with CSCs inhibitor SAL in PLA based block copolymeric NPs. Prepared dual drug loaded NPs (PIRA:SAL NPs & DOX:SAL NPs) showed hydrodynamic size in the range of 90-100 nm, excellent encapsulation efficiency of around 85-95 % and sustained release profile for both the drugs. Additionally, dual drug loaded NPs showed synergistically improved cytotoxic effect against various cancer cell lines and CSCs rich mammospheres of triple negative breast cancer (TNBC) origin. Consequently, dual drug loaded NPs showed lower IC<sub>50</sub> values and higher cytotoxicity as compared to their respective single drug loaded NPs in both monolayered as well as three dimensionally grown cancer cells respectively. The combination of PIRA:SAL 1:1 & DOX:SAL 3:1 were found to be the most synergistic ratios of dual drug loaded NPs and hence selected as the best combination ratio of primary chemo drug & CSCs inhibitor for the cancer therapy. Most importantly, concomitant delivery of PIRA:SAL 1:1 using PLA based block copolymeric NPs resulted in complete tumor regression without showing any sign of cancer relapse till the end of surveillance period. Altogether, the present studies conclude that developed primary chemo drug PIRA & CSCs inhibitor SAL co-loaded NPs exhibits excellent anti-tumor efficacy and hence have strong potential to be used as combination therapy for the cancer treatment.

## सारांश

कैंसर एक प्रमुख वैश्विक स्वास्थ्य समस्या है जिसमें वर्ष 2020 में लगभग 20 मिलियन निदान मामले हैं। डब्ल्यूएचओ की प्रतिवेदन के अनुसार, कैंसर के कारण इस वर्ष लगभग 10 मिलियन मौतें हुईं, जो 2030 के अंत तक प्रति वर्ष लगभग 12.4 मिलियन कैंसर की मौत के अनुमान के साथ लगातार बढ़ रही है। कीमोथेरेपी कैंसर के उपचार के लिए व्यापक रूप से उपयोग किए जाने वाले दृष्टिकोण में से एक है और रोगी को अकेले या अन्य सहायक या नव-सहायक स्थापित चिकित्सीय रणनीतियों के साथ संयोजन में प्रदान की जाती है। हालांकि, दवा प्रतिरोध, कैंसर का फैलना और पुनर्वृद्धि लगभग सभी कैंसर प्रकारों में कीमोथेरेपी से जुड़ी कुछ गंभीर सीमाएं हैं। नैदानिक प्रगति से पता चला है कि ट्यूमर के भीतर कैंसर स्टेम कोशिकाओं (सीएससी) का अस्तित्व इन बाधाओं का प्रमुख कारण है जो कि कैंसर रोगियों में उपचार विफलताओं का कारण बनता है। इसलिए, पूरे थोक ट्यूमर के साथ सीएससी का उन्मूलन उपचार उच्चतम चिकित्सीय प्रभावकारिता प्राप्त करने के लिए एक बाध्यकारी कार्य है। सैलिनोमाइसिन (एसएएल), एक छोटा अणु आधारित आयोनोफोर एंटीबायोटिक है, जो विशेष रूप से विभिन्न कैंसर प्रकारों के सीएससी को एक साथ उनके कई मार्गों को अवरुद्ध करके लक्षित करने और मारने की क्षमता रखता है। हालांकि, शरीर में एसएएल के प्रत्यक्ष प्रशासन को उसके विषाक्तता और खराब दवा गुणों के कारण मानव उपयोग के लिए अनुमोदित नहीं किया गया है। इसलिए, वर्तमान कार्य मुख्य रूप से कीमोथेरेपी के पारंपरिक दृष्टिकोण से जुड़ी सभी सीमाओं को दूर करने और कैंसर उपचार की चिकित्सीय प्रभावकारिता में सुधार करने के उद्देश्य से एसएएल के प्रणालीगत वितरण के लिए एक कुशल दवा वितरण वाहन के रूप में एक नवीन जैविक रूप से नष्ट होने वाले पॉलिमरिक नैनोकणों (एनपी) के विकास पर केंद्रित है। यहां, हमने एक जैविक रूप से नष्ट होने वाले पॉलीलैक्टिक एसिड (पीएलए) आधारित ब्लॉक कॉपोलीमर विकसित किया है जिसमें एमपीईजी-पीएलए और पीएलए-प्लूरोनिक एल 61-पीएलए का उपयुक्त मिश्रण शामिल है और इसे कैंसर थेरेपी के लिए दवा वितरण नैनो-मंच के रूप में अनुकूलित किया गया है। पीएलए आधारित ब्लॉक कॉपोलीमरिक मंच को एमपीईजी और प्लूरोनिक एल -61 का उपयोग करके एल-लैक्टाइड के रिंग ओपनिंग पोलिमेराइजेशन द्वारा संश्लेषित किया गया है। संश्लेषित ब्लॉक कॉपोलीमर को तब जीपीसी, एनएमआर, एटीआर-एफटीआईआर, डीएससी और एक्सआरडी के माध्यम से उनके भौतिक रासायनिक गुणों के लिए चिह्नित किया गया है और इसका उपयोग इमल्शन विलायक वाष्पीकरण तकनीक का उपयोग करके एसएएल से भरा हुआ पीएलए आधारित ब्लॉक कॉपोलीमरिक एनपी तैयार करने के लिए किया गया है। तैयार एसएएल एनपी ने एक संकीर्ण बहुप्रकीर्णता के साथ लगभग 92 नैनोमीटर का हाइड्रोडायनामिक आकार दिखाया। इसके अलावा, विकसित पीएलए आधारित नैनोप्लेटफॉर्म ने एसएएल के लिए उच्च एनकेप्सुलेशन दक्षता (>70%) और निरंतर रिलीज रूपरेखा (पीबीएस में 15 दिनों में ~ 35%, पीएच 7.4) दिखाया। इसके अलावा, एसएएल एनपी का इन-विट्रो कोशिकाविषी मूल्यांकन विभिन्न कैंसर प्रकारों के खिलाफ किया गया और विश्लेषण के परिणाम ने दर्शाया कि एसएएल एनपी सभी कैंसर सेल लाइनों के खिलाफ खुराक निर्भर तरीके से अपनी कोशिकाविषी प्रदर्शित

करता है। एसएएल एनपी के आईसी 50 मूल्यों को मुक्त एसएएल के समान पाया गया, जिसने पुष्टि की कि पीएलए आधारित ब्लॉक कॉपोलीमेरिक एनपी में एसएएल के एनकैप्सुलेशन का इसकी कैंसर विरोधी गतिविधि और चिकित्सीय प्रभावकारिता पर कोई नकारात्मक प्रभाव नहीं पड़ता है। इसके अतिरिक्त, एसएएल लोडेड एनपी को केमो-प्रतिरोधी कैंसर कोशिकाओं और कैंसर रोगी से प्राप्त सीएससी के खिलाफ भी अत्यधिक प्रभावी पाया गया। सबसे महत्वपूर्ण बात, केमो-प्रतिरोधी कैंसर कोशिकाओं और सीएससी के खिलाफ एसएएल एनपी का कोशिकाविषी प्रभाव मुक्त एसएएल की तुलना में काफी अधिक था, जो विकसित ब्लॉक कॉपोलीमेरिक प्लेटफॉर्म में मौजूद प्लूरोनिक एल -61 की केमो-संवेदीकरण क्षमता को दर्शाता है। पीएलए आधारित ब्लॉक कॉपोलीमेरिक एनपी में एसएएल के एनकैप्सुलेशन ने इसके इन-विवो फार्माकोकाइनेटिक्स और बायोडिस्ट्रीब्यूशन रूपरेखा में भी सुधार किया और एसएएल एनपी ने लंबे समय तक परिसंचरण अचल जीवन, गैर-विशिष्ट जैव वितरण में कमी और मुक्त एसएएल की तुलना में उच्च ट्यूमर संचय दिखाया। नतीजतन, एनपी में इसके एनकैप्सुलेशन के बाद एसएएल की अवांछित विषाक्तता काफी कम हो गई और मुक्त एसएएल की तुलना में स्वस्थ बीएएलबी / सी चूहों में खुराक सहनशीलता में वृद्धि भी देखी। हालांकि, एकल एसएएल एनपी के साथ ईएसी ट्यूमर वाले बीएएलबी / सी चूहों के उपचार से मध्यम ट्यूमर प्रतिगमन हुआ और इलाज किए गए चूहों को कोई जिवन लाभ प्राप्त नहीं हुआ। इस परिणाम का सबसे संभावित कारण यह हो सकता है कि एसएएल सीएससी का एक विशिष्ट अवरोधक है और ट्यूमर के गैर-सीएससी के खिलाफ मध्यम कोशिका विषाक्तता रखता है। सीएससी बहुत छोटे अंश का प्रतिनिधित्व करता है और आमतौर पर विषम कोशिकाओं से घिरे पूरे थोक ट्यूमर के मूल के अंदर रहता है। इसलिए, सीएससी तक पहुंचने और उन्हें मारने के लिए ट्यूमर द्रव्यमान को कम करने और ट्यूमर के मूल को उजागर करने की आवश्यकता होती है। चूंकि एसएएल को ट्यूमर को खत्म करने के लिए अकुशल पाया गया, इसलिए हमने कैंसर के उपचार के लिए एसएएल के साथ संयोजन चिकित्सा में शक्तिशाली ट्यूमर विरुद्ध प्रभावकारिता वाली एक प्राथमिक कीमोथेरेपी दवा का भी विश्लेषण किया। यह शोध प्रबंध विभिन्न टैक्सैन (पैक्लिटैक्सेल) और एंश्रासाइक्लिन (पिरारूबिसिन और डॉक्सोयूरूबिसिन) आधारित छोटे अणुओं को प्राथमिक कीमोथेरेपी दवाओं के रूप में चुनती है और उनकी कैंसर चिकित्सा की क्षमता का मूल्यांकन करती है। चूंकि शरीर में इन सभी केमो दवाओं के प्रत्यक्ष प्रशासन से विषाक्तता होती है, प्राथमिक केमो दवाओं को भी तैयार पीएलए आधारित ब्लॉक कॉपोलीमेरिक एनपी में डाला गया, ताकी उनके अवांछित विषाक्त प्रभावों को कम और साथ ही साथ उपचार के लिए उनकी चिकित्सीय प्रभावकारिता में सुधार किया जा सके। सभी के बीच, पिरारूबिसिन (पीआईआरए) को बेहतर कैंसर विरोधी क्षमता के साथ सबसे आशाजनक उम्मीदवार पाया गया और इसलिए आगे के अध्ययन के लिए सर्वश्रेष्ठ प्राथमिक केमो दवा के रूप में चुना गया। पिरारूबिसिन (पीआईआरए) एंश्रासाइक्लिन का एक अर्ध-कृत्रिम व्युत्पन्न है जिसे इसके पैतृक रूप डॉक्सोयूरूबिसिन (डीओएक्स) की तुलना में कम विषाक्तता और बेहतर नैदानिक परिणाम के लिये जाना जाता है। तैयार पीआईआरए एनपी ने शारीरिक स्थितियों में समान कण आकार वितरण, उच्च एनकैप्सुलेशन दक्षता (~ 95%) और निरंतर दवा रिलीज रूपरेखा (~ 15 दिनों में ~ 20%) के

साथ ~ 130 एनएम का हाइड्रोडायनामिक आकार दिखाया। ट्रिपल नेगेटिव स्तन कैंसर सेल लाइनों और विकसित SUM-149 मैमोस्फीयर के खिलाफ इन-विट्रो कोशिकाविषी मूल्यांकन से पता चला है कि पीआईआरए एनपी डीओएक्स एनपी की तुलना में बेहतर कैंसर-विरोधी गतिविधि प्रदर्शित करता है। इसके अतिरिक्त, प्राथमिक केमो दवाओं से भरी एनपी ने बीएएलबी / सी चूहों में कई गुना अधिक खुराक सहनशीलता दिखाई, जिसने पुष्टि की कि, तैयार पीएलए आधारित एनपी में उनके एनकैप्सुलेशन के बाद मुक्त केमो दवाओं की विषाक्तता काफी कम हुई। इसके अलावा, पीआईआरए एनपी ने ईएसी ट्यूमर के उपचार में मुक्त पीआईआरए, मुक्त डीओएक्स या डीओएक्स एनपी से इलाज किए गए समूहों की तुलना में उच्चतम ट्यूमर प्रतिगमन दिखाया। कुल मिलाकर, परिणाम निष्कर्ष निकालते हैं कि तैयार पीआईआरए एनपी एक उत्कृष्ट कैंसर विरोधी प्रभावकारिता प्रदर्शित करता है और कैंसर चिकित्सा के लिए मजबूत क्षमता रखता है। हालांकि, मुफ्त के साथ-साथ एनपी में लदे हुए प्राथमिक केमो दवाओं ने सभी इलाज किए गए चूहों में अस्थायी विराम के बाद ट्यूमर में पुनः वृद्धि दिखाई। इन परिणामों का निष्कर्ष है कि अकेले प्राथमिक केमो दवा द्वारा ईएसी ट्यूमर का उपचार कैंसर पुनः वृद्धि को रोकने के लिए पर्याप्त नहीं है, यह उपचार से सीएससी अप्रभावित रह सकती है और इसलिए उपचार की उच्चतम चिकित्सीय प्रभावकारिता प्राप्त करने के लिए प्राथमिक केमो दवा- एनपी को सीएससी अवरोधक के साथ जोड़ा जाना आवश्यक है। उच्चतम ट्यूमर प्रतिगमन क्षमता को ध्यान में रखते हुए, संयोजन चिकित्सा में दोनों प्राथमिक केमो दवाओं पीआईआरए और डीओएक्स को चुना गया और उनके विभिन्न वजन अनुपात को पीएलए आधारित ब्लॉक कॉपोलीमरिक एनपी में सीएससी अवरोधक एसएएल के साथ सह-समझाया गया। तैयार दोहरी दवा लोडेड एनपी (पीआईआरए: एसएएल एनपी और डीओएक्स: एसएएल एनपी) ने 90-100 एनएम हाइड्रोडायनामिक आकार, लगभग 85-95% उत्कृष्ट एनकैप्सुलेशन दक्षता और दोनों दवाओं के का निरंतर रिलीज रूपरेखा दिखाया। इसके अतिरिक्त, दोहरी दवा से भरे एनपी ने विभिन्न कैंसर सेल लाइनों और ट्रिपल नकारात्मक स्तन कैंसर (टीएनबीसी) मूल के सीएससी समृद्ध मैमोस्फीयर के खिलाफ भी सहक्रियात्मक रूप से बेहतर कोशिकाविषी प्रभाव दिखाया। नतीजतन, दोहरी दवा लोडेड एनपी ने क्रमशः कैंसर कोशिका और साथ ही मैमोस्फीयर में अपने संबंधित एकल दवा लोडेड एनपी की तुलना में कम आईसी 50 मान और उच्च कोशिकाविषी दिखाई। पीआईआरए: एसएएल 1: 1 और डीओएक्स: एसएएल 3: 1 को संयोजन दोहरी दवा लोडेड एनपी का सबसे सहक्रियात्मक अनुपात पाया गया और इसलिए कैंसर चिकित्सा के लिए प्राथमिक केमो दवा और सीएससी अवरोधक के सर्वोत्तम संयोजन अनुपात के रूप में चुना गया। सबसे महत्वपूर्ण बात, पीएलए आधारित ब्लॉक कॉपोलीमरिक एनपी का उपयोग करके पीआईआरए: एसएएल 1: 1 के सहवर्ती वितरण के परिणामस्वरूप पूर्ण ट्यूमर प्रतिगमन हुआ और निगरानी अवधि के अंत तक कैंसर रिलैप्स का कोई संकेत नहीं दिखा। कुल मिलाकर, वर्तमान अध्ययनों का निष्कर्ष है कि विकसित प्राथमिक केमो दवा पीआईआरए और सीएससी अवरोधक एसएएल सह-लोडेड एनपी उत्कृष्ट एंटी-ट्यूमर प्रभावकारिता प्रदर्शित करता है और इसलिए कैंसर के उपचार के लिए संयोजन चिकित्सा के रूप में उपयोग किए जाने की मजबूत क्षमता रखता है।



# TABLE OF CONTENTS

<b>CERTIFICATE</b>	i
<b>ACKNOWLEDGEMENTS</b>	ii
<b>ABSTRACT</b>	v
<b>TABLE OF CONTENTS</b>	xii
<b>LIST OF FIGURES</b>	xxiv
<b>LIST OF TABLES</b>	xxxii
<b>GLOSSARY OF SYMBOLS AND ABBREVIATIONS</b>	xxxiv

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## **Chapter 1: Introduction and literature survey**

1.1 Introduction	2
1.2 Cancer and its hallmarks	4
1.2.1 Self-sufficient cell proliferation	5
1.2.2 Insensitivity to growth suppressive signals	5
1.2.3 Resisting apoptosis	5
1.2.4 Replicative immortality	5
1.2.5 Angiogenesis	6
1.2.6 Metastasis and tissue invasion	6
1.2.7 Genomic instability	6

1.2.8	Pro-tumorigenesis inflammation	7
1.2.9	Reprogrammed energy metabolism	7
1.2.10	Evading immune destruction	7
1.3	Strategies for cancer treatment	8
1.3.1	Surgery	8
1.3.2	Radiation therapy	9
1.3.3	Hormone therapy	9
1.3.4	Immunotherapy	10
1.3.5	Chemotherapy	10
1.4	Overview of chemotherapy	10
1.4.1	Types of chemotherapeutic drugs	11
1.4.2	Limitations with chemotherapy	15
1.4.2.1	Poor pharmaceutical properties	15
1.4.2.2	High off targeted toxicity	16
1.4.2.3	Multi-drug resistance	16
1.4.2.4	Insensitivity towards cancer stem cells	17
1.5	Overview of cancer stem cells	17
1.5.1	Role of CSCs in cancer	19
1.5.2	Anti-CSCs based cancer therapy	22

1.6 Salinomycin as the inhibitor of CSCs	26
1.6.1 Mechanism of action of Salinomycin	27
1.6.2 Limitations of Salinomycin as an anti-cancer agent	29
1.7 Nanotechnology for drug delivery system	30
1.7.1 Nanoparticles as drug delivery system	32
1.7.1.1 Inorganic NPs	33
1.7.1.2 Organic NPs	33
1.7.2 Mechanism of drug delivery for cancer therapy using NPs	37
1.8 Polylactic acid as a platform for DDS	40
1.9 Strategy for designing PLA based biodegradable NPs for cancer therapy	42
1.10 Rational and objective of the research work	44

**Chapter 2: Development and characterization of PLA based block copolymeric nanoparticles for delivery of SAL as cancer stem cell inhibitor**

2.1 Introduction	79
2.2 Materials	81
2.3 Methods	81
2.3.1 Synthesis of PLA based hybrid block copolymer	81
2.3.2 Characterization of PLA based hybrid block copolymer	83
(i) Gel permeation chromatography (GPC)	83

(ii)	<sup>1</sup> H nuclear magnetic resonance ( <sup>1</sup> H NMR)	83
(iii)	Attenuated total reflectance-fourier transform infrared (ATR-FTIR) Spectroscopy	83
(iv)	X-ray diffraction (XRD)	84
(v)	Differential scanning calorimetry (DSC)	84
2.3.3	Preparation of PLA based hybrid block copolymeric nanoparticles	84
2.3.4	Characterization of PLA based hybrid block copolymeric nanoparticles	86
(i)	Hydrodynamic size, PDI and zeta potential of SAL NPs	86
(ii)	Stability studies of SAL NPs	87
(iii)	Optimization for storage stability of SAL NPs	87
(iv)	HR-TEM and FESEM analysis of SAL NPs	87
(v)	<i>In-vitro</i> release study of SAL from PLA based hybrid block copolymeric NPs	88
2.3.5	Cell culture and mammosphere generation	88
2.3.6	Cellular internalization studies of PLA based hybrid block copolymeric NPs	89
2.3.7	Safety evaluation of PLA based hybrid block copolymeric NPs	90
2.3.8	<i>In-vitro</i> cell proliferation inhibition studies of free SAL and SAL loaded NPs	90
2.3.9	Chemotherapy resistant cancer cell inhibition studies of free SAL and SAL NPs	91
2.3.10	Cancer stem cell identification and quantification	92
2.3.11	Cancer stem cell inhibition studies of free SAL and SAL loaded NPs	92
2.3.12	Animal maintenance and tumor model establishment	94

2.3.13	<i>In-vivo</i> toxicity assessment of free SAL and SAL loaded NPs	94
2.3.14	Pharmacokinetics and biodistribution studies of free SAL and SAL loaded NPs	94
2.3.15	<i>In-vivo</i> tumor regression studies of free SAL and SAL loaded NPs	96
2.3.16	Statistical analysis	96
2.4	Results	97
2.4.1	Synthesis of PLA based hybrid block copolymer	97
2.4.2	Characterization of PLA based hybrid block co-polymer	97
(i)	Gel permeation chromatography (GPC)	97
(ii)	<sup>1</sup> H nuclear magnetic resonance	97
(iii)	Attenuated total reflectance-fourier transform infrared (ATR-FTIR) Spectroscopy	99
(iv)	X-ray diffraction (XRD)	99
(v)	Differential scanning calorimetry (DSC)	100
2.4.3	Preparation of PLA based hybrid block copolymeric nanoparticles	101
2.4.4	Characterization of PLA based hybrid block copolymeric nanoparticles	102
(i)	Hydrodynamic size, PDI and zeta potential	102
(ii)	Stability study of SAL NPs	103
(iii)	Optimization of storage stability of SAL NPs	104
(iv)	HR-TEM and FESEM analysis of SAL NPs	105
(v)	<i>In-vitro</i> release study of SAL from PLA based hybrid block copolymeric NPs	105

2.4.5	Cellular internalization studies of PLA based hybrid block copolymeric NPs	107
2.4.6	Safety evaluation of PLA based hybrid block copolymeric NPs	107
2.4.7	<i>In-vitro</i> cell proliferation inhibition studies of free SAL and SAL loaded NPs	109
2.4.8	Chemotherapy resistant cancer cell inhibition studies of free SAL and SAL NPs	111
2.4.9	Cancer stem cell inhibition studies of free SAL and SAL loaded NPs	113
2.4.10	<i>In-vivo</i> toxicity assessment of free SAL and SAL loaded NPs	114
2.4.11	Pharmacokinetics and biodistribution studies of free SAL and SAL loaded NPs	117
2.4.12	<i>In-vivo</i> tumor regression studies of free SAL and SAL loaded NPs	122
2.5	Discussion	123
2.6	Conclusion	130

### **Chapter 3: Development and characterization of PLA based block copolymeric nanoparticles for delivery of primary chemotherapeutic drugs**

3.1	Introduction	138
<b>PART A: Synthesis and Characterization of PLA Based Block Copolymers</b>		
3.2	Materials	143
3.3	Methods	143
3.3.1	Synthesis of PLA based block copolymers	143
3.3.2	Characterization of PLA based block copolymers	144

(i) $^1\text{H}$ nuclear magnetic resonance ( $^1\text{H}$ NMR)	144
(ii) Attenuated total reflectance-fourier transform infrared spectroscopy	145
(iii) Gel permeation chromatography (GPC)	145
(iv) Differential scanning calorimetry (DSC)	145
(v) X-ray diffraction (XRD)	145
3.3.3 Preparation and characterization of PLA based blend block copolymeric NPs	146
3.3.4 Safety evaluation of PLA based blend block copolymeric NPs	146
3.3.5 Cellular uptake study of PLA based blend block copolymeric NPs	147
3.4 Results	148
3.4.1 Synthesis of PLA based block copolymers	148
3.4.2 Characterization of PLA based block copolymers	148
(i) $^1\text{H}$ nuclear magnetic resonance ( $^1\text{H}$ NMR)	148
(ii) Attenuated total reflectance-fourier transform infrared spectroscopy	150
(iii) Gel permeation chromatography (GPC)	151
(iv) Differential scanning calorimetry (DSC)	152
(v) X-ray diffraction (XRD)	152
3.4.3 Preparation and characterization of PLA based blend block copolymeric NPs	152
3.4.4 Safety evaluation of PLA based blend block copolymeric NPs	155
3.4.5 Cellular uptake study of PLA based blend block copolymeric NPs	157

3.5 Discussion	157
----------------	-----

**PART B (I): Preparation, Characterization & Evaluation of Taxane (Paclitaxel) Loaded NPs for Cancer Therapy**

3.6 Materials	160
---------------	-----

3.7 Methods	160
-------------	-----

3.7.1 Preparation of taxane (PTX) loaded PLA based blend block copolymeric NPs	160
--	-----

3.7.2 Characterisation of the STP loaded PLA based blend block copolymeric NPs	161
--	-----

(i) Hydrodynamic size, PDI and zeta potential	162
---	-----

(ii) HR-TEM analysis of the STP loaded NPs	162
--	-----

(iii) <i>In-vitro</i> release studies of the drugs from PLA based blend block copolymeric NPs	163
---	-----

3.7.3 Cytotoxicity evaluation of STP loaded PLA based blend block copolymeric NPs	163
---	-----

3.7.4 <i>In-vivo</i> tumor regression study of STP loaded PLA based blend block copolymeric NPs	163
---	-----

3.7.5 Statistical analysis	164
----------------------------	-----

3.8 Results	164
-------------	-----

3.8.1 Preparation of taxane (PTX) loaded PLA based blend block copolymeric NPs	164
--	-----

3.8.2 Characterisation of STP loaded PLA based blend block copolymeric NPs	164
--	-----

(i) Hydrodynamic size, PDI and zeta potential	165
---	-----

(ii) HR-TEM analysis of STP loaded PLA based blend block copolymeric NPs	165
--	-----

(iii) <i>In-vitro</i> release studies of drugs from the PLA based blend block copolymeric NPs	166
3.8.3 Cytotoxicity evaluation of STP loaded PLA based blend block copolymeric NPs	166
3.8.4 <i>In-vivo</i> tumor regression study of STP loaded PLA based blend block copolymeric NPs	167
3.9 Discussion	168
<b>PART B (II): Preparation, Characterization &amp; Evaluation of Anthracycline (PIRA/DOX) Loaded NPs for Cancer Therapy</b>	
3.10 Materials	169
3.11 Methods	170
3.11.1 Preparation of PIRA/DOX loaded PLA based blend block copolymeric NPs	170
3.11.2 Characterization of PIRA/DOX loaded PLA based blend block copolymeric NPs	171
(i) Hydrodynamic size, PDI and zeta potential	171
(ii) HR-TEM analysis of PIRA/DOX loaded NPs	172
(iii) <i>In-vitro</i> release of PIRA/DOX from PIRA/DOX loaded PLA based blend block copolymeric NPs	172
3.11.3 <i>In-vitro</i> cell proliferation inhibition studies of PIRA/DOX encapsulated PLA based blend block copolymeric NPs	172
3.11.4 <i>In-vitro</i> inhibition of mammospheres by PIRA/DOX encapsulated PLA based blend block copolymeric NPs	173
3.11.5 <i>In-vivo</i> toxicity evaluation of PIRA/DOX encapsulated PLA based blend block copolymeric NPs	173

3.11.6	<i>In-vivo</i> tumor regression studies of PIRA/DOX encapsulated PLA based blend block copolymeric NPs	174
3.11.7	Chemo sensitising effect of penta-block copolymer	175
3.11.8	Statistical analysis	175
3.12	Results	176
3.12.1	Preparation of PIRA/DOX loaded PLA based blend block copolymeric NPs	176
3.12.2	Characterization of PIRA/DOX loaded PLA based blend block copolymeric NPs	176
(i)	Hydrodynamic size, PDI and zeta potential	176
(ii)	HR-TEM analysis of PIRA/DOX loaded NPs	177
(iii)	<i>In-vitro</i> release of PIRA/DOX from PLA based blend block copolymeric NPs	179
3.12.3	<i>In-vitro</i> cell proliferation inhibition studies of PIRA/DOX encapsulated PLA based blend block copolymeric NPs	180
3.12.4	<i>In-vitro</i> inhibition of mammospheres by PIRA/DOX encapsulated PLA based blend block copolymeric NPs	182
3.12.5	<i>In-vivo</i> toxicity evaluation of PIRA/DOX encapsulated PLA based blend block copolymeric NPs	183
3.12.6	<i>In-vivo</i> tumor regression studies of PIRA/DOX encapsulated PLA based blend block copolymeric NPs	186
3.12.7	Chemo sensitising effect of penta-block copolymer	189
3.13	Discussion	191
3.14	Conclusion	195

**Chapter 4: Development and characterization of PLA based block copolymeric nanoparticles for Concomitant delivery of PIRA/DOX with SAL**

4.1 Introduction	205
4.2 Materials	206
4.3 Methods	206
4.3.1 Preparation of drug loaded PLA based blend block copolymeric NPs	206
4.3.2 Characterization of drug loaded PLA based blend block copolymeric NPs	208
(i) Hydrodynamic size, PDI and zeta potential of drug loaded NPs	208
(ii) HR-TEM analysis of drug loaded NPs	209
(iii) <i>In-vitro</i> release studies of the drugs from PLA based blend block copolymeric NPs	209
4.3.3 <i>In-vitro</i> cell proliferation inhibition assay of drug loaded PLA based blend block copolymeric NPs	209
4.3.4 Cancer stem cells inhibition assay of drug loaded PLA based blend block copolymeric NPs	210
4.3.5 <i>In-vivo</i> tumor regression study of drug loaded PLA based blend block copolymeric NPs	210
4.3.6 Statistical analysis	212
4.4 Results	212
4.4.1 Preparation of drug loaded PLA based blend block copolymeric NPs	212
4.4.2 Characterization of drug loaded PLA based blend block copolymeric NPs	213
(i) Hydrodynamic size, PDI and zeta potential of drug loaded NPs	213

(ii) HR-TEM analysis of drug loaded NPs\	213
(iii) <i>In-vitro</i> release studies of the drugs from PLA based blend block copolymeric NPs	218
4.4.3 <i>In-vitro</i> cell proliferation inhibition assay of drug loaded PLA based blend block copolymeric NPs	218
4.4.4 Cancer stem cells inhibition assay of drug loaded PLA based blend block copolymeric NPs	226
4.4.5 <i>In-vivo</i> tumor regression study of drug loaded PLA based blend block copolymeric NPs	227
4.5 Discussion	230
4.6 Conclusion	238

## **Chapter 5: Summary and future scope of the research work**

5.1 Summary and conclusion	245
5.2 Future scope of the research work	255
<b>Answers to the examiner's comments</b>	256
<b>List of Publication &amp; Patents</b>	258
<b>Curriculum Vitae</b>	260

# LIST OF FIGURES

## **Chapter 1: Introduction and literature survey**

Figure 1.1:	Hallmarks of the cancer	8
Figure1.2:	Different classes of chemotherapeutic drugs and their targeted phases of cell cycle	14
Figure1.3:	Cancer stem cells and their asymmetric division to generate heterogeneity in the bulk tumor.	23
Figure1.4:	Structural representation of cancer stem cell inhibitor Salinomycin	26
Figure 1.5:	Mechanism of action of Salinomycin as a cancer stem cell inhibitor	31
Figure 1.6:	Types of nanoparticles use as drug delivery systems	37
Figure 1.7:	Structural representation of L-& D-lactic acid	42
Figure 1.8:	Schematic representation of the research work. mPEG-PLA: Methoxy polyethylene glycol-Polylactic acid; PLA-Pluronic L61-PLA: Polylactic acid-Pluronic L61-Polylactic acid; CSCs: Cancer stem cells; SAL: Salinomycin; PIRA: Pirarubicin; DOX: Doxorubicin; Primary chemo drugs – Paclitaxel, Pirarubicin & Doxorubicin.	48

## **Chapter 2: Development and characterization of PLA based block copolymeric nanoparticles for delivery of SAL as cancer stem cell inhibitor**

Figure 2.1:	Schematic representation of ring opening polymerization of L-lactide using mixture of polymerizing initiators mPEG and Pluronic L-61.	82
-------------	---	----

Figure 2.2:	Derivatization of SAL using vanillin assay to facilitate its detection by UV-Vis spectrophotometer.	86
Figure 2.3:	GPC chromatogram of (a) THF solvent and (b) PLA based hybrid block copolymer	98
Figure 2.4:	<sup>1</sup> H NMR spectra of PLA based hybrid block copolymer	98
Figure 2.5:	ATR-FTIR spectra of PLA based hybrid block copolymer	99
Figure 2.6:	XRD pattern of PLA based hybrid block copolymer	100
Figure 2.7:	DSC thermogram of PLA based hybrid block copolymer.	101
Figure 2.8:	Characterization of SAL loaded PLA based hybrid block copolymeric NPs. (A) Hydrodynamic size (nm), (B) Zeta potential (mV).	102
Figure 2.9:	Stability study of SAL NPs. (A) Change in hydrodynamic size &, (B) zeta potential of SAL loaded NPs over a period of time at various temperatures (4°C, room temperature & 37°C).	103
Figure 2.10:	Characterization of SAL loaded PLA based hybrid block copolymeric NPs. (A) HR-TEM image of SAL NPs, (B) FE-SEM image of SAL NPs	106
Figure 2.11:	<i>In-vitro</i> release studies of SAL from their NPs at 37°C in PBS (pH 7.4). (A) Per day release of SAL till 15 days. (B) Cumulative release of SAL till 15 days.	106
Figure 2.12:	Cellular uptake study of PLA based hybrid block copolymeric NPs. (A) CLSM image of C6 and Rh-B NPs internalized MDA-MB 231 cells, (B) CLSM image of C6 NPs internalized SUM-149 mammosphere, (C) flow cytometry histogram of C6 and Rh-B NPs internalized MDA-MB 231 cells.	108
Figure 2.13:	Safety evaluation of PLA based hybrid block copolymeric NPs by cytocompatibility assay of blank NPs against HEK 293 and NIH 3T3 cells	109

Figure 2.14:	Safety evaluation of PLA based hybrid block copolymeric NPs by hemocompatibility analysis of blank NPs.	109
Figure 2.15:	Dose dependent non-linear regression curve and IC <sub>50</sub> values of tumor spheroid (generated from chemotherapy resistant SCLC NCI-H69AR cells) after treatment with free SAL and SAL loaded NPs	111
Figure 2.16:	Image of tumor spheroids (generated from chemotherapy resistant SCLC NCI-H69AR cells) after treatment with free SAL and SAL loaded NPs.	112
Figure 2.17:	Dose dependent non-linear regression curve of mammospheres generated from TNBC cell MDA-MB 231 after treatment with free SAL and SAL loaded NPs	113
Figure 2.18:	Images of the mammospheres (generated from TNBC patient's tumor samples) before and after the treatment with free SAL and SAL loaded NPs. Scale bar representing 200 $\mu$ m.	115
Figure 2.19:	Quantification of CSCs of mammospheres (generated from TNBC patient's tumor samples) after treatment with free SAL and SAL loaded NPs.	116
Figure 2.20:	Maximum tolerated dose of free SAL and SAL loaded NPs on healthy syngeneic BALB/c mice. Doses were given once a week for three weeks using I.V. and I.P. routes of administration. Drop in the graph shows day of the deaths of mice.	118
Figure 2.21:	Chromatogram of SAL obtained from LC-MS-MS	119
Figure 2.22:	Pharmacokinetic curve free SAL and SAL loaded NPs denoting time vs concentration of SAL in the blood plasma	120
Figure 2.23:	Biodistribution profile of free SAL and SAL loaded NPs in vital organs (Liver, Kidney, Heart, Lung, and Spleen) and tumor.	121
Figure 2.24:	<i>In-vivo</i> EAC tumor regression curve after treatment with free SAL and SAL loaded NPs. Relative tumor volume = tumor volume on measured day (V)/ tumor volume on first day of dosing (V <sub>0</sub> ). ****p $\leq$ 0.0001.	124

Figure 2.25:	Change in body weight of the mice after treatment with free SAL and SAL loaded NPs.	124
Figure 2.26:	Histopathology analysis of vital organ of mice after treatment with free SAL (2.5 mg/kg) and SAL loaded NPs (7.5 mg/kg) once a week for three weeks. Scale bar represents 100 $\mu$ m.	125
Figure 2.27:	Kaplan meier curve showing survival/death of the mice after treatment with free SAL and SAL loaded NPs	126

### **Chapter 3: Development and characterization of PLA based block copolymeric nanoparticles for delivery of primary chemotherapeutic drugs**

Figure 3.1:	Structural differences in Doxorubicin and Pirarubicin	142
Figure 3.2:	Schematic representation of ring opening polymerization of L-lactide using polymerizing initiators mPEG and Pluronic L-61.	144
Figure 3.3:	<sup>1</sup> H NMR spectra of PLA based di-block (mPEG-PLA) and penta-block (PLA-Pluronic L61-PLA) copolymers	149
Figure 3.4:	ATR-FTIR spectra of PLA based (a) di-block (mPEG-PLA) and (b) penta-block (PLA-Pluronic L61-PLA) copolymers	150
Figure 3.5:	GPC chromatogram of (a) THF solvent (b) di-block (mPEG-PLA) and (c) penta-block (PLA-Pluronic L61-PLA) copolymers.	151
Figure 3.6:	DSC thermogram of PLA based di-block (mPEG-PLA) and penta-block (PLA-Pluronic L61-PLA) copolymers	153
Figure 3.7:	XRD pattern of PLA based di-block (mPEG-PLA) and penta-block (PLA-Pluronic L61-PLA) copolymers	154

Figure 3.8:	HR-TEM image of blank PLA based blend (80% mPEG-PLA+20% PLA-Pluronic L61-PLA) block copolymeric NPs. Scale bar representing 200 nm.	155
Figure 3.9:	Cytocompatibility analysis of blank PLA based blend block copolymeric NPs on HEK 293 and NIH 3T3 cells.	156
Figure 3.10:	Hemocompatibility analysis of blank PLA based blend block copolymeric NPs	156
Figure 3.11:	Cellular uptake of PLA based blend block copolymeric nanoparticles. (A) CLSM images of C6 NPs internalized monolayer cultured SUM-149 cells at 40X and 60X magnifications. (B) Bright field and C6 internalized fluorescent images of SUM-149 generated mammosphere. (C) Flow cytometry histogram of C6 NPs internalized SUM-149 cells.	158
Figure 3.12:	Modification of PTX into a pro-drug molecule by conjugating it with stearic acid	162
Figure 3.13:	HR-TEM image of STP loaded NPs. Scale bar representing 100 nm.	165
Figure 3.14:	<i>In-vitro</i> release study of STP from STP loaded PLA based blend block copolymeric NPs in physiological pH 7.4 and 37°C	166
Figure 3.15:	<i>In-vitro</i> cell proliferation inhibition assay of STP loaded PLA based blend block copolymeric NPs against TNBC cell lines	167
Figure 3.16:	<i>In-vivo</i> tumor regression studies of free PTX and STP loaded PLA based blend block copolymeric NPs. Relative tumor volume = tumor volume on the measured day (V)/ tumor volume on first day of dosing (V <sub>0</sub> ). **p≤0.01.	168
Figure 3.17:	Characterization of PIRA/DOX loaded PLA based blend block copolymeric NPs. (A) Hydrodynamic size of PIRA/DOX loaded NPs acquired by DLS. (B) Zeta potential of PIRA/DOX loaded NPs acquired by DLS.	177
Figure 3.18:	HR-TEM images of PIRA/DOX loaded PLA based blend block copolymeric NPs. Scale bar represents 200 nm.	178

- Figure 3.19: *In-vitro* release profile of PIRA/DOX from PLA based blend block copolymeric NPs at pH 7.4 and pH 5 and 37°C. 180
- Figure 3.20: *In-vitro* cell proliferation inhibition studies of PIRA/DOX encapsulated PLA based blend block copolymeric NPs.(A) Dose dependent non-linear regression curve of human TNBC (a-c) and mouse EAC (d) cells. (B) IC<sub>50</sub> values on cancer cells showing superior anti-cancer activity of PIRA NPs over DOX NPs. \* $p \leq 0.05$ , \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$  181
- Figure 3.21: *In-vitro* inhibition studies of mammospheres by PIRA/DOX and their PLA based blend block copolymeric NPs. \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ , \*\*\*\* $p \leq 0.0001$  184
- Figure 3.22: *In-vivo* toxicity evaluation of free and PLA based blend block copolymeric NPs encapsulated PIRA/DOX. (A) Change in body weight of mice after treatment with (a) 3 mg/kg, (b) 5 mg/kg and (c) 7.5 mg/kg dose of free and NPs loaded drugs. (B) Kaplan meier curve showing survival/death of the mice throughout the treatment period. 185
- Figure 3.23: *In-vivo* tumor regression studies of free PIRA/DOX and their NPs. Graph representing change in tumor volume each day after treatment. Relative tumor volume = tumor volume on the measured day (V)/ tumor volume on first day of dosing (V<sub>0</sub>). \* $p \leq 0.05$ . 187
- Figure 3.24: Histopathological images of cardiac tissue of all treated/untreated mice. Scale bar represents 100  $\mu$ m. 188
- Figure 3.25: Change in tumor volume after 20 days of incubation period. \*\* $p \leq 0.01$ , \*\*\* $p \leq 0.001$ . 188
- Figure 3.26: Effect of PLA-Pluronic L61-PLA block copolymer on survival of TNBC cells treated with PIRA/DOX encapsulated PLA based blend block copolymeric NPs. \*\* $p \leq 0.01$  190

Figure 3.27:	Effect of PLA-Pluronic L61-PLA block copolymer on growth of EAC tumor treated with PIRA/DOX encapsulated PLA based blend block copolymeric NPs. $p < 0.05$ represents that differences are significant	190
--------------	--	-----

## **Chapter 4: Development and characterization of PLA based block copolymeric nanoparticles for Concomitant delivery of PIRA/DOX with SAL**

Figure 4.1:	Hydrodynamic size of single (PIRA/DOX/SAL) and dual drug (PIRA:SAL/DOX:SAL) loaded PLA based blend block copolymeric NPs. $p < 0.05$ representing that difference is significant.	216
Figure 4.2:	HR-TEM image of PIRA:SAL (1:1) co-loaded PLA based blend block copolymeric NPs	217
Figure 4.3:	HR-TEM image of DOX:SAL (3:1) co-loaded PLA based blend block copolymeric NPs	217
Figure 4.4:	<i>In-vitro</i> release behaviour of PIRA & SAL from single and dual drug loaded PLA based blend block copolymeric NPs at 37°C in PBS (pH 7.4)	219
Figure 4.5:	<i>In-vitro</i> release behaviour of DOX & SAL from single and dual drug loaded PLA based blend block copolymeric NPs at 37°C in PBS (pH 7.4)	220
Figure 4.6:	<i>In-vitro</i> cell proliferation inhibition assay of single (PIRA/SAL) and dual drug (PIRA:SAL) loaded NPs. (A) Non-linear regression curve of cancer cells treated with drug loaded NPs. (B) Combination index vs fraction affected graph of cancer cells treated with PIRA:SAL co-loaded NPs.	224
Figure 4.7:	<i>In-vitro</i> cell proliferation inhibition assay of single (DOX/SAL) and dual drug (DOX:SAL) loaded NPs. (A) Non-linear regression curve of cancer cells treated with drug loaded NPs. (B) Combination index vs fraction affected graph of cancer cells treated with DOX:SAL co-loaded NPs.	225

- Figure 4.8: *In-vitro* mammosphere's inhibition assay using single (PIRA/DOX/SAL) and dual drug (PIRA:SAL/DOX:SAL) loaded PLA based blend block copolymeric NPs 228
- Figure 4.9: *In-vivo* tumor regression studies of drug loaded PLA based blend block copolymeric NPs. (A) EAC tumor regression curve after treatment with PIRA NPs (2 mg/kg), SAL NPs (2 mg/kg) and PIRA:SAL (2+2 mg/kg) NPs. (B) EAC tumor regression curve after treatment with DOX NPs (3 mg/kg), SAL NPs (1 mg/kg) and DOX:SAL (3+1 mg/kg) NPs. Relative tumor volume = tumor volume on measurement day (V)/tumor volume on the day of first dose (V<sub>0</sub>). \*\*\*p≤0.001, \*\*\*\*p≤0.0001, ns: non-significant 231
- Figure 4.10: Changes in the body weight of mice after treatment with drug loaded PLA based blend block copolymeric NPs. (A) PIRA NPs (2 mg/kg), SAL NPs (2 mg/kg) and PIRA:SAL (2+2 mg/kg) co-loaded NPs. (B) DOX NPs (3 mg/kg), SAL NPs (1 mg/kg) and DOX:SAL (3+1 mg/kg) co-loaded NPs. 232
- Figure 4.11: Histopathology analysis of heart tissue after treatment with drug loaded NPs. Sections of heart tissue was stained with Hematoxylin and Eosin. Images were captured at magnification of 40X. SAL NPs of upper row & lower row depicting 2 mg/kg & 1 mg/kg doses respectively. Scale bar representing 100 μm. 233
- Figure 4.12: Kaplan meier curve showing survival probability of mice after treatment with (A) PIRA NPs (2mg/kg), SAL NPs (2mg/kg) and PIRA:SAL (2+2 mg/kg) NPs and (B) DOX NPs (3mg/kg), SAL NPs (1mg/kg) and DOX:SAL (3+1 mg/kg) NPs. 234

# LIST OF TABLES

## **Chapter 1: Introduction and literature survey**

Table 1.1:	Cell surface phenotype of cancer stem cells.	18
Table 1.2:	Cancer stem cell signalling pathways and their inhibitors.	24
Table 1.3:	Nanocarrier based drug delivery systems in market or under clinical trials for cancer therapy.	39

## **Chapter 2: Development and characterization of PLA based block copolymeric nanoparticles for delivery of SAL as cancer stem cell inhibitor**

Table 2.1:	Physiochemical properties of the PLA based hybrid block copolymeric NPs	103
Table 2.2:	Selection of cryoprotectant to stabilize the SAL NPs in freeze dried form.	104
Table 2.3:	Optimization of minimum required concentration of glucose to stabilize SAL NPs	105
Table 2.4:	IC <sub>50</sub> values on cancer cell lines treated with SAL NPs and free SAL	110
Table 2.5:	Pharmacokinetics studies of free SAL and SAL loaded NPs	122

## **Chapter 3: Development and characterization of PLA based block copolymeric nanoparticles for delivery of primary chemotherapeutic drugs**

Table 3.1:	<sup>1</sup> H NMR characterisation of PLA based block copolymers	150
------------	---	-----

Table 3.2:	Molecular weight analysis of PLA based block copolymers using GPC	151
Table 3.3:	Optimization of weight ratio of di-block and penta-block copolymer for preparation of blank PLA based blend block copolymeric NPs	154
Table 3.4:	Characterization of the nanoparticles	178
Table 3.5:	<i>In-vitro</i> cell proliferation inhibition studies on monolayer cultured cancer cell	182

#### **Chapter 4: Development and characterization of PLA based block copolymeric nanoparticles for Concomitant delivery of PIRA/DOX with SAL**

Table 4.1:	Characterisation of PIRA, SAL & PIRA:SAL loaded PLA based blend block copolymeric NPs	214
Table 4.2:	Characterisation of DOX, SAL & DOX:SAL loaded PLA based blend block copolymeric NPs	215
Table 4.3:	<i>In-vitro</i> cell proliferation inhibition study of PIRA and SAL single and dual NPs on monolayer cell culture	221
Table 4.4:	<i>In-vitro</i> cell proliferation inhibition study of DOX and SAL single and dual NPs on monolayer cell culture	222
Table 4.5:	Combination index of PIRA:SAL co-loaded NPs evaluated against cancer cells	223
Table 4.6:	Combination index of DOX:SAL co-loaded NPs evaluated against cancer cells	223

# GLOSSARY OF SYMBOLS AND ABBREVIATIONS

<b>%</b>	percent
<b>w/v</b>	weight/volume
<b>v/v</b>	volume/volume
<b>m/z</b>	mass/charge
<b>Å</b>	angstrom
<b>μ</b>	micron
<b>μm</b>	micrometre
<b>μg</b>	microgram
<b>μL</b>	microlitre
<b>μJ</b>	microjoule
<b>μM</b>	micromole
<b>mM</b>	millimole
<b>ng</b>	nanogram
<b>mg</b>	milligram
<b>°C</b>	degree centigrade
<b>mm</b>	millimeter

<b>cm</b>	centimeter
<b>mm<sup>3</sup></b>	cubic millimeter
<b>g</b>	gram
<b>h</b>	hour
<b>Hz</b>	hertz
<b>L</b>	litre
<b>M</b>	molar
<b>N</b>	normal
<b>P</b>	probability
<b>V</b>	volt
<b>W</b>	watt
<b>dL</b>	decilitre
<b>kV</b>	kilovolt
<b>eV</b>	electron volts
<b>kDa</b>	kilodalton
<b>kHz</b>	kilohertz
<b>mA</b>	milliamphere
<b>mmol</b>	millimole
<b>mol</b>	mole

<b>min</b>	minute
<b>mL</b>	milliliter
<b>nm</b>	nanometer
<b>rpm</b>	rate per minute
<b>Rf</b>	retention factor
<b>IU</b>	international unit
<b>NP</b>	nanoparticle
<b>U/L</b>	unit/litre
<b>SEM</b>	scanning electron microscopy
<b>TEM</b>	transmission electron microscopy
<b>HR</b>	high resolution
<b>MWCO</b>	molecular weight cut off
<b>PXRD</b>	powder x-ray diffraction
<b>e.g.</b>	for example
<b>i.e.</b>	that is
<b>ANOVA</b>	analysis of variance
<b>ATR-FTIR</b>	attenuated total reflection-fourier transform infrared spectroscopy
<b>CLSM</b>	confocal laser scanning microscope
<b>CSC</b>	cancer stem cell

<b>C6</b>	coumarin 6
<b>DC</b>	dendritic cell
<b>DDS</b>	drug delivery system
<b>DLS</b>	dynamic light scattering
<b>DMEM</b>	dulbecco's modified eagle medium
<b>DMSO</b>	dimethyl sulfoxide
<b>DNA</b>	deoxyribonucleic acid
<b>DOX</b>	doxorubicin
<b>EAT</b>	ehrlich ascites tumor
<b>EDTA</b>	ethylene diamine tetra-acetic acid
<b>EPR</b>	enhanced permeability retention
<b>FBS</b>	fetal bovine serum
<b>FDA</b>	food and drug administration
<b>FITC</b>	fluorescein isothiocyanate
<b>GPC</b>	gel permeation chromatography
<b>H&amp;E</b>	hematoxylin and eosin
<b>IC<sub>50</sub></b>	inhibition concentration
<b>IP</b>	intraperitoneal
<b>IT</b>	intratumoral

<b>IV</b>	intravenous
<b>MDR</b>	multidrug resistance
<b>mPEG</b>	methoxy polyethylene glycol
<b>mPEG-PLA</b>	methoxy polyethylene glycol-poly(lactic acid)
<b>NMR</b>	nuclear magnetic resonance
<b>NPs</b>	nanoparticles
<b>NS</b>	normal saline
<b>PBS</b>	phosphate buffer saline
<b>PD</b>	pharmacodynamics
<b>PDI</b>	polydispersity index
<b>PEG</b>	polyethylene glycol
<b>PEG-PPG-PEG</b>	polyethylene glycol-polypropylene glycol-polyethylene glycol
<b>pH</b>	potential of hydrogen
<b>PIRA</b>	pirarubicin
<b>PK</b>	pharmacokinetics
<b>PLA</b>	poly(lactic acid)
<b>PLA-PEG-PPG-PEG-PLA</b>	poly(lactic acid)-polyethylene glycol-polypropylene glycol-polyethylene glycol-poly(lactic acid)
<b>PTX</b>	paclitaxel

<b>RBC</b>	red blood corpuscle
<b>Rh-B</b>	rhodamine b
<b>RME</b>	receptor mediated endocytosis
<b>ROP</b>	ring opening polymerization
<b>RT</b>	room temperature
<b>SAL</b>	salinomycin
<b>THP</b>	tetrahydropyranyl
<b>TNBC</b>	triple negative breast cancer
<b>WBC</b>	white blood corpuscle