TARGETED DEI ?GS BASED NANOMEDICI ' TRIPLE NEGATIVE BREAST CANCER

RAJALETCHUMY VELOO KUTTY

(B. Eng., University of Malaysia Pahang, Malaysia)

A THESIS SUBMITTED

FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF CHEMICAL AND BIOMOLECULAR ENGINEERING

NATIONAL UNIVERSITY OF SINGAPORE

2015

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

PR	Progesterone receptor
ER	Estrogen receptor
HER2	Epidermal growth factor receptor 2
TNBC	Triple negative breast cancers
MDR	Multi-drug resistance
TAs	Therapeutic agents
RME	Receptor-mediated endocytosis
P-gp	P-glycoproteins
vitamin E TPGS	D-α-tocopheryl polyethylene glycol succinate
CMC	Critical micelle concentration
PLGA	Poly(lactic-co-glycolic acid)
TPDC	Cetuximab-conjugated docetaxel-loaded vitamin E TPGS micelle
TPD	Docetaxel-loaded vitamin E TPGS micelles
TPM	Coumarin 6-loaded vitamin E TPGS micelles
TPMC	Cetuximab-conjugated coumarin 6-loaded vitamin E TPGS mice
TPF	Did-loaded vitamin E TPGS micelles
TPFC	Cetuximab-conjugated did-loaded vitamin E TPGS micelles
TPMC	Cetuximab-conjugated coumarin 6-loaded vitamin E TPGS
F	Did dye
TP	TPGS micelles without drug
Dox	Taxotere®
pCR	Pathologic complete response
EGFR	Epidermal growth factor receptor
SRC	Proto-oncogene tyrosine-protein kinase src
MET	Met proto-oncogene, receptor tyrosine kinase
PARP1/2	Poly ADP ribose polymerase 1/2
PEG	Polyethylene glycol
RES	Reticuloendothelial system
EPR	Enhanced permeability and retention effect
NP	Polymeric nanoparticles
PLGA-PEG	Poly(d,l-lactic-co-glycolic acid)-block-poly(ethylene glycol)

PVA	Poly(vinyl alcohol)
M-NP	Mitaplatin nanoparticles
MAPK	Mitogen-activated protein kinase
Akt	Protein kinase b
c-kit	Tyrosine-protein kinase kit
FAK	Focal adhesion kinase
EGF	Epidermal growth factor
TGF-α	Transforming growth factor alpha
PI3K	Phosphatidylinositol 3-kinase
PKC	Protein kinase c
GPCRs	G-protein-coupled receptors
NRG4	Neuregulin 4
KRAS	GTPase KRas
FDA	Food and drug administration
HLB	Hydrophile–lipophile balance
CDI	1,10-carbonyldiimidazole
DMSO	Dimethyl sulfoxide
DCM	Dichloromethane
PBS	Phosphate buffered saline
EDC	N-(3-dimethylaminopropyl)-n-ethylcarbodiimide hydrochloride
NHS	N-hydroxysuccinimide
TEA	Triethylamine
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
EDTA	Trypsin-ethylenediaminetetraacétic acid
PI	Propidium iodide
FBS	Fetal bovine serum
TPGS-CDI	Imidazole carbamate intermediate
UP	Ultrapure
MWCO	Molecular weight cut-off
ATCC	American type culture collection
DMEM	Dulbecco's modified eagle's medium
RPMI	Roswell park memorial institute
kcps	Kilo counts per second
rpm	Revolutions per minute
CO_2	Carbon dioxide

NaOH	Sodium hydroxide
DLS	Dynamic light scattering
FETEM	Field emission transmission electron microscope
XPS	X-ray photoelectron spectroscopy
HPLC	High performance liquid chromatography
CLSM	Confocal laser scanning microscopy
EPR	Enhance permeability and retention effect
PDI	Polydispersity
IC_{50}	Drug concentration needed to kill 50% of the cells in a designated time period
ROI	Region of interest
HE	Hematoxylin and eosin stains
H_2O_2	Hydrogen peroxide
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
RT-PCR	Reverse transcription polymerase chain reaction
CCNB1	G2/mitotic-specific cyclin-b1
CCNA2	Cyclin-A2
CDK2	Cyclin-dependent kinase 2
VEGF	Vascular endothelial growth factor
IL1β	Interleukin-1β
IGF-1	Insulin-like growth factor 1
TGFA	Transforming growth factor-alpha
TGFB1	Transforming growth factor beta 1

SUMMARY

Triple negative breast cancers (TNBC) can be classified as one of the most aggressive with a high rate of local recurrences and systematic metastases. TNBCs are insensitive to existing hormonal therapy or targeted therapies such as the use of monoclonal antibodies, due to the lack of estrogen receptor (ER) and progesterone receptor (PR) and the absence of overexpression of human epidermal growth factor receptor 2 (HER2) compared with other types of breast cancers. The absence of targeted therapies for selective delivery of therapeutic agents (TAs) into tumours, coupled with the multidrug resistance (MDR) which prevents their delivery, leads to the two consequences: the ineffectiveness of conventional chemotherapy on TNBC cells and considerable side effects to healthy cells due to non-specificity of the chemotherapeutic agents. Although nanomedicine has shown superior killing effects on TNBC cells compared with free drugs, there is need to identify the possible targeting agents in order to effectively deliver the nanomedicine into metastatic TNBC cells. At the outset of this study, only a handful of publications have reported the possible targeting agents used in clinical studies for the treatment of TNBCs. From clinical studies, TNBC patients have been found to show superior response rates with the combination therapy of chemotherapy and the anti-EGFR monoclonal antibody, cetuximab, compared with chemotherapy alone. However, the conventional method of delivering both cetuximab and the chemotherapeutic agents as free drugs does not show long term efficacy and therefore leads to a high rate of recurrences. We postulated that novel targeted nanomedicines – by virtue of the ability of nanocarriers to transport drugs specifically into the TNBC cells, compared

with free drug molecules – could enhance the delivery of chemotherapeutic agents into cells, thereby improving the killing effects and reducing the rate of recurrence.

In this study, we developed a targeted micellar system of cetuximab-conjugated micelles of D-α-tocopheryl polyethylene glycol succinate (vitamin E TPGS) for targeted delivery of docetaxel as a model anticancer drug for the treatment of TNBCs. The *in vitro* cytotoxicity studies has shown that TNBC cells exhibited a greater degree of drug resistance than the ER/PR or HER2 positive breast cancer cells after treatment with the free docetaxel drug, Taxotere® (Dox) which is used commercially in clinical studies. Interestingly, the drug resistance can be greatly attenuated by the docetaxel-loaded vitamin E TPGS (TPD) micellar formulation and further still by the cetuximab-conjugated, docetaxel-loaded vitamin E TPGS micelles (TPDC) to target the EGFR-overexpressing TNBC cells. Then, we used the EGFR-overexpressing cell line, MDA-MB-231/Luc, to develop the TNBC xenograft model in female CB-17 Severe Combined Immunodeficiency (SCID) mice. The real time *in vivo* biodistribution and tumour targeting ability of the micelles after intravenous injection (i.v.) were studied by the noninvasive IVIS® imaging system. The anti-tumour effects of the TPGS, docetaxel loaded targeting and non-targeting TPGS micelles were evaluated using the TNBC xenograft model, and compared with Taxotere®.

In order to elucidate the behaviours of the cancerous cells after the nanomedicine treatment, we performed explant cultures of the tumours pre-treated with one of our micelle formulations, and attempted to re-examine in

greater detail whether our drug-micelle system was indeed efficacious through *ex vivo* investigation. The *ex vivo* study has demonstrated that tumours treated with targeting micelles exhibited enhanced cell cycle arrest and attenuated proliferation compared with the control and with those treated non-targeting micelles. Furthermore, the *ex vivo* investigation revealed that both the targeting and non-targeting micellar formulations culminated in anti-angiogenesis effects and inhibition of metastases. Overall, both the *in vivo* and *ex vivo* data increased the confidence that our micellar formulations, TPDC, effectively targeted and inhibited EGFR-overexpressing MDA-MB-231 tumours.

References

A.Y.Chow. Cell Cycle Control by Oncogenes and Tumor Suppressors: driving the Transformation of Normal Cells into Cancerous Cells. Nat Educ 2010;3:7.

Arnedos M, Bihan C, Delaloge S, Andre F. Triple-negative breast cancer: are we making headway at least? Ther Adv Med Oncol 2012;4:195–210.

Arvizo RR, Saha S, Wang E, Robertson JD, Bhattacharya R, Mukherjee P. Inhibition of tumor growth and metastasis by a self-therapeutic nanoparticle. Proc Natl Acad Sci USA 2013;110:6700-7.

Baselga J, Norton L, Albanell J, Kim YM, Mendelsohn J. Recombinant humanized anti-HER2 antibody (Herceptin) enhances the antitumor activity of paclitaxel and doxorubicin against HER2/neu overexpressing human breast cancer xenografts. Cancer Res 1998;58:2825–31.

Baselga J. The EGFR as a target for anticancer therapy--focus on cetuximab. Eur J Cancer 2001;37 Suppl 4:S16–22.

Bauer KR, Brown M, Cress RD, Parise C a, Caggiano V. Descriptive analysis of estrogen receptor (ER)-negative, progesterone receptor (PR)-negative, and HER2-negative invasive breast cancer, the so-called triple-negative phenotype: a population-based study from the California cancer Registry. Cancer 2007;109:1721–8.

Bhattacharyya S, Bhattacharya R, Curley S, McNiven MA, Mukherjee P. Nanoconjugation modulates the trafficking and mechanism of antibody induced receptor endocytosis. Proc Natl Acad Sci USA 2010;107:14541–6.

Blanco E, Kessinger CW, Sumer BD, Gao J. Multifunctional micellar nanomedicine for cancer therapy. Exp Biol Med (Maywood) 2009;234:123–31.

Blanco E, Shen H, Ferrari M. Perspective Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat Biotechnol 2015;33:941–51.

Brabender J, Danenberg KD, Metzger R, Schneider PM, Park J, Salonga D, et al. Epidermal growth factor receptor and HER2-neu mRNA expression in non-small cell lung cancer is correlated with survival. Clin Cancer Res 2001;7:1850–5.

Burke AR, Singh RN, Carroll DL, Wood JC, D'Agostino RB Jr, Ajayan PM, et al. The resistance of breast cancer stem cells to conventional hyperthermia and their sensitivity to nanoparticle-mediated photothermal therapy. Biomaterials 2012;33:2961–70.

Cabral H, Matsumoto Y, Mizuno K, Chen Q, Murakami M, Kimura M, et al. Accumulation of sub-100 nm polymeric micelles in poorly permeable tumours depends on size. Nat Nanotechnol 2011;6:815–23.

Carey LA, Dees EC, Sawyer L, Gatti L, Moore DT, Collichio F, et al. The triple negative paradox: primary tumor chemosensitivity of breast cancer subtypes. Clin Cancer Res 2007;13:2329–34.

Chan SK, Hill ME, Gullick WJ. The role of the epidermal growth factor receptor in breast cancer. J Mammary Gland Biol Neoplasia 2006;11:3–11.

Chandrasekharan P, Maity D, Yong CX, Chuang KH, Ding J, Feng SS. Vitamin E (D-alpha-tocopheryl-co-poly(ethylene glycol) 1000 succinate) micelles-superparamagnetic iron oxide nanoparticles for enhanced thermotherapy and MRI. Biomaterials 2011;32:5663–72.

Cohen S, Ushiro H, Stoscheck C, Chinkers M. A native 170,000 epidermal growth factor receptor-kinase complex from shed plasma membrane vesicles. J Biol Chem 1982;257:1523–31.

De S, Cipriano R, Jackson MW, Stark GR. Overexpression of kinesins mediates docetaxel resistance in breast cancer cells. Cancer Res 2009;69:8035–42.

Dent R, Trudeau M, Pritchard KI, Hanna WM, Kahn HK, Sawka CA, et al. Triple-negative breast cancer: clinical features and patterns of recurrence. Clin Cancer Res 2007;13:4429–34.

DeVita VT, Chu E. A history of cancer chemotherapy. Cancer Res 2008;68:8643–53.

Doody JF, Wang Y, Patel SN, Joynes C, Lee SP, Gerlak J, et al. Inhibitory activity of cetuximab on epidermal growth factor receptor mutations in non small cell lung cancers. Mol Cancer Ther 2007;6:2642–51.

Domeselaar GHV, Kwon GS, Andrew LC. Application of solid phase peptide synthesis to engineering PEO- peptide block copolymers for drug delivery. Colloids Surf B Biointerfaces 2003;136:323–34. Duffy MJ, McGowan PM, Crown J. Targeted therapy for triple-negative breast cancer: where are we? Int J Cancer 2012;131:2471–7.

Fan Z, Lu Y, Wu X, Mendelsohn J. Antibody-induced epidermal growth factor receptor dimerization mediates inhibition of autocrine proliferation of A431 squamous carcinoma cells. J Biol Chem 1994;269:27595–602.

Feng SS, Chien S. Chemotherapeutic engineering: application and further development of chemical engineering principles for chemotherapy of cancer and other diseases. Chem Eng Sci 2003;58:4087–114.

Feng SS, Zhao L, Zhang Z, Bhakta G, Win KY, Dong Y, et al. Chemotherapeutic engineering: vitamin E TPGS-emulsified nanoparticles of biodegradable polymers realized sustainable paclitaxel chemotherapy for 168h in vivo. Chem Eng Sci 2007;62:6641–8.

Feng SS. Chemotherapeutic Engineering: concept, Feasibility, Safety and Prospect—A Tribute to Shu Chien's 80th Birthday. Cell Mol Bioeng 2011;4:708–16.

Fjällskog ML, Frii L, Bergh J. Is Cremophor EL, solvent for paclitaxel, cytotoxic? Lancet 1993;342:873.

Foulkes WD, Smith IE, Reis-Filho JS. Triple-negative breast cancer. N Engl J Med 2010;363:1938–48.

Freedman GM, Anderson PR, Li T, Nicolaou N. Locoregional recurrence of triple-negative breast cancer after breast-conserving surgery and radiation. Cancer 2009;115:946–51.

Gan CW, Feng SS. Transferrin-conjugated nanoparticles of poly(lactide)-D-alpha-tocopheryl polyethylene glycol succinate diblock copolymer for targeted drug delivery across the blood-brain barrier. Biomaterials 2010;31:7748–57.

Gaucher G, Dufresne MH, Sant VP, Kang N, Maysinger D, Leroux JC. Block copolymer micelles: preparation, characterization and application in drug delivery. J Control Release 2005;108:169-88.

Gelmon K, Dent R, Mackey JR, Laing K, McLeod D, Verma S. Targeting triple-negative breast cancer: optimising therapeutic outcomes. Ann Oncol 2012;23:2223–34.

Graham J, Muhsin M, Kirkpatrick P. Cetuximab. Nat Rev Drug Discov 2004;3:549–50.

Haffty BG, Yang Q, Reiss M, Kearney T, Higgins SA, Weidhaas J, et al. Locoregional relapse and distant metastasis in conservatively managed triple negative early-stage breast cancer. J Clin Oncol 2006;24:5652–7.

Hao YL, Deng YJ, Chen Y, Wang KZ, Hao AJ, Zhang Y. In-vitro cytotoxicity, in-vivo biodistribution and anti-tumour effect of PEGylated liposomal topotecan. J Pharm Pharmacol 2005;57:1279–87.

Harris RC, Chung E, Coffey RJ. EGF receptor ligands. Exp Cell Res 2003;284:2–13.

Henderson IC, Berry DA, Demetri GD, Cirrincione CT, Goldstein LJ, Martino S, et al. Improved outcomes from adding sequential paclitaxel but not from escalating doxorubicin dose in an adjuvant chemotherapy regimen for patients with node-positive primary breast cancer. J Clin Oncol 2003;21:976–83.

Herbst RS, Shin DM. Monoclonal antibodies to target epidermal growth factor receptor-positive tumors: a new paradigm for cancer therapy. Cancer 2002;94:1593–611.

Honary S, Zahir F. Effect of zeta potential on the properties of nano-drug delivery systems - A review (part 1). Trop J Pharm Res 2013;12:255–64.

Hudis CA. Trastuzumab--mechanism of action and use in clinical practice. N Engl J Med 2007;357:39–51.

Isakoff SJ. Triple-negative breast cancer: role of specific chemotherapy agents. Cancer J 2010;16:53–61.

Ismail-Khan R, Bui MM. A review of triple-negative breast cancer. Cancer Control 2010;17:173–6.

Ismailos G, Reppas C, Macheras P. Enhancement of cyclosporin a solubility by d-alphatocopheryl-polyethylene-glycol-1000 succinate (TPGS). Eur J Pharm Sci 1994;1:269–71.

Johnstone TC, Kulak N, Pridgen EM, Farokhzad OC, Langer R, Lippard SJ. Nanoparticle encapsulation of mitaplatin and the effect thereof on in vivo properties. ACS Nano 2013;7:5675–83.

Kaplan HG, Malmgren JA, Atwood M. T1N0 triple negative breast cancer: risk of recurrence and adjuvant chemotherapy. Breast J 2009;15:454–60.

Karapetis CS, Khambata-Ford S, Jonker DJ, O'Callaghan CJ, Tu D, Tebbutt NC, et al. K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 2008;359:1757–65.

Kennecke H, Yerushalmi R, Woods R, Cheang MCU, Voduc D, Speers CH, et al. Metastatic behavior of breast cancer subtypes. J Clin Oncol 2010;28:3271–7.

Kim KM, Kim HM, Lee WJ, Lee CW, Kim T, Lee JK, et al. Surface treatment of silica nanoparticles for stable and charge-controlled colloidal silica. Int J Nanomedicine 2014;9 Suppl 2:29–40.

Kim KR, Kim DR, Lee T, Yhee JY, Kim BS, Kwon IC, et al. Drug delivery by a self-assembled DNA tetrahedron for overcoming drug resistance in breast cancer cells. Chem Commun (Camb) 2013;49:2010–2.

Kreike B, van Kouwenhove M, Horlings H, Weigelt B, Peterse H, Bartelink H, et al. Gene expression profiling and histopathological characterization of triple-negative/basal-like breast carcinomas. Breast Cancer Res 2007;9:R65.

Kutty RV, Feng SS. Cetuximab conjugated vitamin E TPGS micelles for targeted delivery of docetaxel for treatment of triple negative breast cancers. Biomaterials 2013;34:10160–71.

Kutty RV, Wei Leong DT, Feng SS. Nanomedicine for the treatment of triple-negative breast cancer. Nanomedicine (Lond) 2014;9:561–4.

Kutty RV, Tay CY, Lim CS, Feng S-S, Leong DT. Anti-migratory and increased cytotoxic effects of novel dual drug-loaded complex hybrid micelles in triple negative breast cancer cells. Nano Res 2015;8:2533-47.

Kyndi M, Sørensen FB, Knudsen H, Overgaard M, Nielsen HM, Overgaard J. Estrogen receptor, progesterone receptor, HER-2, and response to postmastectomy radiotherapy in high-risk breast cancer: the Danish Breast Cancer Cooperative Group. J Clin Oncol 2008;26:1419–26.

Lasic DD. Mixed micelles in drug delivery. Nature 1992;355:279–80.

Lawrence MJ. Surfactant systems: their use in drug delivery. Chem Soc Rev 1994;23:417.

Lehmann BD, Bauer JA, Chen X, Sanders ME, Chakravarthy AB, Shyr Y, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. J Clin Invest 2011;121:2750–67.

Letchford K, Burt H. A review of the formation and classification of amphiphilic block copolymer nanoparticulate structures: micelles, nanospheres, nanocapsules and polymersomes. Eur J Pharm Biopharm 2007;65:259–69.

Lee J, Cho EC, Cho K. Incorporation and release behavior of hydrophobic drug in functionalized poly (D , L -lactide) -block – poly (ethylene oxide) micelles. J Control Release 2004;94:323–35.

Liedtke C, Mazouni C, Hess KR, André F, Tordai A, Mejia JA, et al. Response to neoadjuvant therapy and long-term survival in patients with triple-negative breast cancer. J Clin Oncol 2008;26:1275–81.

Liu Y, Li K, Liu B, Feng SS. A strategy for precision engineering of nanoparticles of biodegradable copolymers for quantitative control of targeted drug delivery. Biomaterials 2010;31:9145–55.

Livasy CA, Karaca G, Nanda R, Tretiakova MS, Olopade OI, Moore DT, et al. Phenotypic evaluation of the basal-like subtype of invasive breast carcinoma. Mod Pathol 2006;19:264–71.

Lo HW, Hung MC. Nuclear EGFR signalling network in cancers: linking EGFR pathway to cell cycle progression, nitric oxide pathway and patient survival. Br J Cancer 2006;94:184–8.

Longmire M, Choyke PL, Kobayashi H. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. Nanomedicine (Lond) 2008;3:703–17.

Maeda H, Wu J, Sawa T, Matsumura Y, Hori K. Tumor vascular permeability and the EPR effect in macromolecular therapeutics: a review. J Control Release 2000;65:271–84.

Malumbres M, Barbacid M. RAS oncogenes: the first 30 years. Nat Rev Cancer 2003;3:459–65.

Martin HL, Smith L, Tomlinson DC. Multidrug-resistant breast cancer: current perspectives. Breast Cancer (Dove Med Press) 2014;6:1–13.

Master AM, Qi Y, Oleinick NL, Gupta AS. EGFR-mediated intracellular delivery of Pc 4 nanoformulation for targeted photodynamic therapy of cancer: in vitro studies. Nanomedicine 2012;8:655–64. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer chemotherapy: mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 1986;46:6387–92.

Mi Y, Liu X, Zhao J, Ding J, Feng SS. Multimodality treatment of cancer with herceptin conjugated, thermomagnetic iron oxides and docetaxel loaded nanoparticles of biodegradable polymers. Biomaterials 2012;33:7519–29.

Mi Y, Liu Y, Feng SS. Formulation of docetaxel by folic acid-conjugated d-α-tocopheryl polyethylene glycol succinate 2000 (Vitamin E TPGS(2k)) micelles for targeted and synergistic chemotherapy. Biomaterials 2011;32:4058–66.

Mi Y, Zhao J, Feng SS. Targeted co-delivery of docetaxel, cisplatin and herceptin by vitamin E TPGS-cisplatin prodrug nanoparticles for multimodality treatment of cancer. J Control Release 2013;169:185–92.

Milenic DE, Wong KJ, Baidoo KE, Ray GL, Garmestani K, Williams M, et al. Cetuximab: preclinical evaluation of a monoclonal antibody targeting EGFR for radioimmunodiagnostic and radioimmunotherapeutic applications. Cancer Biother Radiopharm 2008;23:619–31.

Morse DL, Gray H, Payne CM, Gillies RJ. Docetaxel induces cell death through mitotic catastrophe in human breast cancer cells. Mol Cancer Ther 2005;4:1495–504.

Mu L, Feng SS. Vitamin E TPGS used as emulsifier in the solvent evaporation/extraction technique for fabrication of polymeric nanospheres for controlled release of paclitaxel (Taxol). J Control Release 2002;80:129–44.

Muthu MS, Kulkarni SA, Liu Y, Feng SS. Development of docetaxel-loaded vitamin E TPGS micelles : formulation optimization , effects on brain cancer cells and biodistribution in rats. Nanomedicine (Lond) 2011;7:353–64.

Muthu MS, Kulkarni SA, Raju A, Feng SS. Theranostic liposomes of TPGS coating for targeted co-delivery of docetaxel and quantum dots. Biomaterials 2012;33:3494–501.

Muthu MS, Kutty RV, Luo Z, Xie J, Feng SS. Theranostic vitamin E TPGS micelles of transferrin conjugation for targeted co-delivery of docetaxel and ultra bright gold nanoclusters. Biomaterials 2015;39:234–48.

Nguyen PL, Taghian AG, Katz MS, Niemierko A, Abi Raad RF, Boon WL, et al. Breast cancer subtype approximated by estrogen receptor, progesterone receptor, and her-2 is associated with local and distant recurrence after breast-conserving therapy. J Clin Oncol 2008;26:2373–8.

Nielsen TO, Hsu FD, Jensen K, Cheang M, Karaca G, Hu Z, et al. Immunohistochemical and clinical characterization of the basal-like subtype of invasive breast carcinoma. Clin Cancer Res 2004;10:5367–74.

Oda K, Matsuoka Y, Funahashi A, Kitano H. A comprehensive pathway map of epidermal growth factor receptor signaling. Mol Syst Biol 2005;1:2005.0010.

Oh N, Park JH. Endocytosis and exocytosis of nanoparticles in mammalian cells. Int J Nanomedicine 2014;9 Suppl 1:51–63.

Owen SC, Chan DPY, Shoichet MS. Polymeric micelle stability. Nano Today 2012;7:53-65.

Pal SK, Childs BH, Pegram M. Triple negative breast cancer: unmet medical needs. Breast Cancer Res Treat 2011;125:627–36.

Pei XF, Noble MS, Davoli MA, Rosfjord E, Tilli MT, Furth PA, et al. Explant-cell culture of primary mammary tumors from MMTV-c-Myc transgenic mice. In Vitro Cell Dev Biol Anim 2004;40:14–21.

Petit AM, Rak J, Hung MC, Rockwell P, Goldstein N, Fendly B, et al. Neutralizing antibodies against epidermal growth factor and ErbB-2/neu receptor tyrosine kinases down-regulate vascular endothelial growth factor

production by tumor cells in vitro and in vivo: angiogenic implications for signal transduction therapy of solid tumors. Am J Pathol 1997;151:1523–30.

Raff JP, Rajdev L, Malik U, Novik Y, Manalo JM, Negassa A, et al. Phase II study of weekly docetaxel alone or in combination with trastuzumab in patients with metastatic breast cancer. Clin Breast Cancer 2004;4:420–7.

Rakha EA, Ellis IO. Triple-negative/basal-like breast cancer. Pathology 2009;41:40–7.

Rakha EA, El-Sayed ME, Green AR, Lee AHS, Robertson JF, Ellis IO. Prognostic markers in triple-negative breast cancer. Cancer 2007;109:25–32.

Rivera E, Gomez H. Chemotherapy resistance in metastatic breast cancer: the evolving role of ixabepilone. Breast Cancer Res 2010;12 Suppl 2:S2.

Roepstorff K, Grøvdal L, Grandal M, Lerdrup M, van Deurs B. Endocytic downregulation of ErbB receptors: mechanisms and relevance in cancer. Histochem Cell Biol 2008;129:563–78.

Rowinsky EK, Onetto N, Canetta RM, Arbuck SG. Taxol: the first of the taxanes, an important new class of antitumor agents. Semin Oncol 1992;19:646–62.

Sadauskas E, Wallin H, Stoltenberg M, Vogel U, Doering P, Larsen A, et al. Kupffer cells are central in the removal of nanoparticles from the organism. Part Fibre Toxicol 2007;4:10.

Sánchez-Muñoz A, Gallego E, de Luque V, Pérez-Rivas LG, Vicioso L, Ribelles N, et al. Lack of evidence for KRAS oncogenic mutations in triple-negative breast cancer. BMC Cancer 2010;10:136.

Sarin H. Physiologic upper limits of pore size of different blood capillary types and another perspective on the dual pore theory of microvascular permeability. J Angiogenes Res 2010;2:14.

Setyawati MI, Tay CY, Chia SL, Goh SL, Fang W, Neo MJ, et al. Titanium dioxide nanomaterials cause endothelial cell leakiness by disrupting the homophilic interaction of VE-cadherin. Nat Commun 2013;4:1673.

Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA Cancer J Clin 2015;65:5–29.

Sun B, Feng SS. Trastuzumab-functionalized nanoparticles of biodegradable copolymers for targeted delivery of docetaxel. Nanomedicine (Lond) 2009;4:431–45.

Sunada H, Magun BE, Mendelsohn J, MacLeod CL. Monoclonal antibody against epidermal growth factor receptor is internalized without stimulating receptor phosphorylation. Proc Natl Acad Sci USA 1986;83:3825–9.

Swami A, Shi J, Gadde S, Votruba A, Kolishetti N, Farokhzad OC. Multifunctional Nanoparticles for Drug Delivery Applications: imaging, targeting and delivery. 1 ed. Svenson S, Prud'homme RK, editors. New York: Springer-Verlag New York; 2012.

Tan GR, Feng SS, Leong DT. The reduction of anti-cancer drug antagonism by the spatial protection of drugs with PLA-TPGS nanoparticles. Biomaterials 2014;35:3044–51.

Taurin S, Nehoff H, Diong J, Larsen L, Rosengren RJ, Greish K. Curcumin-derivative nanomicelles for the treatment of triple negative breast cancer. J Drug Target 2013;21:675–83.

Taurin S, Nehoff H, Greish K. Anticancer nanomedicine and tumor vascular permeability; where is the missing link? J Control Release 2012;164:265–75.

Vakifahmetoglu H, Olsson M, Zhivotovsky B. Death through a tragedy: mitotic catastrophe. Cell Death Differ 2008;15:1153–62.

Voduc KD, Cheang MCU, Tyldesley S, Gelmon K, Nielsen TO, Kennecke H. Breast cancer subtypes and the risk of local and regional relapse. J Clin Oncol 2010;28:1684–91.

Webster L, Linsenmeyer M, Millward M, Morton C, Bishop J, Woodcock D. Measurement of cremophor EL following taxol: plasma levels sufficient to reverse drug exclusion mediated by the multidrug-resistant phenotype. J Natl Cancer Inst 1993;85:1685–90.

Weiss RB, DeVita VT. Multimodal primary cancer treatment (adjuvant chemotherapy): current results and future prospects. Ann Intern Med 1979;91:251–60.

Wells A, Marti U. Signalling shortcuts: cell-surface receptors in the nucleus? Nat Rev Mol Cell Biol 2002;3:697–702.

Win KY, Feng SS. Effects of particle size and surface coating on cellular uptake of polymeric nanoparticles for oral delivery of anticancer drugs. Biomaterials 2005;26:2713–22.

Wong C, Chen S. The development, application and limitations of breast cancer cell lines to study tamoxifen and aromatase inhibitor resistance. J Steroid Biochem Mol Biol 2012;131:83–92.

Yagata H, Kajiura Y, Yamauchi H. Current strategy for triple-negative breast cancer: appropriate combination of surgery, radiation, and chemotherapy. Breast Cancer 2011;18:165–73.

Yamamoto Y, Yasugi K, Harada A, Nagasaki Y. Temperature-related change in the properties relevant to drug delivery of poly (ethylene glycol)–poly (D ,

L-lactide) block copolymer micelles in aqueous milieu. J Control Release 2002;82:359–71.

Zana R. Critical micellization concentration of surfactants in aqueous solution and free energy of micellization. Langmuir 1996;12:1208–11

Zhang W, Gordon M, Schultheis AM, Yang DY, Nagashima F, Azuma M, et al. FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab. J Clin Oncol 2007;25:3712–8.

Zhang Z, Tan S, Feng SS. Vitamin E TPGS as a molecular biomaterial for drug delivery. Biomaterials 2012;33:4889–906.

Zhao J, Mi Y, Feng SS. Targeted co-delivery of docetaxel and siPlk1 by herceptin-conjugated vitamin E TPGS based immunomicelles. Biomaterials 2013;34:3411–21.

Zhao J, Mi Y, Liu Y, Feng SS. Quantitative control of targeting effect of anticancer drugs formulated by ligand-conjugated nanoparticles of biodegradable copolymer blend. Biomaterials 2012;33:1948–58.

Appendix B: List of relevant publications gained during candidature

1. **Kutty RV**, Feng SS. Cetuximab conjugated vitamin E TPGS micelles for targeted delivery of docetaxel for treatment of triple negative breast cancers. **Biomaterials**.2013.34(38):10160-71. (IF:8.31)

2. **Kutty RV**, Leong DT, Feng SS. Nanomedicine for Treatment of Triple Negative Breast Cancer. **Nanomedicine.**2014. 9(5): 561-564. (IF: 5.26)

3. **Kutty RV**[#], Tay CY[#], Chen SL, Feng SS*, Leong DT. Anti-migratory and increased cytotoxic effects of novel dual drug loaded complex hybrid micelles in triple negative breast cancer cells. **Nano Research** 2015.8:2533-2547. (# the authors are equally contributed)

4. Muthu MS[#], **Kutty RV**[#], Luo Z, Xie J, Feng SS. Theranostic vitamin E TPGS micelles of transferrin conjugation for targeted co-delivery of docetaxel and ultra bright gold nanoclusters. **Biomaterials**. 2013.34:10160-71. (IF: 8.31) (# the authors are equally contributed)

5. Setyawati MI[#], **Kutty RV**[#] ,Tay CY, Yuan X, Xie J, Leong DT. Novel theranostic DNA nano-scaffolds for the simultaneous detection and killing of Escherichia coli and Staphylococcus aureus. **ACS Appl Mater Interfaces.**2014.6:21822-31.(IF: 5.01) (#the authors are equally contributed)

6. **Kutty RV**, Chia SL, Setyawati MI, Muthu MS, Feng SS, Leong DT. *In vivo* and *ex vivo* proofs of concept that cetuximab conjugated vitamin E TPGS micelles increases efficacy of delivered docetaxel against triple negative breast cancer.**Biomaterials**. 2015.63:58-59 (IF: 8.31)