

TARGETED DELIVERY

NANOMEDICINES

FOR THE TREATMENT OF

PERPUSTAKAAN UMP



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TRIPLE NEGATIVE BREAST CANCER

TRIPLE

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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 micelle
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 micelles
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-ethylenediaminetetraacetic 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 ₂ O 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
IL1 β	Interleukin-1 β
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.

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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)