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Genetic engineering of tumour-infiltrating monocytes to inhibit metastatic breast cancer

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Abstract

160 million women develop Breast Cancer (BC) annually with subsequent 500,000 deaths, and therefore it is the second most common cause of cancer related deaths in women. Improved treatments together with earlier diagnosis made the disease more manageable, however 30 – 40% of patients will eventually develop metastasis. Metastasis prognosis remains poor, and new therapies effective against advanced and disseminated breast cancer are needed.

Bone is the third most common site for metastatic cancers, after lung and liver. Understanding the molecular pathways promoting bone metastasis may offer new therapeutic targets. Silencing of interferon-α (IFNα) signalling was recently reported as one of the breast cancer bone metastasis hallmarks. Reversing this, may inhibit bone metastasis formation and progression. IFNα is a potent immune stimulator and anti-tumoural molecule, however, its clinical use is limited by the systemic toxicity associated with its therapeutic doses. Therefore, novel therapeutic strategies are essential for the tumour target delivery of IFNα.

Our lab together with Prof Luigi Naldini (TIGET, Milan, Italy) have developed a novel platform for the target delivery of bio-therapeutic molecules to tumour tissues or metastatic organs. This strategy is based on autologous transplantation of gene-modified haematopoietic stem/progenitor cells (HS/PC) expressing the IFNα gene under the control of a cell-specific promoter: the Tie2 promoter. Transplanted HS/PCs then differentiate into all haematopoietic lineages including a subset of tumour-infiltrating monocytes (Tie2-expressing monocytes, TEMs), which specifically upregulate Tie2 after homing to tumour tissues. They previously demonstrated its ability to inhibit primary tumours and lung metastasis in a mouse model of Luminal B breast cancer. They also developed the human delivery platform and showed inhibition of human primary TNBC in a humanised preclinical model. Of note, tumour targeted delivery of IFNα had a positive toxicity profile unlike its systemic administration.

Here, we show that this IFNα-delivery strategy was also able to inhibit TNBC lung and bone metastasis in an immunocompetent preclinical murine model and in a humanised mouse model. Moreover, we tested its ability to improve the outcome of immune checkpoint modulators, or cancer vaccination. As immune checkpoint modulators, we used a new combination currently under investigation in our laboratory that involves anti-PD-1 and anti-41BB antibodies. As a cancer vaccination strategy, we used a novel platform developed in our laboratory for the *in vivo* delivery of relevant antigen formulations to Clec9A+ dendritic cells and based on tailorable oil-in-water nanoemulsion (Clec9A-TNE). Our results show that this IFNα delivery

strategy was able to improve the outcome of cancer vaccination but did not synergies with anti-PD1 and 41-BB.

Finally, thinking about a possible clinical translation, we explored the use of adoptively transferred Tie2-IFNα mature monocytes for the tumour targeted delivery of IFNα in combination with low doses of chemotherapy (Doxorubicin) and showed a significant improvement in the survival of mice treated with this novel combination therapy.

Overall, our data support the clinical translation of our gene- and cell-based therapy for the tumour targeted delivery of IFNα, which has a good safety profile and can enhance therapeutic efficacy when given in combination with best-matching therapies.

Declaration by author

This thesis is composed of my original work, and contains no material previously published or written by another person except where due reference has been made in the text. I have clearly stated the contribution by others to jointly-authored works that I have included in my thesis.

I have clearly stated the contribution of others to my thesis as a whole, including statistical assistance, survey design, data analysis, significant technical procedures, professional editorial advice, financial support and any other original research work used or reported in my thesis. The content of my thesis is the result of work I have carried out since the commencement of my higher degree by research candidature and does not include a substantial part of work that has been submitted to qualify for the award of any other degree or diploma in any university or other tertiary institution. I have clearly stated which parts of my thesis, if any, have been submitted to qualify for another award.

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Publications included in this thesis

No publications included. A paper is pending a patent approval before submission.

Submitted manuscripts included in this thesis

No manuscripts submitted for publication.

Other publications during candidature

Conference abstracts

Sherif M., Engineering Haematopoiesis for Tumour Targeted Delivery of IFNα Inhibit Breast Cancer Metastases in Murine and Human Haematochimeric Models. Oral presentation: UQ health symposium FOM, Brisbane, 2018.

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Contributions by others to the thesis

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Statement of parts of the thesis submitted to qualify for the award of another degree

No works submitted towards another degree have been included in this thesis.

Research involving human or animal subjects

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List of Abbreviations

ADCC	Antibody-dependent cellular cytotoxicity
ADT	Adoptive Transfer
ANG-2	Angiopoietin-2
APCs	Antigen-Presenting Cells
ARG1	Arginase 1
BCR	B cell receptor
BL	Blood
BM	Bone Marrow
BMD	Bone Mineral Density
BMT	Bone Marrow Transplantation
BC	Breast Cancer
BSA	Bovine serum albumin
Cat#	Catalogue Number
CB	Cord Blood
cGY	Centigray
CSF-1	Colony-stimulating factor-1
CRC	Colorectal Carcinoma
CT	X-ray micro-computed tomography
CTLs	Cytotoxic T lymphocytes
CTLA4	Cytotoxic T-Lymphocyte Associated Protein 4
DCs	Dendritic cells
DMEM	Dulbecco’s modified eagle medium
Doxo	Doxorubicin
DP	Double Positive
DN	Double Negative
ECM	ExtraCellular Matrix
EDTA	Ethylenediaminetetraacetic acid
EPCs	Endothelial Progenitor Cells
ER	Estrogen Receptor

FBS	Fetal bovine serum
EMT	Epithelial to Mesenchymal Transition
ER	Estrogen-Receptor
FACS	Fluorescent activated cell sorting
Fluc	Firefly Luciferase
FLT3	Fms Like Tyrosine kinase 3
FMOs	Fluorescence minus one control
FOXP3	Forkhead box P3
GFP	Green fluorescent protein
Gluc	Gaussia Luciferase
GM-CSF	Granulocyte-Macrophage Colony-Stimulating Factor
GVHD	Graft Versus Host Disease
GzB	Granzyme B
HEK	Human embryonic kidney
HER	Human Epidermal growth factor Receptor
HMDS	Hexamethyldisilazane
HSCs	Hematopoietic stem cells
HSPCs	Hematopoietic stem and progenitor cells
hTEB	human tissue engineered bone
ICP	Immune checkpoint
IF	Intrafemoral
IFNα	Interferon-alpha
IFN-β	interferon-beta
IFN-γ	interferon-gamma
IRG	Interferon regulated gene
IL3	interleukin 3
ICD	Immunogenic Cell Death
iNOS	Inducible nitric oxide synthase
Irf7	Interferon Regulatory Factor7
ISGs	interferon-stimulated genes
IV	Intravenous
IVIS	*In vivo* imaging system

LV	Lentivirus
MHC	Major Histocompatibility Complex
M-CSF	Macrophage Colony-Stimulating Factor
MDA3	MDA-231 TNBC cells transduced with three human cytokines
MDR	multidrug resistance
MDSCs	Myeloid-derived suppressor cells
MET	Mesenchymal to Epithelial Transition
MFI	Mean Fluorescence Intensity
MMPs	Matrix MetalloProteinases
MOI	Multiplicity of Infection
mPB	mobilised Peripheral Blood
NK	Natural Killer cells
NSG	NOD scid gamma
Oas-1a	2′-5′ oligoadenylate synthetase 1a
Ortho	Orthotopic
PADRE	Pan DR-binding epitope
PEG	Poly-ethylene glycol
PBL	Peripheral Blood Lymphocytes
PBMCs	Peripheral blood mononuclear cells
PD-1	Programmed cell death protein 1
PGK	Phosphoglycerate kinase
PR	progesterone receptor
PyMT	Polyoma middle T
RNS	Reactive nitrogen species
ROI	Region of interest
RPMI	Roswell park memorial institute
qPCR	Quantitative Polymerase Chain Reaction
RT	Room temperature
RTB	Relative Tumour Burden
SD	Standard Deviation
SEM	Standard error of mean
SS	Single stained control

STING	Stimulator of interferon genes
TAAs	Tumour-Associated Antigens
TALMs	Tumour Associated Lympho-Monocytes
TAMs	Tumour-associated macrophages
TCR	T cell receptor
Teffs	effector T cells
TEMs	TIE2-expressing monocytes
TERT	Telomerase Reverse Transcriptase
Tg	Transgenic mice
TGF-β	Transforming growth factor beta
Th	T helper cells
TIE2	Tyrosine kinase with Ig and EGF homology domains
TIL	Tumour Infiltrating Lymphocytes
TME	Tumour microenvironment
TNBC	Triple-negative breast cancer
TNEs	Tailored NanoEmulsions
TNF	Tumour Necrosis Factor
TPO	ThromboPoietin
Tregs	T regulatory cells
VCN	Vector copy number