ImmunoTools *special* Award 2015

Stephanie Annett, MPharm MPSNI
PhD-student

Supervisor: Prof. Tracy Robson

Queen's University Belfast, U.K.

ALM201 and its role in targeting ovarian cancer stem cells

Background and our supporting data: FKBPL is a divergent member of the immunophilin family and has highly potent anti-angiogenic activity (*Valentine et al, 2011; Yakkundi et al., 2015).* A novel peptide termed, AD-01, derived from the anti-angiogenic domain of FKBPL, was shown to inhibit blood vessel development and reduce tumour growth *in vivo* (*Valentine et al, 2011*). Since FKBPL and its novel peptides are dependent on CD44 to inhibit angiogenesis (*Yakkundi et al., 2013*), it was hypothesised that FKBPL's peptide derivatives may also target $CD44^+$ breast cancer stem cells (CSCs). As anticipated, AD-01 exhibited highly potent activity against breast CSCs by preventing self-renewal and inducing differentiation, resulting in the eradication of the breast CSCs *in vitro* and inhibiting tumour initiation and growth *in vivo* (*McClements, 2013*). Analysis of the structure, activity and stability of AD-01 led to the selection of ALM201, a 23 – residue peptide comprising amino acids 35 – 58 of FKBPL, as the clinical drug candidate which is now Phase 1/2 clinical trials in high grade serous ovarian cancer patients (EudraCT number: 2014-001175-31).

Ovarian cancer is the most lethal gynecological cancer in women and most patients present with advanced disease (*Hennessy BT, 2009*). Whilst 80% respond to first line therapy, the majority of patients become refractory within 15 months post diagnosis (*Banerjee and Kaya 2013*). The long term cure rate has only improved modestly in the last 20 years.

Evidence has emerged for the role of self-renewing ovarian CSCs in the pathogenesis and chemoresistance of epithelial ovarian cancer *(Shah and Landen, 2014*). The cell surface receptors CD44, CD117 and c–kit, are well characterized CSC markers in ovarian cancer. $CD44^+CD117^+$ cells are resistant to chemotherapy and are able to initiate and serially propagate tumours in mice (*Zhang S, et al 2008*). We have already demonstrated that ALM201 can significantly reduce the CSC

population in ovarian cancer cell lines; its ability to reduce this population in primary tissues will now be investigated as well as studying the role of omental stem cells (MSCs) which promotes ovarian cancer growth and dissemination.

Aim: The aim of this project is to establish ALM201's role in selectively targeting ovarian CSCs and MSCs and to ascertain its mechanism of action. We will ask the following questions:

1. Does ALM201 target MSCs?

MSCs are a multipotent population of stem cells contained in the omentum tissue that promote ovarian tumour proliferation, migration and drug resistance, possible by attracting ovarian CSCs. Furthermore, omentum MSCs have been shown to express the following markers; CD11b, CD14, CD29, CD34, CD44, CD45, CD73, CD90, CD105 (*Salimian RB et al, 2015; Nowicka A et al, 2013; Debnath T et al, 2015).* Previous research suggests ALM201 induces the differentiation of CSCs and its activity on omentum derived MSCs will now be investigated. We will also assess the mechanism associated with MSC-mediated abrogation of CSCs. MSCs have been reported to increase stemness in a wide range of cancers and the mechanism underpinning this is due to the expression of cytokines and inflammatory markers, such as SDF-1/CXCR4 axis, interleukin 6 (IL-6) and interleukin 8 (IL-8). IL-6 is associated with adverse outcomes in ovarian cancer and it is secreted from MCSs to act in a paracrine manor to enhance stem-related genes, tumour initiation and growth (*Wei HJ et al, 2008; Coward J et al, 2011*). Furthermore, IL-6 is capable of producing $CD44^+$ stem cells by inducing epithelial to meschymal transition (EMT) (*Sullivan NJ et al, 2009; Xie G et al, 2009*). IL-8 is secreted by ovarian cancer cells and it is a well-known regulator of CSCs, angiogenesis and EMT (*Wang Y et al, 2012; Yin J et al, 2015*). Our recent data suggests that ALM201 prevents the EMT process and we have preliminary evidence that it can also reduce IL-8 levels. Here, we would like to further investigate its potential role in modulating SDF-1/CXCR4, IL-6 and IL-8 signalling, between ovarian CSCs and MSCs.

2. Does ALM201 inhibit signalling between MSCs and CSCs?

MSCs promote gastric cancer cell growth, migration and invasion through the SDF-1/CXCR4 axis and exosomes from ovarian cancer cells have been shown to increase expression of SDF-1 on omentum (*Zhao BC et al, 2012; Cho JA et al, 2011*). Moreover, SDF-1 has been shown to be the main chemoattractant of $CD44^+$ cancer stem cells in initiating ovarian cancer in mouse models (*Yang – Hartwich Y et al, 2014).* VCAM1 (CD106) expressed exclusively on the mesothelium has been correlated with poor prognosis in high grade serous ovarian cancer (*Huang J et al, 2013*). VCAM-1-VLA4 ligand interactions are key regulators of ovarian cancer cell adhesion and invasion (*Slack – Dvis JK et l, 2009*). VCAM-1 physically associates with CD44 resulting in enhancement of CSC markers and engagement of CD44

induces VLA-4 thus triggering cell migration in the absence of chemokine receptor signalling (*Wang PC et al, 2014;Stackstein R, 2012*).

It is clear from the literature that CD44 has a key role ovarian cancer stemness and metastasis through the SDF-1/CXCR4 axis and VCAM-1/VLA4 ligand interactions. Using a range of in vitro and in vivo models we wish to investigate the role of ALM201 in inhibiting these signalling pathways and thus prevent CSCs related metastasis in this deadly disease.

Antibodies, cytokines and ELISA kits provided by ImmunoTools would be invaluable in this project to unravel the complex signalling pathways in ovarian CSCs which might identify new therapeutic targets for the treatment of these resistant cells and might possibly identify pharmacodynamically useful markers and indeed predictive markers of response to ALM201 which will speed of the clinical development of this drug.

ImmunoTools *special* AWARD for **Stephanie Annett** includes 25 reagents

FITC - conjugated anti-human CD11b, CD14, CD29, CD34, CD45, CD105

PE - conjugated anti-human CD49d, IL-6, IL-8, Control – IgG1, Annexin V

APC - conjugated anti-human CD44

human ELISA-set for 96 wells, human IL-6, human IL-8 (each 3 reagents)

recombinant human cytokines: rh IL-6, rh IL-8, rh SDF-1

recombinant human soluble receptors: rh IL-6rec

APC - conjugated anti-mouse CD44

FITC - conjugated anti-mouse CD90

PE - conjugated anti-mouse CD44, CD117 DETAILS more AWARDS