[NTNU_IBI] [Case number 5]

Institution: Norwegian University of Science and Technology (NTNU)
Administrative unit: Department of Biology (IBI)
Title of case study: **Cellular communication tools show success as novel therapeutics**
Period when the underpinning research was undertaken: 2012-2022
Period when staff involved in the underpinning research were employed by the submitting institution: 2012-2022
Period when the impact occurred: 2015 and 2022

1. Summary of the impact (indicative maximum 100 words)

Hyperproliferation is a characteristic of many pathologic processes, such as cancer and chronic diseases. Understanding the molecular mechanisms regulating normal physiologic processes and how these mechanisms change when pathology is activated, is crucial. The Lipid signalling group has identified and investigated important nodes controlling cellular communication regulating the very neat balance between pro and anti-inflammatory cellular conditions. These studies were enabled and successfully accomplished by development and application of powerful molecular tools carrying node high selectivity and sensitivity. Upon later testing these tools as candidate therapeutics, PoC for treatment of psoriasis was accomplished in 2015, and PoC for Aktinic keratosis was accomplished in 2022.

2. Underpinning research (indicative maximum 500 words)

In basic research performed for understanding fundamental biological process as life and death at the molecular and cellular level, a range of tools are needed like genetic and chemical ones. Our effort on understanding cellular communication mechanisms regulating hyperproliferation and programmed cell death and the balance between them, we were among the first worldwide to determine a very central role of an enzyme modifying membrane phospholipids, namely the phospholipase A2α (cPLA2α) enzyme. This is a member of a large PLA2 enzyme family comprising more than 25 different members. We have shown that two PLA2 enzymes are sequentially activated in cells when cytokine, f.ex TNFα, bind to its receptor that in parallel to initiating its intracellular classical kinase cascade eventually leading to activation and nuclear translocation of the very important transcription factor, NF-κB; a parallel kinase cascade is initiated by extracellular activation of a secretory PLA2 enzyme leading to production of minute amounts of leukotriene B4 (LTB4), LTB4 translocate to the outside of the cell and bind to G-protein coupled receptor and initiate a kinase cascade including atypical PKC and MAPkinase p38, leading to activation of cPLA2α and production of massive amounts of LTB4. Thus, generating an autocrine PLA2-driven kinase loop mediated by LTB which is obligatory for the cytokine induced nuclear translocation of NF-κB (2xAnthonsen, Johansen et.al. J.Biol.Chem., 2001 aug and sept). This research was performed in skin keratinocytes which created the understanding that cPLA2α could act as a therapeutic target in psoriasis? After these findings, basic research was expanded into other cellular models representing fibrosis and cancers (balance between hyperproliferation and programmed cell death) and research identifying cPLA2α as critical regulator of such processes, was and is, performed in collaboration with international experts like Prof Joseph V Bonventre,

Harvard University and Prof Ed A Dennis, UCSD, USA. Professor Dennis is the founder of the NIH-supported Lipid Maps Consortium in the US, where we enjoy very successful collaborations. At Harvard University in collaboration with Prof Bonventre, we enjoy access to highly relevant mouse models.

As part of elucidation of the specific role of the cPLA2α, we together with internationally renowned synthetic chemists (Prof Lars Skattebøl, UiO and Prof George Kokotos, U of Athens, GRE), developed highly selective and sensitive chemical compounds that can enter cells, bind selectively to the cPLA2α enzyme active site, and most successfully inhibit its activity! These molecular tools have helped us and others to understand fundamental biological process at molecular level. After achieving this, we speculated that such molecular tools might even have therapeutic potential in diseases characterized by hyperproliferation and delayed programmed cell death? We have now shown by several successful phase IIA clinical trials in patients that there are proof of concept (PoC) for treatment of psoriasis and actinic keratosis. A new clinical trial for skin cancer (BCC) is underway. Furthermore, these treatments are with less adverse events compared to currently used therapeutics, and therefore more sustainable. Indeed, the independent safety committee following the psoriasis clinical phase IIA trials concluded that the treatment was remarkably safe. Planning of testing this inhibition strategy as novel therapeutics is underway also for leukemia.

Key personnel involved in research, where PhD-students are supervised by Postdocs:

Phd-students:
Randi Sommerfelt, defended thesis 2015
Hanna Maja Tunseth, defended thesis
Eirini Tsirvouli, to defend thesis 2023
Nur Mahammad, to defend thesis 2023

Post.docs:
Dr Astrid J Feuerherm
Dr Felicity Ashcroft

Professor, PI:
Dr Berit Johansen

3. References to the research (indicative maximum of six references)

Huwiler A, Feuerherm AJ, Sakem B, Pastukhov O, Filipenko I, Nguyen T, **Johansen B**. The ω3-polyunsaturated fatty acid derivatives AVX001 and AVX002 directly inhibit cytosolic phospholipase A(2) and suppress prostaglandin E(2) formation in mesangial cells. *Br. J. Pharmacol.* **167:**1691–1701, 2012.

SA Moestue, MT Grinde, E Marangoni, T Sørlie, O Engebråten, GM Mælandsmo, **B Johansen**, TF Bathen. Cytosolic phospholipase A2 (cPLA2) as a therapeutic target in basal-like breast cancer. *Cancer Research* 73:24, P6-04-08, 2013.

Randi M. Sommerfelt, Astrid J Feuerherm, Trine Skuland, and **Berit Johansen**. Cytosolic phospholipase A2 modulates TLR2 signaling in synoviocytes. *PLoS One,* 10;4 (EMID:92836968d9e74a43, 2015.

Feuerherm, AJ., Dennis, EA. and **Johansen, B**. AVX001 and AVX002 inhibitors of cytosolic group IVA phospholipase A2 ameliorate collagen induced arthritis. *Arthritis Research and Therapy,* Jan 21;21(1):29, 2019.

Tunset, Hanna Maja; Euceda, Leslie Romelia; Feuerherm, Astrid Jullumstrø; Rao, Shalini Vasudev; and **Johansen, Berit**; Moestue, Siver Andreas. New insight into anti-metastatic properties of cytosolic phospholipase A2 alpha inhibition: Regulation of migration, transcriptome, and protein networks. *Internat. J. Mol. Sci,*20.19 //doi.org/10.3390/ijms20194800, 2019.

Nur Mahammad, Felicity J. Ashcroft, Astrid J. Feuerherm, Samah Elsaadi, Esten N. Vandsemb, Magne Børset, **Berit Johansen**. Inhibition of cytosolic phospholipase A2a induces apoptosis in multiple myeloma cells. *Molecules*, Dec 9;26(24):7447. doi: 10.3390/molecules26247447, 2021.

4. Details of the impact (indicative maximum 750 words)

The basic research performed identified and documented a novel regulatory node, the cPLA2α enzyme, in intracellular signalling cascades controlling the balance between hyperproliferation and programmed cell death. Such fundamental biological processes are of highest importance also in regulation between physiologic and pathologic conditions, therefore the cPLA2α may be exploited as a novel therapeutic target. During our investigations several molecular, cellular and animal tools, like commercial inhibitors against kinases, antibodies for ELISA or westerns or microscopy, primers for pcr, siRNA and animal KO models were employed. To pinpoint the unique physiological role of the cPLA2α enzyme, highly selective and sensitive inhibitors targeting the active site of the enzyme were developed by us in collaboration with internationally renowned synthetic chemists. These inhibitor molecules were very successful in pinpointing the role of the enzyme in intracellular signalling, AND would it also be beneficial as therapeutics? That was something we asked ourselves, and we made contact to biotechnological entrepreneur experts. In collaboration with these we established the biotech company Avexxin AS, that was successful in attracting about a total of 350 millNOK in capital both private and governmental to support development, preclinical safety testing and successful clinical testing all through phase IIA for psoriasis. The funds were primary made available from Norwegian government supported investment funds like SARSIA Seed and LEN Nyskaping and a large number of private investors in Mid-Norway like Sparebanke1 and others. A part of this funding was also from NFR and Skattefunn. Avexxin existed until 2019 when it was decided to enter the stock exchange, be listed. Then we needed to change name to Coegin Pharma (www.coeginharma.com), and in 2020 we were successfully listed on NGM in Stockholm. The listing enabled access to more private and governmental, international capital including Almi Invest supported by the Swedish government, and the company was valued about 400 millSEK. In several rounds of financing the company has lifted more money and successfully completed the clinical phase IIA testing of AVX001 cPLA2α inhibitor as treatment of Actinic Keratosis. The core company Coegin Pharma is now the platform company developing and performing preclinical safety and formulation work of the different molecular tools that were developed against cPLA2α, and when a new indication is decided a daughter company is established in order to recruit money for clinical testing. Until now, Avexxin Oncology is established for testing AVX420 in leukemia and Reccura Therapeutics is established for testing of AVX001 inhibitor to treat basal cell carcinoma, BCC.

The company strategy has attracted highly competent businesspeople with former international pharma competence. The business model is to license the technologies to big pharma after clinical phase II, for further clinical phase III development and market entry of therapeutic. Such licensing discussions are already ongoing with big pharma.

5. Sources to corroborate the impact (indicative maximum of ten references)

George Kokotos, Astrid J. Feuerherm, Efrosini Barbayianni, Ishita Shah, Mari Sæther, Victoria Magrioti, Thuy Nguyen, Violetta Constantinou-Kokotou, Edward A. Dennis and **Berit Johansen**. Inhibition of Group IVA Cytosolic Phospholipase A_2 by Thiazolyl Ketones *In Vitro*, *Ex Vivo*, and *In Vivo*. *J. Medicinal Chemistry*, 57(18):7523-35, DOI: 10.1021/jm500192s, 2014.

Kim, E., Tunset, HM., Cebulla, J., Vettukattil, R., Helgesen, H., Feuerherm, AJ., Engebråten, O., Mælandsmo, GM., **Johansen, B.,** Moestue, SA. Anti-vascular and molecular effects of cytosolic phospholipase A2 inhibition in a patient-derived basal-like breast cancer model. *BMC Cancer,* 16:191, 2016.

Omland SH, Habicht A, Damsbo P, Wilms J, **Johansen, B**, Gniadecki R. A randomized, double-blind, placebo-controlled, dose-escalation first-in-man study to assess the safety and efficacy of topical phospholipase A2 inhibitor AVX001 in patients with mild to moderate plaque psoriasis. *Eur. J. Derm. Venerol.* Jan 20. doi: 10.1111/jdv.14128, 2017.

Chiorazzo, Michael G., Tunset, Hanna Maja, Popov, Anatoliy V., **Johansen, Berit**, Moestue, Siver, Delikatny, Edward, J. Detection and Differentiation of Breast Cancer Sub-Types using a cPLA2 Activatable Fluorophore - DDAO arachidonate. *Scientific reports*, Apr 16;9(1):6122, 2019.

Ashcroft, FJ., Mahammad, N., Flatekvål, HM., Pinõl, M., Feuerherm, AJ. and **Johansen, B.** cPLA2a enzyme inhibition attenuates keratinocyte inflammation and proliferation. *Biomolecules*, Oct 2;10(10):1402. 2020.

Tsirvouli E, Ashcroft F, **Johansen B**, Kuiper M. Logical and experimental modeling of cytokine and eicosanoid signaling in psoriatic keratinocytes. iScience. 2021 Nov 15;24(12):103451. doi: 10.1016/j.isci.2021.103451. eCollection 2021.

Ortner, Vinzent K; **Johansen, Berit**; Kilov, Kim; Mondragon, AC; Kihl, Jesper; Ashcroft, Felicity; Feuerherm, Astrid Jullumstrø; Laugesen, CP; Espersen, MLM; Manole, I; Isberg, IP; Andersen, AD; Rakvaag, Elin; Zibert, JR; Hædersdal, Merete. The Copenhagen Actinic Keratosis study (COAKS). A decentralized clinical trial to evaluate tolerability, safety and efficacy of daily field-directed topical treatment with cytosolic phospholipase A2 inhibitor, AVX001, in participants with actinic keratosis: Protocol for a randomized controlled phase I/IIa trial. *BMJ Open* Oct 5;12(10):e061012; doi: 10.1136/bmjopen-2022-061012, 2022.

Johansen, Berit; Naini, Said Movahedini; Selvik, Linn-Karina M.; Feuerherm, Astrid Jullumstrø; Ashcroft, Felicity; Wang, C; Yu, S; Yin, WQ; Alevizploulos, Konstantinos; Quehenberger, Oswald; Dennis, EA; Bonventre, Joseph V. Group IVA cytosolic phospholipase A2 (cPLA2α): pharmacological 2 inhibition attenuates kidney inflammation and fibrosis. *JASN, in review* 2022.