Institution: University of Kent and University of Greenwich

Unit of Assessment: 3 Allied Health Professions, Dentistry, Nursing and Pharmacy.

Title of case study:

Novel applications of gold nanoparticles to target autoimmune disorders

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

Sumbayev and colleagues have shown that gold nanoparticles represent an excellent platform for the specific delivery of drugs, targeting the HIF-1 biochemical pathway as a novel therapeutic target for diseases such as allergy, leukaemia and other autoimmune disorders. Two international, non-academic institutions have altered the direction of their work as a result of this research and two SMEs have revised their operational procedures and invested in the applied research that derives from this work.

2. Underpinning research (indicative maximum 500 words)

Since 2007, the work of Sumbayev, Gibbs and colleagues whilst based at Medway School of Pharmacy, has focused on the mechanisms of adaptation of human hematopoietic cells of myeloid lineage to different types of stress associated with low oxygen availability. This includes Toll-like receptor (TLR) dependent innate immune reactions (involved in early responses to infection), pro-allergic responses and progression of acute myeloid leukaemia. They investigated the role of the hypoxia-inducible factor 1 transcription complex (HIF-1) and associated biochemical pathways in adaptation of human myeloid cells to stress. They reported for the first time that stimulation of endosomal TLR7/8 led to the activation of HIF-1 in human myeloid macrophages which supported both cell survival as well as the production of pro-inflammatory cytokines [3.1]. They also found that HIF-1 is crucial for activation of the Nalp3 inflammasome, a multiprotein complex responsible for maturation of interleukin 1 beta (IL-1β), a highly inflammatory cytokine which controls the crosstalk between innate and adaptive immunity [3.2].

These observations were then extended to show that the above mechanisms are not unique for host innate immune defence but also apply to biological responses of myeloid cells. For example, they reported for the first time, that pro-allergic responses of human basophils are HIF-1-dependent. HIF-1 was found to be essential for vascular endothelial growth factor (VEGF) mRNA expression and, consequently, release of VEGF protein (which is known to play a vital role in airways remodelling associated with chronic asthma). Moreover, HIF-1 alters IgE-induced ATP depletion in basophils, thus also supporting the production of IL-4 [3.3], a cytokine which orchestrates allergic immune responses. In an Asthma UK-funded study, Gibbs and Sumbayev showed that HIF-1 also plays a central role in the ability of human mast cells to generate pro-inflammatory cytokines. Furthermore, they found that leukaemic responses of human myeloid leukaemia cells also involve HIF-1 [3.4] and the immunogenic action of IL-1β, which is also known to promote autoimmune disease [3.5]. Taken together, they showed that the HIF-1 pathway, controlled by differential biochemical mechanisms, is essential for both normal innate immune defence and pathophysiological (leukaemia, autoimmune reactions, allergy) responses of human myeloid cells. As such, it is clear that regulation of the HIF-1 pathway represents an excellent therapeutic strategy.

To extend this research further, the group investigated the possibility that inert nanomaterials could act as an excellent platform for the delivery of drugs which interact with this pathway. In the course of this research, the group showed, for the first time, that gold nanoparticles specifically downregulated cellular responses induced by IL-1β both *in vitro* and *in vivo* [3.5]. These results indicate that the known anti-inflammatory activity of gold is associated with an extracellular interaction with IL-1β. In addition, unlike other nanomaterials, gold nanoparticles did not induce activation of inflammasomes [3.2, 3.5, 3.6]. Thus, this research shows, firstly, that the HIF-1 pathway is a good therapeutic target for diseases such as allergy, leukaemia and other

autoimmune disorders and, secondly, that gold nanoparticles represent an excellent delivery platform for the specific delivery of drugs that target this pathway.

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

3.1 **Nicholas, S. A** and **Sumbayev, V. V**. The involvement of hypoxia-inducible factor 1 alpha in Toll-like receptor 7/8-mediated inflammatory response. (2009) Cell. Res. 19, 973-983.

3.2 **Nicholas, S. A.,** Bubnov, V. V., **Yasinska, I. M. and Sumbayev, V. V**. Involvement of xanthine oxidase and hypoxia-inducible factor 1 in Toll-like receptor 7/8-mediated activation of caspase 1 and interleukin-1beta. (2011) Cell Mol. Life Sci. 68, 151-158.

3.3 **Sumbayev, V. V., Nicholas, S. A., Streatfield, C. L., Gibbs, B. F.** Involvement of Hypoxia-Inducible Factor-1 in IgE-Mediated Primary Human Basophil Responses. (2009) Eur. J. Immunol. 39, 3511-3519.

3.4 **Gibbs, B. F., Yasinska, I. M., Oniku, A. E., and Sumbayev, V. V**. Effects of Stem Cell Factor on Hypoxia-Inducible Factor 1 alpha Accumulation in Human Acute Myeloid Leukaemia and LAD2 Mast Cells. (2011) PLoS One.6 (7) e 0022502.

3.5 **Sumbayev, V.V., Yasinska I. M.,** Garcia, C. P., Gilliland, D., **Lall, G.S., Gibbs, B. F**., **Bonsall, D. R**., Varani, L., Rossi, F., Calzolai, L. Gold nanoparticles downregulate interleukin-1β-induced pro-inflammatory responses. (2013) Small 9, 472-477.

3.6 **Nicholas, S. A., Coughlan, K., Yasinska, I., Lall, G., Gibbs, B. F.,** Calzolai, L. and **Sumbayev, V. V.** Dysfunctional mitochondria contain endogenous high-affinity human Toll-like receptor 4 (TLR4) ligands and induce TLR4-mediated inflammatory reactions. (2011) Int. J. Biochem. Cell Biol. 43, 674-681.

Authors who were based at MSOP at time of publication are in bold.

Related Grants held at MSOP:

Gibbs BF. The role of HIF-1 in IgE-mediated allergic responses of human basophils. Asthma UK £48,527 (2010).

Sumbayev VV. The role of hypoxia-inducible factor I alpha in Toll-like receptor 7/1-induced inflammatory reactions. Royal Society £11,460 (2009).

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

The described research shows that the HIF-1 pathway is an excellent therapeutic target and the potential of gold nanoparticles as an excellent delivery platform for the specific delivery of the drugs targeting this pathway. The research has had an impact on both Industry and the workings of International Institutions. Two international institutions have altered the direction of their work by *being influenced by this research* and two SMEs have *revised their operational procedures and invested in the applied research* that derives from this work. Clear collaborative links with all four organisations have resulted directly from the research described which has facilitated technical knowledge transfer.

The European Commission Joint Research Centre, Institute for Health and Consumer Protection, Ispra, Italy (JRC, Ispra) is a department of the European Commission. Its primary mission is to provide independent evidence-based scientific support to underpin European policies in a variety

of areas including Health and Consumer Protection (5.1). Following a presentation of initial elements of this research by Sumbayev at a Nanobiotechnology workshop organised by JRC, Ispra in 2010, the two groups initiated a collaborative programme to take the research further, resulting in joint publications (3.5, 3.6). In 2011, JRC, Ispra invested substantial resources into the establishment of a method to visualise the interactions of nanomaterials with human myeloid cells. Before the interaction with Sumbayev and Gibbs, the focus of JRC Ispra's research in this area was, primarily, a characterisation of the physical and chemical properties of nanomaterials. As a result of the research described here and subsequent collaborative interactions, the department has *"expanded its existing research focus in order to help elucidate the biological effects of nanomaterials related to inflammation, allergy and leukaemia"* (5.2) with a particular interest in supporting the development of safe nanomaterials as part of their Health and Consumer Protection portfolio.

The Istituto di Ricerca in Biomedicina, Bellinzona, Switzerland (Institute for Research in Biomedicine) is a private, non-academic research institute led by Professor A. Lanzaveccia, a world-leading expert in immunology. It was founded in 2000 with the goal of advancing the study of human immunology. Within the Institute for Research in Biomedicine, the laboratory of Dr. Varani specialises on the generation of antibodies against highly virulent pathogens. Following Sumbayev & Gibbs research and subsequent discussions, Varani's laboratory has *"opened a new field of research on the use of functionalised gold based nanomaterials"* for the *"specific delivery of inhibitors"* (both direct and indirect) of the HIF-1 pathway in target cells with a particular focus on *"cells involved in leukaemia allergy and autoimmunity"* (5.3). Varani's laboratory has also *"financed and established a system for production of fully functional human IL-1β and stem cell factor"* to advance this research (5.3).

Two SMEs, IZON Science Ltd (IZON) and BOOST Technologies B.V. (BOOST) have been influenced by this research and *"invested in applied research as a result"*. IZON specialises in the characterisation of nanomaterials. They have *"invested resources in the characterisation of gold nanomaterials in order to specifically target leukaemia cells"*, in light of the research described here (5.4). BOOST specialises in the generation of nanomaterials and have previously focused on the functionalisation of nanomaterials with peptides. Now they have committed resources to generate functionalised nanomaterials and nanovaccines based on the findings" described here (5.5).

All four institutions (JRC Ispra, Institute for Research in Biomedicine, IZON and BOOST) have joined together with Sumbayev, Gibbs and colleagues in Kent and other academic partners in Denmark and Italy as part of the NANOVAC consortium (5.6). The consortium is currently in the process of preparing proposals for the Horizon 2020 EU Framework Programme for Research and Innovation (value > £4 million) in order to develop nanoconjugates and nanovaccines for use as therapies against cancer and allergic diseases. They have also recently applied for an Asthma UK Capacity-Building PhD Studentship entitled "Highly specific targeting of mast cell and basophil function using nanomaterials". These proposals arose directly from the findings of the research described here and subsequent collaborative links between the partners and provide evidence that the impact that arises is dependent on the research links. In addition, the research has clear impact potential for Health and Welfare with innovative therapeutic strategies for the treatment of allergy, leukaemia and other autoimmune disorders.

5. Sources to corroborate the impact (indicative maximum of 10 references)

5.1 European commission IHCP JRC website (http://ihcp.jrc.ec.europa.eu/) explaining the mission of the JRC: "As the Commission's in-house science service, the Joint Research Centre's mission is to provide EU policies with independent, evidence-based scientific and technical *support throughout the whole policy cycle. Working in close cooperation with policy Directorates-General, the JRC addresses key societal challenges while stimulating innovation through developing new methods, tools and standards, and sharing its know-how with the Member States, the scientific community and international partners."*

5.2 Letter from Dr L Calzolai, Group Leader, JRC Ispra. *"expanded its existing research focus*

in order to help elucidate the biological effects of nanomaterials related to inflammation, allergy and leukaemia"

5.3 Letter from Dr L Varani, Group Leader, IRB. *"opened a new field of research on the use of functionalised gold based nanomaterials"* for the *"specific delivery of inhibitors"* on *"cells involved in leukaemia allergy and autoimmunity". "financed and established a system for production of fully functional human IL-1β and stem cell factor"*

5.4 Letter from Dr P Aubert, Group Leader, IZON. *"invested resources in the characterisation of gold nanomaterials in order to specifically target leukaemia cells"*,

5.5 Letter from Dr S Litvinov, CEO, BOOST. directed *"resources towards the generation of functionalised nanomaterials and nanovaccines based on the findings"*

5.6 Nanovac consortium website extract:
http://www.msp.ac.uk/research/research-groups/nanovac-consortium.html