specifically in hypoxic areas of human prostate tumours and that expression of the gene being delivered in the adenovirus can be restricted to prostate tumour cells by placing it under the control of a prostate-specific promoter (PSA). This novel approach employs three distinct levels of tumour-specific targeting; the homing of the macrophages to tumours, the synthesis and release of therapeutic adenovirus only in hypoxia tumour areas, and the targeting of therapeutic gene expression to prostate tumour cells.

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A51
Targeted nanodrug delivery systems for the treatment of tuberculosis

Yolandy Lemmer[1,3,*], Boitumelo Semete[1], Laetitia Booysen[1,2], Lonji Kalombo[1], Lebogang Katata[1], Arwyn T. Jones[4], Cameron Alexander[5], Makobetsa Khati[6], Hulda S. Swai[1], Jan A. Verschoor[3]

[1] *Council for Scientific and Industrial Research, Polymers and Bioceramics, Pretoria 0001, South Africa*

[2] *Department of Pharmaceutics, North-West University, Potchefstroom Campus, Potchefstroom 2520, South Africa*

[3] *Department of Biochemistry, University of Pretoria, Pretoria 0001, South Africa*

[4] *Welsh School of Pharmacy, Cardiff University, United Kingdom*

[5] *School of Pharmacy, University of Nottingham, UK*

[6] *Council for Scientific and Industrial Research, Biosciences, Pretoria 0001, South Africa*

*Corresponding author.

E-mail: ylemmer@csir.co.za (Y. Lemmer).

South Africa currently has the highest incidence of TB in the world at 358 per 100,000 people. In 2007 alone 112,000 people died of TB in South Africa, of which 94,000 (72%) were co-infected with HIV [1]. Although TB treatments exist, poor patient compliance and drug resistance pose a great challenge to programs worldwide. To improve the current inadequate therapeutic management of TB, a polymeric anti-TB nanodrug delivery system, for anti-TB drugs, was developed that could enable entry, targeting, sustained release for longer periods and uptake of the antibiotics in the cells, hence reducing the dose frequency and simultaneously improve patient compliance. The aim was to prepare functionalised polymeric nanodrug delivery vehicles to target TB infected macrophage cells. Successful nanoencapsulation of anti-TB drugs and a targeting agent, mycolic acids (MA) was achieved. MA (a lipid molecule on the cell wall of M.tb) was explored due to its cholesteroid properties [2] that could attract it to the infected macrophages/foam cells. The nanoparticles were characterized and subjected to *in vitro* analyses in THP-1 and U937 cells in order to determine their uptake and localization. Cytotoxicity in different cell lines was also analysed. In another approach targeting was achieved via attaching nucleic acid aptamers [3], onto the surface of drug-carrying PLGA nano-particles. The aptamers were prepared via the SELEX process [4], specifically against the mannose receptor (MR), which is significantly over-expressed during the activation of the macrophages in the presence of M.tb. Uptake of the MA PLGA nanoparticles was achieved where little co localization was observed with endocytic markers, indicating that they could be localized in the cytosol. Vesicles bearing these particles were also observed in the cell membrane of these cells. We will report the uptake of the aptamers to THP-1 cells illustrating the feasibility of using the nucleic acid species for active targeted drug delivery. The success of these two approaches of anti-TB drug targeting will greatly address the challenges of poor bioavailability, reduced efficacy and adverse side effects for diseases such as TB.

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A52
Targeted SAINT-O-Somes, a novel type of liposomes for improved delivery of siRNA

Piotr S. Kowalski[1,*], Niek G.J. Leus[1], Joanna E. Adrian[1], Henriette W.M. Morselt[1], Marcel H.J. Ruiters[2], Grietje Molema[1], Jan A.A.M. Kamps[1]

[1] *Department of Pathology and Medical Biology, Medical Biology Section, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands*

[2] *Synvolux Therapeutics B.V., Groningen, The Netherlands*

*Corresponding author.

E-mail: p.kowalski@med.umcg.nl (P.S. Kowalski).

Gene silencing by siRNA has become a powerful technique with a great potential for therapeutic application. Increased insight in the role of endothelial cells in the pathology of cancer and inflammatory diseases has shifted the interest in the development of siRNA drug delivery devices for pharmacological intervention towards these cells. Additionally, endothelial cells are readily accessible for substances transported by the blood and their heterogeneity allows for specific drug targeting approaches. Liposomes represent a drug-carrier system for the delivery of siRNA that can be tailored on demand to introduce cell specificity. However, unlike in macrophages or in many tumor cells, in endothelial cells the processing of liposomes and subsequent release of drug content is inefficient due to the absence of adequate intracellular processing machinery which limits pharmacological efficiency. Therefore, we developed a lipid based drug delivery system with a superior intracellular release characteristic which is suitable for the *in vivo* delivery of siRNA. The design of the carrier is based on long circulating conventional liposomes that were formulated with a cationic amphiphile, 1-methyl-4-(cis-9-dioleyl)methyl-pyridinium-chloride (SAINT-18). These so-called SAINT-O-Somes have a diameter of 100 nm and showed a 10-fold higher encapsulation efficiency for siRNA compared to liposomes without SAINT and protect the siRNA from degradation for at least 6 weeks. Moreover, SAINT-O-Somes are fully stable in a biological relevant milieu (i.e. presence of serum), but are destabilized in the lower pH in endosomes of endothelial cells, enabling release of siRNA into the cytoplasm of the cell. In order to efficiently target activated endothelial cells, SAINT-O-Somes were equipped with antibodies against E-selectin or VCAM-1 adhesion molecules that are (over)expressed at sites of inflammation.

Coupling of these ligands showed a highly beneficial effect for transfection efficacy to TNF-α activated endothelial cells compared to non-targeted SAINT-O-Somes. The intracellular delivery of anti VE-cadherin siRNA SAINT-O-Somes to activated endothelial cells resulted in a specific, 70% down-regulation of VE-cadherin gene expression. In conclusion, we demonstrated that SAINT-O-Somes are stable, high capacity carriers for effective siRNA delivery into endothelial cells that present the requirements for *in vivo* application.

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A53
Toxin assisted intracellular delivery of gold nanoparticles

Umbreen Shaheen*, Yann Cesbron, Raphaël Lévy
School of Biosciences, University of Liverpool, Liverpool, UK

*Corresponding author.
E-mail: umbreen@liv.ac.uk (U. Shaheen).

Targeted intracellular delivery of biomolecules using nanoparticles has attracted many of the science disciplines. Nanoparticles because of their tuneable size and unique optical properties are emerging not only as imaging probes but also serving as intracellular cargo delivery carriers. Gold nanoparticles are best candidate for all these applications because of their not particularly reported cytotoxicity and ease of biofunctionalization. For intracellular cargo delivery application, it is necessary that a carrier is not only has the capacity to carry the biomolecule efficiently but also able to deliver it to the cytosol which is the main site for all physiological and chemical activities inside the cell. It is well documented that on intracellular delivery, bioconjugated gold nanoparticles are trapped by endolysosomes where their biomolecular coating degrades eventually. For avoiding this fate and for gaining access into the cytosol, we used a new approach, that is, toxin assisted delivery for gold nanoparticles. A bacterial toxin streptolysin-O is a secreted protein of 61 kDa which forms pores in plasma membrane of host cell for gaining access into the cytosol. It has been used as a simple and rapid mean of transfection for intracellular delivery of oligonucleotides and siRNA. Our results confirm that SLO treated cells showed an increased cellular uptake of gold nanoparticles then untreated cells. We also studied the effect of poly ethylene glycol (PEG) on SLO assisted cellular uptake by increasing the PEG amount gradually and found that PEG affects the cellular uptake adversely. We are currently combining fluorescence microscopy, photothermal microscopy and transmission electron microscopy to fully understand the mechanism, localization and fate of gold nanoparticles during SLO assisted uptake.

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A54
Quantifying uptake and distribution of arginine rich peptides at therapeutic concentrations using fluorescence correlation spectroscopy and image correlation spectroscopy techniques

Ben Staley*, Egor Zindy, Alain Pluen
School of Pharmacy and Pharmaceutical Sciences, The University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT, UK

*Corresponding author.
E-mail: ben.staley@postgrad.manchester.ac.uk (B. Staley).

Due to an apparent ability to enter cells in an energy independent manner, cell-penetrating peptides (CPPs) are increasingly being used as vectors for the delivery of macromolecules into cells. But 20 years on, their uptake and intracellular distribution are still debated [1] especially as most studies have been carried out at relatively high concentrations (micromolar), while therapeutic doses more likely to be in the nanomolar range. Thus, we hypothesised that taking advantage of fluorescence correlation spectroscopy (FCS) and image correlation spectroscopy (ICS) should help to understand the delivery mechanisms (especially the intracellular distribution) of arginine rich peptides TAMRA-Tat and TAMRA-nona-arginine (R9) at therapeutic doses. TAMRA-Tat and TAMRA-R9 peptides were incubated for one hour with both Caco-2 and HeLa cells. Initial observation of uptake was carried with a Zeiss LSM510 Meta Confocor 2. FCS and ICS were then used to measure peptide concentrations (density of particles per beam waist area) in distinctive areas and in the whole cell (cartography). ICS, implemented in parallel to FCS, was developed in house based on the work of P. Wiseman's group [2,3]. Subcellular distribution was analysed with confocal microscopy revealing two main areas – punctate and cytoplasmic regions – sampled initially with FCS to obtain diffusion times and concentration. Diffusion times in the punctate areas are very long (300 ± 50 μs) compared to the cytoplasm (26 ± 8 μs) at 500 nM, suggesting a bound component compared to free peptide. As FCS cannot sample the whole cell, ICS provided a more complete view of the distribution of TAMRA-Tat and TAMRA-R9 in which large areas of the cells behave as the 'cytoplasmic' area used in FCS. Our results indicate that arginine rich peptides are observed at nanomolar concentrations in all areas sampled. At concentrations below 500 nM, punctate and discrete areas are clearly labelled suggesting a possible entry via an endocytosis only mechanism. Finally, as the bulk concentration increases the fraction detected in the cytoplasm increases suggesting the simultaneous presence of a non-endocytotic mechanism of entry. Overall, FCS and ICS demonstrate that they provide invaluable information on the cellular delivery of peptides at therapeutic levels.

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A55
Tat-LK15, a Tat-fusion peptide, to deliver therapeutic siRNA in chronic myeloid leukemic cells

Yamini Arthanari*, Costas Demonacos, Harmesh Aojula, Alain Pluen
School of Pharmacy and Pharmaceutical Sciences, University of Manchester, Manchester, United Kingdom

*Corresponding author.
E-mail:
yamini.arthanari@postgrad.manchester.ac.uk
(Y. Arthanari).

Chronic myeloid leukaemia (CML) is caused by the reciprocal translocation of chromosomes 9 and 22 resulting in the formation of the BCR-ABL fusion protein, which exhibits deregulated tyrosine kinase activity. Hence, BCR-ABL would be a key target for developing a therapy for CML. We have used the potential of RNA interference to study the silencing of this oncoprotein. siRNA has been used to target wide range of genes in various cell types using cell penetraing peptides (CPPs). In this study we have evaluated the ability of the Tat fusion peptide, Tat-LK15 [1] to study uptake of