Harnessing the power of multimodal imaging to explore ASO distribution in cells, complex in vitro models and tissue

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Introduction

For the past 8 years, GSK has collaborated with Prof. Stephen Boppart and his team in the Beckman Institute at the University of Illinois, Urbana-Champaign (UIUC), exploring the potential of non-linear optical technologies such as Simultaneous Label-free Autofluorescence Multiharmonic (SLAM) microscopy to provide label free imaging of biological samples.

In 2017, we set up the GSK Centre for Molecular Imaging (COMI) within Prof. Boppart's lab, providing GSK scientists with direct access to the imaging technology and the downstream image analytics. A schematic of the COMI system is shown in figure 1.

In 2018, GSK made the strategic decision to select Bepirovirsen, a non-GalNAc targeted ASO for the treatment of Chronic Hepatitis B (CHB). Since this molecule was preferred to the GalNAc version (GSK3389404), new workstreams were commissioned to explore the differences in cellular distribution of these molecules.

In February 2019, Ionis, GSK and COMI formed a 3-way collaboration to investigate the uptake and distribution of GalNAc-conjugated and non-conjugated oligos utilizing a range of nonlinear optical imaging modalities at COMI lab in cellular, complex in vitro models and tissue microenvironments. Malat1 ASOs (+/- GalNAc, labelled with Alexa488) were kindly provided by Punit Seth (Ionis) and used as surrogates for the GSK HBV oligos for in vitro studies.

In a recent article, Migliorati and colleagues [2022] concluded that cellular uptake and subcellular trafficking and distribution are were knowledge gaps. This poster reviews the work performed in the COMI lab over the past 3 years to address this challenge.

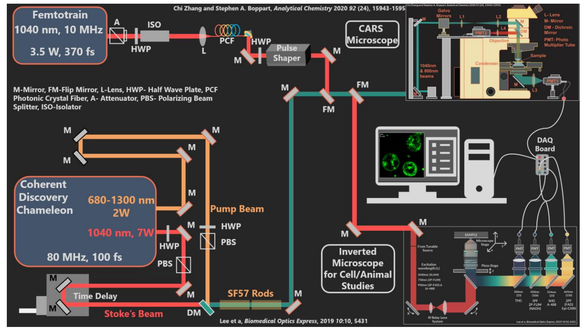


Figure 1 - The configuration of the multimodal label-free imaging system in the GSK COMI lab
(adapted from Mukherjee *et al* 2021).

ASO Distribution in Hepatocytes and Macrophages

The effect of the GalNAc conjugation on oligo uptake was studies in J774A.1 (macrophage) cells and Mouse primary hepatocytes. Cells were transfected in suspension by incubation in media containing ASO and then plated for imaging.

As shown in Figure 2, the kinetics of ASO uptake into macrophages was independent of GalNAc conjugation, reflecting the phagocytic nature of the cellular entry. Conversely there was a clear difference in the uptake into hepatocytes, both in kinetics and spatial distribution.

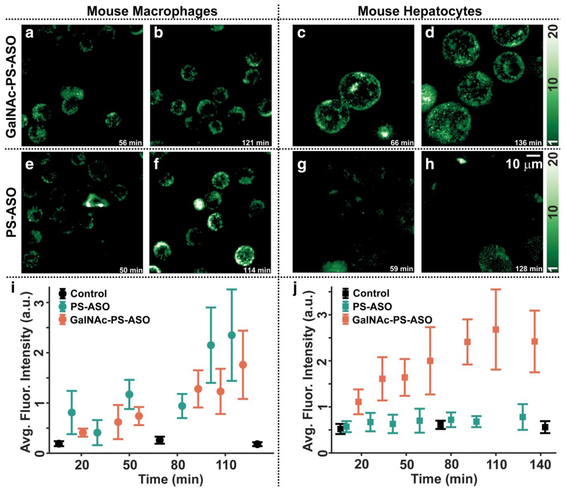
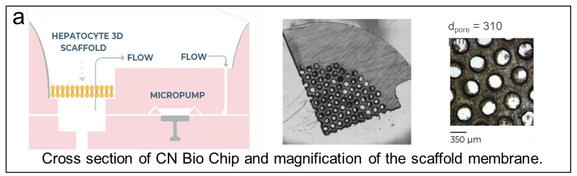


Figure 2. Comparison of the regular PS-ASO and GalNAc-PS-ASO uptake kinetics in mouse macrophages (J774A.1) and PMHs. (a, b) Fluorescence intensity images of GalNAc-PS-ASO-treated macrophages at 56- and 121- min post transfection, respectively. (c, d) Fluorescence intensity images of GalNAc-PS-ASO-treated PMHs at 66- and 136-min post transfection, respectively. (e, f) Fluorescence intensity images of ASO-treated macrophages at 50- and 114- min post transfection, respectively. (g, h) Fluorescence intensity images of PS-ASO-treated PMHs at 59- and 128- min post transfection, respectively. (i) and (j) show the change in the average fluorescence intensity per cell as a function of time after cell treatment with PS-ASOs (teal symbols), GalNAc-PS-ASOs (orange symbols), or PBS-alone (black symbols) (adapted from Mukherjee *et al* 2021).

Imaging ASO Distribution in 3D Complex Invitro Models

Having established that the COMI systems were able to track oligos in monoculture, we proceeded to repeat the uptake experiments in 3 dimensional co-cultures (CN Bio) of human hepatocytes and Kupffer cells (seeded 10:1). After 7 days of continuous culture the Alexa labelled oligos were incubated at a range of concentrations for timepoints up to 48 hours and the discs removed for subsequent microscopic and transcript analysis.

This analysis enabled the study of oligo uptake in Kupfer cells and circular or cuboidal hepatocytes, allowing further understanding of the competitive ASO distribution into these cell types. The preliminary results from this analysis are shown in Figure 3 below and the full data set will be published in 2H22.



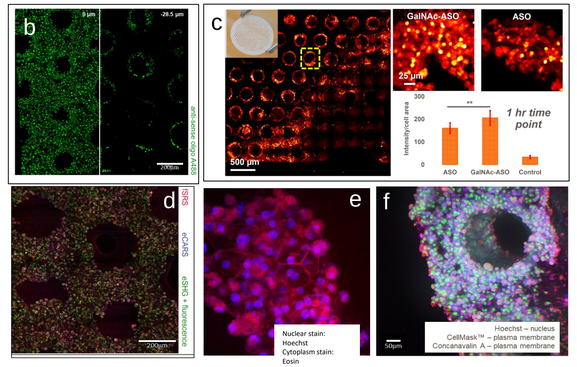


Figure 3. Panel **a** shows a cross sectional diagram of the CN Bio Chip along with enlarged images of the scaffold disc. The cells form a monolayer on the face of the disc and line the insides of the pores, where the fluorescent signal from the oligo can be detected by confocal microscopy (b) and a comparison of uptake of ASO (+/- GalNAc) may be performed (c). Further cellular features were determined using label free CARS and SRS (d) and subsequent staining of the discs identified cuboidal hepatocytes via confocal (e) and light sheet microscopy (f). .

Label free imaging of ASO in tissue samples

In parallel to their efforts to track fluorescently labelled ASOs in cells and CIVMs, the COMI team have been developing protocols to detect unlabeled ASOs through their unique optical signature using Hyperspectral CARS (HS-CARS). Multimodal analysis of kidney samples taken from a 13wk repeat dose toxicology study in mice dosed with Bepirovirsen has yielded some promising results as shown in figure 4. Further work, including livers from the same animals, is ongoing and will be published in due course.

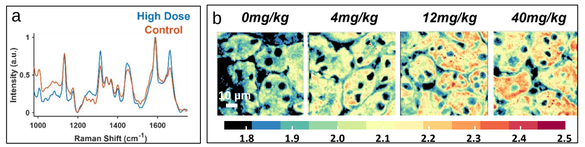


Figure 4 Label-free imaging of Bepirovirsen in mouse kidney cortex. Panel (a) shows the average Raman spectra of the control and high dose mouse kidney tissues fingerprint region. Panel (b) shows images of proximal tubules that have been false color-coded based on the lipid:protein ratio estimated from the HS-CARS images.

Conclusions

Over the past 3 years, the COMI lab have established a range of in vitro and ex-vivo models that can characterize the sub-cellular distribution of ASOs in real time, without disturbing the biological matrix. These systems may now be perturbated with exogenous factors to study the impact on ASO uptake and distribution kinetics.

Furthermore, advances in label free detection of ASOs have opened up the potential to detect therapeutic oligos in their clinical dose form, without the need for additional labels.

GSK and our collaborators are confident that these methodologies will help us understand why GalNAc conjugation does not appear to enhance the efficacy of Bepirovirsen in patients with CHB.

References

1. Migliorati et al (2022) ADME of FDA-approved ASO drugs Drug Metabolism and Disposition February 27, 2022, DMD-MR-2021-000417; DOI: https://doi.org/10.1124/dmd.121.000417
2. Mukherjee P, et al (2021) Differential Uptake of Antisense Oligonucleotides in Mouse Hepatocytes and Macrophages Revealed by Simultaneous Two-Photon Excited Fluorescence and Coherent Raman Imaging. Nucleic Acid Ther. 2021 Nov 19. doi: 10.1089/nat.2021.0059. Epub ahead of print. PMID: 34797690.

Affiliations

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- Peter Watson is an academic collaboration with GSK within the ITN MUSIQ consortium and has no competing financial interests
- Aneesh Alex, Jan Majer and Steve Hood are employees of GlaxoSmithKline and hold stocks there.

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All studies were conducted in accordance with the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals and were reviewed the Institutional Animal Care and Use Committee either at GSK or by the ethical review process at the institution where the work was performed.