IMI1 Final Project Report Public Summary

Project Acronym: COMPACT

Project Title: Collaboration on the Optimisation of Macromolecular Pharmaceutical Access to Cellular Targets

Grant Agreement: 115363
Project Duration: 01/11/2012 - 31/10/2017

Executive Summary

A. Project rationale and overall objectives of the project

The overall goal of the COMPACT consortium is to reduce delivery and targeting bottlenecks for developing novel innovative biopharmaceuticals. In order to reach this main goal, four objectives have been formulated:

- To identify and characterise transport pathways across biological barriers and across cell membranes so that they may be more effectively utilised for drug delivery purposes. The biological barriers investigated are: intestinal (IB), skin (SB), air-lung (ALB), blood-lung (BLB), blood-brain barrier (BBB), including endolysosomal membranes for these selected tissues
- To construct and characterise formulations for non-invasive delivery of peptide- and protein-based drugs with an emphasis on oral, airway and transdermal delivery and for transport over the BBB
- To construct and characterise formulations for delivery of nucleic acid-based drugs with an emphasis on intracellular and transcellular delivery
- To preclinically validate the ability of selected formulations to deliver biopharmaceuticals to specific targets at desired bioavailability including at the intracellular level

B. Overall deliverables of the project

In summary, the overall deliverables for the project are:

- Fully characterized drug delivery systems containing a selection of model payloads (nucleic acids, peptides or proteins) for testing for cellular updates and trafficking
- A manually curated database containing over 1200 entries with useful, searchable information from scientific literature on non-lipid nanoparticles and neurotrophic viruses
- IMI cell banks containing most relevant cell lines and standardized protocols and serum source to be used by all partners throughout the consortium

C. Summary of progress versus plan since last period

COMPACT continued to streamline the number of drug delivery system (DDS) formulations by implementing a DDS evaluation matrix for prioritizing the most promising approaches thereby optimizing the use of the COMPACT resources. This exercise has allowed COMPACT to focus the remaining resources on a small number of formulations with the highest probability of success, and moving some of these formulations into in vivo pharmacology studies as planned for the last year of the project.

The objective to move from “model” payloads (selected for ease of detection) to payloads with more disease relevance endpoints was accomplished in a resource-effective strategic manner.

- Human insulin was used as a disease relevant peptide payload for trans-intestinal delivery;

functionally relevant delivery in a rat intrajejunal instillation model could be demonstrated by using blood glucose levels as biomarker.

• TNF-α was chosen as a disease relevant target for siRNA delivery in in vitro 3D-cellular models consisting of lung cells and macrophages and will be used as a target for in vivo lung delivery in a mouse lung inflammation model that has been established.

• Beta-Secretase -1 BACE was selected as a target for siRNA and Aβ as a target for an antibody to develop DDS's for delivery across the BBB.

• For dermal delivery, an antigen of a pathogen, Listeria monocytogenes, was selected as a disease-relevant payload and used to demonstrate functional relevant delivery of a protein into the skin by hollow microneedles.

These streamlining efforts by implementing a rigorous DDS prioritization process and selecting functionally relevant payloads have led to the identification and characterization of some DDS's that may be pursued in future drug development programs. The two major goals for the last reporting period, the prioritization and optimization of the most promising DDS's and the demonstration of their delivery efficacy by using disease-relevant payloads, have been accomplished.

The generation of transgenic reporter mouse models (Thy1.2-Luc for testing siRNA delivery to the brain; SPC-Luc for testing siRNA delivery to the lung; and chicken β-actin split-Luc for testing ubiquitous delivery of splice-correcting oligonucleotides) was delayed because of breeding problems. Therefore, the originally planned biodistribution studies using these reporter mice could not be performed within the time frame of the project. However, these transgenic mice have now been generated and bred during the last year of the project and will be made available as a COMPACT foreground asset to the scientific community.

Furthermore, the COMPACT management team has strengthened its efforts to ensure efficient communication of the COMPACT accomplishments outside of the immediate project team. COMPACT presentations and posters have featured on the agendas of several significant Conferences and meetings in 2017. This representation, coupled with publications and three patent applications, has ensured that COMPACT was very well represented in 2017. Furthermore, the management team has continued to actively review the foreground to prepare it for potential further exploitation within and outside of the consortium. These efforts have led to the call of a new proposal on the BBB within the IMI2 framework.

D. Significant achievements since last report

• Physico-chemical characterization and up-scaling of DDS's:

o An evaluation matrix to allow objective assessment of individual DDS's was implemented to support the prioritization of DDS's based on the status of research, the quality of data, and the likelihood of success.

o Successful completion of the efforts for the identification of the procedures required to up-scale selected formulations to quantities that will supply repeat dose toxicology and potential first time in human trials. The bottlenecks previously identified for the PLGA-lipidoid

nanoparticles for delivery of siRNAs have been successfully removed and a 10-fold scale-up has been achieved.

- In vitro characterization of DDS's:

  o Completion of the characterization of cellular features enhancing endosomal escape in real time using live cell imaging confocal microscopy.

  o Split protein and biotin ligase reporter assays were fully developed and used to demonstrate delivery of peptides to the site of intracellular action.

  o A "Transcytosis Index" was developed to allow high content monitoring of trafficking of macromolecules in polarized barriers.

  o A novel method to label exosomes was developed for monitoring cell uptake and intracellular trafficking of these DDS's.

- Oral delivery of peptides:

  o Major progress was made in characterizing in vitro optimized formulations for transintestinal delivery in vivo.

  o By using human insulin as a model peptide payload, two SNEDDS formulations were identified that showed in a rat model of intrajejunal instillation promising results in a significant decrease of blood glucose levels after intrajejunal installation.

  o Even more promising results in this model were obtained with insulin in complex with a novel derivative of the cell penetrating peptide (CPP) penetratin.

- Delivery of across BBB:

  o Work to identify a successful brain targeting DDS has continued with several candidates and advanced into in vivo biodistribution studies. However, none of the selected brain-targeting peptides (RVG, g7, EPRNEEK) was able to unambiguously demonstrate brain enhancement.

  o Brain delivery of exosomes was shown using fluorescence as a readout.

  o SLNPs surrounded with a corona were shown to possess enhanced bioavailability and enhanced potential for transcytosis across the BBB.

- Delivery across the air-lung barrier:

  o The development of a 3-D co-culture model for investigating anti-inflammatory treatments was completed. Its suitability was demonstrated with budesonide as a model drug.

  o Lipidioid-PLGA nanoparticles were shown to be very efficacious in delivering siRNA against TNF-α in mouse macrophages; to test these nanoparticles in an in vivo pharmacology study, a mouse inflammation model was established by LPS stimulation.

- Dermal delivery of proteins:

o A preparation method for hollow microneedles with an optimal tip geometry has been developed to completion; the efficacy of these microneedles was demonstrated by immunization of mice with the antigen of Listeria monocytogenes embedded in PLGA nanoparticles.

o A preparation method for dissolving microneedles was developed for two proteins, IgG and ovalbumin, and for ovalbumin-loaded PLGA nanoparticles. Moreover, a new method was set-up to study the protein dose delivered into mice by dissolving microneedles.

E. Scientific and technical results/foregrounds of the project

The foreground generated by the IMI COMPACT project can be divided into 4 main areas:

• Novel Drug delivery systems (DDS) for proteins and nucleic acids – a total of 21 formulations have been evaluated in vitro during the COMPACT programme and 11 have been progressed in vivo. 2 of these formulations (lipidoid PLGA particles for nucleotide delivery (PatNo. WO 2017/158093/A1, & SLNP with corona (Abbvie – patent applied) have suitable novelty and have been filed for Patent protection.

• In vivo and in vitro models developed to evaluate drug delivery systems – methodologies have been established to determine the trafficking of DDS payloads across the cell membranes to the site of action. Reporter gene and split proteins models allow the study of functional delivery of nucleotides and peptides respectively. Multicellular in vitro models of biological barriers have been used to mimic the in vivo environment and these has been complimented by the development of transgenic mice models.

• Data derived for the use of the models with the new DDS formulations – COMPACT has an extensive publication record with over 60 peer reviewed journal articles and several oral and poster presentations at prestigious conferences (see COMPACT website). In addition, an extensive database of the characterisation of the DDS formulations has been compiled during the project and is available on request (Alfresco). Since this database contains unpublished negative data, it is more comprehensive that the published works alone.

• The emergence of novel target for the enhancement of blood brain barrier uptake- following an exhaustive analysis of the protein expressed on the membranes of BBB derived cells, a collection of potential uptake targets has been identified and publicly disclosed. These targets are available for further exploitation after COMPACT has concluded.

COMPACT results have been disseminated through 60+ publications available on the website (https://www.compact-research.org/publications/) and through the presence of key speakers at various conferences along the course of the project. COMPACT results have been shared with the scientific community. Patents have been filed and described above.

F. Potential impact and main dissemination activities and exploitation of results

The COMPACT project has relied on the collaboration of 16 academic groups within the EU, coupled with 7 multinational pharma companies. This has resulted in the combined research effort to be collocated in the heart of Europe while leveraging international expertise. In this fashion, COMPACT has ensured that cutting edge research and innovation is discovered, nurtured and disseminated within

Europe. The results generated in the scope of the project have been largely disseminated within 60+ journals with high impact factor or at international conferences. Through the well recognised papers and oral presentations, the outcomes of COMPACT will guide the European and worldwide research on Drug Delivery and serve as solid foundation for upcoming research/projects and breakthrough discovery in the field of oligo delivery. The immediate outcome of these efforts is to promote efficient collaboration across European boundaries, allowing young scientists to experience scientific practices from many neighbouring countries. Furthermore, tangential collaborations and employment opportunities ensure that future investment in projects and people is retained within the EU.

While it was never the intention of COMPACT to produce novel therapeutic agents, it has laid the foundations for future generations of macromolecular medicines. These efforts reaffirm the European scientific community as key players in the discovery and development of innovative medicines. The direct exploitation of the results led some partners to follow up with new collaboration around testing of delivery technology developed in COMPACT with specific cargo.

COMPACT has achieved a very high level of standardization across multiple labs for cell lines or immunotoxicity and immunogenicity screening. It ensured solid reproducible results that can be internalized outside of the consortium. Methods and protocols have been published and are available on the COMPACT website (under publication section).

Certain of these results are already implemented in the industry partners. Multicellular in vitro models of biological barriers are used to simulate the in vivo environment and support evidence generation for internal research. In keeping with the spirit of IMI, the COMPACT teams have been actively publishing their results for utilisation by the wider scientific community. Furthermore, the methodology developed by individual partners is available for further exploitation, thus ensuring that the legacy of COMPACT continues long after the final report is filed.

G. Lessons learned and further opportunities for research

The COMPACT consortium has shown that precompetitive collaboration between industry and academia can lead to new scientific insights, research tools and nanocarrier formulations to facilitate the delivery of biopharmaceutical drug substances. This collaboration has generated many scientific publications, most of which are openly accessible to stimulate further discussion and scientific collaboration on the topics of drug delivery over biological barriers. In addition, several patents have been filed on technologies and formulations developed within COMPACT that would help future commercial exploitation. Several partners outside COMPACT have shown interest in the nanocarrier formulations that were developed, which may lead to future collaborations between industry and academia at the European level without the need for public funding.

The results generated in COMPACT will without doubt lead to new initiatives for collaborations, some of which have already started. Based on the results obtained around drug delivery to the brain, a new IMI call was launched (Call 12, topic 6: Discovery and characterisation of blood-brain barrier targets and transport mechanisms for brain delivery of therapeutics to treat neurodegenerative & metabolic diseases) in which outcomes generated in COMPACT will be carried over and continued with.

Besides drug delivery to the brain, the COMPACT consortium has generated interesting data and tools to study the process of cytosolic delivery of nucleic acids. Since this topic requires further investigation to

lead to much improved delivery of nucleic acid-based drugs it is our recommendation to stimulate future PPP collaboration on this topic.

Furthermore, research tools and animal models for measuring functional delivery of biopharmaceuticals in a quantitative manner have been developed. Such tools will be very useful for other academic groups and pharmaceutical companies as well.

Lessons learned

Based on our experience with COMPACT we have several recommendations for future IMI-funded

PPP.

- The first recommendation is to keep the size of the consortium as small as possible. COMPACT consisted of 26 partners, which made the decision-making process often slow and cumbersome. The second recommendation is to reserve budget for “unforeseen” tasks that might appear later on in the project. It is very difficult to predict 5 years in advance and. As a consequence, new tasks might arise that require additional funding. Budgets are fixed at the beginning and cannot easily be expanded. Earmarking budget for unforeseen tasks could be a solution for this. Also, make sure that there is a good balance between personnel costs and materials costs.

- The final recommendation is to have frequent updates amongst the partners, either by teleconference or face-to-face. This will keep the coherence in the consortium and keeps the people involved motivated.

- Finally, make sure to disseminate your results not only to the scientific community but also to the main public. PPP can only exist with the support from the tax-payers and they need to be involved as much as possible.