IMI1 Final Project Report Public Summary

Project Acronym: K4DD

Project Title: Kinetics for Drug Discovery (K4DD)

Grant Agreement: 115366
Project Duration: 01/11/2012 - 31/10/2017

1. Executive summary

The executive summary will be made publically available, and therefore should not include information deemed as confidential by the consortium. It should be concise (preferably no more than 40 pages), comprehensive and should capture the updates for the last reporting period as well as the overall outputs of the project and its impact. It shall at least cover the following items**:**

1.1. Project rationale and overall objectives of the project

(max 1 page)

There is mounting evidence that the often ignored kinetic aspects of the interaction between a drug and its target in the body are highly relevant for in vivo efficacy and clinical success. Lack of attention to this important parameter may be one of the reasons for the high attrition rates in drug discovery, as it has been analysed that many recently marketed drugs indeed possess improved kinetic profiles. Within K4DD we strive to develop techniques, structure-kinetic relationships (SKRs), data and models that will be accessible and used by the drug development community and to enable reliable predictions of kinetic properties in silico. All together this would improve the in vitro to in vivo translation of future medicines in terms of efficacy and safety.

Three objectives have been defined that describe the ambition of the consortium:

(i) Gaining molecular understanding of kinetic characteristics to aid the development of predictive kinotypic analysis (WP1).

(ii) Developing technologies to enable the rapid assessment of compounds' kinetic characteristics (WP2).

(iii) Translating these findings to in vivo effects from intact cell to whole animal and man (WP3).

These three objectives are interconnected and the consortium aims to obtain an integrated dataset over the three work packages (WPs) for a set of drug targets. To quickly gain momentum, a few accelerated targets have been selected during the starting phase of the consortium. This serves as a benchmark for other targets, and is expected to enhance the quality of decision making and data interpretation later in the project. The other targets will be selected from multiple prototypic drug target classes to generate knowledge that is relevant for many researchers in the drug development community.

1.2. Overall deliverables of the project

1. The consortium develops tools to study the kinetics of a drug-target interaction i.e. assays, SKRs (Structure-Kinetics Relationships) and kinetic data. The partners of the consortium will work together to develop a set of modeling and molecular dynamics tools, mathematical models of the kinetics of drug-target interactions and *in silico* SKR models.
2. In addition, assays are being developed and compared for soluble and membrane targets and where possible these will become available as kinetic assay kits for the scientific community.

3. To store the collected and generated data, the consortium is developing a database that will also become available for researchers outside of K4DD after the project's end date.

In order to link these kinetic data to the situation in man, mechanism-based PK/PD models will be developed. These models will be based on high-content cellular and *in vivo* data. Ultimately, these mechanism-based PK/PD models may be used in the design of pre-clinical studies.

To achieve the abovementioned goals, experts from many different fields have joined forces within K4DD. For training purposes an Education and Training program for the K4DD fellows has been developed with the aim to make them acquainted with the different aspects of kinetics, relevant techniques and the role of kinetics in drug development.

1.3. Summary of progress versus plan since last period

In period 5, all K4DD objectives were achieved with no large deviations from the project plan often even surpassing the original set of objectives. Not only has a phenomenal amount of data been obtained but valuable findings have been derived from the data, existing technologies have been vastly improved and novel methodology developed.

A major objective for the final period was to establish predictive models and to make these models available in the form of tutorials and a web based tool box. HITS successfully developed and deployed an array of powerful modelling and simulation tools, including Random Acceleration Molecular Dynamics (RAMD) for computational prediction of relative residence times. These and further computational approaches developed and applied by other members of K4DD are collected, along with tutorials and guidelines, in the tool box, which is available online (http://kbbox.h-its.org). The toolbox has been developed along with the WP 4 deliverable of the K4DD database, which captures the work of K4DD over the past 5 years and is the largest for drug binding kinetics. It is being implemented in the EBI-ChEMBL database. Both the toolbox and the K4DD database will be two major legacies from the K4DD consortium.

The previously developed technologies enable K4DD partners to rapidly assess compounds' kinetic characteristics within WP2. Significant achievement this period include work from the Fraunhofer Institute on the H1 receptor, showing tight correlation between the measured recovery time and residence time in clinically relevant cells lines. In addition to this multiple partners have been successful in resolving structures of key targets such as Adenosine A1 (HEP) and A2A (HEP, UL), H1 (ICL, VUA) and IGF1-RK (SAD). K4DD used this final period to extend the large data sets of generated kinetic data for both membrane proteins and soluble proteins. The data have been used to establish multiple SKR models. In general, the experimental data correlated very well with theoretical models, which hypothesis was one of the major goals of the last year of the consortium to be explored.

Regarding the translation from *in vitro* target interaction kinetics to *in vivo* animal PK/PD kinetics a range of predictive modelling approaches were used. With K4DD-generated *in vitro* cAMP data an *in vitro* PK/PD model was developed, providing considerable insight into the relation between BK of the dopamine D2 ligand and that of fluctuating endogenous dopamine on signal transduction as PD biomarker. With the human and rat plasma *in vitro* PT and aPTT data the anticoagulant potency of a series of FXa inhibitors was ranked, with the anti-coagulation potency in human plasma correlating with koff.

An important part of the K4DD project has been its educational programme which has funded more than 20 post-docs and PhD students in the last five years. The programme has provided fellows with the opportunity to gain a thorough understanding of the connection between drug-discovery and drug development by offering them an extensive drug discovery course and several “binding kinetics” oriented symposia. Fellows also got the opportunity to improve their soft skills by taking a scientific writing course, career workshops and a presentation workshop. The first fellows have defended their thesis successfully and more PhD theses are expected. In addition, we have seen many fellows continuing their careers in more senior positions.

1.4. Significant achievements since last report

- The launch of the web based toolbox that provides methods for computing drug binding kinetics has been established (http://kbbox.h-its.org). This toolbox provides also a guide for potential users, examples and tutorials. Further tutorials will be added as the results from K4DD are published.

- The MASS2 SPR machine was launched with several improvements over the MASS1 in response to input from consortium partners. Valuable gains include increased efficiency with individual needle support allowing use of singles channels. The MASS2 also allows greater flexibility in that 4 different buffers can be used within an assay and a huge benefit is the greater overall capacity.

- In collaboration with EBI the K4DD consortium has exported the data items collected during the K4DD project and transferred them to EBI for inclusion in the ChEMBL database. This way, sustainability and open access to the data beyond the project’s lifetime is guaranteed.

- In the final period the first of many K4DD fellows defended her PhD thesis. The thesis focused on binding interactions between neuropeptides, drugs and their target molecules. Indira Nederpelt of Leiden University was the author of the thesis which has already resulted in six peer-reviewed publications, with more to come.

- K4DD published a comprehensive overview article on Drug-Target binding kinetics. In a true Public-Private effort, 9 K4DD partners have joined their knowledge and experience to establish a comprehensive overview article of drug Target-Binding Kinetics. The article appeared in Drug Discovery Today.

- In its final month, the K4DD consortium gathered for one last time to discuss the importance of drug target binding kinetics in drug discovery. For this final meeting the K4DD consortium opened its doors and invited the scientific community to participate in an open scientific meeting. The meeting entitled: 'Binding Kinetics: Time is of the essence' attracted over 180 participants from 18 different countries and consisted of 7 different scientific sessions stretched out over 3 days, hosting almost 30 speakers. The momentum which the field of binding kinetics currently has, was emphasized by the great attention the meeting attracted from all different types of stakeholders. Having representatives from 11 different major pharma companies, 22 SMEs and over 30 universities stretching over the entire globe from China, the US and Europe further

stressed the importance of binding kinetics and public-private partnerships. This final meeting was a great way to conclude the K4DD project.

- Within the last period 17 papers have been published in peer reviewed journals with 20 more being either in submission or under review. The K4DD publications also proved to be of high quality as can be seen by the publication in some of the high impact journals such as: Nature CommunicationsStructure, Scientific Reports, Cell Chemical Biology, Drug Discovery Today and Trends in Neurosciences amongst others. In addition, K4DD members presented K4DD work on a vast number of scientific meetings throughout and outside Europe including of course the *K4DD meeting: Time is of the essence*.

1.5. Scientific and technical results/foregrounds of the project

- In the final period K4DD has achieved the realization of commercial potential from the project's work. Sierra's MASS2 kinetic SPR instrument was developed from an early prototype to a serial product together with WP2 and is now commercialized for high throughput SPR measurement. The results from K4DD partner Bayer have proven to be valuable for an SME "BME Labtech", which was able to commercialize a high throughput reader for the kinetic probe competition assay (kPCA); the development of such an assay was part of WP2. The collaborations between Nottingham University and Promega have led to readily commercially available off-the-shelf assay kits for kinetic analysis. Also, Cisbio has been able to develop similar off-the-shelf assay kits for kinetic analysis based on results generated within K4DD

- Several K4DD partners achieved success in resolving structures of key targets: Heptares with Adenosine A1 and A2A receptors, ICL with olopatidine- and acrivastine-liganded H1 receptors, Leiden University with A2A receptors and Sanofi with IGF1-RK. Further, Heptares have submitted a publication to Nature Scientific Reports on novel methodology used to obtain their structural results.

- For over 15 targets, K4DD has created two or more independent assays, most of which have shown robustness in cross lab experimentation. Studies on for instance the A2A receptor have greatly benefited from the data from 3 independent assay types such as Surface Plasmon Resonance (SPR), Radio Ligand Binding filtration assay (RLB) and Scintillation Proximity Assay (SPA). Moreover, the receptor was presented in the assays in native membrane preparations, in nanodiscs (artificial membrane environment) and in detergent micelles. This allowed to compare ligand binding affinities and binding kinetics across several assay technologies and across different biological environments for a broad range of targets.

- Within WP3, K4DD researchers from Leiden University have presented a mathematical approximation to understand and visualize the effect of drug-target binding kinetics on the duration of target binding. The approximation calculates the duration of a drug effect taking into account two parameters: 1) the rate at which the drug is cleared from the body and 2) the rate of drug-to-target binding and unbinding. In other words, the study demonstrates how the drug concentration in the body and its interaction with the biological target can be analyzed together to predict the timespan of the drug effect. These findings can contribute to make better and safer drugs available for patients.

- The formulation of the recommendations for mechanism-based population PK/PD and PBPK/PD modelling, and the implications for pre-clinical (in vitro and in vivo) study design has been a major achievement of the K4DD project.

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

Within the K4DD consortium, we have strived to establish new techniques, protocols, assays and instruments to aid in the assessment of drug target binding kinetics in a more sensitive, accurate and faster way. This has resulted in several exploitable results which have already been commercialized or are at a stage to be exploitable in the near future. Please find below a list of some examples.

- In collaboration with EBI and after getting the approval from the K4DD consortium, University of Vienna has exported the K4DD database, data items collected during the K4DD project, and transferred them to EBI for inclusion in the ChEMBL database. This way, sustainability and open access to the data beyond the project's lifetime is guaranteed.

- A new SPR system, the MASS-2, has been developed utilizing the input of the K4DD partners. A prototype has been provided to UNIVDUN. It was subsequently brought on the market. The MASS-2 system is a high-throughput, high performance real-time, label-free (RT-LF) analysis system. It has all the features expected from a high-throughput system, including 8 flow cells with 4 detection spots for a total of 32 individually addressable sensors, robust microfluidic sample delivery and high sensitivity SPR+ detection. The MASS-2 utilizes Sierra Sensors' proprietary Hydrodynamic Isolation™ (HI) technology to deliver high-throughput sample analysis with dedicated in-line controls. It is a major improvement to the MASS-1 system and to other kinetic analysis SPR instruments in the following aspects:
    - 32 spots in 4 x 8 array to allow more active reference channels or double the number of interaction measurements
    - Support for 4 different buffers which can be used simultaneously
    - Individual Needle support allows the use of single channels for flexible assay development and efficient use of the sensor chip.

  Details on the MASS to can be found on: http://www.sierrasensors.com

- The Swiss company Genedata has been able to develop a software solution for the evaluation of competition binding kinetics data on the basis of K4DD results from Bayer. This application is already available to all Genedata customers. A poster created with Bayer on the development of this product has been exhibited in different scientific meetings and is downloadable from the Genedata website. https://www.genedata.com/resources/posters/poster/?tx_infores_detail%5Bresource%5D=297&cHash=2a4a700875843decc6cf0efe4517ff20

- Together with the German company BMG Labtech, which manufactures the multi-mode microtiter plate reader PHERAstar, K4DD partners are working on updates of their MARS instrument software. This update is scheduled for late 2017 or early 2018 and will integrate the scripts developed at Bayer to make kPCA measurements with this reader possible. As companion to the update rollout a technical note explaining how to perform kPCA assays using the BMG Pherastar instrument is planned.

- K4DD partners are currently discussing with the French company Cisbio, a world leader in TR-FRET reagents about the publication of technical notes for the use of their products for competition binding kinetics assays. Eventually Cisbio might offer in the future binding assay kits and protocols for the determination of binding kinetics parameters using kPCA.

- K4DD partner University of Nottingham, in collaboration with Promega developed the BRET assay to measure binding kinetics of GPCRs, which has been published in Nature Methods (Nat Methods. 2015 Jul; 12(7): 661–663.) and is currently available at Promega www.promega.com

- The K4DD Computational toolbox is available online and accessible for everybody: A database of methods for computing drug binding kinetics has been built. This includes a guide for potential users , examples and tutorials, extending on a recent review of new approaches to computing drug binding kinetics (https://doi.org/10.1016/j.sbi.2017.10.001). Further tutorials will be added as the results from K4DD are published.

1.7. Lessons learned and further opportunities for research

The added value of a public private partnership to achieve the K4DD objectives

The interaction with many different partners in the K4DD project was indicated as an absolute added value by almost all partners. The partners generated enormous flow of exchange of knowledge in which individuals have had the opportunity to provide contributions, and see their contribution in a much broader context. The biannual meetings were a key element in providing a regular platform for these interactions.

Many partners also recognized the fellow programme to be of particular value to the project since the enthusiastic group of young scientists really formed the cohesive core of the project. The K4DD fellow internships and the dedicated fellow symposia and workshops have created an open atmosphere for discussions and innovation which has been invaluable in terms of training of the K4DD fellows and other members of the different research groups. It has greatly broadened their experience and given them insights that will help them in their later career decisions. A survey among the fellows resulted in a score of 4.5 out of 5 and fellows emphasised the importance of the educational programme. *"I have learned a lot due to the educational programme, and the focus on career perspectives and skills brought me new insight to plan my own career and to decide on the choice between industry and academia."*

The K4DD consortium did not just create a mix between public and private partners but also between partners with broadly diverging scientific backgrounds. Computational scientists benefited from the access to experimental data from the EPFIA partners, for instance by being able to gather crystallographic, thermodynamic and kinetic data on sizeable datasets of drug-like molecules for a range of kinase drug targets. Such exclusive and unprecedented data access has proven to be essential in developing and validating computational methods to study drug binding kinetics. Scientists in charge of translating *in vitro* target interaction kinetics to *in vivo* animal PK/PD kinetics were able to create a range of predictive modelling approaches and benefited from the continued interactions with the partners producing the *in vitro* data.

The K4DD consortium created a wealth of experimental and computational data on a wide variety of targets and a large number of diverse chemical compounds; "*it is hard to imagine how such a broad approach can be realized outside a framework as provided by a PPP like IMI K4DD"* stated one partner.

Multiple SME's pointed out that the different engagements with both academic and EFPIA partners have helped their business case and have already resulted in additional smaller "one to one" collaborations. Building a foundation of trust and collaborative attitude during the K4DD project was essential for these new business opportunities. Developed technologies have already been implemented within the SME core facilities and services as one partners states: "*we will implement the K4DD-developed SPR technologies and the determination of kon and koff values in our drug discovery projects to annotate hit compounds from screening campaigns that we will perform in the future".*

Possible recommendations which could be useful for future PPPs.

At the onset of the project the consortium experienced a slow start due to difficulties in the process of deciding which targets could be worked on in such a big multi partner endeavour. During this stage the consortium benefited from having dedicated program management partners who also offered honest data broker solutions which safeguarded conflicting IP interest and resulted in the establishment of a target list. As one partner commented: "*For a multi-partner scheme such as this, the IP concept should be as simple as possible and it should be developed jointly by the scientific leads and the legal representatives of all partners. Project results should be made open access whenever possible considering the project scope."*

Several partners indicated that the established "rather large" target list worked in great ways through the gathering of a tremendous amount of data on a diverse set of targets, although the project might have benefited from a more focused target list. Such a focused list might have facilitated in taking more targets "end to end" e.g. through all work packages.

The educational programme has been one of the key successes of the K4DD consortium. Not only was the consortium able to educate a future generation of scientist, the Fellow group also formed a cohesive group which facilitated tremendously in the formation of collaborative ties between the different partners. The formation of a group of young dedicated scientists would benefit any public private consortium. While monitoring the fellows in their career paths we have already seen several switches between the public and private side of drug research.

For partners who did not employ a fully dedicated K4DD fellow, a continued focus was a challenge and also made it difficult to meet certain requirements a project like K4DD asks for. Therefore, the consortium would recommend dedicated personnel be allocated to a project thereby ensuring maximum output.

Potential new research to further advance the field.

Within K4DD we see multiple lines of potential new research which could arise from the project's achievements. First of all, continuation and fully exploiting the huge amount of data generated within K4DD will provide, not just for the K4DD partners but for scientists worldwide, with ongoing and new lines of research in drug target binding kinetics. Since a large subset of the K4DD data has just been generated, it will be an impetus for much computational work on method development and tool

development, as the data is publicly released. The computational challenges to studying and understanding how conformational changes affect binding kinetics and to improving force fields for binding kinetics will be topics for further ongoing study. This will lead to improved computational methods for drug binding kinetics and it will lead to a better understanding of drug-target binding kinetics and their implications for drug discovery.

K4DD has delivered on all it deliverables, it has gained molecular understanding of drug target binding kinetics, has developed technologies to enable the rapid assessment of compounds' kinetic characteristics and has even been able to translate these findings to in vivo effects from intact cell to whole animal and man.

During the final biannual meeting, a session was fully devoted to the topic of sustainability. Below follows a summary of this session:

Topics of special interest as follow-up:

- Cellular PK/PD modelling of drug-target binding kinetics and its impact on the downstream pharmacology cascade/network and the time-course of mode-of-action and cellular effect readouts. See also the attached minutes of a separate TC on that topic.

- Translational PK/PD modelling of drug-target binding kinetics to the time-course of cellular effects and in vivo efficacy for the safety aspects of a drug (e.g. hERG & QT prolongation etc.). This could ultimately give rise to a deeper understanding of the potential impact BK may have on the therapeutic window of drugs. In addition, the generation of experimental evidence in support of the hypothesis of 'kinetic selectivity' as novel source of a therapeutic window is regarded as a very attractive topic. (The current IMI project has focussed solely on efficacy.)

- Examination of drug targets that besides binding the agonist or antagonist drug have (multiple) endogenous ligands.

- Behaviour of a target in disease state vs normal physiological conditions and the impact any changes may have on drug-target binding kinetics and subsequent downstream events.

In more general term to maintain the K4DD community alive, a LinkedIn Group has been created which can be used for discussions. In addition, already several one-one collaborations have resulted from the K4DD consortium.