REPOTRIAL

An in silico-based approach to improve the efficacy and precision of drug REPurpOsing TRIALs for a mechanism-based patient cohort with predominant cerebro-cardiovascular phenotypes

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 777111.

Deliverable D2.6:

Publish an open preclinical biobank for preclinical animal models related commROCG and other disease phenotypes for both genders

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Work Package 2

Pre-clinical validation of in-silico trial predictions

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1. Objectives of the deliverable based on the Description of Action (DoA)

One of our key goals within the REPO-TRIAL program focuses on the development of a preclinical biobank platform where blood, urine samples, and all different organs could be stored. This platform will allow direct re-use of the tissue by other groups facilitating the assessment of new potential parameters, and therefore maximally reducing required animal numbers (Task 3). Hence, we here present the development of the Pre-clinicalTrials.org platform where information from preclinical experimentation can be stored and publicly available for the scientific community.

2. Executive Summary

- Methodology: Working Package 2 in close collaboration with Working Package 1 and the IT company Gnome Design & Solutions designed a tissue repository and preclinical data platform called Pre-clinicalTrials.org.
- Results: Through Pre-clinicalTrials.org, researchers all over the world will be able to (i) access to tissue material from already performed animal experimentation, (ii) deposit unpublished negative data, and (iii) store raw data extracted from already published studies.
- Progress beyond the state-of-the-art: The Pre-clinicalTrials.org platform aims to change the current concept of preclinical experimentation and data reproducibly by building an easily accessible data repository where scientists worldwide are able to deposit experimental details, obtain data from already performed experiments, or access to tissue from previously conducted experiments.

3. Introduction (Challenge)

Biomedical research suffers from a dramatically poor translational success. Medical innovation is impacted by major quality and reproducibility issues of basic science data (Prinz et al., 2011, 2013, Ioannidis, 2005) which then impact on further clinical development (Mullard, 2011, Arrowsmith, 2011). In fact, some specific industry sources claim that a shocking percentage of approximately 50-60% of all pre-clinical animal data are, however, irreproducible (Prinz et al., 2011, Loscalzo, 2012, Freedman et al., 2015). This scenario therefore results in a considerably large section of the currently available biomedical literature being considered as unreliable for our particular purpose. Moreover, this

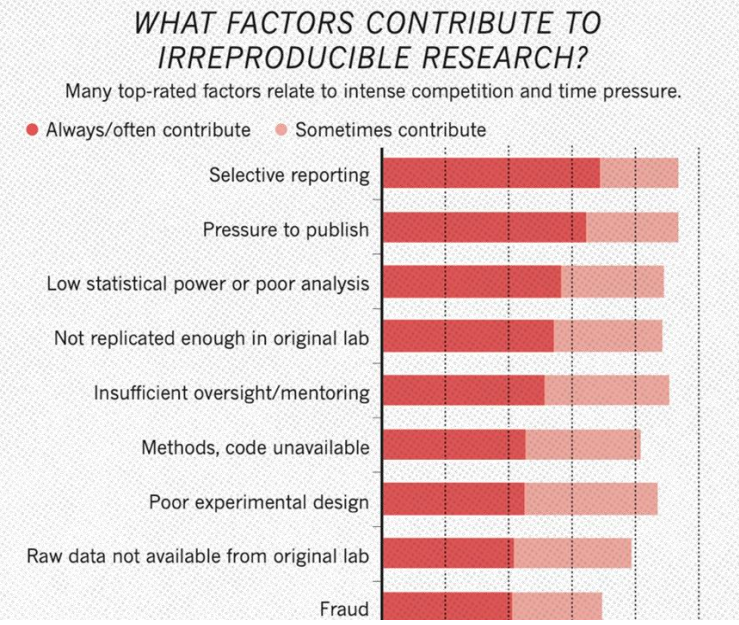

Fig. 1. Top-rated factors leading to irreproducible research in all different scientific disciplines (Baker, 2016)

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situation would get even worse if we would also account for problematic usage of questionable statistical approaches for data analysis in a surprisingly high number of publications (Slutsky, 2013). Reasons for this dramatic loss in trustworthiness includes (i) suboptimal study design, (ii) poor data analysis, i.e. evident statistical flaws (Slutsky, 2013), (iii) pre-selected reporting towards positive data (Dwan et al., 2013), (iv) constant pressure to publish, and (v) not enough detailed methods included in the original publication (Baker, 2016) (**Fig. 1**). Indeed, most papers fail to report many aspects of the experiment together with analysis which are crucial to understanding the result and its limitations, thus forcing to repeating the work (Stark, 2018).

In addition to insufficient power, publication bias has been shown to influence preclinical study results. Therefore, in our last meta-analysis study, we simulate the potential publication asymmetry based on the current original articles published in the same topic identifying a clear presence of publication bias together with an overestimation of the main read-out parameters (**Fig. 2**). Collectively, both issues lead to a distorted state-of-the-art on target-disease relationships and eventually wrong Research & Development investments into eventually ineffective clinical trials and drug attrition.

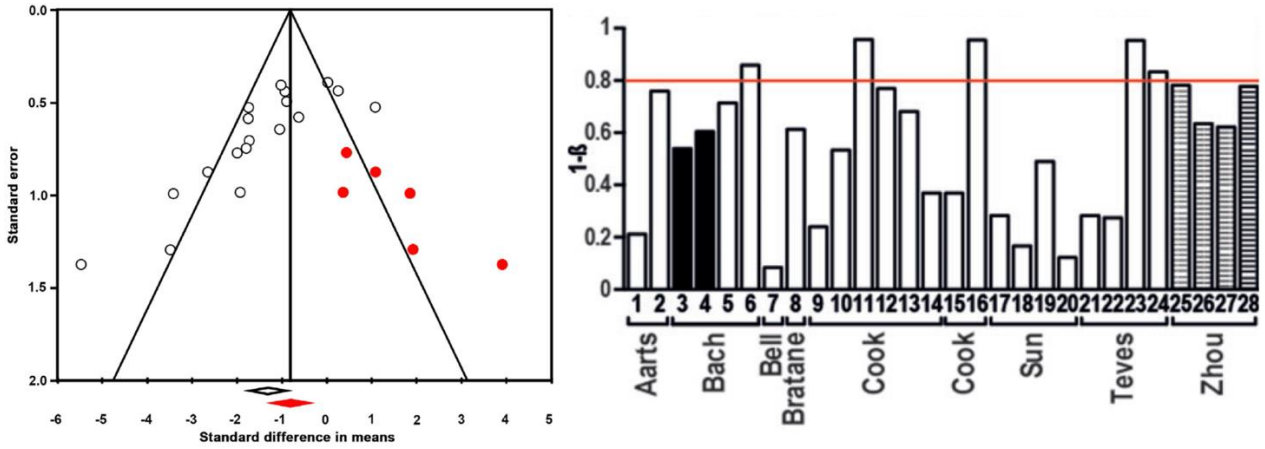

Fig. 2. Publication bias and lack of statistical power in most data sets explain irreproducible results (Kleikers et al., 2015). Left panel, funnel plot (Y-axis precision; X-axis, effect size of individual studies). Using a trim and fill analysis the intervention effect is adjusted for possible missing studies (filled red symbols) amongst published data (open symbols). The asymmetry suggests that studies showing larger effects are missing. These would otherwise shift the mean (open diamond) towards a smaller or no overall effect (closed diamond). Right panel, bar graph of the level of power from several studies and subgroups. The red line indicates the acceptable statistical Power of 0.8, which is reached by only 7 of 28 published data sets (25%).

To overcome this quality and reproducibility crisis in basic biomedical research, we here introduce the Pre-clinicalTrials.org platform, an intended pendant of the well-known, clinicaltrials.gov. Pre-clinicalTrials.org is merely a tool which facilitates (i) the access to tissue material from already performed animal experimentation to other researchers in the field, (ii) deposition of unpublished negative data, and (iii) inclusion of raw data extracted from already published studies. Therefore, data collection under these specific scenarios directly avoids the need to repeat similar experimental designs and facilitate therewith later systemic reviews and meta-analyses, currently a process taking

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several months per target. Thus, both the precision of animal studies will be enhanced, and their total number reduced. Additionally, the Pre-clinicalTrials.org will encourage a large-scale collaborative research aiming to improve translational success rate and validity of preclinical research.

4. Methodology

Platform development

The Pre-clinicalTrials.org platform is based on two separate layers which are the backend API and the frontend for the webpage. The backend API is using .NET Core 3.1 framework where we applied the REST API design, splitting up the functionalities into smaller modules for better code maintainability. The connected database was created with code-first approach and is currently deployed onto an MSSQL database which then is queried by Entity Framework 5.0. The Frontend is using React.js and Redux state management implemented with the Redux Toolkit. After the version 16.8 of React.js the React Hooks where added so these are also used in our platform together with functional components. The UI layout is based on the Material UI 4 which enables our platform to be used seamlessly on mobile phone also. In our next phase the connection and security between the frontend and the backend will be handled by the JWT bearer token authentication which makes our API protected against any misuse.

5. Results

The Pre-clinicalTrials.org website is an easily accessible, user friendly data repository platform (https://repotrialstg.gnd.ro:8197/web/main) specifically focused on preclinical research, animal experimentation, and data collection from already performed studies (**Fig. 3**).

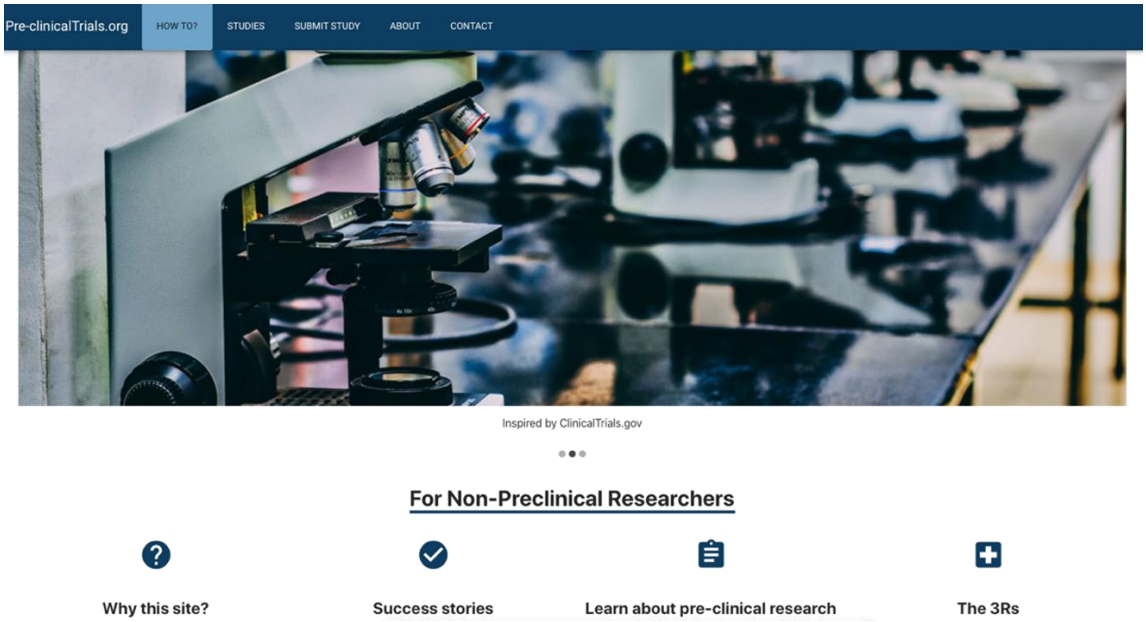

Fig. 3. Current layout of the Pre-clinicalTrials.org website. The platform provides key information regarding preclinical experimentation and data reproducibility offering the option to explore the

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underlying data base, submit new studies, and contact the coordinators for any possible addition or required features.

As previously mentioned, the Pre-clinicalTrials.org platform allows the user to get access to tissue material from already performed animal experimentation specifying where the material is located and the direct contact to the responsible researcher. Therefore, the platform will serve as a pure preclinical biobank avoiding (i) unnecessary repetition of animal experimentation, (ii) maximally reducing animal numbers, and therefore, (iii) facilitating *ex vivo* analysis after animal sacrificing. Thus, when submitting new tissue material, the scientist needs to provide further details regarding the study basic information:

- Contact name and contact email of the responsible researcher.
- Date of study completion.
- Publication of the study and subsequent link (DOI) in case the data has already been released to the scientific community.

Moreover, specific information concerning the parameters used in the study will also be required:

- Primary outcome identified in the study, i.e., improved, no change or worsened.
- Characteristics of the model specifying whether knock-out, knock-in or WT mice are used, also considering possible drug treatments.
- Species used in the study, i.e., cat, dog, Zebra fish, monkey, pig, mouse, rat, and sheep.
- Disease condition experimentally assessed. Information extracted from the OMIM data base
- Target studied. Information extracted from the Uniprot data base.
- Compound used in case of pharmacological treatment. Information extracted from the Drug Bank data base.
- Availability to receive tissue from the responsible researcher (**Fig. 4**).

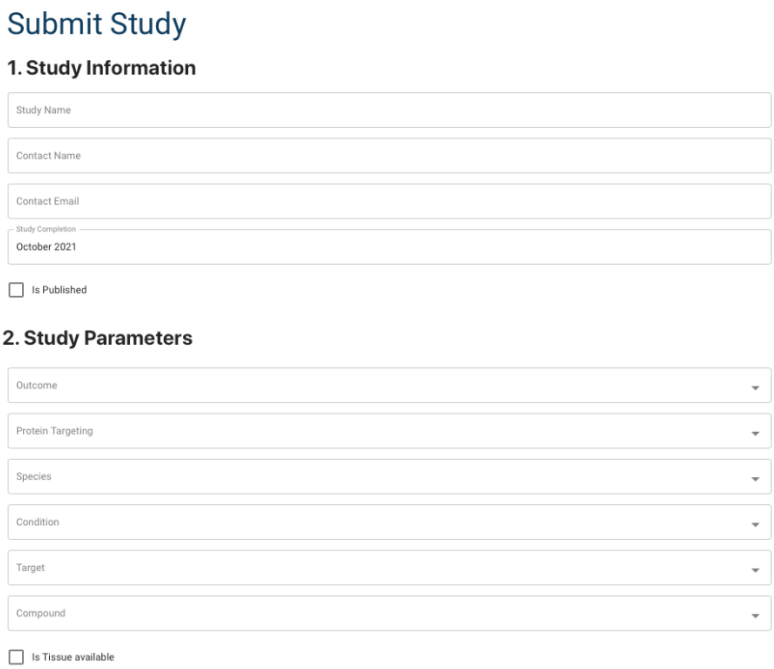

Fig. 4. Submission process of biobank data to the Pre-clinicalTrials.org platform. Users will be able to provide different details regarding general information of the study and related used parameters.

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In addition to a purely tissue repository, the ultimate goal of the Pre-clinicalTrials.org platform focuses on contributing to research quality and data reproducibility. Therefore, users will also have the option to deposit unpublished negative date, and include raw data extracted from already published studies. To maximally simplify this feature, the researcher will have the option to submit:

- Total number of animals used in the study.
- Mean value of the most relevant outcome parameter selected for the study.
- Standard deviation of the collected data (**Fig. 5**).

To ensure further comparison and statistical analysis, outcome parameters from both control and test animals will be submitted. Thus, Pre-clinicalTrials.org will facilitate later systematic reviews and meta-analyses contributing to robust scientific interpretations, effective drug treatments, and reduction of irreproducible preclinical experimentation. Additionally, Pre-clinicalTrials.org will encourage a large-scale collaborative research aiming to improve translational success rate and validity of pre-clinical experimentation.

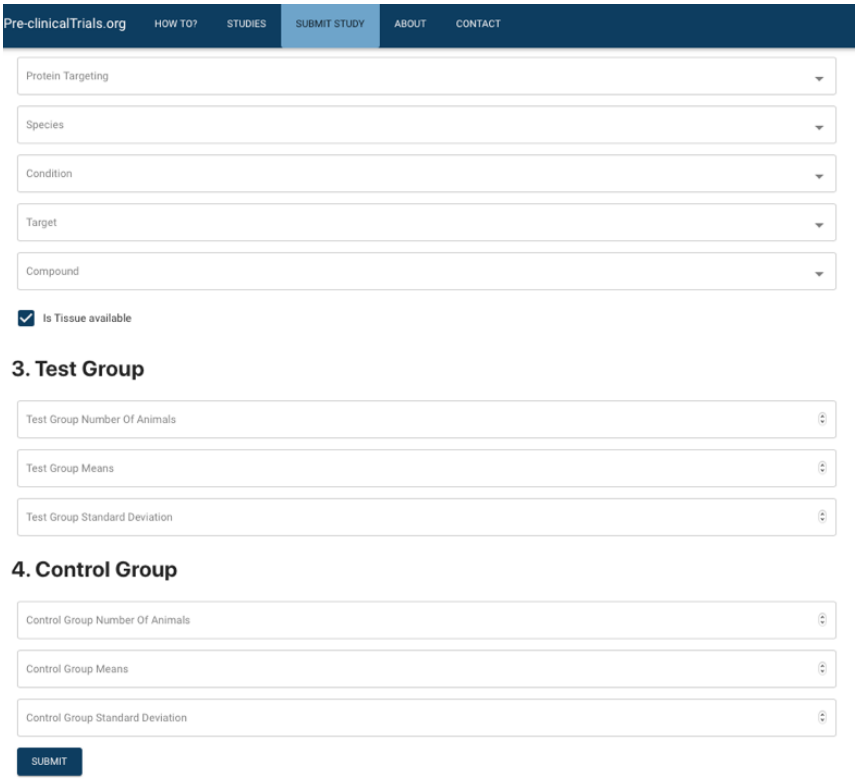

Fig. 5. Submission process of original data to the Pre-clinicalTrials.org platform. Users will be able to submit raw data from previous preclinical experimentation while comparing control animals to test groups.

6. Open issues

Over the next months, we would like to continue engaging different research laboratories worldwide to start using the Pre-clinicalTrials.org platform, and therefore, increase our data collection capacity and number of potential users. Moreover, in close collaboration with Working Package 4 we are

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planning a large dissemination activity including different social media platforms and press releases to further communicate and introduce Pre-clinicalTrials.org to the scientific community aiming to maximize its impact within the biomedical field and encourage new users to explore the website. Ideally, we can convince leading biomedical journals and national animal ethics committees to make a deposition of any target validation animal data of any size into Pre-clinicalTrials.org mandatory.

7. Deviations

Unfortunately, due to technical problems in the university servers hosting the Pre-clinicalTrials.org platform, we had to fully rebuild the Pre-clinicalTrials.org website together with previously submitted datasets. This unexpected scenario led to a certain delay in the submission of Deliverable 2.6 compared to the timeframe proposed in the original REPO-TRIAL program. However, this deviation had no effect on other deliverables or milestones neither in WP2 or other related WP within the REPO-TRIAL program. In fact, we used this reconstruction process to significantly improve the functionality of the platform and included features.

8. Conclusion

Pre-clinicalTrials.org will allow researchers from all over the world to (i) get access to tissue material from already performed animal experimentation, (ii) deposit unpublished negative date, and (iii) include raw data extracted from already published studies. Therefore, the Pre-clinicalTrials.org platform aims to improve the current concept of data reproducibly and quality of science by implementing an easily accessible data repository where scientists are able to deposit experimental information, obtain data from already performed experiments, or access to tissue from previously conducted experiments. Altogether, Pre-clinicalTrials.org will avoid unnecessary usage of animals, facilitate systemic reviews and meta-analyses, and encourage research transparency.

9. References

2013. Facilitating reproducibility. *Nat Chem Biol,* 9**,** 345.

ARROWSMITH, J. 2011. Trial watch: Phase II failures: 2008-2010. *Nat Rev Drug Discov,* 10**,** 328-9.

BAKER, M. 2016. 1,500 scientists lift the lid on reproducibility. *Nature,* 533**,** 452-4.

DWAN, K., GAMBLE, C., WILLIAMSON, P. R., KIRKHAM, J. J. & REPORTING BIAS, G. 2013. Systematic review of the empirical evidence of study publication bias and outcome reporting bias - an updated review. *PLoS One,* 8**,** e66844.

FREEDMAN, L. P., COCKBURN, I. M. & SIMCOE, T. S. 2015. The Economics of Reproducibility in Preclinical Research. *PLoS Biol,* 13**,** e1002165.

IOANNIDIS, J. P. 2005. Why most published research findings are false. *PLoS Med,* 2**,** e124.

KLEIKERS, P. W., HOOIJMANS, C., GOB, E., LANGHAUSER, F., REWELL, S. S., RADERMACHER, K., RITSKES-HOITINGA, M., HOWELLS, D. W., KLEINSCHNITZ, C. & SCHMIDT, H. H. 2015. A combined pre-clinical meta-analysis and randomized confirmatory trial approach to improve data validity for therapeutic target validation. *Sci Rep,* 5**,** 13428.

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 777111.

LOSCALZO, J. 2012. Irreproducible experimental results: causes, (mis)interpretations, and consequences. *Circulation,* 125**,** 1211-4.
MULLARD, A. 2011. Reliability of 'new drug target' claims called into question. *Nat Rev Drug Discov,* 10**,** 643-4.
PRINZ, F., SCHLANGE, T. & ASADULLAH, K. 2011. Believe it or not: how much can we rely on published data on potential drug targets? *Nat Rev Drug Discov,* 10**,** 712.
SLUTSKY, D. J. 2013. Statistical errors in clinical studies. *J Wrist Surg,* 2**,** 285-7.
STARK, P. B. 2018. Before reproducibility must come preproducibility. *Nature,* 557**,** 613.

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