IMI1 Final Project Report Public Summary

Project Acronym: PREDECT

Project Title: New Models for Preclinical Evaluation of Drug Efficacy in Common Solid Tumours

Grant Agreement: 115188
Project Duration: 01/02/2011 - 30/04/2016

1. Executive summary

1.1. Project rationale and overall objectives of the project

Objectives from the original Description of Work:

To improve in vitro models of human disease, through the development of complex, reproducible and robust models that more closely mimic the cellular organisation of tumours (eg in three dimension) and the cellular heterogeneity within human malignancies.

To cross validate, in a reciprocal way, these novel, complex in vitro models against relevant in vivo models which more closely reflect characteristics of human cancer pathology, particularly tumours arising in transgenic mice.

To use a systems biology-based approach to integrate and compare 'omics data derived from the novel models and the public databases, to generate in silico models of the biochemical circuitry associated with potential drug targets.

Cancer remains an area of high unmet medical need, with 770,000 deaths in Europe in 2014 (http://eco.iarc.fr/eucan/Cancer.aspx?Cancer=0). It was estimated that >90% of novel anticancer drugs fail in clinical trials, 66% for reasons of lack of efficacy (Arrowsmith, J. (2011), Bhattacharjee, Y. (2012)). Oncology has the worst attrition rate of any therapeutic area (Bhattacharjee, Y. (2012)). Novel drugs that progress from the laboratory to clinical trial do so on the basis of positive data from preclinical models. These are considered to predict robust clinical efficacy. Current models therefore largely fail to be predictive of drug efficacy in the clinic. A 2016 publication with a "decision theory" analysis of the falling productivity of the Pharmaceutical Industry, in all therapeutic areas, clearly identified inadequate preclinical models as a major contributory factor (Scannell, J. W. and Bosley, J. (2016)). It also identified overly reductionist laboratory tools that address the complexities of human pathology as a cause of failure. Over-reductionist approaches to the understanding of pathology were also identified as a major reason for the discrepancies between the vast financial investments in medical bioscience and the modest output to ameliorate disease (Bowen, A. and Casadevall, A. (2015)).

PREDECT has addressed the failure of current, simplistic, reductionist preclinical models, such as cell lines and some mouse models, to predict drug efficacy and to assess their role in novel target validation (discussed in Section 2.4). It had the objective to characterise alternative models and to determine whether they could better reproduce, with fidelity, aspects of the complexity and heterogeneity of human tumours. This has become particularly important during the lifetime of the PREDECT project, as genome sequencing has revealed that the intercellular heterogeneity of metastatic cancers is a major barrier to effective systemic therapies (Alizadeh, A. A. et al (2015)). The fidelity of *in vitro* models is particularly important for the first stages of drug discovery: target discovery and validation. Tumours from innovative advanced mouse models, including genetically engineered mouse models (GEMMs) were investigated to determine whether they better represented human breast, prostate and lung carcinomas. Patient-derived tumour material, GEMMs and cell line-derived xenografts in mice were used to generate precision-cut tissue slices that captured *in vitro* the native environment and heterogeneity of tumours. Simple and complex three dimensional (3D) models (tumour cells with appropriate stroma), sometimes using bioreactors, were generated to better capture the 3D architecture of cancers. PREDECT then compared, by immunohistochemistry, the patterns of protein expression in the complex models, capturing their heterogeneity. This was achieved using a central tissue microarray (TMA) platform, providing an archive of the models, which can be interrogated by Web-based microscopy.

Novel PREDECT models, now integrated into industry, and the TMA archive should permit further interrogation of their capability to better capture the complex, heterogeneous characteristics of disseminated solid tumours and to promote improved drug discovery and treatment.

Alizadeh, A. A. et al (2015) Nature Med 21: 846-853.
Arrowsmith, J. (2011) Nat. Rev. Drug Disc. 10: 87.
Bhattacharjee, Y. (2012) Science 338: 29.
Bowen, A. and Casadevall, A. (2015) Proc. Natl. Acad. Sci. (USA) 112: 11335-39.

Scannell, J. W. and Bosley, J. (2016) PLoS ONE 11:e0147215.

1.2. Overall deliverables of the project

In order to promote exchange amongst partners sharing similar technologies, pathology-led WPs1-3 were reorganized into Platforms, whilst maintaining expertise in the three pathologies (breast, prostate and lung carcinoma). Here are their overall Deliverables, together with those of WP4 (Molecular Pathology) having achieved the Objectives of PREDECT:

Advanced *in vivo* models (IV Deliverables). Two types of murine models were investigated as possible improvements on existing models: advanced xenografts, particularly using patient derived material (PDXs), and genetically engineered mouse models (GEMMs) permitting syngeneic transplantation. These models, where appropriate, provided material for the *in vitro* platforms and acted as references against which the *in vitro* models could be compared. The EPFL team (Brisken) with University of Tartu (Vilo) made a major breakthrough using the MIND model of ER+ breast cancer (Sflomos, G. et al (2016) and Deliverables IV.3 &4) described in a Commentary in *Cancer Cell* as "a potential game-changer for breast cancer research (Haricharan, S. et al (2016)) The model is ready for exploitation in basic research and drug discovery. One of its key findings was that the ductal microenvironment maintained the phenotype of ER+ breast cancer cells whereas the "standard" so-called orthotopic site, the mammary fat pad did not. The Erasmus Group (Trapman, van Weerden) successfully engineered and published a GEMM of prostate cancer in a *Pten* knockout mouse that showed tumour heterogeneity late in its development (Korsten, H. et al (2016) and Deliverable IV .5). The MIND model of ER+ breast cancer emphasized the important of the microenvironment for tumour cell behaviour and the cross talk with appropriate stroma. Deliverable IV.1 investigated the addition of human fibroblasts to PDXs to provide a more appropriate microenvironment, replacing, in part, mouse stroma which is limited in its ability to provide certain signals to the tumour (eg Hepatocyte Growth Factor).

Precision-cut slices (S Deliverables). The consortium's major paper on the characterization of precision-cut tumour slices and the optimisation of their incubation conditions, to maintain viability and function, was published in Nature Scientific Reports (Davies, E. M. et al (2015)) and suggested that further optimization would require perfusion of slices. Experiments with the oxygen-sensitive slices of the MCF-7 ER+ breast cancer xenograft have shown that viability and function can indeed be extended by perfusion (Deliverable S.2). Slices capture the heterogeneity of a tumour and its potentially heterogeneous response to drugs. Using appropriate pharmacological probes across the three pathologies (breast, prostate and lung) heterogeneous responses were observed (S.3). The slice platform therefore offers the opportunity to observe heterogeneity in an *ex vivo* setting essentially reducing animal usage (20 slices from a single transplanted or GEMM tumour). Using patient derived xenografts (PDX) of non-small cell lung cancer it was possible to compare the pathologies of the clinical material, from whence the PDX was derived, the PDX itself and the precision cut slices (Deliverable S4). Finally, the power of slices to capture the native microenvironment of a tumour was captured in Deliverable S.5 where steroid biosynthesis in prostate cancer was shown to be dependent upon the cross-talk between the native stroma and carcinoma cells: these findings are not possible to make in classic 2D cell cultures.

Complex *in vitro* cultures (2D & 3D Deliverables) Although there has been a recognition that standard two-dimensional (2D) *in vitro* models of cancer, consisting of long established tumour cell lines growing on a plastic substrate, do not capture the three dimensional (3D) architecture of a carcinoma, nor its complexity and heterogeneity, many alternative models remain to be properly characterised. This is particularly pertinent when elements of tumour stroma are co-incubated with tumour cells, either in 2- or 3- dimensional cultures. In a PlosOne publication (Rudisch, A. et al (2015) and Deliverable D.305), we showed that a 2D-mixed fibroblast-non-small cell lung cancer culture (including cancer-associated fibroblasts) initiated a dynamic cross-talk, through cytokine signals that mimicked aspects of the *in situ* pathology. Robust protocols were established by PREDECT for the

preparation and characterization of growth and survival, including by novel imaging techniques (Barbier, M. et al (2016) and Deliverable IA.5) of simple and complex 3D cultures. Expression of protein biomarkers was determined by immunohistochemistry (IHC), *via* preparation of a tissue microarray (TMA) archive (Deliverable MP.1). In addition to static simple and complex, alginate encapsulated 3D cultures were characterized in stirred (mini-) bioreactors (Estrada et al (2016), Santo et al (2016)) and Deliverables D1.10a and 3D.6). Monitoring of the dynamic reorganization of ER+ carcinoma and stromal cells within the mixed spheroids revealed stromal cell stimulated invasion/motility not seen under 2D conditions (Estrada, M. et al (2016)). These small bioreactors are suitable for the large-scale generation of spheroid-based models (Santo et al (2016). Fascinatingly , 3D spheroids of MCF-7 cells harvested from the MIND orthotopic model (see In Vivo above) maintained their "memory" of their microenvironment, with an ER+ breast cancer phenotype, whereas spheroids generated from MCF-7 cells harvested from the fat pad were more basal-like (Deliverable IV.4, manuscript in preparation). The very substantial consortium work to describe robust standard operating procedures for generation and characterization of complex 2D and 3D models has just been accepted for publication in Nature Scientific Reports (Stock et al (2016)) and Deliverable 3D.1).

Molecular and systems pathology (MP and ENSO Deliverables). An early decision of the consortium, permitting capture of the complexity and heterogeneity of the models was to characterise and compare them by a platform of immunohistochemistry and imaging (ENSO award). A systems pathology infrastructure was built based on the archiving of all PREDECT models in tissue microarrays (TMAs) (Deliverable MP.1). After staining for 15 biomarkers analysis was performed through a Web-based microscope (Deliverables IA.4) and image analysis (Deliverable IA.6). Multiplexed immunohistochemistry methods were developed to permit fine analysis of biomarker co-expression (Deliverable IA.3). After an early trial run, a definitive sample collection in 2015 of 2060 fully annotated formalin fixed, paraffin-embedded blocks yielded 81 satisfactory TMA blocks. These provided a total of 35245 images for analysis. Ongoing analysis, using machine learning algorithms (Deliverable IA.6) will provide deeper insights to the ability of PREDECT models to represent aspects of tumour complexity and heterogeneity. Results will be presented as Heat Maps and analysed by tools permitting principle component analysis (Metalu and Vilo (2015)). Aspects of this work have already appeared in the consortium paper on precision-cut slices (Davies et al, 201() and are integrated to the PREDECT paper on 3D models (Stock et al 2016). Publications regarding this molecular pathology infrastructure are under preparation.

PREDECT Publications cited

Barbier, M. et al (2016) PlosOne doi.org/10.1371 :journal.pone.0156942.
Davies, E, J. et al (2015) Nature Scientific Reports 10.1038/srep17187.
Estrada, M. et al (2016) Biomaterials 78 : 50-61.
Haricharan, S. et al (2016) Cancer Cell 29 : 249.
Korsten, H. et al (2016) PlosOne. 10.1371/jpone.0147500.
Metsalu, T. and Vilo, J (2015) Nucleic Acid Res 43: doi: 10.1093/nar/gkv468.

Rudisch, A. et al (2015) PlosOne. 10.1371/journal.pone.0124283.
Santo, V.E., et al (2016) J. Biotechnol. doi: 10.1016/j.jbiotec.2016.01.031.
Sflomos, G. et al 2016) Cancer Cell 29 : 407-422.
Stock, K. et al (2016) Nature Scientific Reports, in press.

1.3. Summary of progress versus plan since last period

The efforts of the final period have been to disseminate the work of PREDECT through publications (see Section 1.2 above citing 9 PREDECT publications and Section 3.2) and to complete a revised set of Deliverables submitted with Amendment 7. These goals have all been achieved. Some publications will appear after the closing date of 30th April 2016. The ten current PREDECT publications are cited throughout this Final Report and detail progress.

There were, in this last period, a few deviations from the original goals. A *direct* cross-comparison of the responses of the different platforms (in vivo, 3D slices and 2D cultures) to standard of treatment

drugs was not technically feasible (Deliverable 3D.1 and S3). Firstly, this would have required data on the pharmacokinetics of all the drugs in the PREDECT *in vivo* models, permitting their approximate reproduction *in vitro.* This was beyond the scope and capabilities of the consortium. Secondly, the optimal incubation conditions of bioreactor cultures with static 3D spheroid models (with or without additional components) compared to slices were different; this precluded direct comparisons. The goal was not a practical one. However, Deliverables 3D.1 and the S3 (Slices) do report on the responses to drugs of these two platforms, across the three pathologies of breast, prostate and lung. Technical issues also arose in WP2 where the stable model of prostate cancer LNCaP (readily forming a xenograft and simple and complex 3D models) could not be sliced. Slices were friable, and rapidly lost viability. Thus, in the WP2 TMA series a complete set of models is not available for comparison, whereas 2D, simple and complex 3D, bioreactor simple and complex 3D, slices and *in vivo* xenograft material is complete for breast carcinoma MCF-7 and non-small cell lung cancer model NCI- H1437. This is commented on in more detail later (Section 2.4)

Finally, the original goal of PREDECT was to generate systems which would better facilitate the validation of targets (not drug responses) and target discovery (see Section 1.6). The latter is well illustrated by the Boehringer-Ingleheim collaborative report on cytokine signalling in mixed 3D cultures (Rudisch et al 2015).The introduction of small bioreactors to permit real-time monitoring of 3D spheroid cultures and their encapsulation with fibroblasts has provided a number of EFPIA companies and academics with a new technology platform to study complex models (Santo et al (2016)). Similarly, industrial partners have embraced precision-cut tumour slices. The recent completion of the TMA collection and on-going analysis by WP members using the Web-microscope, aided by digital pathology and machine-learning tools (ENSO Deliverables), together with proximity ligation assays (PLA, Deliverables MP.4-6) will permit the realisation of the goal to compare the spatial expression of proteins and of cells (including immune cells) in a complex *in vitro* model with that of an *in vivo* tumour.

Rudisch, A. et al (2015) PlosOne. 10.1371/journal.pone.0124283.
Santo, V.E., Estrada, M.F., Rebelo, S.P., Abreu, S., Silva, I., Pinto, C., Velesco, S.C., Boghaert, E., Alves, P.M. and Brito, C. J. Biotechnol (2016) doi: 10.1016/j.jbiotec.2016.01.031.
Stock, K. et al (2016) Nature Scientific Reports, in press.

1.4. Significant achievements since last report

To date, nine open access consortium papers have been published (see Sections 1.2 and 3.2 and the PREDECT Website www.predect.eu) with a substantial manuscript on 3D-models appearing in June's Nature Scientific Reports (Stock, K. (2016)). Other manuscripts are in preparation. As will be seen in Section 1.6, the novel complex models investigated by PREDECT have now been integrated into the cascades of *in vitro* and *in vivo* assays used by our industrial partners and beyond. Medimmune, not a PREDECT partner but an AZ company in Cambridge UK, is active in investigating precision-cut slices for discovery projects in immune-oncology. Servier and AZ have also incorporated precision-cut slices. Roche is using the intra-ductal model of breast cancer, described as a "game-changer". Abbvie, AstraZeneca, Bayer and Boehringer are actively collaborating with iBET to use, in-house, their mini-bioreactor technology for 3D cultures and the academic partner EPFL (Lausanne) has also invested in bioreactor technology (See Deliverables 3D.1 and 3D.6).

The last period has seen the consolidation of the PREDECT database 'MBase' which permits annotation of all of the samples prepared as TMAs and stained for 15 proteins (for numbers see Section 4, Figure 14). In 2016 the infrastructure of WP4 (Molecular Pathology) permitted the completion of the final round of TMA generation and stainings by immunohistochemistry. Currently efforts continue to quality control this TMA collection, and to fill in any gaps, in what is claimed to be a complete set of models from the MCF-7 and NCI-H1437 cell lines (2D, 3D, complex 3D, bioreactor 3D, slices, xenograft). 35,245 high quality samples are currently being subjected to analysis of their heterogeneity by digitalized pathology, using novel algorithms (Deliverables IA.1, 2, 4 and 6).

The TMA platform requires access to the PREDECT Web-based microscope: www.predect.webmicroscope.net. The incorporation of Spotfinder to the site permits users to

interrogate samples prepared as TMAs with ease, as all spots are annotated through MBase (http://predect.cs.vt.ee).
In 2015 members of the PREDECT consortium published standard operating procedures for the preparation and incubation of precision-cut slices (Davies, et al (2015)). We reported that static incubation of slices did not permit prolonged incubation, further than 48h in some examples, before hypoxia was evident in the centre of the slice. These static slices, as such, present a valuable *ex* vivo model of a hypoxic solid tumour. Two approaches have significantly ameliorated the onset of hypoxia (Deliverable S2). First, with the NSCLC GEMM, the use of a rotating incubation unit was able to prevent the onset of hypoxia in a GEMM of NSCLC (see 2.4 below). Secondly, constant perfusion, in an open or closed system, also prevented the appearance of hypoxia in precision cut slices of MCF-7 xenografted tumours. 250 micron slices of MCF-7 were particularly sensitive to changes in oxygen availability and rapidly (48h) expressed HIF1 in the centre of the slice, with reciprocal loss of expression of the ER receptor. This model serves as a useful test-bed for the amelioration of the onset of hypoxia. Experimentation with support material and perfusion permitted extension of the life of MCF-7 slices from 48h to 168h (Details in Deliverable S.2).
On the 14th September 2015, at IMI HQ in Brussels, the PREDECT Executive Committee discussed a sustainability plan and met with our Scientific Officer, Dr Fatiha Sadallah. Details are in Section 5.3. The major actions taken subsequently were to sustain access to the Webmicroscope and to MBase (with updates for two years) and to permit open access to the PREDECT Website protocols and publications, again for two years. Updates of the Website over two years will permit access to new open access publications until May 2018. The work of the PREDECT precision-cut slice platform was the subject of an EU COST application (www.cost.eu) submitted in April 2016 with new partners, principally tissue engineers and immunologists, including four EFPIA companies.

1.5. Scientific and technical results/foregrounds of the project

The IMI Call for proposals, drafted by EFPIA members (with PREDECT responding) was conceived as a precompetitive project to investigate *in vivo* and *in vitro* models (emphasis on the latter) that better represented the architecture, heterogeneity and complexity of solid tumours. The goal was to provide open access to protocols and standard operating procedures. This dissemination exercise is ongoing but publications to date provide these protocols in Open Access journals. All are available on the PREDECT Website www.predect.eu. Regular intellectual property committee meetings and prior scrutiny of all manuscripts submitted for publication has not identified issues of intellectual property. No intellectual property was identified.

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

A major factor driving the origins of the PREDECT project was the high rate of attrition (failure) of anticancer drugs in the clinic (>90%), mainly for reasons of lack of efficacy. For the Pharmaceutical Industry this level of failure is financially unsustainable. Cancer therapeutics is not the only area where there is a high rate of attrition (Bhattacharjee, Y. (2012)). In Europe, these failures have led to substantial job losses as the industry “rationalises” in the face of falling productivity (Scannell et al (2012)). The loss of European talent (and profitability) from this important workplace is of economic concern to the EU. The situation is evidently unsatisfactory for patients with cancer. Cancer incidence is set to rise in Europe as the population ages. Currently, it appears that Industry is addressing the high costs of failure by unrealistically inflating the price of current anticancer drugs. This is unsustainable in the EU and other countries (Ocana, A. et al (2016)). Despite being faced with a process that delivers >90% failure there is little sign that the Industry is willing to assess the reasons for multiple failures (Scannell, J. W. and Bosley, J (2016)). Instead, with respect to cancer, it stampedes to the new approach of immunotherapy where there is great promise but where, currently, the impact is limited. The fundamental question of how to address high rates of attrition and to advance immunotherapy, in PREDECT’s view, is to embrace the realities of the architecture,

complexity and heterogeneity of cancer and to create tools that better serve therapeutics discovery (Hickman et al, (2014)).
PREDECT has advanced, by publication and wider dissemination including a Website, its work to develop and characterise laboratory models of cancer that better capture the biology/pathology of solid tumours of the breast, prostate and lung. The current 10 publications are cited in Section 1.3 above. Most importantly, these tools have been integrated to the laboratories of EFPIA partners. In addition, PREDECT's complete laboratory protocols will be submitted to Nature's open publication *Scientific Data* in the autumn of 2016.
The novel *in vivo* model of ER+ breast cancer (Sflomos et al (2016)) is of particular interest to Astra Zeneca, who pioneered anti-hormonal therapy for breast cancer, and to Roche, who have integrated the model to their battery of in vivo tools (Deliverable IV.3). One of PREDECT's SME partners, Oncotest, has benefited from its proximity to academic groups investigating GEMMs and advanced xenografts supplemented with human stromal cells, recognising the importance of the tumour microenvironment.
When PREDECT started, no company was using precision-cut slices to investigate tumour heterogeneity and its native microenvironment. Astra Zeneca, in particular, has enthusiastically embraced this platform, originating with our partner in Stuttgart, together with their sister company Medimmune who are investigating the native immune response using this important *ex vivo* platform.

Servier and Boehringer are also integrating slices to discovery cascades. Similarly, whilst most companies has expertise (or purchased this) in generating and using 3D models of cancer, none had been aware of the value of the controlled fluidics offered by mini-bioreactors. The expertise and enthusiasm of iBET in Oieras, Portugal, has been transferred to Abbvie (Chicago), Astra Zeneca, Boehringer, Bayer and academic partner EPFL (see also Section 1.3 above).
Will PREDECT platforms "transform" the industry, increase innovation and reduce attrition, to the ultimate benefit of patients? There are strong cultural factors at work that limit this. The culture of "brute force" methods (high throughput assays, that are robust, reproducible and roboticised) despite leading to failure (Scannell, J. and Bosley, J (2016)) are a part of the fabric of the industry. It is now PREDECT's role to promulgate the idea that the complexity of cancer may be better modelled in lower-throughput but higher content (information) formats that we have refined, appropriate particularly for target validation. PREDECT's models are "archived" as TMA's that should be available for interrogation by companies and academics seeking to reduce their reliance on reductionist models.

Bhattacharjee, Y. (2012) Science 338: 29.
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Scannell, J. W. and Bosley, J. (2016) PLoS ONE 11:e0147215.
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Ocana, A. Amir, E. and Tannock, I.F. (2016) Cancer Res 76: 3127-3129.