

IMI1 Final Project Report Public Summary

Project Acronym: BTCURE

Project Title: BeTheCuRE

Grant Agreement: 115142

Project Duration: 01/04/2011 - 31/03/2017

1. Executive summary

1.1. Project rationale and overall objectives of the project

The overall objectives of the BTCure project is on one hand to create an infrastructure to better understand the clinical and molecular features of rheumatoid arthritis (RA) and to align those features with relevant animal models, and on the other hand to develop knowledge within adaptive immunity of RA in such a way that increased understanding can be used for the development of new diagnostics and new therapeutics in RA.

The infrastructure aimed at aligning knowledge of the disease course in RA patients with the generated knowledge on relevant features of experimental arthritis is addressed in WP 1-4. Our strategy has been to assemble knowledge on major longitudinal databases and associated biobanks in Europe (WP2), and make these assets available for analyses of biomarkers (WP3) and allow analyses by bioinformatics tools (WP4). A major emphasis has been on description, standardisation, validation and alignment of animal models with the human disease (WP1). In addition the infrastructure has encompassed major efforts to ascertain the bioethical aspects of both human and animal research and ensure that patient participation helps disseminating the progress of the project (WP5).

The second major aim, learning more about the adaptive immune system, is addressed in WPs 6-9, where the clinical materials and animal models described in WPs 1-4 are used to develop new knowledge about adaptive immunity (WP6), co-stimulation and concerning imaging and other relevant new technologies (WP7). One WP is also assigned to study tissue resident cells, in associated studies in both inflammatory bowel diseases and RA (WP8). Finally one WP is devoted to the development of new mechanistically and molecularly oriented clinical trials (WP9).

1.2. Overall deliverables of the project

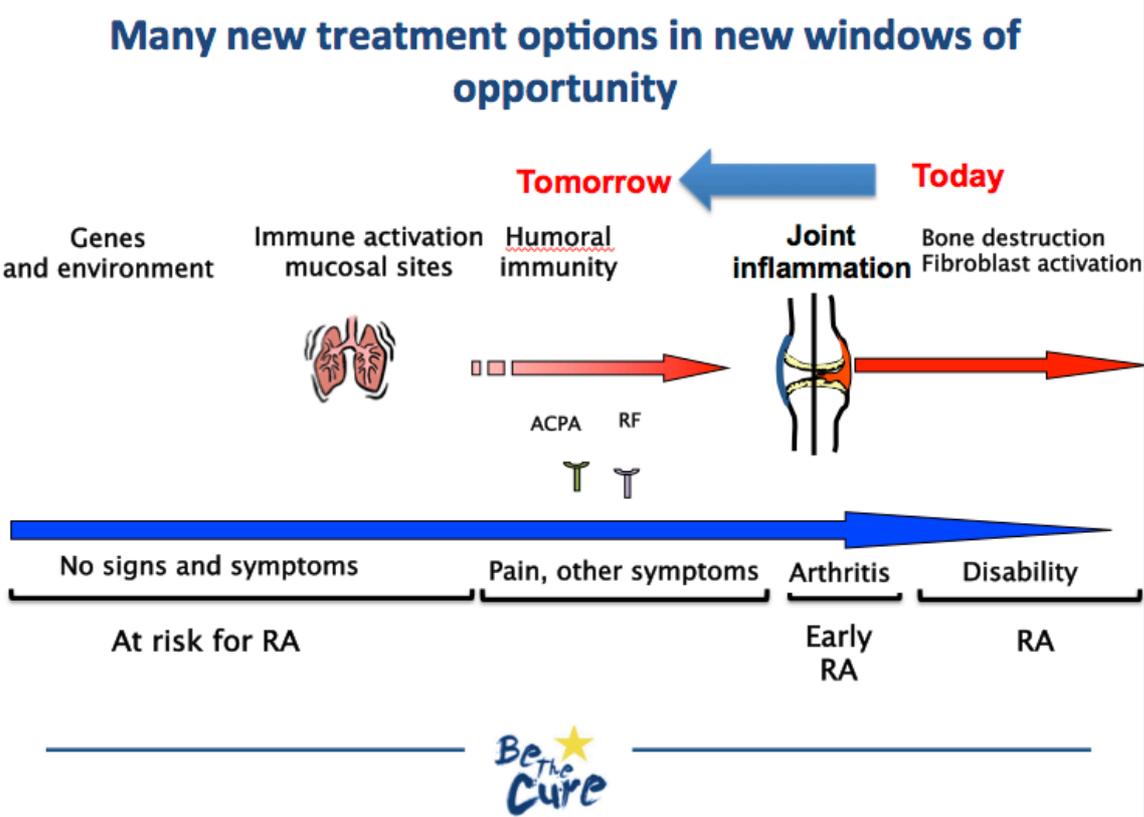
In the last and extended period of BTCure we only had a few deliverables left that has all been delivered now, and much of the work has been to finalise, extend and publish results, as well as to ensure a continuation of the legacy of BTCure in different ways. Also work to ensure the full potential of the extra ENSO funded parts of BTCure have been a large part of the last period, and thanks to the approved extension of the BTCure project, we could optimize our ongoing collaborations with the patient organization PARE as well as with individual patient representatives.

We have continued to develop antibody multiplex platform that can measure ACPA antibodies in serum samples, and we have finalised the last deliverable on B- and T-cell clone sequencing in both human and mouse samples. Several new biomarkers for different stages of RA has been validated. Furthermore, we have continued to fill the Bioretis platform with transcriptome data and developed tools for analysis. Regarding cellular studies, the focus was on detailed immune phenotyping of T and B cell subsets, using protocols that were disseminated across the consortium. These studies extended to detailed transcriptome analysis of lymphocyte sets, including regulatory T cells, analysis of expansions of B cell clones, identification of novel pathways that regulate the generation of pathogenic effector cells and efficient regulatory cells, and a comprehensive analysis of antigenic fine specificity. In addition, we investigated factors that ultimately control and regulate the T cell/APC

interaction leading to the breach of self tolerance and the induction of adaptive responses in RA. These interactions underly the induction of adaptive immunity through influencing the fate decisions (activation/differentiation) of T and B cells. Next to RA patients, light was shed on the complicate mechanisms underlying the chronic inflammatory processes in IBD patients. We also worked on the development of new clinical trials criteria for the recruitment of patients, drawing from existing registries to better reflect patients seen in routine practice.

In summary we have achieved a large collection of RA patient cohorts from several sites in Europe and better, standardised animal models as well as contributed to better collaborations between academia and industry. We have published the most papers among the IMI2 calls with high impact. BTCure has defined and suggested a new nomenclature, in collaboration with PARE, for the disease phase of RA that precedes joint inflammation and contributed to defining the gradual development of RA and the different steps in that development.

BTCure has laid the groundwork for future clinical trials by leading the scientific community in understanding both adaptive and innate immunity in RA and have paved the way for the next generation of earlier, more specific therapies and developed new clinical trial criteria (time line illustrated in the picture below).



1.3. Summary of progress versus plan since last period

WP1

The SOP for PIA has been published. The SOPs for CIA and CAIA, as well as the guidelines for a standardized evaluation of histopathological features, have been finalized. The SOP for GIA has been submitted for publication. The humanized mouse strains have been finalized. The conditional knockin Ncf1 has been validated and a first paper is in revision.

Studies on Treg-based therapies in animal models suggests that a specific T-cell (CD49b) could suppress arthritis in the CIA model (but not colitis). The suppressive function of the CD49b T cells depends on IL-10 and the expression of CTLA-4.

We have developed a nanotechnology for delivery of miR in immune cells and demonstrated that the systemic treatment with antagomir-148a is suitable for the elimination of pathogenic Th1 cells *in vivo*. The intravenous application of antagomir-148a reduced the number of pathogenic Th1 cells in the colon and the severity of inflammation in a murine transfer model of colitis.

WP2

In WP2 we had one deliverable left, linked to the ENSO project within BTCure: D2.12 ENSO: First version to be tested in a complex real life environment with registry data, biobanks, biomarkers and genetic data. This has been going as planned and we have now in fact ensured interoperability between different registres and databases and for example established links between biobanked samples, studies and ethical permissions.

WP3

In WP3 we had two deliverables left; one to analyse TCR and BCR repertoires in mice and one ENSO deliverable where we planned to measure antibodies in serum from at least 2000 blood samples from RA patients that's been followed over time. Both has been achieved successfully and reports are included.

WP4

In WP4 we have continued to work on creating structures for integration of data to the online database, we have now included support for RA data from different cell types and tissues, and to extend our "Immunomap" with tools to access to public data.

WP6

In WP6 from the ENSO project one deliverable was left. The work described under this deliverable resulted into two studies that have been published under open access, describing novel chemokine-dependent molecular mechanism underlying RA associated autoantibody-mediated bone loss and that ACPA induce joint pain independent of inflammation via a chemokine-dependent mechanism.

WP7

In WP7 two deliverables and one milestone was left. During the last reporting period, protocols have been established for imaging of immune cells in animal models. In addition, results from these studies have been submitted for publication.

WP8

In WP8 two milestone reports were left. Last reporting period, we investigated the potential of TREM-2 as a new therapeutic target for modulating chronic inflammatory processes and tumor development associated to the chronic inflammatory processes. In addition, a milestone report was written on the junctional adhesion molecule-A acting as a crucial regulator of epithelial barrier function and recruitment of immune cells in the gut.

WP9

The work for WP9.1.1.1 was completed and the required reports for the set deliverables were submitted with the exception of the last two deliverables (D9.4 – M27 and D9.9 – M44) which were deemed unrealistic and not feasible but were replaced by an extended project proposal. This extended project provided an opportunity to investigate the applicability of RCTs in routine care cohorts represented by a national biologics registry which holds over twenty thousand records of RA patients in the UK. The proposal was approved and has been funded in its entirety by our EFPIA partner, JANSSEN. A new component has been added to the WP9.1.1.1 project since the last period following the notification of the availability of Individual Patient Data (IPD) from one of the RCTs selected for investigation. IPD from the RCT ATTRACT (NCT00269867) is now publicly available through the Yale University Open Data Access (YODA) Project. Previously, only Summary Patient Data (SPD) reported from journal publications from RCTs were to be considered. The start of this work came to a halt due to an 8-month maternity/paternal leave by the assigned research fellow. A no-cost extension was therefore requested and has been approved; the project is to be completed by October 2018. Furthermore, the work for deliverable 9.12 was finalised and a deliverable report describing changes in B and T cell repertoire after B cell depletion in the earliest stages of ACPA+ Rheumatoid Arthritis in the PRAIRI study was written.

1.4 Significant achievements since last report

WP1B efforts during this last reporting period have focused towards generating more phenotyping and large scale data (D1B.1b). In this context, WP1B has generated phenotypic data in the CAIA model upon Enbrel and Dexamethasone treatment, as well as a cytokine profile following *ex vivo* stimulation of arthritogenic splenocytes in the presence of 55 commercially anti-inflammatory drugs in the PIA transfer model (Redoxis). Furthermore, WP1B partners demonstrated the **first systematic examination** of the **pathogenic changes** that occur in **mouse synovial fibroblasts** in progressive TNF-driven arthritogenesis and revealed **significant correlations between mouse and respective human RA SF** data (Fleming/JSSN) (Ntougkos *et al.*, 2017). Finally, some additional pharmacogenomics RNA profiling data in whole joints have been generated in the presence of several treatments (Fleming/Biomedcode) and miRNA expression profiling data in TNF^{ΔARE} mouse model SFs have also been reported (Fleming).

WP1B organised a successful 4th and final Annual workshop on animal models in Athens (D1B.4 and D1B.5) which focused on newly generate data and technologies from established and emerging animal models of RA disease. These included new potential mouse models for RA, identification of interesting pathogenic mechanisms and system level approaches from these models which could lead to a comparison of mouse vs human for the identification of biomarkers and potential drug development, and finally progress on bioimaging applications and potential phenotyping platforms for mouse models.

In WP1c we have worked hard on establishing a vaccination strategy for prevention and treatment of animal models for RA. The last year we managed to stabilize the triple helical structure needed to induce an antibody response, but we still have difficulties to introduce the glycosylation of the T cell epitope which is a prerequisite for the vaccination effect. As an alternative approach we are making recombinant MHC class II molecules complexed with synthetic galactosylated CII peptides – this has been confirmed with a proof of concept in the mouse and is patent protected, and we have obtained support for promoting this to clinical grade and clinical trials.

In WP2 and 5 we have worked to harmonize how and what data to collect in clinical studies with RA, to try to reduce the heterogeneity of data that makes it difficult to combine data from different countries and registries. This has been done through a EULAR task force with the aim to collect a minimum core data set and instruments for data collection.

In WP3 we studied biomarkers for early RA. The onset of seropositive rheumatoid arthritis (RA) is preceded by the presence of specific autoantibodies in the absence of synovial inflammation. Only a subset of these *at-risk* individuals will develop clinical disease. In a prospective cohort study in 21 individuals *at-risk* for RA based on the presence of autoantibodies, we identified a novel marker is significantly associated with arthritis development within three years. In a validation cohort, the positive predictive value was 82%, and the marker clearly added to the recently described clinical prediction rule ($p = 0.024$). This new marker can be used to identify individuals at high risk of rheumatoid arthritis, and thus builds a basis for clinical intervention in this very early stage, when the disease is thought to be more amenable to therapy.

In WP6 we demonstrated that mice lacking HDAC1 in CD4+ T cells (*Hdac1f/fCd4-Cre*) were protected against the induction of collagen induced arthritis, suggesting HDAC1 as a potential therapeutic target in the treatment of RA. Other research from WP6 points to the possibility that key defects may exist in the way that effector T cells respond to Tregs or other modes of immune regulation (eg PD-1 mediated immune suppression) and to the inflammasome, and the production of IL-1 β as a novel checkpoint in Th1 differentiation.

In WP7 the analysis was completed of a kinase inhibitor library and identified a novel series of compounds that inhibit T cell/APC interactions using high throughput imaging approaches. Epigenetic profiles of several types of fibroblasts (synovial, skin, gingival) and monocytes or macrophages after stimulation have been compared. The presence of inflammation and resolution pathways in osteoarthritis (OA) were investigated and identified and demonstrated by using a state-of-the-art technique, for the first time that resolution pathways are present in OA patients. We demonstrated new insights in the RA-HLA connection. Also a role for human mast cells in setting the outcome of T cell responses was shown, through release of caspase-independent IL-1 β . This provides evidence for a novel contribution of MC in boosting the Th17 axis in mucosal immune responses. In addition, mast cells contribute to the preclinical phase of CIA. Depletion of mast cells before disease onset resulted in an altered collagen-specific T cell and cytokine response. These data may suggest that mast cells play a role in the regulation of the adaptive immune response during the development of arthritis.

In WP8 we provided the first analysis of cellular and molecular changes in lymph node stromal cells during the earliest phases of RA. In addition we demonstrated that TREM-2 is a key regulator in maintaining intestinal homeostasis and preventing intestinal tumorigenesis. Another IBD related

study showed that junctional adhesion molecule-A acts as a crucial regulator of epithelial barrier function and recruitment of immune cells in the gut.

In WP9 we developed new clinical trials criteria for the recruitment of patients, drawing from existing registries to better reflect patients seen in routine practice. With the PRAIRI study, changes in B and T cell repertoire after B cell depletion in the earliest stages of ACPA+ Rheumatoid Arthritis were identified.

1.4. Scientific and technical results/foregrounds of the project

Summary of results in WP1A

- All the classical models for RA have now been standardized using SOP protocol and industry partners have joined in the validation process (CIA validation is finalized and manuscript submission is in progress).
- New models were standardized and protocols established.
- New humanized MHCII mice were generated.
- New conditional knock-out mice were generated to study RA specific disease pathway.
- New guidelines for histopathological evaluation of four different arthritis models have been finalized and will be published.

Summary of results in WP1B

- **Development of SOP** for: (i) collecting and handling of biological material for use in “**omics**” studies (Fleming) (**D1B.7**), (ii) **histopathology** with analysis from all four classical models (MUW), (iii) the **TNF^{ΔARE} model**, in order to provide guidance for preclinical evaluation (Fleming) (**D1.E1**), (iv) the collection, storage and transplantation of human **RA synovial tissue in SCID mice** (RUNMC) (**D1.E3**), (v) the characterization of the RA synovial tissue prior to transplantation (RUNMC) (**D1.E4**), (vi) the comparison of various freezing methods for long-term storage of RA synovial tissue (RUNMC), (vii) a panel of serological readout parameters which can serve as biomarkers for human RA synovial tissue prior to transplantation into SCID mice (RUNMC) (**D1.E5**), (viii) **CAIA in the Tg1278TNFKO** animals to obtain a reproducible arthritic pathology (Biomedcode) (**D1.E7**), (ix) **Bioimaging** for *in vitro* high throughput imaging of T cell-APC interaction using INCell Analyzer 2000 (**D1B.2**).

- Generation of **29 phenotypic datasets** which include: (i) SOP validation data for the four classical models (CIA, CAIA, PIA, Tg197), (ii) pre- and post-SOP phenotypic datasets with the use of several treatments (inhibitors and drugs), and **18 Omics datasets** which include (i) Tg197 whole joint pharmacogenomics RNA profiling in the presence of drugs and other treatment profiles, (ii) expression profiling, RNASeq profiling, Methyl-Cap-Seq profiling, proteomic profiling, H3K4me3-Seq (Chip-Seq) profiling, miRNA sequencing and urinary metabolomics profiling in Tg197 and WT+TNF mouse SFs both in the absence and presence of treatments, (iii) proteomics profiles in serum of RA-SCID mice, (iv) miRNA profiling of monocyte subsets in the CIA mouse model and (v) gene expression (RNAseq) and miRNA expression (Small RNAseq) profiling in TNF^{ΔARE} SFs both early and late disease stage (**D1B.1a, D1B.1b**).

- Generation of a **map of gene expression and epigenetic changes occurring in SFs during disease progression in the human TNF-transgenic model of arthritis**, and identification of commonalities

with human SFs. This work presents the **first systematic examination of the pathogenic changes** that occur in mouse synovial fibroblasts in progressive TNF-driven arthritogenesis. (Ntougkos *et al.*, 2017)

- Development of a “**pathogenesis map**”, a table based and adjusted according to Vincent *et al.*, 2012 which summarises each RA model characteristics in an attempt to evaluate the advantages and limitations for each model.

- Development of “**TheRABase**” (www.therabase.eu), a relational database, for archiving and validating data generated from the commonly used murine and rat models (CAIA, CIA, PIA and Tg197), as well as human samples, with the potential to further expand with additional animal models (Fleming) (**D1B.3**).

- Identification of potential **novel biomarkers** for the development of new therapeutic agents for the prevention and cure of RA (**D1B.6**). WP1B partners identified the following candidates which could be further examined towards their biomarker potential which could serve as possible biomarkers for monitoring disease states or the response to specific; PTX3, CCRL2, chemerin, miR-146a, miR-125b and specific metabolites identified through the use of predictive modeling, metabolic differences that allow the distinction between Tg197, WT and Remicade treated animals could be depicted and serve as possible biomarkers for monitoring disease states or the response to specific.

Summary of results in WP1C

- Within BTCure we have developed original Procedure for inducible Tregs based cell therapy in arthritis and colitis with identification of the suppressive and tolerogenic properties of DX5 Treg cells and pDC
- Optimize the engineering of iDC using siRNA-loaded micelles and identify DC subset and the molecules that are involved in antigen-specific Treg induction in vivo
- Within BTCure we have developed original miR-based strategies including nanotechnology for Th1 cells & Th17 cells and lipoplexes for inflammatory monocytes
- We propose novel genes and pathway to target and validated across mouse models and in human samples

Scientific foreground in WP3

We have validated the use of T-cell biomarkers in the management of clinical remission (2 more papers planned by Leeds).

The foreground of WP6 can be divided into three parts:

- Immune regulation

Understanding the molecular and cellular basis of persistence of inflammatory responses is essential if we are going to propose pathways to immune tolerance in a drug free setting. As a first step we must identify the aberrant checkpoints in immune responses. It has been hypothesized regulatory cells do not develop, are insufficient in number and/or are defective in terms of function. This has been hard to prove. Research from WP6 points to the possibility that key defects may exist in the way that effector T cells respond to Tregs or other modes of immune regulation (eg PD-1 mediated immune suppression). This has been born out by in depth transcriptome and functional analysis. In

keeping with this, our work has shown that Th1 switching to a regulatory or resolving phenotype is defective and that this is linked to the integrity of metabolic pathways including the cholesterol biosynthesis pathway. These concepts will underpin a number of key follow up projects.

- Effector T cell responses

Our work points to the inflammasome, and the production of IL-1beta as a novel checkpoint in Th1 differentiation. NLRP3 inhibitors are being developed. These are now being considered for further drug development, targeting Th1 mediated immune mediated inflammatory diseases. In addition, we have shown that TNF blockade can promote expression of the anti-inflammatory cytokine IL-10 in otherwise pro-inflammatory effector T cells, indicating an intrinsic way of self-regulation of effector T cells.

- Autoantibody effector responses

Until recently the pathways whereby autoantibodies contribute to disease pathogenesis in RA have not been defined. WP6 studies provide new insights into how ACPA activate osteoclasts, providing a novel link with bone pathology on the one hand, and how they contribute to activating nociceptive pain pathways before the onset of inflammation.

In WP7, a method has been identified to determine lipid mediators by targeted lipidomics using liquid-chromatography mass spectrometry.

WP8, a collaboration between FHR, ERLANGEN and FLEMING demonstrated similar deregulated pathways in mouse and human IMFs, providing evidence for the function of mesenchymal-specific innate pathways that regulate intestinal homeostasis and inflammation and may underlie IBD susceptibility in humans (Roulis et al., 2014). In collaboration with ERLANGEN, FLEMING further showed that the microbiota is sufficient to drive TNF overexpression and Crohn's ileitis in the genetically susceptible *TnfΔARE/+* model, whereas dysbiosis results from disease-associated alterations (Roulis et al., 2016). Importantly WP8 has provided the first analysis of cellular and molecular changes in lymph node stromal cells during the earliest phases of RA. LNSC of ACPA+ RA risk individuals already showed significant epigenetic, transcriptional and functional changes compared to healthy LNSC. Since these cells are essential for shaping and creating the reticular network for adaptive immune responses, further studies are required to investigate whether we can exploit their immunomodulatory capacities for therapeutic purposes. During the project, WP8 built a comprehensive map of epigenetic marks and coding and non-coding transcripts of synovial fibroblasts (SF) of different joints and different arthritides. We could show that changes in DNA methylation and microRNA expression already occurs at very early stages of RA and that loss of DNA methylation can potentially be restored by therapeutic application of methyl donors. Furthermore, we analysed molecular mechanisms of apoptosis and autophagy in SF, and elucidated pathways, which could be exploited therapeutically to induced apoptosis in SF.

WP9 the still ongoing study will establish how relevant RCT data are when applying to treatment of routine-care patients. It will identify the relationship between patient baseline characteristics and clinical response to better understand the effectiveness of bDMARDs in routine care patients with differing profiles. This will enable more individually tailored prescribing of targeted treatments that

will improve patient clinical outcomes and reduce treatment costs. Furthermore, it will guide the development of future trial design by informing the use of therapies more effectively in any given population. In addition, this project will test whether the usual practice of employing Summary Patient Data (SPD) from published RCTs is robust, by developing a method to select (match) patients from a registry using SPD from published RCTs, and those selected using RCT Individual Patient Data (IPD), and comparing the baseline characteristics and treatment response using the two approaches. This novel methodology will maximise the ability of registries to complement the evidence from RCTs, and have wide reach across different disciplines in addition to rheumatology. For the purposes of addressing rheumatological unmet needs, this methodology could be further applied to other bDMARDs, other RA transition groups (DMARD-naïve, TNF-inadequate response), other registries and other disease areas to similarly assess the clinical effectiveness of therapies from observational data.

In BTCure we have created an infrastructure to better understand the molecular immunity of rheumatoid arthritis with:

- EU wide patient databases & biobanks
- Immune monitoring capabilities (T & B cell & Autoantibody profiling & transcriptomics)
- Animal models & Imaging

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

Our results on animal models will provide novel research tools to the scientific community and thereby facilitate and improve research on rheumatoid arthritis. SOP generation and more importantly, their cross-lab validations will establish a common pipeline in analyzing arthritic phenotypes that will further facilitate the reproducibility of results and will undoubtedly improve research on rheumatoid arthritis.

WP1B has throughout the durations of the program generated phenotypic and omics data from various established RA animal models and from newly developed ones in the context of the ENSO projects. Most of the aforementioned data have further contributed to the generation of knowledge on relevant features of experimental arthritis and thus furthered our understanding on RA disease pathogenesis.

Furthermore, WP1B partners have identified some novel candidates that could be further investigated on their potential of becoming potential biomarkers for the development of new therapeutic agents for the prevention and cure of RA.

Finally, in the context of WP1B, a RA-specific database was created for data archiving, representation and validation (www.therabase.eu). Should TheRABase be further populated and data sharing among researchers increases, further analysis and comparison between models and cross-species (mouse-human) may prove to be beneficial for the discovery of novel biomarkers and improvement of predictions of clinical response to therapies for RA.

(WP1c) These basis results on pathways involved in chronic inflammation will lead to novel strategies for RA and colitis. As such we need to provide vectors to target these new pathways. These results will also identify biomarkers to identify responders. The project outputs have the potential to be rapidly and broadly spread and taken up within the industrial community and healthcare professionals using cohorts a-of RA patients.

The output of WP3, which was based on the development and application of technology, has contributed to the economic benefits for European citizens as several tools, e.g. the Phadia-Thermo Fisher ACPA chip, were analyzed and further developed. The sets of diagnostic assays that have been developed, e.g. high-throughput, multidimensional flowcytometry contribute to the health of European citizens. Clearly all efforts of WP3 in the collaboration with EFPIA partners has contributed to the competitiveness of Europe in the context of biopharmaceutical research and development.

Our (and others) work to harmonize data collection for clinical studies of RA has resulted in the formation a EULAR task force group for Standardizing a Minimum Data Collection for RA Observational Research. This group will work to develop a minimum core dataset (MCD) of data items (i.e. "what to collect") and instruments for data collection (i.e. "how to collect") to 1) harmonize future data collection 2) act as a common data model to which existing databases can be mapped 3) serve as a template for standardized data collection for RA research in routine clinical practice. This is important for future clinical research on RA.

Our experience of working with Patient research partners in BTCure has been presented at the "IMI workshop on how to foster patient engagement in medicines' (April 2016).

Based on our work pointing to the inflammasome and the production of Il-1beta as a novel checkpoint in Th1 differentiation, NLRP3 inhibitors are being developed. These are now being considered for further drug development, targeting Th1 mediated immune mediated inflammatory diseases.

Our functional ACPA studies provide a framework for future studies in at risk RA populations, studying how ACPA contributes to symptoms, and how they subsequently initiate pathways of tissue destruction. Part of this work has been included in an H2020 application.

To increase impact on our obtained results in the scientific community, our results were presented in peer-reviewed high-impact journals and presented by poster- or oral presentation on different meetings, including EWRR, ACR and EULAR. Participants of these meetings are Patient groups, scientists, rheumatologist etc to ensure broad exploitation of the work performed in the project.

Overall, our results provide further insight into disease pathogenesis of RA and IBD and could prove useful both for the development of novel biomarkers and the identification of new therapeutic targets. Indeed, our studies on lymph node stromal cells and synovial fibroblasts help to understand processes that are involved in the initiation and earliest phases rheumatoid arthritis and might in future be used for early diagnosis and preventive treatment of RA patients. These data have been presented at the most important scientific meetings for rheumatologists (EULAR, ACR, EWRR meetings) and for gastroenterologists (ECCO and DDW) and has been published in high impact

journals (e.g. Nat Commun, Ann Rheum Dis). Furthermore, we highlighted our research to a wider community via social media channels (Twitter).

The extended WP9.1.1.1 project aims to develop a novel method to maximise the clinical utility of existing registry data. This will allow the identification of patient profiles, not necessarily included in RCTs, that associate with improved/reduced biologic response in real-world cohorts to establish the degree of applicability of trials to a standard UK patient population. Also, to explore if there are differences between individual-patient data (IPD) and summary patient data (SPD) from trials when comparing outcomes with the routine-care population. This will make a strong case for the sharing of individual patient-level data (IPD) from RCTs, which is advocated by the research community.

1.6. Lessons learned and further opportunities for research

The collaboration in a public private partnership (PPP) has been an added value to achieve the objectives of the project. In addition, most of these relations continue after the project; eg:

- UGLA have initiated collaborations with UCB and AZ directly as a result BTCure
- UGLA, KI and BMS have collaborated on ICOSRA, a project to perform deep phenotyping of inhibition of costimulation in RA
- LUMC have initiated collaborations with BMS for future research
- Public-Private partnership was extremely beneficial at all levels within the WP1B collaborations. Through this partnership, academic partners were familiarised with the needs of pharmaceutical companies, such as the techniques and approaches utilised for pre-clinical evaluation and related platforms involved. Industrial partners on the other hand were introduced to new RA models that could be used for preclinical evaluation of pharmaceuticals, as well as potential novel candidate molecules that could serve as potential biomarkers for monitoring disease states or the response to specific therapies, as well as the development of new therapeutic agents for the prevention and cure of RA.
- The interaction between Phadia-Thermo Fisher and several academic partners with biobanked samples allowed the design of new versions of the ACPA chip. This direct academic – industry interaction has shaped the product.

From our experience, recommendations/ solutions which could be useful for a PPP are that collaborations with industry are incredibly valuable to academic scientists and clinicians, however there is a lag time to set these up. Approaches that could address this and allow more rapid establishment of collaborations would be useful solutions. It is also important to make sure that both the academic and the industry partner are really motivated to work together on a scientific basis.

So after a somewhat slow start with the EFPIA-academic collaborations, in the end also the EFPIA partners valued the benefits from the BTCure project. A summary of the EFPIA partners comments about the BTCure collaborations:

- Improved EFPIA's credibility with European Rheumatology community through scientific engagement.
- Improved knowledge networks & access to Europe's leading RA KOLs.

- Led to new collaborations and access to research technologies
- Influence registration views/discussion
- Created awareness of new therapeutic opportunities for example:
 - Early intervention in pre-RA/VERA patients
 - Role of ACPA auto-antibodies
 - Possibility of specific immunotherapy for drug-free remission.
- Access to RA Biobank samples and patient level data (*with limitations of patient consent & cross border movement of samples*)
- Intelligence: such as the PAD inhibitor project that BMS launched
- FUN!!!!!!

In view of our project achievements, our views on potential new research to further advance the field is that this project and the scientific community in general has increased focus on specific immunotherapy to treat disease. Many biomarkers have been identified in WP3, antibodies, flowcytometry profiles, proteomic signatures etc. In WP7 the following issues, impacts and insights that, in our opinion should be the focus of new research have been identified:

Issue	Impact	Insight
Antigen-specificity of T (and B) cell responses in RA unclear, wide range of specificities detected. Pathogenic role of T cells not clear	Inducing antigen-specific tolerance likely to be challenging! Assessing which cells in a lymphoid or target tissue are pathogenic/tolerogenic and how they recirculate will be key	Need to track antigen-specificities of some suspected relevance (e.g. cit, collagen) and those unrelated (e.g. vaccine antigens) and assess behavior and pathogenic vs tolerogenic contribution and potential
Exactly where tolerogenic (e.g. joint vs LN) or pathogenic T cells, B cells, DC etc localize and exert their influence remains unclear in most scenarios	Employing cell transfer approaches to restore tolerance remains a potentially risky 'shot in the dark' without some fundamental understanding of how cells in different states migrate and interact	Remains an urgent need to use imaging to provide fundamental information on cell migration and interaction
Extremely difficult to tolerise primed cells and/or elicit effective bystander suppression	Antigen-specific prevention of breach of tolerance may require extremely early	Need to assess impact of antigen-specific and non-specific therapeutics on naïve,

(noticeable allergy tends to 'modify' rather than tolerise). Appears that 'tolerance induction' may require 'breach of tolerance' prevention and/or non antigen-specific costimulatory blockade or combination in particular circumstances to e.g. tolerise new thymic emigrants	intervention. Primed response may be 'exhausted' by therapy but true cure/tolerance not established.	primed and tolerised cells of specificities noted above and determine impact on their relative numbers, the numbers (ratios) required to give effective tolerance and the localisation and interaction of cells for e.g. bystander effects etc
What exactly to tolerised T cells look like? How can they be differentiated from exhaustion or other altered/modified states?	How do we demonstrate tolerance induction in clinical trials (aside from waiting for clinical cure).	Surrogate biomarkers of specific tolerance will allow more rapid demonstration of effect evaluation and stratification of cure/non cure patients.

In terms of our experience with involving Patient research partners in BTCure we had a slow start and have learned many things a long the way. Our experience has resulted in recommendations for future involvment of patients in research projects (see also the separate report; BTCure case study that Maarten de Wit presented at the "IMI workshop on how to foster patient engagement in medicines'):

To enhance stakeholder involvement (patients):

- Involve patient organizations and Patient Research Partners right from the start, preferably at the project design phase
- Adapt research and meeting protocols to allow for meaningful patient engagement
- Ensure a governance structure for implementing meaningful and sustainable patient involvement throughout the lifecycle of the project
- Provide guidance and education to both PRPs and researchers
- Provide sufficient resources, for instance to appoint a designated patient engagement coordinator
- Involve patient organisations with an interest in research. Find more information on www.eular.org/pare_patient_research_partners.cfm

We now take our experiences from BTCure and the summarized recommendations from PARE regarding PRP involvement in similar projects as BTCure, into action in our next planned project RTCure. This is done by already from the very beginning identifying a Patient engagement expert, by setting a dedicated budget for PRP work and collaborations, and by asking for input to the research proposal from the PARE-group.