Project n° 281493

TRIAD

Tolerance Restoration In Autoimmune Diseases by selective manipulation of the CD28 costimulatory pathway

Thematic priority: Health-2011.1.4-5 - New therapeutic approaches in chronic inflammatory and autoimmune diseases

Project Final Report

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1. FINAL PUBLISHABLE SUMMARY REPORT

1.1 Executive summary

Deregulated T cell function is a characteristic of autoimmune diseases including rheumatoid arthritis, multiple sclerosis and type 1 diabetes. During T cell activation, co-stimulatory molecules regulate their differentiation into T effector or anti-inflammatory regulatory T cells (Treg). The balance between these two types of T cells is achieved by the concerted action of CD28 and CTLA-4. Targeting the CD28-mediated T cell activation with B7 antagonists (CD80/86) has proven to be a promising alternative to current immunosuppressive treatments. However, this strategy inhibits the entire pathway including CTLA-4 signals that are crucial to the function of Treg cells.

The EU-funded TRIAD (https://www.triad-cd28.eu/) project proposes to correct T cell imbalance through selective inhibition of the CD28-CD80/86 axis, which spares the CTLA4-CD80 interactions. This approach promises to restore or induce peripheral tolerance.

In this context, TRIAD project has dissected the mode of action of selective CD28 antagonists that may conduct to self-tolerance restoration. Results show that antagonizing CD28 increased Treg dwell time with APC and increased Treg suppressive activity, in contrast with the dampening effect on Teff responses. Additional rodent studies suggested that MDSC, myeloid-derived-suppressive cells expressing SIRPα contribute to the tolerance promoting capacities of CD28 antagonists *in vivo*.

Second, TRIAD explored the efficacy of a surrogate CD28 antagonist (PV1-PEG) in murine models. PV1-PEG treatment provided opposite results depending on the experimental model used. In the uveitis setting, PV1-PEG exerts a positive therapeutic effect leading to a decreased eyes injury. However, the protective effect was partial. In the type 1 diabetes (T1D) setting, PV1-PEG administration was rather deleterious and prolonged treatment exacerbated T1D, due to a negative impact of the CD28 antagonist on Treg. However, a positive therapeutic effect was obtained when combining PV1-PEG with Rapamycin, which delayed T1D development.

Third, TRIAD studied the efficacy of FR104 in non-human primate models. Since no cross-reactivity with marmoset leukocytes was found, FR104 efficacy evaluation for experimental autoimmune encephalitis (EAE) and collagen-induced arthritis (CIA) was performed in rhesus macaque models:

- ***In vitro*:** FR104 was confirmed to inhibit function of effector T cells and not of Treg.
- **CIA:** FR104 abrogated CIA symptoms and prevented inflammation and anti-collagen antibody induction. Treatment with FR104 or Abatacept was equally potent in preventing the expression of arthritis, leading to significantly increased disease-free survival compared to placebo-treated animals.
- **EAE:** Moreover, in FR104-treated monkeys, clinical EAE did not develop.

A third baboon model revealed that FR104 (10 mg/Kg) showed preclinical efficacy to a cellular-mediated skin inflammation stimulus in primed animals (DTH). A remnant effect (no erythema after IDR) was observed since hyporesponsiveness was present after total drug elimination for at least 5 months after a single IV injection. Pharmacokinetic analyses revealed that FR104 could be administered on a monthly basis or less in human in order to maintain a full-saturation in vivo of the CD28 target.

Finally, in humanized mice preclinical model relevant to transplant arteriosclerosis (skin graft), FR104 blunted effector T cells and allowed infiltration by Treg cells, a phenomenon not observed with LEA29Y or saline treatment.

Analysis of the potential for reactivation of quiescent viruses after treatment with FR104 in the non-human primate was carried out to ensure noadverse effect. Since no cross-reactivity of FR104 to marmoset CD28 was been observed, the macaque model was substituted and specific tools for analysis of recrudescence of latent viruses in macaque were developed. All samples (incl. control animals) were at baseline at least positive for a single virus. No differences between treated and untreated animals were observed for CMV, SV40 and SA12 and HEV in spleen, brain and/or blood. Cross-sectional measurement of lymphocryptovirus, the rhesus monkey EBV, demonstrated elevated levels in the blood of FR104-treated animals. To date, with respect to the DTH model, no significant reactivation of latent viruses present in the baboons was observed over the experimental time course. For the EAE model, FR104 administration did not appear to facilitate viral reactivation, with the exception of LCV (EBV) indicating that this treatment may potentially reactivated EBV.

All this work is based on the use of a FR104 technical batch. Since then, the development of the best process (PEGylation, purification process, formulation and analytic testing development) has been finalized allowing the release of the FR104 cGMP batch needed for the clinical trials beginning on April 2015.

1.2 Summary description of project & Objectives

1.2.1 Why this project?

A principal function of the immune system is to eliminate infectious agents thereby protecting the host against infection. During their development, immune cells reacting against self-tissues are eliminated providing an immune system that is ‘tolerant’ to self. However, some autoreactive cells do escape into the blood. Mechanisms of control exist in periphery that keeps these autoreactive T cells under control. In some circumstances (genetic predisposition, environmental factors), the immune system is dysregulated, tolerance is broken and AID occurs.

Current immunosuppressive drugs, possessing an important toxicity, suppress immune responses as a whole but also usually suppress the physiological immune regulation supported by Treg. Hence, these drugs, often not able to cure patients, place patients at increased risk for opportunistic infections, immune deficiency-related malignancies, cardiovascular disorders and kidney dysfunction.

New therapeutic agents (anti-TNF) discovered this last decade are very efficient at treating symptoms and crisis in several AID but **are not meant to restore the immune balance between pathogenic and Treg**, which might have the potential to restore normal immunity and cure the disease.

An emerging theory sees peripheral tolerance as an equilibrated system between pathogenic effector T (Teff) and regulatory T (Treg, maintaining the tolerance) cells aimed at controlling immune responses, AID occurring if this balance leans to autoreactive cells. So, understanding the mechanisms that lead to dysregulation of the immune response resulting in AID is necessary to develop better therapies to treat and possibly even prevent these diseases.

In summary, rather than using the current treatments suppressing the immune system as a whole, **finding alternative therapies, suppressing only the parts of the immune system responsible for the autoimmune attack, while sparing Treg, represents a major goal in AID.**

The innovative therapeutic strategy applied within the TRIAD, Tolerance Restoration In Autoimmune Diseases, project is based on the restoration of an equilibrated immune

balance required for peripheral tolerance. The objective is to promote immune regulation, while preventing specifically pathogenic T lymphocyte activation, based on targeting CD28, one of the most important co-stimulatory molecules, required for T cell activation**.**

1.2.2 The TRIAD scientific approach

The activation of T lymphocytes is under the control of costimulatory molecules that regulate differentiation into either *pathogenic Teff* or *anti-inflammatory Treg.* Costimulation through the CD28-B7-CTLA-4 molecular triad helps determine this balance after initial antigen exposure: CD28 leads to T cell activation, whereas CTLA-4 prevents activation and is instrumental in Treg function. Therefore, targeting the CD28-B7 pathway in patients with B7 (CD80/86) antagonists (Orencia®, Abatacept) is a promising alternative to current immunosuppressive treatments. However, this strategy based on the inhibition of the entire pathway inhibits CTLA-4 signals crucial to the function of Treg and to the self-inhibition of autoreactive T cells. Therefore, immune imbalance is not corrected and recovery is not achieved.

The TRIAD approach, characterized by a selective inhibition of CD28, consists in selective blockade of these costimulatory molecules. It has been described for at least a decade, by members of the consortium and other scientists, that CD28 blockade restores or induces peripheral tolerance in AID and transplant experimental models in rodents, since activating interactions (CD28-CD80/86) are inhibited, whereas regulatory interactions (CTLA-4-CD80/86 and PDL-1/CD80) are not only conserved but even amplified (see Figure 1).

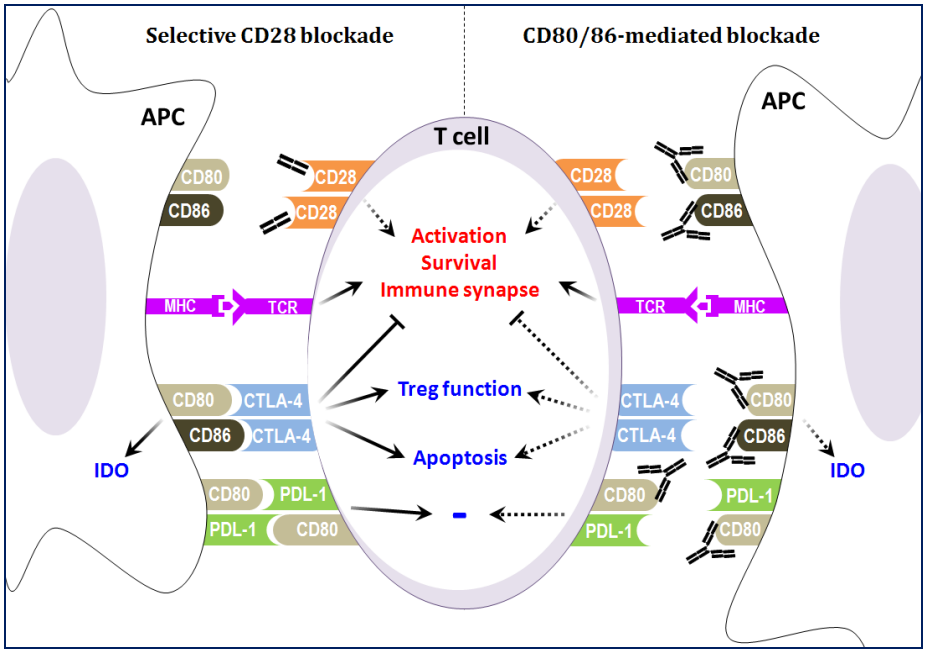


Figure 1.
Costimulatory molecules and biological pathways implicated in the targeting of CD28 vs CD80/86

APC: Antigen presenting cells;

IDO: Indoleamine 2,3-dioxygenase, a potent anti-inflammatory enzyme.

CTLA-4: Cell surface receptor acting as a negative regulator of Teff and required for Treg suppressive function.

CD28: one of the most important co-stimulatory molecules, which are required for T cell activation.
CD28 and CTLA-4 are the ligand for CD80 and CD86 molecules (also called "B7") expressed by antigen presenting cells (dendritic cells, B cells and monocytes).

——— Solid lines/arrows represent active signaling pathways after blockade of CD28 or CD80/86.

▪▪▪▪ Dotted lines/arrows represent disrupted signaling pathways.

From experimental proof of concept in non-human primate transplantation, the coordinator also confirmed that monovalent CD28 antagonist efficiently prevents Teff activation while promoting Treg.

Thus a similar proof of concept in AID would open the way to clinical trials with the potential to restore tolerance by restoring an equilibrated immune balance between pathogenic Teff and Treg cells.

Therefore CD28 antagonists represent a novel class of drugs ("first in class") allowing for the first time a very specific immunosuppression respecting Treg function, immune regulation and hence tolerance induction/restoration and aimed to treat AID by targeting origins of the pathology instead

of consequences. The TRIAD project will be the first testing this innovative strategy in several relevant preclinical models of AID and then in clinical settings.

1.2.3 Description of the S&T objectives

Targeting specifically autoreactive T cells and inducing antigen-specific Treg represents a potentially highly effective therapy in AID with the potential of healing the patients. In collaboration with the INSERM UMR1064 (Nantes, France), we have shown that a monovalent fragment from an antagonist anti-CD28 antibody could fulfil that objective in a transplant setting.

This strategy needed to be formally tested at the preclinical level in AID settings in relevant preclinical models. The ambition was ultimately to allow the clinical evaluation of that therapeutic strategy.

The TRIAD project aimed **at the evaluation of a selective antagonist of CD28,** FR104 - a fully humanized PEGylated monovalent anti-CD28 Fab antibody fragment and of a surrogate antagonist of CD28 in rodents - **in preclinical models** to prevent, treat and/or cure some AID including rheumatoid arthritis, multiple sclerosis, psoriasis, diabetes mellitus type 1, uveitis and arteriosclerosis. We expected that the TRIAD results supported by complementary mandatory toxicological studies and early clinical assessment in humans allow the coordinator of the project to initiate a phase I/II trial in AID patients.

a) Key objectives

In order to reach these goals, the key objectives addressed in parallel and interconnected modules of basic research and preclinical study allowing a further translational clinical development.

Exploratory research objectives

- To study and explore potential expected sensitivity of several AID to a mouse surrogate selective CD28 antagonist in the prevention or treatment of the pathology;
- To confirm previously identified mechanisms of action, such as the induction of Treg and other suppressive cells such as MDSC, in mice models and to further understand these mechanisms of action, in particular how modified cellular interactions favour antigen-specific Treg cell development;

Preclinical studies objectives

- To study the preclinical *in vivo* efficacy of the selective CD28 antagonist, FR104, to prevent or treat some AID in which T-cells play a major role;
- To study the immunological toxicity of the selective antagonist of CD28 *in vivo* in humanised SCID mice reconstituted with human T lymphocytes and in non-human primate models, as well as *in vitro* on human T cells;
- To examine the safety profile of our innovative strategy, such as immunogenicity, viral and malignancy status of primates treated with FR104;
- To confirm previously identified mechanisms of action, such as the induction of Treg, in the context of AID in primate models for a better understanding of these mechanisms of action;

To perform preclinical toxicological studies specific to AID and, in parallel, pharmacokinetics studies.

Translational clinical development objectives

- To perform other formulation and drug development studies (subcutaneous administration);
- To prepare the initiation of an early assessment in humans: a phase I/II trial in confirmed sensitive AID while mandatory preclinical evaluation being performed in parallel by Effimune.

b) Methodology

Study of the selective blockade of CD28

To study the fine and selective CD28 blockade in AID mice model, a surrogate anti-mouse CD28 monoclonal antibody has been used for the prevention of new-onset T1D and for the prevention and/or cure of EAU. A direct comparison with CTLA4-Ig has been performed in the T1D model. Particular attention has been paid to the Treg, TH1 and TH17 T cells inside pancreas and draining lymph nodes. Synergies with other immune-intervention and investigations into the mechanism of action have also been studied. More precise exploration has been checked for other possible *in vitro* mechanisms (i.e. the effect of selective CD28 blockade on Teff, action on MDSC) and cellular interactions (i.e. Treg interaction with APCs and Teff).

Study of the efficacy of the selective CD28 antagonist (FR104)

The efficacy study of the selective CD28 antagonist has been studied:

- ✓ in a non-human primate model: to prevent or cure multiple sclerosis (EAE model) and rheumatoid arthritis (CIA model) to cure immune-related skin diseases such as psoriasis (DTH model). Immunotoxicity parameters such as cytokines secretion after injection have been also analysed and the PK/PD parameters evaluated in parallel.
- ✓ in a preclinical model of human coronary arteriosclerosis grafted in humanized SCID mice reconstituted with human peripheral blood mononuclear cells (PBMC). A back to back comparison with CTLA4-Ig® has been performed.

Pharmaceutical development

The pharmaceutical development has been ensured by Effimune in parallel in order to develop a formulation compatible with a chronic administration in AID. Specific preclinical toxicological and subchronic general toxicity studies have been performed by Effimune to prepare a phase I/II clinical trial in patients suffering from an AID that will be selected. The selection of the disease or a sub-population will be based on the results of the preclinical experiments and the accessibility of the clinical centres.

c) Achievements

Once completed it was expected that the TRIAD project reaches the following achievements:

- ✓ Preclinical animal proofs of concepts that the selective CD28 antagonist could prevent and/or treat certain identified AID in which T-cells act at a central place. This is required to clarify the therapeutic area in AID and to select the pathology for the first phase I/II trial;
- ✓ Study of expected sensitivity of several AID to a surrogate selective CD28 antagonist allowing to enlarge potentially therapeutic indications and to initiate clinical trials in confirmed sensitive AID;
- ✓ Immunological safety data, general and specific to AID toxicity package, required to prepare an Investigational Medical Product application;
- ✓ The final outcome would be a new selective antagonist of CD28 positioned as the only available CD28 antagonist ready to enter clinical studies, making it possible to verify in man the therapeutic hypothesis that CD28 blockade will induce Treg and immune regulation in AID. It constitutes therefore a **"first-in-class"** therapeutic reagent allowing a specific immunosuppression compatible with Treg-supported immune regulation.

Coordinated by a SME, the TRIAD consortium will work in a focused and integrated manner to cover all aspects of the research activity, the preclinical study and the planned early clinical development. The TRIAD project is believed to directly address the unmet medical and socio-economic needs by developing a new therapeutic strategy that can improve the health and the quality of life of AID patients.

1.3 Main S&T results/foregrounds

WP1 – Explorative Research

1.1 - Efficacy of murine selective CD28 antagonist for the treatment of autoimmune diseases

We focused our investigations on two experimental models of autoimmunity: autoimmune (type 1) diabetes (T1D) and experimental autoimmune uveitis (EAU).

a) T1D studies were performed by the team of Lucienne Chatenoud and Sylvaine You (INSERM U1151, team #7, Necker Hospital, Paris, France, TRIAD partner #4b). We used the non-obese diabetic (NOD) mouse model. These mice develop spontaneous diabetes that is very similar to the human pathology. In parallel to *in vitro* experiments, we have administered murine anti-CD28 Fab PEGylated fragment (PV1 Fab') into female NOD mice at different ages that correspond to different steps in disease progression:

- 4 weeks of age (immediately after weaning);
- 6 weeks of age i.e. pre-diabetic stage;
- 10 weeks of age i.e. late pre-diabetic stage;
- NOD mice showing recent hyperglycemia (blood glucose>250mg/dL).

Treatment consisted in 2 intraperitoneal injections per week for 4 consecutive weeks at the dose of 10 mg/Kg body weight. Age-matched PBS-treated NOD mice were used as controls.

Overall, results show that, although anti-CD28 antibodies efficiently inhibit T cell proliferation and responses towards β cell-specific autoantigens *in vitro*, they have no significant protective effect *in vivo* in all groups tested. They neither induced diabetes remission in recently diabetic NOD mice. In contrast, our data show that prolonged treatment in young 6-week-old NOD mice accelerates diabetes development. This is due to the fact that *in vivo*, PV1-PEG targets $CD4^+Foxp3^+$ regulatory T cells (Treg). Blocking CD28 costimulation impairs Treg homeostasis, functions and migration to inflammatory sites (pancreatic islets) but does not significantly impact pathogenic effector T cell activation and homing to target tissues.

Lastly, we performed syngeneic pancreatic islet graft in diabetic NOD mice. Without treatment, grafts are rejected within 7-10 days due to the reactivation of the autoimmune responses. Administration of PV1-PEG was not able to prolong islet graft survival.

b) EAU studies were performed by the team of Luiz Vicente Rizzo (IIEP, Sao Paulo, Brazil, TRIAD partner #5). B10.RIII mice were immunized with the 161-180 IRBP peptide in Complete Freund Adjuvant [CFA] plus *B. pertussis* toxin [PTX] followed by the intra-peritoneal inoculation of anti-CD28 (PV1) or NHS (control) Fab fragments [10 mg/Kg] at 9, 13 and 17 days post-immunization (done 3 times, n=5/group). Animals were sacrificed 21 days after immunization. Disease onset and disease score were decreased in PV1-treated groups compared to NHS-treated and control groups. The lower score was characterized by a lower incidence of vasculitis, granuloma formation, and retinal folding when compared with untreated and NHS-treated animals. The treatment did not inhibit T cell migration to the eye but reduced T cell activation and development of Th1 (but not Th17) responses in the target organ and in peripheral lymphoid organs. As shown in the NOD model of type 1 diabetes, PV1 administration led to a decrease in both frequency and absolute numbers of Treg in both draining lymph nodes and spleen but did not negatively impact on uveitis prevention.

Taken together, the data suggest mPEG PV1-Fab' acts specifically on IFN-γ production and T_H1 polarization and emphasize that this specific CD28 blockade strategy is a potential tool for the treatment of autoimmune disorders in the eye.

1.2 - Efficacy of a combination therapy using anti-CD28 and other drugs (Rapamycine or Tacrolimus)

Since combination of anti-CD28 antibodies (FR104) with rapamycin or tacrolimus was efficient for preventing graft rejection in primates, we tested these combinations in 10-week-old pre-diabetic NOD mice. Administration of rapamycin or tacrolimus alone did not confer protection from diabetes development. However, we observed a synergistic effect of the PV1-PEG / Rapamycin treatment (but not with tacrolimus) which was able to delay disease onset in NOD mice. These results suggest that rapamycin and PV1-PEG have a complementary mode of action allowing the control of the autoimmune responses against pancreatic β cells.

1.3 - Fine understanding of the mode of action of the selective CD28 antagonist and comparison to other costimulatory blockade approaches

Effimune (Nantes, France, TRIAD partner #1) has performed this task from the anti-human CD28 non signaling antagonist monovalent antibody FR104 that it produced. This antagonist was evaluated in an *in vitro* system where human allospecific clonal regulatory or effector T cells were stimulated by their cognate allogeneic B cells to better understand how selective CD28 antagonist *vs* CD80/86 antagonists could differentially control activation of effector (Teff) *vs* regulatory (Treg) T lymphocytes activation at the immune synapse level. We revealed that the three identified ligands of CD80/86, CD28, CTLA-4 and PD-L1, indeed differentially control immune synapse formation and function in human Treg *versus* Teff. Selectively antagonizing CD28 costimulation increased Treg contact time with APC and induced calcium mobilization which translated in increased Treg suppressive activity, whereas it was not the case with CD80/86 antagonists. In contrast we found that selective blockade of CD28 prevent Teff immune synapse with APC by increased motility of Teff. Again, CD80/86 antagonist did not modify Teff motility. This study is published in PLos One (Dilek *et al.* December 2013) and further identified that selective CD28 blockade promoted CTLA-4 signaling which reduced expression of the integrin LFA-1 (CD11a/CD18). This could explain why selective CD28 and not CD80/86 antagonists increase motility of Teff and prevent immune synapse formation. We also identified that Treg activation and prolonged immune synapse of Treg with APC induced by selective blockade of CD28 was also LFA-1 dependent.

We also determined the mechanism of action of selective CD28 blockade in the maintenance of immune tolerance and the accumulation of myeloid-derived suppressor cells (MDSC) *in vivo*. We identified that SIRPα (Signal regulatory protein α) is express by MDSC which accumulate after anti-CD28 treatment. Unexpectedly, SIRPα does not control the suppressive function of MDSC (like CTLA-4 for Treg) but in contrast is mandatory to maintain accumulation of undifferentiated immunosuppressive MDSC, since abrogation of SIRPα signaling induced differentiation of MDSC in effector cells which lost suppressive function and break immune tolerance induce by anti-CD28. This study confirmed that immune tolerance after selective CD28 blockade is maintained by active mechanisms implicating Treg and MDSC and involving at least the SIRPα protein.

1.4 - Conclusion

WP1 partners completed their investigations on the mode of action of selective CD28 antagonists (PV1-PEG) and on their therapeutic efficacy in mouse models of autoimmunity (type 1 diabetes and uveitis). Several conclusions have been reached:

- *In vitro* experiments concur to show the efficacy of selective CD28 antagonists in inhibiting effector T cell activation while favouring Foxp3+ Treg cell functions (in contrast to what observed with CD80/86 antagonists):
- These *in vitro* effects do not systematically translate into a protective effect *in vivo* as treatment with PV1-PEG provided opposite results depending on the experimental model used: partial protection from uveitis (where it inhibits Th1 responses) while it was without effect or even deleterious in T1D;
- *In vivo* investigations in both uveitis and T1D models showed a negative impact of PV1-PEG on Foxp3^{+} Treg cell homeostasis and prolonged treatment leads to an exacerbation of disease occurrence and severity (T1D);
- Myeloid-derived suppressor cells (MDSC), expressing SIRPα (Signal regulatory protein α), may contribute to the therapeutic effect of selective CD28 antagonists;
- Combination of selective CD28 antagonists with immunosuppressive drugs, such as rapamycin, may represent a promising approach to treat autoimmune diseases.

All these *in vivo* and *in vitro* findings should be taken into consideration for clinical applications in human autoimmune diseases.

WP2 – Preclinical Studies

2.1 - In vitro FR104 evaluation on marmoset leukocytes

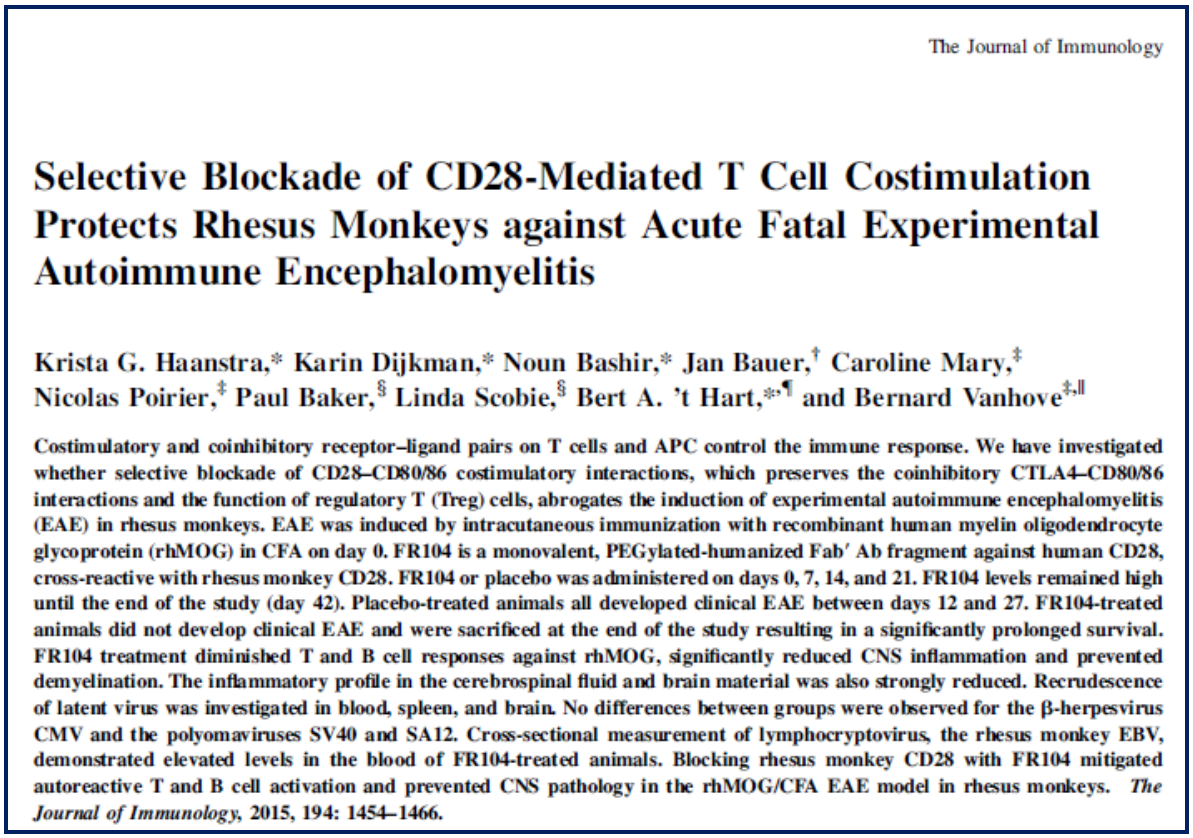
The reason for absence of cross-reactivity of FR104, while it had been shown with the parental, non humanised antibody, is unknown. A sequence comparison indicated that the CD28 sequence of the marmoset is significantly different from human and macaques. One hypothesis is that the humanization process has changed reactivity against marmoset CD28, but this seems unlikely. Another hypothesis is that the first assessment was artefactual (due to the use of a blood sample that had been shipped from Evreux to Nantes, whereas other blood samples tested at that time were freshly drawn). However, an element is intriguing: even with a “positive control” anti-CD28 mAb, already used at the BPRC in the past, there was no clear staining of CD28+ cells in this marmoset PBMC sample. Interestingly, BPRC has been unable to identify a commercially available anti-human anti-CD28 antibody from the approximately six that were tested that is cross-reactive with marmoset T-cells by FACS and that is able to stimulate marmoset T-cells (in conjunction with anti-CD3).

Therefore there is still a doubt whether the marmoset PBMC tested here actually contained CD28-positive cells (for unknown reason that might be related to the immunological status of the animals). It might well be that in these animals the CD28+ T cells represent low to undetectable amount of cells. Another possibility is that most anti-human CD28 mAbs tested so far do not cross-react against the marmoset.

In conclusion, the reason for the absence of binding of FR104 to marmoset PBMC could not be deciphered. It might either be an absence of cross-reactivity of FR104 to marmoset CD28 or an absence of CD28+ T cells in the animals tested here, which seems rather unlikely. Whatever the reason, it seemed not appropriate to assess FR104 in marmoset models. Therefore it was decided instead to use rhesus monkey, in which a clear binding of FR104 could be evidenced.

2.2 - Efficacy evaluation of FR104 in EAE model

In monkeys treated with FR104, clinical EAE did not develop. Histological signs of CNS inflammation were significantly reduced compared to animals receiving placebo treatment and demyelination was not detected. Treatment with FR104 also diminished T and B cell responses against the immunizing antigen rhMOG. The inflammatory profiles of cerebrospinal fluid (CSF) and brain, assayed with Luminex technology and quantitative RT-PCR respectively, were also strongly reduced in FR104-treated animals compared with placebo-treated monkeys. Blocking of rhesus monkey CD28 with FR104 mitigated autoreactive T and B cell activation and prevented the characteristic destructive CNS pathology of the rhMOG/CFA EAE model in rhesus monkeys. To assess the risk of virus reactivation, DNA isolates from blood, spleen, and brain and viral RNA from sera were tested for CMV, LCV, SA12, SV40 and HEV. No major problems were observed, although some evidence of LCV reactivation was seen in both groups with a higher viraemia being noted in those treated with FR104 (reported in WP3 summary). The results have been reported in a publication that has been published in the Journal of Immunology.



The Journal of Immunology

Selective Blockade of CD28-Mediated T Cell Costimulation Protects Rhesus Monkeys against Acute Fatal Experimental Autoimmune Encephalomyelitis

Krista G. Haanstra,* Karin Dijkman,* Noun Bashir,* Jan Bauer,† Caroline Mary,‡ Nicolas Poirier,‡ Paul Baker,§ Linda Scobie,§ Bert A. 't Hart,*,¶ and Bernard Vanhove‡,‖

Costimulatory and coinhibitory receptor–ligand pairs on T cells and APC control the immune response. We have investigated whether selective blockade of CD28–CD80/86 costimulatory interactions, which preserves the coinhibitory CTLA4–CD80/86 interactions and the function of regulatory T (Treg) cells, abrogates the induction of experimental autoimmune encephalomyelitis (EAE) in rhesus monkeys. EAE was induced by intracutaneous immunization with recombinant human myelin oligodendrocyte glycoprotein (rhMOG) in CFA on day 0. FR104 is a monovalent, PEGylated-humanized Fab′ Ab fragment against human CD28, cross-reactive with rhesus monkey CD28. FR104 or placebo was administered on days 0, 7, 14, and 21. FR104 levels remained high until the end of the study (day 42). Placebo-treated animals all developed clinical EAE between days 12 and 27. FR104-treated animals did not develop clinical EAE and were sacrificed at the end of the study resulting in a significantly prolonged survival. FR104 treatment diminished T and B cell responses against rhMOG, significantly reduced CNS inflammation and prevented demyelination. The inflammatory profile in the cerebrospinal fluid and brain material was also strongly reduced. Recrudescence of latent virus was investigated in blood, spleen, and brain. No differences between groups were observed for the β-herpesvirus CMV and the polyomaviruses SV40 and SA12. Cross-sectional measurement of lymphocryptovirus, the rhesus monkey EBV, demonstrated elevated levels in the blood of FR104-treated animals. Blocking rhesus monkey CD28 with FR104 mitigated autoreactive T and B cell activation and prevented CNS pathology in the rhMOG/CFA EAE model in rhesus monkeys. *The Journal of Immunology*, **2015, 194: 1454–1466.**

2.3 - Efficacy evaluation of FR104 in CIA model

Treatment with FR104 or Abatacept was equally potent in preventing the expression of arthritis, leading to significantly increased disease-free survival compared to placebo-treated animals:

- ✓ ***Clinical effect:*** The clinical effect was supported by stable levels of cartilage breakdown products measured in urine and minimal histological changes;
- ✓ ***Biological effect:*** Both treatments also resulted in a suppressed production of the inflammatory markers CRP and IL-6;
- ✓ The immunosuppressive potency of FR104 was illustrated by robust suppression of collagen type II (CII)-induced PBMC proliferation and serum antibody (IgM/IgG) levels;
- ✓ ***No adverse effects:*** Treatment with FR104 prevented the development of clinical arthritis. No acute physical response was observed at the time of intravenous dosing showing that injection of FR104 under inflammatory conditions does not result in adverse effects in this rhesus monkey model of inflammatory arthritis.

A manuscript for publication of the results under the title “Blockade of CD28 with a novel selective antagonist ameliorates Collagen-induced Arthritis in the Rhesus Monkey” has been submitted to European Journal of Immunology.

2.4 - Efficacy evaluation of FR104 in DTH model

Our DTH model tests the efficacy of FR104 in a memory response to BCG vaccination at different doses. After 2 bacillus Calmette-Guérin (BCG) vaccinations, baboons, tested for their immunization against tuberculin, received a first control intra-dermo reaction (IDR). One day before the second IDR, baboons according to their groups (see below) received either control excipient or a single intravenous dose of FR104. Then, several IDR were repeated every month even after elimination of the FR104 (Groups #1, 2, 3, Control group, Figure 2A). In group #4, baboons received similarly to other groups an injection of FR104 at 10 mg/Kg after the first IDR. However, the 2nd IDR was performed only when the receptor occupancy by FR104 was null (saturation 0) (Figure 2B).

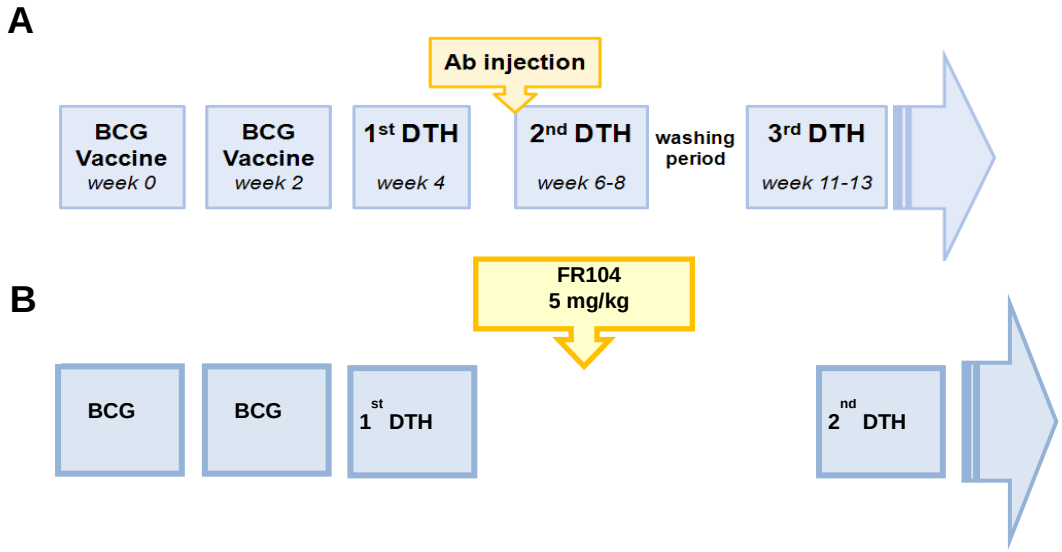


Figure 2.
DTH model in control or FR104 groups
A) Iterative IDR after FR104 injection (Control group, Groups #1, 2, 3); **B**) IDR before FR104 Injection and when receptor occupancy was null (saturation 0) after a wash-out period.

In addition, our model includes also a control of memory humoral response. Two months before drug administration, baboons were immunized with intramuscular injection of 0.6ml of KLH at 9 mg/mL and 0.6mL of complete Freund adjuvant. Baboons were treated once i.v. with either 0,1, 1 or 10 mg/Kg of FR104 or an equivalent volume of excipient. Just after drug administration, baboons received an intravenous administration of 1.5 ml/Kg of sheep red blood cells (SRBC) 10%. Animals were then challenged a second time with SRBC and KLH five months after drug administration using same protocol, excepted that second KLH challenge was performed with incomplete Freund

adjuvant. Sera from animals were collected over time and IgG titers were determined by serial dilution on SRBC by flow cytometry using a fluorescent anti-human IgG or on recombinant protein by Stellar KLH ELISA test kit.

Five groups of baboons have been studied:

- Control group, n= 4, excipient
- Group 1, n =3, FR104, 1 mg/Kg
- Group 2, n =4, FR104, 0,1mg/Kg
- Group 3, n =3, FR104, 10 mg/Kg, at the 2nd DTH
- Group 4, n= 3, FR104, 10 mg/Kg after the 1st DTH, 2nd DTH at FR104 saturation 0.

We evaluated the efficacy of FR104 in the DTH model at different doses: in control group, group #2 (0.1 mg/Kg) and group #3 (10 mg/Kg). The pharmacokinetic and pharmacodynamics (PK/PD) of the molecule was also followed in parallel. In the control group, results showed that iterative IDR with tuberculin consistently elicits a DTH response. Results from group #3 (highest dose - 10 mg/Kg) showed that recipients failed to respond to the cutaneous tuberculin challenge and developed a so called antigen-specific hypo-responsiveness, over at least 5 months. Recipients receiving a low dose (0.1 mg/Kg) responded to Ag challenges showing only a reduction of the IDR in size and time without prevent completely the IDR.

Immune regulation of memory Th1-mediated inflammatory responses seemed dose-dependent, since administration of the CD28 antagonist at 1 mg/Kg to other sensitized animals was also effective during the period of high receptor occupancy in the periphery but inflammatory responses recovered totally after drug elimination. Animals treated with 0.1 mg/Kg antagonist did not display any significant difference with the control group after treatment even at high blood receptor occupancy level. Besides, *in vivo* immune regulation of memory responses was also antigen dependent, since other sensitized animals treated with the highest dose of the CD28 antagonist but not challenged with intradermal tuberculin during the drug exposure, showed erythema when re-challenged after drug elimination (20 weeks after treatment), similar to their first tuberculin challenge.

We demonstrated that these 10 mg/Kg FR104-treated animals were still immunocompetent since they were able to respond to a novel immune challenge performed by intravenous administration of sheep red blood cells

Our data provide the first proof of concept that FR104 can inhibit a TH-1 type cutaneous immune response and may help to promote long-term protection against skin inflammation in non-human primates.

2.5 - Efficacy evaluation of FR104 in skin transplantation, used as a surrogate model for Transplant Arteriosclerosis (TA)

a) Transplant arteriosclerosis model

Arteriosclerosis is an inflammatory response to autoantigens that leads to intimal expansion and occlusion of affected vessels. Transplant arteriosclerosis (TA) is a related disease driven by alloantigen, but is increasingly recognized to be linked to the development of autoimmunity against the transplant. TA is a fibroproliferative disease of the arterial vasculature, characterized by neointimal hyperplasia due to smooth muscle cell proliferation and accumulation of extracellular matrix proteins. Macrophages and T cells play an important role in the pathogenesis.

In this set of assays, we demonstrated the effects of FR104 and CTLA-4 Ig in humanised mice. FR104 and CTLA-4 Ig significantly suppressed the proliferation of human T cells *in vivo.* This

suppression effect was equivalent in both treatment groups. We also demonstrated that FR104 may have a suppressive effect on the development of TA. However, this particular experimental model has been shown to have some critical limitations which preclude us from obtaining further detailed data. We typically require a 3 week period for reconstitution followed by a further 4 week period in which TA can develop. However, XVHD develops between weeks 5 and 7 and whilst FR104 has the capacity to block XVHD, the PEG control and CTLA4-Ig group develop XVHD at an earlier time point. Experiments therefore do not reach the required maturity to allow appropriate assessment of the efficacy of FR104 and its comparison to saline or CTLA-4 Ig at the same time point.

We therefore elected to use a skin transplantation model as a surrogate for the TA model. We have previously demonstrated experimentally that human skin graft alloimmune damage may be prevented by employing human regulatory mechanisms similar to that in the TA model (4,5) and that functional enrichment of Foxp3(+) T cells may delay skin allograft rejection mediated by polyclonal CD4+ effectors (6). In these models, protection of human vasculature within the skin graft by Treg has also been demonstrated and is likely to be important for the survival of the skin graft.

Importantly, this model facilitates the ability to examine mechanistic data that are not possible with the TA model. The assessment of selective CD28 blockade in human skin rejection is relevant to assessing the efficacy and mechanistic activity of FR104 in arteriosclerosis. Therefore assessing CD28 selective blockade in a skin graft rejection is a model as relevant as the TA model to gain translational preclinical information**.**

FR104 may have a suppressive effect on TA development however, due to the limitations of the model the skin transplantation model is more appropriate to continue this investigation and will better help us to determine the effects of FR104.

b) Skin transplantation model

Our aim is to obtain proof of the therapeutic principle for the selective anti-CD28 antagonist FR104 in a preclinical model of inflammatory disorder in humans. Having assessed the viability of the TA model to determine this, we have now selected a skin transplantation model as a surrogate for the TA model.

Using the human skin transplantation model, we have demonstrated that FR104 treatment significantly prolongs human skin allograft survival whereas CTLA4-Ig does not. In addition, we demonstrated the difference between a selective CD28 blockade strategy with FR104 and a direct blocking CD80/CD86 strategy with CTLA-4 Ig. Here, this preclinical transplantation model revealed a difference in the efficacy of FR104 and CTLA-4 Ig. Importantly, for FR104 this was not simply due to a lack of human leukocyte engraftment in the model, as we have confirmed that mice reached an adequate reconstitution level which would normally result in the rejection of human skin (>0.1% human $CD45^+$ cells in the peripheral blood at 3 weeks after adoptive transfer). It is interesting that CTLA-4 Ig does not prolong graft survival (MST= 31 days) and furthermore that this is accompanied with considerable human T cell infiltration into the human skin graft. By contrast, FR104 significantly prolongs skin allograft survival (MST= 56 days) and effectively reduces the infiltration of human T cells into the graft.

Our data demonstrate that FR104 may reduce the development of transplant arteriosclerosis in human vessels in a humanised mouse system. However, the development of XVHD in groups not receiving FR104 limited the ability to further analyse the effect of FR104 in this model. Here, our data also corroborate previous studies demonstrating the efficacy of FR104 at preventing the development of GVHD. Importantly, using a humanised mouse skin transplantation model, FR104 treatment significantly prolongs human skin survival whereas CTLA-4 Ig provides no benefit above treatment with control. FR104 significantly reduced the human leukocytic graft infiltrate, suggesting that this may be responsible for the protection of these skin allografts. Graft protection may also be secondary to a reduced proliferation of human leukocytes in humanized mice.

In summary, FR104 is an effective immunosuppressant *in vivo* which displays greater potency than CTLA-4 Ig. In addition, by sparing CTLA-4 co-inhibitory signals, FR104 may theoretically lead to improved regulatory responses as compared with CTLA-4-Ig treatment.

2.6 - Conclusion

During the TRIAD project, WP2 partners investigated on the mode of action of selective CD28 antagonists (FR104) and on their therapeutic efficacy in non-human primate models of autoimmunity, more precisely Experimental Autoimmune Encephalomyelitis (EAE), Collagen-Induced Arthritis (CIA), Delayed-Type Hypersensitivity (DTH) and skin transplantation, used as a surrogate model for Transplant Arteriosclerosis (TA).

Both primate studies demonstrate a good effect/risk profile of FR104 in valid preclinical models of human autoimmune disease. Although the drug was tested in systems modeled on RA and MS, the results warrant the conclusion that the human CD28 antagonist FR104 has a strong potential as safe and effective treatment of other types of human autoimmune diseases as well.

Summary of deliverables

- Proof that the expected immunomodulatory effect of FR104 on autoreactive T cell activation can be reproduced in rhesus monkeys;
- Proof that prolonged administration of FR104 is well tolerated without major side effects;
- Proof that prolonged administration of FR104 does not cause major problems caused by the reactivation of latent virus infection;
- Proof of a potent clinical effect of FR104 in the rhesus monkey EAE model;
- Proof of a significant clinical effect of FR104 in the rhesus monkey CIA model, which is at least equal in potency as the benchmark treatment with Abatacept;
- The manuscript reporting the clinical effects of FR104 in the EAE model has been accepted in Journal of Immunology;
- A manuscript reporting the clinical effects of FR104 in the CIA model has been written and is in submission;
- Proof that targeting selectively CD28 prevented memory T-cell reactivation and would help to promote long-term protection against skin inflammation in non-human primates;
- In addition, by sparing CTLA-4 co-inhibitory signals, FR104 may theoretically lead to improved regulatory responses as compared with CTLA-4-Ig treatment.

WP3 – Immune Safety

3.1 - Monitoring of a potential immune-related side-effect

Objectives for INSERM A / CHU / EFFI were to confirm:

1. that the injection of FR104 does not induce any cytokine release in primates (baboons and macaques);
2. that the monovalent format of CD28 antagonist does not display any agonist activities on human T Cells;

3. to assess the immunogenicity of FR104.

a) In vitro comparison of human vs baboon T cell responses to anti-CD28 mAbs

First, we confirmed that human PBMC cultured over human umbilical vein endothelial cell (HUVEC) monolayers vigorously proliferated and secreted diverse pro-inflammatory cytokines, including interferon (IFN)γ, tumor necrosis factor (TNF), interleukin (IL)-2, IL-4, IL-5,and IL-6, in a dose dependent manner in response to a superagonist anti-CD28 mAb (clone ANC28.1). In contrast, a conventional agonist anti-CD28 mAb (clone CD28.2) induced a moderate cytokine secretion in this assay and did not induce lymphocytes proliferation. As expected, antagonist Fab' anti-CD28 fragments (FR104) did not elicit any cytokines release or T lymphocytes proliferation.

Baboon PBMC responded similarly to HUVEC with regard to lympho-proliferation, since superagonist anti-CD28 mAbs induced important proliferation in a dose-response manner, while agonistic anti-CD28 mAbs did not. As observed with human PBMC, agonistic anti-CD28 antibody did not elicit measurable IL-2 release with baboon PBMC.

b) In vivo assessment of immunotoxicity of anti-CD28 mAbs in NOD/SCID mice reconstituted with human or baboon PBMC

One week after engraftment, immunodeficient non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice, reconstituted with human PBMC received either vehicle as negative control, superagonist anti-CD28 antibodies as positive control, FR104 or divalent anti-CD28 antibodies. Injection of superagonist anti-CD28 antibodies resulted in a rapid and significant increase of IFNγ, IL-6 and IL-8 and in the induction of TNFα. Injection of divalent IgG anti-CD28 antibodies resulted also in the release of IL-6 and IL-8 but had no effect on IFNγ and TNFα and did not modify significantly the T cell phenotype. As expected, FR104 injection did not result in any cytokine release as compared to the control situation and even reduced release of IFNγ and did not elicit any activation marker on human T lymphocytes.

We conducted similar experiments in NOD/SCID IL-2rγ knockout (NSG) mice reconstituted with freshly isolated baboon PBMC. Similarly, whereas no difference could be observed in term of cytokines secretion between mice treated with FR104 or excipient, administration of a superagonist anti-CD28 mAb resulted in a rapid and significant release of pro-inflammatory cytokines measured in the plasma of NSG mice reconstituted by human or baboon PBMC.

c) In vivo immunotoxicity evaluation of FR104 in baboon

Three baboons were injected once intravenously with FR104 at 20 mg/Kg and immunotoxicity parameters were compared with two baboons that received an equal volume of excipient. The pharmacokinetics and pharmacodynamics analyses showed that C-max concentration in sera reached 160 to 542 µg/mL within one hour. The T1/2 half-life ranged between 8.6 and 9.9 days (mean: 9.3 d) and receptor occupancy in periphery was maintained at 100% over two months, as long as through levels were above 1 µg/mL. Similarly to this previous study, pharmacokinetics and receptor occupancy were also assessed in baboons included in DTH protocol, treated with various FR104 doses (**0.1mg/Kg, 1 mg/Kg, 10 mg/Kg**). Results showed at 10 mg/Kg, a half-life around 10d and a receptor occupancy in periphery maintained at 100% up to two months, as long as through levels were above 2 µg/mL.

Pro-inflammatory cytokines remained undetectable and we did not observe clinical symptoms of cytokine release syndrome based on body temperature, blood pressure, heart rate, oxygen saturation and hematology parameters, which all stayed within physiological ranges for both FR104 and excipient treated baboons.

d) Selective CD28 blockade did not modify memory lymphocytes numbers

The selective CD28 antagonist did not induce general lymphocyte depletion in baboons since no significant modulation of total blood lymphocytes numbers was observed, neither at CD4+ nor CD8+ memory T-cell subsets. No significant modification of Treg cells number or frequency was observed in the blood. The frequency of tuberculin-specific memory T-lymphocytes in the blood remained also similar after treatment. However, while depletion of CD25+ Treg cells from PBMC or exogenous IL-2 to reverse anergy did not significantly modify *ex vivo* restimulation of memory T-cell with tuberculin after treatment, selective blockade of CD28 significantly reduced the IFNγ-secreting cells frequency suggesting CD28 is required for reactivation of these memory cells. Altogether, selective CD28 blockade prevented memory Th1-mediated skin inflammatory response *in vivo* and could be protective on the long-term if T-cells encounter their antigen.

e) Selective CD28 blockade controls humoral memory recall response without disruption of preformed immunity

To investigate the role of CD28 *in vivo* on T-dependent humoral memory responses, we first used the T-dependent sheep red blood cells (SRBC) immunization model. Since baboons have naturally preformed xenoreactive anti-gal IgG which bind to SRBC, we performed a first intravenous SRBC injection 18 hours after excipient or selective CD28 antagonist administration at different doses. All animals significantly increased their sera anti-SRBC IgG titers within 2 weeks, excepted baboons treated with 10 mg/Kg of selective CD28 antagonist. In order to evaluate if selective CD28 blockade could regulate also T-dependent humoral memory responses, we challenged a second time these animals after complete drug elimination. Upon second SRBC challenge, all baboons increased sera anti-SRBC IgG titers, including baboons which did not respond during the first challenge performed under selective CD28 blockade.

To confirm that selective CD28 blockade did not disrupt unchallenged humoral memory immunity, we used a second T-dependent humoral response model. Two months before treatment, baboons were immunized against keyhole limpet hemocyanin (KLH). All animals developed specific anti-KLH IgG within two weeks and titers were monitored over time. Anti-KLH IgG titers were not modified by selective CD28 blockade treatment, even at higher doses, and all animals responded similarly to a second KLH challenge performed after drug elimination. These animals were still immunocompetent since capable to respond to a novel immune challenge performed by intravenous administration of sheep red blood cells.

f) Conclusion

FR104 does not induce, neither *in vitro*, in human and baboon cells, nor *in vivo* in model of NOD/SCID and NSG mice reconstituted with freshly isolated human or baboon PBMC, nor *in vivo* baboon model, any cytokines release or T lymphocytes proliferation as compared to the control situation. Moreover, we did not observe clinical symptoms of cytokine release syndrome based on physiological parameters.

In *in vivo* baboon model, the FR104 half-life is around 9-10 days with a receptor occupancy in the periphery maintained at 100% over two months.

The selective CD28 antagonist did not induce general baboon lymphocyte depletion, without any significant modification of Treg cells number or frequency in the blood. The frequency of tuberculin-specific memory T-lymphocytes in the blood remained also similar after treatment.

Moreover, selective CD28 blockade prevented memory Th1-mediated skin inflammatory response *in vivo* and could be protective on the long-term if T-cells encounter their antigen. However selective CD28 blockade did not disrupt unchallenged humoral memory immunity, since treated animals were

still immunocompetent, still capable to respond to a novel immune challenge perform by intravenous administration of sheep red blood cells.

3.2 - Viral re-activation

Objectives for GCU were to analyse the viral status of primates treated with FR104 and make sure that the consortium's innovative therapeutic strategy did not promote re-activation of quiescent viruses in AID preclinical models. Our aims were to analyse relevant samples (blood, sera, saliva, urine and faeces) collected from the preclinical models pre and post treatment with FR104. A standard operating procedure (SOP) for samples collection and analyses were also to be established.

WP3 was involved in monitoring the potential for virus reactivation following the FR104 administration. Viruses were selected on the basis of their relation to common human viruses reactivated during immune-suppression and known to be representative in non-human primate models (Table 1).

Virus	Clincal Presentation	Confirmation of Diagnosis/Treatment
Cytomegalovirus (CMV)	Weakness, Weight loss, Diarrhea, Fever, Myalgia, Splenomegaly, Colitis	Viral inclusion bodies Ganciclovir
Polyomaviruses (BK, JC, SV40)	Persistent aneamia, Intersitial nephritis, Renal dysfuncton, Ureteritis, PML	Viral inclusion bodies Large T antigen Reduction of immune suppression Interferon / Cidoflovir
Epstein Barr Virus (EBV) **Lymphocryptovirus (LCV)**	PTLD	LN biopsy Discontinue immunesuppression Rituximab
Herpes simplex (HSV) = HVP2	Vesicles / Lesions	Gancicolvir / Cidofovir
Hepatitis E virus	Hepatitis	Virus in feaces/liver Ribavirin Reduction in immune suppression

Table 1. Viruses of concern in human recipients

In particular, members of the herpesvirus and polyomavirus families were the main focus. To facilitate analysis, blood cells and fluids, tissues, urine and feacal samples were collected from treated and control animals as per the agreed SOP set by GCU for sample handling and processing at M6. Baboon and macaque samples were received from BPRC (Macaque – EAE Task 2.2) but only urine was sent from animals utilized in the CIA experiment, Task 2.3) and INSERM A (Baboon – DTH model – Task 2.4). As per protocols for viral analysis, viral DNA and RNA were extracted from these tissues and body fluids at pre and post treatment time points, to identify the viruses present and to monitor any quantitative changes their levels.

Tools were already available for detection of LCV and CMV in the baboon and were initially to be developed for the marmoset. However, since no cross-reactivity had been detected with marmoset samples, the switch by BPRC (Partner 2, WP2, Task 2.2 and 2.3) from the marmoset to the rhesus macaque model changed the focus of development.

Overall, animals were found to have a number of viruses present including CMV, HEV, HVP-2, LCV, SA12 and SV40. In addition, a novel polyomavirus, related to the human JC virus, was identified in a baboon urine sample, albeit the relevance of this virus in immune reactivation is unclear.

For the EAE animals tested, only LCV appeared to exhibit reactivation in both control and FR104 treated groups, albeit the duration of the experiment was quite brief and given the AID (EAE) monitored it was not possible to maintain the animals in this condition for a long period. In this study, even though LCV viral levels increased in the blood, there was no evidence of virus in the brain tissue where EAE lesions are usually observed and high levels of LCV in spleen tissue were only observed in one animal. Other viruses were detectable in the spleen (LCV, SA12 and CMV) and brain (SV40) but did not appear to be significantly reactivated. Given the short duration of these experiments and because the levels in blood were extremely variable between animals, it is not possible to convincingly demonstrate if LCV is indeed truly reactivated in this model and poses a risk for use of FR104. This data is published (*Haanstra et al., J of Immunol, 2015*).

It was not possible to analyse any other viruses due to lack of sample material from WP2, Task 2.3. No samples with the exception of urine were provided from the CIA model. Levels of polyomavirus were assessed in these samples. The results were inconclusive.

For the tested DTH animals, no statistically significant reactivation of latent viruses present in the animals was observed over the time course of the experiment. Copy numbers varied over time for CMV and for HEV but showed no significant changes and no clinical symptoms were observed in the animals. HVP-2 is a ubiquitous virus related to human herpes simplex, and viral shedding is known to occur in the oral cavity (*Eberle et al., Lab Animal Sci 1998*). Variation in copy numbers over time were observed in saliva, however, no oral lesions or clinical observations were made suggesting that there is no significant change in viraemia in the presence or absence of FR104. Overall, reactivation of quiescent LCV has been observed to date in specific animals within the control group and treated groups, albeit, levels are considered low to moderate. Altogether, these results are suggestive that CD28 is required also for reactivation of T-dependent humoral memory responses *in vivo*, but selective CD28 blockade is not long-lasting as it did not induce immune regulation of T-dependent humoral memory response and did not disrupt preformed pathogen immunity.

The previous study in the EAE model demonstrated a more significant reactivation of LCV in both treated and placebo, similar was not observed in the DTH model (*Haanstra et al., J of Immunol, 2015*). In conclusion, reactivation of quiescent viruses did not appear to be significant in both models tested, with only LCV (EBV) demonstrating any increase in the EAE model, albeit this may have resolved over time.

3.3 - Malignancies

Objectives for INSERM A / CHU / BPRC were to monitor primate treated with FR104 and make sure that our innovative therapeutic strategy does not promote malignancies, such as lymphoma in AID preclinical models.

Our different observations in the DTH model, did not show any detection of malignancy induced by the injection of FR104 at 10 mg/Kg at d0 (single injection) as compared to the control group. Similarly, observations in macaques treated by FR104 in EAE model and CIA model at 10 mg/Kg respectively at d0, 7, 14 and 21 and at d0, 7, 14, 21, 28, 35, 42 did not show any malignancy neither in comparison to animals from respective control groups.

In conclusion, the different clinical observations and analyses of animals (baboons and macaques) did not show any malignancy induced by a single injection of FR104 (10 to 20 mg/Kg) or by several (4-7) injections (10 mg/Kg) in comparison to baboon and macaque control groups.

3.4 - Ethics

Objective of INSERM A / CHU was to monitor ethical documents related to animal studies. All participants who have developed animal studies in TRIAD, in WP1, WP2 as well as in WP3, obtained ethical agreements for their respective protocols from their national specific organisms in charge of the ethical evaluation.

It is to note that in France, regulatory ethical documents were not mandatory in 2012, when the TRIAD project started but finally came to application in the beginning of 2013, (European directive 2010/63/UE) and created some deceleration of the animal experimental project.

WP4 – Pharmaceutical Production

4.1 - Production of a FR104 batch

Since the purification process of Fab Intermediate and FR104 production is confidential information, it will not precisely be described here.

To summarize the objective of >50% yield of PEGylated FR104 is met allowing Effimune to follow the production of the technical batch, also named Pilot batch which will be used for the toxicology studies, and a cGMP batch for the clinical trials.

Hence, one technical batch (see annex) has been produced. from 130L batch, 362g of FR104 have been obtained. This batch has been used for the toxicological evaluation. The result of this evaluation is:

- No detection of drug-related event;
- Strong immunosuppression effect;
- No superagonist effect.

Then, the cGMP batch production from 200L batch corresponding to 200g of FR104 has been finalized at the end of December 2014. This product will be used for the Phase I clinical trials beginning in April 2015.

4.2 - Intravenous Formulation

The FR104 drug product obtained at the previous step has been formulated for an intravenous administration.

Then, the stability of this product has been studies during the program period. The Effimune FR104 drug product batch PD13175 was confirmed to be acceptably stable for nine months when stored at the intended storage temperature of +5 °C. From the study data, a shelf life in excess of twelve months could be expected for the product stored at the intended storage temperature of +5 °C following further real time studies.

WP5 – Pharmaceutical Development

Since the pharmaceutical development of FR104 production is confidential information, details will not precisely be given here.

5.1 - Fab' PEGylation

The aim of this task was to manufacture the PEGylated Fab' with 4 PEGylation reagents of different size and structure (y-shaped *vs* comb-shaped) and to purify and assess the resulting PEGylated Fab' antibodies.

In conclusion, the 4 PEG variants can be used. The choice of the lead PEG has been done after the PK/PD studies in mice and primates, as detailed also in task 5.2 "PK/PD in mice".

5.2 - PK/PD in mice

In order to choose the best PEGylation which maintains the activity of FR104, and increase the half-life of the molecule, the task 5.2 carried out experiences:

- To study the pharmacokinetics of the 4 PEG Fab'
- To evaluate the pharmacodynamics of the 4 PEG Fab'

In conclusion, PEGylation influences the distribution and the half-life of the molecule FR104, indeed, FR104 non-PEGylated (Fab Intermediate) had only a half-life of 1.9h in mice (not shown), whereas PEGylated products have a half-life 10 to 20 times longer. For information, we observed that more the bound PEG was large, more the half-life was increased.

The chosen PEG used for FR104 is a good choice of PEGylation reagent because it allows to retain a good anti-CD28 activity to the FR104 product and increase efficiently the half-life of FR104 product.

5.3 - Subcutaneous formulation

The investigation of the FR104 stability at different concentrations without or with surfactant showed that FR104 can be concentrated until **120 mg/mL** (**PEGylated molecule;** i.e. approx. 60 mg/mL protein concentration) without modifying activity and conformation of FR104 molecule. Concentration at 120 mg/mL changed the viscosity of product, but did not alter its anti-CD28 activity, and its conformation. Indeed, we obtain the same profile in SDS-PAGE or in GP-HPLC than FR104 non-concentrated, even after incubation of samples at + 4°C or room-temperature (25°C) for 20 days.

Concerning the administrative way, FR104 can be injected by subcutaneous way after concentration of FR104, because the same bioavailability propriety of FR104 has been obtained than by intravenous injection.

Moreover, Effimune has forecasted to test *in vivo* the subcutaneous formulation with the technical batch. However, since Effimune grants Janssen Biotech an exclusive option to develop FR104, Janssen has decided to carry out these tests.

WP6 – International Cooperation

The main aim of this TRIAD project of three years is to evaluate an antibody that block the costimulation pathway of T Lymphocytes. The studying antibody is FR104, an antagonist monoclonal humanized antibody anti-CD28, it's a PEGylated Fab' fragment. This molecule, that was developed by Effimune, binds with high affinity to CD28, thereby neutralizes its interaction with CD80/86 ligands, without delivering any activation signal to T cells. This molecule seems to be a major therapeutic tool to restore equilibrated immune balance required for peripheral tolerance. The latter is essential in case of auto-immune diseases like rheumatoid arthritis, multiple sclerosis, type I diabetes, psoriasis, uveitis and arteriosclerosis. The objective is to promote immune regulation, while preventing specifically pathogenic T lymphocyte activation.

One specification of the FP7 call is that the cooperation with Brazil was required. Thus, one partner of TRIAD consortium is Brazilian. To capitalize on the strong experience available in Europe in the field of immunology, the TRIAD project relies on the skills of the partner from Brazil to perform the efficacy study in mice models of uveitis.

In order to reflect the **bilateral BRA-EU cooperation**, bilateral exchange has been realized. Initially, training courses were expected on AID modelling in non-human primates (marmoset, macaque) and in the supporting immunological techniques that are used for investigating pathogenic mechanisms in the disease: To perform this task, BPRC had to host in his laboratory a Brazilian scientist to be trained on animal models. Because of administrative procedures, this exchange did not happen. However, a Brazilian fellow has been received in Oxford' lab. Inversely, a European fellow has been welcomed in the Brazilian laboratory in order to transfer the acquired know-how back to their laboratory.

EUmBRella supra-consortium (cooperation with 2 other FP7 projects having Brazilian partners, TIMER and TARKINAID projects) has organized different events:

- Joined meeting on January 2012 in Brussels (at the same time that the KOM);
- Workshop on the *Intellectual Property and technology transfer issues in Health Science and their impact on product development* at the global level on October 2012 in Florence (Italy);
- EUmBRella training course on September 2013 in Rio de Janeiro followed by the 11th world congress on Inflammation in Natal (Brazil);
- Joined meeting on *Course on latest developments in Inflammation research on November 2014* in Nantes (France).

In order to keep momentum and facilitate liaising EU and Brazil, each event had to be an opportunity to **cooperate with Brazilian authorities**. However, Brazilian authorities have attended at only the first joined meeting in Brussels.

1.4 Potential impact, Dissemination and Exploitation of results

The main objective of the project is the evaluation of a selective antagonist of CD28 to prevent, treat and/or cure some AIDs in preclinical models including rheumatoid arthritis, multiple sclerosis, type 1 diabetes, psoriasis, uveitis and arteriosclerosis.

A - Impacts of FR104 treatment as a whole

A.1 Societal impacts

The societal implications of this research are many and varied, including improving the treatment options available for patients who are diagnosed with an autoimmune disease (e.g. psoriasis and on the treatment of patients who receive a transplanted organ). This innovative treatment may have significant benefits to patients in terms of health, wellbeing and quality of life.

Patients may benefit in several ways: as the concept of this research is based not on suppressing the immune system as a whole, but rather only suppressing the parts of the immune system responsible for the autoimmune attack, while sparing and enhancing regulatory T cells this would mean that transplant recipients could experience a reduction in the unwanted side effects of the immunosuppressive regimes which are currently used. These serious side effects can include infection, cardiovascular disease and cancer. If patients were able to benefit from a more selective treatment, this could lead to better long term survival and an improved quality of life for patients, including a reduction in the amount of immunosuppressive drugs required. For patients, it may mean a significant reduction in the number of visits/admissions to hospital. Patients with other conditions such as rheumatoid arthritis, multiple sclerosis, type 1 diabetes, psoriasis and uveitis may benefit from this research and experience an improvement in their morbidity and mortality.

A.2 Economic and scientific impacts

The development of a new drug will also have significant scientific and economic implications for society. The impact of this research will allow the results to be translational and to lead from basic research into pre-clinical applications, again for the future benefit of the patient population.

Economic impacts could include a reduction in the cost of treating AID patients or after organ transplantation if selective immunosuppressive drugs are available, and a reduction in the cost and burden to healthcare providers of treating the side effects of the immunosuppressive regimen followed by patients including less need for drugs and other treatments and less time required in hospital. The success of this strategy could also impact treatments for the other models of disease being investigated; rheumatoid arthritis, multiple sclerosis, type 1 diabetes, psoriasis, uveitis and lead to similar health care cost reductions and savings.

Scientific impacts include advancement in our knowledge of how the immune system behaves; a greater understanding of how therapeutic strategies aimed at manipulating Treg function work and the development of novel therapeutics to help prevent, treat or cure a number of autoimmune diseases.

B- Dissemination

After the end of TRIAD project, every effort will be made to complete experiments, if needed be, and to disseminate the existence of the project, its progress and scientific results to as wide an audience as possible:

- Dissemination of knowledge within principal stakeholders
- Mention of the project EC funding for any publication, poster, and leaflet related to the TRIAD
- Mention of the project during related workshops, scientific meetings…
- Communication of the project internally and externally through a dedicated website.

After the IP potential and regulatory impact has been evaluated, data and results will be made public through the standard scientific community approaches: oral and poster presentation at local and international scientific meetings, publication in peer-reviewed journals ....

C- Specific features of partner

C.1 Partner 1: Effimune

Exploitable knowledge

- ✓ A successful therapy that reflects the relevance of the targeted process in the disease process: This could form the basis of further research and collaborative project for the academic partner.
- ✓ Preclinical results of efficacy of CD28 antagonists to prevent or treat some AID as well as potential new findings in mechanism of action of CD28 antagonist or role of Treg in AID: This has been and will be the subject of publications in peer-reviewed literature, poster and oral presentations at scientific conferences, inside meetings or pharma R&D sites. Also, it could be the beginning of future private-public partnership between SME and academic partners for further preclinical research and efficacy evaluation of other innovative therapeutic strategies developed by Effimune.
- ✓ Relevant results concerning directly a model for evaluation of selective CD28 antagonist and its therapeutic use: Foreground generated is the co-property of Effimune and the academic partner who develops this potential foreground IP. Worldwide & exclusive licences can be exploited by Effimune in the future.
- ✓ Newly generated know-how on the improvement of antibody pharmaceutical processes, and in particular for FR104 production. Foreground generated is the sole property of the Effimune. Effimune grants Janssen Biotech an exclusive option to develop FR104.

Other potential impact

- ✓ Valorisation of Effimune, which is a European SME, via the presentation or scientific publication fundamental and preclinical scientific results generated by TRIAD in each AID animal model in national/international research seminars, research workshops or congress on:
    - Role of costimulation, CD28+ lymphocytes and Treg in the pathogenesis of AID;

- Efficacy of immunoregulatory-intervention therapy based on the use of selective CD28 antagonist for the prevention or cure of several AID.

✓ Recruitment of an IP responsible in order to manage the FR104 IP exploitation, and of the other new molecules developed by Effimune

✓ Choice of rheumatoid arthritis application for the first clinical trial.

✓ Expanding the FR104 application: With the relevant results obtained in the AID model, Janssen Biotech could be interested in test other application

	Rheumatoid arthritis	Transplantation	Uveitis
Phase 1	**2015**	**2017**	**n/a**
Phase 2	**2016**	**2017**	**2017**
Phase 3	**2019**	**2021**	**2019**

✓ Potential commercialization of FR104 in case of successful results at the different clinical trial phases. This suggests return on investment by payment of royalties.

Dissemination

A manuscript is in submission to Nature Communication: “Selective CD28 antagonist blunts memory responses and promotes long-term control of skin inflammation in primates” in ***collaboration with INSERM A, GCU and CHU***.

Published manuscript

1. Poirier *et al.* Advantages of Papio anubis for preclinical testing of immunotoxicity of candidate therapeutic antagonist antibodies targeting CD28. mAbs 2014
   => ***Collaboration with INSERM A and CHU***
2. Dilek *et al.* Targeting CD28, CTLA-4 and PD-L1 costimulation differentially controls immune synapses and function of human regulatory and conventional T-cells. PLoS One. 2013 => ***Collaboration with INSERM A***
3. Mary *et al.* Antagonist properties of monoclonal antibodies targeting human CD28: Role of valency and the heavy-chain constant domain. mAbs 2013
   => ***Collaboration with INSERM A***
4. Poirier *et al.* Preclinical efficacy and immunological safety of FR104, an antagonist anti-CD28 monovalent Fab’ antibody. American Journal of Transplantation 2012
   => ***Collaboration with INSERM A***
5. Poirier *et al.* CD28-specific immuno-modulating antibodies: what can be learnt from experimental models? American Journal of Transplanatation 2012
   => ***Collaboration with INSERM A***

C.2 Partner 2: BPRC

Exploitable knowledge

✓ Reinforcement of the lead position in preclinical evaluation of biologics in non-human primates, in autoimmune diseases;

✓ The effect of FR104 both in the rhesus monkey EAE model and the rhesus monkey CIA model further validated these models as a useful model for T-cell directed therapies;

✓ The focus on research and technology development in the TRIAD project allowed for the development of qPCR technology for brain material and Luminex technology for cerebrospinal fluid as excellent tools for the evaluation of the effect of the FR104 treatment in particular and other immunosuppressive treatments in general;

- ✓ The use of collagen type II specific proliferation responses as a tool for the evaluation, in particular, of costimulation directed therapy;
- ✓ The development of screening methods for the effect of immunosuppressive therapy on Treg-cells.

Other potential impact

Acquisition of an international recognition, including Brazil.The focus of the TRIAD project on dissemination allowed us to present our work on a number of national and international congresses and to publish the results in international journals of high impact. This will further the role of the BPRC as a center of excellence for the testing of therapies in relevant animal models.

Dissemination

A manuscript is in submission to European Journal of Immunology: “Blockade of CD28 with a novel selective antagonist ameliorates Collagen-induced Arthritis in the Rhesus Monkey” in collaboration with Effimune.

Published manuscript

1. Haanstra *et al.* Selective blockade of CD28-mediated T cell costimulation protects rhesus monkeys against acute fatal experimental autoimmune encephalomyelitis. Journal of Immunology 2015 **=> Collaboration with Effimune and GCU**

C.3 Partner 3: University of Oxford

In Oxford, our aim has been to obtain proof of the therapeutic principle for the selective anti-CD28 antagonist FR104 in a model of Transplant Arteriosclerosis.

Exploitable knowledge

- ✓ A reproducible model of TA in a humanized mouse
- ✓ Evaluation of CD28 expression on human leukocytes in the humanised mouse model
- ✓ Examination of the effects of FR104 and CTLA-4 Ig in vivo
- ✓ Determination of the optimum treatment protocol for FR104 in humanized BALB/cRag2-/-cy-/- mice
- ✓ Examination of the effect of FR104 on the development of TA by determining the luminal occlusion
- ✓ A skin transplantation model as a surrogate for the TA model.
- ✓ Assessment of the ability of FR104 and CTLA4-Ig to prevent rejection of a human skin allograft in a PBMC-humanised mouse model
- ✓ Determination of the underlying mechanisms of the beneficial effect of FR104

Other potential impact

The societal implications of this research are many and varied, including improving the treatment options available for patients who are diagnosed with an autoimmune disease (e.g. psoriasis and on the treatment of patients who receive a transplanted organ). This innovant treatment may have significant benefits to patients in terms of health, wellbeing and quality of life.

The development of a new drug will also have significant scientific and economic implications for society. The impact of this research, and in particular, the humanised mouse

model will allow the results to be translational and to lead from basic research into pre-clinical applications, again for the future benefit of the patient population.

We will endeavour to exploit the results in various ways including:

- ✓ **Presentations and Conferences**
  We have presented the data at meetings and conferences, to both internal and external stakeholders, for instance at our group lab meetings and at Departmental research meetings and at the ESOT Basic Science Meeting in Paris in 2013. We plan to continue to exploit the results of the research in this way at forthcoming meetings wherever possible, and to as wide an audience as possible. Networking at professional meetings is also another informal method by which we will exploit the results and in developing collaborations with others where possible.
- ✓ **Website**
  We will post information about the results of the TRIAD project on our group website, with links to the main project website.
- ✓ **Publications**
  Where appropriate, our publications resulting from the work of the project are made "Open Access" to allow for the widest dissemination of the knowledge.
- ✓ **Personnel Mobility**
  We have already undertaken teaching and training of a student from Brazil which was very successful and allowed us to engage in knowledge transfer. Further knowledge transfer will take place when one of the key research personnel on the project returns to Japan and is able to engage with his colleagues in Japan and Asia.

Dissemination

A manuscript is in preparation: ‘‘Prevention of human skin rejection on humanised mice model by selective blockade of CD28 costimulatory signalling" in collaboration with Effimune.

Published manuscript

1. Ferrer *et al.* Induction of transplantation tolerance through regulatory cells: From mice to men. Immunology Review 2014
2. Goto *et al.* Delayed anti-CD3 therapy results in depletion of alloreactive T cells and the dominance of Foxp3+ CD4+ graft infiltrating cells. American Journal of Transplantation 2013 => ***Collaboration with INSERM B***
3. Chandrase-Kharan *et al.* Achieving operational tolerance in transplantation: how can lessons from the clinic inform research directions? Transplantation International 2013
4. Issa *et al.* The where and when of T cell regulation in transplantation. Trends in Immunology 2013
5. Kinnear *et al.* Costimulation blockade: current perspectives and implications for therapy. Transplantation 2013
6. Hester *et al.* Low-dose rapamycin treatment increases the ability of human regulatory T cells to inhibit transplant arteriosclerosis in vivo. American Journal of Transplantation 2012

C.4 Partner 4: INSERM

INSERM A

Exploitable knowledge

- ✓ A reproducible model of DTH in primates;
- ✓ Development of a novel immunotoxicology tool in the humanised mouse model:

- ✓ Development of a PK/PD tool in the primate:
- ✓ Expertise in *in vivo* preclinical non-invasive protocol in primate: PK/PD, DTH, Immune Safety that could be predictive of clinical success of innovative immunosuppressive drug candidates. Our model could early demonstrate the efficacy and the action mechanisms of the drug candidate in a test based in a cutaneous response in NHP to stimuli inducing Th-1 responses. This model is developed upstream of other more invasive preclinical models, like transplantation, and give us lot of information like receptor occupancy, the molecule half-life, phenotypic analyses and numeration of various leucocyte populations and molecule efficiency, leading to a good clinical predictability of the new drug candidate;
- ✓ Determination of the underlying mechanisms of the beneficial effect of FR104 at the cellular level.

Other potential impact

- ✓ International attractiveness has been reinforced**;**
- ✓ By this way, our academic lab represents a good potential partner with SME for further preclinical research and efficacy evaluation of other innovative therapeutic strategies.

Dissemination

A manuscript is in submission to Nature Communication: "Selective CD28 antagonist blunts memory responses and promotes long-term control of skin inflammation in primates" in ***collaboration with Effimune, GCU and CHU***.

Published manuscript

1. Poirier *et al.* Advantages of Papio anubis for preclinical testing of immunotoxicity of candidate therapeutic antagonist antibodies targeting CD28. mAbs 2014 **=>** ***Collaboration with Effimune and CHU***
2. Dilek *et al.* Targeting CD28, CTLA-4 and PD-L1 costimulation differentially controls immune synapses and function of human regulatory and conventional T-cells. PLoS One. 2013 => ***Collaboration with Effimune***
3. Mary *et al.* Antagonist properties of monoclonal antibodies targeting human CD28: Role of valency and the heavy-chain constant domain. mAbs 2013 => ***Collaboration with Effimune***
4. Poirier *et al.* Preclinical efficacy and immunological safety of FR104, an antagonist anti-CD28 monovalent Fab' antibody. American Journal of Transplantation 2012 => ***Collaboration with Effimune***
5. Poirier *et al.* CD28-specific immuno-modulating antibodies: what can be learnt from experimental models? American Journal of Transplanatation 2012 => ***Collaboration with Effimune***

INSERM B

Exploitable knowledge

- ✓ Determination of the *in vivo* effect of selective CD28 antagonists on diabetes development in NOD mice: no protection, exacerbation after a prolonged treatment;
- ✓ Determination of the *in vivo* effect of selective CD28 antagonists on syngeneic islet graft survival in NOD mice: no increase of graft survival;

- ✓ Determination of the *in vivo* effect of selective CD28 antagonists on Treg *versus* pathogenic T cells: perturbation of Foxp3+ Treg homeostasis but no significant impact on autoantigen-specific CD4+ and CD8+ T cells;
- ✓ Evaluation of CD28 expression on NOD mouse T cells: downregulated expression on CD8+ T cells, in particular autoantigen-specific CD8+ T cells infiltrating the pancreas as compared to CD4+ T cells and Treg;
- ✓ Assessment of the therapeutic effect of therapies combining CD28 blockade and rapamycin or tacrolimus: synergistic effect between PV1-PEG and rapamycin but not tacrolimus.

Other potential impact

- ✓ Our results showing a negative impact of CD28 blockade on Foxp3+ Treg cells suggest that caution shall be taken in the context of translation to the clinical arena. They also highlight the importance of the design of the protocol in terms of dosing and duration of treatment.
  Additionally, the PV1-PEG / Rapamycin combination opens new perspectives for the treatment of T1D patients.
- ✓ Results were regularly presented at internal lab meetings.

Dissemination

A manuscript is in preparation: “Therapeutic effect of selective CD28 antagonist in NOD mice”

C.5 Partner 5: IIEP

Exploitable knowledge

- ✓ A reproducible model of uveitis in the B10.RIII mouse developed in a AAALAC certified facility;
- ✓ The uveitis model perfected is similar to what happens in humans, with varied severity and a narrow window after onset where treatment is possible and may revert disease;
- ✓ Analyses of peripheral and infiltrating T cell subpopulations and tracking of regulatory lymphocytes in ophthalmological disease;
- ✓ Determination that the mechanisms involved in the actions of the surrogate PV1 CD28 antagonist occurs by IFNγ down-regulation without inducing Treg cells.

Other potential impact

The favorable result in the rodent model, which emulates the human disease, indicates that FR104 might be beneficial in the treatment of human autoimmune uveitis.

This was the first project developed at IIEP in collaboration with the EU and helped us to get in touch with EU research administrative procedures, and financial and scientific reporting.

Dissemination

A manuscript is in preparation: “Therapeutic effect of selective CD28 antagonist in experimental autoimmune uveitis”

Published manuscript

1. Papotto *et al.* Immunotherapeutic strategies in autoimmune uveitis. Autoimmunity Reviews 2014

Presentations and Conferences
We have presented the data at meetings and conferences, to both internal and external stakeholders, for instance at our group lab meetings, at 4th Lower Saxony International Summer Academy in Immunology held in Hannover, Germany, and at the 39th Congress of the Brazilian Society of Immunology 2014, held in Buzios, Brazil.

Website
The information regarding results of the TRIAD project is present on our website, with links to the main project website.

Personnel Mobility
As a result of the TRIAD project, our student Pedro Papotto has undertaken a training period at TRIG, Oxford University, supervised by Dr. Kathryn Wood which led us to significant knowledge transfer. This student is now currently doing his Ph.D. at Instituto de Medicina Molecular, in Lisbon, Portugal supervised by Dr. Bruno Silva-Santos at the Molecular Immunology Lab.

A Brazilian post-doc student Georgia Porto is developing work in INSERM's lab since June 2014.

C.6 Partner 6: GCU

Exploitable knowledge

- ✓ Evaluation of viral reactivation in NHP models for FR104 for which there has been limited data reported;
- ✓ Provision of tools for viral analysis in baboon, marmoset and macaque primate models;
- ✓ Identification of a novel JC like polyomavirus in baboon now available in Genbank KJ577598;
- ✓ Identification of novel herpesvirus sequence in marmoset.

Other potential impact

This work has provided detailed information on viral reactivation in non-human primate models which has not been presented before. Limited information was available previously on therapeutic molecules in this area, specifically in animal models, before implementation in clinical trial and the value of this work will allow informed trials to take place with specific monitoring relevant to the indicated viruses.

Presentations and Conferences
Attendance at European Society for Clinical Virology, Sept 28-30, 2014, Prague, (Czech Republic) by Paul Baker and poster presented entitled "Viral re-activation in autoimmune disease preclinical models after treatment with a selective antagonist of CD28".

Publications
It is intended that the novel herpesvirus sequences will be further identified and deposited to Genbank as a resource. It is also intended to finalise a brief communication reporting these sequences.

Dissemination

A manuscript is in submission to Nature Communication: "Selective CD28 antagonist blunts memory responses and promotes long-term control of skin inflammation in primates" in ***collaboration with INSERM A, Effimune and CHU***.

Published manuscript

1. Haanstra *et al.* Selective blockade of CD28-mediated T cell costimulation protects rhesus monkeys against acute fatal experimental autoimmune encephalomyelitis. Journal of Immunology 2015
   => Collaboration with Effimune and BPRC

C.7 Partner 7: CHU

Exploitable knowledge

- ✓ A reproducible model of DTH in primates;
- ✓ Development of a novel immunotoxicology tool in the humanised mouse model:
- ✓ Development of a PK/PD tool in the primate:
- ✓ Expertise in *in vivo* preclinical non-invasive protocol in primate: PK/PD, DTH, Immune Safety that could be predictive of clinical success of innovative immunosuppressive drug candidates. Our model could early demonstrate the efficacy and the action mechanisms of the drug candidate in a test based in a cutaneous response in NHP to stimuli inducing Th-1 responses. This model is developed upstream of other more invasive preclinical models, like transplantation, and give us lot of information like receptor occupancy, the molecule half-life, phenotypic analyses and numeration of various leucocyte populations and molecule efficiency, leading to a good clinical predictability of the new drug candidate;
- ✓ Determination of the underlying mechanisms of the beneficial effect of FR104 at the cellular level.

Other potential impact

- ✓ International attractiveness has been reinforced**;**
- ✓ By this way, our academic lab represents a good potential partner with SME for further preclinical research and efficacy evaluation of other innovative therapeutic strategies.

Dissemination

A manuscript is in submission to Nature Communication: “Selective CD28 antagonist blunts memory responses and promotes long-term control of skin inflammation in primates” in ***collaboration with Effimune, GCU and CHU***.

Published manuscript

1. Poirier *et al.* Advantages of Papio anubis for preclinical testing of immunotoxicity of candidate therapeutic antagonist antibodies targeting CD28. mAbs 2014 **=>** ***Collaboration with Effimune and INSERM A***

1.5 TRIAD project website

Project website address	**www.triad-cd28.eu**
Relevant contact	**Dr Bernard Vanhove**, Scientific Director of Effimune SAS
	Tel **+33 (0)2 40 41 28 34**
	Email **bvanhove@effimune.com**

2. USE AND DISSEMINATION OF FOREGROUND

2.1 Dissemination of the knowledge & communication (Section A)

Every effort has been made within the consortium to disseminate the existence of the project, its progress and scientific data to as wide an audience as possible. The following main rules have been followed:

- Dissemination of knowledge within principal stakeholders
- Mention of the project EC funding for any publication, poster, and leaflet related to the TRIAD
- Mention of the project during related workshops, scientific meetings…
- Communication of the project internally and externally through a dedicated website.

After the IP potential and regulatory impact has been evaluated, data and results will be made public through the standard scientific community approaches: publication in peer-reviewed journals (Table 2), oral and poster presentation at local and international scientific meetings,… (Table 3).

Table 2.
List of all scientific (peer reviewed) publications relating to the foreground of the project.
For the open access, visit the site **http://www.openaire.eu**

TABLE 2. LIST OF SCIENTIFIC (PEER REVIEWED) PUBLICATIONS										
No	**Title of Publication**	**Main author**	**Title (Periodical or Series)**	**Number, Date or Frequency**	**Publisher**	**Place of publication**	**Date of Publication dd/mm/yyy**	**Relevant pages**	**Permanent Identifiers[13] (if available)**	**Is/Will open Access[14] provided?**
Coming after the end of the project										
5	Therapeutic effect of selective CD28 antagonist in NOD mice	You S		In preparation						
4	Therapeutic effect of selective CD28 antagonist in experimental autoimmune uveitis	Rizzo LV		In preparation						
3	Prevention of human skin rejection on humanised mice model by selective blockade of CD28 costimulatory signaling	Zaitsu M, Issa F, Hester J, Vanhove B, Wood KJ		In preparation						
2	Selective CD28 antagonist blunts memory responses and promotes long-term control of skin inflammation in primates	Poirier N Chevalier M Mary C Hervouet J Minault D Baker P Ville S Le Bas-Bernardet S	Nature Communication	In submission						

		Dilek N Elarif L Cassagnau E Scobie L Blancho G Vanhove B								
1	Blockade of CD28 with a novel selective antagonist ameliorates Collagen-induced Arthritis in the Rhesus Monkey	Vierboom M, Breedveld E, Kap YS, Mary C, Poirier N, 't Hart BA, Vanhove B	European Journal of Immunology	In submission						
For the 2nd 18-months reporting period										
13	Selective blockade of CD28-mediated T cell costimulation protects rhesus monkeys against acute fatal experimental autoimmune encephalomyelitis	Haanstra K Dijkman K Bashir N Bauer J Mary C Poirier N Baker P Scobie L 't Hart B Vanhove B	Journal of Immunology	Feb15;194(4)	The American Association of Immunologists, Inc.	USA	15/02/2015	1454-1466	doi: 10.4049/jimmunol.1402563. Epub 2015 Jan 14.	After Jan2016
12	Immunotherapeutic strategies in autoimmune uveitis	Papotto PH Blini Marengo E Sardinha LR Goldberg AC Rizzo LV	Autoimmunity Reviews	Sep;13(9)	Elsevier	The Netherlands	12/05/2014	909-916	doi: 10.1016/j.autrev.2014.05.003. Epub 2014 May 12	Yes
11	Advantages of Papio anubis for preclinical testing of immunotoxicity of candidate therapeutic antagonist antibodies targeting CD28	Poirier N Mary C Le Bas-Bernardet S Daguin V Belarif L	mAbs	May-Jun;6(3)	Landes Bioscience	USA	05/03/2014	697-707	doi: 10.4161/mabs.28375. Epub 2014 Mar 5	After July 2015

		Chevallier M Hervouet J Minault D Ville S Charpy V Blancho G Vanhove B								
10	Induction of transplantation tolerance through regulatory cells: From mice to men	Ferrer IR Hester J Bushell A Wood KJ	Immunology Review	Mar;258(1)	John Wiley & Sons Ltd	USA	01/03/2014	102-116	doi: 10.1111/imr.12158. PMID:24517428	Yes
9	Targeting CD28, CTLA-4 and PD-L1 costimulation differentially controls immune synapses and function of human regulatory and conventional T-cells	Dilek N Poirier N Hulin P Coulon F Mary C Ville S Vie H Clémenceau B Blancho G Vanhove B	PLoS One	Dec 23;8(12)	PLOS	USA	23/12/2013	e83139 : 1-14	doi:10.1371/journal.pone.0083139	Yes
8	Delayed anti-CD3 therapy results in depletion of alloreactive T cells and the dominance of Foxp3+ CD4+ graft infiltrating cells	Goto R You S Zaitsu M Chatenoud L Wood KJ	American Journal of Transplantation	Jul;13(7)	Blackwell Publishing	USA	01/07/2013	1655-64	doi: 10.1111/ajt.12272. Epub 2013 Jun 10 PMID:23750800	Yes
7	Achieving operational tolerance in transplantation: how can lessons from the clinic inform research directions?	Chandrasekharan D Issa Fadi Wood KJ	Transplantation International	Jun;26(6)	John Wiley & Sons Ltd	USA	01/06/2013	576-589	doi: 10.1111/tri.12081. Epub 2013 Mar 21 PMID:23517251	Yes

For the 1st 18-months reporting period										
6	The where and when of T cell regulation in transplantation.	Issa F Robb RJ Wood KJ	Trends in Immunology	Mar;34(3)	Cell Press	UK	01/03/2013	107-13	doi: 10.1016/j.it.2012.11.003 PMID:23228885	Yes
5	Costimulation blockade: current perspectives and implications for therapy. Note that this doi is not valid for EC site	Kinnear G Jones ND Wood KJ	Transplantation	Feb 27;95(4)	Lippincott Williams and Wilkins	USA	27/02/2013	527-35	doi: 10.1097/TP.0b013e31826d4672	Yes Review – outside scope of OA
4	Antagonist properties of monoclonal antibodies targeting human CD28: Role of valency and the heavy-chain constant domain.	Mary C Coulon F Poirier N Dilek N Martinet B Blancho G Vanhove B	mAbs	Jan-Feb;5(1)	Landes Bioscience	USA	01/01/2013	47-55	doi: 10.4161/mabs.22697. Epub 2012 Dec 5.	Yes
3	Preclinical efficacy and immunological safety of FR104, an antagonist anti-CD28 monovalent Fab' antibody.	Poirier N Mary C Dilek N Hervouet J Minault D Blancho G Vanhove B	American Journal of Transplantation	Oct;12(10)	Blackwell Publishing	USA	01/10/2012	2630-40	doi: 10.1111/j.1600-6143.2012.04164.x. Epub 2012 Jul 3.	Yes
2	Low-dose rapamycin treatment increases the ability of human regulatory T cells to inhibit transplant arteriosclerosis in vivo.	Hester J Schiopu A Nadig SN Wood KJ	American Journal of Transplantation	Aug;12(8)	Blackwell Publishing	USA	01/08/2012	2008-16	doi: 10.1111/j.1600-6143.2012.04065.x	Yes
1	CD28-specific immuno-modulating antibodies: what	Poirier N Blancho G	American Journal of	July;12(7)	Blackwell Publishing	USA	01/07/2012	1682-90	doi: 10.1111/j.1600-	Yes

	can be learnt from experimental models?	Vanhove B	Transplantation						6143.2012.04032.x. Epub 2012 Apr 4.	

1 - A permanent identifier should be a persistent link to the published version full text if open access or abstract if article is pay per view) or to the final manuscript accepted for publication (link to article in repository).
2 - Open Access is defined as free of charge access for anyone via Internet. Please answer "yes" if the open access to the publication is already established and also if the embargo period for open access is not yet over but you intend to establish open access afterwards.

Table 3.
List of all dissemination activities (publications, conferences, workshops, web sites/applications, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters).

TABLE 3. LIST OF DISSEMINATION ACTIVITIES								
No	**Type of activities[15]**	**Main leader**	**Title**	**Date**	**Place**	**Type of audience[16]**	**Size of audience**	**Countries addressed**
For the 2nd 18-months reporting period								
24	Press release	Maryvonne Hiance	Two major scientific articles validate Effimune's drug candidate and reinforce the proof of concept of the technology aimed at regulating the immune system New hope for multiple sclerosis and kidney transplant patients	Feb 12, 2015	Nantes (France)	Specialized in health or not	n.a.	France and International
23	Poster (Academy of Medical Sciences Winter Meeting)	Fadi Issa	How can we prevent skin rejection?	Nov 17, 2014	London (UK)	Researchers, fellows, other scientists and members of the public		UK
22	Oral comm. (International Society for Neuroimmunology)	Krista Haanstra	Selective manipulation of the CD28 co-stimulation pathway prevents experimental autoimmune encephalomyelitis induction in the rhesus monkeys	Nov 9-13, 2014	Mainz (Germany)	Scientific Community	2 000	World wide
21	Poster (Congress of the Brazilian Society of Immunology 2014)	Pedro H. Papotto	mPEG PV1-Fab', a novel CD28 antagonist, mitigates experimental autoimmune uveitis progression through disruption of IFN-g production	Oct 18-22, 2014	Buzios (Brazil)	Scientific Community		Brazil

20	Poster (European Society for Clinical Virology)	Paul Baker	Viral re-activation in autoimmune disease preclinical models after treatment with a selective antagonist of CD28	Sept 28-30, 2014	Prague (Czech Republic)	Scientific Community	400	World wide
19	Oral comm. (CIMATH2 meeting)	Bernard Vanhove	New immunomodulation paths in transplantation and autoimmunity	Sept 22, 2014	Nantes (France)	Scientific Community	200	France
18	Poster (Lower Saxony International Summer Academy in Immunology)	Luiz Vicente Rizzo	mPEG PV1-Fab', a novel CD28 antagonist, mitigates experimental autoimmune uveitis progression through disruption of IFN-g production	Sept 1-14, 2014	Hannover (Germany)	Scientific Community		World wide
17	Oral comm. (World Transplant Congress)	Masaaki Zaitsu	Anti-CD28 antagonism using a monovalent Fab antibody for the prolongation of human skin allograft survival	July 26-31, 2014	San Francisco (USA)	Scientific Community	6 500	World wide
16	Press releases	Luiz Vicente Rizzo	Inovaçao e doenças autoimunes	Dec, 2013	São Paulo (Brazil)	Civil Society and Health Workers	n.a.	Brazil
15	Oral comm. (Dutch Multiple Sclerosis Society)	Krista Haanstra	Selective manipulation of the CD28 co-stimulation pathway prevents EAE induction in the rhesus monkey ⇨ Rewarded Best Presentation prize	Nov 27-29, 2013	Hasselt (Belgium)	Scientific Community	50	Netherlands & Belgium
14	Poster (ESOT Basic Science Meeting)	Masaaki Zaitsu	Prolongation of human skin allograft survival with FR104, an antagonistic anti-CD28 monovalent Fab antibody	Nov 7-9, 2013	Paris (France)	Scientific Community	150	World wide
13	Oral communication (American College	Michel Vierboom	Evaluation of Selective Manipulation of the CD28 Co-stimulation Pathway in the Rhesus Monkey Model of	Oct 25-30, 2013	San Diego (USA)	Scientific Community	12,000	World wide

	of Rheumatology)		Collagen-induced Arthritis					
12	Poster (Congress on Inflammation)	Pedro H. Papotto	CD28 mPEG PV1-Fab' Inhibits experimental autoimmune uveitis Progression in b10.RIII mice	Sept 21-25, 2013	Natal (Brazil)	Scientific Community	10,000	World wide
11	Oral comm. (Congress on Inflammation)	Stéphanie Le Bas-Bernardet	Preclinical evaluation of FR104, an antagonist anti-CD28 monovalent Fab' antibody, in a skin inflammatory DTH primate model	Sept 21-25, 2013	Natal (Brazil)	Scientific Community	10,000	World wide
10	Poster (Congress on Inflammation)	Bernard Vanhove	Preclinical efficacy and immunological safety of FR104, an antagonist anti-CD28 monovalent Fab' antibody, in humanized mice models	Sept 21-25, 2013	Natal (Brazil)	Scientific Community	10,000	World wide
9	Article in The Parliament Magazine	Maryvonne Hiance	FR104, a novel biologics investigated by the FP7 TRIAD consortium, keeps immune system in check	Nov 12, 2012	Dods	UK	na	Europe
8	Oral comm. (American Transplant Congress)	Caroline Mary	Monovalency and Fc domain isotype are key factors for preserving antagonist properties of anti-CD28 Mabs	June 2-6, 2012	Boston (USA)	Scientific audience	5,000	International congress
For the 1st 18-months reporting period								
7	Workshop	Bernard Vanhove	Intellectual Property and technology transfer issues in Health Science and their impact on product development	Oct 18-20, 2012	Florence (Italy)	Scientific Community	50	World wide
6	Website	Bernard Vanhove	Access to the TRIAD website	July 03, 2012	Nantes (France)	Scientific and other community	na	World wide

5	Oral comm. (American Transplant Congress)	Nicolas Poirier	Preclinical evaluation of FR104, an antagonist anti-CD28 monovalent Fab' antibody, in DTH and kidney transplant primates models	June 2-6, 2012	Boston (USA)	Scientific audience	5,000	International congress
4		Nicolas Poirier	Preclinical efficacy and immunological safety of FR104, an antagonist anti-CD28 monovalent Fab' antibody, in humanized mice models					
3	Press releases	Luiz Vicente Rizzo	Instituto do Einstein participa estudo sobre doenças autoimunes	May, 2012	São Paulo (Brazil)	Civil Society and Health Workers	n.a.	Brazil
2	Press releases	Luiz Vicente Rizzo	Instituto Israelita de Ensino e Pesquisa participa de estudo pré-clínico para nova droga contra doenças autoimunes	May, 2012	São Paulo (Brazil)	Civil Society and Health Workers	n.a.	Brazil
1	Press release	Maryvonne Hiance	Effimune with its lead drug candidate FR104, has been selected through the consortium TRIAD, under the European Union FP7 funding program	Feb13,2012	Nantes (France)	Specialized in health or not	n.a.	France and International

15 - A drop down list allows choosing the dissemination activity: publications, conferences, workshops, web, press releases, flyers, articles published in the popular press, videos, media briefings, presentations, exhibitions, thesis, interviews, films, TV clips, posters, Other.

16 - A drop down list allows choosing the type of public: Scientific Community (higher education, Research), Industry, Civil Society, Policy makers, Medias ('multiple choices' is possible).

Table 4.
Update of the initial plan of dissemination (Annex I)

Identified targets	Disseminating partners	Objectives of the dissemination	Dissemination channels	Comments
Scientific Community	All partners	Spread expertise and new knowledge on pathogenic role of T-cells & immune induced-regulation in inflammatory AID and results of efficacy as well as MoA of CD28 antagonists.	Regular scientific channels such as papers in peer-reviewed & high impact immunology journals, reviews	See Table 2 above
			Poster and oral presentations at international and national conferences/ workshops/ symposium on general immunology or autoimmunity	See Table 3 above
			Cooperation with national or international projects	See Section "WP6 – International cooperation"
			TRIAD website	**www.triad-cd28.eu**
Pharmaceutical sector	Selected project partners	Disseminate the new therapeutic strategy of CD28 antagonist, TRIAD results and findings Disseminate expertise and knowledge tailored to big pharma requirements	Papers in peer-reviewed literature and opinion papers in journals for the pharmaceutical industry	See Press release (Table 3) With TRIAD outside collaborations, the innovative therapeutic strategy of CD28 antagonist has been tested in other applications. See: Reply to "Biologics in organ transplantation". Vanhove B, **Azimzadeh A**. Transpl Int. 2013 Apr;26(4):e25

				doi: 10.1111/tri.12009
			Conferences, seminars and meeting at R&D sites of companies.	Effimune grants Janssen Biotech an exclusive option to develop FR104. Before this deal, several due diligences had been done.
			TRIAD website	**www.triad-cd28.eu**
Medical community	All partners	Create awareness of selective CD28 antagonist as potential therapy in autoimmune-inflammatory disorders	Papers in peer-reviewed and high impact journal for the medical community & reviews	Since Effimune grants Janssen Biotech an exclusive option to develop FR104, the awareness of selective CD28 antagonist as potential therapy in autoimmune-inflammatory disorders will be provided by Janssen Biotech themselves.
			Presentation at medical seminars and conferences specific to AID	
			TRIAD website	
Patient Association	All partners	Disseminate new knowledge on their disease and aware them on new therapy possibilities	Presentation at patient association meetings and press-release to journalistic community	No contact has been taken with patient association
			Lay book writing and leaflet	
			TRIAD website	
Regulatory Agency	EFFI	Spread TRIAD results of efficacy and safety	Presentation to regulatory agency (*AFSSAPS, EMEA, and FDA*) of preclinical results to prevent, treat or cure selected AID with monovalent selective CD28 antagonist before clinical trials	Effimune has followed the regulatory process and obtain the approval of the regulatory agencies in order to begin the Phase I clinical trial in April 2015

2.2 Exploitation of the foreground (Section B)

2.2.1 Patents, trademarks, registered designs

Note that no patents, trademarks, registered designs has been provided during TRIAD project.

Table 5.
Applications for patents, trademarks, registered designs, etc…

TABLE 5. LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS, ETC.						
No	**Type of IP rights[2]**	**Confidential (Yes/No)**	**Foreseen embargo date (dd/mm/yyyy)**	**Application reference(s)**	**Subject or title of application**	**Applicant (s) (as on the application)**
Ex	*Patent*	*Yes*	*24 nov 2014*	*EP123456*		
4	**Not applicable**					

1 - Note to be confused with the "EU CONFIDENTIAL" classification for some security research projects.
2 - A drop down list allows choosing the type of IP rights: Patents, Trademarks, Registered designs, Utility models, Others.

2.2.2 Exploitable foreground

The general assembly (GA) had the responsibility for reviewing the scientific progress of the TRIAD project and for identifying the Foreground IP developed in the project. Then, the GA should assess the value of the potential foreground IP and the optimum route for its protection and subsequent dissemination and/or exploitation. Main expected foreground could be listed in the table 6 below.

Table 6.
Exploitable foreground and plans for exploitation.

TABLE 6. EXPLOITABLE FOREGROUND AND PLANS FOR EXPLOITATION.									
No	**Type of Exploitable Foreground[1]**	**Description of exploitable foreground**	**Confidential (Yes/No)**	**Foreseen embargo date (dd/mm/yyyy)**	**Exploitable product(s) or measure(s)**	**Sector(s) of application[2]**	**Timetable, commercial or any other use**	**Patents or other IPR exploitation (licences)**	**Owner & Other Beneficiary(s) involved**
Ex.		*Ex: New superconductive Nb-Ti alloy*			*MRI equipment*	*1. Medical 2. Industrial inspection*	*2008 2010*	*A materials patent is planned for 2006*	*Beneficiary X (owner) Beneficiary Y, Beneficiary Z, Poss. licensing to equipment manuf. ABC*
1	General advancement of knowledge	New findings in mechanism of action of CD28 antagonist or role of Treg in AID	No		Results on CD28 mechanism of action and Treg role in AID	Scientific research			Effimune
2	General advancement of knowledge	Improvement of pharmaceutical processes for FR104 production	**Yes (Production process)**		cGMP batch of FR104	Pharmaceutical industry			Sole property of Effimune, exploiting Worldwide & exclusive licences
3	Commercial exploitation of R&D results	Successful therapy that reflects the relevance of the targeted process in the disease process	No		CGMP batch of FR104	Pharmaceutical industry	April 2015: Phase I clinical trial	Exclusive option to develop FR104 granted by Effimune to Janssen Biotech	Effimune

4	Exploitation of results through innovation	Preclinical results of efficacy of CD28 antagonists to prevent or treat some AID	No		Preclinical results of efficacy of CD28 antagonists	Pharmaceutical research			Effimune INSERM A
5	Exploitation of results through innovation	Improvement in animal models	No		T1 diabete Uveitis DTH CIA EAE Skin allograft	Medical research			The animal model is the unique property of the academic stakeholder partner who will exploit it for further research. Collaborative project can be set up according to Janssen Biotech
6	Exploitation of results through innovation	Positive results concerning directly a model for evaluation of selective CD28 antagonist and its therapeutic use: exploited by EFFI	**Yes**	Up to the publication of the results	Combination of FR104 with Rapamycin or Tacrolimus	Pharmaceutical research			Co-property of EFFI and the academic stakeholder partner who develops this potential foreground IP ⇨ Case of INSERM B
7	Exploitation of results through innovation	Improvment of tools for the evaluation of the effect of immunosuppressive treatments (incl. FR104)	No		1. Development of qPCR technology for brain material and Luminex technology for cerebrospinal fluid 2. Screening methods for the effect of immunosuppressive therapy on regulatory T-cells.	Biotechnology			Unique property of the academic partner who will exploit it for further research ⇨ Case of BPRC

					3. Use of collagen type II specific proliferation responses as a tool for the evaluation of costimulation directed therapy				
8	Exploitation of results through innovation	Improvment of immunological tools: or collaborative project	No		Detection of macaque viruses	Biotechnology			Unique property of the academic partner who will exploit it for further research ⇨ Case of GCU

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1 - A drop down list allows choosing the type of foreground: General advancement of knowledge, Commercial exploitation of R&D results, Exploitation of R&D results via standards, exploitation of results through EU policies, exploitation of results through (social) innovation.

2 - A drop down list allows choosing the type sector (NACE nomenclature) : http://ec.europa.eu/competition/mergers/cases/index/nace_all.html

2.2.3 Exploitation of results and societal impact

a) Explorative studies

Data obtained from the type 1 diabetes and uveitis autoimmune models provided key insights into the therapeutic effect and *in vivo* mode of action of selective CD28 antagonists, which may be "disease-dependent". Indeed, the partial but significant efficacy of PV1-PEG to reduce uveitis severity and score suggests that specific CD28 blockade could be considered as a promising strategy for the treatment of autoimmune disorders in the eye, which, so far, relies on global immunosuppression associated with a number of undesirable side effects.

In contrast, CD28 antagonist monotherapy was not efficient in the T1D mouse model. However, the fact that combination of PV1-PEG to the immunosuppressive drug rapamycin could prevent from diabetes development may argue for considering this immune intervention approach as a potential treatment of type 1 diabetes as well as other human autoimmune diseases.

In the context on clinical translation, our findings raised concerns about the potential negative impact on selective CD28 antagonists on $Foxp3^+$ Treg cells when prolonged *in vivo* treatment is applied (such effect was not observed *in vitro*). Therefore, our results call for caution and recommend further investigations to precisely assess the impact of blocking CD28 on Treg cells.

Finding a cure for autoimmune diseases is a real health medical challenge since their incidence steadily increases in developed countries. Treatments are either palliative or use immunosuppressive drugs, are associated with major constraints, side effects and lack of effectiveness over long term. In this difficult context, our approach based on short-term course of selective CD28 antagonist monotherapy or biotherapy with rapamycin may provide a promising alternative for a better disease management and quality of life of patients presenting autoimmune diseases.

b) Preclinical studies

UOXF

In Oxford, our aim has been to obtain proof of the therapeutic principle for the selective anti-CD28 antagonist FR104 in a model of Transplant Arteriosclerosis.

The societal implications of this research are many and varied, including improving the treatment options available for patients who are diagnosed with an autoimmune disease where this new treatment may have a real impact on their health and wellbeing. The development of a new drug will also have significant scientific and economic implications for society.

Patients may benefit in several ways: as the concept of this research is based not on suppressing the immune system as a whole, but rather only suppressing the parts of the immune system responsible for the autoimmune attack, while sparing and enhancing regulatory T cells this would mean that transplant recipients could experience a reduction in the unwanted side effects of the immunosuppressive regimes which are currently used. These serious side effects can include infection, cardiovascular disease and cancer. If patients were able to benefit from a more selective treatment, this could lead to better long term survival and an improved quality of life for patients, including a reduction in the amount of immunosuppressive drugs required. For patients, it may mean a significant reduction in the number of visits/admissions to hospital. Patients with other conditions such as rheumatoid arthritis, multiple sclerosis, type 1 diabetes, psoriasis and uveitis may benefit from this research and experience an improvement in their morbidity and mortality.

Economic impacts could include a reduction in the cost of treating patients after organ transplantation if selective immunosuppressive drugs are available, and a reduction in the cost and burden to healthcare providers of treating the side effects of the immunosuppressive regimen followed by patients including less need for drugs and other treatments and less time required in hospital. The success of this strategy could also impact treatments for the other models of disease being investigated; rheumatoid arthritis, multiple sclerosis, type 1 diabetes, psoriasis, uveitis and lead to similar health care cost reductions and savings.

Scientific impacts include advancement in our knowledge of how the immune system behaves; a greater understanding of how therapeutic strategies aimed at manipulating Treg function work and the development of novel therapeutics to help prevent, treat or cure a number of autoimmune diseases.

GCU

At GCU, the objectives were to analyse the viral status of primates treated with FR104 and make sure that the consortium's innovative therapeutic strategy did not promote re-activation of quiescent viruses in AID preclinical models. This work has provided detailed information on viral reactivation in non-human primate models which had not been presented before. Limited information was available previously on therapeutic molecules in this area, specifically in animal models, before implementation in clinical trial, and the value of this work will allow informed trials to take place with specific monitoring relevant to the indicated viruses.

3. REPORT ON SOCIETAL IMPLICATIONS

A. General Information

*Completed automatically when **Grant Agreement number** is entered.*

Grant Agreement Number:	**FP7-281493**
Title of Project:	**Tolerance Restoration In Autoimmune Diseases by selective manipulation of the CD28 costimulatory pathway**
Name and Title of Coordinator:	**Dr Bernard Vanhove - Effimune**

B. Ethics

1. Did your project undergo an Ethics Review (and/or Screening)? • If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final project reports? Special Reminder: the progress of compliance with the Ethics Review/Screening Requirements should be described in the Period/Final Project Reports under the Section 3.2.2 *'Work Progress and Achievements'*	**No**
2. Please indicate whether your project involved any of the following issues (tick box)	***Yes/No***
Research on Humans	
• Did the project involve children?	**No**
• Did the project involve patients?	**No**
• Did the project involve persons not able to give consent?	**No**
• Did the project involve adult healthy volunteers?	**Yes**
• Did the project involve Human genetic material?	**No**
• Did the project involve Human biological samples?	**Yes**
• Did the project involve Human data collection?	**No**
Research on Human embryo/foetus	
• Did the project involve Human Embryos?	**No**
• Did the project involve Human Foetal Tissue / Cells?	**No**
• Did the project involve Human Embryonic Stem Cells (hESCs)?	**No**
• Did the project on human Embryonic Stem Cells involve cells in culture?	**No**
• Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?	**No**
Privacy	
• Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?	**No**
• Did the project involve tracking the location or observation of people?	**No**
Research on Animals	
• Did the project involve research on animals?	**Yes**
• Were those animals transgenic small laboratory animals?	**Yes**
• Were those animals transgenic farm animals?	**No**
• Were those animals cloned farm animals?	**No**
• Were those animals non-human primates?	**Yes**

Research Involving Developing Countries	
• Did the project involve the use of local resources (genetic, animal, plant etc)?	**No**
• Was the project of benefit to local community (capacity building, access to healthcare, education, etc)?	**No**
Dual Use	
• Research having direct military use	**No**
• Research having the potential for terrorist abuse	**No**

C. Workforce Statistics

3. Workforce statistics for the project:
Please indicate in the table below the number of people who worked on the project (on a headcount basis).

Type of Position	Number of Women	Number of Men
Scientific Coordinator	**0**	**1**
Work package leaders	**3**	**3**
Experienced researchers (i.e. PhD holders)	**12**	**10**
PhD Students	**1**	**1**
Other	**9**	**4**
4. How many additional researchers (in companies and universities) were recruited specifically for this project?		**4**
Of which, indicate the number of men:		**3**

D. Gender Aspects

5.	**Did you carry out specific Gender Equality Actions under the project**?	**X** ○	**Yes** No

6. Which of the following actions did you carry out and how effective were they?

		Not at all effective				Very effective
❑	Design and implement an equal opportunity policy	○	○	**X**	○	○
❑	Set targets to achieve a gender balance in the workforce	○	○	**X**	○	○
❑	Organise conferences and workshops on gender	**X**	○	○	○	○
❑	Actions to improve work-life balance	○	○	**X**	○	○
○	Other:					

7. Was there a gender dimension associated with the research content – i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?

○ Yes- please specify

X No

E. Synergies with Science Education

8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?

○ Yes- please specify

X No

9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?

○ Yes- please specify

X No

F. Interdisciplinarity

10. Which disciplines (see list below) are involved in your project?

1.5 Main discipline[1]: **Biological sciences**

3.1 Associated discipline[1]: **Basic medicine** | **3.2** Associated discipline[1]: **Clinical medicine**

[1] Insert number from list below (Frascati Manual).

G. Engaging with Civil society and policy makers

11a	**Did your project engage with societal actors beyond the research community?** *(if 'No', go to Question 14)*	○ **X**	Yes **No**

11b **If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?**

- ○ No
- ○ Yes- in determining what research should be performed
- ○ Yes - in implementing the research
- ○ Yes, in communicating /disseminating / using the results of the project

11c	**In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?**	○ ○	Yes No

12. **Did you engage with government / public bodies or policy makers (including international organisations)**

- ○ No
- ○ Yes- in framing the research agenda
- ○ Yes - in implementing the research agenda
- ○ Yes, in communicating /disseminating / using the results of the project

13a **Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?**

- ○ Yes – as a **primary** objective (please indicate areas below- multiple answers possible)
- ○ Yes – as a **secondary** objective (please indicate areas below - multiple answer possible)
- ○ No

13b **If Yes, in which fields?**

Agriculture		Energy		Human rights	
Audiovisual and Media		Enlargement		Information Society	
Budget		Enterprise		Institutional affairs	
Competition		Environment		Internal Market	
Consumers		External Relations		Justice, freedom and security	
Culture		External Trade		Public Health	
Customs		Fisheries and Maritime Affairs		Regional Policy	
Development Economic and Monetary Affairs		Food Safety		Research and Innovation	
Education, Training, Youth		Foreign and Security Policy		Space	
Employment and Social Affairs		Fraud		Taxation	
		Humanitarian aid		Transport	

13c	**If Yes, at which level?** ○ Local / regional levels ○ National level ○ European level ○ International level

H. Use and dissemination

14	**How many Articles were published/accepted for publication in peer-reviewed journals?**	**13**
	To how many of these is open access[2] provided?	
	How many of these are published in open access journals?	**13**
	How many of these are published in open repositories?	**13**
	To how many of these is open access not provided?	**0**
	Please check all applicable reasons for not providing open access:	
	❑ publisher's licensing agreement would not permit publishing in a repository ❑ no suitable repository available ❑ no suitable open access journal available ❑ no funds available to publish in an open access journal ❑ lack of time and resources ❑ lack of information on open access ❑ other[3]: ……	
15.	**How many new patent applications ('priority filings') have been made?** *("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).*	**0**
16.	**Indicate how many of the following Intellectual Property Rights were applied for (give number in each box).**	Trademark: **0** Registered design: **0** Other: **0**
17.	**How many spin-off companies were created / are planned as a direct result of the project?**	**0**
	Indicate the approximate number of additional jobs in these companies:	

18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:

❑	Increase in employment, or	❑	In small & medium-sized enterprises
❑	Safeguard employment, or	❑	In large companies
❑	Decrease in employment,	**X**	**None of the above / not relevant to the project**
X	**Difficult to estimate / not possible to quantify**		

[2] Open Access is defined as free of charge access for anyone via Internet.
[3] For instance: classification for security project.

19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (*FTE = one person working fulltime for a year*) jobs:	*Indicate figure:* **18**
Difficult to estimate / not possible to quantify	❑

I. Media and Communication to the general public

20. As part of the project, were any of the beneficiaries professionals in communication or media relations?

○ Yes **X No**

21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?

○ Yes **X No**

22 Which of the following have been used to communicate information about your project to the general public, or have resulted from your project?

X	**Press Release**	**X**	**Coverage in specialist press**
❑	Media briefing	❑	Coverage in general (non-specialist) press
❑	TV coverage / report	❑	Coverage in national press
❑	Radio coverage / report	❑	Coverage in international press
X	**Brochures /posters / flyers**	**X**	**Website for the general public / internet**
❑	DVD /Film /Multimedia	❑	Event targeting general public (festival, conference, exhibition, science café)

23 In which languages are the information products for the general public produced?

❑	Language of the coordinator	**X**	**English**
❑	Other language(s)		

***Question F-10*:** Classification of Scientific Disciplines according to the Frascati Manual 2002 (Proposed Standard Practice for Surveys on Research and Experimental Development, OECD 2002):

FIELDS OF SCIENCE AND TECHNOLOGY

1. NATURAL SCIENCES

- 1.1 Mathematics and computer sciences [mathematics and other allied fields: computer sciences and other allied subjects (software development only; hardware development should be classified in the engineering fields)]
- 1.2 Physical sciences (astronomy and space sciences, physics and other allied subjects)
- 1.3 Chemical sciences (chemistry, other allied subjects)
- 1.4 Earth and related environmental sciences (geology, geophysics, mineralogy, physical geography and other geosciences, meteorology and other atmospheric sciences including climatic research, oceanography, vulcanology, palaeoecology, other allied sciences)
- 1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences)

2 ENGINEERING AND TECHNOLOGY

- 2.1 Civil engineering (architecture engineering, building science and engineering, construction engineering, municipal and structural engineering and other allied subjects)
- 2.2 Electrical engineering, electronics [electrical engineering, electronics, communication engineering and systems, computer engineering (hardware only) and other allied subjects]

2.3. Other engineering sciences (such as chemical, aeronautical and space, mechanical, metallurgical and materials engineering, and their specialised subdivisions; forest products; applied sciences such as geodesy, industrial chemistry, etc.; the science and technology of food production; specialised technologies of interdisciplinary fields, e.g. systems analysis, metallurgy, mining, textile technology and other applied subjects)

3. MEDICAL SCIENCES

3.1 Basic medicine (anatomy, cytology, physiology, genetics, pharmacy, pharmacology, toxicology, immunology and immunohaematology, clinical chemistry, clinical microbiology, pathology)

3.2 Clinical medicine (anaesthesiology, paediatrics, obstetrics and gynaecology, internal medicine, surgery, dentistry, neurology, psychiatry, radiology, therapeutics, otorhinolaryngology, ophthalmology)

3.3 Health sciences (public health services, social medicine, hygiene, nursing, epidemiology)

4. AGRICULTURAL SCIENCES

4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects)

4.2 Veterinary medicine

5. SOCIAL SCIENCES

5.1 Psychology

5.2 Economics

5.3 Educational sciences (education and training and other allied subjects)

5.4 Other social sciences [anthropology (social and cultural) and ethnology, demography, geography (human, economic and social), town and country planning, management, law, linguistics, political sciences, sociology, organisation and methods, miscellaneous social sciences and interdisciplinary , methodological and historical S1T activities relating to subjects in this group. Physical anthropology, physical geography and psychophysiology should normally be classified with the natural sciences].

6. HUMANITIES

6.1 History (history, prehistory and history, together with auxiliary historical disciplines such as archaeology, numismatics, palaeography, genealogy, etc.)

6.2 Languages and literature (ancient and modern)

6.3 Other humanities [philosophy (including the history of science and technology) arts, history of art, art criticism, painting, sculpture, musicology, dramatic art excluding artistic "research" of any kind, religion, theology, other fields and subjects pertaining to the humanities, methodological, historical and other S1T activities relating to the subjects in this group]

4. FINAL REPORT ON THE DISTRIBUTION OF THE EUROPEAN UNION FINANCIAL CONTRIBUTION

For the coordinator:

This report shall be submitted to the Commission within 30 days after receipt of the final payment of the European Union financial contribution.

Table x: Report on the distribution of the European Union financial contribution between beneficiaries

Name of beneficiary	Final amount of EU contribution per beneficiary in Euros
1. Effimune	
2. BPRC	
3. Univ. Oxford	
4. INSERM	
5. IIEP	
6. GCU	
7. CHU Nantes	
Total	