PROJECT FINAL REPORT

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FINAL REPORT

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Final publishable summary report

- Executive summary

The SADEL consortium, which stands for "Scaffolds for Alternative DELivery", initiated a collaboration to develop oral-formulation of a Nanofitin®-based drug in Inflammatory Bowel Diseases (IBD). The two main phenotypes of IBD, Crohn's Disease (CD) and Ulcerative Colitis (UC), are chronically relapsing intestinal inflammatory conditions with a typical onset in young adulthood and with an unpredictable disease course that may lead to debilitating complications. The clinically validated target chosen by the Consortium is TNFα, a cytokine considered a key regulator of immune cells and involved in systemic inflammation. The objective of the SADEL project is to advance existing Nanofitins® hits against TNFα towards the preparation of Phase I Clinical trials in UC.

Nanofitins® constitute a new class of non-antibody affinity ligands able to selectively bind a wide range of targets. As an addition to their antibody-like properties, Nanofitins® have a small size (optimal tissue penetration), pH resistance (favorable stomach passage), resistance to human intestinal fluids (long half-life in digestive track) and high affinity (low effective concentration needed). They also demonstrate strong potential for optimizing pharmacological properties, including reducing immunogenicity.

Consequently, the SADEL project is designed to address unmet technical challenges by making optimal use of the Nanofitins® protein scaffold. The Nanofitins®-based drugs will progress through routes not travelled by antibodies while interacting with targets not modulated by chemical compounds. They will be administered orally, reducing the systemic exposure and avoiding the safety issues reported with systemic administration of antibodies. This requires large quantities of proteins for frequent administration, which is compatible with Nanofitins® manufacturing in bacterial systems, broadly adopted in the industry with a low cost of goods.

To achieve this mission, the project is assembling the key competencies along the drug development value chain : engineering and optimization of the product (Affilogic), analytics, control and pre-formulation (University of Geneva), process development and GMP manufacturing (iBET and Genibet), preclinical testing (Intestinal Biotech Development and Fleming Research Institute), clinical oversight (European Crohn and Colitis Organization) and industrialization capability (Ferring International SA). This group is managed by a professional project team (Innov'Hub) and is ideally equipped to move the Nanofitins® from concept to product not only within the framework of the project but also beyond SADEL.

- A summary description of project context and objectives

A need for new targeted therapies compatible with oral administration. Efforts made in understanding disease mechanisms allowed the identification of specific therapeutic targets, such as cytokines, that are involved in many biological cascades (immune response, inflammation…) by the mean of their interaction with a specific receptor or partner. All these cascades are established by a fine and tight balance between the different cytokines. Disregulation (up- or down-regulation) of the balance may result in a particular disease phenotype. In the case of an up-regulation, significant improvements in diseases management were made possible by the use of therapeutic antibodies capable of neutralizing a define cytokine, as opposed to traditional medicines having a broader spectrum of action and so potential side-effects.

Although antibodies have provided a breakthrough in the therapeutic world, they are injectable only which remains a burden for patients that have to suffer multiple injections (especially for chronic diseases) and may lead to treatment dropout. An oral formulation would greatly improve patient compliance, but the hostile environment of the gut (acidic pH of the stomach, high content in proteases that degrade proteins) restricts the oral bioavailability of conventional biologics such as antibodies. This limitation becomes particularly critical for diseases localized in the gut like intestinal bowel diseases (IBD, whose Crohn's disease and ulcerative colitis are the two main phenotypes), which would benefit from a targeted therapy at the very site of the disease (Figure 1). An oral delivery would drastically reduce the systemic exposure of the therapeutic and largely avoids the known safety issues reported with systemic administration of targeted therapies in these indications (e.g. anti-TNFα monoclonal antibodies).

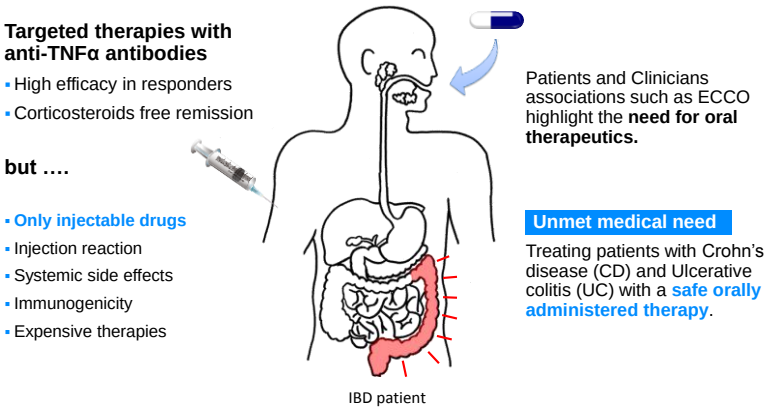


Figure 1: Highlight of the need for need for targeted therapeutics that can be administered orally in IBD

The Nanofitin technology as an orally deliverable alternative to antibodies. The Nanofitin (NF) technology is based on the Sac7d protein scaffold from the hyperthermophilic *Sulfolobus acidocaldarius* (Figure 2). This bacteria was found in the sulfur-rich hot acid springs in Yellowstone National Park (85°C and pH 2). Sac7d is a small protein composed of 66 amino acids (7 kDa), naturally able of molecular recognition. Engineering of the Nanofitin scaffold by randomly substituting the 14 amino acids that composed the natural binding surface allows the generation of libraries of Nanofitin mutants. Each of these mutants will exhibit a specific combination of the 14 position at the binding surface that can be compatible with a specific target, following the key-lock principle. So far, these libraries allowed the identification of series of high affinity (pM to nM range) binders against all the different protein targets tested (> 50). The large majority of protein binding NFs keep the favourable biophysical properties of the wild type Sac7d, in particular a very high resistance to temperature (Tm>70°C), stability under a broad range of pH (including stomach acidic conditions) and high resistance to degradation in human stomach, small intestine and large intestine conditions.

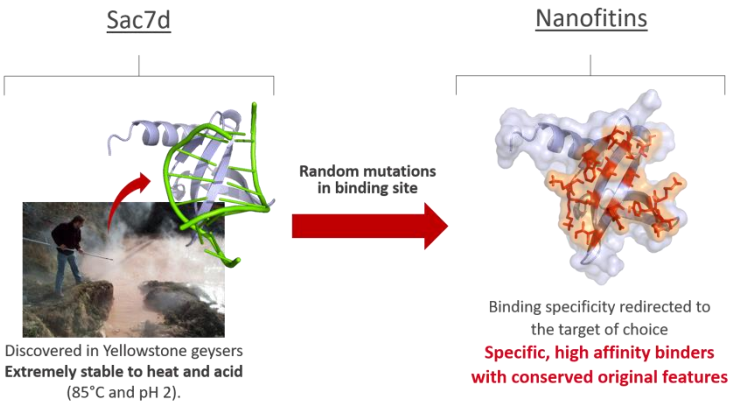


Figure 2: From natural proteins to efficient and hyperstable Nanofitin therapeutics. Structure of sac7d obtained from the pdb 1azp (Robinson et al., Nature 1998)

The SADEL consortium radically chooses to make optimal use of the strengths of this new scaffold. As an addition to their antibody-like properties, Nanofitins have a small size (optimal tissue penetration), pH resistance (favourable stomach passage), resistance to human intestinal fluids (long half-life in digestive track) and high affinity (low effective concentration). Consequently, SADEL proposed the development of Nanofitin therapeutics to solve the unmet technical need in medicines to tackle disease targets present in the digestive tract with a biological. These targets can be present on the surface of digestive tract epithelium (e.g. receptors), in the lumen (e.g. bacteria, peptides), or even at the basal side of the epithelium in case the mucosa is damaged (e.g. cytokines, receptors). To avoid adding risks on top of those related to the innovative scaffold behaviour itself, the consortium consciously decided to engage on a clinically validated target (TNFα) for the ulcerative colitis (UC) indication to provide the clinical Proof Of Concept (POC) of the technology.

The rational of the consortium was as follow:

- There is definitely an unmet medical need to treat UC patients with a safe orally applied drug.
- There is a very solid business case to choose for this disease.
- The disease is localised at the surface of the gut lining and does not penetrate deep in the gut lining. So, the topical administration of our product has a good chance for success. Actually, pre-clinical data confirm that orally applied anti-TNF blockers are able to reduce the inflammation parameters of colitis in mice.
- The disease presents itself in 95% of cases in the rectum, which is easier to target with oral formulation than a disease which is spread over the entire intestine. On top of that, in case the project fails to develop an oral formulation, there will still be the option to develop an enema formulation for UC based on our product.
- The lead target in the program is human TNFα

Ahead of the beginning of the SADEL programme, Affilogic had already identified anti-TNFα NFs ready to be introduced in the project. The NFs show high affinity for human TNFα and cross reactivity with mouse TNFα, enabling the use of both gold standard and specialised (genetic) wild type mouse models, as well as more predictive humanised models. Affilogic showed that different epitopes on TNFα could be targeted by different Nanofitins, allowing for multiple combinations and a variety of potential mechanisms of action. The NFs have the capacity to be further formatted, i.e. engineered to match the requirement of a pharmaceutical drug.

A unique consortium organisation. To carry the project over, SADEL assembled as a midsized biotech company gathering team task force with the key expertise necessary to streamline the development of anti-

TNFα Nanofitins from the bench to the clinic (Figure 3). The work packages were designed like departments in the virtual company. The selected work package leaders have the skills of senior directors of a biotech department, with input from scientific excellence of the partners, academic and private, as "internal" R&D experts. As in a matrix-structured company the project moved between the work packages, guided by the Steering Group acting as project leader under chairmanship of Affilogic, the partner responsible for development of the Nanofitin scaffold technology.

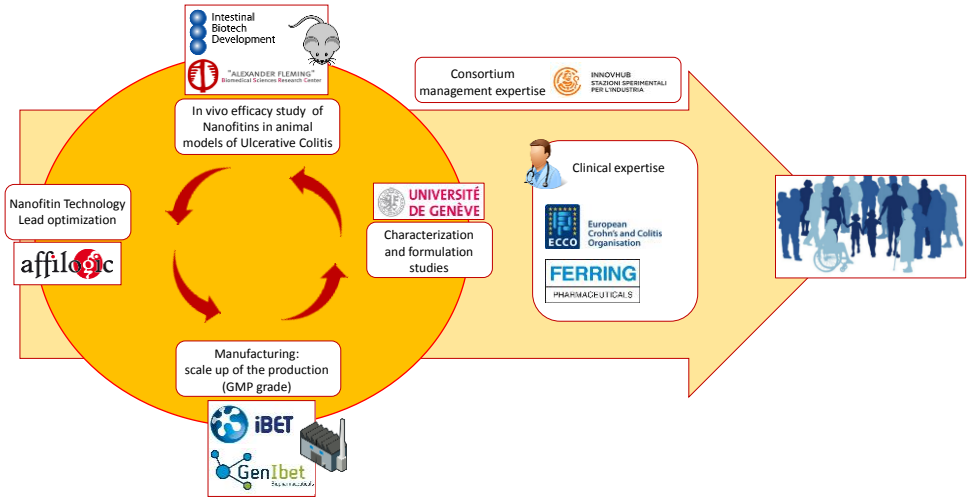


Figure 3: SADEL, a SME-like consortium gathering the key expertise to streamline the development of Nanofitins from the bench to the clinic

Objectives of the programme. There are two general objectives of the project. The first objective is to build a case study supporting the Nanofitins as a technology platform to deliver stable biological protein binders in the human digestive tract. The second objective is to bring a therapeutic product as close as possible to the clinic that solves important unmet needs for IBD patients.

To support the general objectives, science and technology objectives were set as follow:

- **In vivo validation of mechanism**: A proof of concept that locally applied anti-TNFα NFs induce statistical improvement of macroscopic and histological colitis parameters. To achieve this goal, the endpoints will be checked in the gold standard TNBS-induced colitis on wild type mice.
- **Second generation – optimized NFs**: Optimization of the amino acid composition for higher binding efficiency, adequate immunogenicity and chemical stability for long term storage.
- **Lead candidate for development**: To select a lead molecule and one back up molecule based on proof of concept in mouse colitis models.
- **Manufacturing process**: To obtain before optimized downstream and upstream NF production process that fits the requirements of the maximum production cost price.
- **Final formulation development**: To obtain optimized NF formulation process that fits the requirements of the maximum production cost price.
- **Regulatory Tox/PK**: To prove pre-clinical safety of the formulated lead NF.
- **IMPD preparation**: To compile a dossier gathering the available data supporting subsequent IMPD filing.

- A description of the main S&T results/foregrounds

From hits to Lead Candidates. At the beginning of the project, a set of anti-TNFα Nanofitins (> 30 hits) were made available by Affilogic to the SADEL development programme. All the different Nanofitins differ by a maximum number of 14 amino acids, which correspond to the binding surface. This starting diversity enables the implementation of a funnel-like screening process aiming at sorting out the relevant hits that match the requirement of the project. Sequence homology, specificity, level of cross reactivity between mouse and human TNFα, intrinsic affinity for the target and productivity yield were amongst the parameters allowing the selection of 9 hits, herein after named from N1 to N9.

To be suitable for oral administration, a biologic requires both gastric stability and therapeutic efficacy in a relevant environment, which in the case of ulcerative colitis indication is the colon. While formulation can help circumventing gastric instability, therapeutic efficacy relies only on the drug. With this in mind, the 9 hits were first evaluated for *in vivo* efficacy in a mouse model of colitis (TNBS-induced) with an intrarectal instillation, resulting in the local application of the Nanofitins at the site of the disease (ie. colon). This model can be operated under a preventive format (application of the drug before induction of the disease phenotype) or curative format (application of the drug after the induction of the disease phenotype). For this first evaluation a preventive set up was decided, and the Nanofitins were applied on a daily basis, starting from 5 days before induction to 2 days after, at a dose of 10 mg/kg (no specific formulation involved). The Nanofitins were then scored for efficacy based on the macroscopic evaluation (level of inflammation of the colon, Wallace's score), as well as histologic evaluation (evaluation of the integrity of colon wall, mucosa, presence of cell infiltrates..., Ameho's score) of the colon. In this assay, 2 Nanofitins (N9 and N1) did stand out of the others with a reduction of the lesion in the same range of the positive control Pentasa®. Efficacy of these 2 Nanofitins was also demonstrated in another mouse model of colitis (DSS-induced), still using intrarectal instillation. These results provided evidences on the *in vivo* anti-inflammatory efficacy of these two hits and their stability in the hostile environment of the intestine. The latter was further confirmed by *in vitro* evaluations of the stability of the Nanofitins in US Pharmacopeia (USP) simulated fluid mimicking intestinal fluid (slightly acidic pH and presence of pancreatin protease). Both N1 and N9 have shown astonishing stability in the USP simulated fluid with little to no degradation visible after 60 minutes of incubation in presence of protease, as revealed by mass spectrometry.

Using the same *in vivo* set up, therapeutic efficacy of N9 was demonstrated to be very similar when compared side by side between intrarectal instillation and oral gavage - still no specific formulation involved, the Nanofitin was applied as a liquid formulation composed of a very standard buffer (PBS) - meaning that the Nanofitin biologic can travel clean through the gut while maintaining its properties of active ingredient. Subsequently, a dose escalation study showed that the minimal dose for a maximal effect was of 100 mg/kg for N9 applied by oral gavage in the TNBS-induced mouse model of colitis.

During the course of the project, it was brought to the knowledge of the consortium that many drugs under development in IBD failed at demonstrating efficacy in a curative set up while doing so in a preventive one. Hence, it was important for SADEL to validate that Nanofitins can also be found efficient in a curative set up. This was investigated for N9 and N1 in the TNBS-induced model of colitis, with an application of the Nanofitins on a daily basis at 100 mg/kg by oral gavage, from the day of induction up to 4 days afterwards. Both N1 and N9 were found able to heal the lesions in this set up at a level similar to what was observed for Pentasa® (150 mg/kg, *ad libitum* in the food), demonstrating their efficacy both in preventive and curative set up when given by the oral route.

The demonstration of the oral efficacy of the anti-TNFα Nanofitins in absence of any specific formulation was an important milestone in the project. Nevertheless, the consortium agreed that a dose of 100 mg/kg was a

matter for further optimizations, mainly for two reasons. First, at least a 10 times decrease of the dose would be necessary to become compatible with a packaging as a pill, envisioned as the preferred formulation. Second, the consortium engaged in making all the possible efforts toward the development of an affordable drug, whose cost is directly related to dose. Optimization efforts have focused on the affinity improvement of the best hit from the first anti-TNFα generation (N9), resulting in a lead of second generation named N11. N11 was found able to neutralize TNFα interaction with both of its receptors TNFRI and TNFRII with an efficacy almost 500 times better than N9 in biochemical assay. This increase of the neutralization potency translated *in vivo*, in the TNBS-induced model of colitis, with a level of efficacy similar than N9 at a 10 times lower dose.

Characterization of the anti-TNFα biology. Together with *in vivo* efficacy, it is important to document a proper understanding of the mode of action of the selected Nanofitins. This is all the more important that the TNBS- and DSS-induced mouse models are not exclusively TNF-driven. The standard mechanistic assays developed over the years to test antibodies were thus implemented and Nanofitins were compared with state-of-the-art monoclonal antibodies. Namely, the L929 assay was implemented.

In the L929 assay, L929 cells are challenged by the presence of TNFα and actinomycin, the combination of both triggering apoptosis (death of the cell). Neutralization of TNFα is expected to result in an increase of cell survival. Surprisingly, no NF was found active in this assay even at very high concentrations, which is not consistent with the biochemical competition assays performed earlier.It has been brought to the knowledge of the consortium that similar observations were also made with other non-antibody therapeutics, potentially pointing a specificity of these assays towards antibody constructs. The consortium decides to investigate other possible assays that would still demonstrate a TNF-driven mechanism of action for NFs in a cell-based assay. Resources were therefore allocated to the development of an alternative assay involving the systemic co-administration of TNFα and anti-TNFα Nanofitins in mice followed by the monitoring of IL6 concentration in blood over time. Free pro-inflammatory TNFα cytokine induces a raise of IL6 level in blood in a dose dependant manner, which can be quantified. In this assay, the neutralization of TNFα either with anti-TNFα mAb or Nanofitins resulted in a decrease of IL6 secretion, whereas irrelevant Nanofitin did not impact IL6 secretion level. This provided a validation of the anti-TNFα neutralization biology of Nanofitins in an *in vivo* context, with a higher potency of N11 than N9 as expected.

Analytics. A set of analytical methods were developed during the course of the SADEL programme aiming at finely characterizing the identity of the final Nanofitin product. Molecular weight, oxidation, deamidation, meric homogeneity were all investigated with dedicated methods developed on purpose.

The meric homogeneity of the Nanofitins as a function of the concentration was checked by dynamic light scattering (DLS). For a concentration up to 50 mg/mL in PBS, solutions of Nanofitins are completely homogeneous and are composed of one meric population as shown in Figure 4.

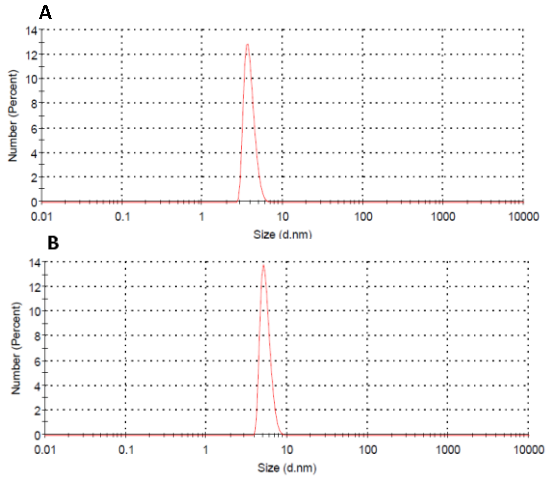


Figure 4: Analysis by DLS of N9 at 5 (A) and 50 (B) mg/mL in PBS

An analytical Nanofitin quantification method was developed using reversed-phase UPLC with UV detection (Figure 5). Quantification was performed using over a broad concentration range (from 5 to 100 µg/mL, six concentration levels, and triplicate measurements). Coupling UPLC method with tandem UV and MS (SIR) detection allowed a 10-fold decrease of the limit of quantification to 0.5 µg/mL. Linearity of the method was found excellent for both detection modes, with correlation coefficients greater than 0.999. Furthermore, precision and repeatability were good over the total calibration range, with variation coefficients below 2 % (n=3 injections).

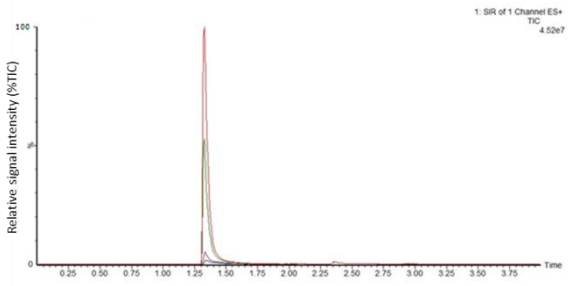


Figure 5: UPLC-MS (SIR) chromatogram of N5 standards from 0.5 to 10 µg/ml. Concentrations of 0.5 (black), 1 (purple), 5 (green) and 10 ug/ml (red) were analyzed for the calibration curve.

This UPLC-UV method is able to resolve the different forms of oxidized NFs containing from one to four oxidized methionine residues. N1 being composed of four methionine residues, this Nanofitin was submitted to forced oxidation conditions to evaluate the resolution power of the method. As shown in Figure 6, a clear separation of the different oxidized forms was achieved. Mass spectrometry studies confirmed that the additional peaks correspond to oxidized forms, validating the method for the identification of chemical modifications on the Nanofitin scaffold.

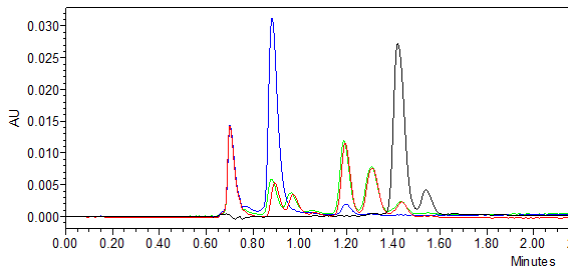


Figure 6: overlay of UPLC-UV chromatograms of N1 containing four methionine residues (100 µg/ml) before (black), after 1 hour oxidation (orange, green) and overnight oxidation (blue). Injection peak is in red.

Thus, in a routine manner, detection of the formation of oxidized NFs could be detected using a simple UPLC-UV for quality control of Nanofitins batches or if degradation occurs during the stability studies. However, if other peaks would appear on the chromatograms, additional MS analyses can be easily performed to identify the side degradation products.

Fine and systematic analytical investigation of each produced batch of Nanofitins was implemented to confirm the molecular weight of the product and its purity. The conjugation of UPLC-UV to mass spectrometry allowed highlighting a problem of heterogeneity in the products at the very early phase of the project, which was later solved by reformatting the Nanofitin expression vector. This was notably involving a differential sensitivity of the Nanofitins to Nterminal methionine removal; even though all the Nanofitins are sharing a similar Nterminal amino acid sequence.

UPLC with UV detection is a common tool for the detection and quantification of drug in serum samples. Method development was performed to find a way to precipitate serum protein without precipitating Nanofitins. Acetonitrile (from 20 to 50%) and acid (TCA, 10%) were tested as precipitating agent. The performance of the method was assessed using serum samples spiked with N9. Acetonitrile as organic precipitating agent was selected as the most adequate method, when the proportion of organic phase in the final sample is kept below 20% of the total volume. The analytical method conditions were derived from analytical methods previously developed for the quantification of NFs. The limits of detection and quantification determined for N9 as a model NF are detailed in the Table 1.

UV detection at	**214 nm**		**280 nm**	
Limit (µg/ml)	LOD	LOQ	LOD	LOQ
Clean Std	0.03	0.1	0.15	0.5
With serum	0.15	0.5	0.15	0.5

Table 1: Limit of detection (LOD) and limit of quantification (LOQ) determined for NF9.

While decent level of detection was achieved with the UPLC-UV method, the consortium was afraid that the sensitivity might not be high enough and dedicated resources to the development of an alternative ELISA based Nanofitins quantification method. It is notorious that ELISA can achieve very low detection limit, but it requires both a capture and a detection agent. If TNFα can serve as the capture modality, the detection modality that could be a generic anti-Nanofitin antibody was yet to be generated. Such generic anti-Nanofitin

antibody has to bind a region of the Nanofitin that will not compete with the binding to TNFα. Considering the small size of a Nanofitin and the low immunogenicity of the scaffold itself, the generation of such an antibody has been a challenge that the consortium overcomes.

N11 Nanofitin has been spiked directly in the rat serum (1/3 dilution) at the highest concentration point and serial diluted to evaluate the detection limit of the system (Coating of TNFα for the capture of N11 and revelation with anti-Nanofitin antibodies, Figure 7). With this method an LOD of about 10 pg/mL and a LOQ of about 100 pg/ml was obtained.

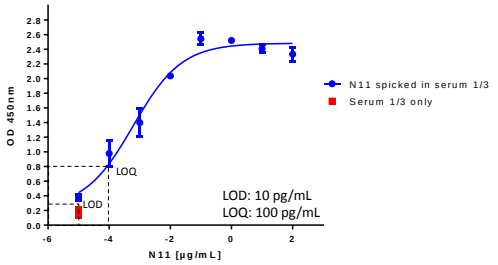


Figure 7: LOD and LOQ by ELISA based Nanofitin detection method

Stability in US Pharmacopeia gastric fluids. Using biorelevant gastric fluids, stability experiments confirmed that Histag disfavors the stability of Nanofitins with regards to sensitivity to proteases degradation. The stability of histagged N1 was compared to untagged N1 in fasted simulated gastric fluid. The results presented in the Figure 8 clearly demonstrated the lower stability of the histagged Nanofitins compared to its untagged equivalent. In the biorelevant gastric fluids, we confirmed that degradation of Nanofitins is enzyme-dependent. Three pepsin concentrations (0, 0.01 and 0.1 mg/ml) were compared in fasted simulated gastric fluids. Untagged N1 displayed a high stability even at the highest pepsin concentration of 0.1 mg/ml, which correspond to the physiological concentration recommended by the Pharmocopeia. These results prompted the consortium to involve untagged Nanofitins only in oral administration experiments, and to adapt the manufacturing process accordingly for the preparation of tag free material.

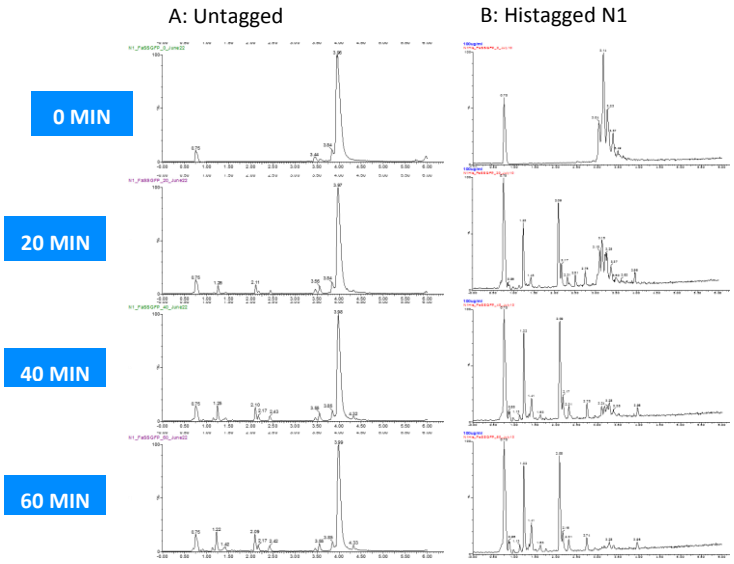


Figure 8: Stability of untagged N1 (A) compared to histagged N1 (B) in fasted simulated gastric fluid (0.1mg/ml pepsin). The histagged version of the NFs displays a lower stability compared to the untagged version of the Nfs.

Manufacturing. The procedures for small scale production of histagged Nanofitins at laboratory scale were made available to the consortium. These included the conditions for recombinant protein overexpression in *E. coli* DH5α cells using complex culture medium. Optimization of critical parameters, namely growth medium and time of harvest, were performed using culture flasks to define the initial conditions for scale-up tests in 10 L and 30 L bioreactors. The replacement of complex growth medium by a chemically defined medium was found to not not significantly affect protein productivity. This finding was important because the chemically defined medium is compatible with GMP, and thus it could be used as a starting point for further optimization.

Batch tests were performed initially in 10 L and later in 30 L bioreactors, using chemically defined medium. In these conditions a productivity of 50 mg of Nanofitins per liter could be obtained. Further work involved the optimization of a fed-batch strategy, in order to attain high cell densities and, consequently, significantly increase the productivity of the process. In this culturing mode, specific nutrients are supplied to the growth medium while they are being depleted by growing cells. A series of analytic methods have been used for the quantification of the main nutrients, namely glucose, phosphate, ammonia and sulfur, and in this way we could determine the consumption profile of each. The definition of a feeding strategy based on these results led to a dramatic increase of the volumetric productivity of the model Nanofitin N5 by 80 fold (up to 4 g per liter, see Figure 9). The same optimized procedure applied to the production of other Nanofitins allowed reaching high yields (1 g/L).

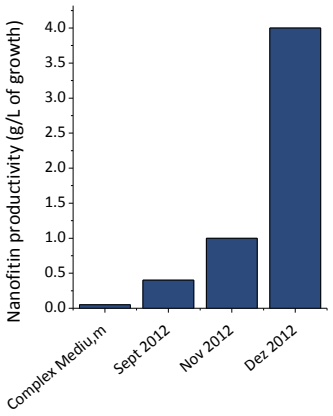


Figure 9: Optimization progress for the production of Nanofitin N5

The available N5 material was used to optimize some aspects of downstream process for Nanofitin production. Optimized procedures for medium-scale production (1-2 kg of starting biomass) included clarification steps by centrifugation and filtration, nickel affinity chromatography, desalting by size exclusion chromatography and concentration by tangential flow filtration. Particular attention was given to the methods for endotoxin elimination. Besides the use of pyrogen-free material and reagents, it was found that column washing steps using triton X-100 effectively removed the majority of endotoxins, as determined by the limulus amebocyte lysate test. Further removal of endotoxins to fairly low levels (below 1 EU/mg protein) was performed by anionic exchange chromatography (Sartorius).

As expected by the consortium, the productivities initially obtained with histagged Nanofitins (~1g/L) were not affected by the removal of the histidine tag sequence and allowed us to develop and optimize a purification scheme that permitted to obtain purified Nanofitins at the required quantities for the *in vivo* studies. Cell disruption tests were carried out using thermal or mechanical lysis, being the latter adopted due to better scalability. Thermal disruption, although promising, was not further pursued due to increased risk of target modifications induced by heat. The development of a tangential flow ultrafiltration/diafiltration strategy as a first line purification step was implemented because of its associated high purification factor. In

comparison with other purification techniques it offers relatively low costs and straightforward scale-up. An additional ionic exchange chromatographic step was then required to remove both protein and non-protein contaminants (nucleic acids, endotoxins, etc). The final products could be obtained at a fairly good purity (Figure 10), as accessed by measuring common contaminants (host cell proteins, endotoxins, nucleic acids).

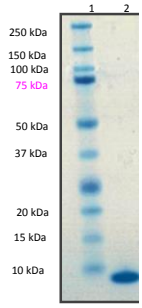


Figure 10: SDS-PAGE analysis of purified untagged Nanofitin.

First generation Nanofitins were further optimized for increased binding, originating the lead candidate N11. The manufacturing process as optimized for first generation NFs was successfully used for producing lead Nanofitin N11. The production of an R&D Master Cell Bank of the E. coli strain expressing N11 lead molecule was performed. Immediately after producing the R&D Master Cell Bank, the production process was applied for the production of a GMP-like batch of the lead Nanofitin candidate. This lot was performed using a 30 L pilot bioreactor permitting to obtain sufficient amounts of lead candidate as required for the completion of the toxicology studies in animal models performed. This active substance was quantified and characterized using the set of analytical methods developed in course of the program.

Formulation. Formulation efforts planned in the SADEL programme were significantly revised in view of the impressive stability of Nanofitins demonstrated both by *in vitro* incubation in simulated gut fluid and *in vivo* experiments by oral gavage. Following the drug development expertise within the consortium, the large non-GMP batch of API produced was formulated as simple Drug Product. The consortium has agreed on a formulation consisting of Phosphate buffered saline buffer (PBS) at pH 7.4, as i) the selected Nanofitin shown to be stable in this buffer ii) this formulation is commonly used for animal testing due to its negligible effects. Quality Control of the produced product was performed based on the selected analytical analysis methods developed for quantification and characterization (Protein extinction coefficient, SDS-Page, Molecular Mass and purity by UPLC-MS, endotoxin content by PTS-LAL, Host Cell DNA and Host Cell Protein content, Appearance, among others), allowing the release of the product for the animal testing.

Toxicology study/PK. Preliminary Toxicological studies were run on N9 and N11 in agreement with the guidelines for regulatory toxicological evaluation. Based on the fact that N11 derived from N9, and so have a very similar structure, a similar toxicity profiles were expected. The potential genotoxic and cardiotoxic effects were investigated *in vitro* with MLA and hERG assays respectively. With respect to the assessment of a genotoxic effect, the MLA assay was preferred to the Ames, knowing that the Ames can provide false positive in the presence of histidine; which is part of the amino acid composition of the Nanofitins. Those *in vitro* assay were also completed by a serie of *in vivo* evaluations including: i) a pharmacokinetic evaluation in rats after oral and IV administration and ii) evaluation of the maximum dose tolerated (MTD) via *in vivo* dose ranging in rats after oral administration of NFs and evaluation of toxicity effects in 6 different organs. Although an oral application is targeted in the current project, it has been the choice of the consortium to

explore the toxicity with systemic injection as well. This choice was mainly motivated by the fact that toxicity is more likely to occur after systemic exposure to the drug and impact and vital organs, if it has to be. Considering that the systemic exposure will be low with an oral administration, the consortium decided to evaluate the Nanofitins in the worst case scenario.

N9 and N11 did not increase the mutation frequency in the mouse lymphoma TK assay (MLA) test system under the experimental conditions (dose level of up to 2000 µg/mL), leading to the conclusion that these molecules are not genotoxics. In the human Ether-à-go-go-Related Gene (hERG) test, no inhibition of $K_v11.1$, the alpha subunit of a potassium ion channel was found with range of concentrations going up to 3000 µg/mL. This ion channel is best known for its contribution to the electrical activity of the heart that coordinates the heart's beating. This result led to the conclusion that such a high level of Nanofitin concentration could be safely, with regards to cardiotoxicity, implemented in clinical phase study.

Pharmacokinetics and MTD study were not terminated at the end of the SADEL programme but preliminary results suggested a very fast clearance from the systemic circulation of the Nanofitins, hence low systemic exposure. Moreover, no mortality or clinical signs were observed for the animals that received Nanofitins (even with daily repeated administration at a dose of 206 mg/kg), giving a first hint on the safety of these molecules.

The SADEL project demonstrated the principle of efficacy of orally administered Nanofitins to interact with their target and elicit an *in vivo* response in IBD models. The industrial viability is also demonstrated: scale-up, control, formulation. Finally, important steps towards regulatory approval for use in humans have been achieved: favourable preliminary toxicity, low immunogenic profile, confirmed TNFα-specific mechanism of action. However, the finding that this mechanism is not identical to that of monoclonal antibodies, added to the novelty of the Nanofitin scaffold in itself, raises the regulatory complexity for such products to be accepted. More work is still ongoing after the project to reach a clinical milestone within two years.

- The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

Potential impact:

SADEL. The SADEL programme focuses on the development of an oral formulation of anti-TNFα Nanofitins for the treatment of IBD, with the ultimate goal of establishing a generic proof of concept around the Nanofitin technology as a targeted oral drug platform. By concentrating on the well-known and clinically validated TNFα target for ulcerative colitis, the consortium consciously restricted the risks on the project only on the behaviour of the innovative Nanofitin scaffold itself with regards to the oral route of administration, and not on the biology of the disease. This careful, yet ambitious, decision was made to streamline the development of a Nanofitin-based drug, while filling an unmet medical need in an indication where the demand for new therapeutics is very high.

IBD incidence, prevalence and treatment. IBD are chronic disabling gastrointestinal disorders, with an early onset, requiring lifelong treatment and impacting every aspect of the affected individual's life (physical suffering and pain, impaired function, and diminished quality of life). These persistent relapsing diseases have a significant influence on individual employment status and work-related productivity. In addition to the significant burden on patients and their families, IBD represent a sizable burden to society due to high healthcare and non-healthcare related costs. An estimated 2.5–3 million people in Europe are affected by IBD, with a direct healthcare cost of 4.6–5.6 bn Euros/year. Non-healthcare related, or indirect, costs - primarily associated with decreased work productivity, disability payments, and early retirements - are typically greater contributors than direct healthcare costs to the total costs associated with IBD.

The Datamonitor reports (2010) "Pipeline Insight: Inflammatory Bowel Disease" and "Commercial Insight: Inflammatory Bowel Diseases" revealed that the IBD market will grow from $3.5 billion in 2009 to $5.6 billion in 2019. The earlier treatment of patients with anti-TNFα drugs, continued uptake of biologics in UC, and raise in patient numbers fuel the sales growth. 890,000 prevalent cases of CD were estimated in 2009 and a 10% increase is envisioned from 2019 onwards. 1,387,000 prevalent cases of UC were estimated in 2009 and 42% increase of the UC patient population is envisioned from 2019 onwards. Increasing incidence and prevalence of IBD in many regions of the world point to its emergence as a global disease.

Though there is currently no drugs available to cure IBD, the three main goals of IBD treatment are achieving remission, maintaining remission (prevention of disease flares), and improving quality of life. The care of a patient can be either medical or surgical in nature or, in many cases, a combination of both. The medical approach generally covers both symptomatic care (ie. relief of symptoms) and mucosal healing following a stepwise approach to medication, with escalation of the medical regimen until a response is achieved. The management algorithm will be highly dependent of the phenotype (Crohn's disease or ulcerative colitis) and severity of the disease.

A major breakthrough in the management of IBD patients came with the venue of neutralizing antibody specific to TNFα. Initially engaged in the late phase of a step-up treatment scheme after failure of, or intolerance to conventional aminosalicylates and corticosteroids, anti-TNFα therapy brought an ultimate alternative to surgery. More recently, TNFα neutralizing antibodies were also shown highly effective when used in first line, so called top-down approach, opening the possibility for corticosteroid-free remission with a more rapid and higher rate of mucosal healing. The shift from the traditional step-up treatment paradigm to a top-down algorithm is supported by the result of Centocor's SONIC clinical study, which offers a strong incentive that physicians will start using early combined anti-TNFα and immune-modular therapy.

Anti-TNFα mAbs. In Europe, infliximab (Remicade®, a chimeric mAb) and adalimumab (Humira®, a fully human mAb) are approved for the treatment of moderately to severely active Crohn's disease and ulcerative colitis. More recently, golimumab (Simponi®, a fully human mAb) was approved for the treatment of

moderately to severely active ulcerative colitis. Infliximab is administered intravenously in the hospital whereas adalimumab and golimumab are administered subcutaneously and are therefore often preferred by the patient for practical reasons. Of the patients initially responding to the anti-TNFα targeted therapy, 30 - 60% will lose clinical benefit during maintenance therapy, which is often attributed to suboptimal dosing and/or immunogenicity. Loss of response is managed clinically by decreasing the interval between administrations of drug and/or by increasing the dose. Immunogenicity - that can be defined as the ability of an antigen to elicit a humoral or cell-mediated immune response - leads to the development of anti-drug antibodies that either block the drug's activity directly or cause a faster elimination from the systemic circulation of the drug. Though all anti-TNFα antibodies are immunogenic to a certain extent, treatment regimens have been optimised over the years to minimize this issue, notably with the use of the mAb in combination with immunosuppressors (such as azathioprine and methotrexate) instead of monotherapy.

Despite the significant impact of the anti-TNFα mAb agents on patients with refractory CD and UC, the variable magnitude and duration of response to these agents, the adverse effects related to their administration by injection (systemic side effects, injection reaction, poor compliance) and high costs continue to prompt the development of new biologic agents for IBD.

Unmet medical needs in IBD. Datamonitor draws conclusions and performs predictions based on its own knowledge of a topic and based on interviewing key opinion leaders in the USA, Europe and Japan. For CD and UC the unmet needs identified by Datamonitor are listed (in decreasing level of importance) in table 2.

Crohn's disease	Ulcerative colitis
Effective agents for maintaining remission without causing immunosuppression	**Powerful, well-tolerated agents for inducing remission quickly**
Drugs for mild Crohn's disease	**Oral maintenance agent after steroid induced remission**
A non-biologic OR a **safe oral agent that is not as costly as the current biologics**	Effective agents for refractory patient population
An effective treatment for fistulising disease	Disease modifying drugs
Predicting response to therapy	Simple blood tests that indicate disease activity
Better activity measures in clinical trials	

Table 2: unmet medical needs in IBD listed following a decreasing level of importance

By keeping a similar biology – anti-TNFα neutralization – while moving with the Nanofitin technology to an oral delivery route, the SADEL consortium invested in the development of a drug that proposes to solve the top unmet medical needs in IBD (*labelled in bold in table 2*). The oral route restricts the systemic exposure of the Nanofitins leading to a safe oral product that has no or little influence on general immunosuppression. Supposed that Nanofitins could reach the systemic compartment in patients bearing heavy damages at the intestinal barrier, their fast elimination from the blood will result in a minimal exposure as well. The manufacturing of Nanofitins involves a process by bacterial fermentation, which is cheaper than the mammalian production system used for antibodies; though that also mammalian production systems will become cheaper over time. With these perspectives in mind, the SADEL programme was fully in line with highlights from Datamonitor stating that cost and safety will dictate the future of the IBD market. Not to mention that on top of that, an oral drug will significantly improve patient comfort and compliance to treatment.

Within this programme, we did not focus on CD but it is comfortable that once our product is approved for UC, human Proof Of Concept of active ingredient will be available and it will be easier to also explore CD as therapeutic indication.

Unique innovative treatments for digestive tract diseases. Convenient oral delivery of biologicals would definitely open up a new set of opportunities to treat many different diseases. With the oral delivery technology developed in this consortium, Nanofitin based biologics could be delivered to diseased areas ranging from the mouth, pharynx, stomach, small intestine, large intestine and anus. Diseases of the digestive tract include mucositis, aphthous stomatitits, esophafitis, inflammatory bowel disease, irritable bowel syndrome, celiac disease, trauma to the digestive tract, infections of the digestive tract and cancers of the digestive tract. For some digestive tract diseases, there are currently no effective treatments available.

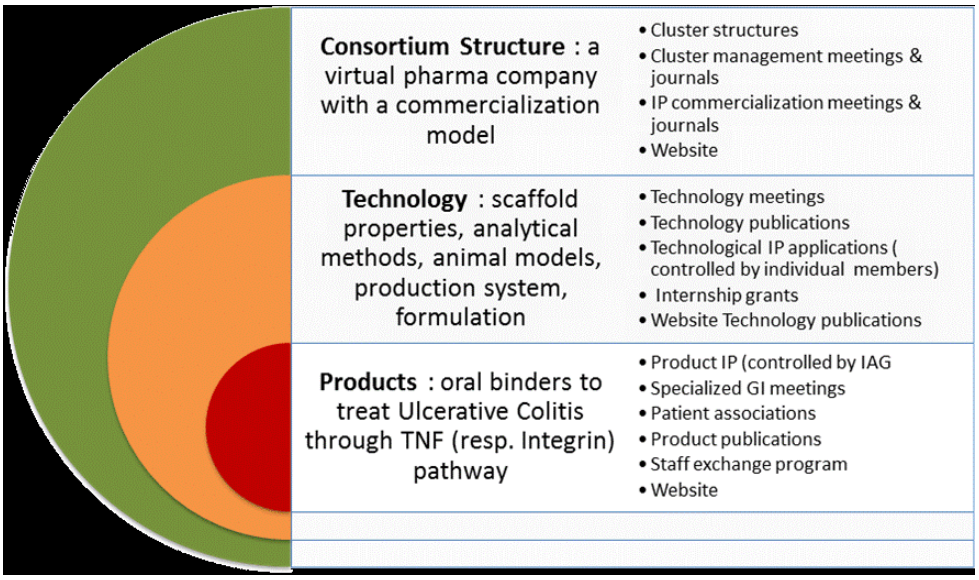
One example of such unmet medical need is mucositis, a serious and painful condition that results from radiation therapy and/or chemotherapy for cancer treatment. Mucositis can affect up to 95% of bone marrow transplant patients, and is the most frequent serious side effect of therapy in the first 100 days after transplant and is the most common condition requiring system analgesics during cancer. Oral mucositis also affects up to 80% of patients receiving radiation for head and neck malignancies and approximately 40% of patients undergoing chemotherapy. Damage can occur throughout the digestive tract and frequently results in cessation or dose reduction of the cancer therapy. Oral mucositis presents with pain, erythema and deep, diffuse ulcers that can cause difficult speaking, eating and swallowing. Intestinal mucositis presents with nausea, vomiting, abdominal pain and diarrhea. There is a single medication approved for the treatment of mucositis, palifermin, but it is only used in a limited subset of patients. Anti-inflammatory Nanofitins might be used to develop novel therapies in this area.

Another example of an unmet medical need is recurrent aphthous stomatitis (RAS), a common oral disease, affecting 5-20% of the normal population. RAS presents with recurrent bouts of rounded, shallow painful ulcers on the mucosa of the mouth. The cause of RAS is unknown and there are no approved pharmacotherapies. Once the molecular mechanisms of this disease are known, Nanofitins might be used to develop novel therapies.

A solid consortium. One key benefit from working as a group for 5 years is the quality of the relationships built over time and effort in this project. The partners have established deep relationships that already led to other one-to-one collaborations (research and business), and a general agreement to share the information and know-how generated during SADEL with an aim to continue moving ahead with Nanofitins in IBD. Besides the human component, this is a testimonial to the high degree of complementarity between the partners and to the level of commitment from each participant. The project did not stop at the end of the EU funding, and novel actions are being led to keep this opportunity going forward for patients, for the development of SMEs, and for the progress of knowledge.

Main dissemination activities

When SADEL project was designed, the consortium decided to divide the dissemination of the project in three categories: the consortium structure related dissemination, the technology related dissemination and the product related dissemination (highest level of confidentiality (+++)). The product related dissemination is really the core of the consortium and gets the highest control from the IP Advisory Group (IAG).



From an operational point of view, the dissemination actions have been the object of a full work package (WP7) and directly managed by project coordinator with the support of whole consortium.
At the project start, the Coordinator has managed the development of:

- A logo and visual identity of the Consortium
- A consortium PowerPoint template to be used by the members for consortium presentations
- A website to explain the consortium set up and publish public results (for the general public)
- A restricted area (within the website) used by partners to contain all key data related to the product (but no data related to technology inventions), all materials used during meetings (presentations, summaries, minutes etc.) and all standard operating protocols
- A web conferencing system to allow the partners arranging for virtual meeting (in addition to physical ones)

To describe the dissemination procedures the Coordinator has prepared a dissemination plan and a Standard Operating Protocol (SOP) to guarantee confidentiality and respect of IP protection.The SOP is a common procedure that involves a strict control of published material in order to grant security and accuracy.

The reason for raising the level of attention on communication issues has always been related to project activities and future outputs, due mainly to their confidentiality and high potential of innovativeness. Any incautiously published piece of information could indeed potentially hamper project results and activities, this in particular under intellectual property point of view.

The process starts from each partner willing to propose a publication (website, press release, article, interview…). The main body involved in this process is the IP advisory board, which will be responsible for the analysis of proposed material and its validation/correction.

This procedure has been used during the whole project duration and put the partners in the position of promoting/disseminate the project and its results in several context as described below:

a) Oral presentations

On December 11th 2014, UniGeneve attended the annual meeting of the French Society for Nanomedicine (SFNano) in Nancy and Magali Zeisser from Uni Genev presented within the session "Nanomedicine and inflammation" and oral presentation titled: DEVELOPMENT OF NANOFITIN® PROTEIN SCAFFOLD FOR THE TREATMENT OF INFLAMMATORY BOWEL DISEASES.

In Barcelona, during the 10th ECCO Congress held on February 21st 2015, Affilogic had the chance to make an Oral presentation titled "Oral delivery of a new class of non-antibody protein scaffold Nanofitins targeting TNF-alpha shows a strong preventive and curative anti-inflammatory effect in models of inflammatory bowel diseases". The abstract was selected as one of the best 28 abstracts and thus invited for presentation in the Scientific Plenary Programme.
(https://www.ecco-ibd.eu/index.php/abstracts-search.html?q=cinier&Itemid=698)

On November 3rd 2015, Affilogic attended the PEGS Europe Congress in Lisbon (which hosted 700 attendees from more than 30 countries) and Mathieu Cinier made a presentation titled: "Oral Delivery of Anti-TNF-A Nanofitin Shows a Strong Preventive and Curative Anti-Inflammatory Effect in Models of Inflammatory Bowel Diseases".

During the 11th ECCO Congress in Amsterdam, on March 16th 2016, Sadel project was invited to participate to the ECCO-EU project forum during which an oral presentation about the project and its results has been made.
The presentation titled "Oral delivery of a new class of non-antibody protein scaffold Nanofitins targeting TNF-alpha shows a strong preventive and curative anti-inflammatory effect in models of inflammatory bowel diseases" was made by Olivier Kitten from Affilogic, Christel Rousseaux from IBD and Magali-Zeisser Labouèbe from the university of Geneva and provided information about project's results and updates. The presentation has been also webcasted and uploaded on ECCO website and can be viewed at the following link https://www.ecco-ibd.eu/images/7_Public_Affairs/SADEL%20ECCO%20March2016_web.pdf

b) ECCO Newsletters (ECCO News)

ECCO News is the society magazine of ECCO – European Crohn's and Colitis Organisation. The ambition of ECCO News is to reflect what's going on within the organisation and to report on IBD activities at large within Europe. With this periodical publication ECCO opens a new way to maintain the information-flow on the activities taking place within the society - keeping its members up-to-date as well as all gastroenterologists, surgeons, paediatricians, basic scientists and nurses worldwide interested in IBD.

In 2014 Sadel Project was summarized in the ECCO News Issue 04/2014 (page 17) from the perspective of Dr. Johannes Meier who had been employed by ECCO as a research fellow for the project:
https://www.ecco-ibd.eu/images/6_Publication/6_2_ECCO%20News/ECCO_News_Issue4_2014_Content_Update.pdf

In 2015, Sadel Project was also described reported in the ECCO News Issue 4/2015 (page 7) from the perspective of Dr. Raja Atreya who had been employed by ECCO as a project research fellow:
https://www.ecco-ibd.eu/images/6_Publication/6_2_ECCO%20News/2015_Nr4_insert_ECCO_NEWS_4_2015_CORRIGENDUM_29_01_2016.pdf

c) Posters

Thanks to ECCO, it has been possible to prepare some promotional material such as posters, to be shown during important events and congresses.
In the context of the 9th ECCO Congress in Copenhagen, in February 2014, which had a record number of participants of 5175, the FP7 SADEL Project was firstly presented with a promotional poster, right next to one of the major entrances of the plenary hall which accommodated an audience of over 3500 persons
(https://www.ecco-ibd.eu/images/7_Public_Affairs/MASTER_sadel_2014_Webversion.pdf)

The poster, duly updated, has been shown also during the other 10th and 11th ECCO Congresses in Barcelona (2015) which counted a record number of 5.420 participants from 80 countries and in Amsterdam (2016) with 6.265 delegates from 91 countries.
The 2 versions of SADEL posters can be viewed at the following links.
(https://www.ecco-ibd.eu/images/7_Public_Affairs/MASTER_sadel_2015_Webversion.pdf)
(https://www.ecco-ibd.eu/images/7_Public_Affairs/MASTER_2016_POSTER_FP7_Sadel_Project.pdf)

In addition to ECCO Congresses, some partners had the chance to present a Sadel poster during important international events.

During the "Digestive Disease Week Congress" in Washington DC, May 16-19th 2015, Christel Rousseaux from IBD presented a poster titled "Oral delivery of a new class of non-antibody protein scaffold Nanofitins targeting TNF-α shows a strong preventive and curative anti-inflammatory effect in models of IBD" .

On June 15-17th 2015 during the "EUFEPS annual meeting" in Geneva, Magali Zeisser-Labouèbe from UniGe presented a poster with the title "Development of Nanofitin protein scaffold for the treatment of inflammatory bowel diseases".

On March 25th 2016, during the "Sanofi R&D days" in Le Port-Marly – France, Mathieu Cinier from Affilogic presented the poster titled "Oral delivery of a new class of non-antibody protein scaffold Nanofitins targeting TNFα shows a strong preventive and curative anti-inflammatory effect in models of IBD".

Last but not least, Mathieu Cinier from Affilogic had the chance to present during the "PEGS Europe Congress" in Lisbon, October 31st - November 4th, a poster with the title "Oral delivery of Nanofitins targeting TNF-alpha shows a strong preventive and curative anti-inflammatory effect in models of inflammatory bowel diseases".

d) Cordis
Cordis (Community Research and Development Service) is the primary repository and website for EU-funded projects and results. In September 2015, SADEL project has been published on Cordis "Result in Brief" and "Cordis Monthly Magazine". Sadel's Results in Brief has been published in six languages to support the dissemination and exploitation of EU research results. The Result in Brief and publishable summary (referred to as a Report Summary) have been published and linked to Sadel project's permanent factsheet at: http://cordis.europa.eu/projects/rcn/102101_en.html

e) Articles
ECCO JCC
The Journal of Crohn's and Colitis is the official journal of the European Crohn's and Colitis Organisation (ECCO JCC) and is concerned with the dissemination of knowledge on clinical, basic science and innovative methods related to Inflammatory Bowel Diseases (IBD).The journal publishes original articles, review articles, editorials, ECCO Guidelines, viewpoints, short reports, abstracts and letters to the Editor. JCC is being published every month (12 issues per year) and covers the knowledge and science related to Inflammatory Bowel Diseases: the aims are to update, innovate and challenge.

A Viewpoint article on Sadel has also been published in JCC with the title "How to apply for and secure EU funding for collaborative IBD research projects" (Authors: O. Kitten – Affilogic; Ilaria Bonetti – Innovhub SSI; J.Satsangi, M.Chavez, R.Kalla, S. Scott, N. Ventham, E Louis).
IMPACT
Sadel project has been also promoted on Impact (https://impact.pub/) a quarterly publication designed to provide an open access platform for researchers to communicate the aims, objectives and potential socioeconomic and academic impact of their research.
It has been distributed in printed and digital format in November 2016 to 35'000 readers worldwide and will be read by all key stakeholders related to the health and medical research areas within universities, research institutes, national and regional funding agencies, policy, NGOs, government, hospitals and health authorities and public and private sectors.

Impact is published under a Creative Commons licence and is completely open access and freely available. The full article is available at this link:
http://www.ingentaconnect.com/content/sil/impact/2016/00002016/00000002/art00029

The article has been drafted according to the collaboration of all partners and has been distributed to all consortium's contacts.

f) Webcast
During the ECCO Congress in Barcelona on 21st February 2015, the oral presentation delivered by Mathieu Cinier from Affilogic (on behalf of Sadel consortium) within the "Scientific session 11: Managing the manageable: Chronic pain and fatigue" has been also webcasted and uploaded on ECCO website. It can be viewed at the following link
https://www.ecco-ibd.eu/index.php/public-affairs/eu-research-projects/fp7-sadel-project.html

- The address of the project public website, if applicable as well as relevant contact details.

http://www.sadelproject.eu

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