Project no. LSHC- CT-2006-037874

VITAL

DEVELOPMENT OF OPTIMIZED RECOMBINANT IDIOTYPIC VACCINES FOR SUBSET-SPECIFIC IMMUNOTHERAPY OF B CELL LYMPHOMAS

SPECIFIC TARGETED RESEARCH PROJECT (STREP)

Thematic Priority 1:
Life Sciences, Genomics and Biotechnology for Health (LifeSciHealth)

Publishable Final Activity Report

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Dissemination Level		
PU	Public	**X**
PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Contents

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1. Abstract

Therapeutic vaccines targeting B cell non-Hodgkin lymphoma (NHL) idiotype (Id) represent a promising approach against these malignancies. A broad use of Id-based vaccination, however, is hampered by the complexity and costs due to the individualized production of these vaccines. Recent evidence indicates that these limitations may be overcome. In fact, distinct sets of stereotyped immunoglobulins have been identified in various B-NHL, suggesting that patients share Id with a higher frequency than appreciated previously. We found that the VK3-20 protein, frequently expressed by HCV-related B-NHL, induces specific T cell responses cross-reacting with related VK proteins commonly used by different B-NHL. These findings provide the rationale to develop recombinant vaccines using Id shared by different B cell malignancies. Through the complementary and synergistic work of academic partners and three SMEs, we plan to develop and produce optimized recombinant Id vaccines for subset-specific immunotherapy. A database of VH and VL sequences expressed by different B-NHL will be constructed to identify subgroups of tumors expressing molecularly correlated Id proteins. Selected Id proteins will be characterized for their immunogenicity and, particularly, for the ability to induce cross-reactive immune responses against related Id proteins. B and T cell epitopes will be identified using innovative approaches (Pentamer libraries, MATRIX-SCAN) and dedicated assays for immunomonitoring will be developed. Optimized versions of selected Id vaccines will be produced using new strategies (Gly-Ala repeat insertion, peptide sequence optimization) and validated in animal models. New adjuvants and delivery systems for improved Id vaccine formulations and administration will be also evaluated and validated. The most promising Id proteins will be produced and purified according to GMP standards and included in new vaccine formulations for innovative trials of "cross-reactive" immunotherapy.

2. Introduction

Non-Hodgkin's lymphomas (NHL) are a heterogeneous group of malignancies that still substantially contributes to the incidence of and mortality associated with cancer in Europe. In 2006, NHL accounted for 3.2% of new cancer cases and 2.8% of cancer deaths in Europe, making it the 8th leading cause of new cancer cases and the 10th leading cause of cancer deaths. Moreover, European cancer registry data indicate that the incidence of NHL has significantly increased in recent decades. Several lymphoma histotypes, such as mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL), are diseases predominantly found in the elderly population and it has been reported that >50% of new NHL cases in Europe and North America occur in patients older than 65 years. Moreover, NHL are emerging as disorders of clinical relevance also for HIV+ patients, as they constitute one of the major causes of death in this setting despite the therapeutic improvement introduced by highly active anti-retroviral therapy. Although the outcome of patients with aggressive and indolent lymphomas has improved considerably in the Rituximab era,

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patients who experience relapse after salvage regimens have a poor prognosis, thus stimulating the search for novel and more effective therapies.

Therapeutic vaccines targeting B cell lymphoma idiotype (Id) represent a promising immunotherapeutic approach for a better clinical control of these malignancies. This strategy is based on the observation that clonal immunoglobulins (Ig) expressed by neoplastic B lymphocytes carry unique determinants in their variable regions (idiotypes), which can be recognized as tumor specific-antigens. Indeed, vaccination using the tumor-specific Ig as whole molecules or a portion thereof has been shown to elicit both protective and therapeutic immunity in murine models. In addition, both protein- and dendritic cell-based vaccines that use the patient-specific Id have resulted in clinically significant tumor-specific cellular responses with very little toxicity. Notably, one phase III trial successfully reached its primary endpoint showing a significant improvement in disease-free survival for patients vaccinated in first complete remission after standard chemotherapy.

A broad use of Id-based vaccination for B cell lymphomas, however, is hampered by the fact that these approaches are patient-specific so that the vaccine must be individually produced for each patient. Recent evidence however suggests that the limitations related to the individualized production of Id vaccines may be overcome. In fact, distinct sets of "molecularly similar" immunoglobulins have been identified in various B cell malignancies, suggesting that a much higher frequency of idiotype sharing exists among patients than appreciated previously. In particular, the molecular characterization of Hepatitis C Virus (HCV)-related lymphomas, showed that more than 70% of these cases preferentially use light chains of the VKIII family, with a high degree of homology across different lymphomas. These molecular features are consistent with a role of a sustained antigenic stimulation in the development of these lymphomas. Notably, molecularly similar immunoglobulins are also frequently expressed in non-HCV-associated cases, suggesting that VKIII-derived vaccines could be used in a considerable fraction of patients with different B cell lymphoproliferations.

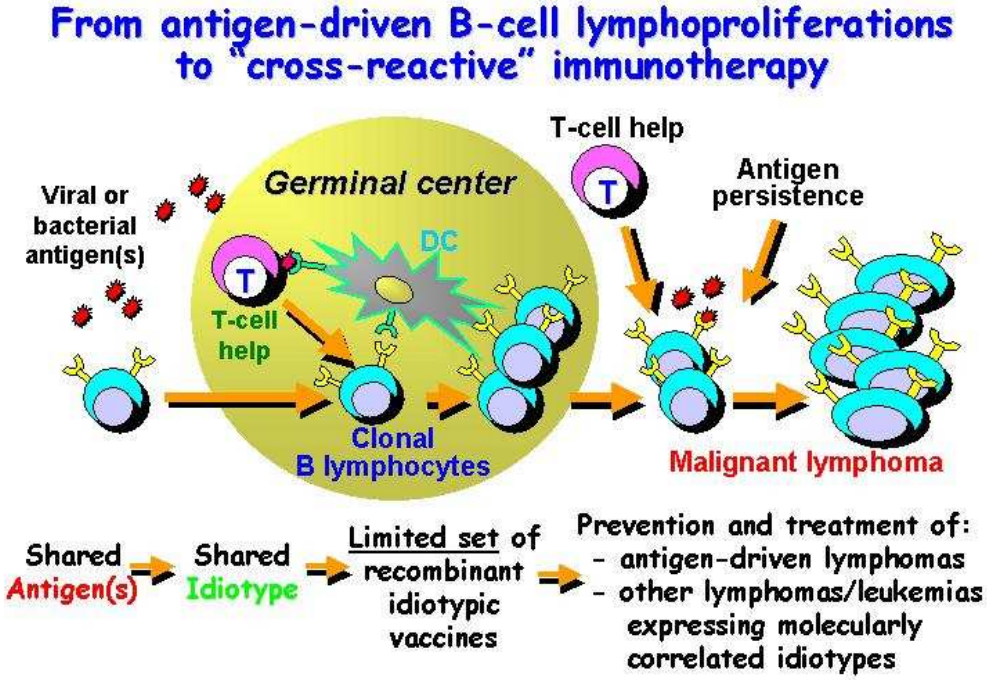

Figure 1. Rational background for the VITAL project.

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These molecular features are consistent with a role of a sustained antigenic stimulation in the development of these lymphomas (***Figure 1***). Notably, molecularly similar immunoglobulins are also frequently expressed in non-HCV-associated cases, suggesting that VKIII-derived vaccines could be used in a considerable fraction of patients with different B cell lymphoproliferations.

Since the idiotype proteins are "self" Igs, several methods have been used to enhance the immunogenicity of Id vaccines. The most frequently used approach is the chemical conjugation to a highly immunogenic carrier protein such the keyhole limpet hemocyanin (KLH). The idiotype-KLH conjugate may then be administered along with an immunogenic adjuvant to further boost the immune response. Almost all clinical trials carried out so far in humans have used idiotype-KLH conjugates. Although Id vaccines may show clinical efficacy in a proportion of patients, these are first-generation vaccines, whose potency is still limited. Therefore, new formulations, including the use of more potent adjuvants, are needed to improve the efficacy of these vaccines.

3. Project execution

3.1 Project Objectives

The main objective of VITAL was the development and production of optimized recombinant Id vaccines for the immunotherapeutic treatment of distinct subset of B cell lymphomas and leukemias. In particular, the following main objectives and achievements have been pursued:

1. ***Identification of candidate idiotipic proteins for "cross-reactive" immunotherapy***. This aim required the establishment of a large database including the sequences of idiotypic immunoglobulin genes expressed by a wide spectrum of B cell lymphoproliferations including low grade B-NHL, autoimmunity-associated lymphoproliferations (HCV-related NHL, mixed cryoglobulinemia, Sjögren's syndrome, etc.), and chronic lymphocytic leukemia (CLL). The analysis of the database was instrumental for the identification of subgroups of lymphomas/leukemias characterized by the expression of molecularly similar immunoglobulins.

2. ***Pre-clinical characterization of the immunogenicity of selected idiotipic proteins.*** Particular efforts were made to verify whether the candidate vaccines were able to induce "cross-reactive" immune responses against tumor cells expressing molecularly similar immunoglobulins.

3. ***Design and validation of optimized idiotypic vaccines.*** New strategies were planned to be used to improve the immunogenicity (i.e. optimize) of selected Id vaccines.

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4. ***Evaluation and validation of new adjuvants and innovative delivery systems for improved vaccine formulations and administration***. The project also included the analysis of new adjuvants and delivery systems to further enhance the strength of vaccination.

5. "***Clinical-grade" production and purification of "optimized" idiotipic proteins for patient vaccination***. The project also aimed at developing protocols for the production and purification of the most promising idiotypic proteins according to good manufactory practice (GMP) standards in order to make them available for clinical studies.

3.2 Contractors Involved & Coordinator Contact Details

The VITAL Consortium consists of four research organisations and universities and three industrial participants. Contractors are given in ***Table 1***.
The Project website is: http://www.cro.sanita.fvg.it/progetti/vital/index.htm

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3.3 Work Performed

The VITAL project consisted of eleven workpackages. Organization and management of the Consortium was carried out in **WP0**, research and development work in **WPs 1-9**, exploitation of results in **WP10**. The workflow of the VITAL project is represented in ***Figure 2***. Globally, the project run according to the workplan as far as the achieved results are concerned.

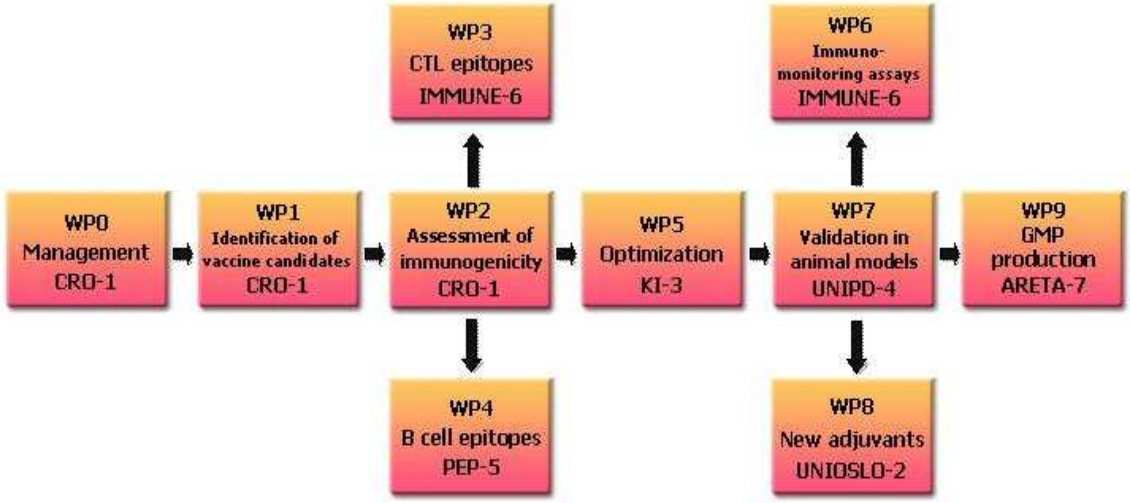

Figure 2. Workflow of the VITAL project

The work carried out in **WP1** allowed the setting up of a dedicated database including sequences of Id immunoglobulin genes expressed by a wide variety of B cell tumors, including in particular, low grade B-NHL, and autoimmunity-associated lymphoproliferations. Careful analysis of the sequences obtained allowed the identification of subgroups of B cell lymphomas/leukemias characterized by the expression of Id proteins with high sequence homology: besides HCV-related B-NHL, "shared" Id proteins were also expressed by autoimmunity-associated lymphomas (Sjögren's syndrome, rheumatoid arthritis, etc), HCV-unrelated low-grade lymphomas), and subsets of chronic lymphocytic leukemias (CLL). Also a proportion of B cell lymphomas arising in HIV-infected patients show the expression of molecularly similar Id proteins, opening thus the possibility to extend our proposed vaccination approach also in this setting. Notably, clonal B cell expansions related to HCV infection (i.e.: mixed cryoglobulinemia) at high risk of evolution to malignant lymphoma were also found to express Id proteins similar to those of HCV-associated lymphomas. The data obtained provide convincing evidence supporting the relevance of VK3-20 and additional Id proteins as "subset-specific" vaccines for the prevention and treatment of different B cell malignancies. Considering that the proposed vaccines should be able to elicit effective immune responses against a relatively broad spectrum of lymphomas/leukemias, the amino acid sequences of Id proteins targeted by these vaccines should be sufficiently conserved among the different cases. This issue was directly assessed by the analysis of the degree of interpatient variability of selected Id proteins within tumor subsets of interest. This work provided a body of data that proved to be useful to

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estimate the degree of immunogenic epitope variations occurring in VK3-20+ normal and malignant B cells (see also **WP3**).

In **WP2**, the VITAL Partners performed an extensive characterization of the immunogenic properties of selected recombinant Id proteins. In particular, evidence was obtained demonstrating that VK3-20 and other "shared" Id proteins can be used in recombinant form *ex vivo* as pre-made vaccines able to induce specific immune responses from different donors. Molecular characterization of a large panel of tumor cell lines allowed the identification of 5 different B cell lymphoma-derived cell lines naturally expressing the Id protein of interest, which proved to be suitable models for both *in vitro* and *in vivo* studies. It has been also shown that the T cell responses induced by the selected prototypic VK3-20 Id protein are also effective against target cells expressing VK3-20 proteins derived from unrelated lymphomas. Notably, these T cell responses were also "cross-reactive" against a number of molecularly similar Id proteins expressed by a relatively broad spectrum of lymphoid tumors. These experiments provided the "proof of concept" for the use of "shared" Id proteins as pre-made vaccines for the treatment of distinct subsets of patients with B cell lymphoproliferations. We also demonstrated that VK3-20 and other Id proteins of interest are also naturally immunogenic *in vivo*, as shown by the presence of specific memory T cell responses in lymphoma patients. The extensive molecular characterization of the amino acid sequences of the If proteins of interest allowed the identification of the best candidate sequences to be used as vaccines. This selection was made also on the basis of the immunogenic epitopes (see **WP3**) carried out by each Id protein, thus leading to the design of "consensus" sequences. Genes encoding these "optimized" Id proteins were synthesized and cloned in expression vectors suitable for the "clinical-grade" (GMP) production and purification of the recombinant candidate vaccines. These vectors were transferred to one SME Partner (**ARETA-7**), who produced aliquots of recombinant proteins for *in vitro* and *in vivo* studies.

The activity of **WP3** allowed the identification and validation of a number of new relevant immunogenic T cell epitopes within Id proteins of interest. Both HLA class I and II epitopes were identified. This has been accomplished by the novel and fast REVEAL & ProVE® methodological approach of Partner **IMMUNE-6.** Identification of these highly immunogenic sequences allowed the synthesis of further optimized versions of the vaccine and the development of immunomonitoring assays suitable for both HLA class I and II T cell responses and for different HLA backgrounds. Pentamers specific for these epitopes were synthesized and made available to the Consortium for testing. With these reagents and using ELISPOT-based tests, assay development was performed for monitoring immune responses in the clinical setting. All the Id sequences collected in the database were analyzed to assess the degree of conservation of validated immunogenic epitopes among different cases. The results obtained indicate that the load and type of mutations affecting VK3-20 light chains probably do not constitute a major obstacle to the use of the prototypic VK3-20 molecule as a vaccine for the treatment of VK3-20+ lymphoproliferative disorders. The ability of VK3-20 to induce T cell responses cross reacting also against similar but unrelated immunoglobulin light chains stimulated studies to verify whether the immunogenic epitopes of VK3-20 are also shared by other VK genes. This with the aim to better define the spectrum of lymphoid tumors against which VK3-20-

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based vaccines could be effective. The analysis showed that most of the VK3-20 epitopes are substantially conserved in most light chains of the VKIII family and shared also by a number of other VK families, which are extensively used among B cell tumors. The ability of vaccine induced T cell responses to cross react against slightly modified epitopes carried by similar but unrelated Id proteins was directly demonstrated by functional experiments.

The work carried out in **WP4** focused on studies on the identification and validation of B cell epitopes within Id proteins of interest. Six mouse monoclonal antibodies selected as reactive against VK3 light chains have been obtained so far. Two of these antibodies are able to stain these light chains expressed at the cell surface of lymphoma cells, being thus reagents of possible diagnostic value. Moreover, one of these antibodies was also shown to mediate cytotoxic effects *in vitro* in the presence of immune effectors, thus deserving attention for the possible therapeutic activity. Using libraries comprising cyclic and linear peptides 20-mers and linear 10-mers, Partner **PEPTHER-8** identified one specific antigenic region in the VK3-20 protein. The epitope was characterized to a very high resolution using a so-called full replacement analysis and the amino acid side chains involved in the protein binding identified. Although the dominant epitope was found in mapping studies of all anti VK3-20 mAbs, it is largely hidden at the interface of the light and heavy chains in the complete IgG protein. Therefore, additional antibodies to VK3-20 were elicited with rational designed peptides based on a three dimensional structure of VK3-20 in its native context with a VH chain. These peptides were used to immunize mice that were boostered with recombinant VK3-20. Polyclonal anti-VK3-20 peptide antibodies were obtained, and were shown to bind to the VK3-20 protein as a recombinant protein. Work is in progress to develop additional anti-Id monoclonal antibodies with potential diagnostic and therapeutic relevance.

The work carried out in **WP5** focused on new means to optimize the immunogenicity of Id proteins of interest. To this end, the VK3-20 protein was chosen as a model. Introduction of particular domains within the sequence of the prototypic VK3-20 protein resulted in modification of its immunogenic features and in changes of the type of T cell responses induced. These results have been obtained also through an exchange of personnel between Partner **CRO-1** and Partner **KI-3**. Moreover, analysis of the epitope sequences found in **WP3** led to the identification of sequence variants able to enhance the induction of T cell responses. Exploitation of these results may lead to a further optimization of the identified vaccines.

The **WP6** was aimed at developing and validating Id-specific assays useful to monitor immune responses in the clinical setting, particularly in vaccinated patients. For Id-specific T cell responses, general assay validation was achieved. Epitope-specific assays have been also developed, and their full validation will be completed shortly after the end of the project. Partner **IMMUNE-6** has been able to set up validated immune monitoring assays for intracellular cytokine staining, and for ELISPOT analysis for IFN-γ and IL-2. These assays have been set up in compliance with good laboratory practice (GLP) standards.

The **WP7** was aimed at validating patient-derived Id vaccines in animal models. B cell lines expressing target Id proteins were tested for *in vivo* growth and reliable

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models have been set up. This work took more time and efforts than planned, due to the unexpected down-modulation of MHC class I and II molecules occurring *in vivo*. This problem was solved through the ectopic expression of HLA.A2.1 in target B cell lines. Characterization of Id-specific cytotoxic T cells (CTLs) generated from different donors demonstrate that both CD4+ and CD8+ cytolytic T cells capable of specific lysis can be isolated, an aspect that may open important clinical applications. Moreover, evidence has been provided demonstrating that VK3-20-specific CTLs have a tumor growth inhibitory activity *in vivo.* Similar findings were also obtained in other mouse models further supporting the immunogenicity *in vivo* of Id proteins. Using Epstein-Barr-associated B cell lymphoproliferations as a model Partner **UNIPD-4** tried also to address the importance and the efficacy of Ag-specific cytolytic CD4+ T lymphocytes in adoptive therapy protocols. Data obtained in the EBV-PTLD mouse model indicate that EBV-specific cytotoxic CD4+ T cells are therapeutic in mice bearing PTLD-like tumors, even in the absence of CD8+ T cells. These findings pave the way to use cultures of pure CD4+ T cells in immunotherapeutic approaches not only for EBV-related malignancies, but also for other different tumors, including EBV-unrelated lymphomas. Recombinant Id proteins were also shown to induce humoral responses, although repeated immunizations were needed to obtain antibodies able to detect the native light chain expressed on surface of lymphoma cells and thus potentially able to mediate clinically relevant responses.

The activities related to **WP8** started were aimed at identifying suitable adjuvants and new delivery systems for Id vaccination. Tests for immunogenicity and immunizations for antibody production have been carried out by Partner **UNIPD-4** using the classical CFA/IFA adjuvant, which represents a reference for such kind of approaches but nevertheless has limited or no use for clinical purposes. Despite initial agreements with external collaborators who should have provided the IC31 and CoVaccine HT adjuvants, these reagents were not made available for testing to the VITAL Consortium. As an alternative, innovative option, Partner **UNIPD-4** extensively characterized a natural polysaccharide, namely Hyaluronic acid (HA), a biodegradable, biocompatible, non-toxic, non-immunogenic and non-inflammatory anionic biopolymer, which plays pivotal roles in wound healing, cell motility, angiogenesis as well as construction and integrity of extracellular matrix. The results obtained demonstrate that HA can act as a powerful "natural" adjuvant and could be exploited for the development of new vaccination strategies. Partner **UNIOSLO-2** extensively characterized recombinant vaccines (vaccibodies) using chemokines as tools to target antigens to antigen presenting cells. Vaccibodies equipped with LD78β isoform of human CCL3 have been constructed and tested *in vitro* demonstrating that this chemokine targets antigen delivery to both mouse and human antigen-presenting cells affording augmented T-cell responses as compared with non-targeted control vaccibodies. Thus, LD78β vaccibodies have been constructed having prototypic Id proteins assembled as single chain variable region fragment (scFv). Immunogenicity of this vaccine has been characterized in balb/c and D10B2 mice. DNA vaccination with electroporation as a delivery system was also investigated. Partner **PEPTHER-8** will provide a new adjuvant based on sugars and detergent for vaccination studies that will be carried out on a collaborative basis beyond the time frame of the present project.

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The work carried out in **WP9** focused on the development of a GMP-ready production process based on the use of disposable bioreactors and able to generate titers of recombinant Id proteins up to 20 mg per liter, ready for the scale-up to the clinical phases. The process was studied by Partner **ARETA-7** comparing the fermentation process in the new disposable bioreactor with the traditional system of shake flasks. The results obtained so far indicate that it is possible to utilize the disposable bioreactor for the fermentation of bacteria and the production of the Id proteins of interest. The protocols for the production of the recombinant VK3-20 have been optimized, resulting in a detailed set of procedures for the upstream and downstream process of production. The product is then subjected to treatment for endotoxins removal in order to generate a clean and safe product, and then it can be formulated as a KLH conjugate if necessary. Partner **Areta-7**'s Quality Operations group started the preparation of the Drug Master File, a document describing the characteristics of the product, the manufacturing process and the quality control tests to be performed in order to assess the safety, quality and potency of the product. Partner **Areta-7** also defined the characteristics and the set of tests necessary for the preparation of the Master Cell Bank.

The work carried out in **WP10** was mainly devoted to the definition of the best strategies to disseminate and exploit the results of the project. Intellectual property issues were also carefully analyzed. Some patents have already filed and other promising leads for Id vaccination and diagnostics will be patented. Collaborations among Partners beyond the timeframe of the project were also discussed and defined. A final report and a second leaflet highlighting the work performed as well as the outcome and the concrete results of the project were prepared.

3.4 End Results & Impact

The most innovative aspect of the VITAL project was constituted by the idea to exploit the molecular features of Id proteins of distinct B cell lymphomas/leukemias, particularly those pathogenically associated with a chronic antigenic stimulation, to develop pre-made, recombinant Id proteins to vaccinate subgroups of lymphoproliferative disorders expressing molecularly correlated idiotypes. Through the synergistic activity of Academic and SME Partners, the VITAL project has led to the precise identification of subsets of B cell lymphoproliferative disorders characterized by the expression of molecularly similar Id and thus targetable by pre-made Id vaccines for prevention and treatment purposes. A first set of Id proteins to be used as "shared" vaccines have been identified and characterized both *in vitro* and *in vivo*. Notably, the extensive immunologic characterization of Id proteins of the VKIII family provided the "proof of concept" that selected Id proteins may elicit immune responses that cross-react against a broad spectrum of molecularly similar Id expressed by various lymphoproliferative disorders. These findings indicate that it is now possible to overcome the current complexity and costs due to the individualized production of Id vaccines. The subset-specific vaccines developed within the frames of the VITAL project retain a high selectivity for tumor cells combined with a good tolerability and more affordable costs. A newly developed

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"natural" adjuvant, hyaluronic acid, was included in a first vaccine formulation ready for clinical studies. New delivery systems (vaccibodies) able to enhance antigen targeting to antigen presenting cells have been also characterized. The project also led to the development of reagents suitable for the monitoring of idiotype-specific immune responses in the clinical setting (Pentamers, ELISPOT assays, monoclonal antibodies, etc.). More importantly from an industrial perspective, the VITAL results open new avenues for European SMEs active in the market of cancer immunodiagnostics and therapeutics.

The VITAL Consortium made a great effort to characterize the ability of selected Id proteins to induce specific T cell responses. This is a clinically relevant issue since most of B-NHL patients are currently treated with anti-CD20 monoclonal antibodies, which are known to deplete host B cells thus impairing humoral responses.

The results obtained also indicate that some of the Id vaccines developed may be used not only for the treatment of overtly malignant lymphomas, being administrable also for preventive purposes in defined clinical settings. This is the case of HCV-related cryoglobulinemia, a pre-lymphomatous condition sustained by B cell clones frequently expressing the same Id proteins of HCV-associated lymphomas. Therefore, vaccines targeting these "shared" Id proteins may reduce the burden of the expanded B cell clones, thus mitigating the symptoms and preventing the evolution of the disease to an overt lymphoma.

Identification of several subsets of B cell lymphoproliferations expressing shared Id proteins allowed the estimation of the size of the potential market for the Id vaccines developed. The results so far obtained, particularly those relative to subsets of CLL, suggest that the number of patients treatable with subset-specific Id vaccines is probably higher than previously hypothesized. A GMP-ready production and purification process of these recombinant idiotypic proteins has been developed and a first phase I/II clinical trial is expected to start soon after the end of the project.

These pre-made vaccines, once validated as drugs in clinical trials, will have the advantage to be easily distributed to all Hematology and Oncology Departments, including those of peripheral Hospitals/Universities. Thus, results obtained in the present project will have an important strategic impact in solving, at least in part, the dramatic social and health problem represented by NHL.

Finally, a strong network between European research institutions and industry has been established opening new co-operation possibilities in the field of cancer vaccines.

4 Dissemination and Use

Dissemination activities

Dissemination activities are very important to support the deployment of project results, as they can adequately present the findings obtained with style and format suitable for the potential users and customers and can spread such information to a wide audience of interested partners, in order to create a network of contacts. During the VITAL project, all involved Partners presented the main objectives and some updates on the intermediate results to the scientific community. The dissemination was performed at several national and

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worldwide events that covered a wide range of audiences, being purely scientific meetings or business partnering events. Particular efforts have been made by participating SMEs to highlight the features of the VITAL project at International BIO events. In these occasions, current medical needs and the concrete possibility of generating innovative products for the market have been emphasized. The results of the VITAL project and, particularly, their impact in terms of therapeutic and diagnostic innovation will be also presented in the near future at international meetings gathering both scientists and clinicians involved in the treatment of lymphoproliferative disorders.

In terms of scientific publications, a first set of papers have been published in international journals. Most part of publishable results has been obtained in the last part of the project. After careful selection of publishable results, we have concluded and submitted to major journals several other papers describing the main results of the VITAL project. Other manuscripts are currently in preparation. It is also expected that the collaboration beyond the time frame of the VITAL project will allow to obtain additional publishable results.

The final publishable report will be made available on the public VITAL website.

Exploitation of results

The VITAL Consortium has thoroughly discussed the strategies to exploit the results obtained so far as well as those that are expected to be achieved after the end of the project. As a first step, a phase I/II clinical study based on the use of recombinant VK3-20 (Patent PCT/IB2008/001936) Id vaccine for patients with low-grade B-NHL will be activated. Partners **CRO-1** and **Areta-7** are producing the documents necessary for the required authorizations by competent regulatory Agencies. The design of the study is currently in progress. A second trial will be activated with the same Id vaccine, but administered to patients with symptomatic cryoglobulinemia and clonal expansions of VK3+ B lymphocytes. This study will be carried out in collaboration with the Rheumatology Unit of the University of Udine and will have as primary endpoints the reduction of symptoms and the prevention of possible B-cell malignant lymphomas. Additional optimized Id vaccines can be patented in the near future.

During the VITAL project, Partner **Areta-7** worked at the optimization of the process for the production of the candidate Id proteins. The manufacturing of such products requires an innovative approach that must take into consideration the possible "personalization" of the drugs and therefore be very flexible and sustainable in terms of costs even at the small scale. The work resulted in the set-up of a manufacturing process using single-use, plastic bag bioreactors for the preparation and fermentation of bacterial cultures. To date, this is one of the first application of this new technology for the production of recombinant proteins in bacteria. Its major advantage is the possibility of reducing the risks of the process and the cost associated to the cleaning validation. Partner **Areta-7** will benefit from this acquired knowledge having the possibility to use this technology to expand and improve its manufacturing activities in the field of innovative biological drugs.

The protein candidates identified during the VITAL project are very interesting products that are currently under evaluation for the possibility of patents. The protection of the intellectual property of these proteins will allow the generation of potential value and assets that will be used to attract investments

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to support the clinical development of the new drug products. The possibility to generate a spin-off based on the exploitation of these innovative vaccines is carefully considered at the present time and is being discussed by the Partners involved.

The interaction of Partner **Areta-7** with the academic Partner **CRO-1** resulted in the consolidation of a partnership that will continue after the VITAL project with the aim of making available to the patients the new candidate vaccines as soon as possible with the activation of clinical trials. Moreover, the VITAL project allowed Partner **Areta-7** to meet and interact with several other players and leaders in the sector of therapeutic proteins development. Some of these entities perform activities that might be well supported by the services offered by Partner **Areta-7**, therefore resulting in the expansion of this SME across Europe.

Apart from the specific results produced in the project, Partner **IMMUNE-6** has added significant internal experience to the use, running and quality standards of its unique REVEAL Rapid Epitope Discovery System through the work conducted as part of VITAL. Since the REVEAL Platform is already commercially available, Partner **IMMUNE-6**'s broad customer base will be able to benefit immediately from numerous technical improvements made to this Platform. With respect to the specific deliverables of the project, the parties intend to continue to cooperate to complete the picture further on epitope validation and characterization beyond the scope of the project and to build a more complete picture of the antigenicity of the Id sequences under investigation for the purpose of defining the best possible set of final vaccine candidates. Partner **IMMUNE-6** will discuss publication and protection of any relevant knowledge with the other partners as appropriate. Where appropriate and with agreement of the other partners in the consortium, Partner **IMMUNE-6** will also use its own newsletter platform, which has a circulation of approximately 100,000 scientists to disseminate the results of is participation in the project. The readership of this newsletter also includes a significant number of scientists working in leading pharmaceutical companies, which increases the likelihood that the results of the project will lead to further collaboration with such companies. This could be of significant help in taking the technologies developed in the project further forward towards clinical applications.

A patent application "HOMODIMERIC PROTEIN CONSTRUCTS", inventors Pier A. Ruffini, Agnete B. Fredriksen, B. Bogen has been filed by Partner **UNIOSLO-2** via the employer of the inventors, Medinnova at the Oslo University Hospital - Rikshospitalet. The patent application describes that the human chemokine CCL3 (LD78β) can be used as targeting unit in vaccibodies.

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