

# Development of anti-idiotype vaccine for human follicular lymphoma

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Non-Hodgkin's lymphomas (NHL) constitute a heterogeneous group of malignancies whose incidence has significantly increased in recent decades. In the year 2000, more than 145, 000 cases of NHL were diagnosed in developed countries, representing thus the sixth most common cancer occurring among men and the eighth among women. Low-grade B-cell NHLs, in particular, are incurable diseases characterized by relatively slow growth and excellent initial responsiveness to chemotherapy but also by continuous relapses. In particular, for patients with follicular lymphoma, median overall survival (7-10 years) has not improved over the past 30 years.

Although in the vast majority of patients complete or partial remissions can be obtained with either single agents or combination chemotherapy, the clinical course is characterized by a high relapse rate. After relapse, both the response rate and relapse-free survival after subsequent salvage treatment regimens steadily decrease, resulting in a median survival of only 4-5 years after the first relapse. These clinical findings, coupled with the substantial toxicities of standard treatments, have stimulated the search for novel and more tumor-selective therapies.

Follicular lymphoma is a clonal B cell malignancy that expresses a unique antigen that is formed by the immunoglobulin light and heavy chains that possess highly variable regions at their amino termini. These variable regions combine to form the antigen recognition site, which can itself be recognized as an antigen, termed the idiotype. The antigen-binding site is a structural feature of each immunoglobulin that distinguishes it from other immunoglobulins. The idiotype of a particular clonal B cell lymphoma

represents a tumor-specific antigen. Idiotype is a target of interest in human lymphoma.

Therapeutic vaccines targeting B cell lymphoma idiotype (Id) represent a promising immunotherapeutic approach for a better clinical control of these malignancies.

Immunoglobulin (Ig) molecules are composed of heavy and light chains that possess highly variable regions at their amino termini. B-cell malignancies are clonal proliferations of Ig-producing cells. The idiotypic determinants of the surface Ig can thus serve as a tumor-specific marker for the malignant clone.

Indeed, 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. 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. On these grounds, new strategies obviating the need to produce customized vaccines would further simplify clinical applications of idiotypic vaccines.

Goals:

Goal 1:

Establishment of a large database including sequences of idiotypic VH and VL genes expressed by a variety of lymphoproliferative disorders, including low grade B-NHL, autoimmunity-associated lymphoproliferations, and chronic

lymphocytic leukemia. This will allow the identification of candidate Id proteins for “cross-reactive” immunotherapy.

#### Goal 2:

Pre-clinical characterization of the immunogenicity of selected natural Id proteins, with particular regard to their ability to induce immune responses against lymphoma cells expressing molecularly correlated Id proteins. The characterization will include the identification of B cell epitopes and HLA Class I-restricted cytotoxic T cell epitopes using innovative approaches and will allow the development of dedicated assays for immunomonitoring.

#### Goal 3:

Design and validation of optimized Id vaccine.

#### Goal 4:

Evaluation and validation of new adjuvants and innovative delivery systems for improved Id vaccine formulations and administration.

#### Goal 5:

“Clinical-grade” production and purification of optimized Id proteins for patient vaccination.

#### Introduction

There are approximately 65, 000 new cases of non-Hodgkin’s lymphoma diagnosed each year in the US with a comparable number in Europe. Despite the use of aggressive chemotherapy and recent advances in therapy such as monoclonal antibodies (Rituxan, TM), the disease is almost invariably fatal.

Follicular lymphoma (FL) patients, in particular, can have an indolent but <https://assignbuster.com/development-of-anti-idiotypic-vaccine-for-human-follicular-lymphoma/>

ultimately fatal clinical course. The median relapse time for FL patients is three years, with 90% of patients dying of a tumor-related mortality within 7 years of the date of diagnosis.

The clinical course is usually characterized by a series of remissions and relapses. Good response rates are seen with treatments such as chemotherapy, radiation, lymphocyte transplantation, and monoclonal antibodies. However, following initial response to treatment, the cancer invariably returns and the majority of patients relapse with resistance to all available therapy. Related B-cell derived neoplasms include multiple myeloma (approx. 15, 000 cases/year in the US) and chronic lymphocytic leukemia (approx. 10, 000 cases/year in the US).

Isolation of tumor-specific antigens (TSAs) has been a long sought-after goal for scientists involved in both basic and clinical research. Whereas tumor-associated antigens (TAAs) are localized on both normal and tumor cells, TSA are peculiar to tumor cells. This characteristic makes TSA a very desirable target for immune therapy strategies aiming to spare normal cells, or at least the indispensable ones.

As regards effectors mechanisms, although some indirect evidence exists for participation of both natural killer (NK) cells (especially those activated by IL-2, known as lymphokine- activated killer, or LAK cells), and TNF-secreting macrophages in tumor immunity, most interest has been focused on the role of antigen-specific antibodies and T lymphocytes. This is particularly true among scientists developing anti-Id vaccines for human FL, even though no substantial agreement has yet been reached on which of the two main effectors pathways is most important.

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FL conforms to the general rule that tumors have several mechanisms to escape the attention of the immune system. The risks that Ig somatic hypermutations could result in aminoacid residue replacements leading to substantial changes within the fine immunogenic structure of the Id do not seem to be so relevant. Indeed, no such occurrence has been reported in any of the several dozen patients who have been immunized over the last decade. A much more relevant issue is the very limited ability of FL cells to present their own antigens.

Although ontogenetically very close to normal mature B-lymphocytes, with respect to their normal counterparts FL cells are very poor as antigen presenting cells (APCs). This makes it rather difficult to evaluate any vaccine-induced, tumor-specific cytotoxicity even in vitro. On the other hand, no such problems exist for ELISA-based detection of the tumor-specific and vaccine-induced humoral response.

The first study of anti-Id vaccinations in humans dates only from 1992. Until then, all the work had obviously been confined to animal models. However, the accumulation of experimental data has led to the development of several promising strategies that are currently being investigated in clinical trials. These include the utilization of the Id in the form of a soluble protein or as a DNA sequence, either used to pulse dendritic cells (DCs) or else to be administered in combination with immunologic adjuvants.

Soluble protein Id vaccine production is based on a hybridoma technique, which in vitro allows production of exactly the same Ig as that present on the surface of the clonal B cells of FL, or in other words the tumor-specific Id. The suspension of single cells obtained from a biopsy specimen almost invariably

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contains a residual population of normal B-lymphocytes alongside the tumor cells. Screening of the hybridomas by means of Ig heavy chain CDR3 PCR identification is therefore required in order to make sure that the Ig of the selected hybridoma is truly identical to the tumor-associated one. 31 Once the cultured hybridoma has yielded enough purified Id, the TSA needs to be made far more immunogenic than it is in its free form. For this purpose, it may either be conjugated with a highly immunogenic carrier such as keyhole limpet hemocyanin (KLH), or else used to pulse autologous DCs.

The association of a soluble protein Id vaccine with immunologic adjuvants monocyte colony-stimulating-factor has also proved extremely important. Granulocyte factor (GM-CSF) currently seems to be the best such adjuvant both in animal models and humans, probably because of its capacity for local recruitment of DCs in vivo at the site of vaccine injections. This step would appear to be superfluous when autologous DCs are loaded with Id ex vivo and then re-injected into the patient.

A completely different alternative approach involves administration of the patients' Id-encoding DNA sequence. With the rise of molecular technology, such DNA vaccines are beginning to come into their own. For instance, exploitation of appropriate molecular vectors (ie containing both a leader and promoter sequence) for insertion of the nucleotide sequences responsible for biosynthesis of both the Ig heavy and light chains variable regions is now relatively easy.

Between the heavy- and light-chains variable regions sequences, an intertwined linker peptide must also be inserted to allow the ultimate Id-containing molecule (scFv) to fold properly. Furthermore, the vaccine can be <https://assignbuster.com/development-of-anti-idiotypic-vaccine-for-human-follicular-lymphoma/>

further strengthened by adding other DNA sequences encoding for immunologic adjuvants or powerful immunogens to the vector. Finally, intramuscular injections allow progressive release of the Id following synthesis by muscular cells. In addition, this administration route seems to be associated with prolonged conservation of the genetic information within the cells without any apparent signs of integration into their genome.

### Experimental Design

#### Goal 1:

Establishment of a large database including sequences of idiotypic VH and VL genes expressed by a variety of lymphoproliferative disorders, including low grade B-NHL, autoimmunity-associated lymphoproliferations, and chronic lymphocytic leukemia. This will allow the identification of candidate Id proteins for “cross-reactive” immunotherapy.

In establishing a database, there will be steps to follow in order to support the evidences claimed.

I. Finding the cases and the occurrence of VH and VL in lymphoproliferative disorders in different hospitals and institutions that could provide valuable information for the said disorders. The facts and information should have the confirmed consent of the persons involved.

II. Subjecting the cases to thorough analysis to provide the essential information needed in documenting the cases.

III. Testing the subject under the identification of Id proteins.

IV. Organizing the information and establishing the database.



## Methods:

Establishing a database to easily organize information and data needed in making the information available readily whenever they will analyze situation in which there is a suspected occurrence of lymphoproliferative disorder. By providing the information needed, they could develop system that would make things easier for them to do actions required in addressing such situation.

Primary step is consolidating all the available facts and information provided that they have the consent from the owner of the information. By having the desired facts available for the reorganization of it, they could classify it according to the general category they want to use in creating their database. It could be based on severity of the case or could be base on gender or any factor that could greatly affect the situation.

Then, by gathering the information they needed, the analysis of the data should be carefully done for them to eliminate excess and negligible data for an easier organization of the structure of their available resources. By implicating the main thrust of the database to the core concept of having advance cases of NHL, the higher chance they could get the information and the data based on the clinical findings of actual patients and people who suffered from that.

By simply opening the way of introducing different vaccines in addressing the situation, they could develop a system of transferring and managing information that could make things easier especially in developing new technology and medicinal advancement in creating a better and more

effective ways of treating such disease. By making the information more manageable, they could likely innovate an advanced communication that would lead them in establishing better information and data management for the use of the development of vaccines and cures.

Since they have the information but they should examine carefully every bit of information that will be a part of their set of data and facts. By looking closely to the subjects result and the specification of the action done, the development of such process in introducing a new finding on the matter should be considered. Since the goal is to establish a database that will focus on the information that could provide the facts needed on the cases diagnosed with a NHL, it is important to screen the cases as important and not negligible for them to be able to use it as a case.

At the end of the process, they would go back to their primary goal and that is to establish a functional database that the core information and the key factors are integrated in a way that it would make the processing of facts and vital data would be efficient and effectively handled.

Also, it will introduce technology that would compensate the fast rising of development in technological advancement even in the field of medicine. Because there are ready to use valuable information for them to handle and initialize their desired action, they would be able to commend the different opportunities in which they could get specimens and studied it for future discoveries and researches.

In all, by their incorporation of the cases of NHL and their desired goal of making the information available for them to be able to easily study and

review the situations and cases they previously have for them to execute and evaluate the validity of the existing tests in the current occurrence of the disease in the real place.

Goal 2:

Pre-clinical characterization of the immunogenicity of selected natural Id proteins, with particular regard to their ability to induce immune responses against lymphoma cells expressing molecularly correlated Id proteins. The characterization will include the identification of B cell epitopes and HLA Class I-restricted cytotoxic T cell epitopes using innovative approaches and will allow the development of dedicated assays for immunomonitoring.

In dealing with the pre-classification of the immunogenicity of selected natural Id proteins, the processes involved are:

- I. Accumulating soluble proteins to be tested.
- II. Testing them with hybridoma essential in testing the equivalence of the tumor inducing material that leads to development of the tumor.
- III. Inducing the effect of the proteins and identifying its effect on B cells.
- IV. Using advance technique in analyzing the result and implicating with the use of the modern testing equipment and processes.

Accumulation:

Testing the proteins for it to be classified will be the first step. From the patients who are suffering from FL, different samples will be getting for the medical technology to be applied. Then by cultivating natural proteins, they will use it to further test the capacity of the natural cell in penetrating and

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deeply interacting with other Id proteins in the development of resistance to such substance.

By eradicating some external factors such as the presence of other organisms, they could screen the protein level for them to be able to produce and test the Id proteins by exposing it to toxoids that could develop resistance on the desired solution. Then, the Id proteins gathered will be stored for further testing.

Testing:

Then, preparation of the Id protein to be tested will be carefully done in a controlled environment. Since the tumor development can not be detected by the immune system, the development of inducing material will be necessary for them to penetrate the basic defenses of the tumor.

By exposing it to NKL, tumor will exhibit a different behavior but will not be extinguished. Since B cells epitope derived multiply myeloma that had been the major cause of the return of the behavior of the tumor cells, the gradual exposing it to be classified by soluble Id proteins will be dedicated.

The allowance of certain percent productivity will be the basic goal of the clinical testing for them to be able to derive the pre-classification scheme that will determine substances that induce immunity on certain level with the use of soluble and Id protein present in the environment.

Effect and its Identification

After the testing had been carefully done, they will examine its effect on various elemental positions by trying the substance on the possible outcome.

Then, FL cells will be isolated then proteins will be added to see the effect on the neoplasms produced by B-cells. Since the outcome would produce certain behavior that will exhibit a different expected one, the process will be repeatedly associated with soluble proteins to target the development of TSA since it target tumor cells.

### Analyzing the Result

Then, the result will be analyzed in a way that it consistently produces same output. Then after looking closely and making sure that no other substance induced the effect, the validity of the result will be the next concern in analyzing the data. For it to be valuable, the result should consist the scientific analysis of the vaccine to be introduced for them to be able to adopt a real one.

### Goal 3:

Design and validation of optimized Id vaccine.

Since the protein had been introduced in TSA that would target tumor cells, it is important to develop the next stage wherein it will pay attention to that. Tumor cells, after being extinguished by some other methods, always come back and provide a worse situation than before. It is a common problem of the development of cure because as soon as they introduce stronger antigens and antibiotics, the cells develop stronger immunity to them, making them more powerful and gave them the power to come back and come back whenever they are defeated.

The result of the previous testing of the material will be used as the raw data in determining the precise development of the vaccine needed for the tumor

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cells. Toxoids produced by microorganisms will be introduced to the tumor cells for them to create an astounding reaction with the cells to help the antigens produce a better shield to the tumor cells.

They would also address the production of its own immunity by targeting the B cells epitopes produced by the tumor cells for them to be able to weaken the effect of the tumor cells in the body. By simply having the same effect on the cells, they would establish the immunity desired.

In addition, since soluble proteins produces amino acid residue, the effect of it to the development of various outgoing tumor cells will be beneficial in the sense that it would catch up the screening proves by a hard core stimulation of heavy chain CDR3 PCR. Then, the use of material that would likely predict the behavior will also introduce for the existing antigen to determine it.

Goal 4:

Evaluation and validation of new adjuvants and innovative delivery systems for improved Id vaccine formulations and administration.

After having the result of the vaccine being tested hand-on on the tumor cells, the activation of the production of antigens will be manipulated for those to be able manage the outcome of the result. By having the systematic chain of micro toxoids that will enter the entire system of the body, they would likely produce different kinds of reactions that would benefit the production of self stimulating antigens.

By exposing it to different procedures that would attest the certainty and validity of the desired production, after introducing different sets of toxoids

and NK cells, the development of the self inducing multiple protein will help in eradicating the symptoms and the effect of FL.

It is known that FL is fatal in terms of its effect on the human entire system. So it is important to devise a precise way of handling and dealing with it for them to be able to have an outer perspective of the natural phenomena.

Id proteins will act as binders to the solutions that will be used in strengthening NK cells and TSA to promote the development and inhibit the further production of malignant development of tumor cells. By preventing the further growth of it, they would have larger revenue in which they could satisfy the needs and the improvement for having a stronger antigen.

Then, natural growth of TSA will be affected by the inducing of soluble protein to target dendritic cells for them to be able to manifest the basic function of fighting foreign toxins that could affect the development of the tumor as a vital implication of the vaccine. The use of different methods in determining the feasibility of inducing the growth and the development of natural antigens that would be sufficient enough to fight the invading tumor cells will be of great use for them to be able to produce more antigens that will prevent the further worsening of the situation.

Validating the use of the vaccine as one of the potential sources of defense against the foreign material invading the system would be beneficial if they could handle the needs of having a more systematized and organized level of founding a solution that would focus on the elimination of cell processes that inhibits the growth of malignant tumor like FL that is fatal to humans.

Subjecting enough NK cells to further strengthening process will help them in making the process worthwhile. Making a protein Id that would address the situation as founding solution to the antigen development.

Goal 5:

“ Clinical-grade” production and purification of optimized Id proteins for patient vaccination.

After developing the vaccine the process would involve the following:

I. Purifying the Vaccine to be prepared.

II. Final Verification

III. Mass Production

IV. Patient Vaccination

Purification

After devising the vaccine, the next step is purifying it by eliminating microorganisms that would have effects on the vaccine. By continuously subjecting the vaccine into different microorganism killing environment, they would lessen the potential of having such. Radiating and constantly developing processes will be sufficient in terminating such microorganisms.

Final Verification

After the purification of the vaccine, a method will be done for them to be able to test if the results are really valid by having it tested for final verification. It is important to deal with it because the importance of verifying the vaccine would greatly affect its validity in the medical society. By



having it tested through lab rats or animals that have developed FL tumors; they would be injected with such vaccine for them to see if the previous results will be the same.

### Mass Production

After the verification process, the next process will involve producing the vaccine enough for human consumption. The proteins that deal with the development of TSA would have a various report on its validity and essentialism for them to be able to have a developed system of introducing vaccines.

### Vaccination

After the production and the vaccine is ready to use, it would be given to the patients, as long as it is approved by the medical board, to be used as vaccine against the development of FL into malignant tumors that endangered the lives of many people. Then, by having the system of production of certain involvement of the NK cells within the hybridoma of dendritic cells, the vaccine will be of much use since it will introduce antigens that will prepare the body for the possible FL development.

Since there are certain kinds of toxoids that will be introduced, there will be a harsh reaction at first to the place where it is injected because of the behavior of the toxoids and the T cells of the body. This is a sign that the vaccine is effective and doing a reaction that would strengthen the immune system of the body.

### Discussion

After the development of the vaccine in FL, it is important to understand the need of developing such because of its adverse effect on the development of humans. It endangered the lives of many people without having the prior notification of the said disease. This is a vital step in the clinical world.

Considering the existence of the natural antigens present in the environment, by the use of Id proteins that inhibits the growth of tumor cells; it would be beneficial to mankind if the continuous development will take place. By exploring the kind of the interaction ventured in this kind of process, the elemental composition of the vaccine would be developed to address the needs of the people in having the desired implication of the subject process.

The processing of vaccine would include the development of stages in which it would acknowledge the presence of the cells responsible for the development of the disease. In effect, they would have a better understanding on the subject, matter and would increase the possibility of having a curable state.

By implicating the notion of having a different technique in addressing the development of the vaccine, the question left for it is how long would it last for them not only to develop vaccine but also to develop a cure that would forever block the negative effects of the disease. By using and ensuring the safety of the user, they would have a proper citation of the needed plan for them to be able to execute the importance of the vaccine and its use in the modern life.

Furthermore, by examining the application of the vaccine in the curing of the disease, we would see the importance of development and use of innovating techniques in determining the possible outcome of the curing of the disease.

Finally, the consideration of the process if it fits the standards of the medical consideration despite the fact that there exist different processes that involve much medicinal advancement should take into consideration the impact of the introduction of this vaccine prior to the ethical understanding of the matter. Since FL can be considered as one of the deadliest disease that one can have, the help of having a vaccine against it is beneficial to the human industry.