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Future of idiotypic vaccination for B-cell lymphoma

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During the last three decades, idiotypic vaccination has cleared all the hurdles between preclinical studies and randomized clinical trials, assuming the forerunner role among human therapeutic cancer vaccines. However, over the last few months, the very same Phase III clinical trials that were supposed to consecrate idio type vaccines as the first such products to reach the market have, one after another, begun failing to achieve their main end points. While their negative outcome had been predicted long ago, it must be underlined that most clinical studies on idiotypic vaccination have shown a substantial correlation between induction of an idio type-specific immune response and improvement of clinical outcome. Failures of late-stage clinical trials were due to severe pitfalls in their study design and not necessarily to idiotypic vaccination itself. Therefore, it is desirable that dedicated scientists and clinicians persevere until confirming ultimately the clinical benefit of a broadly and readily available idio type vaccine.

KEYWORDS: clinical trial • idio type • lymphoma • vaccine

The term idio type refers to the whole collection of idiotopes displayed on an individual immunoglobulin molecule. Idiotopes can be found exclusively in the hypervariable regions of the immunoglobulin variable domain, are somatically generated and are recognized as foreign due to the fact that the tiny amount of them typically present in any individual is insufficient to elicit the activation of any self-tolerance mechanism [1]. Idiotopes may be classified into two categories: public and private idiotopes. The former is largely derived from the immunoglobulin's framework region sequences, whereas the latter arise from the unique immunoglobulin's complementarity-determining region sequences. The implications of this different localization are extremely important, particularly when we consider the whole immunoglobulin no longer as an antibody, but rather as an antigen in the context of cancer vaccine-related, anti-idiotypic immune activation. In particular, only humoral responses against the private idiotopes will have value for tumor suppression, since other antibodies, if at all raised, will be absorbed by the serum immunoglobulins. Similarly, only private idiotopes will ultimately function as a collection of clonal markers for each tumor [2]. Indeed, since each immunoglobulin features its own idio type and identical idiotypes define identical immunoglobulins, the clonal idio type of a B-cell

malignancy can serve as a complete, tumor-specific antigen for vaccine therapy, provided that the tumor cells express it intact as their functional B-cell receptor on the cell membrane and in the form of idiotopes associated with the HLA molecules for epitope presentation [3].

Basic features of an idio type vaccine

An idio type vaccine must be powerful enough to break immune tolerance. In this respect, it may seem appalling that the most advanced human cancer vaccines have been developed against tumors such as lymphoma and myeloma. These tumors originate in the very immune system and, as such, should be among those that most induce immune tolerance and least warrant harnessing the immune system's potential against them. Yet, several observations have demonstrated that, in the presence of a *bona fide* complete and tumor-specific antigen, it is possible to produce a vaccine capable of breaking such a biological barrier, indicating that tolerance must be either incomplete or possible to circumvent [2].

Whichever the method used to obtain the tumor-specific soluble protein idio type, the most developed vaccine formulations employ it after chemical conjugation with the clinical grade, powerful immunogenic carrier keyhole limpet hemocyanin (KLH) [4]. The function of this molecule is that of enhancing idio type

immunogenicity, which, despite the specificity of the private idiotope collection, remains substantially low. Furthermore, clinical grade granulocyte–monocyte colony-stimulating factor (GM-CSF) has emerged as the best immunological adjuvant to complete the vaccine formulation [5]. Idiotypic and KLH doses (0.5 mg of each per vaccination) have remained unchallenged from the original Phase I studies, whereas the dose of GM-CSF has ranged from 100 to 500 µg per vaccination, depending on the study design, but with no evidence of different outcome among different trials [6]. So far, both impressive clinical results and looming controversies associated with idiotypic vaccination have been confined to follicular lymphoma [7]. Therefore, this report will be mostly devoted to this particular setting in detriment of other potential applications, such as mantle cell lymphoma, for which the information available is still very limited [8].

Idiotypic vaccine production

In recent years, at least three independent methods have been used to obtain the clonal, patient- and tumor-specific idiotype: large-scale culture of hybridomas, recombinant technology and DNA vaccines [3]. The first two methods aim at reproducing the soluble protein idiotype in the laboratory. In most cases, the ultimate product is the whole, idiotype-containing immunoglobulin, although protein fragments corresponding to the single-chain variable region fragments of the immunoglobulin have also been both reproduced and utilized in Phase I clinical trials [9,10]. On the contrary, the last technique focuses on the generation of the idiotype by the patient himself, through the direct transfection of some of his somatic cells by the administration of the DNA sequences encoding for the idiotype.

The hybridoma-based methodology [11] used to rescue tumor-specific, idiotype-containing whole immunoglobulins has been responsible for most if not all idiotype vaccine-related achievements over the last two decades. A large number of tumor cells are required for the fusion with a purposely selected cell line termed K6H6/B5 (ATCC CRL 1823). Among the growing hybridomas, one is ultimately selected according to a number of morphological, genetic, immunological and quantitative features. In particular, this hybridoma should grow relatively rapidly and give rise to a single colony of clonal cells, to avoid contamination from other clones either not producing any idiotype or not producing the same idiotype as the tumor cells. Therefore, the hybridoma's idiotype molecular fingerprint must fully overlap with that of the corresponding tumor cell's idiotype, to ensure complete identity between the tumor-specific soluble protein idiotype and that synthesized by the hybridoma. In fact, even when dealing with a massively tumor-infiltrated lymph node, it remains possible that the stochastic cell fusion process may involve a residual patient's normal B lymphocyte, whose idiotype-containing immunoglobulin would by definition be different from that specific for the tumor. Furthermore, the hybridoma must also secrete the synthesized immunoglobulin as determined by standard ELISA. Finally, a sufficient amount of the tumor-specific immunoglobulin must be produced by the hybridoma, to guarantee enough material for all vaccine doses, as well as for the post-vaccine immunological tests [12].

Over the years, this method has allowed idiotypic vaccination to deliver many proofs of principle in humans. However, it has also suffered considerable setbacks both from the logistical and financial standpoint, since it may take up to 6 months to actually produce a single idiotype vaccine, whose use is then limited to a single patient [3].

To circumvent both problems, while preserving the single-patient specificity, which in principle remains a virtue of this therapeutic strategy, several different production techniques have been developed to generate idiotype proteins more effectively. In particular, most of them share a recombinant technology aiming at a molecular rescue of the idiotype. This process involves PCR-based amplification and cloning of the genes encoding for the tumor-specific immunoglobulin variable regions, followed by their ligation into plasmid or viral vectors for protein expression in mammalian (e.g., murine lymphoma), insect (e.g., sf9), bacterial (e.g., *Escherichia coli*) or plant (e.g., tobacco) cells [13]. Depending on the method utilized, the ultimate recombinant and still custom-made idiotype can consist of a full-length tetrameric immunoglobulin with either a human IgG3 or IgG1 backbone, in an antigen-binding fragment, or in a single-chain variable region fragment. Finally, a novel way to rescue tumor-specific idiotypes might stem from the first report on the use for this purpose of an *E. coli*-based, cell-free *in vitro* system [14].

DNA vaccination against idiotype-related antigens is also based on the sequences encoding for both tumor-specific immunoglobulin's variable regions, since they can be readily identified and assembled in a variety of molecular forms. For instance, it is possible to encode the two-chain, idiotype-containing immunoglobulin molecule within one cassette. Alternatively, it is also possible to assemble the complete idiotype as a single-chain variable-region fragment, which, as mentioned previously, represents another convenient format to express the idiotype epitopes. Then, other sequences encoding pathogen-derived, immunogenic molecules, such as the tetanus toxin Fragment C, can be incorporated into the DNA vaccine formulation, in order to enhance idiotype immunogenicity [2]. So far, this methodology has not been employed in late-stage clinical trials [2], and as such will not be further discussed in the present report.

Clinical applications

Soluble protein idiotype vaccines obtained from hybridomas

Almost 20 years ago, an idiotype vaccine achieved the first unprecedented success in human cancer vaccine development (TABLE 1). Researchers at Stanford University (CA, USA) proved for the first time that a custom-made idiotype vaccine was capable of inducing an idiotype-specific humoral response in seven out of nine follicular lymphoma patients. The main characteristics of this vaccine formulation were represented by the use of KLH as an immunological carrier for the idiotype, and that of Syntex adjuvant formulation (SAF)-1 as an immunological adjuvant [15].

The same pilot study, 5 years later, had been expanded into a full-scale Phase II clinical trial enrolling 41 follicular lymphoma patients. Their long-term follow-up results provided some tentative evidence

concerning the possible clinical impact exerted by idiotypic vaccination in follicular lymphoma patients who experienced an idiotype-specific immune response. In particular, the 20 out of 41 patients who mounted a humoral and/or a cellular, idiotype-specific, vaccine-induced immune response obtained a significantly better cumulative overall disease-free survival than those who did not. These results also suggested that the idiotype vaccine is more likely to play an important role in human follicular lymphoma treatment

when administered to immunocompetent patients while in a clinical complete response of their disease to chemotherapy regimens. Moreover, the overall survival curves of this study showed that, in spite of the apparent clinical success of idiotypic vaccination in the responding patients, they seemed still destined to relapse, although later than the nonresponders [16]. Of course, the study was not designed to assess clinical benefit and, as such, did not overcome the general criticism that the different outcome between immune responders and nonresponders could be due to patient selection rather than to a direct effect of vaccination.

In 1999, researchers at the National Cancer Institute provided very strong data supporting the notion that idiotype vaccines are also capable of frequently eliciting idiotype- and tumor-specific cellular immune responses. More importantly, they demonstrated for the first time that cellular and/or humoral responses induced by idiotypic vaccination could kill *in vivo* follicular lymphoma cells that had survived prevaccine chemotherapy. In particular, this was the first study documenting how patients with minimal residual disease, although in clinical complete response after conventional chemotherapy, could achieve a molecular remission status of their follicular lymphoma through idiotypic vaccination alone [17]. These results were later confirmed in an independent, smaller study [18]. Both results of this trial were attributed to a possible idiotype vaccine's efficacy augmentation through the replacement of SAF-1 with GM-CSF. The follow-up of this Phase II clinical study has been subsequently updated and, with a median follow-up of 9 years, median disease-free survival was 8 years, with a 95% overall survival rate [19].

Finally, our group has recently reported the outcome of 25 patients with follicular lymphoma after induction of a second complete response with standard chemotherapy without rituximab and subsequent extensive idiotypic vaccination [20]. A vaccine-induced, humoral and/or cellular, idiotype- and/or tumor-specific immune response was detected in 20 out of 25 patients. The median duration of the second complete response among these patients was statistically significantly longer than the median duration of their first complete response. Moreover, in all cases it was also conspicuously longer than 13 months, which is the unchanged median duration of a second complete response induced by standard chemotherapy without rituximab over the last three decades. On the contrary, the five patients who did not respond to vaccination from an immunological standpoint had

Table 1. Major clinical trials published in the history of idiotypic vaccination for follicular lymphoma.

Phase	Vaccine	Production	Type of study (pts)	Result	Ref.
I	Id-KLH+SAF-1	Hybridoma	Proof of principle (7/9)	Biological efficacy	[15]
II	Id-KLH+SAF-1	Hybridoma	Confirmatory (20/41)	Biological efficacy	[16]
II	Id-KLH+GM-CSF	Hybridoma	Proof of principle (8/11)	Clinical efficacy	[17]
II	Id-KLH+GM-CSF	Hybridoma	Confirmatory (8/9)	Clinical efficacy	[18]
II	Id-KLH+GM-CSF	Hybridoma	Proof of principle (20/25)	Clinical benefit	[19]

Id: Idiotype; GM-CSF: Granulocyte-macrophage colony-stimulating factor; KLH: Keyhole limpet hemocyanin; pt: Patient; SAF: Syntex adjuvant formulation.

a second complete response shorter than both 13 months and their first complete response. This Phase II nonrandomized trial proved for the first time a clinical benefit associated with the use of a human therapeutic cancer vaccine [20,21]. Currently, all conclusions of our study are confirmed. The median duration of the second complete response of the 20 patients who responded to vaccination has not been achieved, but exceeds 50 months. A total of 12 patients (60%) remain in a continuous second complete response. Seven of the eight immune responders who later relapsed did so within 6–12 months from the last vaccination boost, while the last patient relapsed more than 4 years thereafter (FIGURE 1).

Previous studies could not prove clinical benefit because either no comparison was included in their design or it was limited to historical controls that were not as informative as the same patients in first and then second complete response. Any tentative conclusion on idiotype vaccine-associated clinical benefit derived from simple comparisons with historical controls would be based on a form of analysis that has been discredited. Patients who fail to make a response may be sicker. Thus, the fact that they did not fare as well cannot be said to reflect a positive treatment effect in those who responded. The successful trial conducted at the University of Navarra (Pamplona, Spain) also showed that, when it comes to customized, patient- and tumor-specific immunization, a formal, systematic, inpatient comparison can be far more telling than a simple randomization, in which all patients in the control arm receive the same placebo, while each and every patient in the experimental arm receives a different compound, none of whose ingredients are *per se* active against the tumor [22]. Of course, our trial was aimed at a proof-of-principle, rather than at market approval and, similar to the other two such studies, showed that proofs of principle do not require a large number of patients, but rather a sharp study design [15,17].

Nonetheless, a series of Phase III, randomized clinical trials was launched in the ensuing years (TABLE 2): the first of them at the National Cancer Institute 9 years ago [23]. Although its results have not yet been fully released, the recent public disclosure of most analyzed data [101] indicates that, as predicted [22], this study has failed to achieve its preliminary end point. In particular (TABLE 3), this trial: was supposed to enroll 629 patients, but enrolled only 177 patients; was supposed to randomize 375 patients, but randomized only 117 patients; was supposed to administer vaccination and placebo to 250 and 125 patients,

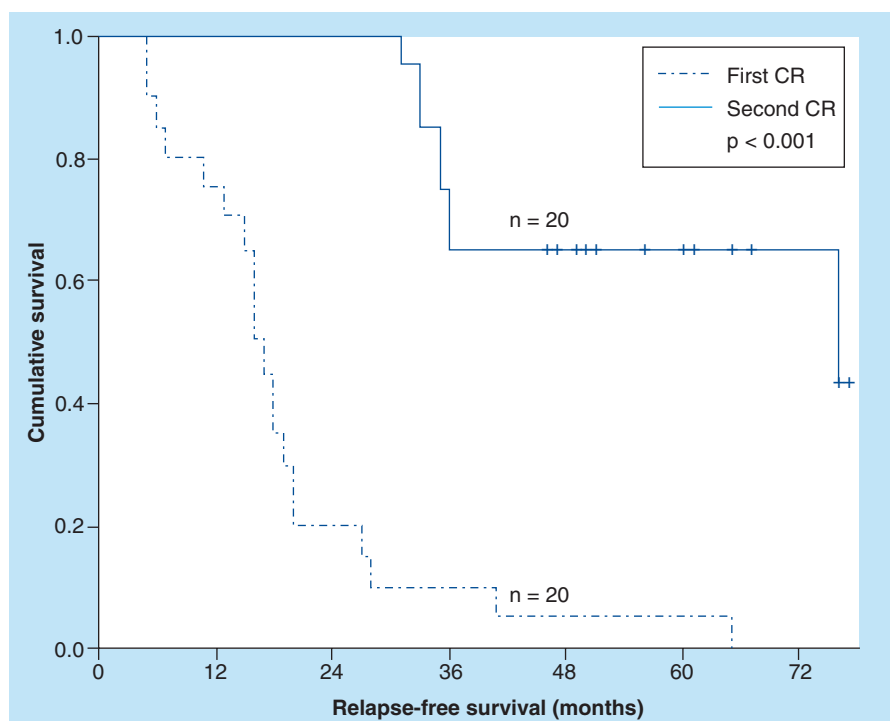


Figure 1. Updated relapse-free survival after the first and second complete response of 20 patients who made an immune response to idiotype vaccination. Data is from the first trial to prove clinical benefit associated with the use of this therapeutic option in follicular lymphoma. Redrawn with permission from [20].

respectively, but did so only to 72 and 39 patients, respectively, while six patients remain unaccounted for at the time of this writing; aimed at achieving a high statistical significance in disease-free survival measured from the date of randomization ($p < 0.01$), but failed to do so, while claiming barely statistical significance in disease-free survival measured from the date of first vaccination ($p = 0.047$). As we wait for the release of complete data, an in-depth discussion on the overall outcome of this trial is required. After the first promising years, the sponsoring company failed to complete patient enrollment despite later opening the trial to a number of Russian centers, mainly due to the fact that it soon became evident that the addition of rituximab to any chemotherapy regimen dramatically improves response rates in follicular lymphoma patients [24–27]. As such, the prevaccine cyclophosphamide, doxorubicin, etoposide and prednisone (PACE) regimen chosen for this trial became a substandard treatment. Considering the public claim of an idiotype vaccine-production failure rate as low as 3%, which is dramatically better than that of any previous idiotype vaccine trial, it must be assumed that approximately 60 patients failed to either achieve or maintain for 6 months a first complete response with the PACE regimen. Therefore, it must be assumed that those belonging to the latter

group were randomized but never had a chance to actually receive the assigned treatment, arguably affecting the intent-to-treat analysis. In fact, randomization in this study took place upon achievement of the clinical complete response, that is, approximately 6 months prior to the first vaccination. In this respect, the attempt of retrospectively measuring disease-free survival from the date of first vaccination appears to be futile and arbitrary. Since it typically takes three to five idiotype vaccine administrations to elicit, if at all, a detectable immune response to it, disease-free survival measured from the date of first vaccination is gravely misleading. In fact, for patients who did not exhibit a specific immune response, the date of any vaccination could be considered instead. On the contrary, for patients with a detectable immune response, using the date of first immune response detection would be far more consistent with the underlying intention of directly correlating clinical benefit with documented vaccine activity. However, either option would be subject to a degree of variability and uncertainty that is inconceivable in a randomized clinical trial.

Therefore, the only acceptable time point from which disease-free survival can be consistently measured is indeed the date of randomization. In this respect, being both the date of randomization and that of first vaccination readily available to the investigators, the fact that no disease-free survival data were released as measured from the former time point seems to indicate that disease-free survival measured from the date of randomization was not statistically significant. Indeed, the addition of 6 early and, most probably, event-free months to the disease-free survival of each patient in both arms would probably push the barely significant p -value described earlier (0.047) above the limit of statistical significance (0.05). Of course, the only reasonable way to ascertain whether or not credible evidence of statistical significance is within reach would be to complete the study. The

Table 2. Comparative overview of randomized Phase III clinical trials on idiotype vaccination for follicular lymphoma aiming at market approval.

Sponsor	Pretreatment	Patients	Random	Boosts	End point	Status
Biovest	6–8 × PACE q 3w	CR	2:1	No	DFS	Failed
Genitope	8 × CVP q 3w	CR, PR	2:1	No	PFS	Failed
Favrille	4 × rituximab q 1w	CR, PR, SD	1:1	Yes	TTP	Failed

CR: Complete response; CVP: Cyclophosphamide, vincristine and prednisone; DFS: Disease-free survival; PACE: Cyclophosphamide, doxorubicin, etoposide and prednisone; PFS: Progression-free survival; PR: Partial response; q 1w: One dose weekly; q 3w: One cycle every 3 weeks; SD: Stable disease; TTP: Time to progression.

addition of more than 250 new patients to the two study arms, as originally planned, would indeed conclusively clarify these results either way. Unfortunately, as mentioned before, the advent of rituximab makes this option unrealistic in terms of feasibility. No patient would accept being enrolled in a clinical trial whose first-line prevaccine treatment does not include rituximab, and no ethical committee would consider the PACE regimen without rituximab as an acceptable choice in that context.

Soluble protein recombinant idiotype vaccines

In order to possibly lessen the burden of laboratory work necessary to systematically produce idiotype vaccines through hybridomas, recombinant idiotype vaccine-based strategies have emerged as an appealing alternative. The first Phase I/II clinical trial on idiotypic vaccination for follicular lymphoma based on the use of recombinant, custom-made and tumor-specific idiotypes showed both safety and immunogenicity were comparable, although only in historical terms, with those already observed when using hybridoma-generated idiotypes [13].

Despite the amount of clinical data regarding recombinant idiotype vaccines for the treatment of follicular lymphoma being limited, two independent Phase III clinical trials were competitively launched at the beginning of this decade. However, once again, as predicted in detail long before their failure [22], they have both been unable to achieve their primary end points [102,103], and the sponsoring companies have renounced to further pursue idiotype vaccine regulatory approval. Since full, peer-reviewed publication of the negative data is still awaited, it is not currently possible to exactly pinpoint the relative impact on them of each and every pitfall previously detected in their clinical trial designs. In fact, beside the too simplistic recourse to standard randomization to prove clinical benefit of a customized manipulation of the individual, tumor-bearing host immune system, other caveats (TABLE 2) involved patient selection, prevaccine treatment and vaccine schedule [22].

The first study had a successful vaccine production rate of approximately 90% and showed no statistical differences in progression-free survival between vaccinated patients and those in the control arm [28,102]. Both arms' overlapping progression-free survival curves feature a sharp drop of approximately 25% over the second trimester of follow-up, which is uncharacteristic of

follicular lymphoma. This occurrence might depend on the fact that in this trial patients were randomized after achieving either complete or partial response with chemotherapy not including rituximab. Therefore, such a sharp drop could be mostly related to patients who were randomized with disease still clinically detectable, although no clear-cut data are available to confirm this hypothesis. Moreover, in this trial, randomization took place shortly prior to the first vaccination. The nonpredetermined analysis of some patient subsets showed instead statistical significance in favor of those with a detectable, vaccine-induced, anti-idiotype, humoral response compared with either those who did not mount such an immune response and those in the control arm. This result was even more pronounced whenever a robust immune response, that is, an anti-idiotype titer greater than 1.8 µg/ml, was elicited [102]. Other claims of this study appear to be less sustainable; in particular, that both vaccination and control (KLH plus GM-CSF) were capable of inducing some clinical response improvements and to achieve a plateau in their respective progression-free survival curves [102]. As for the former, the number of patients experiencing it is too small to make an impact. As for the latter, the few cases on the alleged plateaus have been mostly progression free yet over less than 5 years. Therefore, since they are follicular lymphoma patients in first continuous complete response, a far longer follow-up is required to confirm that those plateaus are real.

The second study had a vaccine-successful production rate of approximately 84% and also failed to show statistical differences in time to progression between vaccinated patients and those in the control arm [13,103]. As in the other randomized trial using recombinant idiotype vaccine, randomization was carried out shortly before the first vaccination. However, contrary to the other trials, in this study patients were randomized after achieving complete or partial response, or even just stable disease, following four weekly administrations of the single-agent rituximab: that is, no chemotherapy was given prior to vaccination. As a consequence, the complete response rate before randomization and vaccination was lower than 5%. Therefore, most vaccinated patients still had measurable tumor against which even a strong vaccine-induced immune response would have been unlikely to be clinically beneficial. Moreover, it is generally considered that the prolonged, rituximab-induced

Table 3. Detailed analysis of randomized trials failure.

Sponsor	Total numbers of pts enrolled	Randomization	End point	p-value
Biovest (expected)	629	Vaccine: 250 pts; placebo: 125 pts	DFS from random date	<0.01
Biovest (actual)	177	Vaccine: 72 pts; placebo: 39 pts	DFS from first vaccination*	0.047*
Genitope (expected)	360	Vaccine: 240 pts; placebo: 120 pts	PFS from random date	<0.01
Genitope (actual)	360	Vaccine: 192 pts; placebo: 95 pts	PFS from random date	NS
Favrille (expected)	342	Vaccine: 171 pts; placebo: 171 pts	TTP from random date	<0.01
Genitope (actual)	349	Vaccine: 174 pts; placebo: 175 pts	TTP from random date	NS

*The actual DFS from the date of randomization might be not be statistically significant (p = NS).

DFS: Disease-free survival; NS: Not significant; PFS: Progression-free survival; pt: Patient; random date: Date of randomization; TTP: Time to progression.

B-cell depletion is mechanistically at odds with the desirable early induction of idiotype-specific humoral responses through idiotypic vaccination. On the other hand, this was the only randomized trial on idiotype vaccines aiming at elucidating the possible clinical impact of boost vaccine administration. All in all, it does not come as a surprise that not even the analysis of any subset of patients provided the slightest indication of statistical significance [103].

Summary & conclusion

Some clear-cut conclusions on idiotypic vaccination can already be drawn, since they have been formally proven beyond reasonable doubt. In particular:

- It is possible to immunize a cancer patient against an antigen solely expressed by his/her own tumor [17,20,29];
- An idiotype vaccine can elicit idiotype-specific humoral responses, also implying the activation of specific T helper cells [17,20,29];
- The addition of GM-CSF to the vaccine formulation appears to be able to improve both frequency and quality of the idiotype- and tumor-specific T-cell responses, including the induction of specific cytotoxic T lymphocytes, without reducing idiotype-specific B-cell responses [17,20];
- Vaccine-induced B- and/or T-cell responses are capable of killing tumor cells *in vivo* that had survived prevaccine chemotherapy [17,20,30];
- Soluble protein idiotype vaccines are both virtually nontoxic and substantially harmless to patients diagnosed with follicular lymphoma [17,20,29,30];
- Simple, traditional randomization is not the best method to prove the clinical benefit of a customized, patient- and tumor-specific active immunotherapy in which no ingredient is *per se* active against the tumor.

This last characteristic, shared with many other customized immunotherapies, makes idiotype vaccines unlike common drugs, which must be active *per se*. With customized idiotypic vaccination, the ultimate tumor control or eradication depends on the actual intervention and active participation of each patient's own, different and variably responsive immune system every time each patient receives a vaccine dose or boost [6,22]. These facts are at the basis of the failures of all randomized studies conducted so far with idiotype vaccines and will have strong implications on how regulatory agencies prepare to address future market approval trials for these types of treatment.

Expert commentary

A number of Phase II and III clinical trials have provided formal [20] or at least somewhat compelling [17,101,102] evidence of clinical benefit associated with idiotypic vaccination in follicular lymphoma patients who make a specific immune response to it. However, the sole formal proof comes from a study that never

had market approval as a target and, as such, did not have the power to establish idiotypic vaccination as a therapeutic option for most patients with follicular lymphoma [20]. *Vice versa*, the trials designed with market approval as a goal failed to meet the end points that would warrant it [101–103]. Therefore, it is probable that follicular lymphoma patients will not have a readily available idiotype vaccine at their disposal for several years. In this respect, the failure of much awaited but poorly designed or poorly conducted randomized trials is likely to affect the general confidence of both patients and clinicians toward idiotypic vaccination, independently to whether the vaccines that were used may have had a great potential to provide clinical benefit or not. Moreover, the pharmaceutical market for follicular lymphoma is extremely competitive, with nearly as many competing therapeutic options available as are the potential patient's needs. There is still hope for idiotypic vaccination, but the next trials to be conducted will probably be the last and, therefore, cannot afford to fail again.

Five-year view

The future of idiotypic vaccination will depend on whether any clinical trial designed to both confirm its clinical benefit and to obtain market approval from the competent regulatory authorities will be able to succeed in both aims. At the time of this writing, such a possibility may be envisioned only as the result of brand new clinical studies whose design is still in the making. In fact, none of the other currently ongoing trials on idiotype vaccines are structured to achieve either of the aims above [104]. Not even the study we are conducting [105], whose ambitious goal is the cure [7] of some patients with poor-prognosis follicular lymphoma, rather than that of confirming clinical benefit of idiotypic vaccination, is structured in this way [20].

New trials should be designed keeping in mind the very nature and the peculiar features of the therapeutic approach under scrutiny. An idiotype-specific vaccine:

- Is customized;
- Is *per se* inactive against the tumor;
- Does require each and every immunization to be processed by an immune system that may not necessarily be as prone to be engaged into delivering an effective immune response as it was at previous immunizations or as it might be at subsequent immunizations: it might be more or less reactive to the idiotype each and every time.

As a result, simple randomization may not be an adequate enough tool to assess clinical benefit of such an active immunotherapy. Moreover, follicular lymphoma is not a homogenous disease either, and newly diagnosed patients with this disease may have an extremely variable prognosis [7]. As such, they do not provide an optimal study setting for customized treatment trials [7].

New trials on idiotypic vaccination for follicular lymphoma should be designed seeking preliminary agreements with the regulatory authorities on a number of issues such as:

- First-relapse patients constituting the most informative study setting for this disease [7];
- Nonrandomized comparison of second complete responses obtained through chemotherapy alone and maintained by idiotype vaccines, with first complete response obtained through chemotherapy plus rituximab being granted the same value of randomized comparisons, provided that statistical significance is achieved in either type of analysis;
- Predetermined comparisons being allowed both on an intent-to-treat ($p < 0.05$) basis and on a immune responders versus nonresponders ($p < 0.01$) basis.

In this scenario, it would be possible to design Phase II/III clinical trials in which either/both the nonrandomized and/or the randomized part would have independent chances to prove clinical benefit and obtain market approval for idiotypic vaccination.

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Key issues

- First among human therapeutic cancer vaccines, idiotypic vaccination has proved biological and clinical efficacy, as well as exhibiting clinical benefit in follicular lymphoma.
- Proof-of-principle trials are not suitable for the approval of idiotype vaccine-based products, mainly due to the small number of patients enrolled.
- The idiotype vaccine is not a drug but rather a customized tool attempting to harness the power of the patient's immune system: a different tool for each and every patient, acting on a different immune system at the time of each and every immunization.
- Simple randomization applies poorly to late-stage clinical trials of idiotypic vaccination because patients in the placebo arm all receive the same placebo, whereas patients in the experimental arm each receive a different vaccine.
- Recently, all randomized trials on idiotypic vaccination failed, not necessarily due to poorly effective vaccines, but rather due to the predictable and predicted pitfalls and caveats of their respective study designs.
- Clinical trials aiming at formal approval of a customized, active immunotherapy, such as the idiotype vaccine, should contain both a nonrandomized comparison (e.g., comparison of the length of the second complete response maintained by vaccination with that of the first complete response achieved with the best standard treatment) and a randomized comparison: both with sufficient statistical power and independent value as main end points.

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