

OPINION

Idiotypic vaccines for lymphoma: proof-of-principles and clinical trial failures

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Abstract | The clonal immunoglobulin idiotype displayed on the surface of most malignant B cells is a patient- and tumour-specific antigen that can be used for therapeutic vaccination. Several studies have confirmed the biological efficacy of soluble protein idiotypic vaccination and two clinical trials have shown the clinical efficacy of this procedure. One study has demonstrated clinical benefit associated with idiotypic vaccination. However, three randomized clinical trials have recently failed to achieve their main end points for reasons that are probably unrelated to the vaccine. While scepticism towards this type of non-toxic medical intervention is mounting, such patient-specific treatments might yet see the light of day through better designed clinical trials.

Unlike most other proteins, which, from an immunological standpoint, can function only as antigens, antibodies can function both as antigen-recognizing and -binding molecules and as antigens by virtue of several types of epitopes found on their heavy and light chains¹. In particular the idiotype (Id) — that is, the collection of antigenic determinants called idiotopes, which are located in the hypervariable regions of the antibody — defines the immunoglobulin's clonality^{2,3}. Therefore, the Id of the immunoglobulin found on the surface of a B cell in its capacity as a B cell antigen receptor is a clone-specific antigen, and when that B cell happens to be neoplastic the Id represents a tumour-specific antigen — arguably one of the few tumour-specific antigens currently known in humans⁴.

The tumour-specific Id is a weak self-antigen, so soluble protein Id vaccine formulations typically include an immunogenic carrier and an immunological adjuvant (BOX 1) to increase Id immunogenicity and overcome immune tolerance. The Id is a suitable tumour-specific target for most non-Hodgkin's lymphomas, with follicular lymphoma (FL) currently being the most studied disease for the clinical application of customized Id vaccines⁵. It is precisely owing to the limited availability of other tumour-specific antigens that human therapeutic cancer vaccines have been developed sooner against tumours of the immune system than against other tumours. And it is owing to its peculiar relationship with the rest of the patient's immune system⁶, and to

its generally indolent behaviour⁷, that FL is the preferred field of application of Id vaccines despite its variable and unpredictable clinical course⁷.

After two decades spent on the validation of this approach in preclinical models^{8–10}, soluble protein idiotypic vaccination has provided proof-of-principle of biological efficacy, clinical efficacy and even clinical benefit in small studies conducted in humans. In this Opinion article, I use the following terms to describe the effects of idiotype vaccination in people: biological efficacy is the capacity to elicit a specific immune response, clinical efficacy is the capacity to induce anti-tumour effects *in vivo*, and clinical benefit is the capacity to influence disease end points, such as disease-free or overall survival. Therefore, after a biologically successful vaccination there may be clinical efficacy without clinical benefit, but there cannot be clinical benefit without clinical efficacy. In fact, although in general improved survival does not occur without tumour shrinkage or control, tumour shrinkage or control can be observed without improved survival. Soluble protein Id vaccines are biologically efficacious because they induce Id- and tumour-specific immune responses in most patients with FL^{11–15}, they are clinically efficacious because their immunologically successful use in patients with FL is associated with anti-tumour activity — that is, the *in vivo* clearance of FL cells that had survived pre-vaccine chemotherapy^{12,15} — and they are clinically beneficial because the immune

responses that they elicit are associated with improved disease-free survival in patients with FL¹⁴. Abundant data have already shown a lack of any significant side effects associated with the continuous clinical use of Id vaccines over several years¹⁶. However, the studies discussed above are too small to support the approval of these agents; therefore, clinical benefit from soluble protein idiotypic vaccination needs to be proven in larger studies.

However, 2008 saw the failure of two randomized clinical trials aimed at confirming these proof-of-principles in a large-scale setting, and although a third study showed encouraging results in this respect it is unlikely to lead to regulatory approval for the marketing of an Id-based vaccine owing to insufficient patient accrual and statistical significance. Although disappointing, the results of these clinical trials were not entirely unexpected owing to several important flaws intrinsic to their respective study designs¹⁷. Therefore, although the field of idiotypic vaccination is now likely to face increased scepticism from clinicians and scrutiny from regulators, it is also fair to state that the same clinical trials that failed to confirm clinical benefit from Id vaccination also failed to prove that the negative results were due to the failure of the vaccine rather than to defects in study design. In other words, as I argue in this article, in both trials that failed to show statistical significance between patients receiving vaccination and those receiving the control, it is not possible to attribute the negative results to the failure of Id vaccination¹⁸.

Preclinical development

A comprehensive analysis of all preclinical studies of idiotypic vaccination conducted over nearly half a century to develop both concepts and applications for this process far exceeds the scope of this article, which is focused on its use in humans. However, there are at least three studies that over time have maintained their conceptual uniqueness and the conclusions of which should be taken into account now that it is time to interpret recent clinical trials.

Shortly after the first demonstration that antibodies made by BALB/c mice against myeloma proteins 315 and 460 were specific for the respective Ids of these two immunoglobulins¹⁹, formal proof was provided by Lynch and colleagues that both proteins can function as tumour-specific antigens and that the administration of each protein can induce a humoral immune response leading to the specific rejection of the

corresponding tumour but not the non-corresponding one⁸. However, the same study also showed that protein 460 induced less intense resistance to tumour MOPC-460 than protein 315 did to MOPC-315, probably because the vaccine-induced Id-specific antibodies had a lower affinity for protein 460 than the corresponding antibodies had for protein 315 (REF. 8). Differences in the *in vivo* biology of the tumours could also have contributed to the differences. This observation (namely the heterogeneity of the elicited immune response to Id) may influence any attempt to use simple randomization to prove the clinical benefit of Id vaccines, because there is no guarantee that each customized vaccine can activate each patient's immune system to the same degree²⁰. Indeed, the immune response mounted by each patient may also differ greatly from that of any other patient at the time of each and every immunization. In addition, patients with FL who have the same stage of disease, histological grade, prognostic score, age, treatment and so on may present with varying clinical behaviour⁷. Although this last issue may be mostly countered by balancing the experimental and control arm patient populations using clinical prognostic factors⁷, such as the Follicular Lymphoma International Prognostic Index (FLIPI) score, pure randomization cannot solve the problem posed by the fact that patients in the control arm of an Id vaccine trial all receive the same, presumably inactive, compound, whereas

patients in the experimental arm each receive a unique and individually different immunogen²⁰.

Approximately 15 years after the pioneering studies of Lynch and colleagues, a new study showed that soluble idiotype protein vaccination conferred tumour-specific immunity against the 38C13 murine B cell lymphoma to C3H/HeN mice through an Id-specific humoral response. This immunity occurred only when the Id was conjugated to a powerful immunogenic carrier, such as keyhole limpet haemocyanin (KLH)^{9,21}, the vaccination preceded tumour challenge⁹ and the free Id protein was not circulating at the time of vaccination⁹. This work led to the common use of Id conjugated to KLH (Id-KLH) in the vaccine formulation. It also suggested that the induction of clinically relevant tumour-specific immunity was less likely to be achieved if vaccination was performed in animals with a sizable tumour burden⁹. This observation in mice predicts that patients who achieve a clinical complete response (CR) after standard chemotherapy represent the best clinical setting for optimizing immunogenicity and evaluating the clinical effects of the vaccination^{12,14}. However, patients without a CR after chemotherapy can also mount an immune response and have been included in clinical trials^{16,22}. Moreover, circulating tumour Id that could inhibit a soluble Id protein vaccine is typically undetectable in patients with FL either in CR or in partial response (PR), unlike in patients with myeloma.

Finally, another study on the same murine lymphoma model conducted a decade later showed that the addition of granulocyte-macrophage colony-stimulating factor (GM-CSF) to the Id-KLH vaccine formulation substantially improved anti-tumour protection by increasing T cell immunity¹⁰. GM-CSF has been incorporated into all soluble protein Id vaccine regimens (Id-KLH plus GM-CSF) used in humans ever since, replacing other less reliable immunological adjuvants such as the Syntex adjuvant formulation 1 (REF. 23).

Proof-of-principle in humans

The administration of a few doses of Id-KLH plus Syntex adjuvant formulation 1 to patients with FL in clinical CR or with measurable residual disease after chemotherapy resulted in Id-specific humoral and/or cellular immune responses in most of the patients studied²³. These results were subsequently confirmed by other groups using the Id-KLH plus GM-CSF formulation¹²⁻¹⁵ (BOX 1), indicating biological efficacy (the capacity of inducing an antigen-specific immune response).

However, biological efficacy does not necessarily translate into clinical efficacy (the induction of an anti-tumour effect). Previous idiotype vaccination studies have indicated that patients who do not respond to the vaccine from an immunological standpoint seem to have a worse outcome, and this could relate exclusively to the fact that these patients might be more unwell^{17,18}. Therefore, the ability to respond to vaccination with an Id-specific immune response might represent a favourable prognostic factor rather than the immune response being the cause of a better outcome²². In any case, clinical efficacy was proved several years later by showing that successful idiotype vaccination can elicit an immune response associated with the *in vivo* clearance of FL cells that survived prior chemotherapy¹². In other words, patients with FL who are in clinical CR but who have minimal residual disease still detectable at the molecular level several months after completion of chemotherapy became minimal residual disease-negative only following a vaccination programme that succeeded in eliciting an Id- and tumour-specific immune response¹². These results were confirmed by others¹⁵.

Clinical efficacy does not necessarily translate into clinical benefit (increased remission duration or survival). Therefore, clinical benefit was demonstrated (TABLE 1) by showing that the duration of a second CR in patients with FL — who were treated

Box 1 | Idiotype vaccine formulation

The virtually non-toxic¹⁶, customized soluble protein idiotype (Id) vaccines currently in use are based on the formulation including 0.5 mg of patient- and tumour-specific Id¹ conjugated to 0.5 mg of keyhole limpet haemocyanin (KLH)²¹. This is administered subcutaneously on day 1 of the vaccination schedule together with a dose of granulocyte-macrophage colony-stimulating factor (GM-CSF) that may differ slightly from trial to trial¹². The same dose of GM-CSF is subsequently delivered subcutaneously at the same site as the first injection over the following 3 days¹⁰. The vaccination schedule may also vary from trial to trial³⁵, but recent safety¹⁶ and efficacy¹⁴ data seem to encourage the prolonged administration of maintenance boosters.

The amount of Id to be used for immunization has never been the subject of a dose-finding process. The 0.5 mg of Id per vaccination was chosen at a time when hybridoma rescue was the sole method by which soluble protein, tumour-specific Id could be reproduced in the laboratory, and only small amounts can be produced by this method over several months of specialized and tedious work^{36,37}. As at least half of patients vaccinated at this dosage mount an Id-specific immune response¹¹⁻¹⁵, even the most recent clinical trials, including those based on recombinant soluble protein Id vaccines, have been conducted using this historical dose⁴³. However, because molecular technology allows faster production of greater amounts of the same Id, and in light of those recent clinical trial failures, Id vaccine dose adjustment may have a substantial role in future early-phase clinical studies.

Clinical grade KLH and therapeutic GM-CSF are both commercially available and function as an immunogenic carrier²³ and an immunological adjuvant^{12,14}, respectively. Importantly, neither has ever shown activity against follicular lymphoma nor been proposed for follicular lymphoma treatment. For this reason, with or without KLH, GM-CSF was chosen as the placebo control in all recent randomized clinical trials on Id vaccines.

Table 1 | Main features and interpretation of clinical trials assessing the clinical benefit of idiotype vaccines for follicular lymphoma

Sponsor	Idiotype production	Type of comparison	Pre-vaccine treatment	Patient status before vaccination	Number of patients (actual out of expected)	End point	Results	Potential caveats	Refs
University of Navarra, Spain	From hybridoma	Same patients: second compared with first CR	Six cycles of CHOP or CNOP	Second CR	25 out of 25	Second CR longer than both first CR and 13 months in vaccine immune responders	20 of 20 immune responders achieved both end points; 5 of 5 immune non-responders did not	Small proof-of-principle study, not aimed at ascertaining cause and effect or regulatory approval	14
Genitope	Recombinant	First line 2/1 randomization	Eight cycles of CVP	First CR or PR	Vaccine: 192 out of 240; control (KLH plus GM-CSF): 95 out of 120	PFS ($p < 0.01$)	$p = \text{n.s.}$	More patients than expected (at least one-quarter) relapsed or progressed within 9 months from randomization	25, 30, 35
Favril	Recombinant	First line 1/1 randomization	Four doses of rituximab	First CR, PR or SD	Vaccine: 174 instead of 171; control (GM-CSF): 175 instead of 171	TTP ($p < 0.01$)	$p = \text{n.s.}$	Too few patients achieved a CR before vaccination. Most if not all patients were vaccinated while under severe B cell depletion from rituximab	18
Biovest	From hybridoma	First line 2/1 randomization	Six cycles of PACE or rituximab plus CHOP	First CR or CRu	Vaccine: 76 out of 250; control (KLH plus GM-CSF): 41 out of 125	DFS ($p < 0.01$)	$p = 0.045$	The trial randomized only 31.2% of the patients (modified ITT) planned. The achieved p value is not highly statistically significant	25, 32, 35

CHOP, cyclophosphamide, doxorubicin, vincristine and prednisone; CNOP, cyclophosphamide, mitoxantrone, vincristine and prednisone; CR, complete response; CRu, complete response unconfirmed; CVP, cyclophosphamide, vincristine and prednisone; DFS, disease-free survival; GM-CSF, granulocyte-macrophage colony-stimulating factor; ITT, intent to treat; KLH, keyhole limpet haemocyanin; n.s., not significant; PACE, prednisone, doxorubicin, cyclophosphamide and etoposide; PFS, progression-free survival; PR, partial response; SD, stable disease; TTP, time to progression.

with standard chemotherapy (and without the CD 20-specific monoclonal antibody rituximab) and then successfully vaccinated (from an immunological standpoint) with their Id vaccine — was systematically (in 20 of 20 patients) and statistically significantly longer than the duration of the same patient's first CR obtained through standard chemotherapy with or without rituximab¹⁴. Moreover, all 20 patients with a vaccine-induced, Id-specific immune response had a second CR substantially longer than 13 months¹⁴, which is the median duration of a second CR in patients with FL induced by any salvage chemotherapy programme without rituximab²⁴. Both facts (that the second CR is longer than the first and that the second CR is longer than 13 months) are conspicuously contrary to the common clinical knowledge gathered on FL over the decades^{7,20,24}. These results have recently been updated²⁵ (FIG. 1), and patients who responded to their Id vaccine continue to

show prolonged remission after vaccination. The most straightforward interpretation of these data is that the Id-specific immune response that is induced by vaccination is responsible for the improved disease-free survival, as all five patients without an Id vaccine-induced immune response experienced a typical second CR shorter than their first CR, and their second CR was shorter than 13 months¹⁴. Others have argued that it cannot be formally ruled out that the second chemotherapy could have been more effective than the first chemotherapy regimen²⁶. However, such an objection seems to ignore that at least 16 of the 20 patients who experienced a clinical benefit had a second-line treatment known to be of similar or inferior efficacy to that received in first-line treatment^{7,14}. It also ignores 50 years of clinical research into the treatment of FL^{20,24}. All in all, this study shows the strongest association yet published between specific immune responses induced by a cancer vaccine

and improved relapse-free survival in the patients that demonstrated an immune response. To also show a cause-and-effect relationship between the two findings, a control arm should have been added in which patients vaccinated with an irrelevant Id vaccine mounted an irrelevant Id-specific immune response and failed to experience an improved relapse-free survival. However, this type of prospective study design might have to overcome some preliminary and elementary ethical hurdles, particularly if randomization were performed shortly before vaccination start, as it would require nonspecific vaccination of some patients for whom a tumour Id-specific vaccine had been prepared and deliberately not used.

The major limitation of all these proof-of-principle studies is that they have been conducted in a limited number of patients and, as such, they are not suitable for regulatory approval of the whole procedure²⁷. Regulatory approval justly requires a clear-cut

demonstration of longer survival and/or better quality of life associated with any medical intervention carried out in a sizable patient population^{27,28}.

The failure of three Phase III trials

The last Phase III trial launched to test an Id vaccine is the first to provide peer-reviewed, published data¹⁸. This study (TABLE 1) failed to show a statistically significant difference between the time to progression of vaccinated patients and that of those who received the control product. This study also showed a statistically significant difference in favour of the patients treated with the control product¹⁸. However, the authors of this trial have indicated that a statistically significant imbalance in terms of the FLIPI score between the two randomized patient populations might account for these differences — the vaccinated patients had a worse prognosis than those receiving the control product¹⁸. Of the 349 patients randomized in a 1/1 fashion, around one-tenth did not receive study therapy for various reasons, and 315 were treated with either the recombinant vaccine formulation or GM-CSF alone¹⁸. Besides the imbalance in terms of the FLIPI score, several other flaws in the study design have clouded interpretation of

the overall results^{17,18}. This trial was launched after rituximab had become a key player in the management of newly diagnosed patients with FL⁷ and as such was designed to show that vaccination might further improve the clinical results obtained with rituximab, including CR, PR and stable disease²⁹. However, pre-vaccine treatment consisted solely of four weekly administrations of standard-dose rituximab without any chemotherapy. As a consequence, most of the subsequently randomized patients failed to achieve a CR before vaccination was started¹⁸. Moreover, it is well known that rituximab causes nearly complete B cell depletion in the peripheral blood, which can last up to 12 months⁷. Therefore, it is likely that most if not all of the patients started vaccination with severely depleted numbers of circulating B cells¹⁷. It is unclear whether defective responses to the Id vaccine and/or early disease progression were responsible for the study being negative¹⁸.

The first randomized trial launched to test recombinant Id vaccines (BOX 2) managed to enrol a sufficient number of patients (TABLE 1) in a timely fashion to allow completion of the predetermined statistical analysis. However, to date the data from this trial have not been published in the peer-reviewed literature.

Based on information from a published synopsis³⁰, 192 patients were randomized in a 2/1 fashion to receive seven Id vaccine doses, while 95 received the KLH plus GM-CSF control. Unfortunately, no statistical significance was found between the progression-free survival of vaccinated patients and that of those in the control arm. Once again, vaccinated patients who failed to mount an Id-specific humoral response and those who received the control formulation had a statistically significantly poorer progression-free survival, but as discussed above these are not acceptable criteria for proving clinical efficacy and/or benefit of Id vaccines. Several factors independent of the vaccine effects may explain this negative result^{17,18,25}. For example, the results may have been compromised by the decision to pool together and randomize not just patients who had achieved a pre-vaccine CR but also those who had achieved only a PR^{18,25,30} after receiving eight cycles of a cyclophosphamide, vincristine and prednisone (CVP) regimen. Around one-quarter of all the randomized patients relapsed or progressed between the sixth and eighth month after randomization, which was performed shortly before the first vaccination²⁵. This is uncharacteristic of FL⁷ and might reflect the fact that the quality of at least one-quarter of all CRs and/or PRs obtained through CVP was not sufficient to actually give vaccination a chance to succeed in preventing relapse or progression. To a lesser extent, similar considerations may apply to the fact that, like the other two randomized trials, this study enrolled not only patients with grade 1 and 2 indolent FL but also patients with grade 3 FL, who probably have a worse prognosis³¹ and for whom CVP is not an effective regimen⁷.

The only Phase III trial conducted to test hybridoma-derived Id vaccines (BOX 2) enrolled only newly diagnosed patients with FL who achieved a clinical CR before vaccination (TABLE 1), in line with the findings of the study that had shown clinical efficacy¹². However, the decision to retain the same chemotherapy regimen used in that single-centre trial¹² proved inappropriate for a multi-centre setting¹⁷. The regimen, which includes prednisone, doxorubicin, cyclophosphamide and etoposide, was neither popular nor widely used in the United States when this trial started and became less popular a few years later as rituximab became a mandatory regimen addition for all patients with FL receiving combination chemotherapy⁷. A late switch to pre-vaccine conventional chemo-immunotherapy including

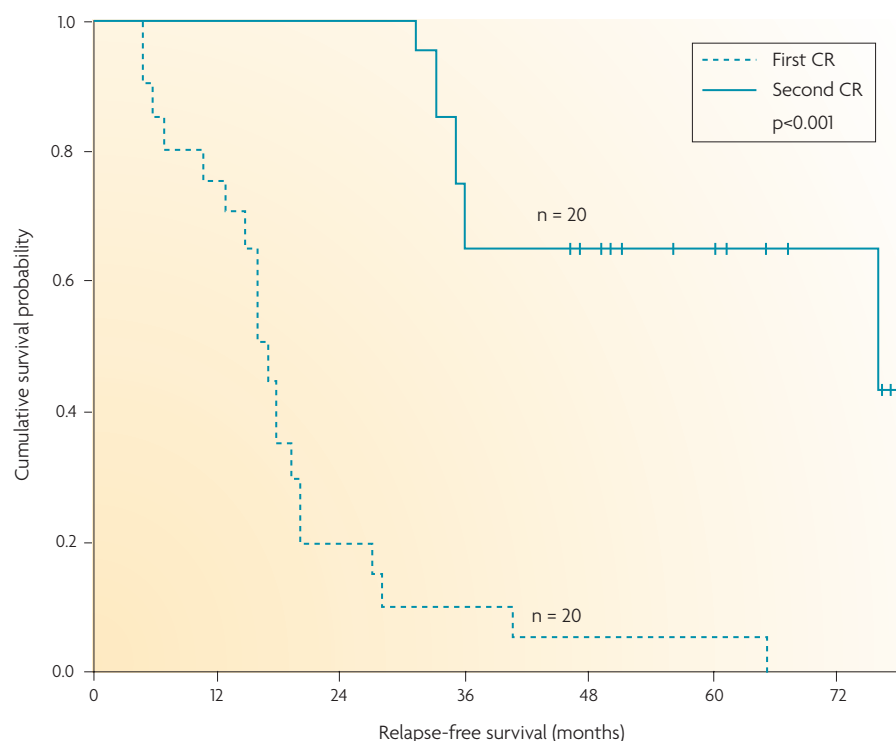


Figure 1 | Relapse-free survival curves of the first trial to prove clinical benefit of idiotype vaccination. All 20 patients with follicular lymphoma who had a vaccine-induced, idiotype-specific immune response experienced a standard chemotherapy-induced second complete response (CR) that was statistically significantly longer than their corresponding first CR obtained with standard chemotherapy with or without rituximab¹⁴.

rituximab, cyclophosphamide, doxorubicin, vincristine and prednisone, and an attempt at expanding recruitment to a number of Russian centres²⁵, did not reverse the slow patient accrual. As a consequence, over nearly a decade this trial randomly assigned less than half of the patients that had been targeted for entry within 3 years¹⁷. According to the published synopsis, on a modified intent-to-treat basis only 117 patients were evaluable instead of 375. Among them, only 76 instead of the hoped for 250 patients received at least 1 of the 5 doses of Id vaccine, and just 41 rather than 125 received the KLH plus GM-CSF control³². Therefore, less than one-third of the targeted patient accrual goal was actually reached. Statistical significance ($p = 0.045$) was shown in disease-free survival, favouring vaccinated patients (median was 44.2 months compared with 30.6 months for controls³²). However, given the facts that the trial accrual was largely incomplete and that the targeted level of statistical significance ($p < 0.01$) was clearly missed, it can be concluded that this trial failed to achieve the primary end point. The study does, however, support the concept of clinical benefit associated with the use of hybridoma-derived Id vaccines¹⁴.

All in all, the sobering conclusion is that three large-scale, Phase III clinical trials have failed to achieve their primary clinical end points and we cannot reject the null hypothesis that Id-based vaccines are ineffective.

Conclusions and future directions

Two clinical trials have shown clinical benefit of hybridoma-derived Id vaccines in FL. However, one of them was a small proof-of-principle study not designed for regulatory approval, and the other, although aimed at approval, fell short of its desired patient accrual and did not meet its main clinical end point. The other two trials aimed at approval failed to show clinical benefit of different versions of recombinant Id vaccines. As discussed above, each of the three Phase III clinical trials had several flaws in its protocol and so was predicted to fail long before its data were unblinded¹⁷. As, in my opinion, possible study protocol pitfalls cannot be credibly assessed in preclinical models in which, among many other non-reproducible features, the same Id is used to immunize all animals, the only way to further address the efficacy of Id vaccines is to conduct new and better designed clinical trials.

As mentioned previously, the use of standard randomization to assess the clinical benefit of a patient-specific vaccine^{17,20}

Box 2 | Soluble protein idiotype vaccine production

Regardless of the method used to produce the soluble protein Id in the laboratory, the only source of patient- and tumour-specific idiotype (Id) template is the tumour cell itself, as it is solely within this cell that the genomic material encoding the fully rearranged immunoglobulin that contains the Id can be found in its native form. Remarkably, Id production is amenable to and should be carried out under good manufacturing practice conditions.

In the case of Id hybridoma rescue, lymphoma cells are fused with a mouse and human heterohybridoma^{36,37} and one or more newly created hybridomas are selected that synthesize and secrete the same whole Id-containing immunoglobulin as that expressed on the tumour cell surface. The Id is subsequently purified from the supernatant of large-scale cultures of selected hybridomas^{36,37}.

In the case of recombinant Id production, tumour cells are indispensable for the cloning of both heavy and light chain variable regions of the Id-containing, tumour-specific immunoglobulin. Both cloned sequences are subsequently inserted into a plasmid that contains the sequence of a shared immunoglobulin constant region, and the complete plasmid is finally transduced into different living structures such as mammalian cells, insect cells, tobacco plants and bacteria³⁵. The Id is later purified from either the corresponding transfected cells or the culture supernatant³⁵.

After purification, the Id is partly conjugated to keyhole limpet haemocyanin using glutaraldehyde²³, and stored for later clinical use, and partly stored directly for subsequent assay of Id-specific immune responses¹⁴.

All things considered, recombinant technology is superior to hybridoma rescue from most standpoints, including time to production, quantity of Id obtained, variety and size of initial tumour samples, and successful production rate^{35,43,51}. However, although hybridoma-derived and recombinant Id vaccines have clearly shown immunogenicity⁴³, unlike hybridoma-derived Id vaccines recombinant Id vaccines have not shown clinical efficacy or clinical benefit so far^{12,14,18}.

has several intrinsic problems, some specific to FL and some common to other types of cancer. First, there are at least five sources of uncontrollable variability that make idiotypic vaccination in FL unlike a standard cancer drug regimen and dictate alternative approaches to the standard Phase III randomized trials: the heterogeneous natural history of the disease⁷, the heterogeneous influence of the tumour-bearing state on host defences⁷, the heterogeneous responsiveness of the host to the Id as an antigen (variable affinity of the Id antibodies for the tumour immunoglobulin)⁸, the heterogeneous anti-tumour effects of an anti-Id immune response (differences in cellular immune responses and differences in the outcome of signalling a tumour through its surface immunoglobulin)¹⁴, and the possible emergence of variant immunoglobulin over the course of the disease^{33,34}. The second issue reflects the entirely appropriate regulatory agency requirement for high statistical significance ($p < 0.01$) of the difference in survival (or possibly disease-free survival, given the long median overall survival) between vaccinated and non-vaccinated patients. Although the Phase III trials had similar vaccine formulations, each produced them in a different way (BOX 2), used a different vaccination schedule and used a different pre-vaccine treatment^{25,35}. There was no back-up trial planned to confirm the possibly positive findings of any of these trials. Therefore, since their onset it has been

known that the results of these competing clinical studies could not be used to either support or contradict each other in a case of simple statistical significance ($p < 0.05$). Meta-analysis was out of the question in light of the many variations among the studies.

It could be argued that differences between hybridoma-derived and recombinant Id vaccines might account for some of the trial findings. The differences between these vaccine types involve the constant region of the Id-containing immunoglobulin on the one hand and the glycosylation patterns of the resulting Ids on the other hand. The hybridoma-derived immunoglobulin containing the patient- and tumour-specific Id inherits the original heavy chain constant region as synthesized in the original tumour cells^{36,37}. Therefore, the actual Id contained in the vaccine formulation can be displayed on an immunoglobulin constitutively featuring a μ -, γ - or α -heavy chain³⁷. In recombinant Id vaccines the patient- and tumour-specific Id is inserted into a plasmid containing a predetermined, non-tumour cell-derived γ -heavy chain³⁵. The glycosylation patterns of human FL cell Ids^{38–40} are virtually impossible to reproduce on either hybridoma-derived or recombinant Ids. In hybridoma-derived Ids this is because the fusion partner of each patient's FL cells, the K6H6/B5 cell line, is a mouse and human heterohybridoma, not a fully human cell^{36,37}. In the case of recombinant Ids it is because the Id-containing plasmid is transduced into

other mammalian or insect cells, bacteria or plants³⁵. However, the limited data available seem to confirm that neither the variations in the heavy chain nor those affecting Id glycosylation between the original FL cell and the reproduced Id create any immunogenicity problem or make the anti-Id immune responses elicited through vaccination irrelevant to the actual FL cell-borne Id^{41,42}. As recombinant Id vaccines are easier to produce than their hybridoma-derived counterparts⁴³, new trials should probably concentrate on recombinant Id vaccines, but with better study designs. Preclinical research is underway to further improve the Id vaccine formulation. It is possible that Id vaccine immunogenicity can be further increased through different procedures of conjugating the Id with KLH, such as that based on the substitution of glutaraldehyde with maleimide^{44,45}, or by introducing chemokines in the vaccine formulation⁴⁶.

Compared with the Id-specific monoclonal antibodies⁴⁷ (abandoned long ago because of both the technical challenges and the emergence of Id variants^{33,34} that would not be recognized by such a patient-, tumour- and idiootype-specific passive immunotherapy), Id vaccines are meant to elicit polyclonal, Id-specific humoral and cellular immune responses. Whether an Id-specific humoral or cellular immune response is always necessary to achieve a survival benefit remains controversial³⁵. In fact, experimental^{9,10,48,49} and clinical^{22,50} evidence suggests that cellular^{10,50} and humoral^{9,48} immunity can each contribute to the efficacy of soluble protein Id vaccines, but their relative effect has never been fully ascertained⁵⁰. In my opinion, until this crucial piece of information is generated, eliciting and detecting both types of immune response^{12,14,49} should be a highly desirable biological goal for any new trial of Id vaccines. For this reason, and in spite of some evidence that the profound B cell depletion induced by rituximab⁷ affects humoral but not cellular anti-Id immune responses⁵⁰, future clinical trials on idiotype vaccination, particularly in patients who have relapsed following a rituximab-containing regimen, should probably not include the addition of rituximab to pre-vaccine chemotherapy or should delay immunization until documented B cell recovery. Furthermore, since Id availability is not a limiting factor of recombinant idiotype vaccination^{43,51}, higher doses of Id, as well as more frequent and/or extended booster administration^{14,16}, should be tested. Currently, new clinical trials with soluble protein idiotype vaccination are being developed to address these hypotheses.

Another point of major interest would be to develop the capacity to distinguish which patients are likely to benefit from vaccination and which are not, based on some pre-vaccination clinical characteristics. So far, prognostic factors for response have not been described. Although retrospective analyses have suggested better immune responses and clinical outcomes for patients with a particular immunoglobulin G Fc receptor genotype^{52,53}, this finding has not been confirmed by others¹⁴ or in a prospective manner^{18,30}. Furthermore, it would be desirable to better ascertain the actual relationship between patient disease and immune status on the one hand, and vaccine immune response and clinical activity on the other hand. The available data are still inconclusive regarding whether the most unwell patients with FL can still benefit from idiotype vaccination^{18,22}. It is also unclear whether the Id-specific immune response is necessary to see the clinical benefit.

The design of future clinical trials will be crucial. Considering that most newly diagnosed patients with FL who are actively treated receive rituximab with chemotherapy⁷, and that pre-vaccine rituximab seems not to be appropriate because of the associated B cell depletion⁷, a better setting in which to assess clinical benefit of Id vaccines could be in patients with FL who are in first relapse and/or progression^{7,14}. A few hundred such patients could be treated with a chemotherapy regimen generally considered similar or not superior to any rituximab chemotherapy administered as first-line therapy. If the addition of the customized Id vaccine confirms that the duration of the second clinical response is longer than the first in a substantial proportion of patients, that should at least allow consideration for regulatory approval without facing the specific complications of a randomization^{14,17,20,27,28}. Alternatively, in case this new approach were to face considerable opposition, the same main clinical end point could be independently integrated into a similar study design in which a patient population, after receiving chemotherapy, could be randomly assigned to receive either idiotype vaccination or maintenance rituximab. In this case, a second independent main clinical end point, possibly allowing regulatory approval, would be the non-inferiority of Id vaccines compared with rituximab. This goal could indeed be within reach for idiotype vaccination, given the fact that approximately half of the patients receiving rituximab for a second time for recurrent FL develop resistance⁵⁴.

Ultimately, idiotype vaccination is facing a crucial nexus. It will take determination and intelligence to avoid wasting four decades of study on a safe and possibly efficacious type of active specific immunotherapy. The mechanisms that lead to regulatory approval also need to be addressed given that efficacy could be missed because the system of drug development is tailored to agents that essentially do the same thing in every patient. This is not applicable to an intervention that is different in every patient and may have distinct effects on the subset of patients in whom it works.

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