# Biological Efficacy, Clinical Efficacy and Clinical Benefit of Idiotypic Vaccination for B-Cell Lymphoma

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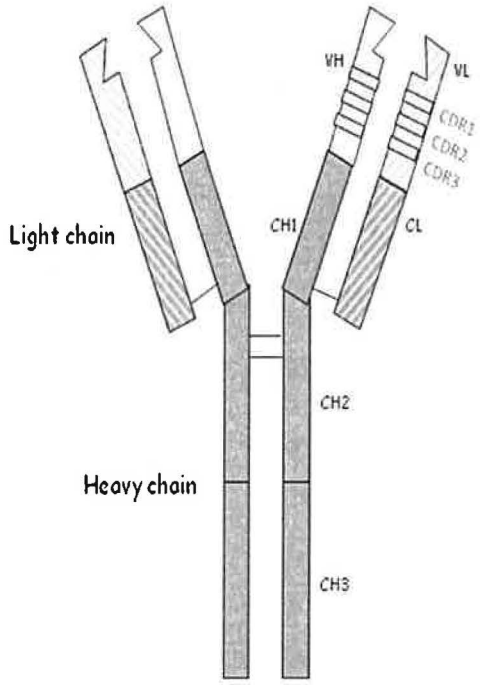
# INTRODUCTION

An idiotype is the entire collection of antigenic determinants termed idiotopes and contained in an antibody molecule. Idiotopes are found solely within the hypervariable regions of the immunoglobulin variable domain. These epitopes are somatically generated and can be recognized as foreign because they are present only in small amounts in an individual. Therefore, they are quantitatively insufficient to induce self tolerance mechanisms (1).




Schematic structure of a monomeric immunoglobulin. Legend: VH, heavy chain variable region; VL: light chain variable region; CH: heavy chain constant region; CL: light chain constant region. Idiotopes are exclusively contained in VH and VL.

Idiotopes may be classified as public or private. Public idiotopes are typically found within the immunoglobulin's framework regions, whereas private idiotopes are generally localized within the unique immunoglobulin's complementarity determining regions. The consequences stemming from this sub-classification are very important, given the fact that through idiotypic vaccination we strive to use the immunoglobulin no longer as an antibody, but as an antigen. On one hand, as far as anti-idiotype humoral responses are concerned, tumor suppression must ultimately rely on those against the private idiotopes, because any other anti-idiotope antibodies, even in the hypothesis that they are actually elicited, would be absorbed by the many other serum immunoglobulins. On the other hand, solely private idiotopes can actually function as a whole as a clonal marker of each tumor (2). As a matter of fact, each immunoglobulin features an extremely specific idiotype or, in other words, identical idiotypes prove identity among individual immunoglobulins. Therefore, in the case of a B-cell malignancy, the clonal idiotype featured by the tumor cell-synthesized immunoglobulin can be used as a tumor-

specific antigen for vaccine therapy. In this context, it is of paramount importance that the tumor cells express a correctly-shaped B-cell receptor on their cell membrane, or at the very least as many of its private idiotopes as possible within the HLA molecules for epitope presentation (3). After all, the ultimate biological goal of idiotypic vaccination is that of eliciting both a humoral and cellular, polyclonal immune response against several idiotype epitopes (4), in order to later prevent the potential tumor immune escape caused by the described susceptibility of some idiotypes to spontaneously undergo replacing mutations and/or amino acid insertions/deletions over time (5-7).

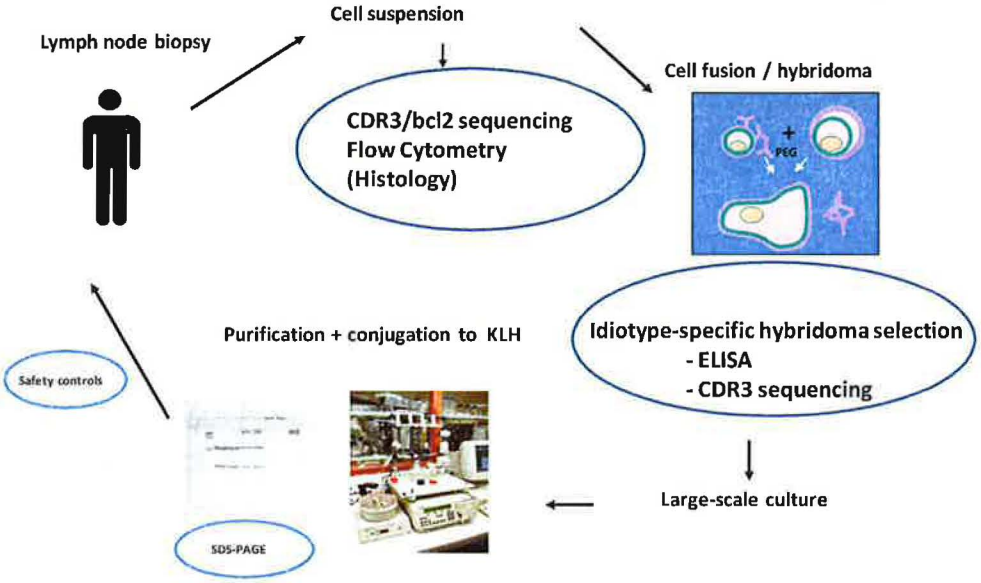
## IDIOTYPE VACCINE TYPES AND GOALS

Central to the production of any type of idiotype vaccine (8) is the timely availability of immunotherapeutic amounts of tumor-specific, private idiotopes. Not surprisingly, obtaining this material is exceedingly complicated in B-cell lymphoma, since the tumor cells do not shed their surface immunoglobulin, as compared to most cases of multiple myeloma, whose circulating paraprotein is vice versa readily available for purification from a common blood sample.

Idiotype vaccine strategies that have reached clinical application so far include the following: soluble protein idiotype vaccines (9-10), idiotype fragment-based vaccines (11-13), idiotype DNA vaccines (14-15), dendritic cell-based idiotype vaccines (16-17), liposomal idiotype vaccines (18), and transfer of allogeneic immunity achieved through soluble protein idiotypic vaccination (19-20).

Soluble protein idiotype vaccines for lymphoma utilize an idiotype protein obtained either from hybridomas (21-22) or via recombinant technology (23). The former methodology has been the sole to clear all proofs of principle in humans (8-9), though it is not equally feasible in different subsets of B-cell malignancies (22,24), while the latter has been seemingly successful in delivering a customized vaccine for most if not all patients in a faster and more standardized fashion (25). As mentioned before, soluble protein idiotype vaccines for myeloma utilize instead the purified clonal immunoglobulin freely circulating in the serum of the vast majority of patients.

## Vaccine Production




Most soluble protein idiotype vaccines used in lymphoma patients and not relying on the complete, tumor-specific immunoglobulin are rather based on its corresponding single chain variable fragment (scFv). This immunoglobulin fraction can be reproduced in the lab in a variety of manners, including those based on its synthesis in tobacco plants (26), cell-free systems (27) or Escherichia coli (28).

Idiotype DNA vaccines also feature the scFv only, but are typically injected as naked scFv-containing plasmid DNA into the patient's muscular cells, in which the expressed idiotype protein is supposed to create a depot of antigen (29).

In dendritic cell (DC)-based idiotype vaccines, either the whole idiotype-containing clonal immunoglobulin (16-17,20) or some of its idiotype peptides (17) can be employed to *ex vivo* pulse autologous (16-17) or allogeneic (20) DCs which will be subsequently infused to the patient with a B-cell malignancy. DC-based idiotype vaccines have been studied extensively over the last decade, particularly in multiple myeloma (30-36). However, their use seems to be fading lately, possibly due to some reports stressing the fact that DCs harvested from these patients are functionally defective (37-38). Moreover, even at the preclinical level different tumor models have lead to apparently contradictory conclusions about the meaning and the mechanisms of DC-based idiotype vaccine protective immunity (39-40).

Liposomal idiotype vaccines consist of a formulation in which both the soluble protein idiotype and a lymphokine, for instance recombinant human interleukin-2, are incorporated into liposomes to enhance immune recognition of the relevant antigen (41).

Finally, idiotypic vaccination can be safely used not just in an autologous, but also in an allogeneic setting. In particular, the soluble protein idiotype has been used, with limited clinical impact so far, to vaccinate healthy hematopoietic progenitor cell donors of siblings with multiple myeloma. The transfer of an allogeneic, tumor idiotype-specific immunity can be later carried out through an allogeneic stem cell transplant (42), as well as through donor lymphocyte (43) or idiotype-pulsed DC (20) infusion to the recipient of a previous allotransplant (20).

Given the fact that the tumor-specific idiotype is per se a weak antigen (8), in most vaccine formulations a primary role is typically played also by a limited number of carrier and/or adjuvant molecules (8-9). For example, in most soluble protein vaccines the idiotype is conjugated to the immunogenic carrier keyhole limpet hemocyanin (KLH) and administered together with granulocyte-macrophage colony-stimulating factor (GM-CSF), which has emerged as the best immunologic adjuvant so far (44), while similar considerations can be made for the use of the tetanus toxin in idiotype DNA vaccine formulations (29).

Crucial to the potential success of idiotypic vaccination for B-cell malignancies is a preliminary and thorough awareness of both its distinctive features and its actual goals. In particular, three different types of effect can be assessed in the evaluation of any human vaccine, including customized idiotype vaccines. The ability to elicit a specific immune response is called biological efficacy. The ability to induce anti-tumor effects in vivo is called clinical efficacy. The ability to influence disease end-points such as disease-free or overall survival is called clinical benefit. As for the latter two concepts, it is self evident that while there may be clinical efficacy without clinical benefit, yet there cannot be clinical benefit without clinical efficacy (8).

## BIOLOGICAL EFFICACY

A conspicuous number of studies have confirmed that different idiotype vaccine formulations and approaches can biologically succeed in humans. Idiotype-specific humoral and cellular immune responses can indeed be elicited in patients with lymphoma (Table 1) and myeloma (Table 2).

The first proof of principle of biological efficacy of idiotypic vaccination dates back two decades ago. Researchers at Stanford University demonstrated that a limited number of administrations of a soluble protein idiotype vaccine following chemotherapy sufficed to elicit idiotype-specific immune responses in a small number of follicular lymphoma patients (45-46). This study was later expanded and the proof of principle confirmed in 20/41 such patients (47).

Subsequently, other studies confirmed biological efficacy of idiotypic vaccination in lymphoma. In particular, variable degrees of idiotype- and tumor-specific, humoral and/or cellular immune responses were elicited in other studies employing soluble protein following autologous stem cell transplantation (48) as well as conventional treatment including the anti-CD20 monoclonal antibody rituximab (49). Similar results

have been also achieved using recombinant idiotypes (50) as well as substantially different idiotype vaccine formulations, including those based on its scFv (28,51), naked DNA (52), or liposomal delivery (53). Over time, it has not been clarified whether both idiotype-specific humoral and immune responses are essential for idiotypic vaccination to have a chance at aiming at clinical efficacy and benefit (54-55). A couple of retrospective analyses had raised the possibility that the induction of idiotype-specific humoral responses and a specific immunoglobulin G Fc receptor genotype might be independently correlated with better clinical outcome in follicular lymphoma patients (56-57), but these data have not been confirmed in subsequent prospective trials. Finally, once elicited, idiotype-specific immune responses can be safely maintained over several years by means of the prolonged administration of idiotype vaccine boosts (58).

Few years after the first evidence of biological efficacy of idiotypic vaccination in lymphoma, several clinical studies were carried out in multiple myeloma as well: once again, using either the whole soluble protein idiotype vaccine formulation (59-61) or just the customized idiotype to ex-vivo pulse autologous DCs (62-65). Various degrees of idiotype-specific humoral and/or cellular responses were reported in most if not all these trials conducted at independent centers (59-65). However, only tentative correlations have been possible between the duration of such immune responses and their impact, if any, on disease progression and survival (66-68).

## CLINICAL EFFICACY

Among the published papers showing biological efficacy of idiotypic vaccination for B-cell malignancies, many fewer also claim its clinical efficacy either in lymphoma. In particular, the first proof of principle of clinical efficacy of idiotypic vaccination in a small number of patients with follicular lymphoma dates back a decade ago. Researchers at the National Cancer Institute showed that most (8/11) follicular lymphoma patients who responded to idiotypic vaccination from an immunologic standpoint experienced the in vivo clearance of minimal residual disease that had persisted several months after successful completion of standard pre-vaccine chemotherapy (69). These results were later confirmed by others (70-71).

Beyond the clearance of relatively small amounts of circulating follicular lymphoma cells, immunologically successful idiotypic vaccination has been also occasionally associated with tumor shrinkage in up to 20% of treated patients, independently on whether the vaccination was based on pulsed DCs (72) or on the recombinant, soluble protein idiotype (73). However, this is also roughly the frequency by which clinically measurable follicular lymphoma has been described to undergo spontaneous regressions in the absence of any treatment (74-75). Therefore, these data are arguably less compelling in further supporting the clinical efficacy of the procedure.

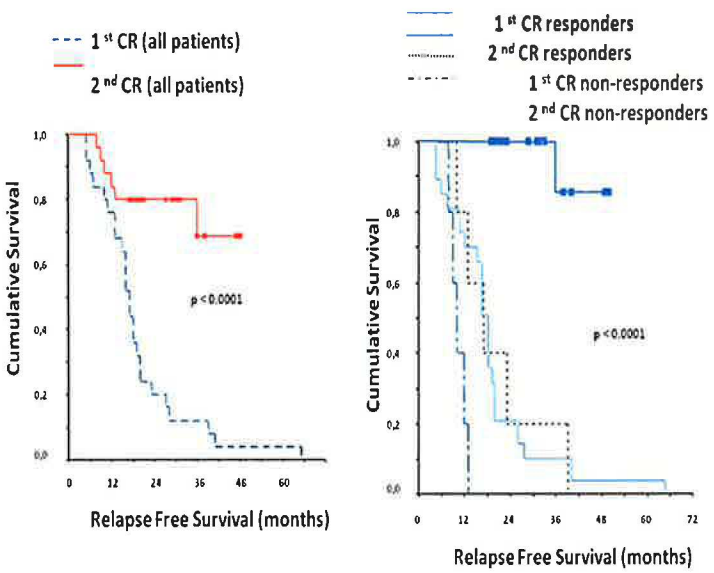
Tentative evidence of clinical efficacy associated with the use of idiotypic vaccination has been also provided in multiple myeloma patients. In a couple of small studies, the successful induction of idiotype-specific T cells by idiotypic vaccination

correlated with a decrease in circulating myeloma cells (76-77). However, neither significant changes in the serum paraprotein concentration nor any impact on the disease outcome were reported.

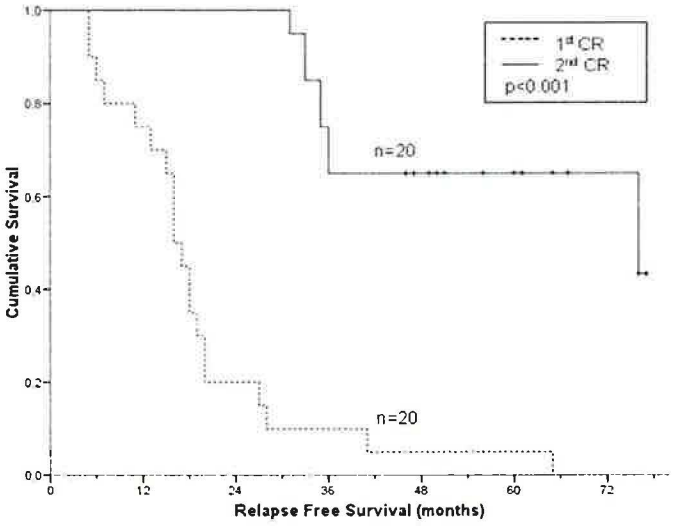
## CLINICAL BENEFIT

As it has been typical for idiotypic vaccination in follicular lymphoma, the first proof of principle of its clinical benefit has been provided through another small clinical trial conducted by researchers at the University of Navarra (78). In this study, all twenty patients in second clinical complete response after standard chemotherapy who mounted a post-vaccine idiotype-specific immune response experienced a second complete response statistically significantly longer than their first complete response (8,9,78), as well as systematically longer than the typical average duration of a second complete response obtained through standard chemotherapy (78). Both these clinical results cannot be explained in follicular lymphoma unless a beneficial idiotype vaccine effect is considered (8,75,79). Vice versa, all five patients in second complete response who did not respond to vaccination from an immunological standpoint experienced a second complete response both shorter than their first compete response and than the average duration of a second complete response in follicular lymphoma induced by standard chemotherapy (78). Others have argued that in this study it cannot be formally ruled out the possibility that chemotherapy could have been more effective than the first chemotherapy regimen (25). However, this objection is not sustainable (8), considering that at least 16/20 of the patients who responded to idiotypic vaccination in that study had indeed received a second line treatment known to be either non superior (79) or in many cases even frankly inferior (79) to that received in first line (T able 3), and that the highly statistical significance of the differences between second and first relapse-free survival was not at all affected by the 4/20 patients for whom such a claim may be arguable or speculative. Another unsustainable objection is that patients enrolled in this study were subjected to a selection bias, since only patients achieving a pre-vaccine second complete response were subsequently vaccinated (80). In facts, that was exactly what the study was meant to prove: unprecedented second complete responses longer than first complete responses in the very same follicular lymphoma patients. A patient cannot be selected if his/her results with an experimental therapy are compared to his/her own previous results with a gold standard treatment, because by definition a patient cannot be selected when he/she is also his/her own matched control.

## Survival curves




## Current update




Disappointingly but as predicted well before their clinical data were unblinded (81), all three independent phase-III randomized clinical trials on idiotypic vaccination for follicular lymphoma aiming at regulatory approval of the procedure have failed to achieve their main endpoints for reasons most likely unrelated to the different vaccines

they used (8-9). In particular, two trials using differently-produced versions of a customized, recombinant, soluble protein idiotype did not show any statistical difference in terms of disease control between vaccine and placebo study arms (82-83). However, given the important flaws in their independent study designs, their negative results may or may not have depended on the actual efficacy of the vaccines they used (8-9). The third randomized trial, based on hybridoma-derived idiotypes like those used in all successful proof-of-principle studies mentioned before, was well designed instead, but unsatisfactorily performed (8-9). Contrary to the two competing trials above, it failed to enroll enough patients and did not even manage to randomize over nearly a decade one third of the patients that was supposed to enroll within three years (84). However, it tentatively confirmed clinical benefit of idiotypic vaccination by showing a statistically significant advantage ($p=0.045$) in relapse-free survival for vaccinated patients compared to patients receiving a placebo (84). Unfortunately, the statistical significance achieved by this study was not as stringent ($p<0.01$) as required for regulatory approval (8-9) and this fact, together with the conspicuous deficit in patient enrollment, will likely condemn the trial to be yet another promising failure of idiotypic vaccination. Besides, it has to be also acknowledged that, with a disappointing (~30%) of the expected accrual to which base the statistical analysis on (8,84), there is no guarantee that, had the enrollment be completed, the ultimate p value of this study might have been even more statistically significant on one hand, but also non-statistically significant at all on the other.

## NHL idiotype vaccine: previous endpoints

| Sponsor | Enrollment | Randomization | Endpoint | p value |
|---|---|---|---|---|
| | | | | |
| **Biovest (expected)** | **563 pts** | **Vaccine: 250 pts<br>Placebo: 125 pts** | **DFS from random date** | **<0.01** |
| *Biovest (actual)* | *177 pts* | *Vaccine: 72 pts<br>Placebo: 39 pts* | *DFS from random date* | *=0.045** |
| | | | | |
| **Genitope (expected)** | **360 pts** | **Vaccine: 240 pts<br>Placebo: 120 pts** | **PFS from random date** | **<0.01** |
| *Genitope (actual)* | *315 pts* | *Vaccine: 192 pts<br>Placebo: 95 pts* | *PFS from random date* | *ns* |
| | | | | |
| **Favrille (expected)** | **342 pts** | **Vaccine: 171 pts<br>Placebo: 171 pts** | **TTP from random date** | **<0.01** |
| *Favrille (actual)* | *349 pts* | *Vaccine: 174 pts<br>Placebo: 175 pts* | *TTP from random date* | *ns* |

All in all, evidence of clinical benefit of idiotypic vaccination has been shown so far only in follicular lymphoma and only with hybridoma-derived idiotype vaccines. Even long-term, retrospective correlations between idiotype-specific immune responses and overall survival seem to support that notion (85). However, this does not necessarily mean that recombinant idiotype vaccines are less or not at all efficacious. The two failed randomized trials using recombinant idiotype vaccines were not designed to clarify the

reasons of their eventual failure, and as they indeed failed, further studies will be needed to give recombinant vaccines a fair chance to succeed, given the fact that they would be both easier to produce and more readily available to patients (8).

Finally and as briefly mentioned above, no data is currently available to support the notion of clinical benefit of idiotypic vaccination in multiple myeloma and in other B-cell malignancies.

## A ROAD MAP FOR THE FUTURE

Idiotypic vaccination for B-cell malignancies is one of the boldest and most complex therapeutic strategies under development in human oncology. Contrary to most if not all other treatment options, it is not directly active against tumor cells (58), and yet is meant to be tumor-specific (8), uniquely tailored for each patient's immune system (8) and virtually non-toxic (58). All these features in general, and its extreme specificity in particular, as it is the case for virtually any form of truly personalized medicine, pose a serious intellectual challenge to historical ways of possibly proving its clinical benefit: even the otherwise unbeatable randomized trials (8,75). As many as three of these studies, for different reasons, have recently and sadly confirmed this concept. An alternative approach to both confirm clinical benefit and aim at regulatory approval might be that of conducting another clinical trial similar to that performed at the University of Navarra (78), but with about ten times the accrual (8). To achieve such an ambitious goal, idiotype vaccine production via the recombinant technology seems however preferable to traditional hybridoma rescue (8).

Meanwhile, since irrespective of the actual outcome of previous studies there is no reason to halt the developmental process of idiotype vaccines, further research is warranted in order to possibly improve and enhance the immunogenicity of this weak antigen. For instance, very little is known about the implications of the natural addition of sugar molecules to the variable regions of the idiotype-containing clonal immunoglobulin (8). We know that the variable regions of follicular lymphoma-associated clonal immunoglobulins are characteristically rich in acquired potential N-glycosylation sites (86-88) and that actual idiotype glycosylation may vary substantially depending on the type of glycosylation machinery inherently used during the idiotype production process by mouse/human hetero-hybridomas, mammalian or insect cells and tobacco plants (89). Yet, no data is available to ascertain whether any type of idiotype vaccine should be preferred to others based on these biological features. Since hybridoma-derived idiotype vaccines are less feasible but have cleared all the hurdles of clinical development, while recombinant idiotype vaccines are far more feasible but have not gone beyond proofs of biological efficacy, this type of knowledge should be eagerly pursued.

Another interesting way to possibly improve the immunological quality of our idiotype vaccine formulations may depend on an improved methodology of conjugation between the small size idiotype and the large size KLH molecule. In this respect, some

seemingly successful attempts have been carried out recently to replace glutharaldehide with maleimide in this process (90-92).

Currently, several lines of original translational research are in progress to further enhance idiotype vaccine biological activity in clinically meaningful ways. They include but are not limited to the addition of chemokines (93-95) or immunostimulatory CpG oligodeoxynucleotides (96-97) to the vaccine formulation, as well as the delivery of the tumor-specific idiotype together with other putative tumor-specific and tumor-associated cell membrane antigens through proteoliposomes (98-99). Moreover, further attention is dedicated on ways to streamline and make more reproducible the tests by which post-vaccine, idiotype-specific immune responses are assessed and reported. Idiotype-specific T-cell responses are still documented by too many lab tests (78), while the arbitrary four-fold increase in post-vaccine, anti-idiotype antibody titer currently defining a positive, idiotype-specific humoral response, could be replaced by an arguably more stringent, though equally arbitrary, definition. For instance, an anti-idiotype humoral response could be considered specific provided that the following conditions are both met: a) the optical density ratio between post- and pre-vaccine sera is at least 4-fold in one dilution and 2-fold in another dilution, or at least 3-fold in two dilutions, or at least 2-fold in three dilutions; b) the same optical density ratio above, compared with that of an irrelevant, isotype-matched Id control is also at least 4-fold in one dilution and 2-fold in another dilution, or at least 3-fold in two dilutions, or at least 2-fold in three dilutions.

Finally, two recent preclinical reports are mentioned here because their results seem to go against the established common knowledge and, as such, they deserve to be kept in mind and followed up. The first study indicated that idiotype-specific humoral responses can delay myeloma cell growth despite the well-known virtual absence of immunoglobulins on the clonal plasma cells (100), while the second study showed that a favorable environmental and psychological setting dramatically increases the efficacy of idiotypic vaccination (101).

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