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REVIEW

Anti-idiotype antibodies in cancer treatment

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As a cancer immunotherapy tool, idiotypes (Ids) have been used in different ways over the last three decades, depending on the actual human tumor cell target. It all started with passive, monoclonal, anti-Id antibody treatment of B-cell lymphoma, a setting in which results were tantalizing, but logistics unsustainable. It then moved toward the development of anti-Id vaccines for the treatment of the same tumors, a setting in which we have recently provided the first formal proof of principle of clinical benefit associated with the use of a human cancer vaccine. Meanwhile, it also expanded in the direction of exploiting the antigenic mimicry of some Ids with Id-unrelated, tumor-associated antigens for the immunotherapy of a number of solid tumors, a setting in which clinical results are still far from being consolidated. All in all, over the years Id-based immunotherapy has paved the way for a number of seminal therapeutic improvements for cancer patients, including the development of most if not all Id-unrelated monoclonal antibodies that have recently revolutionized the field.
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Introduction

The idiotype

The term idiotype (Id) refers to the entire collection of idiotopes contained in a single immunoglobulin (Ig) molecule. Idiotopes are one of the two types of Ig epitopes identified by monoclonal antibodies (mAbs), the other being that of allotopes. As opposed to what happens with allotopes, which are mostly localized within the heavy- and light-chain constant regions of the Ig, idiotopes can be found only in the hypervariable regions of the Ig variable domains. Moreover, they are somatically generated, rather than derived from the inherited germ line like the allotopes. Finally, idiotopes can be recognized as foreign because of the fact that the tiny amount of them normally present in any individual is insufficient to elicit self-tolerance, whereas allotopes can be also recognized as foreign, but rather because they are not shared by different individuals (Bendandi, 2001). To date, the patient-specific Id borne by a number of different B-cell lymphoma subtypes is the sole, complete, tumor-specific antigen we know (Bendandi, 2004).

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Id-based immunotherapy strategies for the treatment of cancer

The most obvious application of such a tumor-specific antigen would be of course that consisting in targeting it by means of therapeutic antibodies. This whole issue of *Oncogene Reviews* is all but a tribute to the extraordinary clinical results achieved in so little time by means of targeting tumor-associated – not even tumor-specific, with all limitations ensuing – antigens. It is intuitive that such a strategy might prove even more efficacious when targeting a tumor-specific, rather than just tumor-associated antigen. The main problem, though, is that the Id is not only tumor-specific: it is also patient-specific, with all imaginable consequences when it comes to reconcile independent factors such as science, logistics, large-scale production, business and social costs. Still, few nearly unfeasible studies on anti-Id mAbs have ultimately paved the way for the clinical development and diffusion of other such therapeutic mAbs. The target changed. The concept has not. To such an extent, that in the case of solid tumors even just Id mimicry with certain Id-unrelated, tumor-associated antigens is being tentatively exploited in therapeutic terms.

In this review, we shall discuss the main clinical applications of Id-based cancer immunotherapy. Most methodological and preclinical issues can be further studied by means of the pertinent bibliography that follows this text.

Anti-Id antibodies as a passive immunotherapy for B-cell lymphoma

In 1982, Ron Levy and co-workers were the first to report on the treatment of human B-cell lymphoma by means of a custom-made, patient-, tumor- and Id-specific mAb (Miller *et al*., 1982). What might have

seemed merely anecdotal – although revolutionary indeed – at that time, evolved into a pilot study in which 11 patients with B-cell malignancy received their respective, customized, murine, anti-Id mAbs. Nearly half of these patients experienced objective remissions with clinical significance, although it was immediately clear that a number of features concerning this new therapeutic approach needed to be refined. For instance, patients with circulating, soluble, tumor-specific Id protein had to be considered less prone to respond from a clinical standpoint, and human anti-mouse antibodies were produced over time by a considerable number of patients treated with anti-Id mAbs (Meeker *et al*., 1985).

In Europe, similar anecdotal attempts were performed shortly thereafter. The number of patients treated with anti-Id was very limited, so that the evidence could not be regarded as conclusive. Still, it is noteworthy that a group from the University of Nijmegen reported that, on one hand, despite its strong potential, passive anti-Id immunotherapy seemed incapable of eradicating the tumor clone in humans (Allebes *et al*., 1991); and on the other, tumor recurrences with phenotypic and functional changes were unlikely to be prevented (Allebes *et al*., 1990). In this respect, even more robust data on these issues were once again provided by scientists at Stanford University. In fact, they clearly showed that the first combination of a short course of chemotherapy (CHT) and immunotherapy ever used – a decade before that became standard practice with anti-CD20 mAbs and polyCHT in lymphoma – was both effective and safe, although it did not solve all problems associated with the use of both a nonspecific treatment like CHT and a 'too specific' (being monoclonal) tool like the anti-Id mAb. In particular, the addition of chemotherapy did not interfere with the anti-tumor effect of anti-Id mAbs, nor prevented the emergence of Id-negative tumor cell variants (Maloney *et al*., 1992). This latter fact, together with the *in vivo* emergence as well of Id variants on the clonal cells of human B-cell malignancies – regardless of whether they had been previously treated with anti-Id mAbs (Meeker *et al*., 1985) or not (Raffeld *et al*., 1985) – and with the unsustainable logistical problems, ultimately decreed the downfall of passive immunotherapy of B-cell malignancies based on anti-Id mAbs. What we are left with is a largely unfeasible therapeutic approach, by which as many as 45 indolent B-cell lymphoma patients were treated in a single center between 1991 and 1993. Of them, six maintained their complete response for up to 10 years without further treatment, but also without eradication of their minimal residual disease (MRD) as monitored by polymerase chain reaction (Davis *et al*., 1998).

Anti-Id vaccines as an active immunotherapy for B-cell lymphoma

Soluble protein Id vaccines for follicular lymphoma

Id vaccines cannot overcome the methodological difficulties associated with the issue of customizing each therapeutic tool, which is to provide one immunotherapeutic formulation per patient. However, no matter in which way such vaccines are ultimately produced (Caspar *et al*., 1997), they can at least dramatically limit the pitfalls associated with the use of a monoclonal treatment directed to a single – potentially and not seldom changing due to somatic mutations – Id epitope. In fact, when immunologically successful, they are indeed able to elicit polyclonal immune responses that cover mutated tumor Ids.

Following the intriguing though frustrating results obtained with anti-Id mAbs, the same group at Stanford University was the first to prove biological activity of a human cancer vaccine. In particular, after a decade spent testing the same hypothesis in murine models – mainly aiming at inducing both specific and effective anti-Id humoral responses – they showed that 7/9 follicular lymphoma (FL) patients treated with customized anti-Id vaccines were indeed able to produce anti-Id Abs as a direct consequence of such immunization. Clinical efficacy could not be assessed, nor a thorough analysis of specific, anti-Id cellular responses induced was carried out, but the results were undoubtedly solid enough to pave the way for further development of Id vaccines (Kwak *et al*., 1992). This study was expanded over time, with Id vaccines being ultimately administered to 41 patients with B-cell lymphoma. In nearly half of them the procedure proved capable of eliciting Id-specific immune responses, with a generic trend, but no formal proof of improved clinical outcome for immune responders (Hsu *et al*., 1997). In this line, over the years, the same group has kept focusing on the possible correlation between Id-specific humoral responses induced through idiotypic vaccination and patients' clinical outcome, always with compelling results (Weng *et al*., 2004, 2006), but also with a substantial failure to prove clinical efficacy and/or benefit, as well as to thoroughly document vaccine-induced, Id- and/or tumor-specific cellular immune responses. Moreover, the idea of pulsing autologous dendritic cells with the soluble, tumor-specific Id protein (Hsu *et al*., 1996), although feasible and promising, did not generate enough evidence in terms of possibly improved biological and clinical results as to warrant further development (Timmerman *et al*., 2002).

Meanwhile, scientists at the National Cancer Institute showed in preclinical models of idiotypic immunization against B-cell lymphoma that the use of granulocyte macrophage colony-stimulating factor (GM-CSF) as an immunologic adjuvant substantially improved vaccine efficacy in terms of ability to elicit Id-specific cellular responses (Kwak *et al*., 1996). This fact, translated to a phase-II clinical trial, led to the second formal proof of principle: that of clinical efficacy. In fact, besides documenting the expected Id-specific humoral response in 75% of vaccinated patients, this trial showed an unprecedented 95% of Id- and tumor-specific, vaccine-induced cellular response. Even more importantly, most tested patients with MRD after CHT and before vaccination had their MRD cleared upon completion of the vaccination schedule. In other words, this study

showed that tumor cells that had survived CHT were indeed killed by immune effector mechanisms induced through vaccination (Bendandi *et al*., 1999). Similar results were later reproduced on a smaller scale by scientists at the Puerta de Hierro Hospital of Madrid (Barrios *et al*., 2002).

Finally, idiotypic vaccination has recently achieved the ultimate goal, never achieved before by any human cancer vaccine: clinical benefit (Inogés *et al*., 2006). All first-relapse FL patients with a CHT-induced second complete response who responded to their post-CHT, customized Id vaccine maintained that clinical result over a time far longer than both the expected, well-known median duration of it in typical FL patients in the same setting, and than their own first complete response. This latter piece of evidence is unprecedented. Even more compelling was the fact that the study was designed to give idiotypic vaccination any chance to possibly fail. In particular, being the typical median duration of a standard CHT-induced second complete response in FL about 13 months, all patients underwent Id vaccine treatment during 26 months. Had any immune responder relapsed while both receiving such an immunotherapy and responding to it, we would have proved the opposite concept: that Id vaccines, although biologically and clinically active, are not clinically beneficial to FL patients. Yet, this fact never happened, providing the ultimate conclusive evidence of how Id vaccine clinically benefits each and every patient who responds to it from an immunologic standpoint. This proof of principle is particularly important at this time, as due to both conceptual and design-related crucial pitfalls, it is quite unlikely that any of the three ongoing, randomized clinical trials on idiotypic vaccination, including one based on soluble protein Id vaccines (Neelapu *et al*., 2005a, b, c), will be able to prove the same clinical benefit (Bendandi, 2006). Regardless, other hurdles wait for Id vaccines clearance: first and foremost, it might become paramount to verify whether CHT plus idiotypic vaccination can withstand efficacy and benefit comparisons with other combination regimens such as those including CHT plus anti-CD20 mAbs (Longo, 2006). Given the huge difference in logistic and large-scale production problems, Id vaccines could indeed survive such comparisons only if providing a clear survival advantage over the latter therapeutic strategy at least in a substantial subset of lymphoma patients. Moreover, it remains to be fully elucidated whether B-cell depleting treatments such as those based on anti-CD20 mAbs – which leave most if not all patients without any chance of anti-Id humoral response for up to 12 months after completion of such a passive immunotherapy – may or may not be conveniently coupled with subsequent idiotypic vaccination of patients with B-cell lymphoma (Neelapu *et al*., 2005a, b, c).

Recombinant Id vaccines for FL

Over the last two decades, less than 200 patients with B-cell lymphoma have been treated worldwide with soluble protein Id vaccines. This fact does not come as a surprise, taking into account that manufacturing each and every such a customized therapeutic tool typically takes around 6 months, if at all feasible (Inogés *et al*., 2003).

For these reason, both the academic environment and few biotechnology companies have begun exploring safety, feasibility and now even clinical significance of administering custom-made Id vaccines produced by means of recombinant technology (Timmerman, 2002).

Currently, three independent approaches to recombinant Id vaccines have reached the stage of clinical trials: two of them have indeed entered already the arena of phase-III, controlled clinical trials (Bendandi, 2006). After extensive preliminary work carried out at Stanford University, Genitope Corp (Fremont, CA, USA). has completed enrollment of patients destined to be randomized at receiving either cyclophosphamide vincristine prednisone (CVP) CHT plus the customized Id vaccine or CVP CHT alone. Both annual interim analyses conducted so far have failed to show statistical significance between the two arms (Longo, 2006), and only a final analysis is left, less than a year from now. Meanwhile, Favrille Inc. (San Diego, CA, USA) is essentially doing the same (Hurvitz and Timmerman, 2005), although patients are randomized to receive or not the customized Id vaccine following anti-CD20 mAb passive immunotherapy, rather than after CHT. The only interim analysis conducted so far has recently failed to show statistical significance in achieving the study's secondary goal, whereas primary goal was not the object of such analysis. As mentioned above, both these studies are also flawed by a number of both conceptual and design-related pitfalls that may ultimately endanger their chances of success, irrespective of the actual value of the Id vaccines they are based on (Bendandi, 2006). Indeed, at least in the case of Favrille's Id vaccine, recently published data from a phase-II clinical trial, in which previously treated patients were treated at relapse with Id vaccine alone, are particularly promising (Redfern *et al*., 2006).

Finally, scientists from the University of Freiburg have recently reported on the clinical application of a recombinant Id vaccine consisting of the sole tumor-specific Ig's Fab produced in *Escherichia coli* (Bertinetti *et al*., 2006a, b). Specific immune responses were documented in the first phase-I clinical trial conducted with this new Id vaccine formulation, although it goes without saying that further studies are warranted to better evaluate the possible clinical relevance of such an alternative approach (Bertinetti *et al*., 2006a, b).

DNA-based Id vaccines

An even more alternative approach to soluble protein Id vaccines is that which aims at administering to B-cell lymphoma patients the tumor-specific Id in the form of its corresponding DNA sequence (Stevenson *et al*., 1995a, b). This field, both in the preclinical and clinical setting, has being mostly pioneered and further explored by a single group of scientists working at the University of Southampton (Stevenson *et al*., 1995a, b).

In general, clinical experience with intramuscular injections of DNA-based Id vaccines in FL patients remains extremely limited and with results which definitely warrant further increase of the procedure's immunological potency (Hawkins *et al*., 1997). However, some impressive preclinical data seem to predict that the addition of powerful adjuvant DNA sequences – such as fragment C of tetanus toxin (King *et al*., 1998) or potato virus X coat protein (Savelyeva *et al*., 2001) – to the nude Id DNA sequence might indeed be crucial for this strategy to induce clinically efficacious immune responses (Zhu *et al*., 2001). Moreover, it seems also well established that, as it happens with soluble protein Id vaccines, to maximize chances of both immunologic and clinical efficacy of a DNA-based idiotypic vaccination strategy, both Ig's variable region genes must be included in the vaccine formulation (Benvenuti *et al*., 2000).

Soluble protein Id vaccines for multiple myeloma

Multiple myeloma (MM) likely represents the clinical setting in which Id vaccines have collected the most disappointing results. Whether this may be due to the intrinsic clinical differences between myeloma and lymphoma, or rather to the fact that after facing for a long-time huge amounts of circulating tumor-specific Id paraprotein, the MM patient's immune system is far more unlikely to respond to idiotypic vaccination, it is largely unknown (Bendandi, 2004). Yet, over the last decade, a considerable amount of clinical data has been published.

Scientists from the Karolinska Hospital were the first to report that GM-CSF-containing idiotypic vaccination of a limited number of patients with IgG MM systematically elicited type-I, human leukocyte antigen (HLA)-restricted, $CD8^+$- and $CD4^+$-specific T-cell responses (Osterborg *et al*., 1998), occasionally translating in an encouraging reduction of circulating clonal myeloma cells (Rasmussen *et al*., 2003). However, specific anti-Id humoral responses were never documented, serum paraprotein levels remained unaffected in all cases and an actual clinical benefit could never be proven.

Similar results have been also reported in a slightly larger number of patients with MM treated at the University of Turin with Id vaccines after autologous stem cell transplantation conditioned with high-dose CHT. In spite of the expected, short-lived but profound immune suppression, vaccine-induced Id-specific cellular – but not humoral – immune responses were documented even in this setting (Massaia *et al*., 1999). Although idiotypic vaccination was unable to clear MM MRD in any immunized patient, a median progression free and overall survival of 40 and 82 months, respectively, seem to warrant further investigation on this kind of combined (high-dose CHT-conditioned autologous stem cell transplant followed by idiotypic vaccination in MRD patients) treatment approach (Coscia *et al*., 2004).

To possibly make idiotypic vaccination more effective from an immunological point of view, soluble protein Id-pulsed dendritic cells have been used as an immunotherapeutic approach in a number of clinical settings involving MM patients. However, the data generated by a few independent groups do not allow for firm conclusions to be drawn even just on a clear-cut issue like the actual ability to induce Id-specific immune responses, which possibly depending on slight differences on the ultimate method used to purify autologous dendritic cells, has resulted being at times absent (Cull *et al*., 1999), and other times occasional (Reichardt *et al*., 1999), relatively frequent (Reichardt *et al*., 2003), and even associated with anecdotal, unprecedented Id-specific humoral responses (Titzer *et al*., 2000).

Finally, in the allogeneic setting, two different and groundbreaking attempts have been carried out to possibly work the potential of donor-derived immune cells in favor of sibling, HLA-matched MM patients. On one hand, soluble protein Id-pulsed, allogeneic dendritic cells have been administered to MM patients with as poor a prognosis as it is intrinsic for those who have relapsed following reduced-intensity conditioning allogeneic stem cell transplantation. The procedure was safe and feasible, but overall results were substantially disappointing (Bendandi *et al*., 2006). On the other hand, scientists at the National Cancer Institute have published the long-term results of a pilot study in which sibling, HLA-matched hematopoietic stem cell donors were immunized by idiotypic vaccination before harvest and high-dose CHT allogeneic stem cell transplantation of their corresponding MM patients. Specific anti-Id immunity transfer was formally demonstrated (Neelapu *et al*., 2005a, b, c), although it remains difficult to establish the possible correlation with the generally favorable clinical outcome, which of course might depend on the transplant procedure *per se*.

Anti-Id antibodies as an immunotherapy for solid tumors

General aspects

The use of Id vaccines to stimulate anti-tumor immunity against a number of non-B-cell cancer varieties has shown promising results (Bhattacharya-Chatterjee *et al*., 2002), and it is fundamentally based on the fact that some anti-Id mAb – used indeed as vaccines in this setting – function as true surrogate for selected tumor-associated antigens that have nothing to do from a functional point of view with any Id *per se*. In other words, the stochastic tumor antigen mimicry of these anti-Id mAbs is exploited to elicit specific immune responses against both antigenic structures. In most cases, this mimicry depends far more on the two proteins' (the anti-Id mAb and the Id-unrelated tumor-associated antigen) structural rather than amino-acid sequence homology, although in a limited number of settings the opposite may actually occur (Bhattacharya-Chatterjee *et al*., 2002). Occasionally, such an antigen mimicry can even involve non-proteic structures such as carbohydrates (Sugiyama *et al*., 1991).

The results obtained through this anti-Id mAb-based approach in phase-I and -II trials are eagerly awaiting possible confirmation from a number of randomized trials currently ongoing or planned.

Melanoma

Over the last two decades, a number of melanoma-associated antigens have been identified, whose mimicry with selected murine, anti-Id mAbs could be used for therapeutic aims. For instance, mAb MK2-23, which bears the internal image of high molecular weight-melanoma-associated antigen (HMW-MAA), has been used to vaccinate a small number of patients with advanced melanoma. With all limitations of either small-scale pilot studies or retrospective analyses, scientists at New York Medical College were able to document temporary metastases regressions (Mittelman *et al*., 1994), as well as survival prolongation possibly correlating with the kinetics of vaccine-induced, Id- and HMW-MAA-specific humoral responses (Mittelman *et al*., 1995). With respect to the same antigen mimicry, it has been recently shown that it strictly depends on the peculiar homology – in terms of both structure and amino-acid sequence – existing between the HMW-MAA core protein and the portion of mAb MK2-23, resulting from the juxtaposition of its heavy chain's complementarity-determining region 3 and its light chain's complementarity-determining region 1 (Chang *et al*., 2005).

Other clinical applications of different anti-Id mAbs mimicking HMW-MAA have been also subsequently attempted in small multi-center trials. Among them, Melimmune is a dual preparation of two such murine mAbs, which has proved capable of inducing antigen-specific humoral and cellular immune responses (Saleh *et al*., 1998), including HMW-MAA-specific cytotoxic T lymphocytes (Murray *et al*., 2004).

Finally, other phase-II clinical trials based on anti-Id mAb-based vaccines, whose encouraging results need further confirmation in controlled studies, are those respectively utilizing the so-called TriGem formulation (Lutzky *et al*., 2002) and the 1E10 γ-type, anti-Id mAb, whose mimicry concerns *N*-glycosyl-containing gangliosides as well as other antigens expressed on human melanoma and breast carcinoma cells (Alfonso *et al*., 2002). In both cases, anti-Id mAb-based vaccination elicited strong and specific humoral responses directed against all antigens involved in the mimicry pattern. Clinical implications of these results, if any at all, remain to be fully elucidated.

Colorectal cancer

Clinical investigation on mimicry of colorectal cancer-associated antigens by anti-Id mAbs has focused over the last two decades fundamentally on two independent tools such as: colorectal carcinoma (CRC)-associated GA733 antigen (also known as CO17-1A, KS1-4, KSA or EpCAM) and the carcinoembryonic antigen (CEA). The latter is known to be expressed also by a wide variety of adenocarcinomas other than CRC.

Over about 20 years, scientists at the Wistar Institute have tenaciously brought to sequential clinical application passive immunotherapy with murine CO17-1A mAb and active immunotherapy with polyclonal goat anti-Id antibodies produced against the same CO17-1A mAb, which mimics the CRC-associated antigen above (Birebent *et al*., 2001a, b). Results of the former approach were substantially modest, whereas with the latter strategy anti-anti-Id humoral immune responses were systematically documented ever since the first trial was concluded, and encouraging partial responses were also described. However, in most cases, it was not possible to single out the exact contribution to these results of anti-Id antibodies boosts on one hand and adjuvant CHT on the other (Herlyn *et al*., 1989). Further development involved the transition from intradermal to subcutaneous administration, with the first evidence of specific cellular, together with humoral immune responses induced by this immunotherapeutic approach. Once again, the actual clinical impact of the strategy could not be completely ascertained, inasmuch as all patients had undergone primary tumor and lymph node metastases surgical removal before receiving immunotherapy (Somasundaram *et al*., 1995). More recently, a rat mAb directed against CO17-1A has shown clear superiority over the goat polyclonal antibodies above in inducing antigen-specific, humoral and cellular immune responses; these responses also benefited of conjugation of such a mAb with keyhole limpet hemocyanin (Birebent *et al*., 2001a, b). Subsequent studies have also proved that when molecularly cloned in a baculovirus, the GA733 epitope is more immunogenic than its mimicking anti-Id mAb counterpart, both in terms of humoral and cellular immune responses elicited (Birebent *et al*., 2001a, b). These results have also been independently confirmed by scientists at Karolinska Institute, who have showed that Ep-CAM protein induces more pronounced and longer lasting, specific humoral and cellular immune responses compared with a human anti-Id mimicking Ep-CAM, when both immunogens are administered in combination with GM-CSF (Mosolits *et al*., 2004). In particular, such cellular responses are characterized by an increased usage of the T-cell receptor BV19 by antigen-specific $CD8^+$ T cells, and of BV12 by antigen-specific $CD4^+$ T-cells (Mosolits *et al*., 2004). All in all, the overall impact of this immunotherapeutic approach remains somewhat unclear and definitely warrants further clinical investigation.

Finally, it has to be noted that the encouraging preliminary results from independent pilot studies on patients with respectively advanced (Foon *et al*., 1997) and resected (Foon *et al*., 1999) colorectal cancer treated with an anti-Id mAb that is the internal image of CEA still await further confirmation. In those trials, most patients developed both anti-anti-Id polyclonal antibodies and cellular immune responses specific for both the anti-Id used for treatment and the actual CEA expressed by the tumor of the patients. In this respect, it is not clear whether the use of an anti-Id single-chain fragment variable, rather than the whole Ig (Pignatari

et al., 2006), or that of CpG motifs as immune adjuvants (Saha *et al*., 2006) might prove in any way advantageous. Similarly, further clinical trials are warranted to assess the therapeutic potential of anti-Id mAbs mimicking CD55, which have also recently entered the clinical arena with intriguing immunologic results (Ullenhag *et al*., 2006).

Ovarian cancer

The use of an anti-Id mAb mimicking the tumor antigen CA125 (abagovomab) has been recently reported in a limited number of clinical trials primarily designed for patients with ovarian cancer. On an intention-to-treat basis, scientists at the University of Marburg enrolled 119 patients with advanced ovarian cancer who received a mean of about 10 administrations of abagovomab. A CA125-specific humoral response was elicited in nearly half of the patients, whereas antibody-dependent, cell-mediated cytotoxicity was documented in about a quarter of all patients. Interestingly, a correlation between specific humoral response to abagovomab and patients' survival has also emerged, so that randomized, controlled studies are now warranted to possibly prove clinical benefit (Reinartz *et al*., 2004). However, it seems fair to underline that other subsequent clinical trials on recurrent ovarian cancer (Pfisterer *et al*., 2006) and on a miscellanea of gynecologic/peritoneal primary tumors (Sabbatini *et al*., 2006) seem to confirm the encouraging results above. Moreover, pre-clinical data suggest that the integration of IL-6 within the abagovomab formulation – as a fusion protein – might improve efficacy by eliciting even more robust, CA125-specific humoral responses (Reinartz *et al*., 2003).

Breast cancer

Available results of treatment of breast cancer patients with anti-Id mAbs are still very preliminary and conclusions about them go no further than mere biologic proofs of principle.

A pilot study exploiting the so-called TriAb formulation following autologous stem cell transplantation showed that despite the high-dose CHT conditioning of the transplant procedure, most patients were able to mount an antigen-specific humoral and cellular immune response (Reece *et al*., 2000). Apparent clinical efficacy, though, was somewhat modest (Reece *et al*., 2001).

As mentioned above, the 1E10 γ-type, anti-Id mAb is also a potential therapeutic tool for breast cancer patients, owing to its mimicry with *N*-glycosyl-containing gangliosides and other antigens expressed on this tumor cells, as well as on those of malignant melanoma. The two small clinical studies reported so far seem to agree on the fact that specific humoral responses are more likely to be elicited than cellular immune responses, and that different mAb doses do not seem to produce different immunologic results (Diaz *et al*., 2003; Guthmann *et al*., 2006). Once again, clinical relevance remains unproven.

Finally, over the last 5 years, preclinical research has begun targeting Her-2/neu-positive breast tumors to establish whether it might be worthwhile to develop anti-Id mAbs mimicking this antigen for possible clinical use. Results are extremely preliminary and await further characterization (Baral *et al*., 2001; Mohanty *et al*., 2006).

Conclusions

Patient- and tumor-specific, anti-Id mAbs as a passive immunotherapy for B-cell malignancies are safe and potentially quite effective. Still, they have failed to reach the multicenter application because they are not feasible on a large production scale.

Patient- and tumor-specific Id vaccines capable of inducing Id- and tumor-specific polyclonal Abs and cellular immune responses have been also developed. They are always safe, but effective only in patients who respond to them from an immunologic point of view. Large-scale feasibility remains however questionable at the time of this writing.

Anti-Id antibodies mimicking Id-unrelated, tumor-associated antigens are also being actively investigated in a number of clinical settings. They are safe and feasible, but their efficacy remains to be ultimately proven.

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