

Introduction to monoclonal antibodies

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The communicative enthusiasm of Lloyd Old

Before starting this introduction on the development of monoclonal antibodies for cancer therapy and the role of Lloyd Old in the field, in parallel with his central interest in T cell activation by tumor antigen vaccination, let me say a few subjective words about a unique human quality of Lloyd: his extraordinary *communicative enthusiasm*. That quality is sometimes called charisma, but there is more to it than that; it is a capacity to communicate your interest to your fellow scientists (in the sense of “love your neighbor” in the scriptures) and to give to your interlocutor the impression of being fully understood. The result was that, when you came out of Lloyd’s office after a scientific dialogue, you felt “boosted” to develop your project with renewed energy and more imagination.

Lloyd had this kind of positive influence on so many fields and so many personalities that it is not possible to mention them all. I will first mention, very briefly, the two most representative well-known examples by which Lloyd stimulated and contributed to the field of cancer immunotherapy, and then follow with several other examples in the field of monoclonal antibodies by mentioning the central personalities who worked with him or independently, including myself and a few co-workers from my group who enjoyed the privilege of having interacted with him. While writing this kind of personal review, I kept in mind this question: would Lloyd enjoy reading it and would he agree with the points I am trying to underscore?

The first example in the field of immunotherapy was the discovery of the *MAGE* genes as the source of antigens recognized by tumor-reactive T cells, which was initiated and developed by Thierry Boon’s group in the Brussels Branch of the Ludwig Institute for Cancer Research (LICR) (1, 2). Lloyd himself had pursued passionately the identification of T cell-defined tumor antigens. Together with one of his fellows, Alexander Knuth, they systematically analyzed the tumor-reactive CD8 T cells, which could be expanded in autologous mixed lymphocyte-tumor cell culture (3). It was subsequently a collaboration of Knuth with Boon that allowed the discovery of *MAGE-A1* (1). Furthermore, Lloyd, along with Yao-Tseng Chen, had a central role in the field by the discovery of NY-ESO-1 antigen (4) and its broad extension by the definition of the concept of Cancer/Testis (CT) antigens (5).

The second major example is the SEREX method, using a bacterial expression library for detecting patients’ serum antibodies reacting with tumor antigens (6). This methodology was described independently by Lloyd’s former collaborator, Michael Pfreundschuh, but Lloyd had already for many years a major interest in the use of patient serum for autologous typing

and immensely broadened the application of the SEREX method, up to the description of the immunome (7) and the SEREX database, in collaboration with the late Matthew Scanlan. The antibodies discovered by this technology were not used for therapy, but they represented precious evidence of patients’ immune responses against their own tumors, and, most importantly, SEREX-detected antibodies led to the identification of several new Cancer/Testis antigens, including the most important, NY-ESO-1. In addition to these two emblematic examples, we can say, without risk of contradiction, that since he took over the direction of the LICR in 1988, Lloyd spread his enthusiastic and liberal spirit within all the different branches of LICR.

Radiolabeled antibodies

My initial contacts with Lloyd were through work in the field of radiolabeled antibodies. As early as 1974, in collaboration with Stefan Carrel, we had shown in a nude mouse/human colon carcinoma xenograft model that ^{131}I -labeled, immunoabsorbent-purified, high-affinity polyclonal antibodies against carcinoembryonic antigen (CEA) could specifically localize in significant amounts in tumors (8). The subsequent clinical studies, performed by David Goldenberg’s group (9) and ourselves (10), both with ^{131}I -labeled anti-CEA polyclonal antibodies, gave precise evidence of specific tumor localization, but we considered the usefulness of tumor detection by the so-called *immunoscintigraphy* more cautiously than our competitor.

Soon after the discovery of the monoclonal antibody technology by César Milstein and Georges Köhler, we produced, with Roberto Accolla, the first anti-CEA monoclonal antibodies (mAbs) (11), and in 1981, we reported the first clinical trial of radiolabeled mAb injection (12). Twenty-eight patients with CEA-producing carcinomas were injected with ^{131}I -labeled anti-CEA mAb and tested by external photoscanning and tomoscintigraphy (SPECT). The tumor-specific localization of radiolabeled mAb was confirmed, but the absolute amounts of radioactivity delivered to the tumor were low. This initial clinical trial was followed by several more with second generation anti-CEA mAbs and fragments labeled with ^{123}I (13), by ^{111}In (14), and later, using a chimeric anti-CEA mAb labeled with different fluorescent molecules, allowing the direct tumor visualization and opening the field of immunophotodetection (15, 16).

Interestingly, it was at the time of the first clinical evaluation of radiolabeled anti-CEA mAb that Richard Miller and Ron Levy reported the first treatment of patients with cutaneous T cell lymphomas by injection of an anti-T cell mAb (17), soon followed by the anti-idiotypic mAb treatment of B cell lymphoma by Levy’s group (18).

In parallel, we performed a clinical study of colon carcinoma localization of the ^{131}I -labeled mAb CO17-1A, in collaboration with Hilary Koprowski and Jean-François Chatal (19). There were definite positive tumor uptakes of radioactivity, but the tumor localization was less contrasted than with our anti-CEA mAbs. Interestingly, mAb CO17-1A was the same mAb that was later injected in large amounts without labeling by Koprowski's group for the treatment of gastrointestinal carcinomas (20), and later by Gert Riethmüller for adjuvant treatment of Dukes C carcinoma patients, in order to prevent relapse or metastases by elimination of undetectable residual disease (21).

Lloyd was actively involved in the field of radiolabeled anti-tumor antibodies through very efficient and productive collaborations with different scientists and clinicians (including Sidney Welt and Gerd Ritter from the New York LICR Branch and Steve Larson from the Nuclear Medicine Department of the Sloan-Kettering Institute, who had already performed pioneering radioimmunotherapy with a ^{131}I -labeled anti-melanoma mAb (22), as well as Andrew Scott and Anthony Burgess from the Melbourne LICR Branch).

Within a few years, these collaborations resulted in the selection of mAb A33, specific for an antigen expressed by malignant and normal gut epithelium, and a series of clinical studies of colorectal carcinoma patients for evaluation of mAb A33, labeled either with ^{131}I for diagnosis and radioimmunotherapy (23), with ^{125}I for Auger particle emission (24), or later, using the humanized huA33 mAb labeled with ^{124}I for immunoPET quantitative imaging (25).

In parallel, the same groups evaluated the tumor localization of the anti-ganglioside GD3 mAb KM871 in melanoma patients (26), as well as the targeting of the mAb G250 (anti-renal cell carcinoma, developed by Dutch scientists from Leiden) with diagnostic (27) and therapeutic doses of ^{131}I (28).

However, despite the highly contrasted tumor images demonstrated clinically, and the very encouraging radioimmunotherapy results obtained in human tumors xenografted in experimental animals by David Goldenberg (29) and by Franz Buchegger in our group (30), as well as by Sidney Welt in Lloyd's group (31), the clinical radioimmunotherapy of solid tumors still did not produce significant satisfactory results. This is a personal conclusion based on our own last clinical experience with ^{131}I -labeled anti-CEA $\text{F}(\text{ab}')_2$ fragments (32) and on a broad review of the literature (33). Indeed, the high radioresistance of solid tumors compared to the radiosensitivity of the bone marrow, which receives relatively high radiation doses from circulating radiolabeled antibody, remains a difficult problem to resolve, despite the use of antibody fragments with a short half-life in the circulation (30), or new methods of two-step tumor targeting, developed by Jean-Marc Le Doussal and Jacques Barbet (34, 35).

In contrast, radioimmunotherapy of more radiosensitive target tumors, such as lymphomas, was more successful, as demonstrated by the use of ^{131}I -labeled or ^{90}Y -labeled anti-CD20 mAbs in the treatment of non-Hodgkin B cell lymphomas (36, 37). Interestingly, it was when the doses of ^{131}I isotopes used to label the B1 anti-CD20 mAb were progressively lowered and a good part of the anti-tumor effect was maintained (38) that the intrinsic therapeutic properties against lymphomas of the original B1 anti-CD20 mAb from Stuart Schlossman were discovered. This led to the selection of the anti-B020 rituximab, mimicking the previously used B1 mAb. Rituximab became the first FDA-approved mAb for treatment of patients with B cell lymphomas (39) and, importantly, also for the treatment of several forms of autoimmune disease. From this example, we

can say that radiolabeled antibodies paved the way for successful tumor treatment by unlabeled mAbs (16).

Unlabeled monoclonal antibodies for cancer therapy

The success of rituximab should not make us forget that not all patients with lymphoma, even in the indolent form, respond to the unlabeled antibody, and that a higher percentage of patients respond to the different forms of radiolabeled anti-CD20 mAbs (36, 37). Furthermore, different positive experiences in the treatment of lymphomas with radiolabeled anti-CD20 mAbs, as well as our local observation of remissions of more than ten years in half of the patients with relapsed or refractory indolent B cell lymphoma treated with ^{131}I -labeled antibody, speak in favor of maintaining the interest for this form of radioimmunotherapy (40).

The next unlabeled mAb which was approved by the FDA, for the treatment of HER2-positive breast carcinoma, was the humanized anti-HER2 mAb trastuzumab (41), followed by the chimeric anti-EGFR mAb cetuximab (42). It is important to note, however, that despite the widespread clinical use and commercial success of these mAbs, as well as of other mAbs with similar specificities, the unlabeled anti-solid tumor mAbs have almost always had to be used in conjunction with chemotherapy.

Lloyd's group was also extremely productive in the selection and testing of unlabeled mAbs for tumor therapy, as described in more detail in this issue of the journal by Gerd Ritter, his central collaborator in the field. I just want to underscore here the particular interest of Lloyd in the selection of a more tumor-specific mAb, directed against the mutated form (delta 2-7) of EGFR commonly expressed in glioma. The discovery of the new anti-EGFR mAb 806 allowed its experimental evaluation not only in comparison with conventional anti-EGFR, but also in combination with the latter. Interestingly, the coinjection of the two mAbs, directed against two different epitopes of EGFR, enhanced the anti-tumor activity in human glioma subcutaneous or intracranial xenograft models (43). Similarly, the group of Yosef Yarden at the Weizmann Institute had demonstrated that coinjection of two mAbs directed against different epitopes of HER2 was more efficient than a single mAb in the treatment of HER2-positive xenografts (44). The latter observation may have led the way to the recent strategy of Genentech to treat HER2-positive early breast cancer by injection of two anti-HER2 mAbs, pertuzumab and trastuzumab, known to recognize different HER2 epitopes. The recently reported phase II clinical trial showed higher therapeutic benefit of coinjection of the two mAbs than injection of either mAb alone (45).

In this context, I had the pleasure to collaborate with Christel Larbouret, Bruno Robert, and André Pèlerin from Montpellier, who demonstrated in three different human pancreatic carcinoma xenograft models that the coinjection of two clinically approved mAbs directed against EGFR and HER2 had a definite synergistic therapeutic effect, despite the fact that the three target tumors expressed very low levels of HER2 (46, 47). The latter point suggests that the coinjection of anti-HER1 and anti-HER2 mAbs may be beneficial in treating carcinomas with a low surface expression of HER2, if they coexpress EGFR, which is relatively common. Another point of interest of this study is that the *in vivo* therapeutic synergism of the two mAbs was demonstrated on two human pancreatic carcinoma lines, MIA PaCa-2 and Capan-1, which both have a mutant KRas phenotype. The synergistic therapeutic effect may be due to an

inhibition of HER2 heterodimerization, as demonstrated by a TR-FRET assay (48).

Furthermore, most interestingly, the synergy in anti-human pancreatic carcinoma BxPC-3 xenografts between the anti-HER1 and -HER2 mAbs could be also demonstrated by coinjection of their F(ab')₂ fragments, clearly indicating that the anti-tumor effect was, at least in part, due to the direct reactivity of the fragments with the two types of HER receptors on the surface of the target cells, without the need for an Fc-dependent effector mechanism (47). As a confirmation of this point, the synergy against the human pancreatic carcinoma xenograft of the same F(ab')₂ fragments were reproduced in a model of immunodeficient SCID/Beige mice, lacking NK cells (48). In this context, one should acknowledge that despite the very large number of cancer patients who have been treated with mAbs, we still don't know the exact mechanism of the therapeutic activity of each mAb.

The activation of complement by anti-tumor mAbs has been a subject of great interest for Lloyd. Indeed, the activation of the complement proteolytic cascade could help mAb therapy, not so much for its relatively weak capacity to lyse solid tumor cells *in vivo*, but for its properties to opsonize tumor cells by deposition of C3 component and release anaphylatoxins, such as C3a and C5a, resulting in increased vascular permeability and better penetration of antibodies at the tumor site. However, except for anti-lymphoma and for some anti-ganglioside mAbs, such as the anti-GD2 from Nai-Kong Cheung, now in a phase I trial for the treatment of neuroblastoma (49) and the well-known anti-GD3 from Lloyd's group (26), complement activation was not retained as a major anti-tumor effector mechanism.

There is a consensus, based mostly on the work of Raphael Clynes and Jeffrey Ravetch (50), as well as on clinical observations of a correlation between FcγRIIIA genetic polymorphism and response to mAb therapy (51), suggesting that most mAbs act by "antibody-dependent cell-mediated cytotoxicity" (ADCC). However, there may be exceptions to this general rule, as demonstrated by the successful synergistic treatment of a pancreatic carcinoma by two F(ab')₂ fragments directed against EGFR and HER2 (47). Furthermore, it is important to underscore that the two mAbs already approved for solid tumor therapy are directed against important tyrosine kinase receptors, while other mAbs such as CO17-1A or CHL6, from Hilary Koprowski and Ingegerd Hellström's laboratory, respectively, directed against other types of surface antigens (20, 52) did not induce significant tumor remissions despite repeated injections of up to 400 mg/m².

Promising directions for improvement of monoclonal antibody therapy

This leads me to continue this introduction by presenting six directions that appear to me the most promising for the improvement and optimal use of mAbs for cancer therapy. These proposals do not pretend to be new; most of them were the subject of discussion with Lloyd and will be the topics of reports by specialists in this issue. The lengths of the paragraphs are dependent on the interest of Lloyd and ourselves on the different subjects.

Coinjection of different monoclonal antibodies

It appears that the coinjection of two or more mAbs directed against different relevant receptors or different epitopes of receptors, expressed on the surface of cancer cells, as mentioned above, may lead to synergistic anti-tumor activity. This may look

too expensive, and the commercial companies are difficult to convince, but if we take the historical model of chemotherapy, it appears obvious that it is the combination of drugs attacking cancer cells by different mechanisms which led to success of many cancer treatment modalities.

Temporary enhancement of the tumor vasculature permeability

Improving the accessibility of antibodies and other drugs to cancer cells, by the induction of a temporary increase in permeability and blood flow in the tumor vessels, also appears to be an important factor in improving any form of cancer therapy, particularly with molecules as large as antibodies. Some results in this direction were obtained by our group, through the injection of tumor necrosis factor (TNF) (53), and by the group of Dario Neri, by injection of antibody fragments conjugated to cell-permeating HIV-1 TAT peptides (54). Furthermore, the group of Erkki Ruoslahti has recently published a series of provocative papers, suggesting that several peptides with an arginine at the C-terminus (CendR) can increase tumor vascular permeability through binding to neuropilin-1, and also improve tumor penetration of coinjected drugs including mAbs (55).

In this context, the use of the anti-VEGF bevacizumab for tumor therapy (56) appears paradoxical. How can a mAb that decreases tumor blood flow and neutralizes a permeability factor like VEGF nevertheless improve the efficacy of chemotherapeutic drugs, which requires the permeability of tumor blood vessels to reach the tumor cells? Rakesh Jain, who made important contributions to the demonstration that cancer drugs penetrate very poorly into tumors because of the excess interstitial fluid pressure within the tumor, made the hypothesis that bevacizumab and other anti-angiogenic therapies were active mostly by "normalizing" the tumor vessels (57). However, more recent clinical results, obtained in non-small cell lung carcinoma patients, demonstrated a rapid decrease in delivery of chemotherapy (using radiolabeled docetaxel) to tumors after bevacizumab injection (58).

Modification of the Fc structure of monoclonal antibodies

Modifying the Fc structure of the anti-tumor mAbs, both through selected mutations (59) and/or alteration of their glycosylation (60) in order to increase the affinity of Fc for the activating receptors expressed by the effector cells, offers potential for therapeutic improvement. Despite the above-mentioned demonstration of the direct anti-tumor growth effect of the F(ab')₂ fragments of anti-HER1 and -HER2 (47), the majority of anti-tumor mAbs are supposed to induce their anti-tumor effect through the activation of effector cells engaged by their Fc receptor (50). This subject will be presented by the specialist in the field, Jeffrey Ravetch, in this issue's commentary on optimizing antibodies.

Bispecific anti-tumor anti-effector cell antibodies

Another approach to bring effector T cells/NK cells in contact with target tumor cells is the use of bispecific antibodies with one arm directed at the tumor cells and the other at the effector cells. This strategy will be presented by Gert Riethmüller, who played a central role in bringing these types of bispecific antibodies to useful clinical applications (61). The question is why were these bispecific mAbs, described over 25 years ago in two *Nature* papers by the groups of Michael Bevan and David Segal (62, 63), so slow to become efficient *in vivo* and clinically useful?

We had shown that our locally produced bispecific anti-EpCAM x anti-CD3 hybrid mAb was extremely efficient in

cytotoxicity induction *in vitro*, and could localize very specifically after trace labeling with ^{125}I in tumor xenografts (64), but its *in vivo* activity was not demonstrated. The groups of Reinder Bolhuis in Holland and of Maria Colnaghi in Italy conducted therapy trials in ovarian carcinoma patients by intraperitoneal coinjection of bispecific mAbs with activated lymphocytes, with very modest results (65). One possible explanation is that the affinity of the anti-CD3 arm of the early bispecific antibodies was too high, leading to an initial binding to the circulating T cells before they had reached the tumor, which would inhibit the subsequent targeting to the tumor cells. Indeed, Antonio Lanzavecchia's group demonstrated that a lower affinity of the anti-CD3 arm of the bispecific mAb, induced by selected mutations, helped to avoid binding to effector T cells in the circulation. Binding of the T cells to the low-affinity anti-CD3 becomes possible only at the tumor site, by an avidity effect due to the presence of multiple copies of the bispecific antibody oligomerized at the surface of the tumor cells (66). Gert Riethmüller will tell us if that strategy was instrumental in the excellent activity of their recombinant single-chain bispecific antibody *in vivo* (61).

The beauty of this bispecific single-chain variable fragment (scFv) anti-CD19 x anti-CD3, called blinatumomab, produced by Riethmüller and the company behind him, is that it can induce tumor regressions in patients with non-Hodgkin lymphomas after injection at very low doses, in the range of less than 0.1 mg. This looks like a great advantage, as compared with the injection doses of rituximab, in the range of 50 to 100 mg, and suggests that the bispecific scFv induces a much more efficient mechanism of target cell killing by the CD3 effector cells than do the monospecific intact mAbs by an Fc-dependent ADCC mechanism. Whether these excellent results obtained against lymphomas can be also obtained by a similar strategy against well-established solid tumors, known to be resistant to active immunotherapy, still needs to be demonstrated.

Furthermore, the small size of the bispecific scFv, leading to a very short circulating half-life, and thus requiring several days of intravenous (i.v.) injection, still represents a problem. Maybe larger forms of bispecific antibodies that redirect T cells against tumors, such as the tribodies with one arm directed against the T cells and two against the tumor (67), or the so-called trifunctional bispecific antibodies with a functional Fc fragment (68), will compete with the bispecific scFv.

Antibody-mediated tumor targeting of antigenic MHC complexes

Another strategy for retargeting the T cells to the tumors consists in coating the tumor cells with an antigenic major histocompatibility complex (MHC)-viral peptide complex linked to an anti-tumor antibody fragment. We developed this strategy in collaboration with Bruno Robert from my group, as well as with Pedro Romero, Philippe Guillaume, Immanuel Luescher, and Jean-Charles Cerottini from the LICR Lausanne Branch, and reported it in one of the first research articles of *Cancer Immunity* (69). I take the liberty to describe this strategy in some detail, since it was supported by a Cancer Research Institute (CRI) grant, awarded through Lloyd, and we had several discussions about it, and also because it represents a real bridge between antibody- and T cell-mediated immunotherapy.

Fab fragments from anti-CEA, -HER2, or -CD20 mAbs were chemically linked to recombinant HLA-A2 molecules, loaded with Flu matrix peptide, and coated on the target tumor cells expressing one or the other differentiation marker (LoVo/CEA+, SKBR3/HER2+, and Daudi/CD20+). When anti-influenza T cell clones were added, at effector-to-target cell

ratios of 10 to 20, we obtained, in a 4 h ^{51}Cr release assay, specific lysis (ranging from 60-90%) of the target tumor cells expressing the relevant marker recognized by the antibody Fab fragment of the conjugate, used at 10 to 100 picomolar concentrations. The beauty of the system is that, like the bispecific antibody described by Lanzavecchia, mentioned above, the monomeric HLA-A2 molecules in solution had a very low affinity for the T cell receptors (TCRs), but when the conjugate was oligomerized on the tumor cells through the Fab fragment, they induced a high avidity binding to the T cell receptors, resulting in activation and lysis. In brief, our conjugate had the capacity to replace a differentiation marker expressed by tumor cells and recognized by an antibody with an antigenic viral antigen recognized by a T cell receptor.

Interestingly, at that time, there was no publication in this field, except for the group of Philip Savage from Oxford, who used, for the same goal, a two-step tumor coating system involving first a biotin-labeled anti-CD20 antibody, followed by biotinylated HLA-A2/gag complexes, bridged by an avidin molecule (70).

At this point, it was not certain that this immunotherapy strategy would function *in vivo* in a syngeneic tumor system. Alena Donda and Valérie Cesson, in our group, gave a positive answer to this question. They first showed that injection of anti-CEA-H2K^b/OVA peptide conjugate could induce specific growth inhibition and regression in a model of established syngeneic carcinoma, transfected with human CEA and grafted in OT-1 C57BL/6 mice expressing a transgenic anti-OVA TCR. The results were confirmed in a model of CEA-transgenic mice which received anti-OVA T cells from OT-1 mice (71).

One year later, the group of Yoram Reiter from Israel presented a similar strategy of antibody-mediated tumor cell-coating of antigenic MHC complexes, but with the use of a recombinant fusion protein consisting of an HLA-A2 molecule fused with an antigenic Epstein-Barr virus (EBV)-derived peptide and an anti-tumor scFv. The results, which confirmed our approach with more modern tools, were published in a *Proceedings of the National Academy of Sciences* article communicated by Lloyd Old, confirming his interest in the field (72).

Our group further demonstrated in a fully immunocompetent murine model that a physiological immune response against lymphochoriomeningitis virus (LCMV) or influenza virus was sufficient to provoke the growth inhibition of tumor coated with anti-tumor-H2K^b conjugates loaded with the relevant immunodominant viral peptide (73).

In recent years, Alena Donda, who has now created her own research group with Pedro Romero at the Ludwig Cancer Center of Lausanne University and with whom I have the pleasure to collaborate, developed a novel related strategy, allowing the activation and recruitment at the tumor site of NKT cells, known to be at the junction between the innate and the adaptive arms of the immune response. For this purpose, she synthesized a recombinant, MHC-related, CD1d molecule fused to anti-HER2 scFv fragments and showed that, when loaded with the CD1d ligand superagonist α -galactosylceramide (α -GalCer), this fusion protein, injected i.v., could induce a potent inhibition of lung metastases, produced by an i.v. injection of syngeneic HER2-transfected B16 melanoma cells, 2 to 7 days before treatment. Interestingly, it was discovered during these immunotherapy experiments that the α -GalCer, when loaded on CD1d-scFv, induced a sustained NKT cell activation, while injection of free α -GalCer induced an acute NKT cell activation, rapidly followed by the well-known NKT cell anergy, and in the present model, no anti-tumor effect (74). These new forms of

immunotherapy, which may contribute to the enhancement of adaptive anti-tumor responses, were developed with the scientific and financial support of Maurice Zauderer and his company, demonstrating the usefulness of collaboration between the University, the LICR, and private companies, as recommended, in recent years, by Lloyd.

Blocking of regulatory pathways by monoclonal antibodies

The last promising role of antibodies in improving cancer immunotherapy is the development of mAbs directed not against the tumor cells' antigens, but against coinhibitory receptors expressed on effector T cells. Indeed, well-organized tumor tissues are part of our immunological self. Thus our organism has multiple mechanisms and regulatory molecules to avoid autoimmune reactions against our own tissues. These regulatory molecules unfortunately inhibit our efforts to raise an immune reaction against our own tumor. Therefore, in order to trigger weak anti-tumor T cell responses in the host or to reinforce our vaccination strategy against the selected tumor-specific or differentiation antigens, aimed at rejecting our tumors, several mAbs have been derived to block the regulatory molecules that prevent tumor rejection. The first one, directed against the cytotoxic T lymphocyte-associated protein 4 (CTLA-4) coinhibitory receptor expressed by activated and regulatory T cells was developed by James Allison (76), who, with Jedd Wolchok and Andrew Scott, will describe it in more detail in this issue's commentary on antibodies in immunomodulation.

Furthermore, additional coinhibitory receptors are overexpressed on "exhausted" lymphocytes during chronic inflammation, such as T cell immunoglobulin mucin 3 (Tim-3) and programmed cell death 1 (PD-1). These were found to be coexpressed in 50% of tumor-infiltrating lymphocytes, by Ana Anderson. Her group reported that simultaneous blockade of both Tim-3 and PD-1, by coinjection of two antibodies against Tim-3 and PD-L1, was highly effective in restoring T cell immunity in a model of CT26 carcinoma in BALB/c (76). One should mention also, that in parallel with the development of the above blocking antibodies against coinhibitory receptors, a series of agonistic antibodies directed against activating receptors, such as CD137, expressed on effector NK or T cells, are presently tested for enhancement of anti-tumor activity with encouraging experimental results (77, 78).

At the clinical level, ipilimumab, the fully human IgG1 form of anti-CTLA-4, was shown to prolong survival in a phase III trial of metastatic melanoma patients and thus was approved as a single agent for the first-line treatment of this condition (79). Ipilimumab was also found to enhance the CD4 and CD8 T cell responses against NY-ESO-1 CT antigen, in patients with durable objective clinical response or stable disease (80).

Finally, I would not like to end this introduction without a brief mention of yet another strategy, which is at the edge between antibody and T cell therapy, consisting in the design of chimeric antigen receptors (CARs). This approach was pioneered by Zelig Eshhar at the Weizmann Institute, who demonstrated the possibility to generate antigen receptor chimeras composed of the antigen recognition domains of an anti-tumor antibody, fused with the CD3 zeta chain, one of the signaling components of the TCR for antigen (81). Retroviral or lentiviral transduction of T cells with CARs confer to T cells the recognition capabilities of antibodies, which have the advantage of being MHC-independent, but are limited to the specific recognition of antigens expressed on the surface of tumor cells. CARs have been refined over the years and today are designed to contain the signaling modules of costimulatory receptors, such

as those from CD137. Recent phase I clinical trials of cellular therapy with CAR-reprogrammed autologous T cells, expressing for at least six months functional CARs at high levels, have shown great promise. For instance, adoptive transfer of T cells carrying a CD19-specific CAR led to impressive complete responses in two out of three patients reported with treatment-refractory chronic lymphocytic leukemia (82).

Conclusion

It is evident that monoclonal antibodies directed against particular receptor structures or differentiation markers overexpressed on tumor cells, but also present on normal cells, have had an enormous impact on present day cancer therapy. The fact that mAb therapy for solid tumors still needs to be given in conjunction with chemotherapy shows some of its limitations. In particular, it is not yet known to what extent the recruitment of innate immune effector cells at the tumor site, by the targeting of massive amounts of antibody molecules, can help in the induction of an active T cell response of the patient against his or her own tumor cells. Indeed, the development of an active immune response against the patient's own tumor, expressing mutated antigens or CT antigens, is the ultimate goal of tumor immunologists like Lloyd, since it represents the best chance to prevent the development of relapsing tumor cells derived from tumor stem cells, often lacking the differentiation markers and/or remaining insensitive to chemotherapy.

In order to enhance an active anti-tumor response, I would definitely favor antibody strategies that bring effector T cells to the tumor site, like the bispecific anti-CD3/anti-tumor strategy or the tumor targeting of MHC, or MHC-related, antigenic complexes. The experience acquired with the tumor targeting of mAbs labeled with radioisotopes or fluorescent probes showed us that many other molecules, such as cytokines (83) or drugs (84)—subjects that I have not covered here—can be selectively delivered to tumors.

I am grateful to have belonged to this generation of scientists, who were guided by the support and enthusiasm of Lloyd Old, who had the chance to witness the first success of cancer immunotherapy, and who feel entitled to expect many more successes in this field in the near future.

Abbreviations

scFv, single-chain variable fragment; mAb, monoclonal antibody

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