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Anti-GD2 Strategy in the Treatment of Neuroblastoma

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Abstract

The prognosis for advanced neuroblastoma remains poor with high risk of recurrence after consolidation. Therapies based on monoclonal antibodies that specifically target disialoganglioside GD2 on tumor cells are improving treatment results for high-risk neuroblastoma. This article reviews the use of anti-GD2 antibodies either as monotherapy or as part of a larger and more complex treatment approach for advanced neuroblastoma. We review how anti-GD2 antibodies can be combined with other treatments or strategies to enhance their clinical effects. Tumor resistance and other problems that decrease the efficacy of anti-GD2 antibodies are discussed. Future developments in the area of anti-GD2 immunotherapies for neuroblastoma are also addressed.

A. Neuroblastoma

1. Significance, Standard of Care, Clinical Strategies

Neuroblastoma is the most common malignancy in infants, the most common extracranial solid tumor of childhood, and the third most common cancer in children (1–5). The average age at diagnosis is 17 months with 50–60% of patients having metastatic disease when diagnosed (6–8). Overall treatment has improved in children under 15 years of age with 5-year overall survival rates for newly diagnosed patients increasing from 52% in the 1970s to 69% in the last decade (9,10).

Despite advances in the treatment of low- to intermediate-risk neuroblastoma, outcomes for patients with advanced disease remain poor. Standard treatment for high-risk patients includes surgery, radiation, and/or myeloablative chemotherapy with autologous stem cell transplantation, followed by cis-retinoic acid (CRA). CRA, an anti-proliferative agent, when given following completion of chemotherapy has been shown to have an increased survival effect in patients with stage 4 disease (4,11–12). With current standard therapy, most high risk patients achieve remission with no clinically evident disease (NED) status. However, complete eradication of tumor cells has remained elusive. Microscopic residual tumor cells (minimal residual disease) survive treatment and cause recurrent refractory disease. The 3-year event-free survival of these high risk patients remains as low as ~30% (4,6,13–14). Fortunately, a recent COG randomized trial has shown that a combination of anti-GD2 antibody and cytokines in this setting can help prevent recurrence (15,16).

In this review, we examine several current strategies using monoclonal antibodies (mAbs) against the disialoganglioside GD2, and their derivatives, for the treatment of high risk neuroblastoma, either as primary therapy or as part of a multifaceted treatment approach, in clinical trials. We review the pitfalls of this treatment approach, including tumor resistance and the development of blocking antibodies that may interfere with mAb therapy. Finally, we look ahead at potential future therapies.

2. GD2-Importance, Rationale

Surface antigens expressed on neuroblastoma that have been used as targets for mAbs include the gangliosides GD2, GD3 and GM3, and the glycoproteins CD56 (NCAM), L1-CAM, GP58 and GP95 (17). GD2 is a disialoganglioside antigen that is expressed on tumors of

neuroectodermal origin including neuroblastoma and melanoma (18–19). These tumors express GD2 with relatively little heterogeneity between cells (20–21). Patients with neuroblastoma were found to have significantly elevated free GD2 levels in serum compared with normal children and children with other tumors (20). Also, GD2 expression is not lost from the cell surface of neuroblastoma cells even when bound to antibody, unlike other tumor antigens described previously (21).

In normal tissues, GD2 expression is largely limited to neurons, skin melanocytes, and peripheral pain fibers (22), making it well suited for targeted antitumor therapy. Recently, GD2 has been “ranked” 12th in priority of all clinical cancer antigens by an NCI workshop (23). In addition to neuroblastoma and melanoma, GD2 is expressed on some soft tissue sarcomas, osteosarcomas, and small cell lung cancers (24,18). In all, GD2+ diseases account for ~8% of all cancer deaths in the US (25).

GD2 has been used extensively as a target in mAb therapy and has been the primary target of antibody recognition in neuroblastoma. In 1984, a murine mAb (mAB126) was produced against cultured human neuroblastoma cells (LAN1). The original murine anti-GD2 mAbs described were 3F8, 14.18 and 14.G2a (18–19). Clinical testing has been performed with 3F8, 14.G2a, and ch14.18 (the human-mouse chimeric variant of 14.18) in neuroblastoma and melanoma (26–33).

B. Single Agent Antibodies

1. ADCC and CDC

An ideal anticancer agent would specifically target tumor cells and minimize injury to healthy cells (24). Monoclonal antibody (mAb) therapy creates specificity to tumor cells through its recognition of cell surface antigens found exclusively on tumor cells or that are found in much greater amounts on tumor cells compared to normal cells (34–35). Currently, mAbs are in use in the detection, diagnosis, and treatment of neuroblastoma (14,36–38). Antibodies can mediate destruction of tumor cells through several mechanisms including antibody-dependent cell-mediated cytotoxicity (ADCC). After the variable region of the antibody binds to antigen on the tumor cell, the Fc portion of the antibody can bind to the Fc receptor on monocytes, macrophages, neutrophils and/or natural killer (NK) cells and stimulate tumor cell lysis via ADCC (39–40).

In addition, complement-dependent cytotoxicity (CDC) may be induced after an antibody binds to the tumor cell surface (24). However, dose limiting toxicities (DLT) caused by anti-GD2 mAb do occur and include fever, chills, anaphylactoid reactions most likely from cytokine and complement activation, and transient neuropathic pain, which are controllable with analgesics. These toxicities are mostly likely the result of mAb recognition of GD2 on peripheral pain fibers and complement deposition (40–42,22,29).

2. 3F8 Clinical Testing

The first mAb tested in clinical trials was the anti-GD2 mAb 3F8 (26,43–46). In the initial Phase I and II trials using 3F8 in patients with stage 4 neuroblastoma, there was no significant antitumor effect on bulky disease but some response in microscopic bone marrow disease (17,47–50). Side effects included pain most commonly, hypertension, hypotension, fever, vomiting, diarrhea, and urticaria. Pain can be dose limiting and has been attributed to antibody recognition of peripheral pain fibers expressing GD2 (40–42). Also, human antimouse antibodies (HAMA) can develop in patients treated with 3F8. As these neutralize the function of 3F8, development of HAMA has resulted in termination of therapy (51). 3F8 has been shown to activate tumor cell destruction by both CDC and ADCC *in vitro* (52–53).

3. 14.G2a

The 14.18 antibody is a separate IgG3 murine mAb targeted to the GD2 antigen (18). In an effort to enhance ADCC, a class switch variant called 14.G2a has also been prepared (54). The 14.G2a antibody activates complement and mediates ADCC with monocytes, neutrophils, NK cells, and lymphokine-activated killer (LAK) cells (55–56). The 14.G2a antibody has undergone clinical testing both as single-agent therapy and in combination approaches. Its toxicities and induction of HAMA responses were similar to that seen with 3F8.

4. Ch14.18 Clinical Testing

A human-mouse chimeric form of the 14.18 murine anti-GD2 mAb, designated ch14.18, was subsequently created to reduce the immunogenicity associated with the murine antibody (Fig. 1). The chimeric antibody is less immunogenic and is more effective than 14.G2a in mediating lysis of neuroblastoma cells with NK cells (57). The ch14.18 antibody has undergone clinical testing as a single-agent therapy. Simon et al. have published their results using standard induction treatment (chemotherapy with autologous stem cell rescue) for children and infants with stage 4 neuroblastoma followed by consolidation with chimeric 14.18 antibody for 5 days every 2 months, versus 12 months of oral maintenance chemotherapy or no further therapy (58). In patients <1 year old, there was no significant difference in event-free survival or overall survival in the three consolidation groups, with an overall survival of >90%. In patients >1 year old, the 3-year overall survival of ch14.18 treatment was superior to maintenance therapy or no additional therapy ($P = 0.018$) (59), although there was no difference in event free survival.

5. Hu14.18K322A Clinical Testing

A Phase I clinical trial is now underway at St. Jude Hospital using a novel hu14.18K322A anti-GD2 mAb, which was made using the same variable region as the ch14.18 mAb. However, this mAb has 3 major differences from the ch14.18 mAb. First, it is a humanized, not chimeric, mAb and thus could be less immunogenic with less allergic toxicity than ch14.18. Second, there is a single amino acid switch, from K to A at position 322 in the Fc region, which nearly abrogates complement activation, hopefully resulting in less neuropathic toxicity than ch14.18. Third, this mAb is produced in the YB2/O cell line, rather than CHO or NS/O lines, eliminating the normal fucosylation of the Fc region, and hopefully augmenting interaction with FcRs to increase ADCC (60). Thus, this novel hu14.18K322A is designed to cause less allergic reactions, less complement dependent toxicity, and more ADCC-mediated antitumor effects than ch14.18.

C. Antibodies Combined with other Agents

1. Antibody plus ADCC-Augmenting Cytokines

As the mechanisms of mAb-based tumor cell lysis were discovered, it was evident that the antibody must accomplish three separate jobs to kill a tumor cell. First, the antibody must recognize and bind to the tumor cell. Second, it must bind long enough and avoid internalization to adequately signal immune effector mechanisms. Third, the activated immune effector cells or effector proteins must be able to create a destructive signal (24). Since mAb-mediated tumor cell destruction relies on ADCC and/or CDC to kill tumor cells, strong effector functions are required. However, effector function, particularly ADCC, is often compromised in cancer patients due to immune suppression from metastatic cancer and/or chemotherapy (17,53,61). It is thought that the addition of cytokines that activate cells to mediate enhanced ADCC to mAb therapy will augment effector cell function and improve the overall antibody therapy efficacy (24).

a. 14.G2a + IL-2 Trial—Interleukin 2 (IL-2) is a strong pro-inflammatory cytokine with effects on both innate immunity, increasing the number and activation state of NK cells, and adaptive immunity, stimulating antigen-specific T cells (62–63). A Phase I trial through the Children's Cancer Group involved 33 patients. IL-2 was administered by three 96 hr infusions on days 1, 8, and 15 over consecutive weeks and 14.G2a was given as a daily 2 h infusion between days 9–13 (64). The treatment timing sought to take advantage of IL-2 induced lymphocytosis and maximal NK cell cytotoxic activity seen in several previously conducted *in vitro* analyses (65). One patient had a partial response with a 70% size decrease in an abdominal tumor facilitating complete resection. Three additional patients had a transient reduction in microscopic bone marrow disease but no overall reduction in tumor burden. Serum samples from these patients were found to have sufficient levels of 14.G2a to result in ADCC of GD2-positive tumor cells in *in vitro* assays (66). HAMA responses were also noted.

b. Ch14.18 + GM-CSF + IL-2 + CRA Pilot Trial—Testing of ch14.18 in refractory neuroblastoma included co-administration of GM-CSF in studies done by the Pediatric Oncology Group (67,68). Also, the Children's Cancer Group conducted a Phase I clinical trial of ch14.18 with GM-CSF in children with neuroblastoma immediately after hematopoietic stem cell transplant (69). Results of this trial determined the MTD of ch14.18 in combination with GM-CSF to be 40 mg/m²/day for 4 days in the early post-transplant period. A subsequent Phase I study found the MTD of ch14.18 to be 25 mg/m²/d for 4 days given concurrently with 4.5×10^6 U/m²/d of IL-2 for 4 days with alternating cycles of IL-2 and GM-CSF. Though two patients experienced DLTs from ch14.18 and IL-2, this combination was deemed tolerable in the early post-transplant period. This study also found that cis-RA can be safely administered between courses of ch14.18 and cytokines (70).

c. Phase III Neuroblastoma Ch14.18 + GM-CSF + IL-2 + CRA Trial—Preliminary data led to the design of the Children's Oncology Group (COG) Phase III trial, ANBL0032, which prospectively examined this ch14.18 + GM-CSF + IL-2 + CRA combination therapy in patients after myeloablative chemotherapy and autologous stem cell rescue. CRA was added to the regimen because it was shown previously in a Phase III clinical trial to improve overall survival in patients with stage 4 neuroblastoma (4). Following autologous transplant, patients were randomized to receive CRA alone or CRA in combination with ch14.18 and GM-CSF (in courses 1, 3 and 5) and IL-2 (in courses 2 and 4) (16).

From 226 patients with high-risk neuroblastoma, the results showed a two-year event-free survival of 66% in the immunotherapy group versus 46% in the standard treatment group ($p=0.0115$). Overall survival at two years was 86% for the immunotherapy group versus 75% for the standard treatment group ($p=0.016$). The results from this phase III trial have been recently reported (16).

This study shows a substantive increase in survival for high risk neuroblastoma. It is the first clinical trial to document that a combination of an anti-cancer mAb with ADCC-augmenting cytokines is an effective anticancer therapy. Also, it is the first time an antibody targeting a non-protein antigen (as GD2 is a glycolipid) has proven to be effective for immunotherapy of cancer. The 20% improvement in 2-year prevention of relapse for children with neuroblastoma receiving the experimental immunotherapy represents an advance in treatment that is being regarded now as the treatment of choice for high risk patients that achieve remission, in order to decrease the chance of relapse (16).

This study also shows the use of a monoclonal antibody combined with cytokines (GM-CSF and IL-2) to enhance antibody dependent cell-mediated cytotoxicity (ADCC) having made an impact on increasing survival in neuroblastoma in a minimal residual disease setting. Other monoclonal antibodies also mediate ADCC (Rituxan, Erbitux, Herceptin), but have yet to be

tested with cytokines in a minimal residual disease setting. This trial may portend future clinical trials testing cytokine combinations in more common malignancies that are currently treated with monoclonal antibodies (16).

d. 3F8 plus β -glucan—3F8 therapy is enhanced in mice when used in combination with the glucose polymer β -glucan (71). β -glucan sugars act as strong signals to the innate immune system, are well tolerated, and have been shown to stimulate TNF- α secretion and ADCC mediated by NK cells, monocytes, and neutrophils (72–76). 3F8 mAb binds to a tumor cell and coats tumor cells with iC3b. Soluble β -glucans can be used to prime CR3, the iC3b receptor, on the leukocytes, and cause dual ligation of the CR3 receptor on leukocytes to both iC3b and soluble β -glucan, which enhances tumor cytotoxicity (71,72). *In vivo*, oral or intraperitoneal β -glucan has been shown to be effective against neuroblastoma in mice. In nude mice bearing human neuroblastoma tumors, β -glucan and 3F8 mAb therapy resulted in near-complete tumor resolution while either agent alone had less effect. Survival was also increased compared with control animals and this effect was lost when tested on GD2-negative tumors (44,77). The use of β -glucan in conjunction with 3F8 is currently in clinical investigation.

D. Conjugated Antibodies

1. Antibodies Linked to Toxic Agents (Toxins, Chemotherapeutics, Radionuclides)

Antibodies are fairly easy to manufacture and can be linked to toxic agents. Conjugating mAbs to agents for selective delivery to tumor cells has included toxins, chemotherapeutic agents, radioactive isotopes, and immunological agents. Preclinical and some clinical work have been performed with these agents.

2. Radioimmunoconjugates

Radiolabeled mAbs have been used for both disease detection and targeted treatment of a variety of adult cancers but very few childhood tumors. However, radioimmunotherapy is attractive in neuroblastoma because of extensive studies on GD2 directed mAbs and because its tendency to be radiosensitive (78). The only widely studied radio-labeled mAb for treatment of neuroblastoma is ^{131}I -labelled 3F8. A Phase I dose-escalation study performed at Memorial Sloan-Kettering Cancer Center (MSKCC, New York) enrolled 23 patients with refractory stage 4 neuroblastoma. Out of ten patients evaluable, two had complete response (CR) of bone marrow disease and two had a partial response (PR) of soft-tissue disease (78). Based on these results, ^{131}I -labelled 3F8 was added to a multimodal treatment regimen under study at MSKCC for children with high-risk neuroblastoma (79).

3. Immunocytokines (ICs) - Antibodies Linked to Cytokines

a. ch14.18-IL2—The ch14.18-IL2 is an immunocytokine (IC) formed by linking IL-2 to the carboxyl end of the constant region of the chimeric mouse-human IgG1 ch14.18 mAb (80–82). Preclinical data in mice show that treatment with ch14.18-IL2 is far superior to comparable doses of ch14.18 mAb combined with IL-2 in mediating antitumor effects. In general, ADCC depends on the number and function of Fc receptors (FcR) on effector cells including activated NK cells (83,24,61,84). However, activated NK cells also have augmented IL-2 receptor (IL-2R) expression (85) leading to a dramatic *in vitro* response to IL-2 (86). In mouse models, the IL-2 component of this IC can activate NK cells without FcR, through their IL-2R (87). Thus, it is thought that effector cell binding to tumor is mediated in T-cells via IL-2Rs and in NK cells via FcRs and IL-2Rs (82,88). Data suggest that ch14.18-IL-2 could function as both a T-cell inducing vaccine as well as an activator of NK mediated ADCC. These data provided the basis for initiating clinical testing of this 14.18 based IC molecule as therapy for neuroblastoma (83) using an immunocytokine based on the humanized, rather than chimeric, form of the mAb; this IC is hu14.18-IL2.

b. hu14.18-IL2

i. Preclinical Development: When murine (14.G2a) or chimeric (ch14.18) anti-GD2 IgG mAbs are injected intravenously (IV) in mice, half-life is 2–5 days (29,62). In contrast, the half-life of the ch14.18-IL-2 and hu14.18-IL2 is only ~4hrs (89) when injected intravenously into mice. These data led to hu14.18-IL2 being given frequently (daily) to maintain both IL-2 and hu14.18 *in vivo* activity (83).

ii. Phase I Testing in Neuroblastoma: The Children's Oncology Group has completed a Phase I trial using hu14.18-IL2 in 27 pediatric patients with recurrent neuroblastoma using four courses of hu14.18-IL2 for patients with stable disease (90). The MTD was 12 mg/m²/day with dose limiting toxicities (DLTs) of hypotension, allergic reaction, blurred vision, neutropenia, thrombocytopenia, and leukopenia. No CR or PR was noted, but three patients had clinical changes suggestive of antitumor activity with radiographic and bone marrow response. Immune activation was noted with elevated sIL-2R α and lymphocytosis. All toxicities were reversible, and there were no treatment-related deaths.

iii. Phase II Study: A Phase II study (COG-ANBL0322) of hu14.18-IL2 in children with recurrent or refractory neuroblastoma was designed to evaluate the clinical antitumor activity and *in vivo* immunological effects of hu14.18-IL2. Also, this study sought to differentiate between patients with bulky disease and patients with minimal evaluable neuroblastoma. Patients received 3 daily IV doses of 12.0 mg/M²/d hu14.18-IL2 in each of 4 monthly courses (91). Fifteen patients had disease measurable by standard radiographical criteria (stratum-1) and 24 patients had disease evaluable only by meta-iodobenzylguanidine (MIBG) scanning and/or bone marrow (BM) histology (stratum-2). Responses were confirmed by independent radiological review and immunocytochemical (ICC) evaluation of the bone marrow.

No responses were seen in the 15 stratum-1 patients. In the 24 stratum-2 patients, 5 showed CR (MIBG and BM/ICC resolution). These response data support the conclusion that this agent and regimen have clinical activity in stratum-2 but not in stratum-1 patients (92). As all patients in this study had recurrent/refractory disease to prior multi-modality therapy, these responses are of interest to pediatric oncologists (91).

E. Anti-Idiotypic Antibodies

1. Mechanism of Tumor Resistance to Anti-GD2 Ab (HAMA, HACA, HAHA)

A problem with mouse mAb therapy has been the development of blocking antibodies to the mAb itself, called a HAMA (human anti-mouse antibody) response (80,57). The development of a HAMA response has been detected within 7 days of treatment and can neutralize any further treatments with the mouse anti-GD2 antibody (83,80). This led to the development of increasingly humanized versions of these mAbs. Chimeric antibodies have linked the GD2 specific variable ends of the immunoglobulin light and heavy chains from the mouse antibody to the human constant regions of the immunoglobulin light and heavy chains from the human antibody to create a less immunogenic mAb. Unfortunately, human "antichimeric" antibody (HACA) responses can still be detected (80,69).

The current humanized mAb, hu14.18, was developed retaining only the complementarity-determining regions (CDRs) of the original mouse antibody. It is ~98% human amino acid sequence (Fig. 1) (83,80,65). This humanized form of IC, hu14.18-IL2, was made with hopes of reducing immunogenicity of the IC in patients and has been studied in recently completed Phase I and II trials (80,90). The humanized mAb making the hu14.18-IL2 less immunogenic typically does not stimulate a neutralizing HACA or human antihumanized antibody (HAHA) response (24).

2. Anti-IC Antibodies and Antibody-Response Networks

Normally, the HAMA response inhibits antitumor effect. However, a HAMA response has been associated with increased antitumor effect that was also associated with enhanced survival (12). Current thinking suggests that an antibody-response network mechanism may be responsible for providing antitumor benefit. The antigen binding component of an anti-GD2 mAb (Ab-1) serves as an antigen for another antibody (Ab-2) generated in response to Ab-1 treatment. This binding region of Ab-2 may be “immunologically similar” to the GD2 antigen itself (as both bind to the antigen binding portion of the anti-GD2 mAb) and may serve as an additional antigen source for induction of a third antibody (Ab-3). Ab-3 in certain cases can bind to GD2 as well as Ab-2 and can generate antitumor responses similar to those elicited by Ab-1 (24,93,94).

In patients receiving 3F8 antibody, presence of Ab-3 was a predictor of overall survival (6, 94). Ab-3 is not seen in all patients. Anti-idiotypic antibodies (Ab-2) have been used as an antigen source in clinical trials (6,95–97). Also, similar to Ab-2, peptide mimics that bind to the therapeutic Ab-1 have been used in place of GD2 or Ab-2 molecule in an effort to induce an active antitumor response following vaccination (98,99). Currently, efforts at inducing ADCC are focused on patients entering remission, which typically requires intense immunosuppressive treatment to achieve. Therefore, as of now, the paradigm of immunotherapy is to avoid the HACA/HAMA (Ab-2) response.

F. T-cell Engineering in the Treatment of Neuroblastoma

T-cell activation and tumor-specific memory responses have been shown in response to mAbs in animal models and clinical settings (100). T-cell cytotoxicity can be enhanced through manipulation of the T-cell receptor (TCR) to redirect its specificity toward tumor antigens (101). T cells have been genetically altered to express chimeric TCRs consisting of a variable domain of an anti-GD2 antibody linked to a cytoplasmic signaling domain. Engagement of the TCR complex initiates cytotoxic effector function and release of pro-inflammatory cytokines including GM-CSF and IFN- γ upon incubation with GD2-positive tumor cells. These modified T cells mediate antitumor killing with minimal effects on GD2-negative targets (102).

Isolation of CD8⁺ T cells with altered TCR specificity from plasmids encoding engineered antigen receptors has been shown in human patients (103,104). Incorporation of DNA encoding the novel antigen receptors has been achieved via uptake of naked plasmid DNA by electroporation and retrovirus transfection (102,105). Typically, infusions of autologous tumor-specific T cells had half-lives of 1–42 days with minimal toxicity. Although this approach has been used more extensively for leukemia and lymphoma, human clinical trials targeting neuroblastoma are also under way (104–108). Patients who undergo stem cell transplantation require months to regenerate a functional immune system. Thus, the infusion of large numbers of tumor-specific effector T cells is an attractive alternative to waiting for an autologous immune response, especially in a minimal residual disease setting.

G. Summary

Current conventional therapy of high-risk neuroblastoma (surgery, radiation therapy and multi-agent chemotherapy) can put most children into remission. However, the majority of these patients eventually succumb to recurrent or refractory disease. The current strategy for treatment development is to utilize separate therapeutic approaches for patients in remission but harboring minimal residual disease. The use of anti-GD2 mAbs in this setting has been a promising approach under investigation. Preclinical data using these mAbs show strong antitumor effects in the minimal residual disease setting, and antitumor efficacy preclinically can be enhanced by using cytokines that stimulate ADCC. This has potential clinical implications

for patients, who have already undergone conventional surgery, radiation and/or chemotherapy, and who are in remission but suspected to carry minimal residual disease. A recent Phase III trial of this approach by the Children's Oncology Group has shown a 20% increase in event-free survival after two years. Novel approaches, using genetically engineered mAb derivatives, alone or combined with other agents, are even more effective in preclinical testing. Clinical trials of these concepts are underway to determine how best to integrate these approaches into an overall multi-modality treatment that can provide improved long-term disease free survival.

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Figure 1.

Monoclonal Antibodies and Immunocytokines. (a) A chimeric monoclonal antibody (mAb) combines the constant region of a human antibody with the variable domain of a murine antibody. The antigen specificity is conferred by the murine variable domain. (b) In the humanised mAb, the murine framework determinants of both the heavy and light chains are replaced with human framework determinants, but the antigen specificity of the original murine mAb is retained. (c,d) Fusion proteins or immunocytokines combine the mAb with covalently linked cytokines, such as molecules of interleukin 2 (IL-2), to the end of each of the heavy chains at the C-terminus.