Antibody-based immunotherapy in high-risk neuroblastoma

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Although great advances have been made in the treatment of low- and intermediate-risk neuroblastoma in recent years, the prognosis for advanced disease remains poor. Therapies based on monoclonal antibodies that specifically target tumour cells have shown promise for treatment of high-risk neuroblastoma. This article reviews the use of monoclonal antibodies either as monotherapy or as part of a multifaceted treatment approach for advanced neuroblastoma, and explains how toxins, cytokines, radioactive isotopes or chemotherapeutic drugs can be conjugated to antibodies to enhance their effects. Tumour resistance, the development of blocking antibodies, and other problems hindering the effectiveness of monoclonal antibodies are also discussed. Future therapies under investigation in the area of immunotherapy for neuroblastoma are considered.

Neuroblastoma is the most common malignancy in infants and the third most common cancer in children. The average age at diagnosis is 17 months and 50–60% of patients have metastatic disease when diagnosed (Refs 1, 2, 3). Despite advances in the treatment of low- and intermediate-risk neuroblastoma, the outcome for patients with advanced disease remains poor. Patients diagnosed after 18 months of age are at especially high risk for aggressive disease. The majority cannot be cured with standard combined therapy.

Standard treatment for these high-risk patients includes surgery, myeloablative chemotherapy with autologous stem cell transplantation, and radiation. Chemotherapy, when combined with 13-cis retinoic acid (CRA), an antiproliferative and differentiating agent, has been shown to have some effect in a high percentage of patients with stage 4 disease (primary tumour with spread to distant lymph nodes, bone, bone marrow, liver, skin and/or other organs) (Refs 4, 5, 6). However, the 3-year event-free survival of these patients remains low, at ~30% (Refs 1, 6, 7, 8).

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Recurrences develop from microscopic residual tumour cells ('minimal residual disease') that are resistant to local or systemic treatment. Complete eradication of these tumour cells has remained a therapeutic challenge.

Chemotherapy and radiation are constrained by dose-limiting toxicities and little tumour specificity. The ideal anticancer agent would be directed specifically to the tumour with minimal injury to healthy cells/tissue (Ref. 9). Immune therapy holds great promise as a treatment modality because it takes advantage of the specificity of immune effector cells targeted to the tumour, potentially reducing the systemic side effects observed with other forms of treatment. Monoclonal antibodies (mAbs) derive their specificity from recognition of antigens expressed on the tumour cell surface that are either found only on tumour cells and not on normal tissue or are found in much greater amounts on tumour cells compared with normal cells (Refs 10, 11). Antibodies are also advantageous because they are fairly easy to manufacture and can be linked to other molecules such as cytokines, toxins and chemotherapeutic drugs to enhance their antitumour effect.

mAbs are currently in use in the detection, diagnosis and treatment of neuroblastoma (Refs 8, 12, 13, 14). There are several mechanisms by which antibodies can destroy tumour cells. Antibodies by themselves can stimulate antibody-dependent cell-mediated cytotoxicity (ADCC) of tumour cells: after the variable region of the antibody binds to antigen on the tumour 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 tumour cell lysis (Refs 15, 16). In addition, complement-mediated cytotoxicity (CMC) may be induced after an antibody binds to the tumour cell surface (Ref. 9).

In this review, we examine several current strategies using mAbs for the treatment of high-risk neuroblastoma, either as primary therapy or as part of a multifaceted treatment approach, in clinical trials and also discuss newer molecules that combine mAbs with toxins, cytokines, radioactive isotopes or chemotherapeutic drugs. In addition, we review the pitfalls of this treatment approach, including tumour resistance and the development of blocking antibodies that make mAb therapy less effective. Finally, we look ahead at potential future therapies. In the 20 years following the first clinical trial using mAb therapy for neuroblastoma, significant progress has been made and there is great promise for the future (Ref. 17).

Murine and chimaeric mAbs as single agents

Target antigens

Neuroblastoma expresses a wide variety of antigens on its surface. Antigens 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 (Ref. 16). GD2 is a disialoganglioside antigen that has been used extensively as a target in mAb therapy and has been the primary target of antibody recognition in neuroblastoma. It is expressed on tumours of neuroectodermal origin that include neuroblastoma, melanoma, osteosarcoma, and small-cell lung cancer (Refs 9, 18). In normal tissues, GD2 expression is limited to neurons, skin melanocytes, and peripheral pain fibres (Ref. 19), making it well suited for targeted antitumour therapy.

In 1984, a murine mAb (mAB126) was produced against cultured human neuroblastoma cells (LAN1). This antibody was directed to the GD2 antigen on neuroblastoma and melanoma cells. Patients with neuroblastoma were found to have significantly elevated free GD2 levels in serum compared with normal children and children with other tumours (Ref. 20). In addition, unlike other tumour antigens that have been described in the past [e.g. CALLA in acute leukaemia (Ref. 21)], GD2 expression was not lost from the cell surface of neuroblastoma cells, even when bound to antibody (Ref. 22). (This concept is further detailed below in 'Mechanisms of tumour resistance to antibody therapy'.)

3F8

The first mAb tested in clinical trials was the anti-GD2 mAb 3F8 (Refs 17, 23, 24, 25, 26). This murine IgG3 antibody has been shown to have significant antitumour effects on both primary tumours and bone-marrow metastases. Labelled 3F8 has demonstrated selective localisation to tumours expressing GD2, with very little nonspecific uptake in the liver or spleen (Ref. 26). In the initial Phase I and Phase II trials using 3F8 in patients with stage 4

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neuroblastoma, there was no significant antitumour effect on bulky disease but some response was seen in bone marrow disease and microscopic bone disease (Refs 16, 27, 28, 29, 30) (Table 1). Side effects included hypertension, hypotension, fever, vomiting, diarrhoea and urticaria. The most common complaint was pain, which can be severe (grade 3–4) and dose limiting. Pain with the use of anti-GD2 antibodies has been attributed to recognition by antibodies of GD2 expressed on peripheral pain fibres (Refs 31, 32, 33). HAMA (human antimouse antibody) can develop in patients treated with 3F8 and has resulted in termination of therapy (Ref. 34) (see section 'Mechanisms of tumour resistance to antibody therapy').

The mechanism of tumour cell lysis using 3F8 antibody is similar to that of other IgG mAb therapies. 3F8 has been shown to activate tumour cell destruction by both CMC and ADCC in vitro (Refs 35, 36). As a result of the lack of complement-inhibitory proteins CD55 and CD59 on neuroblastoma cells, this tumour is particularly sensitive to complement-mediated tumour cell lysis in vitro (Refs 35, 37).

14.18

14.18 is a separate IgG3 murine mAb targeted to the GD2 antigen (Ref. 18). In an attempt to enhance ADCC, a class switch variant called 14.G2a has also been prepared (Ref. 38). The 14.G2a antibody activates complement and mediates ADCC with monocytes, neutrophils, NK cells and lymphokine-activated killer (LAK) cells (Refs 39, 40). A chimaeric anti-GD2 antibody, ch14.18, was subsequently created to reduce the immunogenicity associated with the murine antibody (Fig. 1; see also section 'Mechanisms of tumour resistance to antibody

Table 1. Clinical trials of monoclonal antibodies (mAbs) as single agents

mAb	Clinical trial	Dose	Number of patients	Results
3F8	Phase I (Ref. 17) (Memorial Sloan Kettering Cancer Center)	5–100 mg/m^2	8 (neuroblastoma)	1 complete response bone marrow; 1 partial response microscopic bone disease
	Phase II (Ref. 27) (Memorial Sloan Kettering Cancer Center)	50 mg/m^2	16 (neuroblastoma)	1 complete response; 3/8 complete marrow response; 2/7 response in microscopic bone disease
14.G2a	Phase I (Ref. 42) (Tübingen, Germany)	100–400 mg/m^2	9 (neuroblastoma); 6 evaluable for response	2 complete response; 2 partial response; 2 no response
	Phase I (Ref. 43) (M.D. Anderson)	50–200 mg/m^2	18 (neuroblastoma/ melanoma); 5 evaluable for response	2 partial response; 2 mixed response
	Phase I (Ref. 44) (University of Alabama)	10–120 mg	12 (neuroblastoma/ melanoma); 3 evaluable for response	1 mixed response; 1 complete response; 1 stable disease
ch14.18	Phase I (Ref. 45) (Tübingen, Germany)	150, 200, 250 mg/m^2	9 (neuroblastoma)	2 complete response; 2 partial response; 1 minor response; 1 stable disease; 3 progressive disease
	Phase I (Ref. 46) (Pediatric Oncology Group)	10–200 mg/m^2	10 (9 neuroblastoma; 1 osteosarcoma)	1 partial response; 4 mixed response; 1 stable disease

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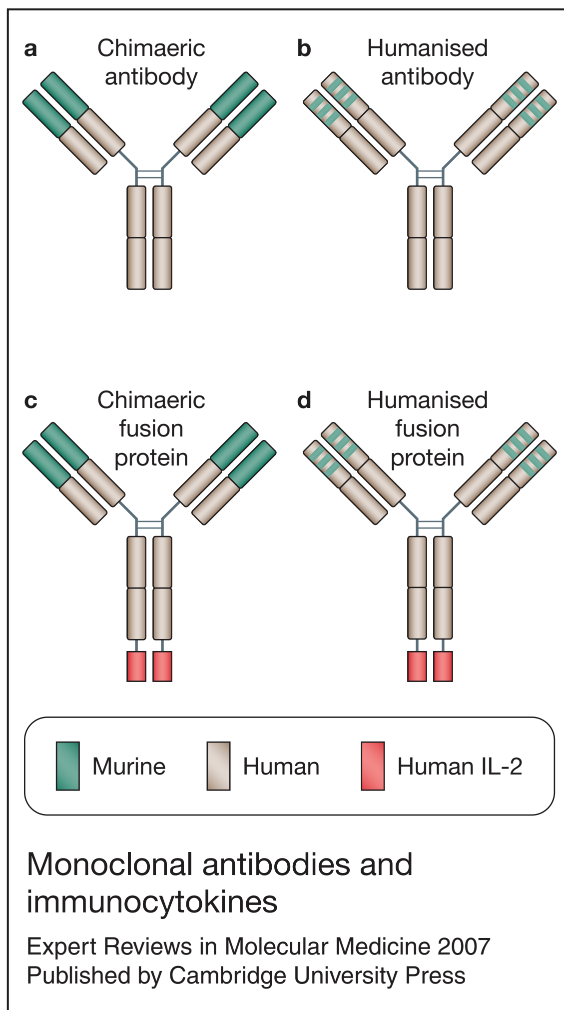

Figure 1. Monoclonal antibodies and immunocytokines. (a) A chimaeric monoclonal antibody (mAb) combines the constant region of a human antibody with the variable domain of a murine antibody. The antigen specificity is conferred by the murine variable domain. (b) In the humanised mAb, the murine framework 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. Adapted, with permission, from Ref. 58 (© 1999 Elsevier).

therapy'). The chimaeric antibody is less immunogenic and is more effective than 14.G2a in mediating lysis of neuroblastoma cells with human effector cells such as granulocytes and NK cells (Ref. 41). The ch14.18 and 14.G2a antibodies have undergone clinical testing both as single-agent therapies and in combination approaches; in this section, we focus on the former.

As a single agent, 14.G2a has been used in three separate Phase I clinical trials. In 1992, in Tübingen, Germany, nine patients with stage 4 neuroblastoma were treated with 14.G2a: all patients developed HAMA during or shortly after therapy; six patients were evaluable for response (Ref. 42). In a trial conducted at M.D. Anderson (Houston, USA), 18 adults with melanoma or neuroblastoma received the 14.G2a mAb in a dose-escalation trial by continuous intravenous (IV) infusion over 5 days: the maximum tolerated dose (MTD) was 100 mg/m^2/day (Ref. 43); 16/18 patients developed HAMA. Lastly, at the University of Alabama (Birmingham, USA), 12 adults with melanoma or neuroblastoma received the antibody by 1 h daily infusion on days 1, 3, 5 and 8: all patients developed HAMA (Ref. 44). Responses were variable among all patients but 14.G2a did show promise as a therapeutic agent (Table 1). The toxicities seen in these trials included fever, diarrhoea, nausea, vomiting, transient neuropathy, tachycardia, hypotension, anaphylactoid reactions and pain (Refs 42, 43, 44).

The chimaeric antibody has also been tested as a single-agent therapy in two separate trials involving patients with stage 4 neuroblastoma. In a Phase I trial in Tübingen, Germany, nine heavily pretreated patients with stage 4 neuroblastoma were treated with 19 courses of chimaeric anti-GD2 antibody at dose levels 30, 40 and 50 mg/m^2/day for 5 days per course. The MTD was 50 mg/m^2/day. No HAMA responses were seen (Ref. 45) (Table 1). Similar results were seen in ten patients with refractory neuroblastoma and one with osteosarcoma. Patients in this study received 20 courses of chimaeric antibody at dose levels of 10, 20, 50, 100 or 200 mg/m^2. Dose escalation was permitted for a single patient if tolerated. The MTD was not reached in this study. Ten patients were evaluable for response; results are summarised in Table 1 (Ref. 46). The chimaeric antibody was well tolerated in both studies with few side effects seen at dosages of 10 mg/m^2 or less, and encouraging results were seen in bone marrow and microscopic bone disease. Toxicities in each were similar to those seen with the murine antibody, including pain,

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tachycardia, hypertension, fever, urticaria and transient neuropathy (Refs 45, 46). Optic nerve atrophy was seen in two patients in the Tübingen study. Both patients had received prior radiotherapy and this was implicated as the cause of this adverse event. The effect was reversible in both patients (Ref. 45). Seven out of ten patients in the latter study did develop a HACA (human antichimaeric antibody) response as measured in post-treatment serum samples (Ref. 46). Pharmacokinetic and immunological studies in association with these trials have shown that the chimaeric antibody has a longer plasma half-life and is less immunogenic than its murine counterpart, potentially improving its clinical utility (Ref. 47).

Use of mAbs with augmenting agents

The ADCC effect of mAbs on tumour cell lysis can be augmented by the addition of other agents that can prime or boost effector cell function. The anti-GD2 antibodies have been used in conjunction with various cytokines and β-glucans, with favourable experimental results.

Use of mAbs with cytokines

As the science of mAb-based tumour cell lysis was elucidated, it became clear that the antibody must accomplish three separate tasks to kill a tumour cell: first, the antibody must recognise and bind to the tumour cell; second, it must bind long enough to the tumour cell without being internalised to properly signal immune effector mechanisms; and third, the activated immune effector mechanisms must be able to create a destructive signal (Ref. 9). Since mAb therapy relies on ADCC and/or CMC to kill tumour cells, strong effector functions are required. However, owing to immune suppression from metastatic cancer and/or chemotherapy, effector-cell function may be compromised in cancer patients (Refs 16, 36, 48). The addition of cytokines to mAb therapy can augment effector-cell function and improve the overall effect of immune therapy (Ref. 9).

Although chemotherapy may be strongly immunosuppressive, the effect on granulocytes is more transient (Ref. 49). GM-CSF (granulocyte–monocyte colony-stimulating factor) has been found to both increase granulocyte cell populations and increase adhesion molecules on lymphocytes, which enhances ADCC against neuroblastoma cells (Refs 36, 50, 51, 52). It has been used in clinical trials in combination with the mAbs ch14.18 (Ref. 53), 14.G2a (Ref. 54) and 3F8 (Refs 34, 36). The results of all four trials suggest that GM-CSF in combination with anti-GD2 mAb improves the antitumour response, particularly in bone marrow disease (Refs 34, 36, 53, 54). However, this has not yet been tested directly in a randomised controlled clinical trial.

Interleukin 2 (IL-2) is a strong pro-inflammatory agent with effects on both the innate and adaptive immune system. IL-2 increases both the number and activation state of NK cells. Activated NK cells bind the Fc portion of the mAb through their FcγRIII and enact ADCC (Refs 55, 56). IL-2 also stimulates antigen-specific T cells to kill tumour cells, an example of breaking tolerance to tumour antigens (Refs 56, 57). In a Phase I trial through the Children's Cancer Group (predecessor to the Children's Oncology Group, which conducts multi-institutional clinical trials in paediatric oncology in the USA) involving 33 patients, IL-2 was administered by three 96 h infusions over successive weeks, on days 1, 8 and 15 (Ref. 54). The mAb 14.G2a was given as a daily 2 h infusion on days 9 through 13. This was timed to take advantage of the lymphocytosis and maximal NK cell cytotoxic activity seen in several in vitro analyses carried out prior to the study (Ref. 58). One patient had a partial response with a 70% decrease in size of an abdominal tumour over 3 months, facilitating complete resection. Three additional patients had a transient reduction in microscopic bone marrow disease but no reduction in tumour burden in other locations. Serum samples were obtained from these patients and were found to contain sufficient levels of 14.G2a to result in ADCC of GD2-positive tumour cell targets in vitro (Ref. 59).

Use of mAbs with β-glucans

3F8 therapy is enhanced when used in combination with the glucose polymer β-glucan, which is homologous with polysaccharides normally found on the cell wall of fungi or yeast (Ref. 60). These sugars act as strong signals to the innate immune system, much like lipopolysaccharide (LPS) found on the cell envelope of Gram-negative bacteria. While LPS has been found to be too toxic to use in human patients, β-glucans are well tolerated and have been shown to stimulate ADCC mediated by NK cells, monocytes and

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neutrophils, as well as increase production of tumour necrosis factor α (TNF-α) (Refs 61, 62, 63, 64, 65). When mAb binds to a tumour cell, the complement cascade is activated and coats the tumour cell with the complement fragment iC3b. The receptor for iC3b is the CR3 molecule on leukocytes. Soluble β-glucans can be used to prime CR3 on the leukocytes, leading to dual ligation of the CR3 receptor on leukocytes to both iC3b on the tumour and soluble β-glucan; this dual ligation subsequently enhances tumour cytotoxicity (Refs 60, 61) (Fig. 2).

In vivo, the addition of oral or intraperitoneal β-glucan has been shown to be effective against neuroblastoma. In nude mice bearing human neuroblastoma tumours NMB7, LAN-1, SK-N-ER, SK-N-MM or SK-N-JD, the addition of β-glucan to 3F8 mAb therapy resulted in near-complete or complete tumour resolution. By contrast, either agent alone had significantly less effect. Survival was also increased by 2.6–5.5 times compared with control animals depending on the tumour type. The effect was lost when tested on a GD2-negative tumour (Refs 24, 66). Depletion of NK cells resulted in a depressed antitumour effect in nude mice, but synergy was still observed in severe combined immunodeficient (SCID-beige) mice, suggesting that some of the antitumour effect was mediated in an NK-cell-independent manner (Ref. 67). This synergy in combination with oral β-glucan has been seen in other mAb systems using the antigens GD3, CD20, EpCAM and HER-2 as targets for mAb therapy (Ref. 67). The use of β-glucan in conjunction with 3F8 is currently under Phase I clinical investigation.

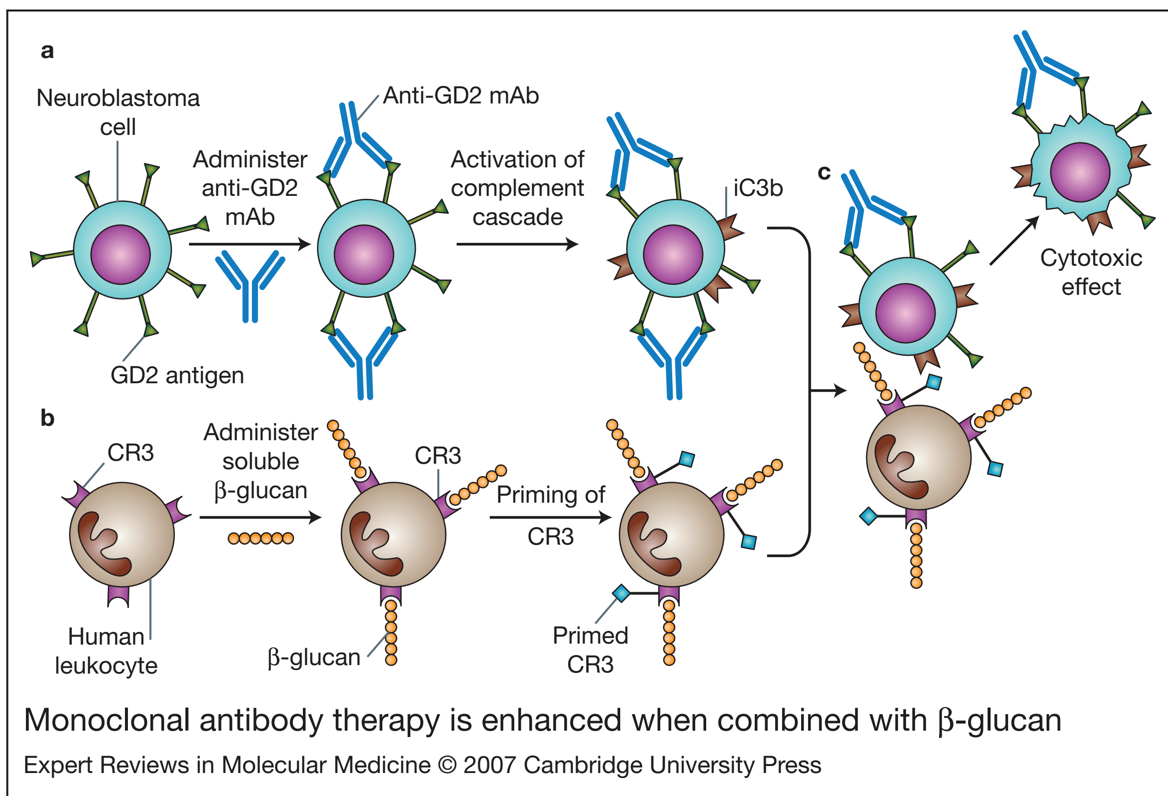

Figure 2. Monoclonal antibody therapy is enhanced when combined with β-glucan. (a) When anti-GD2 mAb binds to the GD2 antigen on neuroblastoma cells, the complement cascade is activated. While many molecules are involved in the complement cascade, one that becomes displayed on the neuroblastoma surface is the complement fragment iC3b. (b) β-glucan is a glucose polymer normally found on the surface of yeast. Soluble β-glucan binds to the iC3b receptor (CR3) on human leukocytes, priming it for interaction with the iC3b on tumour cells. (c) When the β-glucan-primed CR3 recognises iC3b on the neuroblastoma cell, tumour cell cytotoxicity results.

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Conjugated mAbs

The idea of conjugating mAbs to other agents for selective delivery to tumour cells has been expanded to include toxins, chemotherapeutic agents, radioactive isotopes, and immunological agents. Preclinical and some clinical work has been performed with these agents.

mAbs linked to toxins

mAbs have been conjugated to toxins as a means of selective toxin delivery. These agents kill tumour cells via antigen-directed internalisation of the toxin and can work independently of immune effector cells (Ref. 68). Several antibody–toxin pairs have been tested. For example, anti-GD2 mAb has been linked to either *Pseudomonas* exotoxin A (Ref. 69) or diphtheria toxin (Ref. 70). Both agents have shown cytotoxicity against GD2-positive neuroblastoma cell lines but neither has yet undergone clinical testing (Refs 69, 70). Noncytotoxic antibody fragments, such as the Fab fragment of 3F8, can be made increasingly cytotoxic through linkage of the Fc fragment to cobra venom factor (CVF), which is a functional homologue of activated human complement. In the presence of human complement, these antibodies have been shown to mediate complement-dependent lysis of neuroblastoma cells, with up to 100% killing in some in vitro assays (Ref. 71). Furthermore, linkage of ch14.18 mAb to the superantigen staphylococcal entertoxin A demonstrated effective T-cell-mediated killing of GD2-positive neuroblastoma cells, independent of major histocompatibility complex (MHC) class II expression (Ref. 72). Ricin A has also proven effective against GD2-positive neuroblastoma cell lines in both in vitro studies and in IMR5-tumour-bearing SCID mice (Ref. 68). Although all of these agents have shown promising in vitro activity, few have moved into preclinical testing.

mAbs for targeted chemotherapy delivery

Antigen-specific drug delivery is another possible mechanism of killing tumour cells selectively. Anti-GD2 or -GM2 antibody fragments have been linked to liposomes containing adriamycin and have been shown to result in significant cytotoxicity when placed in culture with GD2- and GM2-positive cell lines (Ref. 73). In a second model, the synthetic retinoid fenretinide, which is known to induce apoptosis in neuroblastoma cell lines, has also been incorporated into liposomes and bound to anti-GD2 mAb. In vivo, this combination prevented the development of metastatic disease in nude mice bearing human HTLA-230 tumours, and was more effective in survival analyses than either empty liposomes or anti-GD2 mAb alone (Ref. 74). Further in vivo work is required to determine the efficacy of antibody-directed chemotherapy, but this method may prove helpful in reducing the side effects of systemic administration, especially in heavily pretreated patients.

Radioimmunoconjugates

Radiolabelled mAbs have been used for both disease detection and targeted treatment.

Detecting disease

While [^{131}I]metaiodobenzylguanidine (mIBG) continues to be used as the standard of care for clinical detection of metastatic disease in patients with neuroblastoma, 14.G2a, chimaeric 14.18 and 3F8 have all been used for radioimmunodetection of neuroblastoma in clinical trials, with excellent sensitivity and specificity. ^{131}I-labelled 14.G2a was used in a Phase I clinical trial designed to determine the MTD of 14.G2a in 18 patients with refractory melanoma, neuroblastoma or osteosarcoma (Ref. 43). Patients received the radiolabelled antibody prior to treatment to determine GD2 positivity. The radioimmunoconjugate had a sensitivity of 86% in detecting known metastases (Ref. 43). In comparison studies of mIBG versus ch14.18 labelled with technetium-99m (Tc99m) used for detecting recurrence in 18 patients with stage 4 neuroblastoma, radiolabelled antibody demonstrated superior sensitivity to detect local tumour recurrence, skeletal metastases and soft-tissue/lymph-node metastases. Specificity was 100% for both modalities. An additional advantage of Tc99m-labelled ch14.18 antibody was earlier detection of metastatic disease than with [^{131}I]mIBG (Ref. 75). Similarly, ^{131}I-labelled 3F8 showed superior sensitivity in the detection of metastatic disease in comparison with [^{131}I]mIBG, computed tomography or magnetic resonance images. In this study, 14/26 patients with bone marrow involvement based on antibody scans had confirmation of bone marrow disease by

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bone marrow aspirate or biopsy examinations (Ref. 26). Other radiolabelled antibodies have been used in the detection of neuroblastoma, including ^{131}I-labelled UJ13A (an antibody against NCAM, which is another antigen found on neuroblastoma), ^{131}I-labelled CE7 (a murine mAb against cell-surface glycoprotein on human neuroblastoma cells) and Tc99 m-labelled BW575 (an IgG1 mAb against cell-surface glycoprotein on small-cell lung cancer carcinoma, neuroblastoma, brain tumours and nervous tissue) (Refs 76, 77, 78).

Treatment

Radiolabelled mAbs have been used in the treatment of a variety of adult malignancies, but very few childhood tumours. However, because of the extensive studies on GD2 and mAbs directed against it, neuroblastoma is an exception. Radioimmunotherapy is attractive in neuroblastoma, as it tends to be a radiosensitive tumour (Ref. 79). The only widely studied radiolabelled 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, USA) enrolled 23 patients with refractory stage 4 neuroblastoma. All patients receiving ^{131}I-labelled 3F8 developed grade 4 myelosuppression requiring either GM-CSF or autologous stem cell rescue if no response to GM-CSF. Extramedullary toxicity was limited to hypothyroidism. Patients did receive thyroid protection prior to treatment. Ten patients were evaluable for response: two had a complete response of bone marrow disease and two had a partial response of soft-tissue disease (Ref. 79). Based on these results, ^{131}I-labelled 3F8 was added to the N7 program, a multimodal treatment regimen under study at MSKCC for children with high-risk neuroblastoma (see section ‘Combining antibody treatment with conventional treatment’) (Ref. 80).

The central nervous system (CNS) is a potential site of recurrence in neuroblastoma, with incidences ranging from 1% to 16% (Ref. 81). One explanation is that the blood–brain barrier blocks the access of chemotherapeutic and immunotherapeutic agents to residual cancer cells in the CNS. Investigators at MSKCC noted an increase in CNS recurrence among patients treated on the N6 or N7 protocols using 3F8 (6.4 and 14%, respectively) versus those treated on the N4 or N5 protocols (0 and 3.2%, respectively). Subsequent retrospective analysis of all patients with metastatic neuroblastoma who were treated on protocols N4, N5, N6 and N7 revealed an overall incidence of CNS recurrence of 6.3% (Ref. 81). This observation prompted investigation in treatment of CNS disease using 3F8. To overcome the blood–brain barrier, direct delivery of immune therapy into the cerebrospinal fluid has been attempted, with promising results. In a rat model, intrathecal delivery of the ^{131}I-labelled 3F8 antibody has been effective (Refs 82, 83). In primates, intraventricular delivery of this agent can ablate tumour with no long-term side effects (Ref. 81). This delivery method has undergone a Phase I trial. In eight patients with meningeal GD2-positive neuroblastoma, focal uptake of the agent was seen in seven patients, with minimal radiation to other body organs (Ref. 84). The ^{131}I-labelled 3F8 antibody for use in CNS disease is now undergoing Phase II trials [N.K. Cheung (MSKCC, New York USA), pers. commun.]. A second antibody, ^{131}I-labelled 8H9, is also being tested in neuroblastoma with CNS involvement. 8H9 is a murine IgG1 mAb developed at MSKCC that recognises the B7-H3 antigen found on a variety of solid tumours, including neuroblastoma (Ref. 85).

Immunocytokines

While the addition of cytokines to mAb therapy has shown a synergistic effect, systemic cytokine administration has been limited by toxicity arising from the nonspecific activation of the inflammatory cascade, with no specific activity against tumour cells (Ref. 11). To limit the toxicity from cytokines, a new therapeutic agent was developed that linked the cytokine protein to the Fc end of the mAb (Fig. 1). This ‘immunocytokine’ retained the antigen-binding specificity of the original mAb and delivered the cytokine directly to the tumour microenvironment. As a single agent, both the antibody effector function and the cytokine co-signal are provided (Ref. 86). The dose of cytokine required is much lower than that required with systemic administration and its half-life may be significantly increased (Refs 9, 87, 88).

One of the best-characterised immunocytokine molecules is ch14.18–IL2, which was formed by linking the gene sequence of IL-2 to DNA

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encoding the carboxyl end of the constant region of the human IgG1 molecule that composes the ch14.18 mAb (Refs 87, 89). The advantage of this agent is that it can activate cells without Fc receptors (which includes a subpopulation of cytotoxic NK cells) (Ref. 90) and in vivo data suggest that activated NK cells have augmented IL-2 receptor expression (Ref. 91). Local T cells also may be activated through their IL-2 receptor if the T-cell receptor (TCR) does not recognise the tumour antigen initially (Ref. 89).

Several preclinical studies have been completed using the ch14.18–IL2 molecule (Refs 89, 92, 93, 94). In SCID mice bearing neuroblastoma with bone marrow and liver metastases, intravenous administration of ch14.18–IL2 resulted in a significant reduction in tumour burden. This was not seen in animals that received ch14.18 and IL-2 as separate intravenous infusions (Ref. 93). In animals depleted of NK cells, the effect of ch14.18–IL2 was lost, suggesting that the effect was dependent on NK cells. The addition of NK-stimulating agents such as interferon γ (IFN-γ) further increased the antitumour effect. $CD8^{+}$ T-cell depletion, however, did not significantly affect the antitumour response (Ref. 94). In a second study, using immunocompetent mice, treatment with ch14.18–IL2 prevented metastatic spread to the liver and bone marrow, whereas giving both agents separately did not prevent metastatic disease. Mice given the ch14.18–IL2 molecule survived twice as long as the other treatment groups; however, the effect on bulky tumours was minimal (Refs 93, 94).

The ch14.18–IL2 molecule has been refined to a humanised form, known as hu14.18–IL2, in the hope of further reducing its immunogenicity in patients (Fig. 1). This agent has recently completed Phase I trials (Ref. 95). Twenty-seven patients with stage 4 neuroblastoma were given infusions for three consecutive days. The maximum tolerated dose was 12 mg/m^2/day. Toxicities included hypotension (requiring use of dopamine in two patients), allergic reaction, blurred vision, neutropaenia, thrombocytopaenia and leukopaenia. All toxicities were reversible. Although there were no complete or partial responses, the patients did respond with lymphocytosis and elevated levels of soluble IL-2 receptor, and three patients had some radiographic and/or bone marrow response. No patient, however, had a reduction in bulky disease in other sites. This agent is now being tested in a Phase II clinical trial through the Children's Oncology Group (study number ANBL0332) in patients with recurrent or refractory stage 4 neuroblastoma.

Immunocytokines have also been developed using the cytokine GM-CSF (Ref. 96). This has been linked to ch14.18 to create a molecule that has been shown to be very effective at stimulating ADCC against the neuroblastoma cell line NMB7 (Ref. 96). When used in combination with granulocytes or monocytes from patients with neuroblastoma, the immunocytokine was equivalent in cell killing to equal amounts of free GM-CSF and ch14.18 mAb. Although the immunocytokine was not found to be superior in vitro, it is advantageous to be able to provide targeted cytokine delivery to stimulate both granulocyte and monocyte proliferation and migration. The ch14.18–GM-CSF immunocytokine has not yet entered clinical testing.

Combining antibody treatment with conventional treatment

Treatment of high-risk neuroblastoma is generally viewed in three phases: intensive induction chemotherapy with radiation therapy and surgery to reduce tumour burden, marrow ablation followed by autologous stem cell rescue to target residual and possibly resistant disease, and therapy aimed at eliminating or keeping minimal residual disease in check. Immunotherapy theoretically works best in the setting of minimal residual disease. In fact, in the trials using anti-GD2 antibodies described above, the most demonstrable and consistent effect was in microscopic marrow disease (Refs 17, 34, 53, 95). When interpreting these results, one must keep in mind that detection of microscopic marrow disease is difficult to evaluate because of test limitations and sampling error (i.e. false negative bone marrow biopsies). However, based on the observations made in the Phase I and II clinical trials performed with 3F8 and chimaeric 14.18 as single-agent therapies, there have been several studies aimed at combining conventional treatment for neuroblastoma with immunotherapy.

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A pilot study published in 1998 demonstrated the feasibility of this combined approach. Eleven patients with stage 4 neuroblastoma were treated with mIBG and high-dose chemotherapy (melphalan, carboplatin and etoposide) followed by autologous stem cell rescue. All patients subsequently received either murine anti-GD2 antibody (14.G2a) or chimaeric 14.18. Four children experienced no change in disease status, three achieved a complete response, one achieved a partial response, and three had progressive disease (with two subsequent deaths) (Ref. 97).

Simon et al. have published their results using standard induction treatment for infants and children over 1 year of age with stage 4 neuroblastoma followed by consolidation treatment with chimaeric 14.18 antibody alone. Patients received six to eight cycles of induction chemotherapy followed by either four cycles of low-dose oral chemotherapy or high-dose chemotherapy with autologous stem cell rescue. Consolidation consisted of six cycles of antibody at 20 mg/m^2/day for 5 days in six cycles every 2 months, versus 12 months of oral maintenance chemotherapy or no further therapy (Ref. 98). In patients less than a year of age, there was no significant difference in event-free survival or overall survival in the three consolidation groups, with an overall survival in all groups of >90%. In patients older than 1 year of age ($n = 334$), univariate analysis found similar 3-year event-free survival for all three groups. For 3-year overall survival, ch14.18 treatment was superior to maintenance therapy or no additional therapy (68.5% $\pm$ 3.9%, 56.6% $\pm$ 5% and 46.8% $\pm$ 6.2%, respectively; $P = 0.018$) (Ref. 99).

While the results of the Simon trials did not demonstrate a definitive survival advantage in using mAb as a single agent for maintenance chemotherapy, the results of the studies using mAb in combination with cytokine therapy (both separately and as fusion proteins) have spurred several clinical trials in stage 4 neuroblastoma through the Children's Cancer Group, Pediatric Oncology Group and the merged Children's Oncology Group.

The Children's Cancer Group conducted a Phase I clinical trial of ch14.18 with GM-CSF in children with neuroblastoma immediately after haematopoietic stem cell transplant (Ref. 53). Results of this trial determined the MTD of ch14.18 in combination with GM-CSF to be 40 mg/m^2/day for 4 days in the early post-transplant period. A subsequent Phase I study examined the MTD of ch14.18 with the addition of alternating cycles of IL-2 and GM-CSF (Gilman, A.L. and Sondel, P.M., unpublished). Although the results of this study have not yet been published, preliminary data led to the design of the current Children's Oncology Group Phase III trial, ANBL0032, which will prospectively examine this combination therapy in patients who have received myeloablative chemotherapy followed by autologous stem cell rescue and who are receiving CRA. CRA has been added to the regimen as it was shown in a previous Phase III clinical trial to improve 3-year event-free survival and overall survival in patients with stage 4 neuroblastoma (Ref. 6). Following autologous transplant, patients are randomised 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).

3F8 has also been used in combination with conventional therapy (Ref. 80). The N7 treatment protocol conducted at MSKCC for children with high-risk neuroblastoma consisted of seven courses of chemotherapy (cyclophosphamide, doxorubicin and vincristine alternating with cisplatin and etoposide). Patients subsequently underwent surgical resection and 7 days of external beam radiation therapy for local control (21 Gy in 1.5 Gy fractions twice daily). Consolidation therapy consisted of myeloablation with ^{131}I-labelled 3F8 followed by autologous stem cell rescue with marrow cells purged using 3F8. Patients were subsequently treated with 3F8 at 10 mg/m^2/day for 5 days on and 2 days off per week for 6 weeks. Progression-free survival using this regimen was approximately 40% (Ref. 80). The most recent protocol utilising 3F8 is the N8 protocol. In this regimen, patients receive five cycles (versus seven) of induction chemotherapy. Consolidation therapy consists of mAb 3F8 with GM-CSF given as separate infusions [N.K. Cheung (MSKCC, New York USA), pers. commun.].

In addition to the N7 and N8 regimens, the investigators at MSKCC have also used the 3F8 antibody together with cyclophosphamide and irinotecan, as well as 3F8 with cyclophosphamide, topotecan and vincristine. The goal was to use an immunosuppressive

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regimen that would allow passive use of mAb (i.e. reduce the development of a neutralising HAMA response) as well as one with little risk to major organs in a group of heavily pretreated patients. A major response was seen in 15% of patients treated with cyclophosphamide, topotecan and vincristine, and in 17% of patients treated with cyclophosphamide and irinotecan. This regimen also had relatively little toxicity to nonhaematologic organs. Another advantage of this regimen is the radiosensitising nature of topotecan (Ref. 100).

Use of mAbs in purging bone marrow for autologous marrow/stem cell transplant

As outlined above, autologous stem cell transplantation currently plays a role in consolidation therapy of patients with high-risk neuroblastoma. Analyses of patients receiving genetically 'marked' autologous marrow transplants for acute leukaemia have shown that recurrent leukaemia can be derived from microscopic, undetected, residual leukaemia in the marrow infusate (Ref. 101). Thus, for neuroblastoma, if the conditioning therapy could effectively destroy any residual neuroblastoma in the patient, it would seem prudent to ensure that marrow/stem cells re-infused into a patient after myeloablative therapy were rid of neuroblastoma contaminating cells. Several strategies have been employed for purging contaminating tumour cells, including chemical purging, photoradiation, and the use of a viral-directed enzyme prodrug (Refs 102, 103, 104, 105). Immunomagnetic depletion of tumour cells from harvested marrow/stem cells makes use of mAb for the detection and subsequent purging of neuroblastoma cells (Refs 14, 106, 107).

Cheung et al. have reported the use of 3F8 for purging efficacy. As previously stated, 3F8 mediates CMC and ADCC. By incubating harvested bone marrow with mAb 3F8 for 1 h, the investigators were able to demonstrate effective purging of tumour contaminating cells (Ref. 106).

It remains unclear as to whether purged bone marrow or peripheral blood stem cells will add a survival advantage in patients treated with transplantation. Evidence to date suggests that it is the presence of residual tumour in the bone marrow at the time of harvest that correlates with clinical outcome (Ref. 14). This may or may not support a role for purging. In fact, one study has provided results that suggest purging may be deleterious. Handgretinger et al. reported that graft-contaminating tumour cells in patients with high-risk neuroblastoma receiving autologous stem cell transplant may confer a survival advantage (Ref. 108). They speculate that contaminating tumour cells may elicit a protective antitumour response after transplantation. The results of a randomised clinical trial comparing purged versus unpurged peripheral blood stem cell transplant after myeloablative chemotherapy for patients with high-risk neuroblastoma (Children's Oncology Group protocol A3973) will hopefully bring a resolution to this question.

Mechanisms of tumour resistance to antibody therapy

One proposed method of tumour resistance to mAb therapy is down-regulation of the target antigen on the cell surface. However, it appears that GD2 expression does not change significantly after treatment with anti-GD2 mAb. Patients who had been treated with 3F8 at MSKCC between 1987 and 1997 were analysed at a later date by immunohistochemistry and bone marrow immunofluorescence for GD2-positive neuroblastoma cells. In 62 patients who had refractory or recurrent disease after treatment with 3F8, 61 (98%) tested positive for GD2. This suggests that the complete loss of the GD2 target antigen is uncommon in neuroblastoma and is not a likely cause of treatment failure (Ref. 22). However, GD2 down-regulation remains a possibility.

While GD2 expression may remain relatively constant in the setting of mAb treatment, MHC class I molecules may be up-regulated as a mechanism of tumour-escape from NK cells (Ref. 109). The Ly49 inhibitory receptor on murine NK cells recognises MHC class I molecules and normally prevents NK-cell-mediated cytotoxicity. Increased MHC class I expression by tumour cells has been shown to decrease NK-cell-mediated tumour lysis and ADCC in vitro (Ref. 110). To block this inhibitory interaction, a Ly49-inhibitory antibody has been used in combination with immunocytokine therapy, which increases NK-cell-mediated ADCC of tumour cells to some extent (Ref. 110). Up-regulation of MHC class I on tumour cells is stimulated by IFN-γ released

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into the tumour microenvironment by NK cells during ADCC (Ref. 109). In contrast, MHC class I expression on neuroblastoma tumour cells may be down-regulated in response to treatment with Flt3-L, which induces cytotoxicity primarily via T cells. Down-regulation of MHC class I makes tumour cells more resistant to T-cell-mediated destruction (Ref. 110). This variability in antigen expression demonstrates that select tumour cells may be resistant to mAb therapy and illustrates the need for combination therapy to effectively eliminate all remaining tumour cells. A recent preclinical analysis suggests that combining Flt3-L and hu14.18–IL2 treatment can result in synergy (Ref. 111)

Other surface antigens can also inhibit the antitumour immune response. For example, tumour cells can induce apoptosis of tumour-infiltrating T cells through expression of the Fas ligand on the tumour cell, surface (Ref. 112). Expression of CD46, CD55 and CD59 may also be increased on tumour cells, thereby inhibiting complement-mediated cell lysis (Ref. 113). Secreted factors from tumour cells may also facilitate tumour escape. Ganglioside GD1a can be released by tumour cells and interfere with antigen-presenting cell function, specifically disrupting both dendritic-cell maturation and T-cell function (Refs 114, 115, 116). This result has been confirmed in in vivo models in which neuroblastoma gangliosides were injected with splenocytes. Examination of the draining lymph node 4 days post-injection showed a decreased lymph-node mass and lymphocyte number compared with controls (Ref. 117).

One potential hurdle in the effective use of mAb therapy is the development of blocking antibodies to the therapeutic agent itself. The development of a HAMA response with murine-derived antibodies has been detected within 7 days of treatment and can neutralise further treatments with the mouse anti-GD2 antibody (Ref. 9). This prompted development of increasingly human antibodies (Fig. 1). A chimaeric human–mouse antibody that retained the Fab fragment of the mouse antibody with its binding specificity, bound to the Fc portion of a human antibody was the first to be introduced. This was a less immunogenic form, but a HACA (human antichimaeric antibody) response could still be detected (Ref. 53). The current therapeutic form is a humanised mAb that retains only the complementarity-determining region of the original mouse antibody but is otherwise 98% human. This has significantly reduced the immunogenicity of the therapeutic agent (Ref. 9). In addition, the development of antibody to the humanised form does not appear to completely neutralise the therapeutic agent's effect (P.M. Sondel, unpublished). However, antibody development to the mAb is still a therapeutic challenge.

Novel uses of antibody: vaccines to induce the antibody-response network

Although the HAMA response can inhibit mAb efficacy by blocking the antibody–antigen interaction, somewhat surprisingly, a HAMA response has been correlated with survival, through a potential antitumour effect (Ref. 5). This mechanism might also be manipulated to provide antitumour benefit. The basis of the antibody-response network is that the antigen-binding component of the original mAb (Ab-1) can also serve as an antigen. This antibody generated in response to mAb treatment is often designated Ab-2. This is a subset of the HAMA antibody response, as antibodies can be generated against many components of the mAb or immunocytokine structure. Ab-2 is specific for the variable domain of the original mAb (Ab-1). However, this second antibody's binding region is also very similar to the original tumour antigen and can serve as an antigen source for a third antibody, Ab-3. A subset of this third antibody (Ab-3′) also binds to the original tumour antigen and can generate antitumour responses like the original mAb (Ab-1) (Refs 9, 118, 119). If the original tumour antigen was a self-antigen, as is the case for GD2, the presence of Ab-3′ represents the loss of self-tolerance (Fig. 3).

These antibodies can be measured in the serum of patients who have received mAb therapy and may correlate with remission (Ref. 16). In patients who received the 3F8 antibody, the presence of Ab3 and Ab3′ was a predictor of both progression-free survival and overall survival (Refs 1, 119). However, Ab3 is not seen in all patients and may require optimal timing of mAb (Ab1) after myeloablative chemotherapy to be induced. High serum levels of mAb (Ab1) during the period of immune recovery after

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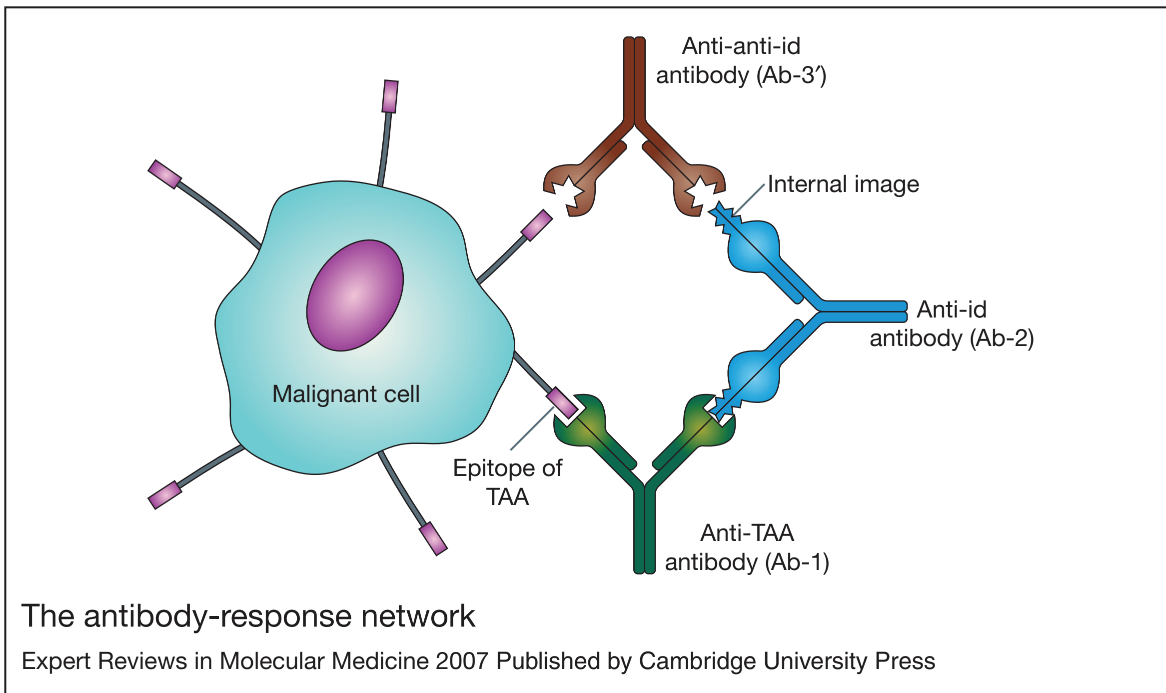

Figure 3. The antibody-response network. The antigen-binding regions of monoclonal antibodies (mAbs) can serve as sources of antigen themselves, as part of a complex binding network. The original antigen on the tumour cell (tumour-associated antigen; TAA) binds the antigen-specific (idiotypic; id) region of Ab-1. A HAMA (human antimouse antibody) response often develops in response to mAb therapy, and a subset of these HAMA antibodies (Ab-2) bind to the antigen-binding domain of the original Ab-1. In order for Ab-2 to bind to the antigen-binding domain of Ab-1, however, Ab-2 must have a similar structure to that of the original tumour antigen. Therefore, when a third antibody (Ab-3) is generated against Ab-2, a subset of Ab-3 can also bind tumour (Ab-3′). Both Ab-3′ and Ab-1 bind tumour antigens and generate antitumour effects. If the original tumour antigen was a self-antigen (such as GD2), the generation of Ab-3′ represents a break in self-tolerance. Adapted, with permission, from Ref. 9 (© 2001 Elsevier).

chemotherapy may influence the immune system to generate higher levels of Ab3, and thereby shift the immune response to generate high levels of anti-GD2 antibodies and break self-tolerance. The advantage of using Ab2 as an antigen source is that these proteins are relatively easy to synthesise, compared with the original complex carbohydrate antigen present on the tumour cell. There is also evidence to suggest that a stronger T-cell response can be generated against Ab2 compared with the original tumour antigen (Ref. 16). Peptide mimics that bind to the therapeutic Ab1, analogously to Ab2 molecules, have also been used in place of complex antigen (like GD2) or in place of the Ab2 molecule to induce an active immune response following vaccination (Refs 120, 121). In addition, HAMA antibody has been used as an antigen source in clinical trials (Ref. 1).

With respect to HAMA and HACA, there thus appear to be two somewhat conflicting perspectives. If induction of an ‘immune-response network’ is actually beneficial, then clinical trials of therapeutic mAbs might be designed to enhance this HAMA/HACA response. This mechanism appears to require active immunity, with an active T-cell component. However, if the antitumour effect obtained from mAb treatment is largely the result of ADCC, mediated by cells of the innate immune system, then any HAMA/HACA would limit the levels and function of the therapeutic antibody. Preclinical data and some clinical data are supportive of, but do not prove, both conflicting theories. More data will be required to clarify this. For now, as most neuroblastoma patients require intensive, immunosuppressive treatment to achieve

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remission, and our efforts at inducing ADCC are focused on patients that are entering remission, the goal is to avoid the HACA/HAMA response.

Future prospects

T-cell engineering

The antitumour response seen with mAbs is not limited to the innate immune system, as T-cell activation and tumour-specific memory responses have also been shown in animal models and in certain clinical settings (Ref. 122). Even though neuroblastoma may appear to be MHC class I and II negative in some instances, MHC molecules can be upregulated, making these cells recognisable for T-cell cytotoxicity. T-cell cytotoxicity can be enhanced through manipulation of the TCR to redirect its specificity toward tumour antigens (Ref. 123). One common target is GD2. T cells can be genetically altered to express chimaeric TCRs that consist of the variable domain of an anti-GD2 antibody linked to a cytoplasmic signalling domain. Engagement of this TCR complex initiates cytotoxic effector function. These cells release a variety of pro-inflammatory cytokines including GM-CSF and IFN-γ upon incubation with GD2-positive tumour cells. These modified T cells are also capable of tumour-specific cytotoxicity with minimal effect on GD2-negative targets (Ref. 124). Furthermore, the feasibility of isolating $CD8^+$ T cells and altering the TCR specificity with plasmids encoding engineered antigen receptors has been shown in human patients (Refs 125, 126). Infusions of autologous tumour-specific T cells had half-lifes of 1–42 days with minimal toxicity, and their lifespan was inversely proportional to the patient's disease burden, with particularly long lifespans in patients with minimal disease. Incorporation of DNA encoding the novel antigen receptors has been achieved via a variety of methods, including uptake of naked plasmid DNA via electroporation and retrovirus transfection (Refs 124, 127). Virtually any tumour antigen may be targeted with this method. Although this has been used more extensively for leukaemia and lymphoma, human clinical trials targeting neuroblastoma are also under way (Refs 126, 128, 129, 130). Survival and proliferation of engineered T cells can also be enhanced by modification of the intracellular signalling domain. By attaching the additional elements normally provided by costimulatory molecules to the intracellular domain, a single TCR can engage a GD2-positive target and stimulate the intracellular signals necessary for cytotoxicity, T-cell survival and proliferation (Ref. 131).

T-cell purification and stimulation can be made more effective when combined with adjunctive immunotherapy. Patients who have undergone stem cell transplantation require several weeks to months to regenerate a fully functional immune system. The infusion of large numbers of tumour-specific effector cells purified ex vivo is an attractive alternative to waiting for an autologous immune response, especially in the critical period of minimal residual disease. Human γδ T cells are a subset of the T-cell population that have been shown to be particularly cytotoxic to tumour cells, including neuroblastoma (Ref. 132). Their survival is enhanced when infused with IL-7 (Ref. 133). The use of combination therapy of allogeneic γδ T cells with IL-7 and the anti-GD2 mAb hu14.18 has shown a significant survival benefit in a murine model of disseminated neuroblastoma using tail-vein injected NB-1691 cells (Ref. 134).

Single-chain variable antibody fragments

One limitation of mAb therapy is poor penetration into solid tumours. Single-chain antibodies consisting of a single variable heavy chain and light chain, also known as single-chain variable fragments (ScFvs), have better tumour penetration and reduced antigenicity (Ref. 135). These agents can be easily linked to toxins to improve their therapeutic effect. In addition, antibody binding and toxin administration can be divided into two steps in a method known as multistep targeting. In this technique, tumours are labelled with mAb conjugated to a second molecule. A toxin with affinity for the tumour-bound antibody is then administered. In this way, the toxin is more selective and there is less systemic exposure to the toxic agent. An anti-GD2 mAb bound to streptavidin has shown excellent targeting when coupled with radiolabelled biotin (Ref. 136). Making a dimer out of two 'single-chain antibodies' further enhances their affinity and results in slower clearance compared with ScFvs (Ref. 137).

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Antibody engineering

Other modifications of immunoglobulin structure and function are being considered in the production of therapeutic reagents. Amino acid substitutions in the antigen-binding portions of the variable region of a humanised antibody can be designed to make the molecule even less immunogenic to the human immune system (Ref. 138). The desired result is preventing the development of a neutralising antibody against the therapeutic antitumour mAb. Intentional modifications in the Fc end of the immunoglobulin can alter effector functions of the mAb. Changes in glycosylation or amino acid substitution can enhance interactions with Fc receptors on effector cells, thus augmenting ADCC (Ref. 139). Other modifications can alter the interaction with complement, either enhancing or inhibiting complement activation. As complement activation by anti-GD2 antibodies is thought to play an important role in the neuropathic pain associated with anti-GD2 mAb treatment, but complement activation may not be critical in the observed antitumour effects, some effort is now being directed into the testing of a mAb modified to lose its ability to activate complement while retaining its ADCC capability.

Summary

Current conventional antineoplastic therapy (surgery, radiation therapy and multi-agent chemotherapy) can put most children with high-risk neuroblastoma into remission. Sadly, the majority of these patients still succumb from recurrent or refractory neuroblastoma. The current philosophy is to utilise separate therapeutic approaches for patients in remission, yet still harbouring minimal residual disease, in order to eliminate any residual neuroblastoma. The use of mAbs in this setting is clearly a promising approach under active investigation. Preclinical data show that strong antitumour effects can be obtained in the minimal residual disease setting using tumour-reactive mAbs. In the clinical setting, then, this would apply to patients who have already undergone conventional surgery, radiation and chemotherapy, and who are presumed to carry some minimal residual disease that may respond to immunotherapy. A potentially definitive Phase III trial of this concept is currently under way by the Children's Oncology Group. In addition, more novel approaches, using genetically engineered mAb derivatives, alone or combined with agents that afford synergy, are even more effective in preclinical testing. Clinical trials of these concepts will be needed to best integrate this approach into overall multimodality treatment that can provide improved long-term disease-free survival.

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Further reading, resources and contacts

The Children's Oncology Group site provides general information for patients, families and providers in the field of paediatric oncology. There are links to information specific for neuroblastoma as well as information about ongoing clinical trials in neuroblastoma:

http://www.curesearch.org

The National Cancer Institute maintains a website dedicated to neuroblastoma, providing comprehensive information on epidemiology, prevention, treatment, ongoing research and further resources:

http://www.cancer.gov/cancertopics/types/neuroblastoma/

Features associated with this article

Figures

Figure 1. Monoclonal antibodies and immunocytokines.
Figure 2. Monoclonal antibody therapy is enhanced when combined with β-glucan.
Figure 3. The antibody-response network.

Table

Table 1. Clinical trials of monoclonal antibodies (mAbs) as single agents.

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