

Antitumor Immunity: Easy as 1, 2, 3 with Monoclonal Bispecific Trifunctional Antibodies?

John Maher^{1,2,3} and Antonella A. Adami¹

Abstract

Monoclonal antibodies occupy an increasing niche in the arsenal available to treat cancer. Several developments have rendered this the fastest growing sector in the pharmaceutical industry. Traditionally, antibodies were developed to block key signaling molecules implicated in tumor progression. However, antibodies also recruit additional immune effector mechanisms against tumors, a property that may be exploited for clinical benefit. Bispecific antibodies represent one such strategy in which elements derived from two monoclonal antibodies are incorporated into a single molecular species. Commonly, the bispecific approach is used to achieve simultaneous cross-linking of CD3 and a tumor antigen such as epithelial cell adhesion molecule (EpCAM), thereby recruiting T-cell activation to the tumor cell surface. A further sophistication involves the engineering of trifunctional derivatives such as the clinically approved agent, catumaxomab. Catumaxomab has antigen-binding arms that engage CD3 and EpCAM and a constant domain that recruits Fc receptor-bearing cells, notably monocytes, dendritic cells, and natural killer cells. Owing to this triangular binding capability, catumaxomab can activate both innate and adaptive immune effector mechanisms in addition to promoting immunologic memory. Recent data indicate that this agent can also promote immunogenic cell death, particularly when used in combination with selected chemotherapeutic agents such as oxaliplatin. *Cancer Res*; 73(18); 5613–7. ©2013 AACR.

Introduction

Monoclonal antibodies and their derivatives are the fastest growing class of pharmaceutical agents. The exquisite specificity that can be achieved using antibody variable domains renders these targeting moieties suitable for several applications to counter malignant disease. Antibodies may be used to engage cell-surface molecules that are lineage restricted (e.g., CD20) or that signal directly (e.g., HER2), or indirectly (e.g., VEGF) to promote tumor progression. Alternatively, they can be harnessed to deliver toxic payloads in a targeted manner or to disrupt immune checkpoints that suppress endogenous antitumor immune responses. Earlier problems with immunogenicity of murine-derived sequences have led to a drive to progressively "humanize" these agents. Consequently, the majority of antibodies in current clinical development are fully human in sequence. Nonetheless, problems remain with high production costs and the challenge of enabling the efficient penetration and diffusion of antibodies within solid tumors, where interstitial

pressure is generally elevated. Reflecting this, most clinically-approved antibodies are used to treat hematologic malignancies, which collectively account for only 15% of cancers.

Among their diverse actions, monoclonal antibodies co-opt elements of the innate immune system in a manner that depends upon the antibody Fc domain. This may lead to complement fixation or activation of IgG Fc receptor-bearing cells [notably natural killer (NK) cells, monocytes, macrophages, and dendritic cells], which in turn promotes antibody-directed cell-mediated cytotoxicity (ADCC) or phagocytosis. The importance of innate immune-cell recruitment is illustrated by several clinical associations between Fc receptor-polymorphisms and therapeutic efficacy of many monoclonal antibodies. Nonetheless, it should be emphasized that such relationships are not uniformly consistent across datasets and are further complicated by the fact that either high- or low-affinity polymorphisms may confer benefit depending upon context (1).

The Move to Bispecific Antibodies

Considerable evidence suggests that in the most effective immune responses, innate and adaptive arms of the immune system operate in an interdependent and ultimately synergistic manner (2). T cells constitute the most numerically important mediators of adaptive antitumor immunity. Clinical studies using infused T cells with natural (3) or genetically engrafted specificity for antigen (4–6) can achieve striking clinical responses in selected cancer types. However, T cells do not express stimulatory Fc receptors and thus are not harnessed by tumor-specific monoclonal antibodies. To circumvent this,

Authors' Affiliations: ¹King's College London, King's Health Partners Integrated Cancer Centre, Department of Research Oncology, Guy's Hospital; ²Department of Allergy and Clinical Immunology, King's College Hospital NHS Foundation Trust, London; and ³Department of Immunology, Barnet and Chase Farm NHS Trust, Barnet, United Kingdom

Corresponding Author: John Maher, CAR Mechanics Group, Department of Research Oncology, Division of Cancer Studies, Third Floor Bermondsey Wing, King's College London School of Medicine, Guy's Hospital Campus, Great Maze Pond, London SE1 9RT, United Kingdom. Phone: 44-207-188-1468; Fax: 44-207-188-0919; E-mail: john.maher@kcl.ac.uk

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bispecific antibody reagents have been engineered that combine within a single molecule the capacity to juxtapose a tumor-associated cell surface molecule and CD3, the signaling complex associated with the T-cell receptor. The resultant colocalization of these cells results in T-cell activation, bypassing the natural specificity of these cells for antigen or their HLA-restriction.

The history of bispecific antibodies has recently been described in eloquent detail by Riethmüller (7). Their early development was hindered by difficulty in producing these reagents at sufficient yield and purity for clinical purposes. Since their inception, multiple bispecific antibody-formats have been described. One of the more effective formats is the bispecific T-cell engager (BiTE), in which a tumor-reactive single-chain variable antibody fragment (scFv) is translated in tandem with a second scFv that binds CD3 (8). Owing to their compact structure, bispecific antibodies of the BiTE-class promote the formation of immunologic synapses and mediate serial triggering of tumor cell cytotoxicity. The most clinically advanced BiTE, blinatumomab, consists of scFvs that can simultaneously engage CD3 and the ubiquitous B-cell antigen, CD19. In early phase clinical testing, this agent has shown compelling efficacy in both B-cell lymphoma (9) and acute B-cell malignancy (10). However, administration of BiTE antibodies requires continuous intravenous infusion and may occasionally be complicated by cytokine release syndrome, which can manifest clinically as haemophagocytic histiocytosis (11). Despite these caveats, several additional BiTEs are undergoing clinical development (12). Alternatively, similar bispecific designs may be used to colocalize tumor cells with effector cells that express CD16a (FcγRIIIa). Such reagents act mainly by recruitment of NK cell-mediated ADCC and have the theoretical advantage that regulatory T cells are not engaged.

Catumaxomab: The Prototype Monoclonal Bispecific Trifunctional Antibody

While these recent developments are exciting, the only bispecific antibody that has achieved marketing authorisation for clinical use was originally described more than 14 years ago (13). Catumaxomab is a highly distinctive bispecific reagent in which a mouse IgG anti-CD3 hemi-antibody is paired with a rat IgG2b hemi-antibody that binds epithelial cell adhesion molecule (EpCAM). Because catumaxomab contains an Fc domain, it is "trifunctional," reflecting the three discrete binding interactions that it can promote. Several solid tumors exhibit upregulated expression of EpCAM, including virtually all types that give rise to malignant ascites. In contrast, lower levels of EpCAM may be found in healthy tissues, primarily at poorly accessible intercellular tight junctions. In keeping with this more limited pattern of expression, toxicity induced by systemically delivered EpCAM-specific monoclonal antibody-therapy has generally been mild and manageable, although there have been concerns in some cases regarding pancreatic toxicity (14). Following a successful pivotal phase II/III clinical trial, catumaxomab was approved in 2009 by the European Medicines Agency for the intraperitoneal treatment of malignant ascites resulting from EpCAM-expressing carcinomas (15). Patients received catumaxomab in four divided

escalating doses (to minimize impact of cytokine release syndrome), administered over a 10-day period. The primary endpoint in that study was paracentesis-free survival and this was significantly prolonged from a baseline median of 11 days to 46 days in the catumaxomab group. Although catumaxomab required inpatient administration, tolerability was considered acceptable. Most patients suffered febrile responses because of cytokinemia (see below), whereas gastrointestinal symptoms and transient hepatic enzyme-disturbances were also commonly observed. Following completion of therapy, temporary clearance of EpCAM⁺ tumor cells from ascites was observed (including cells that expressed the putative stem cell marker, CD133), accompanied by a reduction in ascitic levels of VEGF (16).

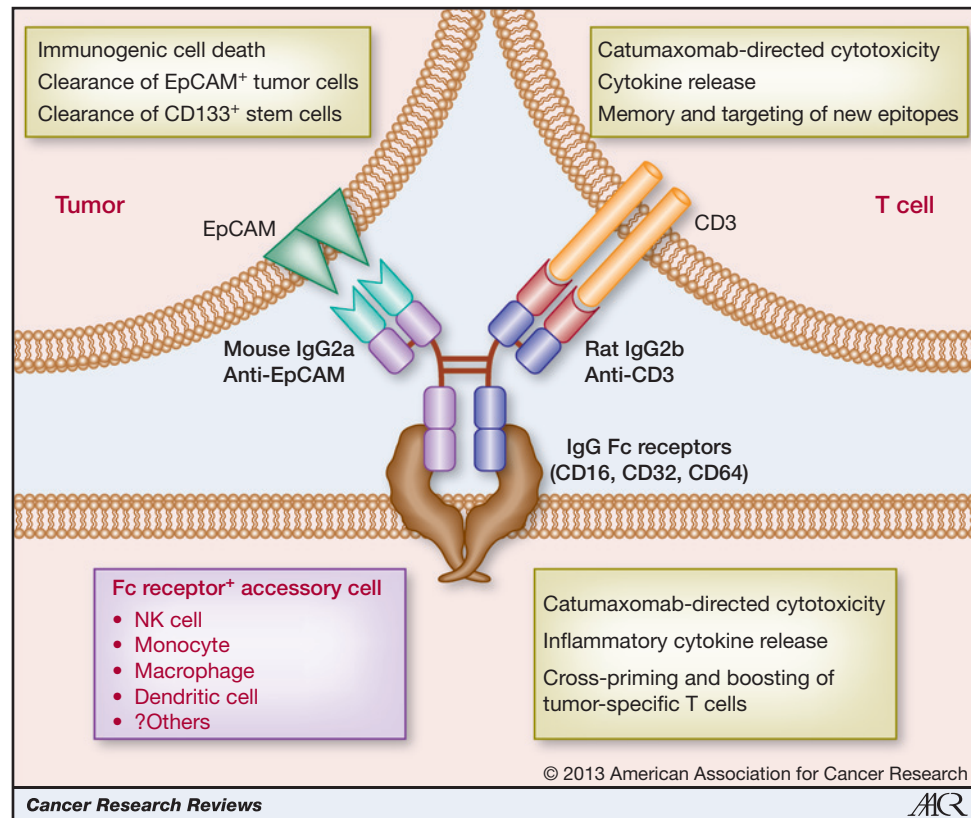
The distinctive nature of catumaxomab has provided fertile ground for preclinical investigation of its antitumor activities. Much evidence supports a model whereby catumaxomab promotes the coassociation of T cells, tumor cells, and Fc receptor-expressing accessory cells in a tricellular complex (Fig. 1). Importantly, the hybrid murine Fc domain in catumaxomab preferentially binds to activating-Fc receptors, including FcγRI (CD64), FcγRIIa (CD32a), FcγRIIIa (CD16a), and FcγRIIIb (CD16b) and less well to those with inhibitory properties such as FcγRIIb (CD32b; ref. 17). Uptake of liberated tumor-antigen or tumor-cell phagocytosis may be achieved by antigen-presenting accessory cells recruited in this manner (17). Furthermore, T cells and accessory cells can provide reciprocal positive signals to each other within this triangular arrangement (13). This may account for the preclinical observations that catumaxomab can induce protective immunity accompanied by B-cell and T-cell memory (18, 19).

Catumaxomab is produced by a "quadroma." This comprises a fusion of the two parental hybridomas that secrete the constituent monoclonal antibodies from which it is derived. Two fortuitous properties make the purification of catumaxomab much easier than might otherwise have been predicted. First, the association between the heavy and light chains found in catumaxomab is tightly species-restricted. In other words, mouse or rat heavy (H) chains will only associate with light (L) chains from the same species. This eliminates the formation of unwanted crossspecies H/L hybrids, which would be wasteful and contaminate the desired product. Second, although the resultant half-antibodies (e.g., H/L chain pairs) are homologous, these can pair within or across the species barrier to generate intact antibodies that comprise mouse/mouse, rat/rat or mouse/rat hybrids. The latter chimeric species is the desired therapeutic reagent and this can be collected using protein A and separated to high-purity from unwanted mouse/mouse antibodies by application of a pH-gradient during elution (20). As a result, it has been possible to produce catumaxomab at acceptable efficiency in compliance with good manufacturing practice, paving the way for clinical development of this agent.

Dissecting the Immunomodulatory Activities of Trifunctional Antibodies in Human Models

Recently, the immunomodulatory effects of catumaxomab have been investigated in two clinical studies, either in patients

Figure 1. Multipronged antitumor immune attack triggered by catumaxomab. Evidence supports the formation of a tricellular complex in which catumaxomab causes colocalization of T cells, Fc receptor-expressing accessory cells, and EpCAM-expressing tumor cells. Multiple direct and delayed immunologic consequences ensue from this triangular crosstalk.



who have received this agent (16) or using biologic material derived from patients with EpCAM⁺-malignancy (21). In both cases, it was shown that catumaxomab promotes the activation of both ascitic CD4⁺ and CD8⁺ T cells in addition to CD16 (low affinity Fc receptor)-expressing accessory cells in a strictly EpCAM-dependent manner (21). Immune cell-activation was accompanied by release of prototypic type 1 T-cell cytokines [interferon (IFN)- γ , interleukin (IL)-2] and the inflammatory cytokines, tumor necrosis factor- α and IL-6, despite the presence of regulatory T cells at that site (16, 21). Accessory cells comprised NK cells, which upregulated the cytolytic effector TRAIL, in addition to monocytes, which upregulated the expression of costimulatory ligands (CD40 and CD80).

One of the most notable findings to emerge from these studies concerns the nature of tumor cell-death induced by catumaxomab-activated immune effector cells. This question was studied using catumaxomab alone or in combination with oxaliplatin, a chemotherapeutic agent that is known to promote immunogenic cell death in its own right. When these agents were combined, tumor cells exhibited an increase in three cardinal hallmarks of immunogenic cell death, namely translocation of calreticulin from endoplasmic reticulum to cell surface, accompanied by release of high mobility group box 1 (HMGB1), and adenosine triphosphate (21). Respectively, these provide a combination of "eat me," danger signals, and inflammasome activating/chemotactic signals to monocytes and dendritic cells that together favor the crosspriming of tumor-reactive T-cell responses (22).

Clinical testing of this synergistic interaction is a tantalizing proposition.

Implications and Future Perspectives

The confirmation of the potent and multifunctional stimulatory action of catumaxomab using clinical material reinforces the therapeutic potential of this approach. Nonetheless, questions remain about the tumor-destructive and immunologic consequences of exposure to this agent. Markers of immunogenic cell death induced by catumaxomab in isolation were limited despite the known ability of TRAIL-expressing NK cells to achieve this effect (21). It is clear that steric factors are highly important in the optimum activity of antibody-targeted lymphocytes. In the original report, in which catumaxomab was described, tumor cytotoxicity by CD2⁺ cells (e.g., T cells and NK cells) was far less potent than that achieved by unfractionated PBMC (13). Evidence from studies using bispecific antibodies and chimeric antigen-receptor engrafted T cells collectively suggests that optimal efficacy requires engagement of an epitope that lies close to the tumor cell-membrane, ideally located within an antigen of small size (5, 23). Taken together, these findings raise the possibility that catumaxomab (and indeed trifunctional antibodies in general) may not achieve such optimal distancing from the tumor cell-surface but that reciprocal positive interactions between innate and adaptive immune cells within the tricellular complex can compensate for this. A further related question is whether T cells and NK cells corecruited to the tumor cell-

surface by catumaxomab can cause destruction of each other in this arrangement, thereby limiting efficacy. Study of synapse formation between these participating cell types and capacity for serial killing would be of considerable interest.

An important concern relates to the highly immunogenic nature of the mouse/rat hybrid Fc domain in catumaxomab. As expected, the majority of patients develop human anti-mouse (HAMA) and human anti-rat antibody (HARA) responses following treatment with this agent (24). Counter-intuitively however, those patients who mounted HAMA responses exhibited significantly-improved outcome. A trite explanation for these findings is that HAMA/HARA formation represents a surrogate measure of immunologic "fitness," akin to the measurement of antibody-responsiveness to test immunization in clinical practice. However, an alternative and much more intriguing possibility is that immune-complex formation promotes the enhanced cross-presentation of antigen, leading to the priming of additional tumor-reactive T-cell responses. Of additional importance in this regard, preliminary studies in which catumaxomab has been readministered to patients with HAMA have shown proof of concept of the safety of this approach with maintenance of efficacy (25).

A third issue concerns the prospect of intravenous administration of catumaxomab for the treatment of EpCAM⁺ malignancy. The molecular mass of catumaxomab is similar to that of IgG-based monoclonal antibodies. Both cetuximab and herceptin have demonstrable efficacy when delivered in this manner to treat selected solid tumors. Nonetheless, an important limiting factor may be the ability of these agents to penetrate effectively into solid tumor masses where interstitial pressure is generally elevated. This may curtail the applicability of the trifunctional approach against other potentially attractive solid tumor targets, such as prostate-specific membrane antigen.

Finally, there is a clear need to explore further the capacity of trifunctional agents such as catumaxomab to elicit immunologic memory and epitope-spreading in patients with malignancy. Preclinical studies using a ganglioside-specific trifunctional antibody have recently shown the induction of T-cell memory directed against a panel of other antigens expressed by the targeted tumor. Furthermore, this immunizing effect was sufficient to protect mice against subsequent challenge

with a lethal dose of antigen-negative parental tumor (19). Intriguingly, some clinical experience with catumaxomab also reinforces this possibility. Several patients developed T-cell responses against autologous tumor, indicated by IFN- γ release when cells were cocultivated with autologous tumor (26). A small study has suggested that circulating and bone marrow EpCAM⁺ cells are reduced in some patients with ovarian cancer following intraperitoneal administration of this agent for malignant ascites (27). Anecdotal reports have also described regression of metastases outside the peritoneal cavity in some patients (28, 29). It is conceivable, however, that some of this effect might have resulted from a direct action of the antibody following absorption from the peritoneal cavity (30). Ongoing clinical studies are exploring the therapeutic value of catumaxomab in combination with a variety of chemotherapeutic regimens. In that setting, it will be important to assess carefully whether administration of this agent can prime and/or boost immune responsiveness against defined tumor antigens. The intriguing demonstration of the synergistic induction of immunogenic cell death with agents such as oxaliplatin provides a rationale for the design of further clinical studies to investigate, optimize, and maximize this interaction for patient benefit.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Disclaimer

The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health.

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Writing, review, and/or revision of the manuscript: J. Maher, A.A. Adami

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