EDITORIAL

BJC

British Journal of Cancer (2016) 114, 1177–1179 | doi: 10.1038/bjc.2016.84

Countering immunotoxin immunogenicity

David J Flavell*,[1]

[1]The Simon Flavell Leukaemia Research Laboratory, Southampton General Hospital, Southampton SO16 6YD, UK

The entry of antibody-based drugs into mainstream medicine has provided the oncologist with new therapeutic tools that have begun to transform the treatment of cancer (Scott *et al*, 2012; Lee *et al*, 2013). Toxicity profiles and cell killing mechanism(s) exhibited by antibody and small-molecule cytotoxic therapies are largely non-overlapping, thus providing positive clinical advantages relating to drug resistance and therapy side effects. Additionally, in some instances, combinations of antibody given together with conventional chemotherapy show synergistic activity manifest as significant improvements in treatment outcomes (Hallek *et al*, 2010). However, not all naked antibodies possess therapeutic activity *per se*, a property that is determined in part by the antibody's particular target molecule. In instances where the antibody has only weak or no observable therapeutic activity, the strategy has been to 'arm' the antibody with a protein toxin or a highly potent small-molecule cytotoxic drug to create two closely related classes of therapeutic, namely immunotoxins (IT) and antibody drug conjugates, respectively (Kreitman, 2006; Lambert, 2013).

Immunogenicity presents challenges for all antibody-based therapies where multiple administrations of drug over a protracted period are required to achieve a therapeutic effect (Kuus-Reichel *et al*, 1994). Immunogenicity in this context is defined as the property of the therapeutic to elicit an unwanted immune response when the patient's immune system recognises epitopes displayed by the drug as non-self. The first generation of therapeutic antibodies were based on mouse monoclonal antibodies whose non-human peptide sequences proved highly immunogenic, provoking an immune response in the patient and the resultant production of human anti-mouse antibodies (HAMA) (Norman *et al*, 1993). The clinical consequences of HAMA responses are variable. HAMA can be inconsequential, but can also lead to a reduction in antibody therapeutic efficacy due to the generation of neutralising antibodies by the host that block the antigen binding site of the therapeutic antibody and/or an increase blood clearance rates. HAMA responses may also have immunopathological consequences that contribute to the drug's dose-limiting toxicity with immune hypersensitivity reactions being frequently observed (Baldo, 2013). Devising strategies that avoid these problems are a necessary prerequisite to the successful development of any antibody-based therapy.

Humanisation of murine antibodies is one way of overcoming the HAMA problem where murine polypeptide sequences are replaced with human ones. This was achieved initially through the generation of chimeric antibodies in which the entire constant region of the murine antibody was replaced with the corresponding human constant region while the entire murine variable domain (Fv) was retained (Morrison *et al*, 1984). This still left significant amounts of murine sequence in the Fv region that remained immunogenic, and so this prompted the next evolutionary step with generation of 'humanised' antibody molecules where only the murine complementarity-determining regions (CDRs) are grafted onto a wholly human immunoglobulin framework (Jones *et al*, 1986). Further advances came with the production of entirely human antibodies using phage display technology (McCafferty *et al*, 1990) or transgenic mice carrying human immunoglobulin genes (Lonberg *et al*, 1994). While the full humanisation of therapeutic antibodies has succeeded in dramatically reducing the incidence and extent of human anti-human antibody (HAHA) responses (Foon *et al*, 2004), idiotypic paratopes that are unique to each and every antibody of defined specificity reside in the CDR regions of the antigen binding site and are still capable of provoking a humoral anti-idiotype immune response (Harding *et al*, 2010). It is therefore general opinion that complete elimination of HAHA responses will be difficult to achieve through antibody engineering alone and that other strategies will be needed.

As a class of antibody-based drug, ITs present additional special challenges when it comes to immunogenicity. Being comprised of two separate protein components, an antibody and a toxin linked covalently either through a chemical bond or as a genetic fusion means that there are potentially two macromolecular structures displaying epitopes that may be recognised as non-self. The resultant highly immunogenic nature of IT severely limits their clinical usefulness where repeated treatments may be necessary to achieve a therapeutic effect. Various approaches have been adopted to overcome this and include a variety of immunosuppressive agents given concomitantly with IT. These include cyclophosphamide (Oratz *et al*, 1990), cyclosporine A (Selvaggi *et al*, 1993), anti-CTLA4 Ig (Siegall *et al*, 1997), 15-deoxyspergulin (Pai *et al*, 1990), anti-CD4 antibody (Jin *et al*, 1991) and rituximab (Saleh *et al*, 2002). Site-specific modification of IT with polyethylene glycol has also been shown to reduce immunogenicity and increase the plasma half-life (Tsutsumi *et al*, 2000). More recently de-immunisation of ITs based on either *Pseudomonas* exotoxin or diphtheria toxin by engineering out epitopes identified as likely

*Correspondence: Dr DJ Flavell; E-mail: davidf@leukaemiabusters.org.uk.

to provoke an immune response (Liu *et al*, 2012; Schmohl *et al*, 2015) have proven largely successful and this approach would seem the most likely way ahead in the further clinical development of these chimeric therapeutic molecules.

Andersson *et al* (2015) describe the effects of oral or i.v. administered cyclosporine (Sandimmune) on the development of neutralising antibodies in cancer patients receiving the anti-EpCAM IT MOC31PE. MOC31PE is based on the anti-EpCAM (CD326) IgG_1 murine monoclonal antibody MOC31 linked covalently via a thioether bond to *Pseudomonas* exotoxin A and is designed to target EpCAM-positive epithelial cancers. The MOC31PE molecule utilises unmodified murine antibody and PE, and is therefore expected to be highly immunogenic in humans, affected only by the immune status of the individual patients. Attempts to use immunosuppressant doses of CsA to reduce the immune response against IT is not new, having first been used in combination with the ricin-based anti-melanoma IT XOMA-ZYME-MEL, but with little success in this instance (Selvaggi *et al*, 1993). Andersson *et al* (2009) have previously shown in preclinical studies that cyclosporine also augments the cytotoxic and therapeutic activity of MOC31PE in preclinical models of human cervical cancer. The phase I dose-escalation study reported by Andersson *et al* (2015) was undertaken to firstly establish how well the combination of MOC31PE plus CsA was tolerated in patients with EpCAM-positive epithelial cancers and secondly to evaluate the extent to which CsA inhibited the generation of neutralising anti-MOC31PE antibodies. In this dose-escalation study, a total of 34 patients received MOC31PE alone in escalating doses, while 29 received a combination of MOC31PE plus CsA (23 patients received CsA i.v. and 6 orally). Within the patient groups, there were a total of ten grade 3 and three grade 4 adverse events (AE) in the MOC31PE monotherapy-treated group compared with twelve grade 3 and six grade 4 AEs in the MOC31PE plus CsA combination group. The authors used an *in vitro* MTS cytotoxicity assay to determine whether serum from MOC31PE-treated patients contained antibodies that neutralised the IT. Their assay was not capable of discriminating between anti-MOC31PE antibodies that blocked MOC31PE binding to its EpCAM target or those which neutralised the catalytic activity of PE. After three cycles of treatment (each cycle given every 14 days), 93% of patients treated with MOC31PE alone developed neutralising antibodies compared with only 50% treated with the combination of MOC31PE plus CsA given on a 5-day schedule commencing 1 day before MOC31PE administration. The authors therefore conclude that CsA given on this schedule had an acceptable toxicity/safety profile and was capable of reducing the incidence of anti-IT antibody responses that should allow for multiple doses of IT to be given effectively. While the relatively small patient numbers reported in this dose-escalation study with MOC31PE IT do not allow for any meaningful analysis of any therapeutic benefits of using CsA in combination with the IT, it has provided important safety information that paves the way ahead for subsequent phase II and III trials to explore this very issue.

CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

Andersson Y, Engebraaten O, Fodstad O (2009) Synergistic anti-cancer effects of immunotoxin and cyclosporin *in vitro* and *in vivo*. *Br J Cancer* **101**(8): 1307–1315.

Andersson Y, Engebraaten O, Juell S, Aamdal S, Brunsvig P, Fodstad O, Dueland S (2015) Phase I trial of EpCAM-targeting immunotoxin MOC31PE, alone and in combination with cyclosporin. *Br J Cancer* **113**(11): 1548–1555.

Baldo BA (2013) Adverse events to monoclonal antibodies used for cancer therapy: focus on hypersensitivity responses. *Oncoimmunology* **2**(10): e26333.

Foon KA, Yang XD, Weiner LM, Belldegrun AS, Figlin RA, Crawford J, Rowinsky EK, Dutcher JP, Vogelzang NJ, Gollub J, Thompson JA, Schwartz G, Bukowski RM, Roskos LK, Schwab GM (2004) Preclinical and clinical evaluations of ABX-EGF, a fully human anti-epidermal growth factor receptor antibody. *Int J Radiat Oncol Biol Phys* **58**(3): 984–990.

Hallek M, Fischer K, Fingerle-Rowson G, Fink AM, Busch R, Mayer J, Hensel M, Hopfinger G, Hess G, von Grunhagen U, Bergmann M, Catalano J, Zinzani PL, Caligaris-Cappio F, Seymour JF, Berrebi A, Jager U, Cazin B, Trneny M, Westermann A, Wendtner CM, Eichhorst BF, Staib P, Buhler A, Winkler D, Zenz T, Bottcher S, Ritgen M, Mendila M, Kneba M, Dohner H, Stilgenbauer S (2010) Addition of rituximab to fludarabine and cyclophosphamide in patients with chronic lymphocytic leukaemia: a randomised, open-label, phase 3 trial. *Lancet* **376**(9747): 1164–1174.

Harding FA, Stickler MM, Razo J, DuBridge RB (2010) The immunogenicity of humanized and fully human antibodies: residual immunogenicity resides in the CDR regions. *MAbs* **2**(3): 256–265.

Jin FS, Youle RJ, Johnson VG, Shiloach J, Fass R, Longo DL, Bridges SH (1991) Suppression of the immune response to immunotoxins with anti-CD4 monoclonal antibodies. *J Immunol* **146**(6): 1806–1811.

Jones PT, Dear PH, Foote J, Neuberger MS, Winter G (1986) Replacing the complementarity-determining regions in a human antibody with those from a mouse. *Nature* **321**(6069): 522–525.

Kreitman RJ (2006) Immunotoxins for targeted cancer therapy. *AAPS J* **8**(3): E532–E551.

Kuus-Reichel K, Grauer LS, Karavodin LM, Knott C, Krusemeier M, Kay NE (1994) Will immunogenicity limit the use, efficacy, and future development of therapeutic monoclonal antibodies? *Clin Diagn Lab Immunol* **1**(4): 365–372.

Lambert JM (2013) Drug-conjugated antibodies for the treatment of cancer. *Br J Clin Pharmacol* **76**(2): 248–262.

Lee CS, Cragg M, Glennie M, Johnson P (2013) Novel antibodies targeting immune regulatory checkpoints for cancer therapy. *Br J Clin Pharmacol* **76**(2): 233–247.

Liu W, Onda M, Lee B, Kreitman RJ, Hassan R, Xiang L, Pastan I (2012) Recombinant immunotoxin engineered for low immunogenicity and antigenicity by identifying and silencing human B-cell epitopes. *Proc Natl Acad Sci USA* **109**(29): 11782–11787.

Lonberg N, Taylor LD, Harding FA, Trounstine M, Higgins KM, Schramm SR, Kuo CC, Mashayekh R, Wymore K, McCabe JG, Munoz-O'Regan D, O'Donnell SL, Lapachet ESG, Bengoechea T, Fishwild DM, Carmack CE, Kay RM, Huszar D (1994) Antigen-specific human antibodies from mice comprising four distinct genetic modifications. *Nature* **368**(6474): 856–859.

McCafferty J, Griffiths AD, Winter G, Chiswell DJ (1990) Phage antibodies: filamentous phage displaying antibody variable domains. *Nature* **348**(6301): 552–554.

Morrison SL, Johnson MJ, Herzenberg LA, Oi VT (1984) Chimeric human antibody molecules: mouse antigen-binding domains with human constant region domains. *Proc Natl Acad Sci USA* **81**(21): 6851–6855.

Norman DJ, Chatenoud L, Cohen D, Goldman M, Shield 3rd CF (1993) Consensus statement regarding OKT3-induced cytokine-release syndrome and human antimouse antibodies. *Transplant Proc* **25**(2 Suppl 1): 89–92.

Oratz R, Speyer JL, Wernz JC, Hochster H, Meyers M, Mischak R, Spitler LE (1990) Antimelanoma monoclonal antibody-ricin A chain immunoconjugate (XMMME-001-RTA) plus cyclophosphamide in the treatment of metastatic malignant melanoma: results of a phase II trial. *J Biol Response Mod* **9**(4): 345–354.

Pai LH, FitzGerald DJ, Tepper M, Schacter B, Spitalny G, Pastan I (1990) Inhibition of antibody response to Pseudomonas exotoxin and an immunotoxin containing Pseudomonas exotoxin by 15-deoxyspergualin in mice. *Cancer Res* **50**(24): 7750–7753.

Saleh MN, Bookman M, Mauer A, Posey J, Rinhart J, Fleming G, Meropol N, Khazaeli M, Thornton J, Sprague E, Schol J, Yeslow G, Lorenz J, Siegel C, Sing A, LoBuglio AF (2002) Rituximab delays human anti-toxin antibody onset:phase I study of SGN-10 (BR96 scFv-PE40) in patients with advanced stage carcinoma. *Proc Am Soc Clin Oncol* **21**: 28a.

Schmohl JU, Todhunter D, Oh S, Vallera DA (2015) Mutagenic deimmunization of diphtheria toxin for use in biologic drug development. *Toxins* **7**(10): 4067–4082.

Scott AM, Wolchok JD, Old LJ (2012) Antibody therapy of cancer. *Nat Rev Cancer* **12**(4): 278–287.

Selvaggi K, Saria EA, Schwartz R, Vlock DR, Ackerman S, Wedel N, Kirkwood JM, Jones H, Ernstoff MS (1993) Phase I/II study of murine monoclonal antibody-ricin A chain (XOMAZYME-Mel) immunoconjugate plus cyclosporine A in patients with metastatic melanoma. *J Immunother Emphasis Tumor Immunol* **13**(3): 201–207.

Siegall CB, Haggerty HG, Warner GL, Chace D, Mixan B, Linsley PS, Davidson T (1997) Prevention of immunotoxin-induced immunogenicity by coadministration with CTLA4Ig enhances antitumor efficacy. *J Immunol* **159**(10): 5168–5173.

Tsutsumi Y, Onda M, Nagata S, Lee B, Kreitman RJ, Pastan I (2000) Site-specific chemical modification with polyethylene glycol of recombinant immunotoxin anti-Tac(Fv)-PE38 (LMB-2) improves antitumor activity and reduces animal toxicity and immunogenicity. *Proc Natl Acad Sci USA* **97**(15): 8548–8553.