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SYMPOSIUM-IN-WRITING PAPER

Recombinant IgE antibodies for passive immunotherapy of solid tumours: from concept towards clinical application

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Abstract Therapeutic antibodies have revolutionised treatment of some cancers and improved prognosis for many patients. Over half of those available are approved for haematological malignancies, but efficacious antibodies for solid tumours are still urgently needed. Clinically available antibodies belong to the IgG class, the most prevalent antibody class in human blood, while other classes have not been extensively considered. We hypothesised that the unique properties of IgE, a class of tissue-resident antibodies commonly associated with allergies, which can trigger powerful immune responses through strong affinity for their particular receptors on effector cells, could be employed for passive immunotherapy of solid tumours such as ovarian and breast carcinomas. Our laboratory has examined this concept by evaluating two chimaeric antibodies of the same specificity (MOv18) but different isotype, an IgG1 and an IgE against the tumour antigen folate receptor α (FRα). The latter demonstrates the potency of IgE to mount superior immune responses against tumours in disease-relevant models. We identified Fcε receptor-expressing cells, monocytes/macrophages and eosinophils, activated by MOv18 IgE to kill tumour cells by mechanisms such as ADCC and ADCP. We also applied this notion to a marketed therapeutic, the humanised IgG1 antibody trastuzumab and engineered an IgE counterpart, which retained the functions of trastuzumab in restricting proliferation of HER2/*neu*-expressing tumour cells but also activated effector cells to kill tumour cells by different mechanisms. On-going efficacy, safety evaluations and future first-in-man clinical studies of IgE therapeutics constitute key metrics for this concept, providing new scope for antibody immunotherapies for solid tumours.

Keywords IgE · Tumour immunotherapy · FRα/FBP · Monocytes/macrophages · MOv18 IgE · Allergooncology symposium-in-writing · HER2/*neu* · IgG · Trastuzumab · Basophils · Eosinophils · Mast cells · Solid tumours · ADCC · ADCP · Ovarian carcinomas · Breast carcinomas

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Abbreviations

FBP/FRα	Folate-binding protein/folate receptor alpha
ADCC	Antibody-dependent cell-mediated cytotoxicity
ADCP	Antibody-dependent cell-mediated phagocytosis
FcεRI	Fc epsilon Receptor I
HER2/*neu*	Human epidermal growth factor receptor 2
FcεRIα	FcεRI alpha

Successes and challenges for cancer immunotherapy with antibodies: the case for improving efficacy against solid tumours

Benefiting from their unique specificity for their target antigens, antibodies have been hailed as 'magic bullets' able selectively to seek out and attack tumour cells expressing these antigens [1]. Since the first use of a therapeutic monoclonal antibody in B cell malignancy in the 1980s, the field has benefited from considerable technological advances and scientific breakthroughs, combined with clinical experience in engineering and translating antibodies into cancer therapies [2, 3]. Antibodies have now earned their place in clinical applications and complement conventional treatments for a number of malignant diseases, with 10 agents approved for the therapy of a handful of indications, and hundreds of others currently undergoing evaluation in clinical trials [1]. It is notable that over half of these successful agents are approved for haematological indications, i.e., leukaemias and lymphomas. Despite the superb specificity and high affinity of antibodies for their target antigens and some significant advances in antibody immunotherapy for breast and colorectal cancers, the concept of a 'magic bullet' for the treatment of many solid tumours has produced less impressive outcomes [4, 5].

The human immune system naturally deploys nine antibody classes and subclasses (IgM, IgD, IgG1-4, IgA1, IgA2 and IgE) to perform immune surveillance and to mediate destruction of pathogens in different anatomical compartments. Yet, only IgG (most often IgG1) has been applied in immunotherapy of cancers. One reason may be that IgG antibodies (particularly IgG1) constitute the largest fraction of circulating antibodies in human blood. The choice of antibody class is also based on pioneering work in the late 1980s, comparing a panel of chimaeric antibodies of the same specificity, each with Fc regions belonging to one of the nine antibody classes and subclasses [6]. Antibodies were evaluated for their ability to bind complement and their potency to mediate haemolysis and cytotoxicity of antigen-expressing target cells in the presence of complement. IgG1 in combination with human peripheral blood mononuclear cells (PBMC) was the most effective IgG subclass in complement-dependent cell killing in vitro, while the IgA and IgE antibodies were completely inert. Subsequent clinical trials with antibodies recognising the B cell marker CD20 supported the inference that IgG1 would be the subclass best suited for immunotherapy of patients with B cell malignancies such as non-Hodgkin's lymphoma [7]. Since those studies, comparisons of anti-tumour effects by different antibody classes have been confined to IgG and IgM in both murine models and patients with lymphoid malignancies, while IgA has been shown to mediate ADCC in vitro and in vivo in mouse models of lymphoma [8–12]. IgA and IgE antibodies, on the other hand, have never been tested in cancer patients.

Complement-mediated tumour cell death is now known to be only one of several mechanisms by which antibodies may mediate tumour growth restriction [13]. Known mechanisms include engaging immune effector molecules through their Fc regions to induce immune cell-mediated destruction of targeted cells by antibody-dependent cell-mediated cytotoxicity (ADCC) and phagocytosis (ADCP). Antibodies can also act directly on tumour cells to inhibit growth signalling pathways, induce apoptosis, restrict proliferation and cell differentiation of tumour cells, or block tumour cell adhesion and migration. Some antibodies are developed to recognise targets associated with tumour-associated vasculature in order to starve tumours of vital nutrients delivered through blood supply, while others attack immune regulatory targets (e.g., CTLA-4 and PD-1R) to enhance T-cell activation and overcome immunosuppressive elements of the immune response [14–16]. Extensive efforts have also focused on designing antibody conjugates to deliver toxic payloads in the form of drug-activating enzymes, cytokines or radionuclides to tumours [17]. Multiple antibody engineering approaches are also being devised to improve validated therapeutics, such as trastuzumab, with the principal aims to optimise antigen specificity/affinity and effector functions of IgG antibodies [18].

We reasoned that engineering antibodies with Fc regions of a different antibody class may improve antibody effector functions, if antibodies of this class can exert natural immune surveillance in anatomical locations where tumours may be found. This concept may be particularly relevant in the case of solid tumours, since these are frequently refractory to treatment with IgG antibodies. With a serum half-life of 21–24 days, compared to a half-life of 2–3 days in tissues, IgG antibodies may be the most effective antibody class to target blood-resident tumours and circulating tumour cells, while their ability to exert tumour surveillance in tissues may be less potent [19, 20].

Other parameters that may negatively modulate IgG anti-tumoural functions could be slow or ineffective recruitment and/or local suppression of immune effector cells by tumour cells in lesions and the presence/induction of immunoregulatory cells by tumours in situ [21]. For antibodies of the IgG class that do localise in tumour lesions, overcoming these immunomodulatory environments may be challenging. Additionally, factors such as the low affinity of IgG for its Fc gamma receptors and the presence of the inhibitory receptor FcγRIIb in tumour-infiltrating immune cells such as macrophages may negatively influence the efficacy of IgG antibodies in tissues [22, 23]. Immune responses may be often weak, and many antibody engineering strategies to enhance tissue bioavailability, increase the affinities of antibodies for their receptors and repolarise tumour-infiltrating immune effector cells to target tumours have all been devised to overcome these difficulties.

Our conviction has been that since each antibody class operates in different anatomical compartments, and functions through unique Fc receptors and immune effector cells, there are strong grounds for evaluating whether, in certain circumstances, antibodies of the tissue-resident IgE class may be advantageous in immunotherapy of solid tumours.

Antibodies of the IgE class function through unique Fc receptors and potent immune effector cells in tissues

Antibodies of the IgE class are key mediators of the allergic response and confer protection against parasitic infections through high-affinity Fc receptors on a different spectrum of effector cells to IgG. Their natural residency and local immune surveillance in tissues translates into activation of immune responses in situ.

The receptors for IgE expressed on immune effector cells are the high-affinity receptor, FcεRI, and the low-affinity receptor, FcεRII (CD23), both specific for the Cε3 region of IgE, which they bind to with affinities of 10^9–10^{11} and 10^6–10^8 M^{-1}, respectively [24, 25]. The structures of these receptors differ hugely: FcεRI exists as a tetramer, with subunits $\alpha\beta\gamma_2$, on mast cells and basophils and as a trimer, with subunits $\alpha\gamma_2$, on eosinophils, monocytes and APCs—in both of these receptors the IgE binding domain is on the extracellular α-chain [24–27] (Fig. 1). FcεRII (CD23) expression is splice-variant dependent, with CD23a expressed on antigen-activated B cells and CD23b being expressed on inflammatory cells such as monocytes/macrophages. CD23 is a functional trimer and has a cytosolic C-terminal tail, an extra-cellular stalk supporting a c-type lectin domain, binding IgE in a non-Calcium-dependent fashion [24, 28] (Fig. 1).

IgE binding to each receptor has a markedly different signalling response (Fig. 1). Cross-linking of IgE bound to FcεRI causes receptor aggregation and downstream signalling through a Syk-dependent pathway [29]. The net result of this is degranulation by mast cells and the release of inflammatory mediators (histamine, leukotrienes and proteases) and cytokines (IL-3, IL-4, IL-5, IL-6, IL-9, IL-13, GM-CSF and TNF-α) that recruit T cells, monocytes, basophils, APCs and eosinophils to the site of inflammation, resulting in an enhancement of the response to the allergen or parasite [24, 25, 30]. The same mediators also activate infiltrating cells in situ to induce ADCC through release of pro-inflammatory cytokines, enzymes and other cytotoxic mediators (e.g., TNF-α, lysozyme, nitric oxide, H_2O_2 and other reactive oxygen species) [31–33]. IgE binding to CD23 promotes macrophage/monocyte activation via the adhesion molecules CD11b-CD18 [34]. Expression of CD23 on the surface of monocytes/macrophages is greatly enhanced in Th2-biased microenvironments, and particularly in response to IL-4 and IL-13, and it is linked to ADCP of parasites and clearance of IgE–antigen complexes [35–39]. So, it can be seen that differential methods of cell killing are promoted by the binding of IgE to the receptors FcεRI and CD23: degranulation with release of pro-inflammatory mediators and cytokines by mast cells and basophils, ADCC, mediated by the release of mediators such as NO, enzymes and cytokines resulting in target cell lysis, and ADCP, mediated by the activation of macrophages and monocytes. We postulated that the properties of IgE commonly described in allergy and protection from parasitic infections, if redirected against cancer cells, may translate to effective targeting of tissue-resident tumours (Fig. 2).

Could IgE antibodies confer advantages for the treatment of solid tumours?

The particular properties that make IgE a contributor in the allergic response also permit protection against parasitic infections and point to its potential value as a therapeutic agent in cancer. Well-documented manifestations of allergic disease and immune surveillance in parasitic infection, namely local immune stimulation, with the ensuing cascade of 'allergic' inflammation at the site of antigen provocation may be harnessed to re-direct immune effector cells to induce tumour rejection:

High affinity for IgE receptors

The affinity of IgE for its high-affinity receptor, FcεRl, is 10^2–10^5 times higher than that of IgGs for their receptors,

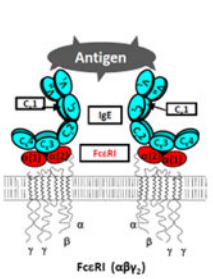
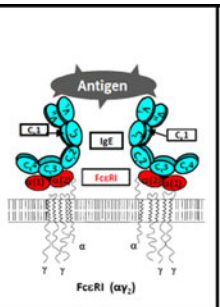
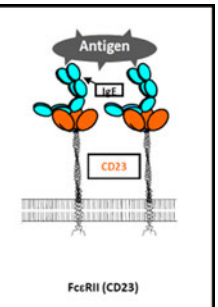
				
Affinity for IgE (K_a)	$10^9 - 10^{11}$ M^{-1}	$10^9 - 10^{11}$ M^{-1}		$10^6 - 10^8$ M^{-1}
Key receptor expressing immune effector cells	Mast cells Basophils	Eosinophils	Monocytes	Monocytes
Expression density (molecules/cell)	130,000-150,000	>2,000	2,000 - 5,000	0-20,000
Immune Mechanisms	Degranulation	ADCC	ADCC/ ADCP	ADCP

Fig. 1 Interactions of IgE with Fcε receptors mediate effector cell functions in response to cancer cells. Schematic of IgE binding to FcεRI tetrameric ($\alpha\beta\gamma_2$) (*left*) and FcεRI trimeric forms ($\alpha\gamma 2$) (*middle*) through the extracellular immunoglobulin alpha (α) chain and interaction with the low-affinity receptor CD23 trimer is through recognition of the lectin domain (*right*). Expression, affinities for IgE and expression densities of IgE receptors of key effector cells and immune mechanisms each mediates. The presence of the β chain on the FcεRI tetramer is responsible for high cell surface expression densities on mast cells and basophils and is associated with induction of degranulation. In the presence of IgE, FcεRI trimmers are known to induce ADCC, while CD23 expression on monocytes and macrophages can trigger ADCP of parasites

uniquely resulting in strong retention of this class of antibodies on effector cells without bound antigen [20, 24, 25].

Tissue residency

The concentration of IgE in the serum of normal individuals is minute (<150 ng/ml, i.e., 1/10,000 the concentration of IgG), and unlike IgG, the presence of IgE in the blood is short lived (half-life of 1.5 days) [20, 24, 25]. Yet, as stated above, the half-life of IgE in tissues (2 weeks) is proportionately longer than that of IgG (2–3 days) [19, 25]. The result is local retention by powerful IgE receptor-expressing resident cells such as mast cells, macrophages and dendritic cells and longer immune surveillance that could be beneficial in the context of cancer.

No inhibitory receptors

Interestingly, unlike IgG, IgE is not subject to an inhibitory receptor (c.f. FcγRIIb for IgG), implying that the suppressive effects of the tumour microenvironments may not bear as heavily on the effector functions of a tumour antigen-specific IgE [24, 25].

Tissue-resident immune effector cells in tumours

A large proportion, as much as 50%, of tumour lesions are made up of infiltrating immune cells that are also concentrated round the tumours [40]. Some of these infiltrates are known powerful FcεR-expressing effector cells such as monocytes/macrophages, mast cells, dendritic cells and eosinophils. In the absence of tumour antigen-specific IgE, these cells may lack the required activity to target tumour cells due to immunosuppressive signals in the tumour microenvironment [41, 42]. The key question would, therefore, be whether the presence of tumour antigen-specific IgE, tenaciously retained through high affinity for FcεR on effector cells, would be sufficient to overcome immune suppression and re-direct these cells against tumours.

Powerful effector functions

As part of their protective role in parasitic infections, IgE antibodies are known to trigger both antibody-mediated cellular cytotoxicity (ADCC) and antibody-mediated cellular phagocytosis (ADCP) of parasites. Both IgE receptors

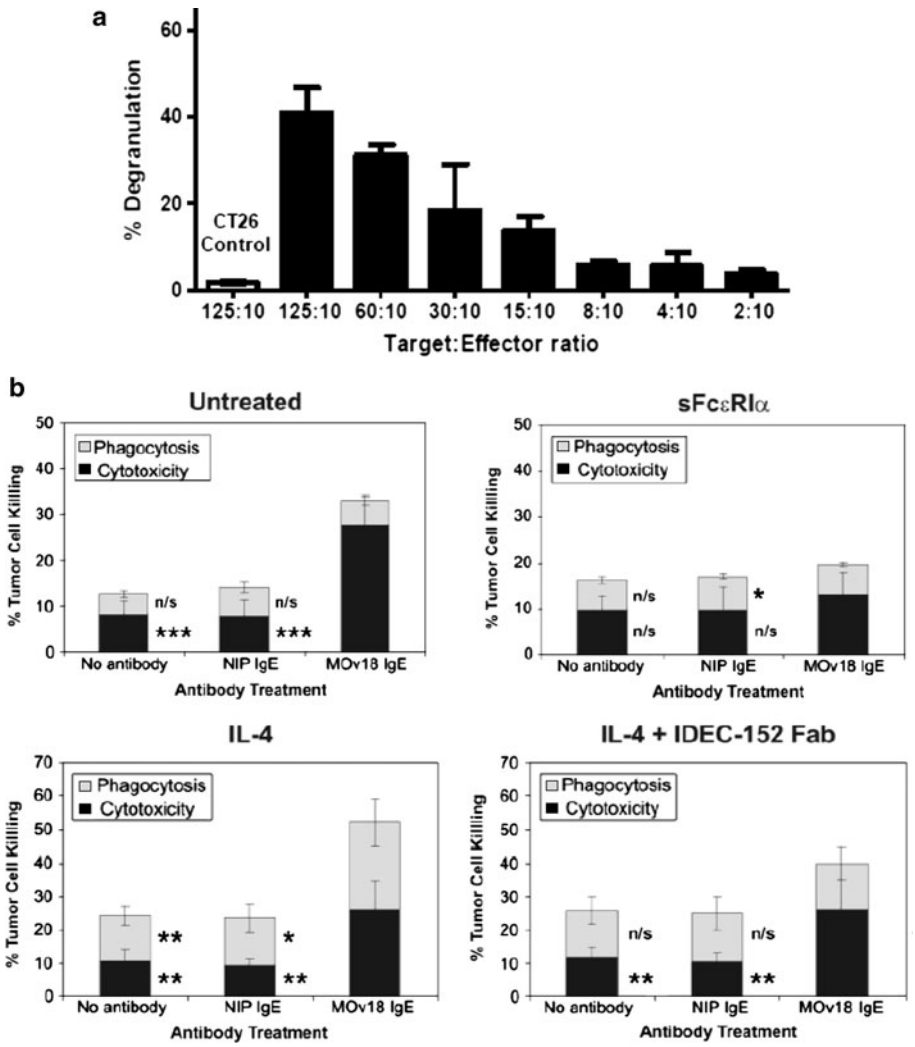


Fig. 2 Known mechanisms of action are mediated by FcεRs in response to antigen-specific IgE and cancer cells. **a** Anti-FRα antibody MOv18 IgE triggered degranulation of a rat basophilic leukaemia mast cell line (RBL SX-38) expressing human FcεRI ($\alpha\beta\gamma_2$) in the presence of increasing densities of FRα^+ tumour cells but not in the presence of FRα^{neg} tumour cells. *Bars* indicate mean values ± standard deviation (SD) of $n = 3$ experiments. **b** *Top* Human monocytes expressing trimeric FcεRI ($\alpha\gamma_2$) triggered tumour killing by ADCC in the presence of tumour antigen-specific antibody MOv18 IgE (*left*). ADCC of tumour cells was inhibited by blocking IgE-FcεRI interactions with soluble FcεRIα (*right*). *Bottom* IL-4-stimulated human monocytes express cell surface CD23, which enhanced MOv18 IgE-mediated tumour cell killing by ADCP (*left*). The function of CD23 in IgE-mediated ADCP is confirmed by interrupting IgE binding to cell surface CD23 with an anti-CD23 antibody Fab, which specifically blocked IgE ADCP of tumour cells. ADCC, *black bars*; ADCP, *grey bars*. Results shown as means ± SD of 6 independent experiments. Significance of values compared with samples given MOv18 IgE by the Student's *t* test. n/s $P > 0.05$; *$P < 0.05$; **$P < 0.005$; ***$P < 0.0005$. Reproduced with permission from Rudman et al. [64], Karagiannis et al. [76]

are up-regulated by IgE and IL-4 on effector cells in situ and are known to participate in these mechanisms of action. These properties of IgE antibodies may be redirected to enhance cytotoxicity and phagocytosis of tumour cells, as well as initiate IgE antibody-dependent antigen presentation by IgE receptor-bearing antigen-presenting cells such as dendritic cells, B cells and macrophages. Thus, passive and active immunity against solid tumours could act in conjunction in tissues, naturally populated by IgE effector cells.

The slow dissociation of the IgE–FcεRl complex and local retention of IgE in tissues may translate to lower effective therapeutic doses and/or reduced frequency of administration compared to IgG. The combined strength of IgE-mediated immune responses in tissues also carries the expectation of increased potency as well as longevity of immune surveillance by IgE and effector cells against solid tumours. These parameters formed the motivation for designing MOv18 IgE, the first chimaeric IgE antibody against the ovarian carcinoma antigen folate receptor α (FRα).

An antibody of the IgE class against the tumour antigen folate receptor α (FRα): the first example of the concept

Choice of tumour antigen, antibody specificity and tumour target

The hypothesis that IgE antibodies may offer some advantages over their IgG counterparts in the passive immunotherapy of cancer has been explored in a series of studies on mouse/human chimaeric antibodies (MOv18 IgE and IgG1), targeting the tumour-associated antigen folate receptor α (FRα) (also termed folate-binding protein). FRα, a glycosylphosphatidylinositol-anchored membrane protein that binds folic acid, is an emerging therapeutic target. It is constitutively expressed on the cell surface of 72–97% of epithelial ovarian cancers and in a proportion of other tumours including renal, endometrial, lung, breast, bladder, pancreatic and colorectal carcinomas and malignant melanomas [43–45]. The most widely studied FRα-expressing tumour type is epithelial ovarian cancer in which FRα expression level is associated with tumour progression, increased grade and decreased patient survival [44, 46]. Despite being expressed at high densities (up to 10^6 molecules/cell) on ovarian tumour cells, FRα expression in normal tissues is limited to the apical surfaces of only a few epithelia, predominantly in the kidney, lung, choroid plexus, intestine and placenta and, therefore, non-target toxicities related to cross-reactivity of a FRα-specific antibody with normal tissues are expected to be low [47, 48]. Thus, FRα would appear to be a prime molecular target for cancer therapy.

The original clone, murine MOv18 IgG1, was generated by immunisation of mice with a surgical specimen of ovarian carcinoma [49]. The variable regions of the resultant antibody were cloned, and the murine γ1-heavy chains and κ-light chains were subsequently replaced with their human equivalents to make chimaeric MOv18 IgG [50]. Chimaeric MOv18 IgE was then engineered by switching the human γ1-constant regions for human ε constant regions [51].

Previous clinical studies of MOv18 IgG (either murine or chimaeric) administered to ovarian cancer patients have suggested therapeutic benefit with no overt toxicity [52–58]. This and other therapeutic IgG1 antibodies specific for FRα have since entered clinical trials. Scintigraphic images using a ^{131}I-radiolabelled chimaeric MOv18 IgG1 revealed accumulation of antibody in ovarian cancer lesions and decreasing retention in other tissues as a function of time. Similar results have been obtained using analogues labelled with other isotopes (^{111}In) [59]. Most recently, FRα targeted immunotherapy with farletuzumab (MORAb-003), a humanised IgG1 antibody with high affinity for FRα, demonstrated a favourable adverse events profile and was well tolerated in a Phase I trial. In Phase II, farletuzumab in combination with chemotherapy demonstrated greater than expected activity compared to historical controls in patients with platinum-sensitive, recurrent ovarian cancer, and ongoing Phase III trials will further assess the effectiveness of this antibody in combination with chemotherapy [60]. FRα has, therefore, been extensively characterised as a tumour-selective target in man using a variety of approaches including monoclonal antibodies and therefore constitutes a promising biomarker for targeted therapies.

Furthermore, FRα was chosen as a target specifically for IgE immunotherapy because, although a soluble form of the receptor (with the folate-binding site intact) arises from proteolytic cleavage of the membrane-associated FRα precursor, this soluble FRα circulates in a monomeric form and unlike other shed tumour-associated antigen fragments would not be expected to cross-link surface-bound MOv18 bound to FcεRI on circulating basophils and thereby increase the risk of a Type I hypersensitivity response [61, 62].

Efficacy of MOv18 IgE in disparate in vivo systems

We compared the in vivo efficacy of MOv18 IgE and IgG1 antibodies in two disparate human xenograft models of FRα-expressing ovarian carcinoma grown in immunodeficient mice [51, 63–65]. Treatment with IgE afforded superior protection compared with the equivalent IgG1 antibody in both systems.

In our first model, severe combined immunodeficiency (SCID) mice were subcutaneously challenged with FRα$^+$ human ovarian carcinoma (IGROV1) cells followed by intravenous administration of human PBMC, added as effector cells, with either MOv18 IgE or MOv18 IgG1 [51] (Fig. 3a). MOv18 IgE combined with human PBMC had a superior and longer lasting effect in restricting tumour growth compared with the same treatment with IgG1. Both MOv18 IgE and MOv18 IgG1 inhibited tumour growth up to 19 days following tumour challenge compared to control groups. However, only MOv18 IgE maintained inhibition of tumour growth for an additional 16 days (to day 35). Furthermore, mice treated with half of the concentration of IgE (50 μg) compared to IgG1 showed 40% inhibition of tumour growth at day 35 (compared to 62% inhibition at full dose). As the human Fcε region of chimaeric IgE is not recognised by mouse Fcε receptors, MOv18 IgE did not induce an anti-tumour effect in the absence of human PBMC, demonstrating the requirement for human cells to mediate the antigen-specific IgE effector functions. Histological examination of tumour sections from mice treated with MOv18 IgE exhibited larger areas of tumour necrosis compared to controls, further suggesting that anti-tumour effector cell functions were activated in response to IgE treatment.

The anti-cancer effect of MOv18 IgE was further evaluated with a different human xenograft system thought to model the clinical situation more closely than the previous one [63–65]. In this mouse model, a patient-derived FRα^{+} human ovarian carcinoma xenograft (HUA) was grown orthotopically (intraperitoneally) in nude mice (Fig. 3b, c). The day after tumour challenge, human PBMC were injected intraperitoneally alone or in combination with 100 μg MOv18 IgE or MOv18 IgG1 and treatments were repeated once, after 14 days. Treatment with PBMC and MOv18 IgE significantly increased survival to 40 days compared to mice treated with the combination of PBMC

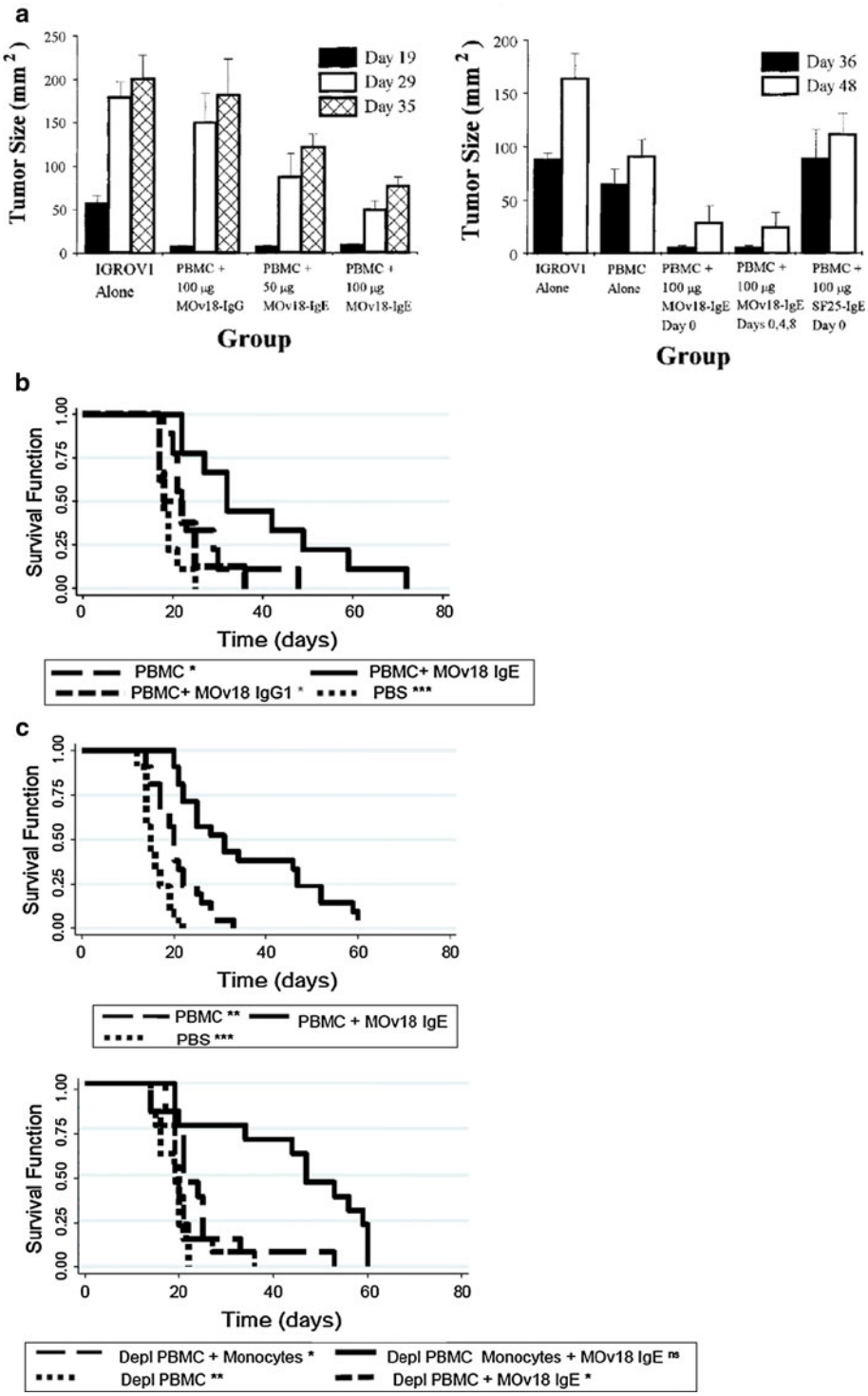


Fig. 3 FRα-specific chimaeric antibody MOv18 IgE demonstrated superior efficacy in two human ovarian xenograft models in immunocompromised mice. **a** Systemic (i.v.) administration of MOv18 IgE and human PBMCs restricted growth of a human ovarian carcinoma grown subcutaneously in SCID mice for a longer period (35 days) compared to the equivalent MOv18 IgG1 and PBMCs (19 days) (*left*), while isotype-matched IgE controls showed substantial tumour growth similar to treatments with PBMCs alone (*right*). *Bars* represent mean tumour sizes ± SEM. Gould et al. [51] reproduced with permission. **b** Effects of MOv18 IgE in an intraperitoneal xenograft model of a patient-derived human ovarian carcinoma (HUA). MOv18 IgE introduced with PBMCs significantly increased survival of nu/nu mice following tumour challenge compared to controls while administration with MOv18 IgG1 with PBMCs conferred no survival advantage. Data from Karagiannis et al. [65] reproduced with permission. **c** Monocytes are important IgE effector cells in vivo: MOv18 IgE and PBMCs restricted i.p. human ovarian carcinoma growth (*top*), and survival advantage was impaired when MOv18 IgE is introduced in combination with PBMCs depleted of monocytes. Increased survival was restored when monocyte-depleted PBMCs were reconstituted with monocytes prior to treatments (*bottom*). Reproduced with permission from Karagiannis et al. [64]

and MOv18 IgG1 (22 days) or compared to mice treated with PBS alone (19 days). MOv18 IgG1 with human PBMC in this model, therefore, offered no survival advantage [65] (Fig. 3b).

These models may in fact underestimate the anti-tumour activity of MOv18 IgE, since the effector cells were limited to exogenously administered human PBMC, which disappear very rapidly from the circulation of immunocompromised mice such as SCID and are widely distributed amongst various tissues [66]. In contrast, in humans, there would be a permanent supply of the entire effector repertoire including PBMC, and thus, the anti-tumour activity of IgE is expected to be superior compared to that observed in this mouse model. Furthermore, the capacity of the IgE to elicit an adaptive immune response could not be evaluated since immunodeficient mice were used. Despite these limitations, this study demonstrates that the anti-tumour activity of MOv18 IgE was superior to its IgG1 counterpart and that the Fc-mediated effector functions of the antibody are essential.

Immune effector cells activated by tumour antigen-specific IgE

By developing therapeutic antibodies of the IgE class, we offer a novel strategy to increase ADCC in solid tumours. While much recent effort has centred on engineering therapeutic antibodies to improve IgG Fc-mediated effector functions by increasing affinities to Fc gamma receptors, we aim to enhance antibody-mediated tumour cell killing by exchanging the Fc region of an antibody for those of a class that binds to unique Fc receptors with a different distribution on effector cells. Many studies of IgG-mediated ADCC have centred around IgG Fc binding to FcγRIIIa on NK cells. However, NK cells potentially have poor infiltration into some solid tumours or reside in the stroma and thus do not come into contact with tumour cells [67]. The location in combination with the low affinity of IgG for FcγRIII may translate into less effective IgG-induced ADCC in solid tumours. However, IgE can bind to different Fc receptors than IgG resulting in activation of different immune cell populations known to be present in many solid tumours such as monocytes, macrophages, basophils, eosinophils and mast cells [68].

Using human immune cells, we developed in vitro, ex vivo and in vivo models in our laboratory to investigate effector mechanisms of therapeutic IgE antibodies targeting tumour cells. In SCID and nude mouse xenograft models of ovarian carcinoma armed with human PBMCs, we strikingly found that MOv18 IgE affords greater protection against ovarian tumour growth in both in vivo models compared to an IgG1 counterpart. These recombinant MOv18 antibodies of the IgG and IgE class did not exert any direct effects on tumour cell viability or proliferation in our in vitro assays allowing for the elucidation of immune-mediated killing. We found that monocytes were key immune effector cells infiltrating the tumour and expressing IgE receptors. Immunohistochemical analysis of HUA xenografts demonstrated that human monocytes infiltrated tumour lesions in MOv18 IgE-treated mice only, suggesting that these IgE receptor-expressing effector cells play an important role in the anti-tumour effect of this antibody [65]. The role of monocytes was confirmed using monocyte-depleted PBMC in this tumour model resulting in loss of survival advantage conferred by tumour antigen-specific IgE [64] (Fig. 3c).

Whereas it is NK cells that are active in IgG-induced ADCC and neutrophils that mediate IgA-induced ADCC, we have shown that IgE-mediated ADCC requires monocytes [69–72]. Mechanistically, MOv18 IgE induced monocytes to kill up to 70% of ovarian carcinoma cells. Tumour killing was mediated by two mechanisms: antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cell-mediated phagocytosis (ADCP) of tumour cells, both known IgE mechanisms of action in protection from parasitic infections [63, 64]. These IgE mechanisms of action were dissected using flow cytometric assays along with corresponding imaging experiments that confirmed IgE-mediated tumour killing [73] (Fig. 2). Additionally, we examined other immune effector cells resident in solid tumours. In vitro, when we introduced MOv18 IgE in mixed cultures of human PBMC and ovarian tumour cells, we observed human monocytes and basophils in contact with these tumour cells in the presence of tumour-specific IgE, highlighting the specificity of the interactions between these cells [65].

We also demonstrated that MOv18 IgE bound to tumour cells is capable of potent cytotoxic killing in the presence of eosinophils, despite exceedingly low FcεRI expression by these cells [64, 74, 75]. Incubation of MOv18 IgE with tumour target cells and the RBL-SX38 rat basophilic leukaemia mast cell line at target-to-effector ratios akin to those in tumour lesions (>2:1) resulted in specific functional degranulation of mast cells [76] (Fig. 2). These observations highlight the diversity of mechanisms available for targeted tumour cell killing and the therapeutic potential for antibodies of the IgE class.

IgE Fcε receptors signal tumour cell targeting and killing

The two IgE receptors, FcεRI and CD23, have been shown to mediate tumour cell killing in vitro in an IgE-dependent manner by both ADCC and ADCP. In three in vitro ADCC assays, MOv18 IgE targeted towards FRα on ovarian cancer cells induced ADCC of these cells, which was

mediated by the FcεRI receptor on monocytes, where there was undetectable expression of the low-affinity receptor, CD23 [64, 65]. Blocking IgE binding to cell surface FcεRI by addition of soluble recombinant FcεRI alpha (FcεRIα) in our assays resulted in complete loss of tumour ADCC by sequestering of the unbound IgE [64] (Fig. 2). Furthermore, freshly isolated PBMCs from allergic patients, previously loaded with non-specific IgEs, mediated lower tumour cell ADCC, confirming the importance of FcεRI recognition by MOv18 IgE in tumour killing [65].

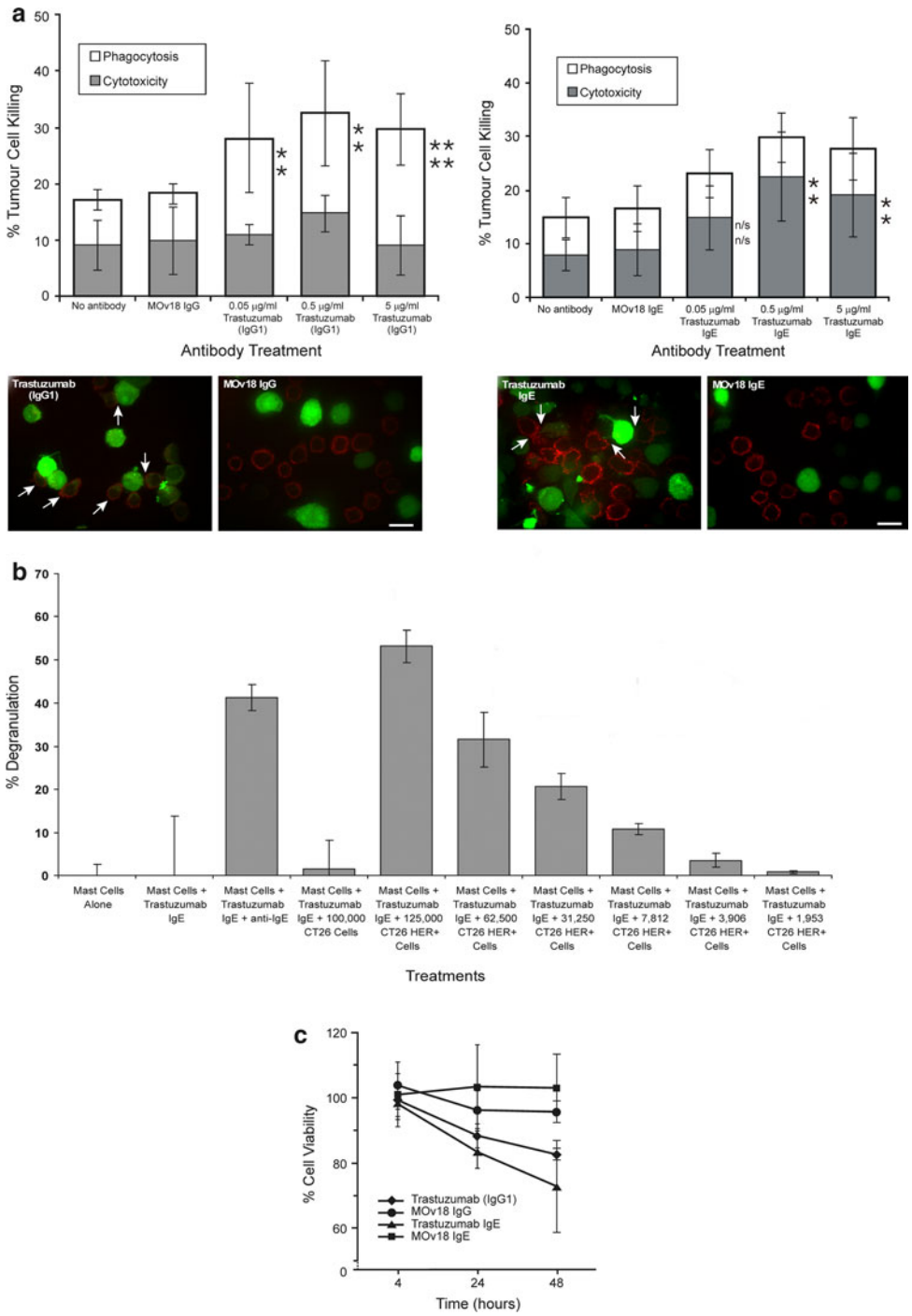
By contrast, tumour cell death by phagocytosis was mediated entirely via CD23 on monocytes. Upon stimulation with IL-4, monocytes expressed CD23 and induced tumour cell killing enhanced by up to 30% entirely attributed to ADCP [63, 64]. The role of CD23 in mediating ADCP was further confirmed by blocking CD23 recognition with the Fab fragment of an anti-CD23 blocking antibody that interfered with the IgE binding site and completely blocked tumour killing by ADCP (Fig. 2). Further evidence of an anti-tumour role for this receptor was shown by enhancing expression of CD23 on monocytic cells by IL-4 stimulation, prior to treatment of ovarian tumour xenograft-bearing mice with stimulated cells and MOv18 IgE, which resulted in prolonged survival of the mice compared to those treated with unstimulated monocytic cells and MOv18 IgE [63].

Both the FcεRI and CD23 IgE receptors potentially play distinct crucial roles in IgE-mediated tumour cell death; these contributions remain to be further elucidated with more tumour-specific IgEs. In view of the emerging emphasis on ADCC and ADCP as important mechanisms of action for passive immunotherapy, evaluation of immunoglobulin classes and immune effector cells in this regard is clearly a matter of considerable importance.

Findings from engineering and testing an IgE antibody counterpart to the anti-HER2/*neu* antibody Trastuzumab

As the above studies focused on a single agent with specificity to one tumour antigen, we wished to explore the principle of engineering an IgE equivalent of an antibody of known specificity against a well-validated tumour target, which has demonstrated efficacy as an anti-cancer therapy. We chose the cell surface tumour antigen HER2/*neu* that is overexpressed by 25% of all invasive breast cancers and 70% of ductal carcinomas. HER2/*neu* belongs to the human epidermal growth factor receptor family which contains four transmembrane tyrosine kinases that mediate cell survival and proliferation. HER2/*neu* is a validated target for antibody immunotherapy and the humanised monoclonal antibody trastuzumab (Herceptin®), an IgG1 antibody raised against the HER2/*neu* extracellular domain. Trastuzumab is approved by the Food and Drug Administration (FDA) for the treatment of HER2/*neu* positive breast cancers, both in the adjuvant and metastatic settings, and represents a benchmark in antibody therapy, as it was the first antibody therapy approved for a non-haematological indication. Well-characterised mechanisms by which trastuzumab IgG functions include the blocking of hetero-dimerisation of HER receptors on the surface of breast cancer cells, which restricts vital tumour cell growth signals, as well as the recruitment of cytotoxic (NK) and phagocytic (monocytes) effector cells that can induce tumour cell death.

To compare potential mechanisms of action mediated by IgE and IgG1 classes, we engineered a trastuzumab IgE antibody by synthesising the cDNA encoding the heavy and light chains of the trastuzumab variable regions, based on the published protein sequence (source: http://www.pdb.org; 1N8Z) [77]. We evaluated the biological properties of our engineered trastuzumab IgE using three functional assays. One assay assessed the ability to mediate tumour cell killing by human effector cells. This three-colour flow cytometric assay simultaneously distinguishes tumour cell killing by ADCC and ADCP, making it an ideal tool to assess effector functions and differentiate between these two major effector cell mechanisms [73] (Fig. 4a). Trastuzumab IgE directed monocytic cells to kill tumour cells expressing the HER2/*neu* antigen by ADCC, a mechanism clearly different from ADCP employed by trastuzumab IgG and monocytic cells. The antibody concentrations (0.5 μg/mL) required to achieve tumour cell killing were the same for IgG and IgE in these in vitro assays, and these findings were validated by confocal microscopy. Interestingly, the concentrations found optimal for the in vitro monocytic cell functions of trastuzumab IgE (0.5 μg/mL) were tenfold lower than our previously reported optimal concentrations required for MOv18 IgE-mediated killing of ovarian tumour cells in equivalent in vitro assays. Since the affinities for FcεRI are equivalent for both IgEs, it is possible that the high affinity of trastuzumab IgE for its tumour cell surface antigen, HER2/*neu*, may be a contributing factor [49, 50, 77]. On the other hand, when each IgE antibody was administered at optimal concentration, MOv18 IgE was capable of mediating a higher proportion of tumour cell death by monocytic cells in vitro compared to trastuzumab IgE; additionally, MOv18 IgE induced monocyte-mediated killing by both ADCC and ADCP, whereas trastuzumab IgE induced tumour cell ADCC only in similar in vitro assays [63, 64, 77]. The reasons for these differences in activities of the two IgE antibodies are not clear. As in the case with IgG antibodies, density and kinetics of target antigens and accessibility of the antigenic epitope to antibody on the tumour cell surface



as well as antibody affinities for their target antigens may influence efficacy and mechanisms of action of different IgE antibodies.

Secondly, cell viability assays demonstrated that trastuzumab IgE maintained the same direct effects on tumour growth arrest reported for trastuzumab over a period of 48 h in culture and at the same effective concentrations as trastuzumab IgG (Fig. 4b). The third assay measured IgE potency through activating IgE receptor-bearing cells by trastuzumab to trigger a functional degranulation upon cross-linking of the high-affinity receptor (FcεRI). By measuring release of the enzyme β-hexoseaminidase by the rat basophilic leukaemia mast cell line RBL-SX38 that expresses the human FcεRI, we showed that trastuzumab IgE induced degranulation in the presence of HER2/*neu*-expressing tumour cells in an antigen-specific manner, perhaps pointing to a potential contribution of IgE-activated mast cells and basophils in tumour lesions in

◀ **Fig. 4** Evaluation of trastuzumab IgE mechanisms of action against HER2/*neu* + cancer cells **a** *Top* quantification of trastuzumab (IgG1, *left*) and trastuzumab IgE (*right*) -mediated killing f HER2/*neu*-expressing target cells by U937 monocytes after 2.5 h by the ADCC/ADCP assay. Cytotoxicity: *black bars*; phagocytosis: *white bars*. Results are means ± SD of six independent experiments. Significance compared to isotype or no antibody control samples by the Student's *t* test: n/s $P > 0.05$, *$P < 0.05$, **$P < 0.005$, ***$P < 0.0005$. *Bottom* Representative confocal fluorescence images of tumour–effector cell interactions potentiated by trastuzumab IgG1 and IgE. CFSE-stained CT26-HER2/*neu* tumour cells (*green*) and CD33-APC labelled U937 cells (*red*) combined at 2:1 E:T ratio after 3 h in culture. U937 cells (*red*) given trastuzumab IgE/IgG (*left*) showed enhanced contact with tumour cells (*green*) and phagocytosis of tumour cells (*green* CFSE inside U937 monocytes, *white arrows*). Neither effector-target cell contact nor phagocytosis was observed when cells were incubated with isotype controls. Original magnification 63× (*Scale bar* 15 μm). **b** Cell viability assays (MTS) demonstrating levels of susceptibility of HER2/*neu* + SKBR3 breast cancer cells to trastuzumab (IgG1), trastuzumab IgE, and control antibodies MOv18 IgG and MOv18 IgE following incubations for 4, 24 and 48 h in culture. Each data point represents mean % cell viability ± SD ($n = 4$). **c** Trastuzumab IgE-mediated degranulation of rat basophilic leukaemia mast cells (RBL SX-38) expressing human FcεRI in the presence of increasing concentrations of HER2/*neu* + CT26 tumour cells. Degranulation was quantified by β-hexoseaminidase release and controls such as trastuzumab IgE cross-linked with anti-IgE polyclonal antibody to confirm mast cell degranulation and HER2/*neu*^{neg} CT26 tumour cells. Data are mean ± SD of three measurements. Reproduced with permission from Karagiannis et al. [77]

recruiting effector cells in tissues through the release of cytokines and chemokines in situ (Fig. 4c).

The IgE responses measured and observed in our assays supported the conclusion that trastuzumab IgE functions with similar potency, but through effector cell mechanisms different from those of trastuzumab IgG, supporting a potential role in complementing or enhancing the known mechanisms of existing antibodies.

Initial evaluations of the potential of tumour antigen-specific IgE antibodies to trigger Type I hypersensitivity in patients with cancer

The main reservation about clinical use of IgE has been a perceived risk of inducing Type I hypersensitivity that may in some circumstances contribute to systemic anaphylaxis in predisposed individuals. Although there is presently little knowledge of what factors predispose some individuals to develop symptoms of anaphylaxis to specific agents, it is known that soluble antigens/allergens, capable of inducing Type I hypersensitivity, must have a minimum two or more epitopes recognised by an IgE antibody. Therefore, Type I hypersensitivity may occur when IgE, attached to FcεRI on circulating basophils and lung/mucosal mast cells, is cross-linked by soluble multivalent antigens (Fig. 5a). To avoid this circumstance, a target antigen that is not shed in a multivalent form into the circulation must be chosen: indeed, the choice of FRα as a target molecule for the design of our first agent, MOv18 IgE, was based on evidence that the molecule is monomeric in its soluble form [61, 62].

A number of metrics, observations and readouts may afford some insight into the possibility that MOv18 IgE may elicit Type I hypersensitivity upon systemic (i.v.) administration:

In vivo human xenograft models

We introduced our FRα-specific antibody MOv18 IgE in two in vivo human ovarian carcinoma xenograft models, one of which was derived from a patient with stage III ovarian adenocarcinoma [51, 63–65]. In these systems, we introduced repeated doses of human immune effector cells with the chimaeric IgE into mice by the intravenous (i.v.) and intraperitoneal (i.p.) routes. Despite the presence of basophils in these effector cell populations and evidence for their function in tumour cell targeting by IgE in these models, we observed no signs of anaphylactic reactions in any of the animals.

Evidence from a syngeneic mouse model of mammary carcinoma

Other groups have independently arrived at similar conclusions. Successful targeting of tumour cells by monomeric anti-mouse tumour IgE antibodies was demonstrated in a syngeneic mouse model of mammary carcinoma [78]. No adverse reactions were observed in the mice, although, like humans, rodents express fully functional tetrameric FcεRI on the surfaces of mast cells and basophils and these cells are found in the expected natural anatomical locations in tissues and blood from which they could trigger Type I hypersensitivity.

Ex vivo readouts of allergy in patient blood and sera

Although our antibody MOv18 IgE binds to a single epitope on the tumour antigen FRα and should not cross-link IgE receptors on basophils, we investigated whether any components in the circulation of healthy individuals and cancer patients might cross-link FRα-MOv18-IgE–IgE receptor (FcεRI) complexes to activate basophils to trigger Type I hypersensitivity [76] (Fig. 5a). Mast cell degranulation in the presence of local tumour antigen-specific IgE may occur upon encountering high densities of antigen-expressing tumour cells in tumour lesions (Fig. 5a). These key events may be pivotal for triggering local immune activation and recruitment of effector cells, kick-starting IgE-mediated immunity in tissues [24, 79, 80]. To examine

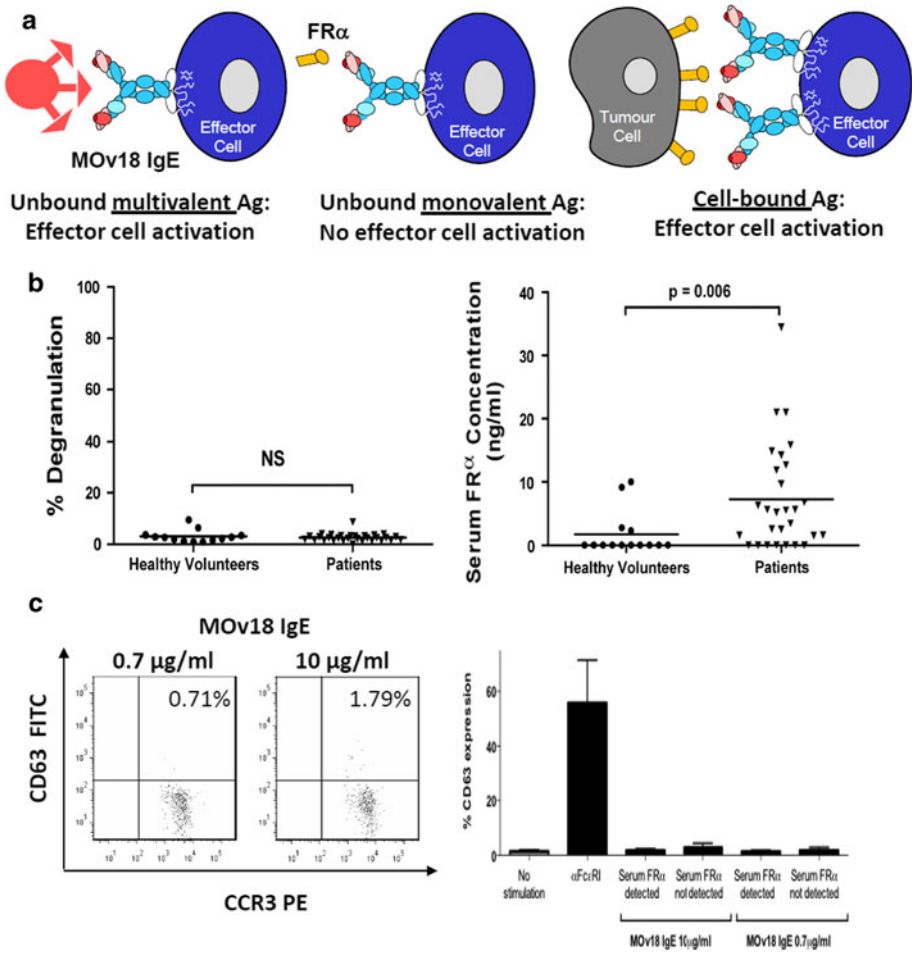


Fig. 5 MOv18 IgE directed against the monovalent cancer antigen FRα does not trigger signs of Type I hypersensitivity when added to the serum or blood of patients with ovarian carcinoma. **a** Schematic diagram of the events leading to IgE-mediated basophil/mast cell activation and degranulation. Unbound multivalent antigen is expected to lead to effector cell degranulation (*left*) whereas unbound monovalent antigen (such as shed FRα) is not (*middle*). Tumour cell-bound antigen, however, is expected to lead to effector cell degranulation and potential tumour cell killing (*right*). **b** β-hexosea-minidase release assays performed with 14 healthy volunteer and 32 ovarian carcinoma patient sera; degranulation was minimal in both groups with no significant differences observed between groups ($P > 0.05$) (*left*). Serum samples from the above cohort were quantified by ELISA: [FRα] increased in ovarian carcinoma patients compared to healthy volunteers ($P = 0.006$) (*right*). **c** Representative two-colour flow cytometric dot plots of % $CCR3^{high}CD63^{+}$ basophils in patient blood given MOv18 IgE (0.7 and 10 µg/mL) (*left*). % $CCR3^{high}CD63^{+}$ basophils in blood of 2 patient cohorts (*right*); one ($n = 5$) with detectable serum FRα titres, and the other ($n = 5$) without detectable serum FRα, illustrating background expression with addition of MOv18 IgE compared to anti-FcεRI and no stimulation controls. *Bars* indicate mean values ± standard deviation (SD) of $n = 10$ experiments, all conditions were tested in triplicate. Reproduced with permission from Rudman et al. [76]

the propensity of a tumour antigen-specific IgE to induce early events that could lead to Type I hypersensitivity, we adopted two ex vivo assays conducted in patient sera and whole unfractionated blood, which are applied in the field of allergy to examine sensitivity to allergens. One assay detects functional degranulation of cells in the presence of human sera (Fig. 5b), while the second, termed basophil activation assay (BAT) is based on detection of cell surface CD63, an early sign of basophil activation that precedes degranulation (Fig. 5c). The latter is an emerging clinical tool to assist diagnosis of sensitivity to a range of agents including medicinal drugs. Upon addition of MOv18 IgE to human sera and whole blood, we observed neither functional degranulation nor significant activation of human basophils. These readouts also yielded no effector cell activation above background in patient or healthy volunteer sera and blood with tumour antigen-specific IgE. No effector cell activation was recorded even in the presence of detectable levels of shed soluble antigen FRα, which we showed to be elevated in a larger proportion of ovarian carcinoma patient sera compared to healthy controls, and despite detection of anti-human FRα auto-antibodies in a proportion of our FRα + patient cohort (Fig. 5b, c). These encouraging results add weight to the hypothesis that an

IgE antibody against a monomeric cancer antigen would not be expected to induce early signs of Type I hypersensitivity once introduced into the blood of patients with cancer or of healthy individuals [81].

On-going evaluations are now aimed at predicting safety in new in vivo models, and the outcomes will inform future clinical testing.

From lab bench to patient bedside: planned metrics and monitoring in the clinical setting

Considerable efforts have so far focused on designing monoclonal IgE antibodies that harness the powerful immune effector mechanisms of IgE against cancer, while predicting and minimising potential toxicities. Specifically, our tumour antigen-specific IgE antibodies were selected against (a) single epitopes on tumour antigens, (b) antigenic targets that are not shed as multimeric complexes in the circulation and (c) antigens highly expressed on tumour cells but with absent or minimal expression and restricted distribution in normal tissues. As discussed above, we have been monitoring safety in three in vivo models of cancer and in ex vivo assays with patient sera and blood. Downstream of this pathway, clinical testing of a tumour antigen-specific IgE antibody will represent a key milestone for the concept. These measures will also provide valuable scientific insights that can advance our understanding of IgE biology, particularly in the context of cancer therapeutics. Clinical trials of MOv18 IgE will be conducted alongside carefully planned metrics and monitoring of patients to inform on safety and efficacy:

Prediction of potential adverse events prior to clinical studies

Aiming to preclude possible adverse events, patients with a known history of anaphylactic reactions, severe allergies or atopic asthma will be excluded from clinical trials. Patients' blood and sera will be examined using established ex vivo readouts such as functional degranulation and BAT assays, as above, and skin prick tests with MOv18 IgE will be conducted prior to inclusion in any clinical trials and patients with positive reactions in these readouts will be excluded from partaking in the trial.

Monitoring patients during clinical studies

Parameters for monitoring and consideration may include:

a. *Monitoring for signs of Type I hypersensitivity*: Alongside known clinical features of allergy or cytokine-release syndrome, titres of histamine and beta-tryptase released into the circulation could represent measurable readouts of Type I hypersensitivity and may be useful monitoring tools during Phase I/IIa studies [82].
b. Ex vivo *assays to assess propensity for Type I hypersensitivity*: Functional degranulation and BAT assays will be conducted with patient sera and blood prior to dosing with antibody and will help monitor for early signs of Type I hypersensitivity [76, 83, 84].
c. *Density of unoccupied FcεR on immune effector cells*: We previously demonstrated that immune effector cells from atopic individuals trigger reduced IgE-mediated tumour killing compared to those from non-atopic controls, and this was attributed to increased occupancy of cell surface FcεR by endogenous IgEs [65]. In light of these findings, density of unoccupied FcεR on effector cells could be proportional to IgE-mediated anti-tumoural functions and may be monitored and considered when assessing efficacy.
d. *Circulating tumour antigen FRα*: This may reduce efficacy of MOv18 IgE. We previously reported that serum levels of circulating FRα (0–34.5 ng/mL) in our ovarian carcinoma patient cohorts were higher than those in healthy volunteer sera [76]. In patients with low circulating levels of FRα, most of the IgE binding sites may be available to bind tumour cell-associated FRα. However, in patients with high serum FRα titres, there may be only partial occupancy of binding sites by tumour cell surface-bound FRα, which may reduce antibody efficacy for the treatment of FRα + carcinomas. This may be addressed by monitoring sFRα, FcεRI-MOv18 IgE occupancy by circulating FRα, and pharmacodynamic endpoints would allow dose up-titration if needed. Similar concerns in patients with HER2/*neu* + carcinomas who have circulating soluble HER2 (>15 ng/mL), which may potentially reduce efficacy for trastuzumab in patients with high soluble antigen titres, have not yielded any clear links between antigen levels in patient serum and clinical responses to trastuzumab [85].
e. *Circulating free FcεRs*: Detectable soluble forms of IgE receptors (sFcεRIα and sFcεRII, or sCD23, with their IgE binding sites intact) in human circulation have been reported [86–88]. The presence of these soluble forms may compete for recognition of IgE by immune effector cells and may reduce activation and ADCC/ADCP. Levels of circulating FcεRs may be monitored and correlated with clinical readouts of efficacy.
f. *Polyclonal IgG auto-antibodies to FRα in patient blood*: Auto-antibodies to FRα have been reported in individuals with a history of neural tube defect pregnancies, oral cleft defects, infertility and in healthy

individuals [76]. High titres of such Abs in patient sera could cross-link FRα bound to IgE on basophils and mast cells triggering degranulation. In our patient cohort, we measured no effector cell activation above background as a result of adding MOv18 IgE in any patient blood sample, although we reported low but detectable levels of auto-antibodies in 6/24 FRα-positive ovarian carcinoma patient sera (<43 ng/mL) [76]. In clinical scenarios, patients may have or could develop anti-FRα antibodies as a result of treatment. We do not propose to exclude patients with detectable anti-FRα antibodies; however, monitoring such Abs following administration of MOv18 IgE may alert us to the risk of potential basophil activation and induction of Type I hypersensitivity.

g. *Auto-antibodies to MOv18 IgE*: Levels of auto-antibodies are routinely monitored in clinical trials of antibody therapeutics. Human anti-chimeric antibodies (or HACA) may neutralise and clear the antibody from the circulation, preventing it from reaching tumour sites and reducing efficacy; HACA, therefore, should be monitored and correlated with clinical readouts.

Clinical management of potential toxicities and interventions

Reliable clinical interventions to counter the effects of any anaphylactic responses to medicines and monoclonal antibody therapeutics should be included in the preparation and risk management of clinical trials [89]. Similarly to the risks inherent in the use of IgG antibodies, those associated with IgE will be monitored and managed. No corticosteroid or anti-histamine pre-medications will be administered as these may suppress immune response triggered by a tumour-specific IgE antibody. However, patients will be intensively monitored during the infusion of antibody and if symptoms suggestive of an allergic reaction or cytokine-release syndrome develop, rapid oxygen, anti-pyretics, steroids, anti-histamine treatment and intravenous fluids can be administered, with the potential to give adrenaline upon development of anaphylaxis.

Designing IgE antibodies for the treatment of solid tumours: lessons and thoughts for future therapeutics

Our findings with the two IgE antibodies against ovarian and breast cancer antigens provide compelling evidence that antibodies of this class may complement or even improve the efficacy of IgG antibodies in clinical applications. The current data lend merit to new explorations with panels of engineered antibodies of the IgE class for the treatment of solid tumour indications. As is the case for IgG antibodies of different specificities, antibody function and efficacy are influenced by many factors; following from this, potential outcomes from our hypothesis may not be easy to predict for the following reasons:

Our research with MOv18 IgE and trastuzumab IgE antibodies

(a) Direct effects on tumour cell proliferation, (b) effector cell-mediated mechanisms of tumour cell killing and (c) effectiveness and optimum doses were different for these two agents. It follows that elucidating whether an IgE antibody against an epitope of a tumour antigen could activate immune effector cells to kill tumour cells required an independent, thorough investigation on a case-by-case basis.

Therapeutic indication

Tumour microenvironments differ quite substantially in various cancers, in relation to Fc receptor expression and distribution, as well as to composition, proportion, localisation and activation of infiltrating immune effector cells. These parameters would have a substantial bearing on the ability of an IgE class antibody to mount immune responses against tumour cells in situ.

Expression and biodistribution of target antigen

An important consideration lies with the biodistribution and expression of different antigens in healthy and malignant tissues greatly influencing on-target toxicities of targeted therapies.

Nature of the antigen and antigen–antibody interactions

A number of variables relate to epitope specificity, including downstream signalling events that may be triggered with engagement of individual receptors/epitopes. Other variables include stoichiometry of the antibody–antigen interactions and resulting affinity, all of which are bound to influence tissue permeability and retention and strength and quality of effector functions.

Presence and form of soluble antigen in circulation

Is the antigen found in soluble form in the circulation of patients and healthy individuals? As discussed above, if the antigen is shed in a soluble form, more than two epitopes recognised by an IgE antibody would be needed for induction of Type I hypersensitivity. To avoid this, it is

important to choose antigens that are not shed in a multimeric form in the circulation.

The above factors would influence the potential therapeutic effectiveness of antibodies and render any predictions of antibody efficacy merely speculative. Therefore, there is, we submit, a strong case for examining not only the concept of an IgE therapeutic for the treatment of ovarian carcinoma, but also for developing a panel of antibodies for testing and selection of the most efficacious antibody and antibody isotype for effective management of solid tumours.

Concluding thoughts

Pre-clinical evidence and clinical studies indicate that the use of antibodies can confer therapeutic benefits for cancer patients. The knowledge that immune effector functions are important contributors of therapeutic efficacy of antibodies such as trastuzumab and others render testing IgE class antibodies a clinically relevant proposal. In our work, we aimed to compare IgE and IgG1 antibodies of the same specificities to elucidate whether efficacy can be improved with the use of the IgE class equivalent. The improved efficacy with the ovarian tumour antigen-specific chimaeric antibody MOv18 IgE in disparate disease-relevant models, and the encouraging preliminary findings that this antibody is unlikely to trigger Type I hypersensitivity in patients with cancer encourage a critical test of the proposition that IgE strategies may be advantageous for cancer therapy either as monotherapies, or in combination with conventional or biological therapeutics.

Collectively, these findings may justify the initiation of Phase I clinical trials. The clinical experience will, however, be the ultimate test of the concept. Without a doubt, the outcomes are expected to enrich our understanding of IgE mechanisms of action in the context of cancer and will contribute new knowledge to the emerging field of 'AllergoOncology' [[90], [81]]. Ultimately, any potential benefits towards improving the design of antibody therapies for cancer and treating patients with cancer for whom few therapeutic options are available render these endeavours worthy of investigation.

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Conflict of interest The authors declare that they have no conflict of interest.

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