

REVIEW ARTICLE

# IgE-based immunotherapy of cancer: challenges and chances

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## Abstract

Passive immunotherapy with monoclonal antibodies is an indispensable cornerstone of clinical oncology. Notably, all FDA-approved antibodies comprise the IgG class, although numerous research articles proposed monoclonal antibodies of the IgM, IgG, IgA and IgE classes directed specifically against tumor-associated antigens. In particular, for the IgE isotype class, several recent studies could demonstrate high tumoricidal efficacy. Therefore, this review specifically highlights the latest developments toward IgE-based immunotherapy of cancer. Possible mechanisms and safety aspects of IgE-mediated tumor cell death are discussed with special focus on the attracted immune cells. An outlook is given on how especially comparative oncology could contribute to further developments. Humans and dogs have a highly comparable IgE biology, suggesting that translational AllergoOncology studies in patients with canine cancer could have predictive value for the potential of IgE-based anticancer immunotherapy in human clinical oncology.

The nascent field of AllergoOncology (1, 2) aims to reveal the inverse associations between atopic and malignant diseases, which have in particular been seen in pancreatic cancer, glioma, and childhood leukemia (3–6), to harness allergic mechanisms, such as degranulation of mast cells or basophils and Fcε receptor (FcεR)-mediated immune effects for therapy of cancer.

Cancer research has aimed for decades to overcome tumor tolerance and instead engage the immune system in defense of cancer. Strategies that have been pursued cover basically the whole spectrum of the immune repertoire, such as vaccines against tumorigenic viruses (7), vaccinations with tumor cells or tumor-associated antigens (TAAs) (8), pulsing of patients' antigen-presenting cells (9), and activating antitumor immunity via blockade of immune checkpoints (10) to passive immunotherapy with monoclonal antibodies (11). More recent experimental approaches propose to use genetically modified immune cells such as natural killer cells (NK cells) to specifically target tumor-associated antigens (TAAs)

(12) or to engage cytotoxic T cells for identification and vaccination against TAA T-cell epitopes (13).

In spite of promising *in vitro* and *in vivo* data of several experimental immunotherapeutic trials and numerous immunotherapeutic approaches in the pipeline (<http://www.cancer.gov/clinicaltrials>), only two approaches are at the moment of practical relevance in public health: prophylactic vaccines against tumorigenic viruses and passive antibody therapy against tumor-associated antigens.

## State of the art: passive immunotherapy of cancer with monoclonal antibodies

Immunotherapy using monoclonal antibodies has found its place in several treatment regimens of malignancies and is at the moment standard of care in, for example, therapy of metastatic breast cancer overexpressing HER-2 (14), metastatic colon cancer overexpressing EGFR (15), or B-cell non-Hodgkin's lymphoma with autonomous growth of

CD20-positive B cells (16). More recent approaches even try to modulate the immune system by attacking immune check-point inhibitors such as the anti-CTLA-4 (cytotoxic T-lymphocyte antigen-4) antibody ipilimumab, which displayed encouraging results in clinical studies of advanced metastatic melanoma (17–21) or the PD-1 (programmed death-1) (22) targeting antibodies nivolumab and lambrolizumab (23). In particular for lambrolizumab, safety and efficacy could be already demonstrated in patients with advanced metastatic melanoma (24).

The target molecules of the established therapies, however, represent either specific markers of malignantly transformed cells, such as CD20, CD33, or CD52 in hematologic malignancies (25), signal molecules promoting the growth of tumors, such as vascular endothelial growth factor (VEGF) (26), as well as growth factor receptors such as epidermal growth factor receptor (EGFR) (27) or human epidermal growth factor receptor-2 (HER-2) (28). An overview of current FDA-approved monoclonal antibody therapies is depicted in Table 1 (adapted from (29)).

Monoclonal antibodies can thus act in two ways: first by interfering via their Fab regions with binding of growth factors to receptors and thus silencing proliferation signals (30, 31) and second by interacting with immune cells via their Fc domains (32), conferring active tumor cell killing by immune cells via antibody-dependent cell-mediated cytotoxicity (ADCC) (33) and antibody-dependent cell-mediated phagocytosis (ADCP) (34). Moreover, the Fc regions do mediate not only cellular responses, but also humoral immune responses like complement activation (35, 36), ultimately resulting in tumor cell lysis (37, 38).

### Fc $\gamma$ -receptor-mediated tumor cell killing

As all monoclonal antibodies currently applied in clinical oncology comprise the IgG class ((39), Table 1), attracted immune cells are Fc-gamma-receptor-bearing cells, such as monocytes, macrophages, granulocytes, NK cells (CD32, CD16) (40), and dendritic or Langerhans cells (41). These cells can lead to ADCC (33) or ADCP (34) of tumor cells, furthermore to antigen-processing, transport, and presentation to T cells.

In humans, three groups of Fc gamma receptors were identified: CD64 (Fc $\gamma$ RI), CD32 (Fc $\gamma$ RIIa, Fc $\gamma$ RIIb, Fc $\gamma$ RIIc), and CD16 (Fc $\gamma$ RIIIa, Fc $\gamma$ RIV) (33). They can be divided into activating and inhibiting receptors, depending on the transduction of their signals via immunoreceptor tyrosine-based activation (ITAM) or immunoreceptor tyrosine-inhibitory motifs (ITIMs), respectively. In humans, only Fc $\gamma$ RIIb acts inhibitory, whereas all others are activating receptors (42). In early studies with monoclonal antibodies directed against TAAs, different efficacy of murine IgG1 or IgG2a could be observed with respect to ADCC (43). This can be explained by the net result of binding capacities to either activating or inhibitory receptors of the two subclasses (44).

These findings are also valid in humans, but as the nomenclature of IgG subclasses differs between the murine and human IgG system, differently labeled subclasses were investigated. How functionally mouse and human IgGs correspond to each other is depicted in Table 2 (45). When Bruhns et al. investigated the binding capacities of different human IgG subclasses to Fc gamma receptors, they could elucidate that IgG1 and IgG3 can bind to all Fc-gamma receptors and that the inhibitory receptor Fc $\gamma$ RIIb has a lower affinity for IgG1, IgG2, and IgG3 than other human Fc $\gamma$ Rs (i.e.  $K_A \sim 2 \times 10^5/M$  compared with, for example,  $K_A \sim 6.5 \times 10^7/M$  for IgG1 to Fc $\gamma$ RI). However, IgG4 has a relatively higher affinity toward Fc $\gamma$ RIIb than to Fc $\gamma$ RIIIa and Fc $\gamma$ RIIIa (46), which led to the present understanding of IgG4 being an anti-inflammatory antibody, supporting the immune system in dampening inappropriate inflammatory reactions (47, 48). In particular, in allergy, IgG4 mediates

**Table 2** Functional correspondence between human and mouse IgG subclasses

Human	Mouse
IgG1	IgG2a
IgG2	IgG3
IgG3	IgG2b
IgG4	IgG1

**Table 1** Overview of FDA-approved monoclonal antibody therapies (adapted from (29))

Antibody	Conjugate	Subtype	Brand name	Target
Cetuximab	–	Mouse/human chimeric IgG1	Erbix <sup>®</sup>	EGFR
Panitumumab	–	Human IgG2	Vectibix <sup>®</sup>	EGFR
Trastuzumab	–	Humanized IgG1	Herceptin <sup>®</sup>	HER-2
Bevacizumab	–	Humanized IgG1	Avastin <sup>®</sup>	VEGF
Ipilimumab	–	Human IgG1	Yervoy <sup>®</sup>	CTLA-4
Rituximab	–	Mouse/human chimeric IgG1	Rituxan <sup>®</sup> /MabThera <sup>®</sup>	CD20
Ofatumumab	–	Human IgG1	Arzerra <sup>®</sup>	CD20
<sup>90</sup> Y-Ibritumomab Tiuxetan	<sup>90</sup> Yttrium	Murine IgG1	Zevalin <sup>®</sup>	CD20
<sup>131</sup> I-Tositumomab	<sup>131</sup> Iodine	Murine IgG2	Bexxar <sup>®</sup>	CD20
Brentuximab Vedotin	Monomethyl auristatin E	Mouse/human chimeric IgG1	Adcetris <sup>®</sup>	CD30
Gemtuzumab Ozogamicin	Ozogamicin	Humanized IgG4	Mylotarg <sup>®</sup>	CD33
Alemtuzumab	–	Humanized IgG1	Campath <sup>®</sup>	CD52

allergy-blocking effects (either on the mast cell or at the antigen-presenting cell), accompanied by increased production of IL-10, induction of T-regulatory (Treg) cells (49), and a decrease in symptoms (48). Hence, in malignant disease, the same IgG4-mediated mechanism rendering IL-10 production and Treg induction could likely prompt tumors to escape immunosurveillance. As demonstrated recently, a monoclonal IgG4 directed against chondroitin sulfate proteoglycan 4 (CSPG4), a surface antigen expressed by >80% of malignant melanomas, was ineffective in triggering effector cell-mediated tumor cell killing *in vitro*. Moreover, when competitively applied with an IgG1 of the same specificity, this IgG4 significantly impaired the tumoricidal impact of anti-CSPG4-IgG1 in a human melanoma xenograft mouse model (50). In line with these findings is another study by Huang et al. (51): when a carcinoembryonic antigen (CEA)-specific IgG4 antibody was converted to IgG1, it significantly gained CDC and ADCC capacity against CEA-expressing tumor cells.

ADCC is one of the most important killing mechanisms harnessed in passive immunotherapy of cancer, underlined by findings that mice deficient for activating receptors Fc $\gamma$ RI and Fc $\gamma$ RIII were unable to mount protective immune responses against a challenge with tumor cells presenting a virus-encoded tumor-specific antigen (52). In contrast, mice deficient for the inhibitory receptor Fc $\gamma$ RIIb showed high capacity of ADCC, resulting in tumor growth arrest of subcutaneously grafted BT474 breast cancer cells. Similar effects could be observed in these knockout mice in a pulmonary metastasis model with B16 melanoma cells, where antibody treatment mediated a 100-fold reduction in pulmonary metastasis load compared with untreated animals (53). In humans, binding of IgG1 is affected by a genetic polymorphism of Fc $\gamma$ RIIIa on position 158 in the IgG-binding domain (phenylalanine F or valine V, with significantly better binding to Fc $\gamma$ RIIIa185V) (54). Accordingly, in a subpopulation analysis of 54 trastuzumab-treated patients with breast cancer, Musolino et al. could depict that individuals homozygous for Fc $\gamma$ RIIIa185V/V showed significantly better objective response rates (ORR) and significantly better progression-free survival (PFS) than heterozygous Fc $\gamma$ RIIIa185V/F or homozygous for Fc $\gamma$ RIIIa185F/F. These findings correlated with significantly higher levels of ADCC in a cytotoxicity assay using peripheral blood mononuclear cells (PBMCs) purified from Fc $\gamma$ RIIIa185V/V patients. For other polymorphisms of Fc $\gamma$ RIIIa (histidine H or arginine R on position 131) and Fc $\gamma$ RIIb (isoleucine I or threonine T on position 232), no clinically significant difference could be found but only a trend toward better ORR and longer PFS for the Fc $\gamma$ RIIIa131H/H genotype (55). Similar effects could be demonstrated in 49 patients with follicular non-Hodgkin's lymphoma treated with the anti-CD20 IgG1 antibody rituximab (56). Also in a patient cohort with metastatic irinotecan-refractory colorectal cancer, treatment with cetuximab resulted in significantly better outcome rates in Fc $\gamma$ RIIIa158V/V homozygous patients with respect to PFS; but also this study failed to display a significant difference for the Fc $\gamma$ RIIIa131 genotype (57).

Another recently discovered regulation mechanism of Fc $\gamma$ R function is high copy number variation in their respective gene loci, which is in clear contrast with the gene loci for other Fc receptors (40). It could be shown that there is an association between gene copy number and surface expression of Fc $\gamma$ RIIIb in neutrophils (58), resulting in enhanced uptake of and adherence to immune complexes (59). Consistently, it was also demonstrated for NK cells from individuals with two or three copies of the FCGR3A gene that also a gene dosage effect for Fc $\gamma$ RIIIa receptor levels as well as for ADCC function exists (60). Several recent studies associated copy number variations of FCGR genes to autoimmune diseases such as systemic lupus erythematosus (SLE), low copy number of FCG3B (61, 62), Sjogren's syndrome (low copy number of FCG3B) (63), rheumatoid arthritis (low copy number of FCG3B) (64), and antglomerular basement membrane antibody disease (anti-GBM disease, high copy number of FCGR3A) (65). In particular for SLE and the deletion of FCG3B, the evidence is clear, as a meta-analysis could confirm this association (66). Clearly, such observations should be included in further studies attempting to identify genetic risk factors for autoimmunity (67). However, the effect of these copy number variations concerning tumor immunology and tumor immunotherapy has not been investigated yet and might also contribute to success or failure of IgG-based immunotherapies.

Altogether, epigenetic modification should be considered as a very important factor in all antibody strategies currently applied for immunotherapy of cancer.

#### Antibody optimization approaches: trials and pitfalls

Different approaches to use the documented effects of Fc $\gamma$  receptor polymorphisms have been pursued therapeutically, for example, by modulation of IgG binding to Fc $\gamma$  receptors via site-directed mutagenesis, mediating significantly higher rates of tumor cell lysis via ADCC (68).

Biochemical studies could reveal that variations in post-translational glycosylation of constant regions in antibodies' heavy chains are also of high relevance for binding to different Fc $\gamma$  receptors (44). So-called glycoengineering of monoclonal antibodies such as the modification of the N-glycosylation pattern at Asn 297 of the IgG heavy chain into reduced fucose content in Fc glycan seems to enhance binding to Fc $\gamma$ RIIIa resulting in higher ADCC levels of cancer cells as well as mediating survival benefits in a CEA-overexpressing xenograft model (69). Another chimeric antibody with low fucose content in its Fc region, ublituximab, directed against CD20, had a marked antitumor effect in intracerebral and intraocular mouse models of lymphoma, resulting again in significantly increased survival rates (70).

First examples of glycoengineered antibodies even made their way into clinical testing, like the humanized anti-CD20 antibody, obinutuzumab (GA101). A recently finished phase I study in Japanese patients with relapsed or refractory B-cell non-Hodgkin's lymphoma exhibited an acceptable safety profile for obinutuzumab, with no dose-limiting toxicities

observed up to doses of 2000 mg ( $c_{\max} = 1910 \pm 156 \mu\text{g/ml}$ ), while end-of-treatment response rates were 58% (71). In a different phase I clinical trial, obinituzumab was administered as maintenance therapy for 2 years, which was again well tolerated (72), leading to current phase III testing of this compound. Another example for a glycoengineered monoclonal antibody in clinical testing is the EGFR-targeting RG7160 (GA201), for which a dose-escalating study showed acceptable safety while exhibiting efficacy in a study cohort of 75 patients with advanced EGFR-positive solid tumors (73).

This also indicates that the expression system for anticancer antibodies is of crucial importance, not just because of efficacy but also for safety aspects. Recently, Platts-Mills et al. observed for cetuximab that it contains galactose- $\alpha$ -1,3-galactose ( $\alpha$ -Gal), an immunodominant glyco-epitope derived from SP2/0 cells used as expression system, leading to a risk of anaphylaxis (74). SP2/0 cells, a murine hybridoma cell line (75), encodes, in contrast to other mammalian expression systems, the gene for  $\alpha$ -1,3-galactosyltransferase ( $\alpha$ -1,3GT), thereby modifying cetuximab post-translationally with  $\alpha$ -Gal residues. Interestingly, nonprimate mammals and New World monkeys decorate glycolipids and glycoproteins with  $\alpha$ -Gal, but not humans, apes, and Old World monkeys, as  $\alpha$ -1,3GT became inactivated in ancestral Old World primates (76, 77). As antigens of the AB0 blood group system are also oligosaccharide moieties, which are closely related to  $\alpha$ -Gal, preformed antibodies against  $\alpha$ -Gal exist in humans (78, 79). Moreover, Platts-Mills et al. could demonstrate that a subgroup of patients with cancer already harbored IgE against  $\alpha$ -Gal prior to cetuximab treatment. Interestingly, a series of those patients also reported episodes of anaphylaxis or severe angioedema 1–3 h after eating red meat (80). Additionally, there was a striking geographic difference in the prevalence of IgE antibodies against  $\alpha$ -Gal with high numbers in the southeast of the USA (Tennessee, Arkansas, and North Carolina) compared with northern or western areas (Massachusetts and California) (74). In a follow-up study, the same group could identify tick bites as the cause of these phenomena. They identified a strong epidemiologic correlation with histories of tick bites and could correlate it with IgE antibodies specific for tick salivary proteins, being  $\alpha$ -Gal decorated, which are potent immunogens (81). The expression of alpha-Gal in red meat explains the potential for associated food-related symptoms.

Overall, oncologists are more and more confronted with hypersensitivity reactions to monoclonal antibodies as well as chemotherapeutics, and pretreatments with antihistamines and cortison belong to their clinical routine. Specifically for that, precise desensitization protocols have been elaborated (82, 83).

### Anticancer IgM, IgA, and IgE

Other optimization approaches aim at engaging different classes of immunoglobulins than IgG. IgM antibodies, physiologically representing the first line of immune response to foreign antigens, could be one option. In particular, it was

discovered that the majority of natural antibodies against cancer cells are IgMs, directed against new carbohydrates on post-translationally modified cell surface receptors of malignant cells (84, 85). Although research in this field is young and recombinant IgMs to peptide epitopes not far developed yet, first results are promising. In a model of metastasizing malignant melanoma, a tumor entity with very limited treatment options, Dobroff et al. (86) could demonstrate that monoclonal IgM antibodies reactive to histone 1 can reduce the number of lung nodules in mice.

Generally therapeutic antibodies are directed against epitopes on cell surfaces; however, especially in autoimmune diseases, early studies suggested an uptake of autoantibodies into viable cells (87), leading to apoptosis (88). As many oncoproteins are located intracellular, for example, phosphatase of regenerating liver-3 (PRL-3) or the polyomavirus middle T (mT) oncoprotein, novel targeting approaches via intracellular antibodies have been evolved recently (89). In addition, combination therapies as antibody–drug conjugates (ADCs) could be highly beneficial using these antibodies as vehicles (90).

As IgM antibodies are formed upon the primary encounter with antigens, their affinity is in general low before affinity maturation occurs during an isotype switch from IgM to IgG, IgA, or IgE (45, 91). For monoclonal antibodies against tumor-associated antigens, affinity values to glycan epitopes have been measured in the range of 0.5 nM/l (anti-human embryonic stem cell monoclonal antibody Hesca 2) (92) to 0.04 nM/l (anti-Sialyl-Lewis<sup>a</sup>, also known as tumor-associated antigen CA19.9) (93).

IgA, however, either in monomeric (94) or dimeric form (95), can attract a similar panel of effector cells as IgG. NK cells, granulocytes, monocytes, or macrophages express the Fc alpha receptor CD89 (96), but IgA could lead to diverse effector mechanisms (94, 97, 98). IgA can trigger substantial amounts of ADCC via Fc $\alpha$ RI, which could be demonstrated elaborately for immature neutrophils, mobilized from the bone marrow upon stimulation with G-CSF (99).

Not only IgA, but also IgE antibodies could be beneficial in this aspect, as IgE is able to mediate high levels of ADCC. Fu et al. (100) could demonstrate that IgE antibodies purified from patients suffering from pancreatic cancer act *in vitro* cytotoxic against pancreatic cancer cell lines. Additionally, IgE can engage a broad panel of effector cells in tumor defense, with a high cytotoxic and phagocytic potential upon binding to IgE receptors (101), as well as restimulate the immune system via IgE-mediated facilitated antigen uptake and consecutive presentation (102).

### Fc $\epsilon$ -receptor-mediated tumor cell killing

Fc $\epsilon$  receptors comprise, in contrast to Fc $\gamma$  receptors, only of two classes: Fc $\epsilon$ RI and Fc $\epsilon$ R2 (CD23), whereupon Fc $\epsilon$ RI is also termed ‘high-affinity IgE receptor’, and CD23 is known as ‘low-affinity IgE receptor’ (103).

Additionally, galectin-3 has IgE-binding properties, but its entire function in the context of IgE remains to be determined. So far it is known that it can have pro-inflammatory



functions in a mouse asthma model (104), via activating mast cells or basophils by cross-linking receptor-bound IgE (105).

However, both 'high-affinity' FcεRI and 'low-affinity' CD23 show outstanding affinity to the Fc domains of IgE. For FcεRI, the affinity is in the range of  $K_a \sim 10^{10}/M$ . CD23 belongs to the C-type (calcium dependent) lectin superfamily of receptors and displays three lectin domains each having a  $K_a \sim 10^6\text{--}10^7/M$  to IgE, thus ranging in the average affinity of Fcγ receptors (106). The avidity of the CD23 trimer increases the affinity to a  $K_a \sim 10^8\text{--}10^9/M$  approaching the high affinity of FcεRI (107) and again exceeding the affinity of IgG to its high-affinity receptor FcγRI (106).

Using recombinant IgE antibodies specific for folate receptor-α on ovarian cancer cells, Karagiannis et al. (101) could demonstrate that monocytic killing of tumor cells via ADCC is FcεRI-dependent: blocking of IgE binding to FcεRI on monocytes with monoclonal antibodies or with a soluble α-chain of FcεRI (108), resulting in substantially decreased ADCC. CD23 on monocytic cells, however, which is upregulated upon incubation with IL-4 and IL-13, has the function to clear IgE-antigen complexes from the circulation, and it could be demonstrated that this mechanism can lead to IgE-mediated phagocytosis (ADCP) of tumor cells (107). In this ovarian cancer model, IgE-armed monocytes killed tumor cells via FcεRI-mediated cytotoxicity, followed by CD23-mediated phagocytosis of the remaining cell fragments (101, 108).

Subsequently, side-by-side comparison studies of ADCC and ADCP of the clinically applied anti-HER-2 antibody trastuzumab (Herceptin<sup>®</sup>, IgG1) and a trastuzumab-like IgE were performed in a breast cancer model, using HER-2-overexpressing cells as targets and the monocytic cell line U937 as effector cells. In this setting, indeed ADCP was the major mechanism of trastuzumab IgG killing, whereas IgE rather triggered monocytes to ADCC of tumor cells (34). The same effect could be observed in a recent study where the clinically used antibody cetuximab (Erbix<sup>®</sup>, again IgG1) and cetuximab-like IgE were compared in the same ADCC/ADCP assay using this time EGFR-overexpressing A431 cells as targets (Plum et al., unpublished observations). The classical cetuximab (IgG1) mediated phagocytosis, as well as cytotoxicity, concentration dependently. In contrast, cetuximab-like IgE samples caused much less phagocytosis, but significantly higher ADCC levels than those of the IgG, in a concentration-dependent manner (109).

## IgE effector cells

### Eosinophils

The IgE-mediated tumoricidal mechanisms of monocytic cells are also valid for eosinophilic granulocytes, being among the most classical IgE effector cells. For long, they were just known for their role in allergy or defense of helminthic parasitic infections (110). However, when human eosinophils were purified from venous blood and armed with the antifolate receptor-α-specific IgE described above, ADCC of ovarian

cancer cells could be measured. In contrast to killing by monocytes, no phagocytosis of ovarian cancer cells could be determined (108). This could be due to low constitutive expression of CD23 on the surface of eosinophils (111) or lack of CD23 expression on the surface of the eosinophils used in these assays (108). Upon IgE activation, eosinophils can release cytotoxic mediators such as eosinophil cationic protein (ECP), major basic protein (MBP), eosinophil peroxidase (EPO), and eosinophil-derived neurotoxin (EDN). These proteins are well investigated in their cytotoxic action against bacteria, parasites, and viruses, but also respiratory epithelium and cancer cells (112). Synthetic eosinophil-derived neurotoxin, slightly modified by adding four extra residues, has even been studied as a therapeutic agent on its own in Kaposi's sarcoma *in vitro* (113, 114). Moreover, eosinophilic granulocytes are able to release TNF-α (115, 116), and it could be demonstrated in a recent study by Legrand et al. (117) that cytotoxic killing of colon cancer cells by eosinophils can be mediated through TNF-α and granzyme A. On the other hand, eosinophils could be ambivalent (118), as they play a role in tissue remodeling in allergic and malignant diseases via mediators such as basic fibroblast growth factor (b-FGF), IL-6, IL-8, granulocyte-macrophage colony-stimulating factor (GM-CSF), platelet-derived growth factor (PDGF), and transforming growth factor-beta (TGF-β) (112).

Eosinophilic peroxidase (EPO) is a haloperoxidase enzyme, whose catalyzed metabolites have been shown to promote oxidative stress and subsequent cell death by apoptosis or necrosis (119). However, even for this eosinophilic enzyme, it could be demonstrated that at noncytotoxic levels, it can drive cell cycle progression and proliferation by signaling via the tumor-associated receptor tyrosine kinase HER-2 (120).

As eosinophils were described to be found in several cancer entities including malignancies of the head and neck region (121), uterine cervix, esophagus, or the gastro-intestinal tract (122), the term 'tumor-associated tissue eosinophilia' (TATE) was introduced (123). It is not yet clear what TATE means with regard to prognosis (124); studies in oral squamous cell carcinoma range from higher overall survival (121), across no significant association with respect to tumor differentiation, perineural, vascular, and muscular invasion or locoregional metastasis (125), to unfavorable prognosis for heavy eosinophilic infiltration and expression of HLA-DR antigen (126). What has been accepted so far is that blood eosinophilia (tumor-associated blood eosinophilia, TABE (123)) in patients with oral squamous cell carcinoma indicates disseminated carcinoma, resulting in poor outcome (127, 128).

Only further studies with recombinant antitumor IgG vs IgE antibodies will give a definite picture about the ambivalent role of eosinophils in cancer.

### Mast cells

The controversy described above is even bigger for another type of IgE effector cells in and around tumors, mast cells (129). Mast cells, named and discovered by Paul Ehrlich in the 19th century (130), have been identified as eminent

players in allergic and anaphylactic reactions of type I hypersensitivity (131). Upon activation via bi- or multivalent antigen-IgE complex binding, which leads to cross-linking of FcεRI, mast cells released within minutes preformed histamine, heparin, and other proteoglycans, several proteases, and cytoplasmic granule-associated cytokines (132), but also a variety of immunomodulatory mediators, such as histamine, serotonin, IL-2, IL-4, IL-21, TNF, G-CSF, and prostaglandins (133, 134). Clinical symptoms of this mediator release include vasodilatation, increase in vascular permeability, contraction of bronchial smooth muscle, mucus secretion, sneezing, itching, and coughing (135). Activation via FcεRI cross-linking also induces the production of cytokines, chemokines, and growth factors, leading to a second wave of allergic symptoms, also called late-phase reactions that typically develop 2–6 h after allergen encounter and peak after 6–9 h (132, 135). Chronic exposure to allergens results in constitutive activation of mast cells leading to tissue remodeling, for example, an increase in mucus-producing goblet cells in the airway epithelium, subepithelial membrane thickening through increased lung collagen deposition, neoangiogenesis, and an increased bronchial smooth muscle mass (132, 136). Similar effects could be demonstrated in a model of human skin, where sonicates of mast cells significantly increased fibroblast proliferation, collagen synthesis, and collagen contraction; surrogates for skin remodeling; and fibrosis (137). These remodeling effects, especially the induction of angiogenesis and neovascularization, are detrimental in malignant diseases (138).

Besides their role in allergy, mast cells are important players in the defense of parasitic infections, such as nematodes and protozoa (139). Furthermore, mast cells contribute to an efficient immune response to bacteria, as it could be demonstrated in an *in vivo* model of skin infection with *Pseudomonas aeruginosa*, where mast cell-deficient mice showed increased lesions due to impaired neutrophil recruitment and bacterial clearance (140).

With respect to tumors, mast cells were reported early in tumor-surrounding tissues of different malignant lesions, even by Paul Ehrlich himself. He assumed that mast cells directly fulfill nutritional requirements of malignant tissues (130, 141). This is definitely not the case, but the distinct role of mast cells in oncology is still a matter of debate (142).

One big research topic is how mast cell-derived proteases act on tumor progression. Aromando et al. (143) could demonstrate in a hamster cheek pouch carcinogenesis model that tumor growth was stimulated by mast cell-specific serine protease-6 (MCP-6, tryptase) through activation of protease-activated receptor-2 (PAR-2) on the surface of carcinoma cells. This finding is in line with a previous *in vitro* study demonstrating that mast cell tryptase stimulates the growth of DLD-1 colon adenocarcinoma cells through PAR-2 and mitogen-activated protein kinase (MAPK)-dependent manner (144). Similar tumor-promoting effects could be demonstrated when investigating mast cell-specific serine protease-4 (MCP-4, chymase), which can activate progelatinase B, thus acting as proangiogenic (145). But when de Souza et al. investigated in a recent study the

expression of mast cell proteases MCP-4, MCP-5, MCP-6, MCP-7, and carboxypeptidase A, they could correlate that all proteases increased during tumor progression in a chemically induced skin tumor model, with the exception of MCP-4. Moreover, they could demonstrate that MCP-6 and MCP-7 were able to induce blood vessel formation *in vitro* (146). Recapitulating these studies, the function of mast cell proteases still remains not fully clear, as it has to be considered that cancerogenesis studies use different chemical compounds for tumor initiation and promotion and different sites, which could also affect the overall susceptibility of the animal to the tumor.

However, there is strong evidence for mast cell-related angiogenesis in tumor growth (138, 145, 146), and also, the multiple immunomodulatory effects of mast cells are intensively investigated, which will clarify the enigmatic role of mast cells in malignant disease.

### Basophils

Other major players of Th2-driven immune responses as well as possible potent effector cells of IgE-based immunotherapies are basophils. Basophils share many features with mast cells, both were initially described by Paul Ehrlich, both express FcεRI, and both release histamine upon IgE binding. Whereas mast cells are located primarily in the tissue, basophils can be found in circulation, but with less than 1% of leukocytes in healthy human beings; basophils are the least abundant immune cell population (147). Apart from their contribution to allergic (148) and anaphylactic reactions (149, 150), basophils play a crucial and nonredundant role in defense of endo- and ectoparasites such as helminths (151) or ticks (152).

As basophils are one of the major sources of histamine and anaphylactic mediators in the circulation during an anaphylactic shock (153), one of the major concerns of passive immunotherapy of cancer with monoclonal IgE antibodies is that intravenously applied IgE sensitizes FcεRI on basophils and could potentially be cross-linked by soluble tumor-associated antigens in the circulation, which are shed by tumors. Therefore, it is crucial to target only epitopes, which are not repetitively expressed on the target antigen and do not occur complexed in the circulation. Such antigens, which form tumor-associated molecular patterns (154) on the cell, would solely lead to degranulation in the tissue, but not in the circulation. Tumor-associated antigens that fulfill these requirements are, for example, EGFR and HER-2, for which we could demonstrate that only the dense and rigid antigen display on the surface of cancer cells leads to degranulation of IgE-loaded rat basophilic leukemia cells (RBL-SX38, transfected with human FcεRI), whereas the soluble, monomeric protein shows no effect (34, 109). Rudman et al. could demonstrate in a recent study that patients with ovarian cancer displayed elevated levels of folate receptor-α not only on cancer cells but also in the circulation (up to 35 ng/ml). Still, sera of these patients could neither trigger degranulation of RBL-SX38 cells loaded with antifolate receptor-α-specific IgE, nor activate basophils of healthy donors in an *ex vivo*

setting again preloaded with antifolate receptor- $\alpha$  IgE (155, 156).

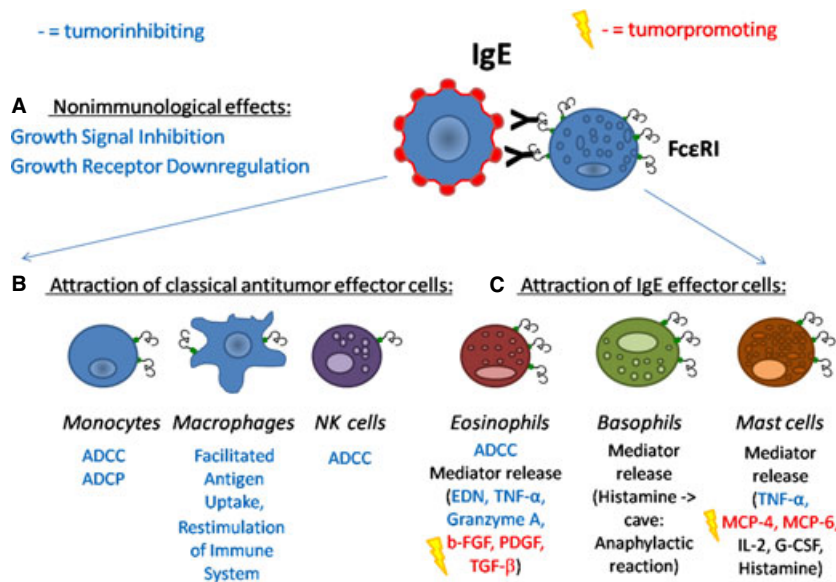
Although this study is very promising, future work in this direction is required, as there are many reports of circulating tumor cells in serum of patients (157–159), and so far it was not investigated how this could affect possible applications of IgE-based immunotherapies.

**How to approach translation – from bench to bedside**

Clearly, more studies with respect to safety and clinical efficacy are needed to clarify the advantages or complementary effects of IgE-based immunotherapy of cancer (Fig. 1). In particular, side-by-side comparison studies with ‘next-generation’ antibodies such as ‘glycoengineered’ IgG or IgA and IgE antibodies of the same specificity could assess the different potential of antibody classes with particular respect to ADCC and ADCP. There is also great demand for further *in vivo* studies above preclinical proof-of-concept in mice, given the fact that *in vivo* efficacy of TAA-specific IgE antibodies has already been demonstrated in severe combined immunodeficiency (SCID) mice with xenografted tumors (101, 160, 161). However, these observed effects do not fully represent the natural picture, which can be expected in cancer patients with spontaneous tumors. To overcome this experimental limitation is not trivial. In contrast to Fc $\gamma$  receptors, which are similarly distributed on human and

murine immune cells, the distribution patterns of Fc $\epsilon$  receptors differ considerably. Whereas Fc $\epsilon$ RI is expressed on human mast cells, basophils, eosinophils, monocytes, Langerhans cells, and dendritic cells, in mice, it could be only found on mast cells and basophils (162). Therefore, mouse strains transgenic for human Fc $\epsilon$ RI have been generated by introducing the human  $\alpha$ -chain of Fc $\epsilon$ RI, which displays the IgE-binding site. Functionality of the receptor could be shown on mast cells (163, 164), as well as monocytes, epidermal Langerhans cells, basophilic and eosinophilic granulocytes (162), making these transgenic mice important models to study the biologic function of IgE (165, 166) and models to investigate the effects and potential of passively applied IgE against grafted tumors (167).

In this context, it is important to note that the dog (*Canis lupus familiaris*) shares a much more similar Fc $\epsilon$ RI expression pattern with humans, with functional Fc $\epsilon$ RI expression not only on mast cells (168), but also on Langerhans cells (169). This results in similar prevalence and pathophysiology of atopic and anaphylactic reactions, underlined by the fact that the historically first described anaphylactic reaction has been observed in a dog model by Paul Jules Portier and Charles Robert Richet (130, 170). In recent years, the value of the dog as a research model has been rediscovered for food allergy and atopic dermatitis (171, 172). As a coincidence, dogs also spontaneously develop tumors, again of striking homology to human disease (173).



**Figure 1** Effects of IgE-based immunotherapy of cancer. (A) Immunotherapy with IgE antibodies can lead to nonimmunologic effects such as growth signal silencing or growth receptor downregulation, due to their epitope specificity. (B) Immunologic effects comprise the attraction of classical antitumor effector cells such as monocytes, macrophages, or NK cells, leading to antibody-dependent cell-mediated cytotoxicity (ADCC) or phagocytosis (ADCP) of cancer cells. Macrophages are also employed to restimulate the immune system, due to their ability for facilitated antigen uptake via Fc $\epsilon$  receptors. (C) Moreover, classical IgE effector cells are

allured to the site of the tumor, that is, eosinophils, basophils, and mast cells. These cells lead again to ADCC of tumor cells, but release additionally specific mediators, which have been shown to act tumor-inhibiting and/or tumoricidal, such as eosinophil-derived neurotoxin (EDN), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), or granzyme A. As these cells are also involved in tissue remodeling, known tumor-promoting agents can be released as well, such as basic fibroblast growth factor (b-FGF), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF- $\beta$ ), or mast cell-specific serine proteases (MCP-4 and MCP-6).

Combining both aspects – IgE pathophysiology and cancer biology – it can thus be anticipated that canine patients would be an ideal natural model, independent of tumor transplants, but developing spontaneous tumors like human patients. Dog patients suffering from cancer simultaneously offer the same IgE effector cell panel, being an ideal model for a potential AllergoOncology trial. They have the same risk of side-effects, but also potentially the same therapeutic benefits as human oncology patients. Such a study could overcome the limitations of the human FcεRI mouse as well as other rodent model organisms, for example, the rat, which although shares IgE receptor biology with humans and is therefore a valuable model in allergy research (174–176), but again would have to get tumors either grafted or artificially induced.

For dogs, however, almost 400 inherited disorders are characterized (177), many of those leading to cancer (178). Moreover, there are several hundred isolated breeds of dogs, and each has a vastly reduced genetic variation. Therefore, several breeds prone to certain malignancies, for example, Golden Retrievers for hemangiosarcoma or Irish Wolfhounds, Siberian Huskies, and Shih Tzus for T-cell lymphoma, could be easily investigated and treated (177).

As dogs live in the same environment like their owners, they share similar risk factors for cancer: age, obesity in early life, and a diet rich of red meat are all associated with higher incidence of mammary carcinoma (179). Also hormonal factors, like reproductive cycles, appear to be similar (180), resulting in expression of estrogen receptors on canine breast cancer cells (181, 182). However, it long seemed that the dog is distinct in its sensitivity to a mammary tumor-promoting effect of progestins. This was due to the fact that the progestin induced growth hormone (GH) in the mammary gland (183), a mechanism that could later also be detected in human mammary tumors (184, 185). But it is still not fully clear, how hormone replacement therapy including progestins changes the risk of breast cancer in women treated with hormone replacement therapy including progestins (186–188).

Furthermore, it could be demonstrated that these malignancies also share biologic properties, as canine homologues of the tumor-associated-antigens EGFR and HER-2 could be detected on canine mammary carcinoma (189–192). This perception that malignancies in companion dogs and humans occur according to very similar biologic principles has attracted attention because it offers a chance to speed up drug development for both, humans and animals, for now peaking in the establishment of the comparative oncology trial consortium (COTC) by the National Cancer Institute (NCI; <http://ccr.cancer.gov/resources/cop/COTC.asp>; an overview of the most recent clinical comparative trials initiated by the COTC is depicted in Table 3). In line with this concept, we could show in a recent work that the canine EGFR and HER-2 homologues are susceptible to cetuximab and trastuzumab targeting, leading to growth arrest due to growth signal inhibition (173). Combining both aspects – that dogs resemble similar biologic properties

**Table 3** Overview of current comparative oncology trials initiated by the Comparative Oncology Trials Consortium of the National Cancer Institute

Study No.	Name	Status
COTC001	Evaluation of RGD Targeted Delivery of Phage Expressing TNF-alpha to Tumor Bearing Dogs	Closed trial
COTC003	Evaluation of the MTOR inhibitor Rapamycin in dogs with osteosarcoma	Closed trial
COTC005	Evaluation of immunocytokine fusion protein in tumor-bearing dogs	Closed trial
COTC006	Evaluation Cryobiopsy Instrumentation And Cellsave Blood Collections In Dogs With Lymphoma	Closed trial
COTC007a	A Pilot Study of Topotecan in Dogs with Lymphoma	Closed trial
COTC007b	Preclinical Comparison of Three Indenoisoquinolines Candidates in Tumor Bearing Dogs	Open
COTC008	Evaluation of the mTOR inhibitor Rapamycin in Dogs with Metastatic Osteosarcoma	Closed trial
COTC010	Evaluation of two immunocytokine fusion proteins in tumor bearing dogs	Closed trial
COTC013	Evaluation of Orally Administered mTOR inhibitor Rapamycin in Tumor Bearing Dogs	Closed trial
COTC016	A Pilot Study to Assess Feasibility of Tissue Collections and Molecular Profiling for future Comparative Oncology Personalized Medicine Studies	Closed trial
COTC018	Evaluation of a novel anticancer agent in tumor bearing dogs to define its pharmacokinetic profile and biological activity	Open

Source:<http://ccrod.cancer.gov/confluence/display/CCRCOPWeb/Clinical+Trials>.

according to the development of malignancies as well as to develop atopic diseases – we suggest the ‘caninization’ of cetuximab and trastuzumab antibodies to canine IgG and IgE antibodies, respectively, in order to more accurately assess side by side the full potential of IgE-based immunotherapies against cancer and important therapy-related safety issues.

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### Conflicts of interest

Both authors declare that they have no conflicts of interest related to this manuscript.



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