Journal of Immunooncology

Nerina, etal., J Immunooncol 2016, 2:1

review Open access

Immunological Effect of Anti-Epidermal Growth Factor Receptor (EGFR) Antibodies in Squamous Cell Carcinoma of the Head and Neck (HNSCC): the Present and the Future

Denaro Nerina[1*], Elvio Grazioso Russi[2], Lo Nigro Cristiana[3] and Marco Carlo Merlano[1]

1Oncology Department Santa Croce e Carle, General Hospital, Via Michele Coppino 21 12100 Cuneo, Italy

2Radiotherapy Department Santa Croce e Carle, General Hospital, Via Michele Coppino 21 12100 Cuneo, Italy

[3]Oncology Translational Laboratory, Oncology Department, Santa Croce e Carle, General Hospital

Via Michele Coppino, 21 12100 Cuneo, Italy

[*]**Corresponding author:** Denaro Nerina, Oncology Department Santa Croce e Carle, General Hospital, Via Michele Coppino 21 12100 Cuneo, Italy, Tel: +39 0171616350; Fax: +39 0171616360; E-mail: nerinadenaro@gmail.com

Rec date: Jan 29, 2016; **Acc date:** Mar 10, 2016; **Pub date:** Mar 14, 2016

Abstract

Targeted therapy with anti Epidermal growth factor receptor (EGFR) monoclonal Antibodies (mAbs) offers the potential to improve outcomes in HNSCC. EGFR is over-expressed in 80 to 90% of HNSCC and leads to tumor cell proliferation and invasion. HNSCC is an immunogenic disease, it has a multiplicity of non-mutually exclusive mechanisms of immune suppression (e.g. reduction CD8+ cell influx and altered function of intra-tumoral CD4+ cells). Monoclonal Abs possess the potential advantage of recruiting immune effector mechanisms, such as complement-dependent cytotoxicity (CDC) and Ab-dependent cellular cytotoxicity (ADCC), as additional mechanisms of tumor cell killing. However immunotherapy with the EGFR-specific mAb Cetuximab is clinically effective in 10-20% of patients. There is a need to further understand the immunological mechanism of the mAbs to optimize the design of a target-based immunotherapy.

Keywords: Anti EGFR; ADCC; Cetuximab; Immune effectors

Introduction

Epidermal growth factor receptor (EGFR) is over-expressed in a wide range of malignancies, including HNSCC, and initiates important signal transduction pathways in HNSCC carcinogenesis. mAbs neutralize EGFR promoting apoptosis or arrest growth of tumor cells merely by binding their target. Monoclonal antibodies (mAbs) anti EGFR are widely used in clinical practice, although their optimal use remain unclear [1]. Ideal patient candidate to mAbs, mechanisms of action and resistance of anti EGFR mAbs, the effect of human papillomavirus status on outcomes, their role when combined with induction chemotherapy or adjuvant radiation, and optimal management of skin toxicity and hypersensitivity reactions. These mAbs can exert direct antiproliferative/cytotoxic effects as they inhibit pro-survival signal transduction cascades but probably they also induce a tumor-targeting immune response [2]. Primary and acquired resistances are serious problems and are responsible for low single-agent response rate and tumor recurrence.

Therapeutic resistance to anti-EGFR therapy may arise from mechanisms that can compensate for reduced EGFR signaling and/or mechanisms that can modulate EGFR-dependent signaling. New anti-EGFR mAbs Nimotuzumab, MEHD7945A, Necitumumab, and RO5083945) are currently under investigation in phase II and III clinical trials in different HNSCC therapeutic settings. The aim of this review is to provide a comprehensive analysis of immuno-modulatory effect of anti EGFR Antibodies.

Antibodies

Cetuximab is an anti EGFR monoclonal Antibody, chimeric IgG1, with multiple approved indications.

It is indicated worldwide for treatment of RAS wild-type, EGFR-expressing, metastatic colorectal cancer in combination with FOLFIRI (irinotecan, 5-fluorouracil, leucovorin) for first-line treatment; in combination with irinotecan in patients who are refractory to irinotecan-based chemotherapy, and as a single agent in patients who have failed OR are intolerant oxaliplatin- and irinotecan-based chemotherapy. It is used in initial treatment of locally or regionally advanced HNSCC with radiation therapy (RT) OR in monotherapy for recurrent or metastatic HNSCC progressing after platinum-based therapy (FDA) OR combined with platinum-based therapy with 5-FU for metastatic HNSCC [3,4].

It binds to an epitope of the domain 3 that is stored in Variant III, so that it is active even in the case of EGFRvIII. It has been shown to improve survival in cancers of the head and neck and colon. Recently Schmitz et al. demonstrated that Cetuximab promotes epithelial to mesenchymal transition and cancer associated fibroblasts (which are implicated in clinical resistance) [5].

Panitumumab is an anti EGFR Antibody, human IgG2 indicated for wild-type KRAS (exon 2 in codons 12 or 13) metastatic colorectal carcinoma (mCRC) as determined by an FDA-approved test for this use (eg, therascreen KRAS RGQ PCR Kit).

In HNSCC phase III trial SPECTRUM did not reach the primary endpoint (HR 0.87) [6]. ADCC utilizes the response of innate immune cells to provide antitumor cytotoxicity triggered by the interaction of the Fc portion of the antibody with the Fc receptor on the immune cell.

Cetuximab exert this activity binding the extracellular domain of EGFR, independent of EGFR mutation status.

On the contrary Panitumumab does not induce ADCC, but can still exert immunological activity for its high binding affinity to Fc receptors (FcγIR and FcγIIRa) expressed on granulocytes, dendritic cells and activated monocytes [7].

Zalutumumab is an anti EGFR Antibody, human IgG1. Machiels et al. investigated its efficacy in a phase III trial after Platinum failure versus best supportive care, but the median survival did not differ significantly (6.7 vs 5.2 m) [8].

Nimotuzumab is anti EGFR Antibody, human IgG1, it is characterized by low affinity to the insulin receptor, which tends to improve in direct proportion to the amount of EGFR present. This differentiates it from Cetuximab whose affinity (high) does not vary based on the amount of EGFR expressed. Positive results in combination with RT, but studied only in small studies [9]. It is registered in South America, East Asia, India, Oceania.

Ficlatuzumab (AV-299) is a humanized hepatocyte growth factor (HGF)-inhibitory immunoglobulin G1 (IgG1) monoclonal antibody. HGF is the ligand of c-Met; the HGF/c-Met signaling pathway converges with the EGFR network at both the PI3K/Akt and MAPK nodes. Laboratory data suggest the ability for reciprocal compensation between EGFR and c-Met [10]. Ficlatuzumab is under investigation in a phase I study in combination with Cetuximab to overcome resistance to Cetuximab in patients with RM HNSCC (NCT02277197). Ficlatuzumab significantly reduced tumor associated fibroblasts derived HGF, mitigating cMet signaling [10].

ABT 806 Highly selective anti EGFR mAb, humanized IgG1. It targets an unique epitope exposed only when EGFR is over-expressed or mutated (EGFRvIII); it has been studied in a phase I study reporting minimal rush; activity was 1 PR/5SD in 6 HNC pts [11,12].

Syms 004 is a new antibody mixture to target EGFR in metastatic colorectal cancer. Preclinical data suggest efficacy in anti-EGFR-resistant tumors, but it remains unclear whether a higher toxicity is outweighed by those advantages [13].

Novel anti-EGFR agents are under investigation such as CetuGEX™, this innovative Ab expresses several anti-tumor modes of action (a very strong ADCC, efficient proliferation inhibition via receptor blockage and apoptosis induction. Based on the optimization of a series of sugar determinants CetuGEX™ is 10 to 250-fold more active in anti-tumor ADCC compared to Cetuximab making it highly potent for patients of all ADCC receptor allotypes and KRAS mutant patients (NCT02052960).

Poziotinib, HM781-36B, is a irreversible pan-HER inhibitor. In preclinical studies, HM781-36B has much lower IC50 values than gefitinib in cell lines engineered to express EGFRvIII mutations and produces tumor growth inhibition in gefitinib-resistant xenografts. A phase I trial of HM781-36B in patients with advanced solid tumors showed clinically significant anti-tumor activity and a phase II trials of HM781-36B in patients with non-small cell lung cancer and advanced gastric cancer are currently ongoing [14].

Immunomodulatory effects

Strong evidence supports the concept of immunosurveillance and immunoediting in HNSCC including the density of T CD8+ and CD45+ lymphocytes infiltration. Metastatic cancers released higher levels of the soluble immune inhibitory mediators and lower levels of IFN-gamma and IL-2 than did primary cancers, although CD34+ cells were similarly present in both primary and metastatic cancers [15].

Animal studies have provided conflicting results concerning the relative contributions of Fab- and Fc-mediated effector mechanisms to the therapeutic efficacy of EGF-R Abs. Hoffman et al. demonstrated that anti-EGFR mAb induces leukocyte infiltration to tumor spheroids by up-regulating chemokine expression. This German group investigated the immunological effects of anti-EGFR mAb, in a three-dimensional spheroid model of EGFR-expressing HNSCC. The blockade of EGFR by anti-EGFR mAb in EGFR-overexpressing HNSCC cells led to differential expression (array) of several cytokines and chemokines, including the chemokine MCP-1/CCL-2. This was confirmed by quantitative PCR and ELISPOT analyses and shown to be functionally relevant [16].

In particular it has been suggested that Cetuximab, in addition to direct inhibition of EGFR, may act *via* antibody-dependent cellular cytotoxicity. ADCC is triggered by the binding of the Fc region of the mAb already bound via Fc regions to the target cell to any of the Fc gamma receptors, i.e. CD16,CD32, and CD64 which are expressed with different patterns by cells of the innate immune system, namely monocytes, macrophages, granulocytes and natural killers [17]. Fc receptor for IgG (FcγRIIIa) enhances production of interferon-γ (IFN-γ) in response to Ab-coated targets. Cetuximab and Nimotuzumab as human IgG1 are particularly effective in triggering Complement-dependent cytotoxicity (CDC) and ADCC by NK cells and is therefore predominantly used for antitumor immunotherapy [18].

Complement-dependent cytotoxicity results in the death of antibody-bound cells as a result of activation of the classical complement cascade. The process is initiated by the binding of c1q to the Fc portion of antigen-bound antibody and culminates in the production of a membrane attack complex in the target cell membrane, causing cell lysis. Resistance to CDC by membrane overexpression of complement regulatory proteins is evident in HNSCC. IgG2 antibodies are less efficient in activating the complement cascade, whereas IgG4 is unable to do so.

ADCC allows the antitumor innate immune response but can also trigger the adaptive immune response [19]. *In vivo*, addition of CpG, a TLR9 agonist able to activate dendritic cells (DCs), increases immune response to Cetuximab and its therapeutic efficacy. The extent of in vitro ADCC of malignant cells is influenced by several variables such as expression of the target antigen and mAb concentration. Schneider-Merck et al. evaluated the difference among IgG1 Ab and IgG2Ab. Zalutumumab and Panitumumab, mediated significant ADCC with myeloid effectors cells in vitro; in contrast, only Zalutumumab was able to induce potent ADCC through mononuclear immune cells (MNCs), most likely by effective triggering of NK cells via FcγRIIIa [20].

More importantly, FcγR IIa and IIIa polymorphisms have been shown to play an important role in the clinical efficacy of mAb-based immunotherapy of malignant disease. Single nucleotide polymorphisms (SNPs) in the coding region of FCγR2A or FCγR3A have been reported to correlate with responses to Cetuximab. The role of FCγR2A H/H or FCγR3A V/V genotypes is especially controversial [21].

Panitumumab is not able to induce NK mediated ADCC but may induce ADCC mediated by polymorphonuclear cells macrophages.

The capacity of mAbs to induce tumor-directed cytotoxic T lymphocyte (CTL) recruitment is intriguing. Intratumoral CTL composition and distribution have been associated with clinical outcomes [22], suggesting the relevance of T cells in anti-tumor immunity.

The Future

Dismal results of EGFR Inhibitors led scientists to focus both on target therapies combination, studying other pathway such as PI3K/Akt/mTOR, and on bio-immune-approach to limit immunoescape mechanism.

Therapy beyond EGFR inhibition means increase immunologic effect in tumor microenviroment.the one hand, simultaneous inhibition of EGFR, as well as pathway components such as Akt or mTOR, could circumvent the feedback activation observed with either approach alone. On the other hand combination of checkpoint inhibitors and EGFR inhibition appear very promising due to their correlation.

Lastwika KJ demonstrated that activation of the AKT-Mtor pathway tightly regulates PDL1 expression,promoting immunoescape. In a mouse model a simultaneous inhibition of mTOR pathway and PD1 axis reduced regulatory T cells increasing cytotoxic T cells [23].

Moreover Tang et al. showed the association between EGFR mutational status and programmed cell death-ligand 1 (PD-L1) expression in lung cancer [24]. Basal levels of ADCC correlates with prognosis (Merlano et al. unpublished data). The number of inducible cytotoxic cells (NK cells) and the level of ADCC activity exerted by these cells from tumor patients in the presence of cetuximab might be useful prognostic or predictive parameters for response to treatment.

Direct cytotoxicity other than signal perturbation

Imaging with radionuclide-labelled anti-EGFR and treatment in combination with external radiotherapy are particularly promising approaches. The conjugation might increase therapeutic efficiency. However liver and kidney radionuclide accumulation and economic costs remain a clinical challenge. It is necessary to select a radionuclide-labelled mAbs considering different characteristics of radiation according to decay characteristics, particle range and physical half-life. Yttrium 90 and Zirconium 89 are the most used radionuclide. Present data support an important impact of the tumour micro-milieu on treatment response that needs to be further validated in patients [25].

Fc mediated immune effector engagement

The impact of ADCC on the efficacy of cetuximab might also be influenced by the occurrence of polymorphic forms of genes coding receptors for the antibody Fc region. Human Fc receptors are expressed by a range of immune cell populations, including B cells, dendritic cells, macrophages, mast cells, NK cells and neutrophils [26]. Fc variants enhanced the cytotoxic potency of an anti-CD20 antibody up to 23-fold against tumor cells in CDC assays, and demonstrated a correlated increase in C1q binding affinity. Kellner et al. reported that antibody Fc engineering improves frequency and promotes kinetic boosting of serial killing mediated by NK cells [27]. Several other Fc-engineered antibodies are currently under investing to boost ADCC and NK activity.

Agonist mAbs (Stimulation of CD137 (4-1BB) and CD134 (OX-40); agonist of Toll like Receptors (TLR) Stimulation of CD137 (4-1BB), CD134 (OX40), and GITR (CD357) promotes impressive tumor-rejecting immunity in a variety of murine tumor models. The mechanisms of action depend on a complex interplay of cytolytic T lymphocytes, helper T cells, regulatory T cells, DCs and vascular endothelium in tumors. Agonist mAbs specific for CD137 have shown signs of objective clinical activity in metastatic melanoma patients while anti-OX40 and anti-GITR mAbs have entered clinical trials [28].

A potentially promising aspect of anti-CD137 mAb immunotherapy is combination with other treatments (both conventional and immunotherapeutic). Combination with CT and RT is clearly synergistic in pre-clinical models and likely dependent on eliciting immunogenic cell death with subsequent cross-priming of tumor antigens. Synergistic combinations with vaccines and virotherapy also rely on the principle that CD137 co-stimulation must act on ongoing tumor-specific immune responses encompassing CD137+ activated lymphocytes. Recently, some intriguing pre-clinical studies have shown that anti-CD137 mAb therapy synergizes with NK-mediated ADCC elicited by antibodies targeting the surface antigens CD20 or HER-2 [29]. Development of anti CD137 (BMS663513) was interrupted for severe toxicities [29]. On the contrary anti-OX 40 appear promising in the preoperative setting (MEDI6469 administration).

TLR agonists enhances anti EGFR effect. Preclinical data demonstrated an augmentation of the ADCC due to NK cells activation when combined with several monoclonal antibodies [30,31]. A randomized, double-blind, placebo-controlled Phase 2 trial of Motolimod (VTX2337), TLR8 agonist, in combination with platinum-based chemotherapy and Cetuximab in patients with recurrent or metastatic squamous cell carcinoma of the head and neck has been initiated. Progression-free survival is the primary endpoint of this study [32].

IL-2 secretion and other cytokines

Cytokine administration could be a useful adjuvant in the cetuximab treatment. Several preclinical trials demonstrated that adding IL2, IL 12, and IL-21 enhance the anti-tumor activity of cetuximab by activating the FcR effector mechanisms of NK cells [33].

Costimulated NK cells also secreted elevated levels of chemokines (IL-8, macrophage inflammatory protein-1alpha, and RANTES) that could direct the migration of naive and activated T cells [34]. More advanced tumors exibit a significant decrease in serum IFN-γ, IL-13, and MIP-1β levels and an increase of IL-10, IL-13 [35].

John et al. demonstrated that Inflammatory cytokines (IL1b) contribute to EMT in HNSCC is investigated. IL-1b -treated HNSCC cell lines demonstrated a significant decrease in E-cadherin mRNA and an increase in the mRNA expression of the transcriptional repressor Snail. IL-1b exposure led to enhanced Snail binding at the chromatin level. Snail overexpression in normal oral keratinocytes and HNSCC cells is sufficient to drive EMT and confers resistance to erlotinib [36].

Bispecific Abs capable of targeting both cancer stem cells and tumor antigens may prove to be effecting in eradicating solid tumors [37].

Deactivation suppressive functions of Treg

Physiological activity of Treg includes prevention of autoimmune diseases by maintaining self-tolerance; suppression of allergy, asthma

and pathogen-induced immunopathology; feto-maternal tolerance; and oral tolerance. Suppressive activities attributed to Treg cells is not exclusive and may in reality, at least in some experimental settings, be exerted by conventional Th cell subsets, such as Th1, Th2, Th17 and T follicular (Tfh) cells. Moreover a truly specific molecular marker for Treg cells is lacking, therefore anti T-reg therapies are difficult to be realized. In HNSCC the effectiveness of cetuximab is limited by Tregs mediated immune-suppression that inhibits NK and other effector cells. A phase II trial NCT01581970 investigated the potentiation of cetuximab by Tregs depletion with metronomic cyclophosphamide; results are awaited. However studies have tested antiCD25, antiFoxP3 with few results. Studies in mice have shown that FoxP3-deficient animals lack Treg cells, whereas overexpression of the FoxP3 protein leads to profound immune suppression.

Promotion of memory

Immunological memory involves both T and B cells and results in a secondary antibody response that is faster, of higher affinity, and results in the secretion of non-IgM isotypes of Ig. Following infection, some of the activated T-cells become memory cells that exist in a state of readiness and have the ability to rapidly expand and fight off recurrence of the same disease. Understanding how memory T-cells work will enable scientists to design more effective vaccines. Table 1 summarizes studies on anti EGFR in combination with immunotherapies.

Therapeutic vaccines for HPV-driven malignancies are currently undergoing clinical investigations, an exhaustive review of HPV targeted therapies (vaccines and gene therapy) is not the goal of this review. Table 1 summarizes recent studies on anti EGFR.

Study	Phase	Targets	Setting	Treatment	Status
NCT01468896	II	Microenvironment	RM HNSCC	Cetuximab+rIL12	recruiting
NCT01935921	I	CTLA4	LA HNSCC	Ipilimumab+Cetuximab+IMRT	recruiting
NCT 01860430	I	CTLA4	LAHNSCC P16- or HR p16 +	Ipilimumab+Cetuximab+IMRT	recruiting
RTOG 3504	II	PD1	LA OPC	IMRT ± cetuximaborcisplatin+Nivolumab	In development
NCT02110082	I	CD137	RM HNSCC	Urelumab	Recruiting
CHECKMATE141	III	PD1	RM HNSCC	Nivolumab vs Cetuximab vs MTX vs TXT	Accrual completed
NCT01836029	II	TLR8	RM HNSCC	PF+cetuximab ± VTX2337	recruiting
NCT02101034	II	CDK4/6	RM HNSCC	Cetuximab+palbociclib	recruiting
NCT02124850	Ib	TLR8	Neo-LA HNSCC	Cetuximab+VTX 2337	recruiting
NCT02282371	I	PI3K	LAHNSCC	Cetuximab+BYL719+IMRT	recruiting
NCT02507154	II	autologous therapy	LAHNSCC	Autologous NK infusion+cetuximab	recruiting
NCT02052960	II	ADCC potentiation	RM HNSCC	PF+Cetuximab or CetuGEX TM	recruiting
NCT02429089	Ib	CDK4/6	RM HNSCC	LEE011+Cetuximab	recruiting
NCT02298595	II	PI3K	LAHNSCC HPV pos	BYL719, cisplatin+Cetuximab? TORS	recruiting
NCT02555644	I	CHK1/2	LAHNSCC	LY2606368+Cisplatin or cetuximab	recruiting
NCT02358031	III	PD1	RMHNSCC	Pembrolizumab+PF vs Cetuximab +PF	recruiting
NCT02105636	III	PD1	RMHNSCC	Nivolumab vs MTX vs TXT vs Cet	recruiting
NCT02551159	III	CTLA4	RM HNSCC	MEDI4736+tremelimumab vs PF Cetuximab	recruiting
NCT02216916	II	panHer2	RMHNSCC	HM781-36B	recruiting
NCT01252628	I-II	PI3K	RMHNSCC	PX866+Cetuximab	completed
NCT01552434	I-II	microenvironment	RMHNSCC	Beva+Temsirolimus+Cetuximab+Valproic Acid	recruiting

Table 1: Recent studies on anti EGFR in HNSCC.

Immunoscore

A major evidence that the outcome of tumors results from genetic and epigenetic modifications of the transformed cells but also from the interactions of the malignant cells with their tumor microenvironment. The composition of the immunological microenviroment differs widely in patients with HNSCC. Some tumors exhibit a poor infiltration by immune cells, and others are highly infiltrated by lymphocytes. Gallon et al. provided evidence that immunoscore (densities, concentrations and location of tumor infiltrating lymphocyte) correlates with prognosis; this score provides ideal targets for the intelligent design of directed preventive or anticancer therapies [38]. In the future immunoscore will probably help clinicians in treatment choices.

Conclusions

EGFR signalling influences immunity in the tumor microenviroment. Monoclonal Abs act not only inhibiting signalling pathways but also through cell-mediated cytotoxicity by innate immune cells and priming of effector cells of adoptive immunity triggered by the tumour-Ag-specific mAb. Immune system plays a key role in human oncology.

To date, no predictive biomarker for HNSCC is available in the clinic. An improved understanding of the molecular mechanisms of resistance to EGFR inhibitors may provide valuable indications to identify biomarkers that can be used clinically to predict response to EGFR blockade and to establish new treatment options to overcome resistance.

A limit of immunotherapy and immune biomarkers is the substantial difference among murine and other artificial models (3D spheroids). It is worth considering, therefore, the substantial differences between the FcR system in humans and their murine counterparts.

References

1. Vermorken JB, Specenier P (2010) Optimal treatment for recurrent/metastatic head and neck cancer. Ann Oncol 21: 252-261.
2. Iberri DJ, Colevas AD (2015) Balancing Safety and Efficacy of Epidermal Growth Factor Receptor Inhibitors in Patients With Squamous Cell Carcinoma of the Head and Neck. Oncologist 20: 1393-1403.
3. Bonner JA, Harari PM, Giralt J, Cohen RB, Jones CU (2010) Radiotherapy plus cetuximab for locoregionally advanced head and neck cancer: 5-year survival data from a phase 3 randomised trial, and relation between cetuximab-induced rash and survival. Lancet Oncol 11: 21-28.
4. Vermorken JB, Mesia R, Rivera F, Remenar E, Kawecki A, et al. (2008) Platinum-based chemotherapy plus cetuximab in head and neck cancer. N Engl J Med 359: 1116-1127.
5. Schmitz S, Bindea G, Albu RI, Mlecnik B, Machiels JP, et al. (2015) Cetuximab promotes epithelial to mesenchymal transition and cancer associated fibroblasts in patients with head and neck cancer. Oncotarget 6: 34288-34299.
6. Vermorken JB, Stöhlmacher WJ, Davidenko I, Licitra L, Winquist E, et al. (2013) Cisplatin and fluorouracil with or without panitumumab in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck (SPECTRUM): an open-label phase 3 randomised trial. Lancet Oncol 14: 697-710.
7. Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, et al. (2014) Final results from PRIME: randomized phase III study of panitumumab with FOLFOX4 for first-line treatment of metastatic colorectal cancer. Ann Oncol 25: 1346-1355.
8. Machiels JP, Subramanian S, Ruzsa A, Repassy G, Lifirenko I, et al. (2011) Zalutumumab plus best supportive care versus best supportive care alone in patients with recurrent or metastatic squamous-cell carcinoma of the head and neck after failure of platinum-based chemotherapy: an open-label, randomised phase 3 trial. Lancet Oncol 12: 333-343.
9. Garrido G, Tikhomirov IA, Rabasa A, Yang E, Gracia E, et al. (2011) Bivalent binding by intermediate affinity of nimotuzumab: a contribution to explain antibody clinical profile. Cancer Biol Ther 11: 373-382.
10. Kumar D, Kandl C, Hamilton CD, Shnayder Y, Tsue TT, et al. (2015) Mitigation of Tumor-Associated Fibroblast-Facilitated Head and Neck Cancer Progression With Anti-Hepatocyte Growth Factor Antibody Ficlatuzumab. JAMA Otolaryngol Head Neck Surg 141: 1133-1139.
11. Cleary JM, Reardon DA, Azad N, Gandhi L, Shapiro GI, et al. (2015) A phase 1 study of ABT-806 in subjects with advanced solid tumors. Invest New Drugs 33: 671-678.
12. Reilly EB, Phillips AC, Buchanan FG, Kingsbury G, Zhang Y, et al. (2015) Characterization of ABT-806, a Humanized Tumor-Specific Anti-EGFR Monoclonal Antibody. Mol Cancer Ther 14:1141-1151.
13. Stintzing S, Heinemann V (2015) Sym004: Truly a New Level of Anti-EGFR Treatment? Cancer Discov 5: 578-580.
14. Noh YH, Lim HS, Jung JA, Song TH, Bae KS (2015) Population pharmacokinetics of HM781-36 (poziotinib), pan-human EGF receptor (HER) inhibitor, and its two metabolites in patients with advanced solid malignancies. Cancer Chemother Pharmacol 75: 97-109.
15. Young MR, Wright MA, Lozano Y, Matthews JP, Benefield J (1996) Mechanisms of immune suppression in patients with head and neck cancer: influence on the immune infiltrate of the cancer. Int J Cancer 67: 333-338.
16. Hoffmann TK (2011) Immunotherapy of head and neck cancer. Identification of a novel mechanism for anti-EGFR mAb anti-tumor effects. HNO 59: 224-229.
17. Monteverde M, Milano G, Strola G, Maffi M, Lattanzio L, et al. (2015) The relevance of ADCC for EGFR targeting: A review of the literature and a clinically-applicable method of assessment in patients. Crit Rev Oncol Hematol 95: 179-190.
18. Dechant M, Vidarsson G, Stockmeyer B, Repp R, Glennie MJ (2002) Chimeric IgA antibodies against HLA class II effectively trigger lymphoma cell killing. Blood 100: 4574-4580.
19. Yang X, Zhang X, Mortenson ED, Radkevich BO, Wang Y, et al. (2013) Cetuximab-mediated tumor regression depends on innate and adaptive immune responses. Mol Ther 21: 91-100.
20. Schneider MT, Lammerts BJJ, Berger S, Rossen K, Berkel PH, et al. (2010) Human IgG2 antibodies against epidermal growth factor receptor effectively trigger antibody-dependent cellular cytotoxicity but, in contrast to IgG1, only by cells of myeloid lineage. J Immunol 184: 512-520.
21. Mellor JD, Brown MP, Irving HR, Zalcberg JR, Dobrovic A (2013) A critical review of the role of Fc gamma receptor polymorphisms in the response to monoclonal antibodies in cancer. J Hematol Oncol 6: 1-10.
22. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, et al. (2006) Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 313:1960-1964.
23. Lastwika KJ, Wilson W, Li QK, Norris J, Xu H, et al. (2016) Control of PD-L1 Expression by Oncogenic Activation of the AKT-mTOR Pathway in Non-Small Cell Lung Cancer. Cancer Res 76: 227-238.
24.
25. Tang Y, Fang W, Zhang Y, Hong S, Kang S, et al. (2015) The association between PD-L1 and EGFR status and the prognostic value of PD-L1 in advanced non-small cell lung cancer patients treated with EGFR-TKIs. Oncotarget 6: 14209-14219.
26. Sihver W, Pietzsch J, Krause M, Baumann M, Steinbach J, et al. (2014) Radiolabeled Cetuximab Conjugates for EGFR Targeted Cancer Diagnostics and Therapy. Pharmaceuticals 7: 311-338.
27. Desjarlais JR, Lazar GA, Zhukovsky EA, Chu SY (2007) Optimizing engagement of the immune system by anti-tumor antibodies: an engineer's perspective. Drug Discov Today 12: 898-910.

28. Kellner C, Peipp M (2014) Fc-optimized antibodies quickly pull the trigger. Blood 124: 3180-3181.
29. Weiner LM, Murray JC, Shuptrine CW (2012) Antibody-based immunotherapy of cancer. Cell. 148: 1081-1084.
30. Melero I, Hirschhorn CD, Morales KA, Sanmamed MF, Wolchok JD (2013) Agonist antibodies to TNFR molecules that costimulate T and NK cells. Clin Cancer Res 19: 1044-1053.
31. Lu T, Gabrilovich DI (2012) Molecular pathways: tumor-infiltrating myeloid cells and reactive oxygen species in regulation of tumor microenvironment. Clin Cancer Res 18: 4877-4882.
32. Stephenson RM, Lim CM, Matthews M, Dietsch G, Hershberg R, et al. (2013) TLR8 stimulation enhances cetuximab-mediated natural killer cell lysis of head and neck cancer cells and dendritic cell cross-priming of EGFR-specific CD8+ T cells. Cancer Immunol Immunother 62: 1347-1357.
33.
34. Chatillon JF, Hamieh M, Bayeux F, Abasq C, Fauquembergue E, et al. (2015) Direct Toll-Like Receptor 8 signaling increases the functional avidity of human CD8+ T lymphocytes generated for adoptive T cell therapy strategies. Immun Inflamm Dis 3: 1-13.
35. Luedke E, Jaime RAC, Bhave N, Roda J, Choudhary MM, et al. (2012) Cetuximab therapy in head and neck cancer: immune modulation with interleukin-12 and other natural killer cell-activating cytokines. Surgery 152: 431-440.
36. Roda JM, Joshi T, Butchar JP, McAlees JW, Lehman A, et al. (2007) The activation of natural killer cell effector functions by cetuximab-coated, epidermal growth factor receptor positive tumor cells is enhanced by cytokines. Clin Cancer Res 13: 6419-6428.
37. Aziz S, Ahmed SS, Ali A, Khan FA, Zulfiqar G, et al. (2015) Salivary Immunosuppressive Cytokines IL-10 and IL-13 Are Significantly Elevated in Oral Squamous Cell Carcinoma Patients. Cancer Invest 33: 318-328.
38. John MA (2015) Inflammatory mediators drive metastasis and drug resistance in head and neck squamous cell carcinoma. Laryngoscope 125: S1-S11.
39. Neves H, Kwok HF (2015) Recent advances in the field of anti-cancer immunotherapy. BBA Clincal 3: 280-288.
40. Galon J, Mlecnik B, Bindea G, Angell HK, Berger A, et al. (2014) Towards the introduction of the 'Immunoscore' in the classification of malignant tumours. J Pathol 232: 199-209.