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Rationale for combination of therapeutic antibodies targeting tumor cells and immune checkpoint receptors: Harnessing innate and adaptive immunity through IgG1 isotype immune effector stimulation

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Abstract

Immunoglobulin (Ig) G1 antibodies stimulate antibody-dependent cell-mediated cytotoxicity (ADCC). Cetuximab, an IgG1 isotype monoclonal antibody, is a standard-of-care treatment for locally advanced and recurrent and/or metastatic squamous cell carcinoma of the head and neck (SCCHN) and metastatic colorectal cancer (CRC). Here we review evidence regarding the clinical relevance of cetuximab-mediated ADCC and other immune functions and provide a biological rationale concerning why this property positions cetuximab as an ideal partner for immune checkpoint inhibitors (ICIs) and other emerging immunotherapies. We performed a nonsystematic review of available preclinical and clinical data involving cetuximab-mediated immune activity and combination approaches of cetuximab with other immunotherapies, including ICIs, in SCCHN and CRC. Indeed, cetuximab mediates ADCC activity in the intratumoral space and primes

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adaptive and innate cellular immunity. However, counterregulatory mechanisms may lead to immunosuppressive feedback loops. Accordingly, there is a strong rationale for combining ICIs with cetuximab for the treatment of advanced tumors, as targeting CTLA-4, PD-1, and PD-L1 can ostensibly overcome these immunosuppressive counter-mechanisms in the tumor microenvironment. Moreover, combining ICIs (or other immunotherapies) with cetuximab is a promising strategy for boosting immune response and enhancing response rates and durability of response. Cetuximab immune activity–including, but not limited to, ADCC–provides a strong rationale for its combination with ICIs or other immunotherapies to synergistically and fully mobilize the adaptive and innate immunity against tumor cells. Ongoing prospective studies will evaluate the clinical effect of these combination regimens and their immune effect in CRC and SCCHN and in other indications.

Keywords

ADCC; Cetuximab; CTLA-4; PD-L1; PD-1; Immunotherapy

Introduction

In recent years, emerging tools for targeting tumor cells via the immune system have shifted oncologists' focus away from cytotoxic chemicals and onto immunotherapy. Almost all of the functions of the immune system may have therapeutic implications, and many have already been widely studied in experimental models and in humans. Among them, antibody-dependent cell-mediated cytotoxicity (ADCC) appears to be a promising field of investigation.

Years of preclinical and clinical work have shown that immunoglobulin (Ig) G1 monoclonal antibodies (mAbs) have the highest capability for stimulating ADCC compared with other isotypes (eg, IgG2) and, furthermore, that ADCC occurs in humans treated with IgG1-based therapies [1–3]. In oncology, several commonly used therapeutic mAbs have the IgG1 backbone and are shown to stimulate ADCC, including trastuzumab (an anti–human epidermal growth factor receptor [EGFR] 2 [HER2] mAb, widely used in breast cancer) [4], necitumumab (an anti-EGFR mAb used in lung cancer), rituximab (an anti–cluster of differentiation [CD] 20 mAb used in non-Hodgkin lymphoma and chronic lymphocytic leukemia) [4], and cetuximab (an anti-EGFR mAb used in *RAS* wild-type metastatic colorectal cancer [mCRC] and locally advanced and recurrent and/or metastatic squamous cell carcinoma of the head and neck [LA and R/M SCCHN]) [4]. These mAbs have the IgG1 backbone and are thought to owe part of their antitumor activity to modulation of immune cells, especially when treating immunologically "hot" tumors [5–8]. Novel immunostimulatory therapies have made possible a new approach to combination therapy with IgG1 isotype mAbs such as cetuximab [9], namely, the synergizing of ADCC (and other possible immune actions) with additional immunomodulatory treatments.

With the emergence of immune checkpoint inhibitors (ICIs) targeting programmed death-ligand 1 (PD-L1), its receptor PD-1, and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4)–along with other immunotherapies–the possibilities for combining various

immunostimulatory drugs are now being explored in clinical trials. ICIs and other immunotherapies have been developed and are being tested in many indications. However, in SCCHN and CRC, ICI monotherapy seems associated with relatively low overall response rates (ORRs; ≤18% in R/M SCCHN and ≈0% in chromosome-unstable CRC [representing the majority of cases] [10–12]) and a lack of dramatic responses in many patients [13] compared with the more impressive ORRs of up to 57% in other advanced/pretreated indications, such as non-small cell lung cancer and melanoma [14–16]. Combination immunotherapy represents a promising approach to boost antitumor activity in indications such as SCCHN and CRC as well as any other indications suitable for immunomodulatory therapy.

As cetuximab is already an established standard of care in both SCCHN and CRC, in this manuscript we focus on cetuximab as a key example of an IgG1 therapy with clinically relevant ADCC and related immunomodulatory activities in order to explore its potential for combination with immunotherapies such as ICIs. We describe the detailed mechanisms for cetuximab-driven immune actions and summarize the available evidence for these effects in CRC and SCCHN. In addition, we provide the scientific rationale for combining ICIs/other immunotherapies with cetuximab to synergistically mobilize the adaptive and innate immune systems against tumor cells, thereby potentially improving upon durable responsiveness and patient survival in challenging indications such as SCCHN and mCRC (Fig. 1). These principles of combining immunostimulatory therapies are also likely to be of interest in indications beyond CRC and SCCHN.

Mechanism of cetuximab-driven immune activity

ADCC is a biological process that contributes to the targeting and killing of antibody-coated cells by immune cells and is triggered by IgG1 isotype mAbs in the presence of natural killer (NK) cells. Cetuximab has strong immunomodulatory activity, in part via ADCC, in addition to inhibition of the EGFR intracellular signaling pathway [17–20]. Briefly, cetuximab stimulates ADCC when its constant region, Fc, binds to a receptor found on NK cells (activating Fc receptor CD16/FcγRIII) [21], resulting in NK cell activation. Active NK cells can carry out their own lytic activity on tumor cells, and each active NK cell can serially lyse multiple target cells [22]. This is the process of ADCC. Importantly, other immune activity also results from the activation of NK cells via the interaction with the Fc region of an IgG1 isotype mAb. NK cells appear to use interferon-γ (IFNγ) and various cytokines to facilitate crosstalk with dendritic cells (DCs) and other immune cells (eg, macrophages, other NK cells). Activated NK cells that lyse tumor cells lead to the release of tumor antigens, which can be cross-presented by DCs to cytotoxic T cells, priming them for additional tumor cell killing activity [19,23–28]. Thus, the binding of an IgG1 isotype mAb to its target and to the CD16 receptor on NK cells can stimulate the priming and activation of both immune effector cells of the innate and adaptive immune systems. Additionally, cytokine-mediated crosstalk with macrophages and other immune cells is essential for bringing into the intratumoral space additional active, cytotoxic T cells, which can then carry out lytic activity on tumor cells and thus generate additional tumor antigens and further stimulate a long-term immune response [29,30]. Thus, cetuximab stimulates immunogenic tumor cell death, involving multiple cytotoxic immune cell types [29,30]. An overview of

the mechanism for mounting a cetuximab-driven, antitumor immune response is shown in Fig. 2A, and current preclinical and clinical evidence for cetuximab-driven ADCC and other mechanisms is summarized in Tables 1 and 2.

Cetuximab elicits tumor cell apoptosis via EGFR inhibition and additional tumor cell death mediated by the distinct mAb-dependent immune actions (cytotoxic T cell recruitment and priming), including mechanisms specific to its IgG1 backbone (NK cell-mediated ADCC) [6,24,31–33]. The existence of an IgG2 isotype anti-EGFR mAb, panitumumab (which does not trigger NK cell–mediated ADCC), has offered researchers the unique opportunity to compare the effects of mAb-mediated EGFR inhibition +/− the attribute of NK cell stimulation. The immunologic distinction between these 2 mAbs has been conclusively demonstrated ex vivo: when all other conditions are equal and optimized, an IgG1 anti-EGFR mAb (cetuximab) stimulates NK cell–mediated ADCC and thus increases immune-mediated tumor cell death to a greater level than does an IgG2 anti-EGFR mAb [19,29,31,34]. This difference in activity may account for the differential efficacy of the 2 mAbs sometimes observed in human patients; for example, in clinical trials, cetuximab has measurable antitumor activity (resulting in overall survival benefits) in SCCHN in combination with radiotherapy or platinum-based chemotherapy, while panitumumab was not able to demonstrate a statistically positive difference [35–38]. Tumor-antigen binding (i.e., to EGFR) and ADCC stimulation are interlinked processes, a phenomenon that may explain why ADCC appears highly relevant for antitumor efficacy in SCCHN (extremely high tumor EGFR expression, i.e., more available targets). Similarly, there are populations of patients with mCRC who may benefit more from an IgG1-based therapy than from an IgG2, potentially due to an increased sensitivity to immunostimulation, including the mechanism of ADCC; discussion follows. Indeed, this may be the case for other indications with high tumor EGFR expression, such as lung cancer [39], or for any patient with cancer who has high basal ADCC activity [2,40].

Experimentally, ex vivo and in vitro assays with patients' purified lymphocyte populations [3,19,25,26,41] from the tumor microenvironment and the peripheral blood are used to directly observe NK cell activation and lytic activity [31,42,43]. Indirect measurements are performed using markers on circulating and tumor-infiltrating T cells, NK cells, and DCs, as well as cytokine levels in the plasma [1,44], including expression of activating receptors such as CD16, CD107a, CD137, NK group 2 member D (NKG2D), and NK cell p46–related protein (NKp46) receptors [26,42,45]. Furthermore, expression of perforin and granzyme B, the functional molecules of NK cell lytic activity, also indicates high tumor cell killing, and their depletion can lead to the eventual dampening of lytic activity [22,42,45]. Conversely, increased levels of transforming growth factor β (TGFβ) or interleukin 10 (IL-10) in plasma, increased expression of CTLA-4 and PD-1 on T cells, PD-L1 expression on tumor or immune cells, or NK group 2 member A (NKG2A) receptor expression on NK cells are considered indicators of immunosuppression, and they work to downregulate NK and effector T cell cytotoxic activity [25,42,45–47]. Finally, increased frequency of $CD4^+$/forkhead box $p3^+$ ($CD4^+/Foxp3^+$) regulatory T cells (Treg), especially in the tumor microenvironment, is associated with suppressed NK lytic activity and reduction of the immune response markers mentioned previously [25,42,46,48], similarly suppressing ADCC activity. The abundance of regulatory mechanisms underline the relevance that ADCC and

other cetuximab-mediated immune activity have in tumor control and eradication, in particular by “priming” innate and adaptive immunity, as well as by inducing a tumor microenvironment that is well suited to further inhibition of ICIs, or to elimination of ICI-bearing dysfunctional lymphocytes, to stimulate better adaptive, T cell–mediated immunity.

Overall, individual patients’ basal ADCC activity, high NKp46 expression, and increased average ADCC-mediated killing have all been shown to correlate with positive clinical outcomes, including longer relapse-free survival, increased likelihood of response to therapy, and prolonged overall survival [2,3,40,45].

Collectively, these observations strongly support the conclusion that ADCC is an important component of cetuximab’s antitumor activity; more generally, studies suggest that ADCC measurement, monitoring, and targeting are of clinical importance during cancer treatment of individual patients with IgG1 isotype mAbs [5,31].

Markers for ADCC and related immune responses

Biological differences between tumor types can be overshadowed by the individual intervariability seen among patients with a given tumor type based on factors such as disease stage, age, genetic markers, and tumor biomarker expression. Individual ADCC activity and CD16 receptor alleles may be predictive for clinical outcomes in response to IgG1-based anticancer therapy [2,49,50]. Furthermore, the additive presence of high levels of baseline ADCC and EGFR expression can have a positive correlation with the rate of complete responses in patients with LA SCCHN who are treated with cetuximab and radiotherapy [40]. Also possibly having an effect on baseline ADCC activity are *KRAS* mutations (although data are conflicting with regard to directionality) [31,51,52], presence of disease (healthy volunteers mount a greater response than cancer patients), and polymorphisms in the CD32A and CD16 Fc receptors [31,33,53,54]. Because increased ability to mount an ADCC response tends to correlate with prolonged overall survival [3,33], it is important that these differences be understood and used to potentially guide personalized treatment decisions. Such information is especially crucial in the first line, when the immune system may be best poised to mount an antitumor response (given that immune depletion often occurs following chemotherapy) [55]. As of this writing, the CD16 polymorphism is the best-studied biomarker for ADCC.

CD16 is not required for endogenous NK cell–mediated tumor cell lysis, but it is necessary for IgG1-mediated ADCC [33,56], and studies suggest that increasing the binding affinity of the Fc region to CD16 can increase NK cell cytotoxic activity [57,58]. A CD16 Fc receptor that has a valine (V) at codon 158 (vs a phenylalanine [F]) has a much higher binding affinity for mAbs. Therefore, patients who carry the V/V polymorphism are more immunologically responsive to IgG1 isotype mAb-based therapy (cetuximab, rituximab, trastuzumab, etc) than patients with the F/F polymorphism; the V/F variant appears to manifest as an affinity phenotype that is intermediate between V/V and F/F or equal to V/V, depending on the study [33,49,50,53,54,59]. Downstream of CD16 activation, CD137 expression (which stimulates recruitment of EGFR-specific cytotoxic T cells to the tumor) correlated with clinical response [44,60]. Interestingly, in an analysis of 107 patients with

SCCHN who received cetuximab, no predictive value for CD16 codon 158 polymorphism was detected for anti-EGFR therapy efficacy (although only 13 patients had the V/V variant); another study in 49 patients with *KRAS* wild-type mCRC found a significant difference in outcomes among patients with different genetic variants of CD16 [26,61]. Therefore, the predictive value of CD16 remains to be fully confirmed.

An additional polymorphism associated with cetuximab immune activity is found on codon 131 (histidine [H] vs arginine [R]) of the CD32A/FcγRIIa receptor on DCs and neutrophils [25]; this polymorphism helps restore tumor immune surveillance and stimulates downstream immunogenic response. The 6-month disease control rate (DCR) was higher in patients with CRC (n = 47) treated with cetuximab and carrying the H/H and H/R variants (67% and 50%, respectively) vs the R/R variant (17%), despite all patients having a mutation in *KRAS*, *NRAS*, *BRAF*, or *PI3K* (suspected to confer resistance to cetuximab in CRC). In the same study, patients carrying the V/V or V/F variant on CD16 (31 patients; 70% of the overall study population) had a combined 6-month DCR of 52% vs 23% in patients carrying the F/F variant (n = 13) [62]. Similarly, in patients with mCRC treated with cetuximab plus irinotecan-based chemotherapy, overall survival was significantly longer in patients carrying a 158 V genotype [63]. A meta-analysis of studies of anti-EGFR mAb-based therapy in CRC (that did not distinguish between cetuximab and panitumumab, a choice that could have confounded the results) concluded that neither the CD32A nor the CD16 polymorphisms are predictive of response during therapy [64]. It should be noted that IgG2-driven immune activity may be associated with polymorphisms on CD32A [65]. Indeed, it appears that any effect of CD32A polymorphisms on baseline ADCC activity is due to linkage disequilibrium rather than direct interaction [66]. Further investigation is required to fully characterize the predictive value of CD16/32 receptor polymorphisms during immunomodulatory therapy.

Treg and other immunosuppressive mechanisms are triggered in the intratumoral space as feedback mechanisms to counteract cytotoxic tumor cell lysis [42,48,67–71]. These negative regulatory mechanisms, detailed in the next section, could become additional therapeutic targets when planning combination treatments with cetuximab.

Immune modulation of ADCC and T cells and implications for cetuximab-based treatment

Treg

An overview of the immunosuppressive pathways activated in response to cetuximab-mediated immunostimulation in the tumor microenvironment is presented in Fig. 2B [42,48,67–71]. Treg activity is one of the most powerful immunosuppressive mechanisms in the intratumoral space. Compared with those of healthy subjects, cancer patients' peripheral blood and tumor-infiltrating lymphocyte populations are enriched in Treg, possibly due to conversion from $Foxp3^{-}$ to $Foxp3^{+}$ in response to increased TGFβ signaling (based on preclinical and ex vivo studies) [48,67,68,72,73]. Treg secrete suppressive cytokines and express membrane-bound TGFβ, thus inhibiting the cytolytic activity of T cells and NK cells, as well as the maturation of DCs [42,68]. Furthermore, highly immunosuppressive $Foxp3^{+}$/CTLA-4^{+} or PD-$L1^{+}$ Treg are found to be more concentrated in the tumor

microenvironment than in the peripheral blood [48]. In the presence of increased $CD4^+$/$CD25^{hi}$/$Foxp3^+$ Treg populations in the intratumoral space, NK cells have lowered expression of biomarkers indicative of ADCC activity, such as granzyme B, perforin, and CD16 [42,46]. In vitro and ex vivo assays demonstrate that the addition of $CD4^+$/$CD25^{hi}$/$Foxp3^+$ Treg suppress cetuximab-driven NK-mediated ADCC in patients with SCCHN via secreted cytokines and membrane-bound TGFβ; TGFβ inhibitors are sufficient to block this Treg-mediated immune suppression in vitro [42,46,68,70,71]. Crucially, in vitro experiments and a phase 1a clinical trial suggest that depleting the $CD4^+$/$Foxp3^+$ Treg population can restore or enhance NK cell cytotoxic activity [68,74,75].

Furthermore, it is conceivable that Treg-mediated suppression of cetuximab-driven immune activity can potentially be a prognostic factor in patients undergoing treatment with cetuximab for LA SCCHN. Cetuximab plus chemotherapy/radiotherapy treatment in patients with LA SCCHN (n = 22) led to a significant increase in the frequency of $CD4^+$/$Foxp3^+$ Treg within lymphocyte populations in both the peripheral blood and tumor microenvironment [42]. Furthermore, 4 weeks of cetuximab monotherapy (n = 18 patients) appeared to increase the frequency of intratumoral $CD4^+$/$Foxp3^+$ Treg expressing markers of immunosuppression such as CTLA-4, CD39, and membrane-bound TGFβ. Peripheral $CD4^+$/$Foxp3^+$ Treg were significantly enriched in CTLA-4, possibly indicating a response to cetuximab-driven immunostimulation (and by extension the conversion of an immunologically “cold” tumor to a “hot” phenotype) [25,42]. When comparing the frequency of Treg in both the periphery and the intratumoral space in clinical responders to cetuximab with that of nonresponders, Jie et al. found that responders have stable Treg populations, while nonresponders have significant increases in $CTLA\text{-}4^+$ Treg within both the peripheral blood and tumor-infiltrating lymphocyte populations [42]. Similar observations regarding the correlation between Treg recruitment to the tumor microenvironment and lower patient survival have been made across multiple tumor types [72,76]. Interestingly, specifically in CRC, tumor-infiltrating Treg can have high (suppressive) vs low (nonsuppressive) Foxp3 expression, and the presence of the latter may be a positive prognostic biomarker of immune response [77]. Overall, it appears that cetuximab-driven ADCC and other immune activity initiate a negative feedback loop of immunosuppression via immune checkpoints; thus, inhibition of suppressive Treg (eg, $CTLA\text{-}4^+$ or $PD\text{-}L1^+$ populations) through ICI treatment is a logical therapeutic strategy to use in combination with cetuximab in both SCCHN and CRC [78]. In addition, experimental data underline the role of the PD-1/PD-L1 axis inhibition in the prevention of the peripherally induced Treg [79] leading to the curtailment of this cell population into the tumor microenvironment. This fact may be of high importance considering that Ghiringhelli et al. showed an inverse relationship between NK cell activation and the extension of the Treg population [68].

Other immunosuppressive mechanisms impacting cetuximab-driven immune activity

Cetuximab monotherapy results in an increased frequency of $CD107a^+$ and $CD137^+$ (i.e., active) NK cells in the tumor microenvironment of patients with SCCHN. Interestingly, cetuximab monotherapy also leads to an increased frequency of circulating vs tumor-infiltrating $perforin^+$ and granzyme B^+ NK cells [42]. As perforin and granzyme B are the

operative molecules of NK cell lytic activity [45], these findings suggest that additional immunosuppressive mechanisms are ongoing in the intratumoral space and that these mechanisms prevent NK cells in the intratumoral space from mounting degranulation and tumor cell lysis. These immunosuppressive processes are likely therapeutically targetable in a way that would further increase the antitumor effects of cetuximab-mediated immune activity (Fig. 2B). Evidence suggests that suppressive activity also occurs in patients with CRC. For example, the presence of CRC and its increasing stage both correlate with higher levels of NK cells present in the peripheral blood vs the intratumoral space; activating receptors such as NKG2D and NKp46 are decreased in expression on NK cells from patients with CRC vs healthy donors [45]. Blocking the immunosuppressive receptor CD32B on DCs, or incubation with IL-2 or IL-15, has been shown to alleviate some of this inhibition on NK lytic activity [22,25,45], and this strategy may therefore be useful in combination with cetuximab treatment in SCCHN and CRC.

Myeloid-derived suppressor cells (MDSCs) are another cell population considered an important hurdle in immunotherapy [69,80]. Their numbers increase in cancer patients vs healthy volunteers [69], and they encourage tumor immune escape by expressing high levels of TGFβ and producing IL-10 in the tumor microenvironment, its periphery, or the lymph node tissue [81]. Additionally, myeloid-derived cytokines suppress antitumor activity of T cells via C-X-C motif chemokine receptors (CXCR3 and 4, for example) [82,83]. Interestingly, the disinhibition of T cells via anti–PD-1 therapy initiates a negative feedback loop, stimulating myeloid cell production of PD-L1 and subsequent T cell reinhibition [82]. These observations suggest that ICI therapy is a good candidate to counter MDSC-mediated suppression of cytotoxic cells via PD-1/PD-L1 in the tumor microenvironment. Like Treg, MDSC development, expansion, and function can be guided by a variety of factors [84,85].

IFNγ is secreted by NK cells in response to the presence of cells coated with cetuximab and stimulates the maturation of DCs; in addition to priming cytotoxic T cells, DCs reciprocally activate NK cells to induce more IFNγ secretion. The blocking of IFNγ with a neutralizing mAb prevents crosstalk between NK cells and DCs [25,26], revealing potentially relevant mechanisms for immune escape. Incubation of human NK cells in the presence of TGFβ also suppresses CD16-mediated IFNγ secretion, and extended treatment inhibits ADCC via reduction of granzymes A and B [86]. In addition, IFNγ can signal via signal transducer and activator of transcription 1 (STAT1) and human leukocyte antigen (HLA) class I to further stimulate cytotoxic T cell activity [87]; defects in this pathway have been associated with impaired T cell–mediated lysis [88], and maintaining HLA class I levels during therapy has been correlated with improved clinical responses to cetuximab-based therapy in patients with SCCHN [87]. Interestingly, EGFR activity works to suppress this pathway, hence facilitating tumor immune escape. HLA class I can thus be upregulated via cetuximab's EGFR-inhibitory activity [87]. Therefore, cetuximab is a logical therapy in that it simultaneously promotes IFNγ secretion and EGFR blockade, both of which are processes that can counteract EGFR-mediated immunosuppression in the tumor microenvironment [87,89,90]. Finally, EGFR signaling and, interestingly, IFNγ aid in tumor immune escape by stimulating PD-L1 expression on tumor cells through the Janus kinase 2 (JAK2)/STAT1 pathway, thus inhibiting active T and NK cells in a PD-1/PD-L1–dependent manner [27,91].

Thus, cetuximab treatment could be useful in potentially priming tumors for better T cell recognition, which would then be enhanced with ICIs.

Optimizing immune action: the promise of combination between cetuximab and immunotherapy

Cetuximab has demonstrated clinically meaningful activity in both SCCHN and *RAS* wild-type mCRC; it is a vital component of the standard of care for both indications in the unresectable setting, and it yields favorable outcomes in clinical trials and in the real-world setting [35,38,92–95]. Furthermore, cetuximab promotes high response rates as evidenced by its addition to prior standard-of-care treatments (e.g., radiotherapy, chemotherapy), which has led to enhanced ORRs and prolonged survival. In addition to the benefits associated with EGFR inhibition, cetuximab-mediated ADCC and the recruitment and priming of cytotoxic T cells to the intratumoral space are powerful attributes. However, as described above, such immunostimulation is necessarily associated with negative feedback loops (Treg, MDSCs, and increased expression of checkpoint molecules such as PD-1, PD-L1, and CTLA-4). Therefore, co-targeting of these immunosuppressive processes, and the potential synergy between the different mechanisms of action of cetuximab and ICIs, holds the potential to improve patient outcomes in SCCHN and CRC. For example, CTLA-4 or PD-1/PD-L1 blockade has the potential to alleviate Treg- or MDSC-mediated inhibition on both T cells and NK cells, thereby restoring cytotoxic activity and fully mobilizing the adaptive and innate immune systems against tumor cells [96–99], because many of these immunosuppressive mechanisms impinge upon negative regulation of T cells and NK cells via PD-1, PD-L1, or CTLA-4 (Fig. 2C) [13,27,47]. As further evidence in favor of this combination of drugs, cetuximab recruits new immune cells to the tumor microenvironment, whereas ICIs disinhibit cells already present. Thus, cetuximab and ICIs complement each other, and cetuximab could serve to prime the immune system in preparation for (or counter T cell and NK cell depletion [protective effect] as a result of) ICI therapy, raising the possibility of true synergistic activity via complementary activation of the innate and adaptive immune systems and the engagement of multiple types of immune cells. Although the known safety profiles of cetuximab and ICIs do not appear to overlap, minimal safety and efficacy data are currently available from trials of cetuximab and ICI combinations. Studies assessing acute and late toxicities of cetuximab and ICI combinations are currently ongoing. On the other hand, compounding of toxicities has been observed in ICI plus ICI combination treatments, with which additive immune-related adverse events can be severe and may preclude the widespread use of dual-ICI therapy [100,101]. Next, we outline the biological rationale for the combination of cetuximab, a logical combination partner due to its various immunostimulatory effects, with emerging immunotherapies in SCCHN and mCRC (cetuximab's approved indications for use), placing special focus on ICIs.

Patients with SCCHN are good candidates for powerful immunostimulatory therapy, because such cancers' possible methods of origin are associated with an immunologically "hot" phenotype [8,13,102–106]. Additionally, the common use of radiotherapy in LA SCCHN provides a unique opportunity to combine the radiosensitizing properties and immunostimulatory activity of cetuximab with T cell disinhibition as well as the

hypothesized abscopal effect with ICIs [21]. Additionally, the combination of cetuximab and avelumab (an anti–PD-L1 IgG1 isotype mAb) is of high interest due to both agents' ability to stimulate ADCC, because the use of 2 ADCC-inducing mAbs could potentially generate a beneficial immune effect by priming and activating NK cells cooperatively.

Although CRC has traditionally been considered an immunoresistant cancer, prognostic factors such as high basal ADCC activity and the presence of tumor-infiltrating T cells suggest this presumption is inaccurate [2,24,107]. However, individual tumor molecular subtypes may be differentially susceptible to ICI monotherapy, and thus far only microsatellite-unstable tumors have shown responses to such an approach [108–110], likely because of their tendency to produce neoantigens. Therefore, research into combination therapy with ICIs plus an agent with already-proven activity in mCRC (i.e., cetuximab) is necessary to determine whether such a combination regimen would possess activity in non-microsatellite-unstable tumors.

Consequently, although CRC and SCCHN are very different diseases, cetuximab plus ICIs may still result in additive activity in CRC tumors by priming them for immunotherapy (e.g., by inducing PD-1/PD-L1 expression on immune cells and by recruiting immune effector cells to the tumor). Additionally, cetuximab can mediate increased immune activity within the tumor microenvironment (e.g., drive crosstalk between NK cells and DCs and recruit cytotoxic T cells to the tumor microenvironment) [19,23–28], thus priming the immune system to be more responsive to ICI treatment. Reciprocally, in vitro research on this combination suggests that ICIs added to cetuximab can overcome cetuximab resistance, such as that mediated by mutations in *RAS* and other genes [43].

Although we have focused on ICIs, cetuximab-mediated immune action drives crosstalk with a variety of immune cell types and processes, and therefore it holds the potential for combination with many additional classes of immunotherapy. From ex vivo studies in CRC, combination treatment with cetuximab plus cytokines such as IL-2 or IL-15 was sufficient to restore the lytic activity of patient-derived NK cells to levels comparable to those of healthy donors [45]. Similarly, in SCCHN, cotreatment with cetuximab and urelumab (a CD137-agonist mAb) in a phase 1b trial led to increased levels of granzyme B and NKp46 on NK cells, although there were no changes in IFNγ, PD-1, CD107a, NKG2D, or CD16 [44]. As mentioned earlier in this review, CD137 is a possible marker for clinical response, and urelumab treatment has been shown to lead to increased IFNγ-driven gene expression and cytokine production and overall enhanced immunologic activity [111]. Furthermore, cetuximab in combination with cytokines or urelumab was also able to exert immune activity on EGFR-expressing CRC cell lines or xenograft models despite the presence of a *KRAS*, *NRAS*, or *BRAF* mutation [43,45,60]. Similar observations have been made for cetuximab in triple-negative breast cancer xenografts and *KRAS* mutant cell lines [112,113].

Additional non-ICI agents currently in clinical trials in combination with cetuximab include monalizumab (IPH2201), an anti-NKG2A mAb that blocks this inhibitory receptor on NK cells in R/M SCCHN (NCT02643550). This combination would stimulate ADCC, inhibit the EGFR, and simultaneously disinhibit NK cells suppressed via TGFβ or IL-10. Motolimod, a toll-like receptor–8 agonist, is being tested in combination with cetuximab for

patients with R/M SCCHN (NCT01836029) and has some available early results indicating a DCR of 54% and increases in circulating cytokines [1]. Combination motolimod plus cetuximab with or without nivolumab is now being tested in patients with LA SCCHN (NCT02124850). Other combinations include systemic immunomodulation via heat-killed mycobacteria (IMM-101, NCT03009058), DNA demethylation via valproic acid (NCT02624128) that has been shown to possess antitumor effects in other indications [114], and stimulation of neutrophil growth and activity with granulocyte-colony stimulating factor (NCT02124148). Finally, several trials are investigating ex vivo–grown and –activated immune cells, including NK and $CD4^+$ or $CD8^+$ T cells, in combination with cetuximab (NCT02028455, NCT02507154) across indications. Therapies for additional novel targets, such as CXCR4 activation, MDSC inhibition, and TGFβ traps, will likely compose the next wave of combination therapies.

Key ongoing trials of cetuximab and ICI combination therapy in CRC and SCCHN are summarized in Table 3.

Conclusions and future outlook

ICI monotherapy is a new and exciting treatment option, but response rates are modest in some indications, including SCCHN and CRC. Fortunately, there is a strong scientific rationale for combining ICIs and the existing standard-of-care mAb cetuximab for the treatment of advanced SCCHN and CRC. In addition to EGFR inhibition, cetuximab mediates clinically relevant ADCC and other immune activity in the intratumoral space, which is associated with tumor cell killing by components of both the innate and adaptive immune systems. Cetuximab can prime the immune system for ICI therapy by recruiting cytotoxic cell effectors of both the innate and adaptive immune systems to the intratumoral space. Additionally, associated negative feedback loops lead to CTLA-4/PD-1/PD-L1–mediated immunosuppression of active cytotoxic cell types, an issue that ostensibly could be overcome successfully via combination therapy with ICIs. Indeed, in some situations such as non-small cell lung cancer, it has been shown that strong PD-(L)1 expression is associated with better outcomes when treated with anti–PD-(L)1. In the case of cetuximab plus avelumab, ongoing prospective studies will evaluate whether using 2 ADCC-inducing mAbs will generate a beneficial immune effect by priming and activating NK cells cooperatively. More generally, by synergistically and fully mobilizing the adaptive and innate immune systems against tumor cells, cetuximab in combination with ICIs or other immunotherapies could hold the key to raising ORRs and durability of response in challenging indications such as SCCHN and CRC. Empirical evidence from currently ongoing clinical trials that are evaluating this hypothesis is eagerly anticipated.

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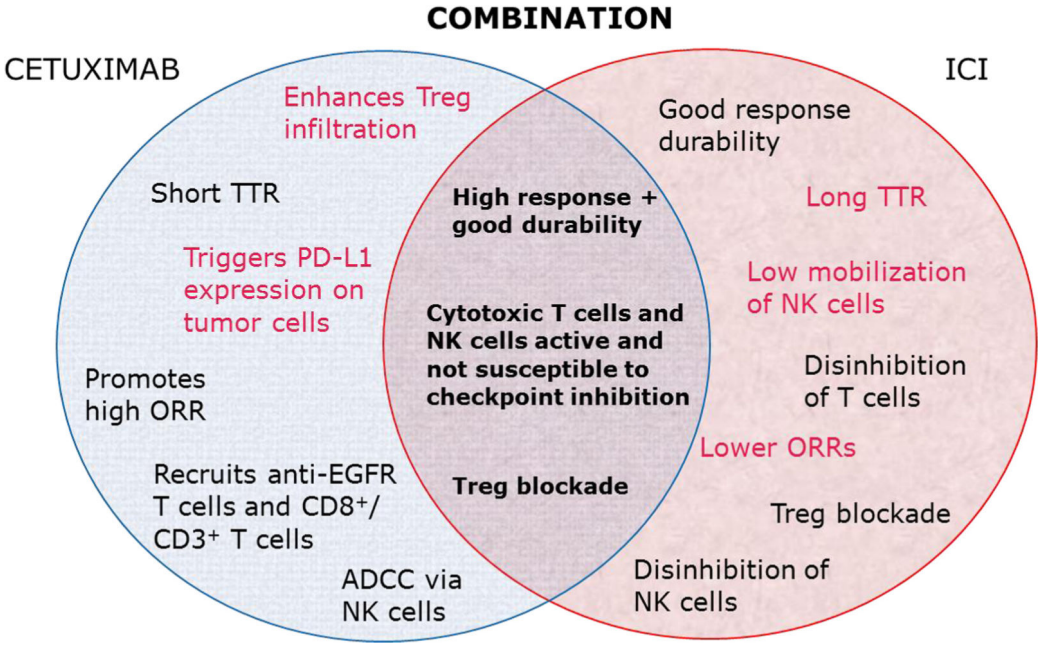

Fig. 1.

Rationale for combination therapy. Complementary and synergistic activities of cetuximab and ICI-based therapies. This Venn diagram describes the known advantages (in black) and challenges (in red) associated with the use of cetuximab and ICIs. The two therapies have complementary properties (eg, when considering TTR and mobilization of Treg), and thus, the combination of cetuximab and ICIs may yield high levels of immunostimulation and a durable response in a high percentage of patients. ADCC, antibody-dependent cell-mediated cytotoxicity; EGFR, epidermal growth factor receptor; ICI, immune checkpoint inhibitor; NK, natural killer; ORR, overall response rate; PD-L1, programmed death-ligand 1; RR, response rate; Treg, regulatory T cells; TTR, time to response. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

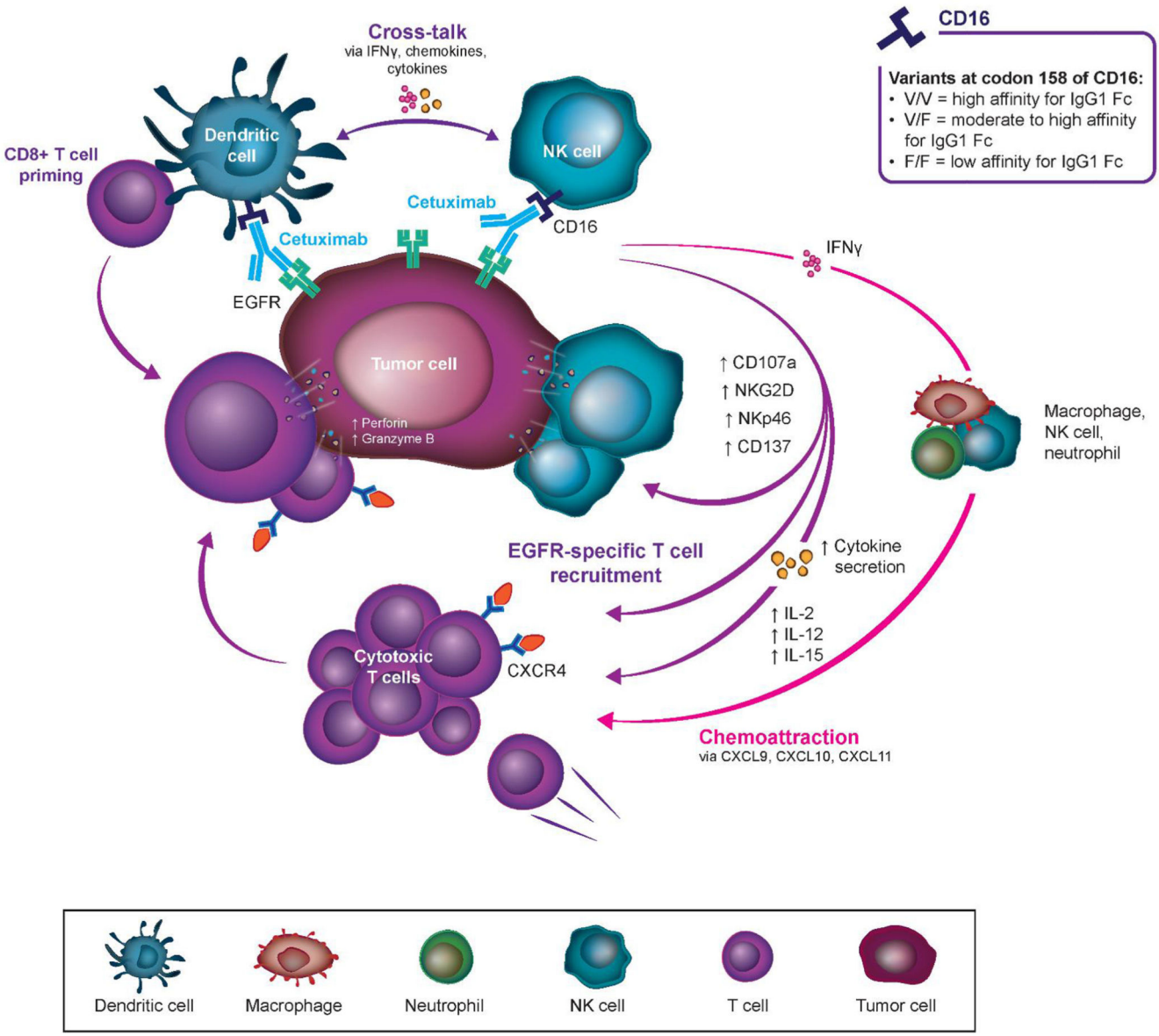

Fig. 2A.

Mechanism of cetuximab-mediated immune activity. The binding of cetuximab to EGFR and to the CD16 receptor on NK and dendritic cells sets off multiple immune actions that can lead to tumor cell targeting and death, including ADCC (innate immunity) and T cell priming (adaptive immunity). CD, cluster of differentiation; CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C chemokine receptor; EGFR, epidermal growth factor receptor; F, phenylalanine; IgG1, immunoglobulin G1; IFNγ, interferon-γ; IL, interleukin; NK, natural killer; V, valine.

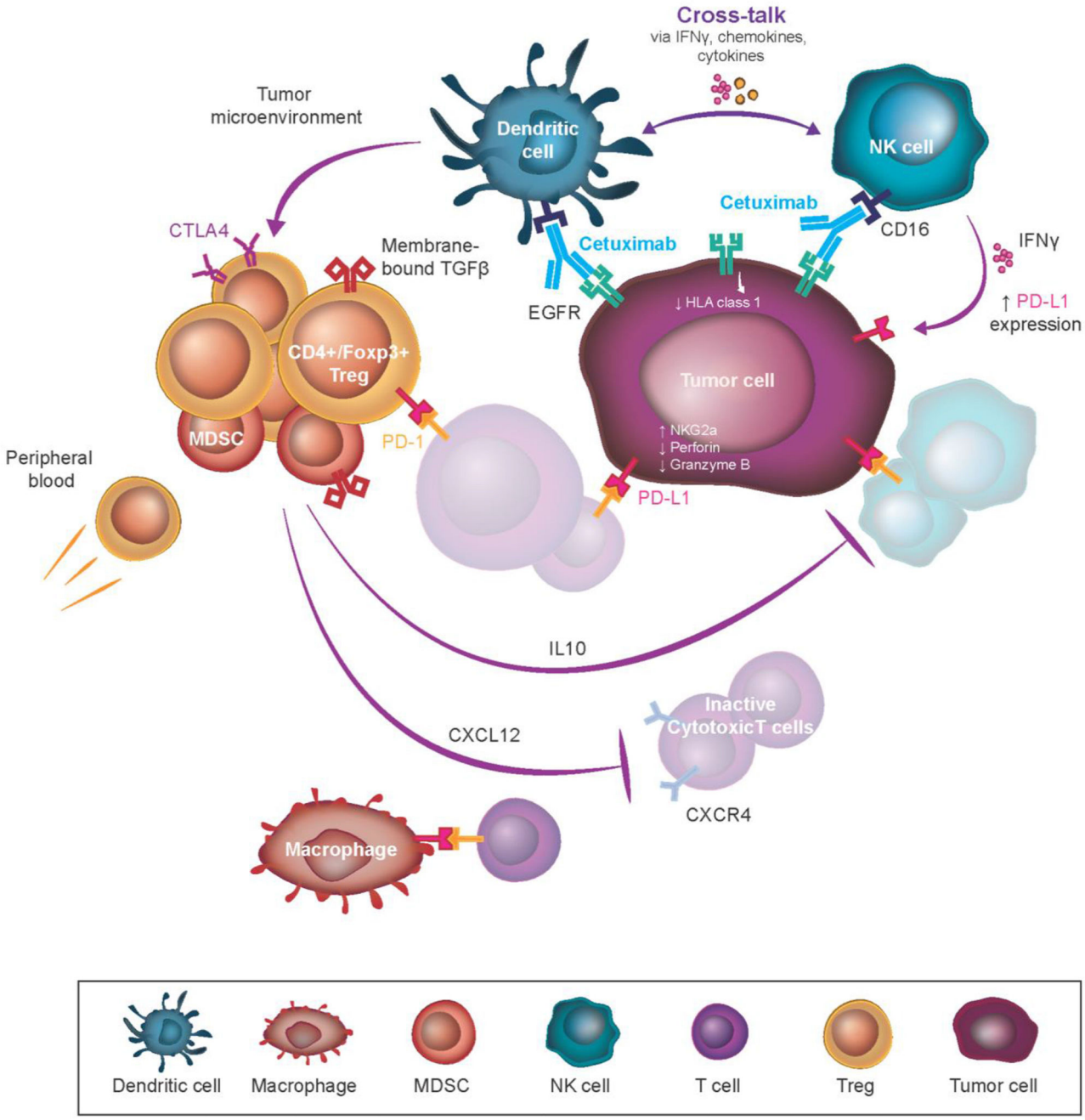

Fig. 2B.

Immunosuppressive mechanisms that can account for the dampening of cetuximab-mediated immune activity. Immunostimulatory activity initiated by the binding of cetuximab to EGFR and to the CD16 receptor on NK and dendritic cells sets off feedback immunosuppressive mechanisms, including Treg tumor infiltration and expression of immune checkpoints on tumor and immune cells. CD, cluster of differentiation; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CXCL, chemokine (C-X-C motif) ligand; CXCR, C-X-C chemokine receptor; EGFR, epidermal growth factor receptor; HLA, human leukocyte antigen; IFNγ, interferon-γ; IL, interleukin; MDSC, myeloid-derived suppressor cell; NK, natural killer;

PD-1, programmed death receptor 1; PD-L1, programmed death-ligand 1; TGFβ, transforming growth factor β; Treg, regulatory T cells.

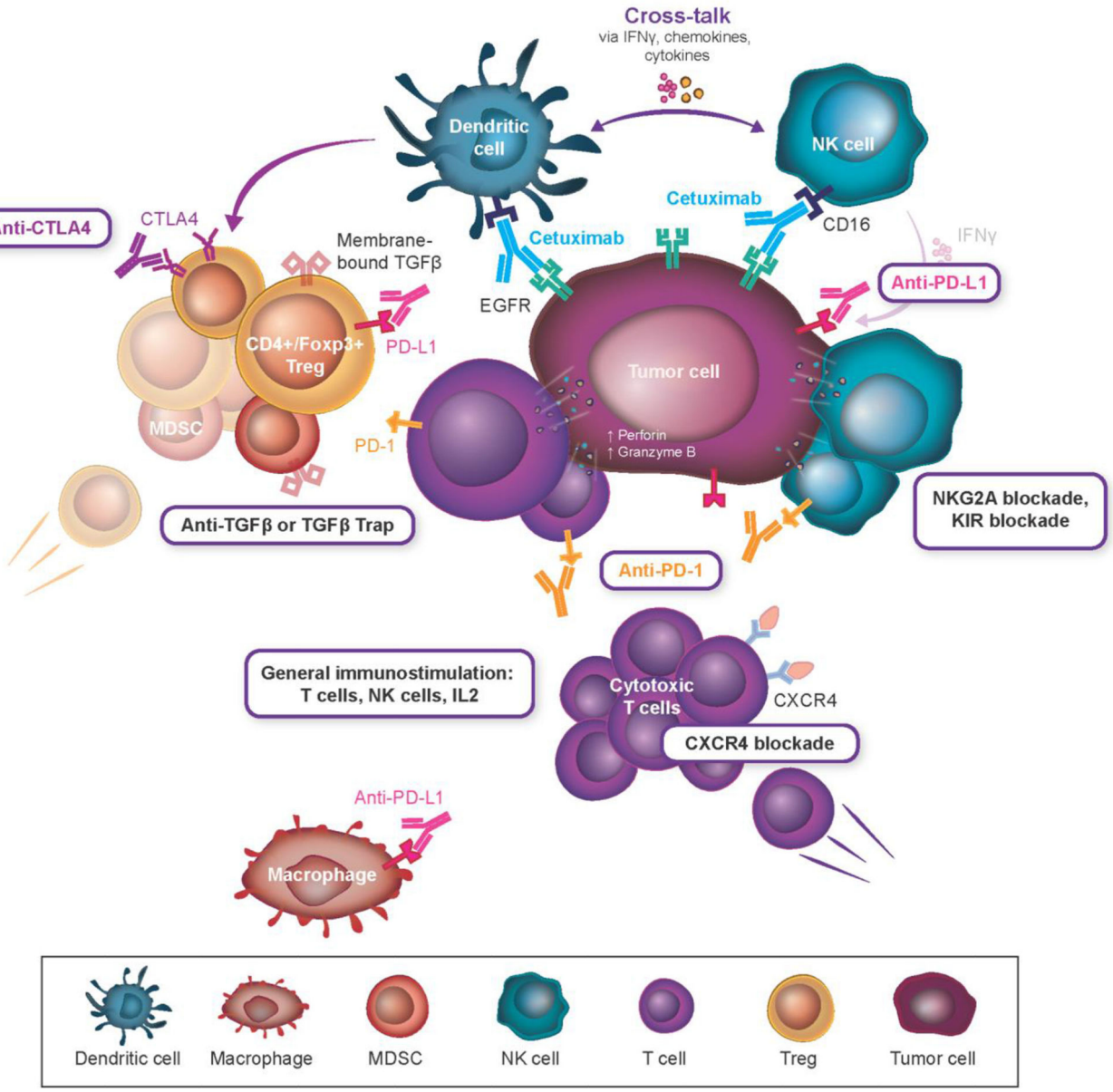

Fig. 2C.

Mechanisms of synergy between cetuximab and ICIs (or other immunotherapies). ICIs may synergize with cetuximab-driven immune activity by disinhibiting immune effector cells present in the intratumoral space. CD, cluster of differentiation; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; CXCR, C-X-C chemokine receptor; EGFR, epidermal growth factor receptor; IFNγ, interferon-γ; IL, interleukin; MDSC, myeloid-derived suppressor cell; NK, natural killer; PD-1, programmed death receptor 1; PD-L1, programmed death-ligand 1; TGFβ, transforming growth factor β; Treg, regulatory T cells.

Table 1

Preclinical evidence for cetuximab-mediated immune effects.

Relevant findings	Indication
Jie et al.: In ex vivo assays, ipilimumab can suppress CTLA-4+ Treg activity and restore cetuximab-driven, NK cell–mediated ADCC [42]	SCCHN
Khort et al.: Cetuximab treatment is associated with increased expression of CD137 on isolated human NK cells; treating with a CD137–agonistic mAb in addition to cetuximab led to increased cytotoxicity. Treating with this combination therapy led to complete tumor resolution in a murine xenograft model [60]	CRC
Kubach et al.:High-dose IgG1 anti–EGFR antibodies induce immune-independent tumor regression, but at low doses, they induce tumor cell killing through $CD8^+$ T cell–mediated ADCC [115]	SCCHN
Trivedi et al.: Panitumumab and cetuximab inhibit the EGFR to a similar extent; however, cetuximab is more effective at triggering NK cell–mediated ADCC [19]	SCCHN
Trotta et al.: Patients carrying the CD16 genotypes 158 V/V and 158 F/V experienced significantly higher ADCC activity after cetuximab treatment than did patients carrying the 158 F/F genotype. Additionally, patients carrying the V allele had longer PFS than patients who did not, although no significant difference in OS was observed [3]	CRC
Yang et al.: Cetuximab antitumor activity is more potent in the presence of adaptive immunity components, including $CD8^+$ T cells [116]	SCCHN
ADCC mechanism	
Chen et al.: In a murine xenograft model, cetuximab increased NK cell–mediated ADCC activity against colorectal cancer cells with high EGFR expression [117]	CRC
Correale et al.: 5-FU, irinotecan, and gemcitabine individually and in combination can induce increased EGFR expression in colorectal tumor cells and increase susceptibility to cetuximab-driven ADCC [118]	CRC
Levy et al.: HLA-E can inhibit cetuximab-driven ADCC and thus interfere with cetuximab-driven immune cell–mediated lytic activity against tumor cells [119]	CRC
Pozzi et al.: Cetuximab in combination with chemotherapy induces immunogenic cell death in EGFR-expressing colorectal cancer cells [30]	CRC
ADCC modulation	
Kondo et al.: EGFR expression levels on tumor cells may influence sensitivity to cetuximab-driven ADCC [120]	SCCHN
Nakadate et al.: Cetuximab-driven, perforin-independent ADCC was observed against colorectal cancer cells only if they have wild-type *KRAS* status [121]	CRC
Taylor et al.: Cetuximab-driven ADCC can kill SCCHN cells in vitro, and is highest in patients carrying a V allele at position 158 on CD16 [122]	SCCHN
Veluchamy et al.: Cetuximab enhances NK cell–mediated lytic activity on EGFR-expressing tumor cells in a CD16–, but not *RAS* mutational status–dependent manner [43]	CRC
Seo et al.: Cetuximab enhances peripheral mononuclear cell–mediated ADCC on EGFR-expressing tumor cells independently of *RAS* or *BRAF* mutational status–dependent manner [123]	CRC
Srivastava et al.: Cetuximab in combination with CD137 agonist mAb urelumab led to increased NK cell survival, DC maturation, and tumor antigen cross-presentation in patients in a phase 1b study [44]	SCCHN

5-FU, 5-fluorouracil; ADCC, antibody-dependent cell-mediated cytotoxicity; CD, cluster of differentiation; CRC, colorectal cancer; CTLA-4, cytotoxic T-lymphocyte-associated protein 4; DC, dendritic cell; EGFR, epidermal growth factor receptor; F, phenylalanine; HLA, human leukocyte antigen; Ig, immunoglobulin; mAb, monoclonal antibody; NK, natural killer; OS, overall survival; PFS, progression-free survival; SCCHN, squamous cell carcinoma of the head and neck; Treg, regulatory T cell; V, valine.

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Table 2

Clinical and in-human, ex vivo evidence for cetuximab-mediated immune effects.

Study	Indication
Bertino et al. A phase I trial to evaluate antibody-dependent cellular cytotoxicity of cetuximab and lenalidomide in advanced colorectal and head and neck cancer. Mol Cancer Ther 2016;15(9):2244–50	CRC
Bibeau et al. Impact of Fc{gamma}RIIa-Fc{gamma}RIIIa polymorphisms and KRAS mutations on the clinical outcome of patients with metastatic colorectal cancer treated with cetuximab plus irinotecan. J Clin Oncol 2009;27:1122–9	CRC
Chow et al. Phase 1b trial of the toll-like receptor 8 agonist, motolimod (vtx-2337), combined with cetuximab in patients with recurrent or metastatic SCCHN. Clin Cancer Res 2016. pii: clincanres.1934.2016	SCCHN
Inoue et al. Cetuximab strongly enhances immune cell infiltration into liver metastatic sites in colorectal cancer. Cancer Sci 2017;108(3):455–60	CRC
Jha et al. Potentiation of cetuximab by inhibition of Tregs in metastatic squamous cell cancers of head and neck. Anticancer Res 2014;34:5975–7	SCCHN
Khort et al. Targeting CD137 enhances the efficacy of cetuximab. J Clin Invest 2014;124:2668–82	SCCHN
Lo Nigro et al. Evaluation of antibody-dependent cell-mediated cytotoxicity activity and cetuximab response in KRAS wild-type metastatic colorectal cancer patients. World J Gastrointest Oncol 2016;8:222–30	CRC
Negri et al. Role of immunoglobulin G fragment C receptor polymorphism-mediated antibody-dependant cellular cytotoxicity in colorectal cancer treated with cetuximab therapy. Pharmacogenomics J 2014;14:14–9	CRC
Rocca et al. Phenotypic and functional dysregulated blood NK cells in colorectal cancer patients can be activated by cetuximab plus IL-2 or IL-15. Front Immunol 2016;7:413	CRC
Srivastava et al. CD137 stimulation enhances cetuximab-induced natural killer: dendritic cell priming of antitumor T-cell immunity in patients with head and neck cancer. Clin Cancer Res 2017;23(3):707–16	SCCHN
Direct link between ADCC and treatment outcomes	
Etienne-Grimaldi et al. Multifactorial pharmacogenetic analysis in colorectal cancer patients receiving 5-fluorouracil-based therapy together with cetuximab-irinotecan. Br J Clin Pharmacol 2012;73(5):776–85	CRC
Inoue et al. FcgammaR and EGFR polymorphisms as predictive markers of cetuximab efficacy in metastatic colorectal cancer. Mol Diagn Ther 2014;18:541–8	CRC
Jie et al. CTLA-4+ regulatory T cells are increased in cetuximab treated head and neck cancer patients, suppress NK cell cytotoxicity and correlate with poor prognosis. Cancer Res 2015;75:2200–10	SCCHN
Jie et al. Increased PD-1+ and TIM-3+ TILs during cetuximab therapy inversely correlate with response in head and neck cancer patients. Cancer Immunol Res 2017;5(5):408–16. doi: 10.1158/2326-6066.CIR-16-0333	SCCHN
Lattanzio L. Elevated basal antibody-dependent cell-mediated cytotoxicity (ADCC) and high epidermal growth factor receptor (EGFR) expression predict favourable outcome in patients with locally advanced head and neck cancer treated with cetuximab and radiotherapy. Cancer Immunol Immunother 2017 Feb 14. doi: 10.1007/s00262-017-1960-8. [Epub ahead of print]	SCCHN
Monteverde et al. 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 2015;95(2):179–90	Review
Rodriguez et al. Fc gamma receptor polymorphisms as predictive markers of cetuximab efficacy in epidermal growth factor receptor downstream-mutated metastatic colorectal cancer. Eur J Cancer 2012;48:1774–80	CRC
Trotta et al. Prospective evaluation of cetuximab-mediated antibody-dependent cell cytotoxicity (ADCC) in metastatic colorectal cancer patients predicts treatment efficacy. Cancer Immunol Res 2016;4:366–74	CRC
Zhang et al. FCGR2A and FCGR3A polymorphisms associated with clinical outcome of epidermal growth factor receptor expressing metastatic colorectal cancer patients treated with single-agent cetuximab. J Clin Oncol 2007;25:3712–8	CRC

Table 3

Key ongoing clinical trials of cetuximab plus another immunotherapy.

Study	Indication	Arms	Endpoints	Institution
NCT02764593 (Phase 1)	LA SCCHN	Nivolumab + cisplatin Nivolumab + high-dose cisplatin Nivolumab + cetuximab Nivolumab + IMRT	Dose-limiting toxicity	Radiation Therapy Oncology Group
NCT02124850 (Phase 1)	LA SCCHN (stage II-IVA)	Motolimod + cetuximab Motolimod + cetuximab + nivolumab	Change in immune biomarkers Antitumor response	University of Pittsburgh Medical Center
NCT01860430 (Phase 1b)	LA SCCHN (stage III-IVB)	Cetuximab/IMRT + ipilimumab	Determining starting dose Clinical response, PFS, potential biomarkers	University of Pittsburgh Cancer Center
NCT01935921 (Phase 1)	High-risk LA SCCHN (stage III-IVB)	Cetuximab/IMRT + ipilimumab	Determining starting dose Clinical response, PFS, potential biomarkers, dose response	National Cancer Institute
NCT02938273 (Phase 1)	LA SCCHN (stage III-IV)	Cetuximab/RT + avelumab	Safety ORR, changes in tumor microenvironment	The Netherlands Cancer Institute
NCT02999087 (Phase 3 REACH trial)	LA SCCHN (stage III-IVb)	Cetuximab/RT + avelumab vs Cisplatin/RT (fit patients) OR cetuximab/RT (unfit patients)	PFS OS, safety	Groupe Oncologie Radiothérapie Tête et Cou
NCT03051906 (Phase 1b DUCRO HN)	LA SCCHN	Cetuximab/RT + durvalumab	PFS Safety	Azienda Ospedaliero-Universitaria Careggi
NCT02643550 (Phase 1, 2)	Pretreated R/M SCCHN	Cetuximab + monalizumab	Safety ORR	University of Pennsylvania
NCT02318901 (Phase 2)	Advanced SCCHN Advanced mCRC (*KRAS/BRAF/NRAS* wt)	Pembrolizumab + cetuximab	Determining recommended dose Safety, ORR, OS, PFS, changes in circulating DNA	Western Regional Medical Center/Cancer Treatment Center of America
NCT02713373 (Phase 1/2)	Unresectable mCRC	Pembrolizumab + cetuximab	Safety, PFS, tumor response rate ORR, OS, changes in blood biomarkers and tumor-immune cell populations	Roswell Park Cancer Institute
NCT03174405 (Phase 2 AVETUX-AIO KRK 0216)	Untreated mCRC (*RAS/BRAF* wt)	Avelumab + cetuximab + FOLFOX	Safety, ORR, PFS, OS, translational research	AIO-Studien-gGmbH

IMRT, intensity-modulated radiotherapy; LA SCCHN, locally advanced squamous cell carcinoma of the head and neck; mCRC, metastatic colorectal cancer; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; R/M SCCHN, recurrent and/or metastatic squamous cell carcinoma of the head and neck; RT, radiotherapy; wt, wild-type.