STAT1-Induced HLA Class I Upregulation Enhances Immunogenicity and Clinical Response to Anti-EGFR mAb Cetuximab Therapy in HNC Patients

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Abstract

The goal of this study was to characterize the molecular mechanisms underlying cetuximab-mediated upregulation of HLA class I antigen-processing machinery components in head and neck cancer (HNC) cells and to determine the clinical significance of these changes in cetuximab-treated HNC patients. Flow cytometry, immunohistochemistry, and chromatin immunoprecipitation (ChIP) assays were performed using HNC cells treated with cetuximab alone or with Fcγ receptor (FcγR)-bearing lymphocytes to establish the mechanism of EGFR-dependent regulation of HLA APM expression. A prospective phase II clinical trial of neoadjuvant cetuximab was used to correlate HLA class I expression with clinical response in HNC patients. EGFR blockade triggered STAT1 activation and HLA upregulation, in a src homology-containing protein (SHP)-2-dependent fashion, more prominently in HLA-B/C than in HLA-A alleles. EGFR signaling blockade also enhanced IFNγ receptor 1 (IFNAR) expression, augmenting induction of HLA class I and TAP1/2 expression by IFNγ, which was abrogated in STAT1−/− cells. Cetuximab enhanced HNC cell recognition by EGFR-specific CTLs, and notably enhanced surface presentation of a non-EGFR peptide (MAGE-3271–280). HLA class I upregulation was significantly associated with clinical response in cetuximab-treated HNC patients. EGFR induces HLA downregulation through SHP-2/STAT1 suppression. Reversal of HLA class I downregulation was more prominent in clinical responders to cetuximab therapy, supporting an important role for adaptive immunity in cetuximab antitumor activity. Abrogating EGFR-induced immune escape mechanisms and restoring STAT1 signaling to reverse HLA downregulation using cetuximab should be combined with strategies to enhance adaptive cellular immunity. Cancer Immunol Res; 3(8); 936–45. ©2015 AACR.

Introduction

The mitogenic activity of the EGFR has provided the rationale for the development of inhibitory strategies to block EGFR signaling, using tyrosine kinase inhibitors (TKI) and EGFR-targeted mAbs. This strategy has been shown to be effective, since the EGFR-specific mAb cetuximab has been approved by the FDA for head and neck cancer (HNC) and colorectal cancer (1), and yet no biomarker of clinical activity has been determined. EGFR signaling also influences the expression of immunologically relevant molecules in HNC cells, including STAT1-mediated HLA and antigen-processing machinery (APM) components (2–4), implying an important impact on adaptive immunity due to EGFR overexpression. However, the precise mechanism, functional effect(s), and clinical significance of these findings have not yet been determined.

Mechanism(s) of HLA class I APM component deficiency are still not clear, despite the importance of avoidance of lysis by cytotoxic T lymphocytes (CTLs; refs. 5–9). Overexpression of EGFR, its ligands, and concomitant downstream signaling facilitates HNC proliferation by activating multiple pathways (10). Previously, we demonstrated the reversal of HLA class I and APM component deficiency in HNC using the STAT1 agonist IFNγ, which enhanced CTL-mediated lysis and induced a higher level of peptide:HLA class I complexes (6, 11–14). EGFR antagonism can also increase expression of HLA class I (14, 15) and proinflammatory cytokines (16). We have recently shown that SHP2, which operates downstream of EGFR and dephosphorylates pSTAT1, plays an important role in HLA-induced immune escape in HNC (17). Thus, we evaluated whether the EGFR–SHP2–STAT1 pathway might regulate HLA downregulation in HNC. The clinical significance of EGFR-induced HLA class I downregulation is important, because recently induction of anti-EGFR T cells has been demonstrated in cetuximab-treated HNC patients (18, 19), supporting the crucial role for tumor cell
downregulation of HLA antigen presentation by EGFR in evading CIL elimination. Thus, the goal of this study was to investigate the mechanism by which EGFR activation inhibits STAT1 activation as well as the HLA class I APM pathway and resulting adaptive antitumor immunity. We also exploited EGFR inhibition in cetuximab-treated HNC patients as a strategy by which this immune escape mechanism can be counteracted, linking HLA upregulation with clinical response in a novel phase II trial of neoadjuvant cetuximab therapy.

Materials and Methods

Cell lines

JHU-022, JHU-028, and JHU-029 were a kind gift from Dr. James Rocco (Harvard Medical School, Boston, MA) in January 2007. SCC90, PCI-13, and PCI-15B were isolated from patients treated at the University of Pittsburgh Cancer Institute (Pittsburgh, PA) through the explant/culture method, authenticated, and validated as unique using STR profiling every 6 months (20, 21). 93-VU-147 T was a kind gift from Dr. Henning Bier (Technische Universität München, Munich, Germany) in October 2013. MCF-7 was a kind gift from Dr. Soldano Ferrone (Massachusetts General Hospital, Harvard Medical School, Boston, MA) in December 2012. 

Patients and specimens

All patients signed an informed consent approved by the Institutional Review Board (IRB #939-06). Peripheral venous blood samples were obtained from HNC patients with stage III/IVA disease (Table 1), receiving neoadjuvant cetuximab on a prospective phase II clinical trial (UPCI 08-013, NCT 01218048). Tumors were biopsied immediately before, and again after 4 weeks of cetuximab therapy. Clinical response was analyzed by comparing paired CT scans pre/post-cetuximab, and quantifying tumor measurement by a dedicated head and neck radiologist blinded to patient status. Anatomic tumor measurements were recorded on June 17, 2017. © 2015 American Association for Cancer Research. cancerimmunolres.aacrjournals.org Downloaded from cancerimmunolres.aacrjournals.org on June 17, 2017. © 2015 American Association for Cancer Research.
**Figure 1.**

Cetuximab-mediated EGFR inhibition differentially enhances expression of HLA class I alleles and APM components in a STAT1-dependent fashion. JHU-029 HNC cells were left untreated or were treated for 48 hours with the EGFR inhibitor mAb cetuximab (10 μg/mL). Levels of HLA-A alleles or HLA-B/C alleles were determined by FACS (A) or by qPCR (B), and levels of surface β2-m were measured by FACS (C). HNC cells were left untreated or were treated for 48 hours with the mEGF (10 ng/mL), and levels of HLA class I (mAb W6/32) were determined by FACS (D). The levels of intracellular LMP2, TAP1, TAP2, tapasin, calnexin, and calreticulin (E) were measured by FACS. F, levels of HLA-A and HLA-B/C were evaluated in parental 2FTGH (STAT1+/+) and derivative U3A (STAT1−/−) cells after treatment with EGFR siRNA plus cetuximab or control siRNA. In Fig. IG, levels of intracellular LMP2, TAP1, TAP2, surface HLA-B/C, and calnexin were examined in 2FTGH (STAT1+/+) and U3A (STAT1−/−) cells. MFI values (EGFR siRNA-control siRNA) were determined by FACS. H and I, cetuximab-induced STAT1 binding to the GAS element (IFNγ activation site) of the TAP1 promoter was measured using a chromatin immunoprecipitation (ChIP) assay. JHU-029 cells were treated with cetuximab (10 μg/mL) for 30 minutes or 36 hours, IFNγ (10 U/mL) and cetuximab plus IFNγ (10 μg/mL, 10 U/mL) and enhanced binding of STAT1 at TAPI promoter was determined by ChIP assay. Results represent mean ± SEM from three independent experiments; *, P ≤ 0.05; **, P ≤ 0.01; ***, P ≤ 0.001; ****, P ≤ 0.0001. MFI, mean fluorescence intensity.

primer (Cell Signaling Technology, Inc.) was used to amplify canonical sequence in the TAP1 promoter for STAT1 binding.

**51Cr cytotoxicity assay**

Cytotoxicity was determined using a 4-hour 51Cr release assay. Untreated and IFNγ-treated JHU-029 HNC cells were incubated in 100 μL of RPMI-1640 media with 25 μCi of Na51CrO4 (PerkinElmer) for 1 hour at 37°C and resuspended in RPMI-1640 media. Cells were washed (twice) and plated at indicated effector/target ratio (E/T ratio 40:1) in U-bottom 96-well plates. Cetuximab (10 μg/mL), anti-HLA class I mAb (50 μg/mL) was added in natural killer (NK) cell: JHU-029 coculture. Plates were incubated for 4 hours at 37°C in a 5% CO2 atmosphere. Controls for spontaneous (cells only) and maximal lysis (cells treated with 5% Triton-X) were included. Each reaction was performed in triplicate. Supernatants (50 μL) were collected and analyzed with a PerkinElmer 96-well plate gamma counter. Results were normalized with the formula of specific lysis = (experimental lysis − spontaneous lysis)/(maximum lysis − spontaneous lysis) × 100.
Control siRNA: 5'-AGUACAGACAAAGGAIACGGt-3' (s) and 5'-CCGUAUCGUUUGCUGUACUt-3'.

Statistical analysis
Data were analyzed statistically using GraphPad Prism 4.0. A two-tailed unpaired or paired t test was used to calculate whether observed differences were statistically significant, defined as P < 0.05.

Results
Cetuximab-mediated EGFR inhibition differentially enhances expression of HLA class I alleles and APM components in a STAT1-dependent fashion
First, we determined the effect of EGFR signaling on the level of expression of two distinct alleles, HLA-A or HLA-B. Interestingly, incubation of HNC cells with the EGFR inhibitor cetuximab (10 µg/mL, 48 hours) led to increased HLA-B expression (~2-fold for protein and 9-fold increased transcription), to a greater extent than the expression of HLA-A (1.25-fold protein level, 2-fold increase in transcription; Fig. 1A and B). Similar upregulation was observed with B2-m expression after EGFR inhibition (Fig. 1C). Although EGFR inhibition did not enhance free surface HLA-A heavy chains (HCA-2 mAb), elevated levels of free HLA-B/C heavy chains (HC-10 mAb) were observed (Supplementary Fig. S1A). Similarly, EGF stimulation inhibited HLA class I expression, inducing inhibition of HLA-B to a greater extent than of HLA-A (Fig. 1D and Supplementary Fig. S1B). In addition to upregulating HLA class I molecules, cetuximab treatment enhanced levels of intracellular APM components LMP2, TAP1/2, tapasin, calnexin, and calreticulin (Fig. 1E).
Figure 3. Cetuximab treatment increases IFNγ receptor 1 expression. A and B, JHU-029, PCI-15B, JHU-028, JHU-022, EGFR+/− HNC cells, and MCF-7 EGFR+/− breast cancer cells were incubated with cetuximab (10 μg/ml, 72 hours), and levels of IFNγ receptor α chain (CD121). A and C, EGFR expression were determined by FACS. Results represent mean ± SEM from three independent experiments: *P ≤ 0.05; **P ≤ 0.001; MFI, mean fluorescence intensity.

We next found that EGFR inhibition led to preferential upregulation of HLA-B/C versus HLA-A molecules, but only in STAT1+/− cells (Fig. 1F). Similar results were observed for expression in APM components LMP2, TAP1/2, and calnexin, indicating the STAT1 dependence of EGFR-driven HLA/APM downregulation (Fig. 1G). Furthermore, EGFR siRNA increased STAT1 activation in STAT1+/− cells, but not in STAT1−/− cells (Supplementary Fig. S1C–S1D). Consistent with these findings, the STAT1 inhibitor fludarabine (20 μmol/L; refs. 23, 24) abrogated the effect of treatment of HNC cells with cetuximab, IFNγ, or cetuximab plus IFNγ, while not affecting EGFR expression (Supplementary Fig. S1E). A chromatin immunoprecipitation (ChIP) assay confirmed that cetuximab indeed induced binding of STAT1 to the TAP1 promoter (GAS element), providing physical evidence for transcriptional activation of APM pathway genes (Fig. 1H and I and Supplementary Fig. S1F).

Inhibition of the EGFR–SHP2 pathway induces STAT1 activation

Having shown that EGFR can regulate HLA expression in a STAT1-dependent fashion, we hypothesized that HLA downregulation due to autocrine or paracrine EGFR activation may drive SHP2 phosphatase activation and resulting STAT1 suppression (13, 17). To investigate this possibility, we treated JHU-029 HNC cells with cetuximab (10 μg/ml, 24 hours) and found that cetuximab treatment significantly decreased SHP2 expression, whereas IFNγ treatment (10 IU/ml) as a positive control had no effect on SHP2 expression. The combination of cetuximab and IFNγ reduced SHP2 levels, when compared with untreated HNC cells (Supplementary Fig. S2A). We then measured the expression of p-STAT1 (Tyr701) and total STAT1 after cetuximab alone or plus IFNγ treatment. After cetuximab treatment, a slight increase in the level of p-STAT1 (Tyr701) was observed (from 1% to 7% p-STAT1−/− cells), whereas a more prominent increase in the level of total STAT1 was observed (~2.7-fold higher mean fluorescence intensity vs. untreated). IFNγ treatment strongly increased expression of both p-STAT1 (Tyr701; 1%–62%) and total STAT1 (~7.9-fold increase in MFI vs. untreated). Interestingly, cetuximab treatment augmented the ability of IFNγ to induce p-STAT1 (from 62% to 81% positive cells) and total STAT1 (~12-fold higher MFI vs. untreated; Fig. 2A–C). A similar observation was confirmed with immunoblotting (Supplementary Fig. S2B) and STAT1 transcript analysis (Supplementary Fig. S2C). We also evaluated STAT1 and HLA expression after co-inhibiting SHP2 and EGFR (using siRNA or cetuximab) to bypass EGFR. SHP2 phosphatase depletion using siRNA (17) enhanced cetuximab-induced p-STAT1 (Tyr701), as well as total STAT1, expression suggesting that high SHP2 expression after coinhibiting SHP2 and EGFR (using siRNA) or cetuximab treatment strongly enhanced cetuximab-mediated STAT1 upregulation (Fig. 2D–F and Supplementary Fig. S2D).

Cetuximab treatment increases IFNγ receptor 1 expression

Because EGFR signaling might downregulate IFNγ receptor1 (IFNγRI; ref. 25), providing a mechanism for synergistic effects of EGFR blockade with IFNγ for STAT1-mediated HLA upregulation, we evaluated levels of IFNγRI after cetuximab treatment. Indeed, cetuximab increased the expression of IFNγRI in several HNC cell lines tested, in an EGFR-dependent fashion (Fig. 3A–C). To test the hypothesis that EGFR density is important in regulating IFNγRI, we used cetuximab and EGFR siRNA to abolish EGFR-proximal signaling (Supplementary Fig. S2E), which independently showed an increase of IFNγRI expression (Supplementary Fig. S2F). As expected, the combination of cetuximab treatment plus EGFR siRNA knockdown showed the most pronounced effect on IFNγRI downregulation (Supplementary Fig. S2F).

Cetuximab-activated NK cells and IFNγ increase expression of HLA class I APM pathway

Because IFNγ increases expression of HLA class I and APM components in HNC cells, and because NK cells secrete IFNγ after recognizing cetuximab-coated HNC cells in the tumor microenvironment (18, 26), we considered the impact of cetuximab-activated NK cells during coculture with HNC cells (19, 27). Under these conditions, even more robust expression of HLA-A and HLA-B/C was observed (Fig. 4A), particularly when both NK cells and cetuximab were present. As shown, IFNγ released from cetuximab-activated NK cells further evoked HLA-A and HLA-B/C upregulation, because an IFNγ-neutralizing Ab abrogated the beneficial effect of NK-cell treatment in both cases (Fig. 4A). An IgG2, anti-EGFR mAb panitumumab failed to activate NK cells under similar conditions (19). Again, HLA-B/C alleles showed a more pronounced enhancement after cetuximab treatment or by cetuximab-activated NK cells (~1.93-fold and ~1.78-fold induction), in comparison with...
HLA-A. In support of a common pathway, the STAT1 inhibitor fludarabine (23) abrogated HLA-A and HLA-B/C upregulation in response to cetuximab, EGFR siRNA, or IFNγ treatment (Fig. 4B). We further evaluated the contribution of IFNγ-induced HLA class I expression to NK-cell–mediated antitumor effects (Fig. 4C). Cetuximab-mediated antibody-dependent cellular cytotoxicity (ADCC) was significantly enhanced against IFNγ-treated HNC targets, and blocking HLA class I with W6/32 mAb (pan-HLA class I mAb) augmented cetuximab-mediated ADCC (Fig. 4D).

SHP2 inhibition robustly enhances cetuximab-mediated tumor antigen presentation

Next, we evaluated the combined effect of cetuximab and IFNγ on the expression of free HLA-A (HCA-2 mAb) or free HLA-B (HC-10 mAb), surface HLA-A and HLA-B/C, surface pan-HLA class I (HLA-A/B/C), or β2-m. Cetuximab alone increased HLA-A expression by approximately 1.25-fold, in comparison with an approximately 1.45-fold increase for HLA-B/C (Fig. 5A); IFNγ alone increased HLA-A expression by approximately 3.9-fold in comparison with an approximately 6.9-fold increase for HLA-B/C when compared with that of no treatment. The most prominent upregulation of HLA-A and HLA-B/C (~5 fold and ~9.5 fold vs. untreated, P < 0.0001) was observed when the combination of IFNγ and cetuximab was used. We also found greater HLA-B versus HLA-A allele transcripts after treatment with cetuximab, with IFNγ alone, or with cetuximab plus IFNγ (Fig. 5B and Supplementary Fig. S3A–S3E).

Reversal of STAT1 Immune Escape by Cetuximab in HNC

Figure 4. Cetuximab-activated NK cells and IFNγ increase expression of the HLA class I APM pathway. A, JHU-029 HNC cells were cultured alone, or JHU-029 plus NK cells in coculture (1:1 ratio) were left untreated or were treated for 48 hours with panitumumab (IgG2, 10 µg/mL), cetuximab (IgG1, 10 µg/mL). Levels of HLA-A (left) or HLA-B/C (right) were determined by FACS. In parallel, polyclonal anti-IFNγ Ab (10 µg/mL) was added at indicated conditions to determine the effect of IFNγ released from cetuximab-activated NK cells. B, HNC cells were pretreated with fludarabine (20 µmol/L), and after EGFR siRNA or cetuximab treatment (48 hours, 10 µg/mL) levels of surface HLA-A, HLA-B/C were determined by FACS. C, JHU-029 HNC cells were cultured alone or were treated with IFNγ (10 U/mL, 36 hours), and enhanced levels of HLA class I (mAb W6/32) were verified with FACS. D, NK cell cytotoxicity (4-hour 51Cr release assay, 40:1 E/T ratio) against untreated or IFNγ pretreated HNC targets (C), were independently evaluated in presence of mAb W6/32 (50 µg/mL), cetuximab (10 µg/mL) or combination of mAb W6/32 plus cetuximab. The ratio of NK cell cytotoxicity against untreated HNC targets, and IFNγ-treated HNC targets is shown. Results represent mean ± SEM from three independent experiments; *, P < 0.05; **, P < 0.001; ***, P < 0.0001. MFI, mean fluorescence intensity.

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Because cetuximab treatment enhanced the expression of LMP2, and TAP1/2 (Fig. 5C and Supplementary Fig. S5A–S5B), we investigated whether the enhanced HLA class I APM components enhanced surface presentation of tumor antigens (TA). We used a novel mAb (12b6), recognizing the HLA-A2:MAGE-3271–279 complex (Supplementary Fig. S5A–S5B), to quantitatively measure levels of surface HLA-TA complexes. Cetuximab enhanced the HLA-A2:MAGE-3271–279 complex (P < 0.001; Fig. 5D and E), which was even more robust after IFNγ treatment (P < 0.0001). Interestingly, the combination of cetuximab and IFNγ treatment evoked the highest level of HLA-A2:MAGE-3271–279–peptide complexes (P < 0.0001). Indeed, the combination of SHP2 siRNA and cetuximab treatment strongly enhanced cetuximab-induced HLA-A and HLA-B/C expression, most prominently in the latter alleles (Fig. 5F and Supplementary Fig. S5C). SHP2 depletion in HLA-A2+ HNC cells also enhanced HLA-A2:MAGE-3271–279–peptide presentation after cetuximab treatment (Fig. 5G and Supplementary Fig. S5D), whereas no binding was observed in HLA-A2− or TA− HNC cells (Supplementary Fig. S5E–S5F).

**Cetuximab neoadjuvant therapy enhances expression of HLA class I in HNC patients**

In a novel phase II prospective clinical trial, tumors from HNC patients were biopsied before and after 4 weeks of single-agent neoadjuvant cetuximab therapy. HLA class I expression was measured semiquantitatively using IHC and digital image analysis, and correlated with clinical response by paired pre/post CT scans to identify clinical "responders." After cetuximab therapy,
both HLA alleles and STAT-1 were upregulated in the clinical responders ($n = 7$) but not in nonresponder HNC patients ($n = 17$; Fig. 6A–D).

### Discussion

In HNC, low levels of HLA class I and APM component expression preclude effectiveness of CTL responses in mediating tumor elimination (11), and this mechanism of immune escape is a consequence of diminished STAT1 activation generated by the overexpression of SHP2 (17). Multiple pathways are linked with SHP2 functions in HNC, primarily the EGFR–SHP2 pathway. Because of the frequent overexpression of EGFR, which is a poor prognostic factor in HNC, constitutive activation of this pathway may greatly facilitate an “immune-escape” phenotype through suppression of p-STAT1–mediated expression of the HLA–APM pathway. This study sheds light on the mechanism(s) responsible for the diminished TA processing and presentation due to suppression of STAT1 and HLA class I APM components in HNC, which may be reversed through EGFR blockade, IFNγ release due to cetuximab-activated NK cells, or both. The effect is likely to have a beneficial impact on the clinical course of the disease in HNC patients treated with cetuximab.

Recently, we have shown that in HNC patients, cetuximab induces cross-priming of EGFR-specific CTLs by NK–DC cross-talk (18, 19). However, the determinants of TA recognition by CTLs
may benefit cetuximab-mediated clinical responses. Intriguingly, processing and presentation of HLA class I peptide complex is an intricate process (28), and polymorphism in HLA class I alleles (29), their differential levels, and the dynamic role of APM components represent important immune-escape mechanisms from adaptive immunity in cancer (19, 29). Using a novel HLA-A2:MAGE-3–specific mAb, we demonstrated quantitatively enhanced TA presentation in HNC cells, which is critical for CTL lysis. Thus, the likelihood of generation of a greater repertoire of TAs (‘antigen spreading’) appears to result from EGFR blockade using cetuximab, perhaps due to IFNγ-induced antigen presentation along with upregulated HLA alleles. Enhanced recognition of the peptide–HLA-A2 complex using the combination of cetuximab and IFNγ could be monitored diagnostically as a measure of Th1-biased immune responses. However, in light of superior restoration of HLA-B with cetuximab, greater characterization of HLA-B–restricted TAs is also warranted, particularly during cetuximab-based immunotherapy. These effects could be overcome by a greater drop in HLA class I expression after EGFR treatment of HNC cells. The effect of HLA upregulation on reduced cetuximab-mediated ADCC supports a moderate impact of NK-cell inhibitory, killer immunoglobulin–like receptors (KIR) and the tumor cell/HLA class I interaction during cetuximab-mediated ADCC.

Polymorphism of HLA class I alleles may play a dominant role in regulating NK-cell effector function. Interaction of a few NK-cell inhibitory receptors with a specific HLA allele plays an important role in NK-cell–mediated antitumor responses. Inhibitory KIRs have higher affinity for HLA-B than for HLA-A (29). This suggests the strong possibility that HLA-B, which interacts with KIR, may have a negative impact on NK-cell stimulation (29), and HNC may become more resilient to further NK-cell attack (30, 31). HLA-B is most often loaded with antigenic peptides (32). In the HNC cell lines used in our study, IFNγ induced HLA-B/C more strongly than HLA-A (32, 33), a result that had been rarely reported previously. This observation reflects the fact that EGFR signaling in combination with p-STAT1 suppression by SHP2 has overwhelmingly negative effects on immunogenic TA-peptide presentation, abrogating the generation, loading, and presentation on surface HLA class I TA peptide complexes, which are necessary for CTL lysis.

Cetuximab induces EGFR–specific CTL responses in some HNC patients (18), whereas clinical response to cetuximab is only observed in a subset of patients (~20%; refs. 34, 35). The effects of concerted antitumor immune responses involving NK cells, NK–DC cross-talk, and CTL responses (18, 19, 36, 37), along with upregulation of HLA class I and IFNRI, may contribute to response to cetuximab therapy. Indeed, we observed STAT1, HLA class I upregulation in cetuximab-treated patients in a novel neoadjuvant trial, suggesting that EGFR inhibition and/or IFNγ release contributes to the reversal of HLA downregulation. Results from recent studies also indicate an immunosuppressive effect of EGFR signaling on STAT1-dependent HLA class I and CIITA genes (38, 39). However, HLA class I and CIITA are modulated by both total STAT1 and p-STAT1 (40, 41), and a recent report also indicates that EGFR inhibitors reduce PD-L1 expression in lung tumors (42), suggesting multiple immune-escape mechanisms mediated by EGFR signaling.

Disclosure of Potential Conflicts of Interest

R.L. Ferris is a consultant/advisory board member for AstraZeneca, Bristol-Myers Squibb, and Merck. No potential conflicts of interest were disclosed by the other authors.

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